

## Breeding System, Flower Visitors and Seedling Survival of Two Endangered Species of *Helianthemum* (Cistaceae)

JAVIER RODRÍGUEZ-PÉREZ\*

Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Miquel Marqués, 21, E-07190 Esporles, Mallorca, Balearic Islands, Spain

Received: 27 October 2004 Returned for revision: 19 January 2005 Accepted: 25 February 2005 Published electronically: 8 April 2005

- **Background and Aims** *Helianthemum marifolium* and *H. caput-felis* are two endangered plant species of the western Mediterranean. Several aspects of the reproduction of both species were examined to determine whether their rarity could be related to factors causing reproductive limitation.
- **Methods** The flowering and fruiting phenology of both species in two non-sympatric island populations (Mallorca, Balearic Islands, western Mediterranean) were compared. Hand-pollination experiments were conducted to determine their fruit and seed production under different pollen sources. The composition of the pollinator assemblage and the effect of temporal variation and sun exposure on reproductive output and seedling survival were also investigated.
- **Key Results** Flowering periods were longer for *H. marifolium* than for *H. caput-felis* in the populations studied. *Helianthemum marifolium* is mostly an outbreeder, i.e. fruit and seed set was three-fold higher when pollen came from other plants. In *H. caput-felis*, neither fruit nor seed set was affected by pollination treatments. Flower visitors were more diverse for *H. caput-felis* than for *H. marifolium*. In both species, most floral visits were made by hymenopterans. The total number of pollinator visits varied significantly between years, decreasing more than two-fold from 2001 to 2002, in both species. In agreement with its outbreeder character, variation in reproductive output between years was also observed in *H. marifolium*. It showed a 50 % decrease in fruit set in 2002, a pollinator-poor year. Finally, seedling survival increased three- to six-fold from 2001 to 2002. A correlation between seedling size and survival had also been detected.
- **Conclusions** Reproductive limitations were detected for neither species (i.e. fruit and seed set, pollination service and seedling survival on natural populations). Hence, the increasing rarity of these two species is probably a direct result of the destruction of their habitat.

**Key words:** Breeding system, conservation, *Helianthemum*, pollinator assemblage, reproductive output, seedling survival, threatened species, western Mediterranean.

### INTRODUCTION

Information on the reproductive biology of endangered plants is crucial for predicting their survival capacity and establishing the appropriate measures for their conservation (Schemske *et al.*, 1994; Delanoë *et al.*, 1996; Bernardello *et al.*, 2001). Such studies may help to identify the factors that reduce the reproductive capacity of individual plants and the subsequent maintenance or regeneration of populations.

Successful plant propagation generally involves three consecutive events that are modulated by three different factors: pollination, seed dispersal (including seed predation) and seedling establishment. Pollination and seed dispersal often depend on mutualistic interactions with animals (Herrera and Pellmyr, 2002). As such, a reduction in pollinator service can directly affect reproductive output, decreasing the quantity and/or quality of fruit and seed set (Ågren, 1996) and promoting selfing in self-compatible species (Erhardt and Jäggi, 1995; Washitani, 1996; Traveset *et al.*, 1998). In addition, seed dispersal may be limited, thus decreasing the probabilities of seed germination, escape from seed predators and/or seedling establishment (Rey and Alcántara, 2000; Traveset *et al.*, 2003). In a broad

sense, an increase in selfing rate or a reduction of seed dispersal results in a reduction in gene flow within and/or among populations and may thus cause an increase in inbreeding depression (O'Brien, 1994; Buza *et al.*, 2000). Finally, seedling survival is considered one of the most critical stages in the life cycle (not only for threatened species) in Mediterranean and arid ecosystems (Escudero *et al.*, 1999; Rey and Alcántara, 2000; Traveset *et al.*, 2003). In such habitats, seedling survival depends strongly on biotic interactions (such as competition and facilitation; Callaway and Walker, 1997) as well as on abiotic factors (e.g. stochastic rainfall patterns; Turner, 1990).

Habitat fragmentation caused by human disturbance is currently considered one of the main factors reducing population viability of rare plants (Schemske *et al.*, 1994). Reduced size may subsequently affect the performance of small plant populations (Kearns *et al.*, 1998). In the Mediterranean basin in particular, over the last 30 years, coastal ecosystems have suffered a massive reduction of size and quality as a consequence of the disproportionate urban development (Greuter, 1995). This process is particularly accentuated in the Balearic Islands due to dramatically increasing tourism (Mus, 1995).

The present work focuses on the study of two endangered plant species of *Helianthemum* (Cistaceae).

\* For correspondence. E-mail jrodriguez@uib.es

*Helianthemum marifolium* (L.) Mill. is a small shrub distributed along the east and south Iberian Peninsula and on the Balearic Islands. All existing populations in the Balearic Islands belong to an endemic subspecies, *H. marifolium* subsp. *origanifolium* (Lam.) G. López (Alomar *et al.*, 1997). Such populations are located in coastal areas, mainly in the Pityusic islands (Eivissa and Formentera), at up to 100 m a.s.l., with a single population in the south-west of Mallorca. This population is close to Palma, the largest city of the island, and it is severely threatened by urban development. *Helianthemum marifolium* subsp. *origanifolium* and its habitat are protected by local and regional laws, and it is considered as 'vulnerable' according to the International Union for the Conservation of Nature (IUCN) (Alomar *et al.*, 1997).

The second species, *H. caput-felis* Boiss., is a rare small shrub distributed throughout the western Mediterranean (Balearic Islands, eastern Iberian peninsula, Sardinia, south Italy and North Africa). In the Balearics it is located only at coastal sites, in the south of Mallorca (Alomar *et al.*, 1997). Pujol (2001) recently reported a dramatic reduction of the Iberian Peninsula populations which he attributed to the direct pressure of coastal urban development. Fortunately, all Balearic populations are located in protected areas, making its local conservation status more favourable. *Helianthemum caput-felis* is included in the Annex of the Berne Convention (1991) and it is protected by European legislation (Habitats Directive, European Community 1992). It is categorized as 'rare' by the IUCN (Alomar *et al.*, 1997).

