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Stress-induced male sterility and mixed mating in the island plant *Cedronella canariensis* (*Lamiaceae*)

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Key words: *Lamiaceae, Cedronella canariensis*; *Apidae, Bombus canariensis*; *Lepidoptera, Macroglossa stellatarum.* – Canary Islands, cross-pollination, gynodioecy, gynomonoecy male sterility, mixed mating system, nectar-robbing, self-pollination.

Abstract: On the Canary Islands, we studied reproductive ecology of the perennial laurel forest herb *Cedronella canariensis* (*Lamiaceae*). Flowers contained small quantities of concentrated nectar. Flower visitors were mainly bumblebees (*Bombus terrestris* subsp. *canariensis*) and lepidopterans (especially *Macroglossa stellatarum*). Their abundance, pollen load, and behaviour are reported. *Cedronella canariensis* was facultatively autogamous. The P:O ratio was higher than expected for a plant with a breeding system of this nature. Seed set was not pollen-limited. Selfed and outcrossed seeds differed in weight. Small, lightly coloured flowers with dysfunctional stamens appeared towards the end of the season. This male-sterility might be induced by drought.

The ability of plants to self-pollinate is regarded as an advantage in island colonisation (Baker 1955). Selfing means independence of the presence of conspecifics and, to animal-pollinated plants, the presence of pollinators. Plants arrive on islands without their pollinators and can only survive sexually by means of a shift to either the local pool of pollinators or to self-pollination. Comparing the same plant species on islands and the mainland, one often finds that island pollinators are less abundant, less reliable, and less effective than on nearby mainland (Heine 1937, Linhart & Feinsinger 1980, Lack & Kevan 1984, Olesen 1992 and references therein). Thus ability to self-pollinate may be a proximate safeguard in an unpredictable pollination environment.

Much of the genetic variation in a population is lost during colonisation of an island, because it occurs through extreme bottlenecks in population size (Mayr 1963). Moreover, plant populations on islands often remain smaller and more isolated than on continents. All of this make insular plants more prone to suffer from loss of genetic variation and reduced adaptability. Thus plant species able to restore and maintain high levels of genetic variation may ultimately have greater success on islands. This requires some level of outcrossing. Carlquist (1974) stressed the fitness premium placed on ability to outcross in an insular environment. How island plants cope with this conflict between the immediate benefits of self-pollination after colonisation and the long-term benefits of outcrossing is little known. Several studies of breeding systems of island plants suggest that the typical solution involves a mixed-mating system of various outcrossing mechanisms combined with spontaneous selfing and vegetative propagation (EHRENDORFER 1979).

The Canary Islands are famous for their endemic, very old plant species. However, only few studies on the reproductive ecology of Canarian plants exist (VOGEL & al. 1984; OLESEN 1985, 1988; WESTERKAMP 1990; FORFANG & OLESEN 1998).

On the Canary island of La Gomera we studied the reproductive ecology of the endemic perennial *Cedronella canariensis* (L.) WEBB & BERTHELOT (*Lamiaceae*). Our paper reports on (i) the flower biology of the species, (ii) its breeding system, and (iii) its present pollinator fauna, and (iv) discusses our findings in relation to the described conflict of island colonisers between the short-term benefits of selfing and long-term benefits of out-crossing.

Materials and methods

Study site. The study was conducted in the Garajonay national park, Gomera (17° 33′ W, 28° 23′ N) during a three month period from May to July 1992 and during 14 days in April 1997. The park protects one of the finest remnants of Macaronesian laurel forest, which is a Tertiary relict plant formation once widespread in the Mediterranean region. It is broad-leaved and evergreen, and of moderately high species diversity. The major trees are *Laurus azorica, Ilex canariensis, Erica arborea* and *Myrica faya.* Since the Pleistocene, the laurel forest has been confined to the Macaronesian islands of Madeira, the Azores, and the five western Canary Islands. These Atlantic islands were less affected by climatic fluctuations than the mainland and thus became a refuge for many plants. Today, much of the Macaronesian laurel forest has been destroyed. At the study site, the dominant species were the trees *E. arborea* and *M. faya,* the shrubs *Adenocarpus foliolosus, Cistus monspeliensis, Hypericum canariensis,* and the herbs *Echium plantagineum, Andryala pinnatifida,* and especially *Cedronella canariensis, Anacyclus radiatus* and *Galactites tomentosa.*

Study plant. *Cedronella canariensis* is a Macaronesian endemic, mainly found in laurel forests (BRAMWELL & BRAMWELL 1990). Its closest relatives are East Asian and North American species (BUDANTSEV 1993). Such a disjunct distribution of the genus supports the notion that *C. canariensis* is an ancient taxon. It probably diverged from an ancestral line in the Tertiary, and may have thrived in the laurel forest ever since (SUNDING 1970). It is a large perennial herb, which may become woody at the base. Its flowering shoots are up to 2 m high, and it forms clones by means of rhizomes. The primary inflorescence is a large spike with over 100 flowers. Only a small fraction of these are open at any one time. Large flowering shoots may develop one or several secondary inflorescences further down the stem, flowering after the primary inflorescence has withered (unpubl. observations).

In Garajonay, *C. canariensis* is very common at the forest edges and along roads and tracks, but it also occurs less frequently below the canopy. It may be regarded as a light-gap species. The population of Garajonay encompasses two chemovarieties, which produce aromatic oils of a distinct smell (WEBB & BERTHELOT 1850, ENGEL 1994). *Cedronella canariensis* var. *canariensis* has an odour like camphor, and var. *anisata* smells like aniseed. Further details about their chemical differences are found in COEN & al. (1995) and PÉREZ DE PAZ & al. (1996). Offspring of between-variety crosses belong to var. *canariensis* (unpubl. obs.). These chemicals are produced by "giant glands" sensu ENGEL (1994) situated between the two thecae of each stamen. On the leaves are much smaller glands which produce oils of almost the same composition (Engel 1994). The shoots of var. *canariensis* are more lax and pubescent and the plants thus appear more sprawling, while var. *anisata* is more upright and glabrescent in habit (Pérez De Paz & al. 1996, and pers. obs.). The varieties grow sympatrically, but var. *canariensis* predominates in the north-western part of the park, while var. *anisata* is most frequent in the south-eastern corner. In the central part, the varieties are approximately equally abundant (Engel 1994, and pers. obs.). Webb & Berthelot (1850) also mention a white-flowered var. *albiflora*, but we suspect this "variety" consists of female plants only – see later.