This paper aims to identify the critical stages in the life history of these two endangered species. The work of the present paper was included as a possible recovery plan for *H. marifolium*. Besides, the comparison with another related species, *H. caput-felis*, could be interesting because *a priori* both species may share the same reproductive limitations. Both species are related phylogenetically and live in similar habitats (Alomar *et al.*, 1997). The following questions are specifically addressed: (a) What is the flowering phenology of these two species? Does it vary between them? (b) Does the reproductive output of both species change under different pollen sources? (c) Does the pollinator assemblage and visitation rate differ between the two species? (d) Do biotic (plant traits) and abiotic factors (sun exposure and year) influence their reproductive potential and seedling survival? The data gathered in this study will be useful for designing more adequate management and conservation practices for these two species.

## MATERIALS AND METHODS

### Study species

*Helianthemum marifolium* subsp. *origanifolium* and *H. caput-felis* are perennial shrubs, with a height up to 35 cm. Flowers are arranged in inflorescences at the tip of new branches. For a summary of floral traits see Table 1. Flowers are yellow and hermaphroditic, open at dawn and close at dusk, and have short lifespans (2–3 d for *H. marifolium* and 3–4 d for *H. caput-felis*; pers. obs.). The anthers are sensitive to touch and spread outwards when

TABLE 1. Floral traits of *H. marifolium* and *H. caput-felis*

Floral traits	<i>H. marifolium</i>		<i>H. caput-felis</i>	
	<i>n</i>	Mean $\pm$ s.e.	<i>n</i>	Mean $\pm$ s.e.
No. of inflorescences	50	16.9 $\pm$ 2.4	50	85.0 $\pm$ 12.3
Flowers/inflorescence	50	7.3 $\pm$ 1.4	50	3.0 $\pm$ 1.0
Flower mass (mg)	45	7.3 $\pm$ 0.4	50	52.8 $\pm$ 1.9
No. of petals	45	4.9 $\pm$ 0.0	50	5.0 $\pm$ 0.0
Petal length (mm)	45	3.93 $\pm$ 0.06	50	7.94 $\pm$ 0.05
No. of sepals	45	3.0 $\pm$ 0.0	50	5.0 $\pm$ 0.0
Sepal length (mm)	45	3.23 $\pm$ 0.05	50	6.91 $\pm$ 0.05
No. of stamens	45	35.4 $\pm$ 1.0	50	46.6 $\pm$ 1.0
Stamen length (mm)	45	1.70 $\pm$ 0.04	50	3.64 $\pm$ 0.04
Anther length (mm)	45	0.46 $\pm$ 0.01	50	0.49 $\pm$ 0.01
No. of ovules	31	10.2 $\pm$ 1.2	49	6.0 $\pm$ 0.0
Gynoceum length (mm)	45	1.78 $\pm$ 0.05	50	4.80 $\pm$ 0.06
Ovarium length (mm)	45	1.04 $\pm$ 0.02	50	1.44 $\pm$ 0.03

Sample size (*n*), plant mean ( $\pm$ 1 s.e.) are given for each trait.

Flowers/inflorescence was determined on a sample of up to 50 inflorescences per plant. Ovule number was estimated for at least 15 flowers per plant. All other measures were taken on one fresh flower per plant. Plant means for flowers/inflorescence and ovule number were averaged for each plant and then across all plants. Flower mass was measured as fresh mass.

the flower is visited by pollinators (pers. obs.), a trait shown by other species in the genus (Proctor *et al.*, 1996). For both species, the main floral reward to pollinators is pollen (Herrera, 1992; Proctor *et al.*, 1996). Fruits are capsules that detach at maturation. Germination takes place in autumn, at the onset of the rainy season (pers. obs.). Seed germination varies widely for both species, being quite high for *H. marifolium* (73 %; Pons, 2002), but very low for *H. caput-felis* (4 %; Tébar *et al.*, 1997), despite the high seed viability of the latter (96 %; Tébar *et al.*, 1997). The low germination of *H. caput-felis* has been attributed to physical exogenous dormancy (impermeable coat), a widespread trait among the Cistaceae (Thanos *et al.*, 1992).

### Study sites

The population of *H. marifolium* is located in a protected area called Es Carnatge, just outside the city of Palma de Mallorca (39°33'N, 2°42'E) and contains approx. 250 adult plants. The annual precipitation (starting in September of the previous year) was 576 and 974 mm for 2001 and 2002, respectively (data from the Balearic Meteorological Institute). This population is found in a relictual thyme scrubland (approx. 200 m<sup>2</sup> in area) that stands on a fixed dune. Vegetation is dominated by *Thymbra capitata*, *Satureja microphylla* ssp. *rodriguezii*, *Fumana thymifolia* ssp. *laevis*, *Cistus clusii*, *Micromeria filiformis* and *H. marifolium*. In 2001, the flowering period of *H. marifolium* overlapped with 19 co-occurring plant species.

The population of *H. caput-felis* is located in a dune system near the town of Sa Ràpita, also in the south of Mallorca, and approx. 30 km south-east of Es Carnatge (39°22'N, 2°58'E). The annual precipitation (same period as above) was 588 and 1201 mm for 2001 and 2002, respectively. Vegetation is dominated by *Juniperus phoenicea* and *Pinus halepensis*; *H. caput-felis* occurs in clearings,

accompanied by *Helicrysum dunense* and *Cistus clusii*. In 2001, *H. caput-felis* flowered simultaneously with one co-occurring species (*Cistus clusii*).

#### Flowering and fruiting phenology

On 15 March 2001, 45 plants of *H. marifolium* and 59 of *H. caput-felis* were tagged in natural populations. An inflorescence was randomly chosen on each plant and periodically examined (every 2–3 d in *H. marifolium* and 3–4 d in *H. caput-felis*) to record the number of receptive flowers (those with petals remaining in the flower). For *H. caput-felis*, most inflorescences produce fewer than three flowers (Table 1), so inflorescences with at least three flowers were tagged, in order to quantify more accurately the flowering phenology.