Spatial distribution. Plant patch size and interpatch distance were mapped along a 250 m track, and numbers of primary inflorescences per patch were counted. Below the canopy, seven patches were found within an area of 100 m^2. The size of these patches was measured and their number of primary inflorescences counted.

Flower biology and sex determination. *Cedronella canariensis* flowers were sampled at 1 m intervals along a transect line and a set of morphological characters important to an understanding of the pollination biology of the species was measured (see Table 1). In a sample of buds, numbers of pollen grains from half an anther were counted. Anthers were sampled randomly. This figure was used to estimate total pollen production per flower, and pollen-to-ovule ratio (P:O ratio) of a single flower was calculated (Cruden 1997). From exposed flowers and from flowers enclosed in perforated polythene bags for 24 hours, nectar was extracted by micropipette and concentration of sucrose equivalents in nectar was measured with a pocket refractometer (Bellingham & Stanley modified for small volumes of nectar, $\geq 0.5\,\mu l$ (concentrations corrected to 20 °C, Corbet 1978)). Single flowers rarely contained enough nectar for measurement, so nectar was pooled from several flowers. Nectar concentration data were gathered on both sunny and foggy days, and throughout the day.

Frequency of female and hermaphrodite flowers was estimated in the field. A subsample of flowers was further investigated in the laboratory to determine the exact number of pollen grains per flower.

Phenology. Four patches of *C. canariensis* with varying sun exposure were selected. In each of these, a transect was established and surveyed weekly from May 8 to July 28. The first 100 primary inflorescences encountered on a census walk along a transect were scored as "flowering" or "non-flowering", the criteria for "flowering" being presence of at least one open flower. On July 21–23, large buds were tagged and inspected for anthesis every half an hour from 7 a.m. to 8 p.m. Flower opening and wilting were noted.

Flower visitors. Visitors to *C. canariensis* were observed on a total of 18 days. Most observations were in favourable weather and were carried out between 10 a.m. and 6 p.m., the main period of visitor activity. Six to twelve inflorescences were tagged. Flower number was counted before each observation census, which lasted 15 minutes. Numbers of visits and visitors were counted. Specimens of each species were caught for later identification. Voucher specimens of plants and insects are deposited at the Department of Ecology & Genetics, University of Aarhus. For bumblebees, it was noted whether the flowers were visited legitimately or not, i.e. nectar-robbed. On June 29 and 30, and July 21, nocturnal flower visitors were observed. A pocket lamp covered with a red cloth was used. On two days in July, 35 flowers of each chemovariety on neighbouring plants were compared simultaneously for visiting insects.

Pollen loads. Butterfly proboscides were placed in glycerol on a slide, which was sealed with nail varnish. Solid glycerine jelly cubes of 8 mm^3 in size were gently rubbed against the body parts of butterflies and bees. The cubes were then placed on slides and warmed until the jelly melted. All slides were examined for pollen. For each visitor, thorax, head, legs and wings were analysed separately. Specimens of other insect groups were washed in small amounts of alcohol. The suspension was centrifuged, and the sediment of pollen examined under the light microscope. Pollen from bee corbiculae was suspended in alcohol and identified. A reference collection of pollen from entomophilous plants in the area was made.

Breeding system. Data on breeding system were obtained by a series of crossings in June. Only the lower flowers on each inflorescence were used in the experiment except in category (v), where all flowers on each inflorescence were scored for fruit set. The chemovariety of all plants used was noted. Flowers used for crossing experiments were enclosed in perforated polythene bags at the bud stage. The experiments were: (i) bagged, unmanipulated flowers (test of spontaneous selfing); (ii) bagged flowers, selfed by hand-pollination (test of self-compatibility); (iii) bagged flowers, emasculated in bud stage (test of agamospermy); (iv) bagged flowers, emasculated in bud stage, crossed by hand-pollination with at least two donors from other patches (test of pollen-limitation of fruit and seed set); and (v) unbagged flowers (seed set caused by spontaneous selfing and legitimate pollinators). Fruit set, and seed set in (v), were estimated when fruits were almost ripe. Seeds from (v) were weighed.

Statistical analysis. Then non-parametric Mann-Whitney U-test was used to compare between two groups of data, to avoid the t-test's assumptions (SIEGEL & CASTELLAN 1998: 128) (comparisons: corolla length of hermaphroditic and female flowers; flower production and flower lifetime of the two chemovarieties; weight of selfed and outcrossed seeds; and flower handling time and pollen load of illegitimate and legitimate bumblebee visitors). Variation in proportion of female flowers and differential visitation by bumblebees and *Macroglossum stellatarum* between the two chemovarieties were analysed using a χ^2-test (FOWLER & COHEN 1990: 111). Dependence of nectar concentration on ambient temperature and time of day was analysed using parametric regression analysis (FOWLER & COHEN 1990: 147). Anther dimensions were compared using a paired t-test (FOWLER & COHEN 1990: 176).

Results

Spatial distribution. *Cedronella canariensis* had a patchy distribution. Forty-two patches of plants ($\geq$1-many plants) were found along the track. Average patch size (plant ground cover) and inter-patch distance were 5.1 m^2 (range 0.04–35.2 m^2) and 6.3 m (range 0.6–36 m) respectively. Each patch contained an average of 29 primary inflorescences (range 1–200). The seven study patches growing below the canopy averaged 15.2 m^2 in size (range 1–30 m^2) and each contained an average of 62 primary inflorescences (range 10–53). Below the canopy, patches thus covered (15.2 × 7 × 100/1000=)11% of the forest floor.

Flower biology. The structure and dimensions of the flower of *C. canariensis* are summarised in Fig. 1 and Table 1. The white-pink/mauve, homogamous flower was bilabiate with a narrow straight corollar tube, bilobed stigma (Fig. 1g) and four stamens situated very close to the stigma and hidden inside the hooded upper lip. The morphology of the pollen grain is shown in Fig. 1d, e. A smaller landing

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Fig. 1. Morphology of the hermaphroditic flower of *Cedronella canariensis*. *a* flowering plant in Garajonay national park; *b* open anther with pollen grains and oil droplets; *c* flower; *d*, *e* pollen grain; *f* ovary and basal part of style with adhering oil droplets; *g* stigma (*b*, *d*, *e*, *f*, and *g* are scanning electron micrographs)

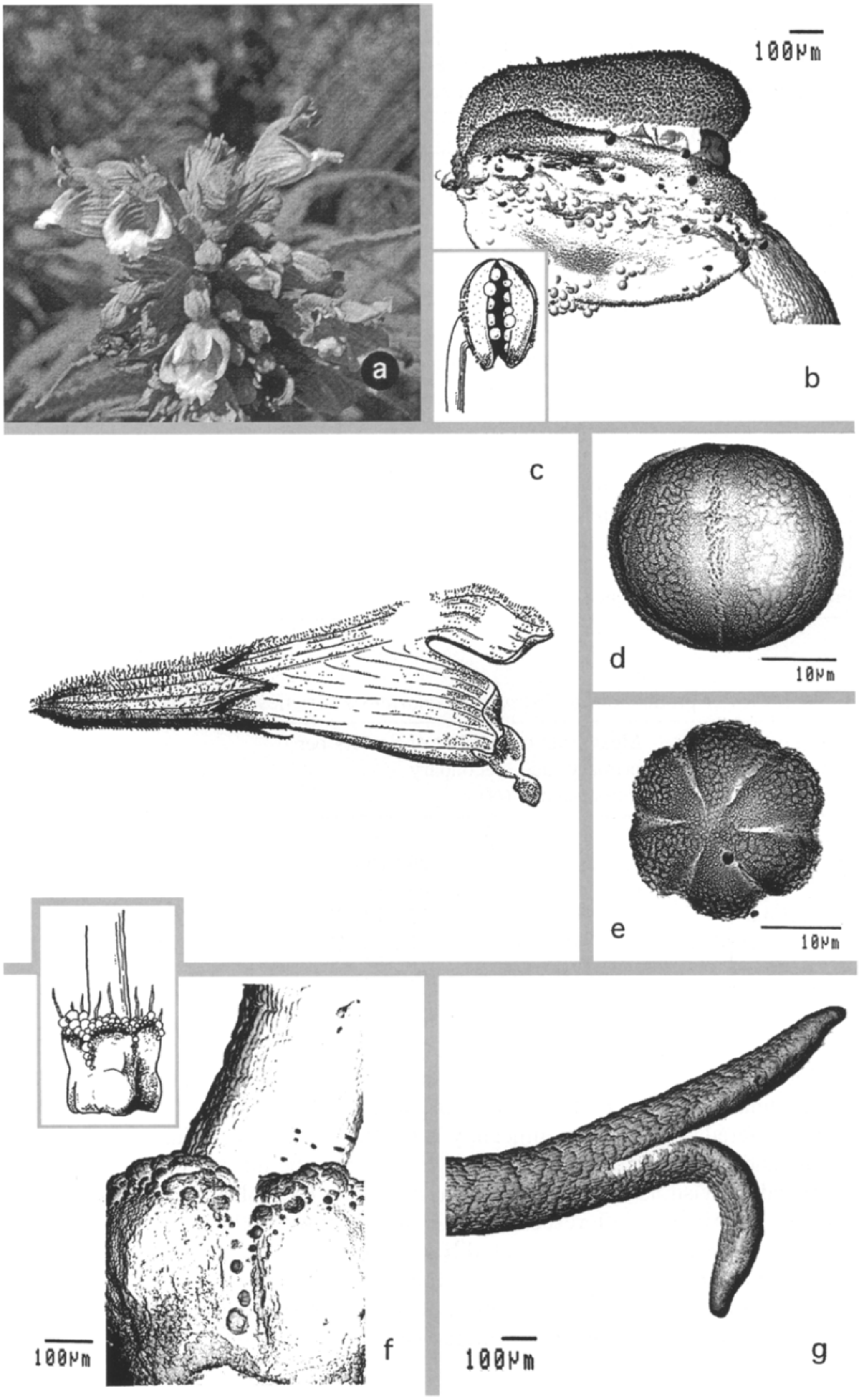
a
100µm
b
c
d
10µm
e
10µm
100µm
f
100µm
g

Table 1. Quantitative characterization of flower traits of *Cedronella canariensis* suspected to be of importance to its pollination biology (N=30, one measurement per flower per plant). Length of front and rear anther (t=8.84, P<0.0001) and of outer and inner anther half (t=2.20, P<0.04) differ significantly from each other upon paired t-test

Flower trait (mm)	Mean	SD
Total flower length	21.9	6.1
Calyx length	12.2	0.8
Basal calyx width	2.1	0.1
Apical calyx width	3.8	0.1
Calyx lobe length	3.6	0.6
Upper-lower lip distance	8.2	0.8
Corolla opening height	5.9	0.8
Corolla tube width just above ovary	1.1	0.1
Corolla tube width 5 mm from basis	1.4	0.2
Corolla tube width 8 mm from basis	3.1	0.3
Front anther length	1.2	0.2
Rear anther length	1.3	0.2
Length of outer anther half	1.3	0.2
Length of inner anther half	1.2	0.2
Front-rear anther distance	1.6	0.7
Stigmatic lobe length	1.1	0.2
Front anther-stigma distance	1.2	0.5

Table 2. Flower production in *Cedronella canariensis* varieties (*N* sample size)

	Mean±SD of number of flowers per primary and secondary inflorescence (N)		Percent female flowers (N)
var. *canariensis*	153±37 (30)	29±16 (30)	31 (231)
	n.s.[a]	n.s.[a]	$P<0.003$[b]
var. *anisata*	157±45(30)	32±17 (30)	12 (691)
Weighted average	155±41 (60)	31±16 (60)	16 (922)

[a]Mann-Whitney U-test, [b]χ^2 -test

platform was present (Fig. 1c), and the interior of the corolla had several parallel longitudinal furrows. The front and rear pair of anthers and the two halves of an anther differed statistically significantly in size from each other (Table 1). Stamens, stigma and pollen were white in colour. Entrance to the interior of the flower was restricted to visitors less than 5.9 mm in height ('Corolla opening height' in Table 1). Primary inflorescences had more flowers than secondary ones. Chemovarieties, however, did not differ in flower production (Table 2). Each stamen contained 2167±485 pollen grains (N=20 stamens sampled from the beginning of the season when no female plants had appeared yet – see later). Hence the P:O ratio in hermaphroditic flowers was (2167 × 4 stamens/4 ovules=) 2167.