#### Breeding system

The reproductive biology of both species was examined in 2002 under controlled conditions. In October 2001, nine and eight adult plants of *H. marifolium* and *H. caput-felis*, respectively, were transplanted into pots filled with soil from the original localities and moved to an experimental greenhouse (free of pollinators) in Esporles (Mallorca). On each plant, four to 26 inflorescences (depending on availability on each plant) were randomly tagged and only one of the treatments assigned to each inflorescence (i.e. to every single flower on that inflorescence). To each treatment and species, one to seven inflorescences were assigned (both species), using  $10.0 \pm 0.5$  and  $2.9 \pm 0.1$  (mean  $\pm$  s.e.) flowers per treatment and plant (*H. marifolium* and *H. caput-felis*, respectively). The experiment was conducted as a complete block design, with the treatments nested within each plant. The treatments were (1) no manipulation (control or spontaneous self-pollination); (2) hand-pollination with pollen of the same flower (induced self-pollination), (3) hand-pollination using pollen from the same plant, but from another flower (individual geitonogamy) and (4) hand-pollination using pollen from a different plant (xenogamy). All flowers used for treatments 3 and 4 were emasculated before they had become receptive. Hand-pollinations were performed once on each a flower as it became receptive.

Fruits from all treatments were harvested as soon as they ripened (mainly during May) and dissected in the laboratory. In both species, and especially for *H. caput-felis*, both developed and undeveloped fruits remain attached to the inflorescence for long periods of time (pers. obs.), hence dissection was necessary to distinguish developed and empty fruits with certainty. To avoid the effect of inflorescence variation, fruits from all inflorescences within each individual plant and treatment were pooled, i.e. within-plant variation among inflorescences was ignored.

#### Flower visitors

Direct observations of pollinators of *H. marifolium* and *H. caput-felis* were made on three different dates in 2001 and 2002 (with a total of 4.75 and 18.0 h for *H. marifolium* and 12.5 and 12.25 h for *H. caput-felis*, for 2001 and 2002,

respectively), to determine the pollinator assemblage and the rate of flower visitation by the different visitors. Observations were made on a total of 42 and 85 plants of *H. marifolium* and *H. caput-felis*, respectively. For each individual plant, observation hours always varied across different observation days. Each observation was mostly performed on sunny days, and lasted for 15 min, during which the following were recorded: (a) the pollinator species, (b) the number of visits made by each pollinator species, (c) the number of contacted flowers per visit, (d) the flower handling time (only for flying pollinators) and (e) exposure of the plant to sun ('sun' or 'shade'). At the end of each census, the number of receptive flowers per plant was noted. Nocturnal observations were not performed because flowers of both species are closed at night.

#### Factors determining reproductive potential

A total of 50 plants of each species of *Helianthemum* were tagged on 15 March 2001, coinciding with those most used for phenology. For each plant, the plant size (average between the maximum and its orthogonal crown diameter) was measured to the nearest centimetre and recorded sun exposure ('sun' vs. 'shade'). Fruits of each plant ( $17.6 \pm 2.0$  for *H. marifolium* and  $26.5 \pm 1.2$  for *H. caput-felis*, respectively) were collected to determine fruit and seed set in the laboratory. Fruit set was estimated afterwards by dissecting fruits and counting the total of developed fruits in relation to collected fruits. Developed fruits were then used to measure: (a) fruit mass (to the nearest 0.1 mg), (b) seed mass (mean mass of one seed), (c) fruit length (to the nearest 0.01 mm) and (d) seed set (i.e. number of seed per total number of ovules), since aborted ovules are easily distinguishable in both species. In 2002, total inflorescence and flower production per plant were also measured in both species (using a subsample of 50 inflorescences per plant to estimate flower production in *H. caput-felis*).

#### Seedling survival

In February 2001, 200 seedlings of *H. marifolium* and 240 of *H. caput-felis* were individually tagged in their natural populations, their maximum crown size and number of leaves measured, and their exposure to sun ('sun' vs. 'shade') recorded. As the age of the seedlings could not be determined, seedlings with more than ten leaves were not considered in order to exclude seedlings that were possibly 2 or more years old (pers. obs.). Seedling survival was determined once in October 2001. In February 2002, a new cohort of 130 seedlings of *H. marifolium* and 214 of *H. caput-felis* (14 and 19 of them respectively had been tagged in 2001) were individually tagged; seedling survival was determined in November 2002.

#### Data analyses

The pattern of flowering phenology of both species was tested using failure-time analysis. A Cox proportional hazard regression model was fitted to the number of days between inflorescence tagging and the opening (receptivity) of each one of the flowers on the inflorescence. Using the



parametric accelerated failure-time model, results were identical. Each individual plant was added as a random or 'frailty' effect in the model. Ties were estimated using the efron method, using the program S-Plus 2000 (Mathsoft, 1999).

Unless otherwise stated, all further analyses were performed using the GENMOD procedure of SAS 9.0 statistical package (SAS Institute, 2000). Differences between both species of *Helianthemum* in the number of receptive flowers at flowering peak was assessed by fitting a generalized linear model (GLIM) with a Poisson distribution and a logarithmical link function. Deviances from the model were scaled using the root-square of the ratio deviance/degrees of freedom to correct the over-dispersion of data.

The effects of the different pollination treatments on fruit and seed set within each plant (i.e. proportion of flowers producing fruits, and the proportion of ovules producing seeds within each fruit) were analysed separately for each species, using GLIMs with binomial error distributions and logit link functions. As all pollination treatments were applied to each individual plant, a repeated measures design was used, with individual plant as random factor and treatment as (within-subject) fixed factor. For seed set, aborted fruits (without seeds) were excluded for further calculations and analyses. Whenever significant differences between treatments were detected, multiple pairwise contrasts were performed, using likelihood ratio statistics and Bonferroni corrections.

Differences in the number of pollinator visits and the number of flowers visited per visit per plant were assessed using GLIMs, with poisson distributions and a logarithmical link functions. Deviances from the model were scaled as above. For the number of visits, pollinator group, year and sun exposure were included as fixed factors and the number of receptive flowers per plant as continuous covariates. For the number of flowers visited per pollinator visit, pollinator group was included as a fixed factor and the number of receptive flowers as a continuous covariate. Flower handling-time per pollinator visit was tested using GLIMs, with normal distribution and a logarithmical link function. The independent variables were: pollinator taxon for *H. caput-felis*; year for *H. marifolium*. Only plants that were visited by pollinators were considered for this analysis. In all the previous analyses for pollinators, only differences between orders of flying insects were considered (Diptera vs. Hymenoptera), in order to have a big enough sample size. In these analyses each plant species was analysed separately.

Difference in reproductive traits in field conditions between years and sun exposure (fixed and independent variables) were tested separately for each plant species. Mean plant size was included as a covariate, performing a previous analysis to detect a homogeneity of slopes between independent variables and the covariate. These analyses were assessed with GLIMs, using binomial distribution and logit link functions for fruit and seed set, and normal distributions and logarithmical link functions for the rest of the variables (fruit mass, seed mass and fruit length). For further references to these analyses applied in an ecological context, see Herrera (2000).

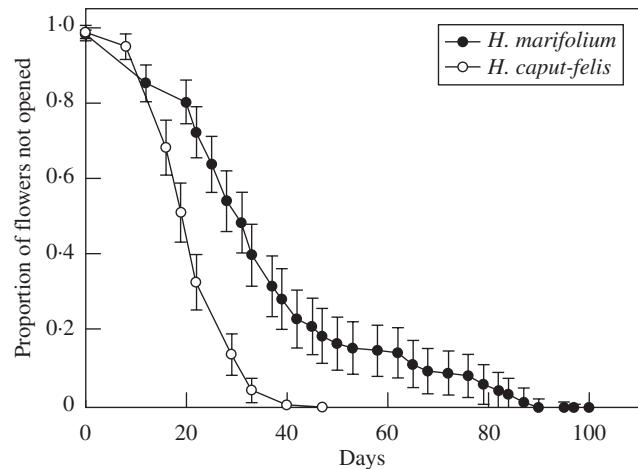


FIG. 1. Survivorship curves of the flowering pattern of *H. marifolium* and *H. caput-felis*, showing the cumulative proportion of receptive flowers that are last to open on census days (mean  $\pm$  s.e.). Census day was considered as the number of days between the tagging day of each inflorescence (for each species) and the opening of each one of the flowers on each inflorescence. Survival curves were fitted using the Kaplan–Meier estimation.

GLIMs were also used to analyse the potential effect of seedling size (number of leaves and maximum crown), year and sun exposure on seedling survival, using a binomial response variable and a logit link function. To choose between both continuous variables and evaluate whether (and which type) a model with heterogeneous slopes was required, all possible models were fitted using the various combinations of these variables and their interactions, and the best model selected based on their AIC (Akaike information criterion; estimates the variation explained by the model but penalizes for the number of parameters estimated by the model; see Akaike, 1973). For this best model, significant factor effects based on type III likelihood-ratio tests were reported. These analyses were performed using STATISTICA 6.0 (Statsoft Inc., 2001) because this program can select the best model based on their AIC.

Unless otherwise stated, average values are reported as mean  $\pm$  standard error ( $\pm 1$  s.e.).

## RESULTS

### Flowering and fruiting phenology

*Helianthemum marifolium* at Es Carnatge started flowering on 24 February and ended on 4 June, whereas *H. caput-felis* at Sa Ràpita began flowering on 25 March and finished by 11 May. Therefore, the flowering period is roughly twice as long in *H. marifolium* as for *H. caput-felis* (103 vs. 47 d, respectively). With regards to flowering pattern, flowers of *H. marifolium* showed a significantly lower chance of opening than flowers of *H. caput-felis* (index of flowering rate:  $-0.675 \pm 0.081$ ;  $\chi^2 = 68.9$ , d.f. = 1.0,  $P < 0.0001$ ; Fig. 1), e.g. *H. marifolium* had a longer flowering phenology than *H. caput-felis*.

At the peak of flowering of both species (29 March in *H. marifolium* and 16 April in *H. caput-felis*), *H. caput-felis*

TABLE 2. Fruit set (proportion of flowers that produced fruits on each plant) and seed set (proportion of flowers that produced seeds within each fruit) of *H. marifolium* and *H. caput-felis* for the different treatments in the hand-pollination experiment (mean  $\pm$  s.e.)

Source of pollen	<i>H. marifolium</i>			<i>H. caput-felis</i>		
	<i>n</i>	Fruit set	Seed set	<i>n</i>	Fruit set	Seed set
SelfS	102	0.153 $\pm$ 0.078 a	0.646 $\pm$ 0.132 a	21	0.833 $\pm$ 0.105 a	0.450 $\pm$ 0.097 a
SelfI	95	0.166 $\pm$ 0.070 a	0.371 $\pm$ 0.031 a	23	0.952 $\pm$ 0.048 a	0.288 $\pm$ 0.055 a
Geit	70	0.045 $\pm$ 0.030 a	0.219 $\pm$ 0.081 a	23	0.929 $\pm$ 0.071 a	0.413 $\pm$ 0.055 a
Xen	102	0.561 $\pm$ 0.071 b	0.560 $\pm$ 0.039 a	43	0.975 $\pm$ 0.025 a	0.666 $\pm$ 0.056 a

Treatments with the same letter do not differ significantly (likelihood pairwise contrasts;  $P < 0.05$ ).

SelfS, not manipulation; SelfI, pollination with pollen of the same flower; Geit, hand-pollination using pollen from the same plant, but from another flower; Xen, hand-pollination using pollen from a different plant; *n*, number of pollinated flowers per treatment.

Mean values (1  $\pm$  s.e.) are shown for each treatment.

showed a significantly higher number of receptive flowers per inflorescence than *H. marifolium* (1.4  $\pm$  1.7 vs. 0.4  $\pm$  0.7;  $\chi^2 = 17.07$ , d.f. = 1,  $P < 0.0001$ ), in spite of the higher flower production per inflorescence of *H. marifolium* (Table 1).

#### Breeding system

Fruit set in *H. marifolium* differed marginally between treatments ( $\chi^2 = 6.27$ , d.f. = 3,  $P = 0.099$ ): outcrossed flowers had a higher fruit set than the rest of the treatments (Table 2). However, significant differences were not detected in seed set ( $\chi^2 = 5.13$ , d.f. = 3,  $P = 0.167$ ).

In *H. caput-felis*, pollination treatment did not affect fruit set or seed set, e.g. *H. caput-felis* produced the same number of seeds and fruits whether or not a pollinator visit took place ( $\chi^2 = 3.44$ , d.f. = 3,  $P = 0.329$ ;  $\chi^2 = 6.25$ , d.f. = 3,  $P = 0.100$ , for fruit and seed set respectively).