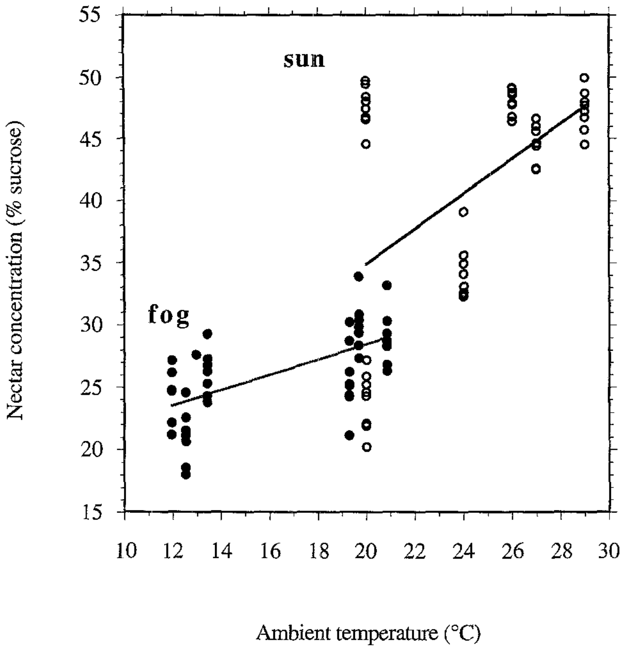

Fig. 2. Nectar concentration of *Cedronella canariensis* plotted against ambient temperature on days with sun (open circles) or fog (closed circles). Linear regressions: Sun $y = 1.42x + 6.36$, $F_{1,46} = 17.9$, $P < 0.005$, $N = 48$, $R^2 = 0.28$. Fog, $y = 0.62x + 16.05$, $F_{1,46} = 31.9, P < 0.005, N = 48, R^2 = 0.41$. R^2 is the coefficient of determination and is a measure of the proportion of the variability in nectar concentration that is accounted for by the variability in the two independent variables; F is the variance ratio and the suffix gives the degrees of freedom for the numerator and denominator of the ratio

Nectar was produced by the nectary situated beneath the ovary. Bagged flowers contained approximately 1 µl of nectar with average sugar concentrations of 26 and 41% on foggy and sunny days respectively (sucrose equivalents, range 18 to more than 50 %, $N=2 \times 48$ plants (sun and fog), a sample unit consisted of nectar from several flowers of the same plant; some of the nectar might have had a concentration above 50 %, but the refractometer had 50 % as its upper threshold value). Concentrations varied with temperature (Fig. 2) and time of day (Fig. 3). The very high levels achieved on sunny days might be due to evaporation. Oils were not only produced by glands between the thecae (Fig. 1b) but also by glands on the ovary (Fig. 1f).

Variation in floral sex. Late in the season, an increasing number of small and more whitish flowers appeared. If they had any anthers at all these were smaller and more irregularly shaped than anthers in hermaphroditic flowers. At the end of the season, these apparently female flowers constituted up to 26 % of all flowers (N=922 flowers). *Cedronella canariensis* var. *canariensis* plants had significantly more female flowers than var. *anisata* (Table 2). Mean corolla length of apparent female and hermaphroditic flowers was 15.7 and 21.9 mm respectively. The difference was highly significant (Mann-Whitney U-test, P<0.0001, N=30 flowers/sex). 25 % of all flowers field-determined as apparent female were "leaking", i.e. they contained some pollen. Likewise, 80 % of all late-season

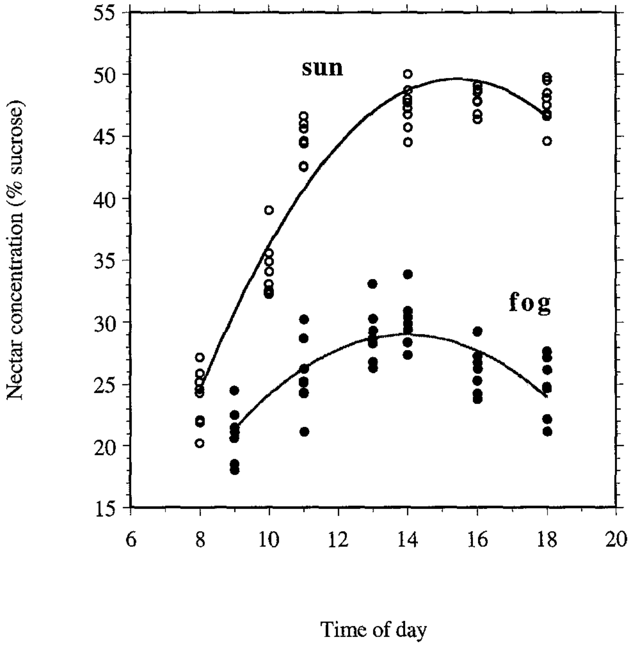

Fig. 3. Nectar concentration of *Cedronella canariensis* plotted against time of day on days with sun (open circles) or fog (closed circles). Polynomial regressions: Sun, $y = -0.31x^2 + 8.69x - 31.64$, $F_{3,45} = 1965.5$, $P < 0.005$, $N = 48$. $R^2 = 0.59$. Fog, $y = -0.46x^2 + 14.11x - 59.14$, $F_{3,45} = 3664.9$, $P < 0.005$, $N = 48$, $R^2 = 0.91$ (for statistics cf. legend to Fig. 2)

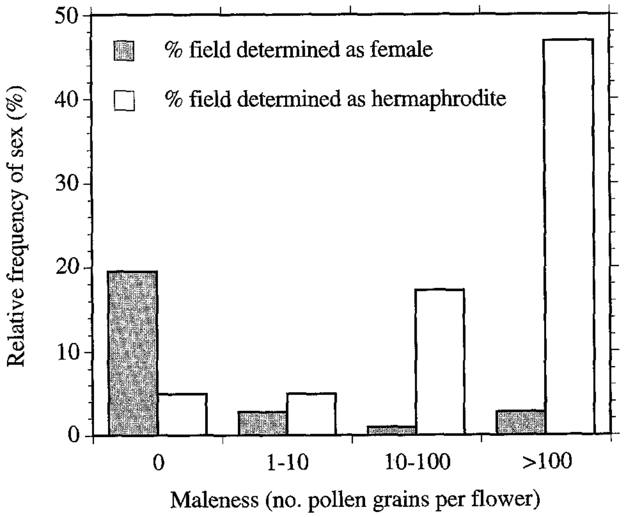

Fig. 4. Frequency distribution of sex in *Cedronella canariensis* late in season. The columns show to which sex the flower was determined in the field. Sum of all columns is 100%, and sum of each pair of dark and white columns is frequency of the different maleness categories

apparent hermaphrodites turned out on inspection in the laboratory to have a substantially lowered production of pollen. The distribution of the actual level of sex expression is given in Fig. 4 as level of maleness, i.e. number of pollen grains per flower. Field determination of flower sex was thus rather crude.