#### Flower visitors

*Helianthemum marifolium* received a lower diversity of flower visitors than *H. caput-felis* (Table 3). In both species, hymenopterans performed most visits (96 % in *H. marifolium* and 66 % in *H. caput-felis*), *Apis mellifera* being the foremost (92 % in *H. marifolium* and 64 % in *H. caput-felis*).

The number of pollinator visits was significantly affected by the year in both plant species (*H. marifolium*:  $\chi^2 = 9.45$ , d.f. = 1,  $P = 0.002$ ; *H. caput-felis*:  $\chi^2 = 345.9$ , d.f. = 1,  $P < 0.0001$ ), being higher in 2001 than 2002 (Fig. 2). In *H. marifolium*, exposure to sun had only a marginal effect (sun: 0.86  $\pm$  0.12; shade: 0.39  $\pm$  0.11;  $\chi^2 = 3.78$ , d.f. = 1,  $P = 0.052$ ) but not with the 'sun exposure'  $\times$  year interaction ( $\chi^2 = 0.98$ , d.f. = 1,  $P = 0.322$ ). In *H. caput-felis*, however, plants in shade received significantly more visits (1.26  $\pm$  0.27;  $\chi^2 = 7.56$ , d.f. = 1,  $P = 0.006$ ) than plants in sun (0.67  $\pm$  0.10). For this species, differences between years were not tested due to the low number of visits in 2002 ( $n = 3$ ); for this reason both years were pooled, the effect of year not being considered in further analyses. In separate analyses, the number of flowers visited was significantly different between pollinator groups (Hymenoptera vs. Diptera;  $\chi^2 = 4.74$ , d.f. = 1,  $P = 0.030$ ) for *H. caput-felis*, being higher for Diptera than for Hymenoptera (2.6  $\pm$  0.4,  $n = 46$  and 2.1  $\pm$  0.2,  $n = 90$ , respectively, both years pooled).

TABLE 3. Taxonomic affiliation (species, order and family) and visiting frequency per year of the different flower visitors of *H. marifolium* and *H. caput-felis*

Order	Family	Species	2001	2002
<i>H. marifolium</i>				
Coleoptera		Coleoptera spp. 1	0	1 (0.06)
		Coleoptera spp. 2	0	5 (0.28)
		Coleoptera spp. 3	0	1 (0.06)
Hymenoptera	Apiidae	<i>Apis mellifera</i>	34 (7.16)	59 (3.28)
	Halictidae	<i>Halictus</i> sp.	0	4 (0.22)
<i>H. caput-felis</i>				
Diptera	Syrphidae	<i>Eristalinus aeneus</i>	15 (1.20)	0
		<i>Eupeodes corollae</i>	17 (1.36)	0
		<i>Sphaerophoria scripta</i>	2 (0.16)	0
		<i>Eristalis tenax</i>	9 (0.72)	0
		Syrphidae spp. 1	1 (0.08)	1 (0.08)
Hymenoptera	Apiidae	<i>Apis mellifera</i>	89 (7.12)	2 (0.16)
	Halictidae	Halictidae spp. 1	3 (0.24)	0
Lepidoptera		Lepidoptera spp. 1	1 (0.08)	0

Numbers in parenthesis refer to the number of visit per hour per each species of pollinator.

Numbers of hours censused totalled 4.75 and 18.0 h for *H. marifolium* and 12.5 and 12.25 h for *H. caput-felis*, for 2001 and 2002, respectively.

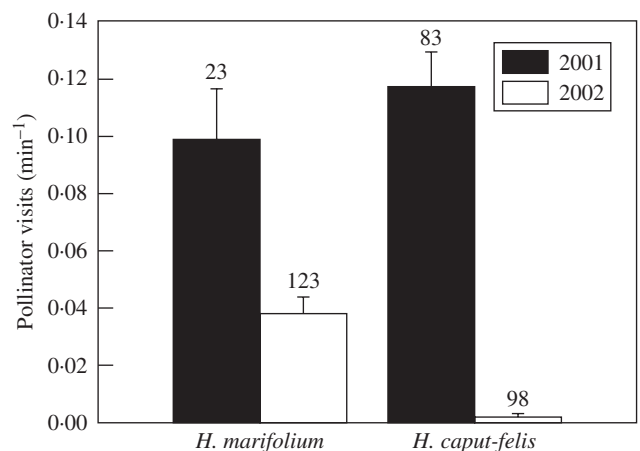


FIG. 2. Visitation rate (number of pollinator visits per minute; mean  $\pm$  s.e.) for each species of *Helianthemum* and year. Numbers over the columns indicate the number of observations (15 min) during which plants were observed for each species and years. For each species, years are significantly different ( $P < 0.05$ ).

TABLE 4. Mean ( $1 \pm s.e.$ ) and results of generalized linear models of the sun exposure (sun vs. shade) and year variation (2001 vs. 2002) on reproductive variables of *H. marifolium* and *H. caput-felis*

Source of variation	<i>H. marifolium</i>					<i>H. caput-felis</i>				
	Sun/2001	Shade/2002	d.f.	$\chi^2$	<i>P</i>	Sun/2001	Shade/2002	d.f.	$\chi^2$	<i>P</i>
Inflorescence production										
Sun exposure	13.7 $\pm$ 1.6 (29)	21.3 $\pm$ 5.1 (21)	1, 47	0.40	0.525	95.1 $\pm$ 14.9 (36)	58.8 $\pm$ 20.5 (14)	1, 47	19.87	<0.001
Plant crown			1, 47	59.27	<0.001			1, 47	131.38	<0.001
Flower production										
Sun exposure	77 $\pm$ 10 (29)	117 $\pm$ 32 (21)	1, 47	0.10	0.750	306 $\pm$ 63 (36)	172 $\pm$ 56 (14)	1, 47	28.74	<0.001
Plant crown			1, 47	57.88	<0.001			1, 47	218.21	<0.001
One seed mass										
Sun exposure	0.35 $\pm$ 0.02 (22)	0.39 $\pm$ 0.02 (19)	1, 57	0.16	0.693	0.81 $\pm$ 0.017(36)	0.88 $\pm$ 0.04 (14)	1, 80	3.57	0.059
Year	0.36 $\pm$ 0.02 (31)	0.38 $\pm$ 0.02 (31)	1, 57	0.02	0.898	0.84 $\pm$ 0.03 (37)	0.83 $\pm$ 0.02 (42)	1, 80	0.84	0.361
Fruit weight										
Sun exposure	3.11 $\pm$ 0.21 (22)	3.05 $\pm$ 0.29 (19)	1, 58	0.17	0.677	4.74 $\pm$ 0.12 (36)	4.14 $\pm$ 0.22 (14)	1, 80	6.23	0.013
Year	3.12 $\pm$ 0.21 (32)	3.19 $\pm$ 0.22 (31)	1, 58	0.02	0.881	4.79 $\pm$ 0.19 (37)	4.47 $\pm$ 0.13 (42)	1, 80	1.49	0.222
Fruit length										
Sun exposure	3.15 $\pm$ 0.07 (23)	3.17 $\pm$ 0.10 (19)	1, 59	0.06	0.813	3.36 $\pm$ 0.03 (36)	3.17 $\pm$ 0.06 (14)	1, 81	15.07	<0.0001
Year	3.18 $\pm$ 0.08 (33)	3.19 $\pm$ 0.07 (31)	1, 59	0.92	0.337	3.27 $\pm$ 0.05 (38)	3.32 $\pm$ 0.03 (48)	1, 81	0.40	0.592
Plant crown			1, 59	3.58	0.059			1, 81	5.20	0.023
Fruit set										
Sun exposure	0.590 $\pm$ 0.070 (25)	0.523 $\pm$ 0.036 (19)	1, 62	1.91	0.168	0.476 $\pm$ 0.032 (36)	0.506 $\pm$ 0.059 (14)	1, 88	0.05	0.819
Year	0.742 $\pm$ 0.058 (35)	0.352 $\pm$ 0.022 (32)	1, 62	13.18	<0.001	0.516 $\pm$ 0.045 (44)	0.463 $\pm$ 0.031 (49)	1, 88	0.56	0.454
Plant crown			1, 62	4.03	0.045			1, 88	0.08	0.771
Seed set										
Sun exposure	0.516 $\pm$ 0.047 (22)	0.383 $\pm$ 0.054 (19)	1, 58	2.04	0.153	0.351 $\pm$ 0.023 (36)	0.307 $\pm$ 0.035 (14)	1, 81	1.05	0.306
Year	0.478 $\pm$ 0.048 (32)	0.432 $\pm$ 0.040 (31)	1, 58	0.10	0.751	0.398 $\pm$ 0.029 (38)	0.309 $\pm$ 0.022 (48)	1, 81	3.61	0.056

Inflorescence and flower production were only compared in 2002 because both variables were only measured in that year.

The numbers in parenthesis refer to sample size for each category of sun exposure and year.

The interaction between variables and/or the covariate (plant crown) were included in the table whenever they were significant ( $P > 0.05$ ).

In *H. marifolium*, virtually all pollinators were hymenoptera; hence, differences between pollinator groups were not tested. *Helianthemum marifolium* showed significant differences between years ( $\chi^2 = 3.99$ , d.f. = 1,  $P = 0.046$ ), with 2001 having the greater visitation (2001:  $2.8 \pm 0.3$ ,  $n = 34$ ; 2002:  $2.5 \pm 0.2$ ,  $n = 64$ ).

Comparing the two pollinator groups in *H. caput-felis*, flower handling time was significantly higher for dipterans ( $40.4 \pm 8.7s$ ,  $n = 46$ ) than for hymenoptera ( $6.8 \pm 0.8s$ ,  $n = 90$ ;  $\chi^2 = 60.40$ , d.f. = 1,  $P < 0.001$ ). In *H. marifolium*, differences between years were detected ( $\chi^2 = 20.91$ , d.f. = 1,  $P < 0.0001$ ), i.e. hymenoptera showed higher handling times in 2001 ( $16.4 \pm 20.7$ ,  $n = 34$ ) than in 2002 ( $10.0 \pm 1.9$ ,  $n = 60$ ).

#### Factors determining reproductive potential

Reproductive traits varied between sun exposure for *H. caput-felis* but not for *H. marifolium* (Table 4). *Helianthemum caput-felis* produced more inflorescences per plant, more flowers per inflorescence and larger fruits in sun-exposed plants.

However, these differences did not result in higher fruit or seed sets in plants located in sunny sites for either species (Table 4). Considering differences between years, fruit set was significantly lower in 2002 for *H. marifolium* (2001:  $0.74 \pm 0.06$ ,  $n = 35$ ; 2002:  $0.35 \pm 0.02$ ,  $n = 32$ ), but not for *H. caput-felis*. None of the interactions between year and sun exposure were statistically significant.

Considering the effect of plant size on reproductive traits, inflorescence and flower production were significantly correlated in both species, and for fruit length for *H. caput-felis* and fruit set for *H. marifolium* (Table 4). None of the reproductive variables showed a significant interaction between sun exposure and/or year with the covariate (plant crown), except for inflorescence and flower production for sun exposure ( $\chi^2 < 60.18$ , d.f. = 1,  $P < 0.001$ , for four analyses). In *H. caput-felis*, as would be expected, plants located in the sun showed a higher slope value in the correlation between plant size and inflorescence and flower production (i.e. as plant size increased, plants in the sun produced proportionally more inflorescences and flowers than plants under vegetation). However, *H. marifolium* showed an inverse trend: larger plants under vegetation produced proportionally higher numbers of inflorescences and flowers.

#### Seedling survival

Seedling survival varied significantly between years for both species (Table 5), being three- to six-fold higher in 2002 for both species (Fig. 3). Though sun exposure did not significantly affect in seedling survival in either species, a significant interaction between year and sun exposure was observed in *H. marifolium* (Fig. 3 and Table 5). As would be expected, maximum seedling crown was positively correlated with seedling survival in both species (Table 5), while the number of leaves was positively correlated with survival in *H. caput-felis* seedlings (Table 5), but not in *H. marifolium*.

TABLE 5. Results of generalized linear models analysing the effect of leaf number, maximum seedling crown, sun exposure and year on the success of seedling survival in *H. marifolium* and *H. caput-felis*

Species	Independent variables	d.f.	$\chi^2$	P
<i>H. marifolium</i>	Maximum seedling crown	1	8.23	0.004
	Year	1	137.5	<0.001
	Sun exposure	1	2.83	0.093
	Year $\times$ sun exposure	1	5.83	0.016
<i>H. caput-felis</i>	Leaf number	1	9.08	0.002
	Maximum seedling crown	1	8.41	0.004
	Year	1	39.5	<0.001

Variables shown are those included in the model with the lowest AIC score. Non-significant variables are omitted from tables.