Flowering phenology. The population flowered from the beginning of May to the beginning of August, with flowering starting in the sunniest tracks (Fig. 5). Flowering of an inflorescence lasted approximately 25 days (range 20–30 days, N=7). Those with the highest number of flowers had the longest flowering. Flower number per inflorescence ranged from 77 to 180 (N=7). Each inflorescence had an average of about 5 open flowers daily. Mean lifetime of a flower was 20.7±6.2 hours (range 5–29 hours, N=38), i.e. from one day to the next. Flowers of the two chemovarieties had the same lifetime (Mann-Whitney U-test: $P<0.9$, N=17 (var. *canariensis*) *and* 21 (var. *anisata*) respectively). All flowers opened between 10 a.m. and 6 p.m.; 53% of them between 11 a.m. and 1 p.m. (N=73).

Flower visitors. Flowers of *C. canariensis* were visited by at least 27 different insect species (Table 3). The most frequent visitors were the endemic Canarian bumblebee species *Bombus terrestris* subsp. *canariensis*, the sphingid *Macroglossum stellatarum*, and various *Halictinae* spp. (*Halictidae*). These visitors accounted for 94.8% of all observed visits (Table 3). They were present during the

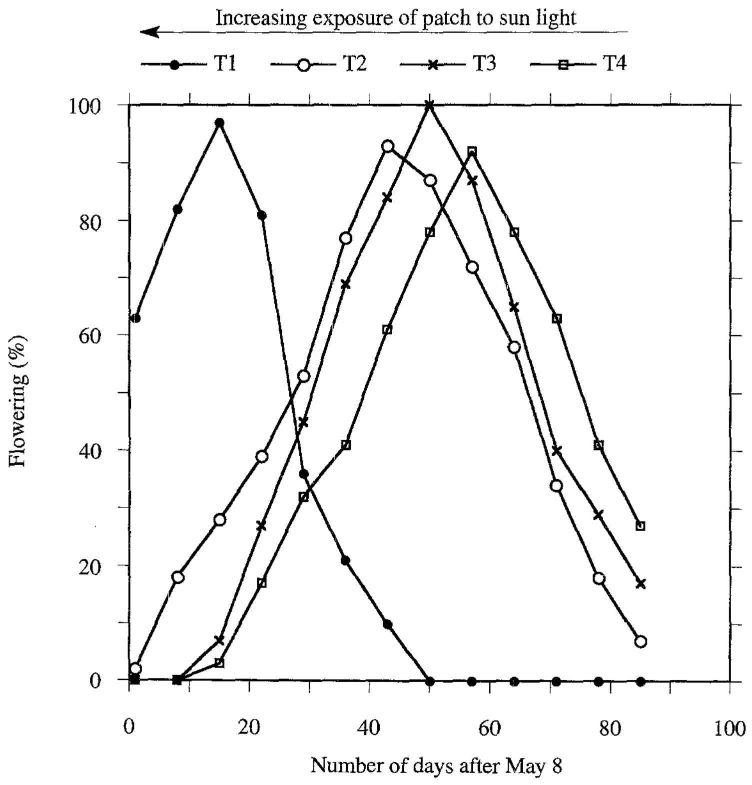

Fig. 5. Flowering phenology in four patches of *Cedronella canariensis* exposed to varying level of sun light (transect 1 was on a sunny slope; transect 4 was below the cannopy; and transects 3 and 4 were intermediates)

Table 3. Flower visitors of *Cedronella canariensis* and relative frequency of their visits and average flower handling time. *p* pollen; *n* nectar; *N* sample size; – frequency <0.1%

Species visitor	Aim of the visitor	Relative visit frequency (%)	Flower handling	
			Time (s)	N
Hymenoptera		**74.6**		
Andrenidae				
Andrena sp.	?	–	–	
Apidae				
Apis mellifera	n	–	–	
Bombus terrestris subsp. *canariensis*	p n	60.1	–	
legitimate foragers	p n	(20.7)	1.7±0.6	62
illegitimate foragers	p?n	(39.4)	0.9±0.5	42
Halictidae				
Halictinae spp. *	p n	10.3	20±29	62
Lasioglossum loetum	p n	–	–	
Anthophoridae				
Anthophora alluaudi	p n	4.2	1.5±0.5	21
Thyreus histrionicus	?	–	–	
Ichneumonidae sp.	?	–	–	
Lepidoptera		**25.1**		
Sphingidae				
Macroglossum stellatarum	n	24.4	<0.5**	50
Nymphalidae				
Pandoriana pandora	n	–	–	
Vanessa atalanta	n	–	–	
Vanessa indica subsp. *vulcanica*	n	–	8.5±2.3	57
Satyridae				
Pararge xiphioides	n	–	–	
Pieridae				
Artogeia rapae	n	–	–	
Gonepteryx cleobule	n	–	6.6±3.0	5
Lycaenidae				
Cyclyrius webbianus	n	–	–	
Lampides boeticus	n	–	–	
Lycaena phlaeas	n	–	–	
Noctuidae				
Cornutiplusia circumflexa	n?	–	–	
Geometridae				
Cleora fortunata	n?	–	–	
Coleoptera		**<0.1**		
Malachiidae				
Attalus sp.	p?	–	–	
Staphylinidae				
Aleocharinae sp.	?	–	–	

Table 3 (continued)

Species visitor	Aim of the visitor	Relative visit frequency (%)	Flower handling	
			Times (s)	N
Diptera		**< 0.1**		
Syrphidae				
Heringia adpropinquans	p?	–	–	
Meliscaeva auricollis	p?	–	–	
Myatropa florea	p?	–	–	
Scaeva albomaculata	p?	–	–	
Empidae				
Empis sp.	?	–	–	
Thysanoptera spp.	?	**<0.1**	–	

* Perhaps identical to *Lasioglossum loetum* or comprising several species of the genera *Halictus* and *Lasioglossum* (see text)