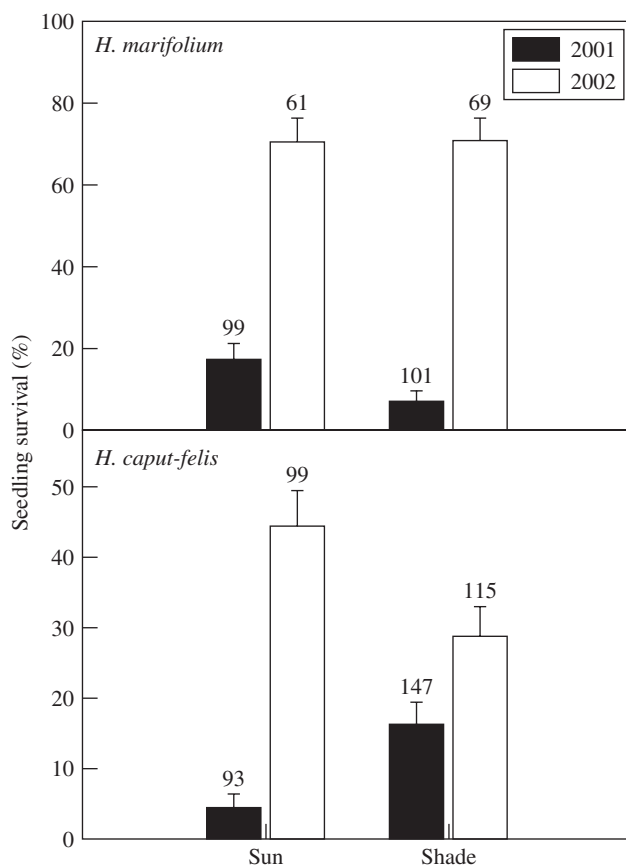


FIG. 3. Percentage of seedling survival per species, year and sun exposure (mean  $\pm$  s.e.). Numbers over the columns indicate the number of tagged seedlings per species, year and sun exposure.

## DISCUSSION

The reproductive stages studied for these two species of *Helianthemum* do not limit the plant reproductive performance of the populations and years studied. So, at least for the short and medium term, both species are able to persist in their natural populations. However, the variation between years detected in seed production, pollinator service and seedling survival, suggests that reproductive output may be affected in adverse years.

*Helianthemum marifolium* is self-compatible, but mostly an outbreeder, i.e. outcrossed flowers produced three times more fruits than self-pollinated ones (i.e. those receiving pollen from the same flower or plant). *Helianthemum caput-felis* shows a comparable fruit and seed set in self-pollinated and outcrossed flowers; hence, fruit and seed set do not depend on the pollen source. Previous studies have suggested that self-incompatibility is common in the Cistaceae (Herrera, 1992; Talavera *et al.*, 1993), and it is generally attributed to gametophytic incompatibility (Nettancourt, 1977). Apparently, this mechanism does not operate so effectively in *H. caput-felis* (as suggested also by Tébar *et al.*, 1997).

The reliance on a single pollinator species in *H. marifolium*, where 95 % of the visits are performed by *A. mellifera*, increases the vulnerability of this species. However, *A. mellifera* is one of the most widespread and generalist insect pollinators (even as an alien species, in many ecosystems); hence, it is more reliable than most other pollinators (Richardson *et al.*, 2000). Considering each pollinator, hymenopterans visited more flowers per plant than dipterans. This does not necessarily imply an increase of fruit and seed set, as it could favour geitonogamy and thus increase selfing rates. Such risk is higher for *H. caput-felis*, given its higher number of receptive flowers per day and higher self-compatibility. A comparable case has been described for *Hormatophylla spinosa* (Gómez and Zamora, 1996). In this Mediterranean high-mountain shrub, 90 % of pollinator flights are produced among flowers of the same plant, suggesting that wind pollination may increase the outcrossing rate of this species. Although the flower handling time was five- to six-fold longer for dipterans than for hymenopterans, dipterans are unlikely to be more effective pollinators in *H. caput-felis*; instead, they rarely touch the stigma surface, often acting as pollen robbers (pers. obs.). Unfortunately, data about pollinator efficiency (pollen load) was not collected and this hypothesis cannot be verified.

The differences between years in plant reproductive performance (decreased fruit set in *H. marifolium* and seed set in *H. caput-felis*, both in 2002), are probably related to the lower number of pollinator visits observed that year, at least for *H. marifolium* whose reproductive output relies on crossing pollinations. Sun exposure appeared to affect flower production, but not reproductive output in *H. caput-felis*; sun-exposed plants produced a larger flower display, but the same fruit and seed set as shaded plants [similar to report by Herrera (1991) for *Lavandula latifolia*]. In contrast, the floral display of *H. marifolium* did not differ between sun exposure, but fruit set was higher in sunny sites; this difference cannot be explained by variation in pollinator visits, since these were not more frequent in sun-exposed sites.

Rainfall patterns determine one of the main resources affecting seedling survival in arid and Mediterranean regions (Fowler, 1988; Escudero *et al.*, 1999; Traveset *et al.*, 2003). In the present study, higher seedling survival of both species coincides with one of the years with the highest rainfall (2002). In contrast to other species (Rey and Alcántara, 2000; Traveset *et al.*, 2003), seedling survival of



both species did not vary with exposure to sun. Larger seedlings of both species showed a higher summer survival rate, as has been previously observed in the congeneric *H. squamatum*, an endemic gypsophile from semi-arid Spain (Escudero *et al.*, 1999).

In short, none of the factors controlling the reproduction of the two endangered species of *Helianthemum* are important for their reproductive performance. In other words, neither biotic or abiotic limitation affects the conservation of these two species. Instead, what seems crucial to explain their rarity is the increasing destruction of their habitat. A large part of the seacoast of the Mediterranean region has been degraded by tourism facilities (19 %); this effect is most dramatic in the island of Mallorca (48 %; Blondel and Aronson, 1999). Thus, the preservation of such habitats is the most critical aspects in any strategies for the conservation of these two species and, more generally, of the Mediterranean coastal flora and fauna.

#### ACKNOWLEDGEMENTS

Amparo Lázaro, Núria Riera and Joan Carles Salom provided assistance during some of the fieldwork. Enrique Descals revised the English text, Angeles Marcos-García identified the syrphid flies and Luis Santamaría provided very useful comments and statistical advice. I am especially grateful to Anna Traveset for her supervision of the design and write up of this work. Thanks are also due to three anonymous reviewers for comments on the final version of the manuscript. This work was partially funded by the Spanish DGIC (PB97-1174) and by a research grant from the Town-Hall of the Ciutat de Palma 2000.

#### LITERATURE CITED

- Akaike H.** 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csaki F, eds. Proceedings of the second international symposium on information theory. Budapest, Hungary: Akademiai Kiado, 267–281.
- Alomar G, Mus M, Rosselló JA.** 1997. *Flora endèmica de les Balears*. Palma de Mallorca: Consell Insular de Mallorca.
- Ågren J.** 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* **77**: 1779–1790.
- Bernardello G, Anderson GJ, Stuessy T, Crawford D.** 2001. A survey of floral traits, breeding system, floral visitors, and pollination systems of the angiosperms of the Juan Fernández Islands (Chile). *Botanical Review* **67**: 255–308.
- Blondel J, Aronson J.** 1999. *Biology and wildlife of the Mediterranean Region*. Oxford: Oxford University Press.
- Buza L, Young A, Thrall P.** 2000. Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. *Biological Conservation* **93**: 177–186.
- Callaway RM, Walker L.** 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* **78**: 1958–1965.
- Delanoë O, Montmollin B de, Olivier L, the IUCN/SCC Mediterranean Islands Plant Specialist Group.** 1996. *Conservation of Mediterranean island plants. 1. Strategy for action*. Gland, Switzerland: IUCN.
- Erhardt A, Jäggi B.** 1995. From pollination by Lepidoptera to selfing: the case of *Dianthus glacialis* (Caryophyllaceae). *Plant Systematics and Evolution* **195**: 67–76.
- Escudero A, Somolinos RC, Olano JM, Rubio A.** 1999. Factors controlling the establishment of *Helianthemum squamatum*, an endemic gypsophile of semi-arid Spain. *Journal of Ecology* **87**: 290–302.
- Fowler NL.** 1988. What is a safe site? Neighbour, litter, germination date, and patch effects. *Ecology* **69**: 947–961.
- Gómez JM, Zamora R.** 1996. Wind pollination in high-mountain populations of *Hornathophylla spinosa* (Cruciferae). *American Journal of Botany* **83**: 580–585.
- Greuter W.** 1995. Origin and peculiarities of Mediterranean island floras. *Ecologia Mediterranea* **21**: 1–10.
- Herrera CM.** 1991. Dissecting factors responsible for individual variation in plant fecundity. *Ecology* **72**: 1436–1448.
- Herrera CM.** 2000. Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. *Ecology* **81**: 15–29.
- Herrera CM, Pellmyr O, eds.** 2002. *Plant animal interactions: an evolutionary approach*. Oxford: Blackwell Publishing.
- Herrera J.** 1992. Flower variation and breeding systems in the Cistaceae. *Plant Systematics and Evolution* **179**: 245–255.
- Kearns CA, Inouye DW, Waser NM.** 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. *Annual Review of Ecology and Systematics* **29**: 83–112.
- Mathsoft.** 1999. *S-Plus 2000. Guide to Statistics, Vol. 2*. Seattle: Mathsoft Inc.
- Mus M.** 1995. Conservation of flora in the Balearic Islands. *Ecologia Mediterranea* **21**: 185–194.
- Nettancourt D de.** 1977. *Incompatibility in angiosperms*. Berlin: Springer.
- O'Brien SJ.** 1994. A role for molecular genetics in biological conservation. *Proceedings of the National Academy of Sciences of the USA* **91**: 5748–5755.
- Pons MA.** 2002. *Estudis sobre la biologia de la reproducció de les brolles i les timonedes (Rosmarinion officinalis Br.-Bl. et Molinier 1934) de les Pitiuses*. PhD Thesis, University of the Balearic Islands, Spain.
- Proctor M, Yeo P, Lack A.** 1996. *The natural history of pollination*. London: Harper Collins.
- Pujol JA.** 2001. La urbanización de la costa alicantina marca el declive de la jarilla de cabeza de gato. *Quercus* **188**: 43–46.
- Rey P, Alcántara J.** 2000. Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. *Journal of Ecology* **88**: 622–633.
- Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmánek M.** 2000. Plant invasions—the role of mutualisms. *Biological Reviews* **75**: 65–93.
- SAS Institute.** 2000. *SAS/STAT<sup>®</sup> software: User's guide*. Cary, NC: SAS Institute.
- Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker IM, Bishop JG.** 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* **75**: 584–606.
- Statsoft.** 2001. *STATISTICA for Windows*. Tulsa: Statsoft Inc. (<http://www.statsoft.com>).
- Talavera S, Gibbs PE, Herrera J.** 1993. Reproductive biology of *Cistus ladanifer* (Cistaceae). *Plant Systematics and Evolution* **186**: 123–134.
- Tébar FJ, Gil L, Llorens L.** 1997. Reproductive biology of *Helianthemum apenninum* (L.) Mill. and *H. caput-felis* Boiss. (Cistaceae) from Mallorca (Balearic Islands, Spain). *Acta Botanica Malacitana* **22**: 53–63.
- Thanos CA, Georghiou K, Kadis C, Panzati C.** 1992. Cistaceae: a plant family with hard seeds. *Israel Journal of Botany* **29**: 22–44.
- Traveset A, Gulias J, Riera N, Mus M.** 2003. Transition probabilities from pollination to establishment in a rare dioecious shrub species (*Rhamnus ludovici-salvatoris*) in two habitats. *Journal of Ecology* **91**: 427–437.
- Traveset A, Willson MF, Sabag C.** 1998. Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. *Functional Ecology* **12**: 459–464.
- Turner RM.** 1990. Long-term vegetation change of a fully protected Sonoran Desert site. *Ecology* **71**: 464–477.
- Washitani I.** 1996. Predicted genetic consequences of strong fertility selection due to pollinator loss and in isolated population of *Primula sieboldii*. *Conservation Biology* **10**: 59–64.