** Handling time of this species is difficult to measure accurately

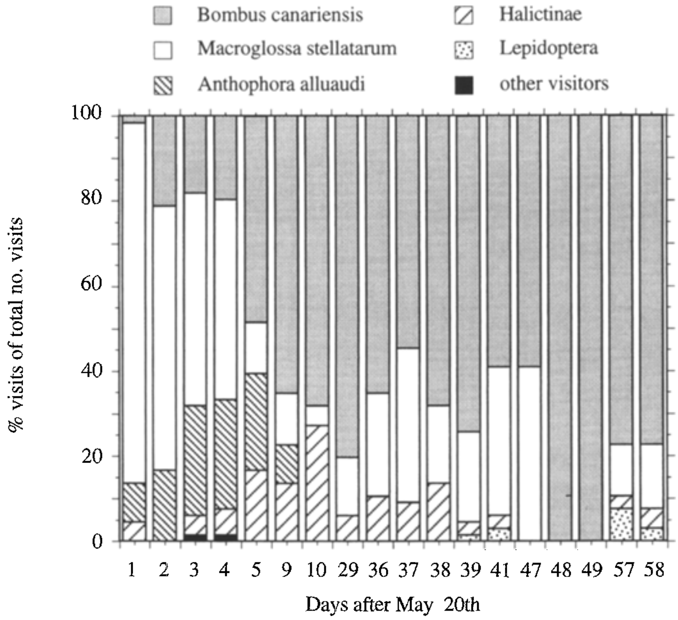

Fig. 6. Relative frequency of different flower visitors to *Cedronella canariensis* during the season

entire flowering season (Fig. 6), and total visitation frequency increased during the season (Fig. 7). At the onset of flowering, *Anthophora alluaudi* was an important visitor, accounting for up to 26% of all visits per day. However, it disappeared after nine days (Fig. 6). Three species of butterflies – *Vanessa indica*, *Gonepteryx cleobule*, and *Pandoriana pandora* – were irregular visitors throughout the season. *Attalus* sp. and *Thysanoptera* spp. were present in the interior of many flowers and buds. They were very frequent during the second half of the flowering season. Only

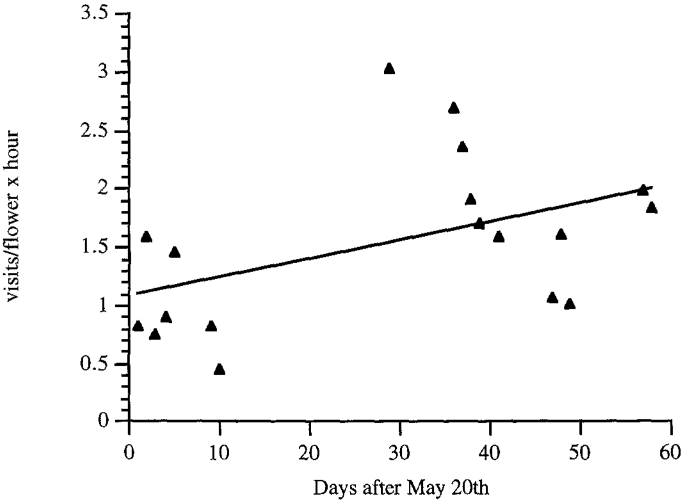

Fig. 7. Increase in total visitation frequency (all visitors pooled) during the flowering season of *Cedronella canariensis*

few nocturnal visitors were observed: Geometrid and Noctuid moths. Thus we suggest that nocturnal pollination of *C. canariensis* was quantitatively unimportant. Flowers of *C. canariensis* were visited for both pollen and nectar (Table 3). Butterflies, moths and most bees utilised the flowers as a source of nectar. *Bombus terrestris* subsp. *canariensis* routinely collected pollen from nearby individuals of *Adenocarpus foliolosus* (*Fabaceae*) and visited flowers of *C. canariensis* for nectar, whilst a few individuals of *B. terrestris* subsp. *canariensis* were seen actively to collect pollen from flowers of *C. canariensis* as well. *Halictinae* spp. collected both pollen and nectar. Syrphids, *Thysanoptera* spp. and *Attalus* sp. were attracted by pollen. Foraging behaviour of *B. terrestris* subsp. *canariensis* changed during the season. First, they collected nectar legitimately by thrusting their head and part of the thorax into the flowers, becoming visibly powdered with pollen. Around mid-season they started to pierce holes mainly along the basal half of flowers and large buds and extracted nectar illegitimately. This was nectar-robbery, since they contacted neither anthers nor stigmas. The shift from legitimate to illegitimate nectar-foraging in *B. terrestris* subsp. *canariensis* was first observed along the track exposed to sun and later along the track in shade (Fig. 8). *Macroglossum stellatarum* hovered in the air in front of the flower, touching it only with its proboscis. Its visiting behaviour was thus different from all others, which spent longer time on each flower and landed on the flowers (Table 3). Individuals of *Halictinae* spp. literally disappeared into the flower to get nectar. When they collected pollen they sat on the lower lip of the flower. Butterflies sat on the inflorescence inserting their long proboscis from the outside of the flower. The difference in handling time between legitimate and illegitimate *Bombus* visitors was significant (Table 3, Mann-Whitney U-test, $P < 0.0001$). The nectar-robbing holes made in buds by bumblebees seemed to function as an easy entrance for *Thysanoptera* spp. On July 18, almost all robbed buds were visited by Thysanopterans, whereas the frequency was much lower for intact buds. They

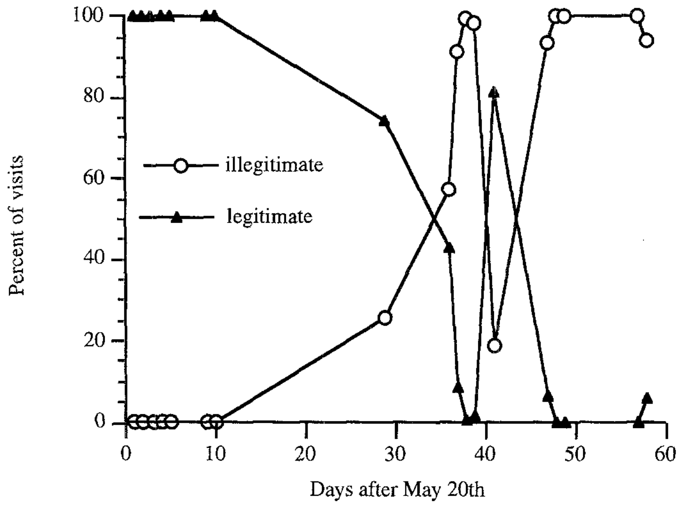

Fig. 8. Relative frequency of legitimate and illegitimate visits made by *Bombus terrestris* subsp. *canariensis* to flowers of *Cedronella canariensis* throughout the season. On June 30 (day 41), observations along the open forest track stopped, and observations were continued below the canopy

might have been responsible for the destruction of some of the stamens. *Bombus terrestris* subsp. *canariensis* preferred var. *canariensis* (398 visits out of 734, $\chi^2 = 5.24$, $P < 0.02$), but *M. stellatarum* did not differentiate (73 visits out of 130, $\chi^2 = 1.97$, $P < 0.16$). None of the visitors was observed to pay any notice to the anther glands and their oil secretions.

Pollen load of flower visitors. Individuals of *Halictinae* spp. contained by far the highest loads of *C. canariensis* pollen (Table 4). Lepidopterans carried significant amounts of pollen on their thorax and only a few grains on other body

Table 4. Pollen load (mean ± SD, exclusively corbicular or scopal load) of visitors to flowers of *Cedronella canariensis*. Sample size is given in parentheses after insect name

	No. of *C. canariensis* pollen grains/ insect[1]	Pollen of other species (% of total load)	% of visitors with *C. canariensis* pollen
Bombus terrestris subsp. *canariensis* (52)			
legitimate (37)	28±31	40±35	92
illegitimate (15)	7±10	50±22	53
Halictinae spp. (14)	774±1337	47±36	100
Anthophora alluaudi (7)	303±213	31±25	86
Macroglossum stellatarum[2](29)	14±13	11±21	48
Vanessa indica ssp. *vulcanica (3)*	267±255	20±33	100
Gonepteryx cleobule (6)	35±36	28±30	100

[1] Only visitors carrying *C. canariensis* pollen are included.

[2] Only the proboscis was inspected for pollen, since it was the only body part that came into contact with flowers.

Table 5. Results of pollination experiments and fruit set in *Cedronella canariensis* varieties. (*N*) sample size

Crossing type	Fruit set (%) var. *canariensis* (N)	 var. *anisata* (N)
i Bagged, unmanipulated (spontaneous selfing)	75.6 (27)	79.3 (29)
ii Bagged, selfed by hand (mediated selfing)	85.2 (27)	84.6 (26)
iii Bagged, emasculated (agamospermy)	6.3 (16)	0.0 (16)
iv Bagged, emasculated, outcrossed by hand		
Between variety	72.7 (11)	88.9 (18)
Within variety	88.9 (18)	88.5 (26)
Unbagged, insect-pollinated	85.2 (27)	84.6 (26)

parts. The two most abundant visitors, *M. stellatarum* and *B. terrestris* subsp. *canariensis*, were covered by moderate amounts of pollen. Legitimate and illegitimate *B. terrestris* subsp. *canariensis* visitors differed significantly in size of pollen load (Mann-Whitney U-test, P<0.008). In two out of 12 corbicular pollen loads from *B. terrestris* subsp. *canariensis*, pollen from *C. canariensis* constituted as much as 60 and 70% of the entire load. In another five, it was rare, 1–10%. Seven loads had pollen from *Adenocarpus foliolosus* as their major pollen source, in one case as the only pollen type. Four had pollen from *Asteraceae* spp. as their major constituent. One out of four loads from *Anthophora alluaudi* contained an almost pure load of *C. canariensis* pollen. Two had mixtures of *C. canariensis* pollen and other species, but with *C. canariensis* as a major fraction (65 and 80%). One had an almost pure load of an unidentified pollen type.

Mating system. The species was not agamospermous, as shown by the very low level of fruit set by enclosed, emasculated flowers (Table 5). None of the other treatments varied in fruit set, and the species was fully self-compatible. Reproduction was not limited by pollination. Seed set did not differ significantly between treatments and varieties. Average number of seeds per fruit for all treatments was 3.6±0.7 (N=187). Outcrossing resulted in significantly heavier seeds than self-pollination (1.7±0.2 mg, N=193, and 1.6±0.3 mg, N=123 respectively; Mann-Whitney U-test, P<0.001).

Discussion

Stress-induced male sterility or gynomonoecy. Female flowers of *C. canariensis* were only seen towards the end of the breeding season, when the weather became hot. Thus stress might lead to a shift in sex. In a greenhouse experiment, two plants were exposed to dry soil conditions. This induced the plants to produce a number of female flowers, while control plants produced perfect flowers of normal size and colour (unpubl. obs.). The production of female flowers in response to stress was surprising: in most studies of sex change, stressed plants produce more male flowers (FREEMAN & al. 1984). In addition, the shift to gynomonoecy at the end of the flowering season may increase the outcrossing rate (BAWA & al. 1982). Only few gynomonoecious populations have been studied with respect to sex variation (Lloyd & Bawa 1984).

Significance of aromatic oil production. None of the flower visitors seemed to pay any attention to the anther glands of *C. canariensis*. The oils may, however, function as a defence against herbivores or parasites. Material from both varieties has been shown to contain powerful agents against both bacteria and fungi (López-Garcia 1992).

Outcrossing. Prolonged flowering of the population and individual inflorescences combined with a short lifespan of individual flowers suggest a strategy, by which the plant maximises outcrossing by offering only a few open flowers at a time and thus forcing visitors to make more inter-inflorescence flights. The prolonged flowering is also a way of hedging the bets against weather unfavourable to forging insects. Fog and low temperatures may prevail in the mountains for weeks.

A balance between pollinators of quantitative and qualitative importance. The visitor spectrum of *C. canariensis* was very similar to that of another *Lamiaceae* species, *Lavandula latifolia* growing in woodland, 1000 m a.s.l. in Southern Spain (Herrera 1987, 1989). This may be due to the similar flower morphology and the low amounts of concentrated nectar found in both species. In *L. latifolia, Macroglossum stellatarum* and *Bombus terrestris* were among the most frequent flower visitors, while a *Halictus* sp. was most effective in stigmatic pollen depositing. The two most abundant visitors of *Cedronella canariensis, B. terrestris* subsp. *canariensis* and *M. stellatarum* carried moderate amounts of pollen. In contrast, some of the less abundant visitors, *Halictinae* spp., *Anthophora alluaudi*, and two butterfly species, carried much larger loads. However, for species with a low number of ovules per flower, a number of pollen grains equal to or even lower than the number of ovules may be sufficient to initiate fruit set (Herrera 1989 and references therein). The variation in pollen load size among visitors might thus only have a minor impact on seed set. Herrera (1987, 1989) concluded that balancing selection by different aspects of pollinator quality in the different visitor taxa (e.g. high deposition rates by *Hymenoptera*, but long flight distances and hence high rates of outcrossing by *Lepidoptera*) would tend to keep *L. latifolia* in a state of promiscuity rather than to push it into specialisation together with one particular pollinator group. In view of its strong capacity for spontaneous selfing and clonal growth, *Cedronella canariensis* may not benefit very much from insect-mediated selfing. Instead, attraction of pollinators promoting outcrossing and thus affecting seed quality more than quantity might have a high fitness premium. The P:O ratio was within the range given by Cruden (1977) for facultatively xenogamous plants, and was surprisingly high for a fully self-compatible species. Higher average seed weight of outcrossed seeds in *C. canariensis* indicates inbreeding depression. In general, butterflies fly longer distances, while bees and flies typically exhibit leptokurtic flight patterns. Butterflies might thus be important as long-distance pollinators influencing the genetic quality of seeds (Schmitt 1980; Herrera 1989; Olesen & Warncke 1989a, b). Therefore we suspect that *C. canariensis* gets seed of a higher quality from pollination by *Macroglossum stellatarum* and other lepidopterans, and only gains little in fitness from attracting bees and flies. Half the visits performed by *B. terrestris* subsp. *canariensis* might even be detrimental to the plant (however, see OLESEN 1997). Only a few centuries ago, very few tracks and no roads traversed the laurel forests. We may visualise a perennial, clonally reproducing plant growing in scattered light gaps in the forest. Under such a scenario visits by short-ranging insects of low specificity would have little significance on outcrossing.

Cedronella canariensis is highly self-compatible and spontaneous selfing might take place frequently. Outcrossing may be mediated by long-distance flying lepidopterans, and devices promoting outcrossing include short lifespan of individual flowers, prolonged flowering season, and male sterility/gynomonoecy. Pinkish flower colour, deep tubular flowers with small amounts of nectar may increase visitation by lepidopterans and decrease visitation by legitimately foraging bumblebees. Thus an ability to self and propagate vegetatively, coupled with some level of outcrossing, may be a typical set of characters in plants which have colonised islands successfully. As a light-gap species it may retain a high level of intrapatch genetic variation by preferentially attracting long-distance pollen agents such as lepidopterans. This mixed reproductive strategy has obviously been successful in *C. canariensis*, judged by its abundance in the laurel forests of the Canaries.

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References

BAKER, H. G., 1955: Self-compatibility and establishment after "long-distance"dispersal. – Evolution **9**: 347–348.

BAWA, K. S., WEBB, C. J., TUTTLE, A. F., 1982: The adaptive significance of monoecism in *Cnidoscolus urens* (L.) ARTHUR (*Euphorbiaceae*). – Biol. J. Linn. Soc. **85**: 213–223.

BRAMWELL, D., BRAMWELL, Z. I., 1990: Flores silvestres de las Islas Canarias. – Madrid: Rueda.

BUDANTSEV, A. L., 1993: Synopsis of the tribe *Nepeteae* (*Lamiaceae*). – Bot. Zhurn. (St. Petersburg) **78**: 106–115.

CARLQUIST, S., 1974: Island biology. – New York: Columbia University Press.

COEN, M., ENGEL, R., NAHRSTEDT, A., 1995: Chavicol β-D-glucoside, a phenylpropanoid heteroside, benzyl-β-D-glucoside and glycosidically bound volatiles from subspecies of *Cedronella canariensis*. – Phytochemistry **40**: 149–155.

CORBET, S. A., 1978: Bees and the nectar of *Echium vulgare*. – In RICHARDS, A. J., (Ed.): The pollination of flowers by insects, pp. 21–30. – London: Academic Press.

CRUDEN, R. W., 1977: Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. – Evolution **31**: 32–46.

EHRENDORFER, F., 1979: Reproductive biology in island plants. – In BRAMWELL, D., (Ed.): Plants and islands, pp. 293–306. – London: Academic Press.

ENGEL, R., 1994: Zusammensetzung und Ontogenese der ätherischen Öle von *Cedronella canariensis* (L.) WEBB & BERTH. ssp. *canariensis* und ssp. *anisata* (*Lamiaceae*) unter besonderer Berücksichtigung der GC-MS-Analytik. – Ph.D. Dissertation, Universität Münster, Institut für Pharmazeutische Biologie und Phytochemie.

FORFANG, A. -S., OLESEN, J. M., 1998: Male-based sex ratio and promiscuous pollination in the dioecious island tree *Laurus azorica* (*Lauraceae*). – Pl. Syst. Evol. **212**: 143–157.

FOWLER, J., COHEN, L., 1990: Practical statistics for field biology. – Chichester: Wiley.

FREEMAN, D. C., MCARTHUR, E. D., HARPER, K. T., 1984: The adaptive significance of sexual liability in plants using *Atriplex canescens* as a principal example. – Ann. Missouri Bot. Gard. **71**: 265–277.

HEINE, E. M., 1937: Observations on the pollination of New Zealand flowering plants. – Trans. & Proc. New Zealand Inst. **67**: 133–148.

HERRERA, C. M., 1987: Components of pollinator "quality": comparative analysis of a diverse insect assemblage. – Oikos **50**: 79–90.

– 1989: Pollinator abundance, morphology, and flower visitation rate: analysis of the "quantity" component in a plant-pollinator system. – Oecologia **80**: 241–248.

LACK, A. J., KEVAN, P. G., 1984: On the reproductive biology of a canopy tree, *Syzygium syzygioides* (*Myrtaceae*), in a rain forest in Sulawesi, Indonesia. – Biotropica **16**: 31–36.

LINHART, Y. B., FEINSINGER, P., 1980: Plant-hummingbird interactions: Effects of island size and degree of specialization on pollination. – J. Ecol. **68**: 745–760.

LLOYD, D. G., BAWA, K. S., 1984: Modification of the gender of plants. – Evol. Biol. **17**: 255–329.

LÓPEZ-GARCIA, R. E., 1992: Essential oils and antimicrobial activity of two varieties of *Cedronella canariensis* (L.) W. et B. – J. Ethnopharmacol. **36**: 207–211.

MAYR, E., 1963: Animal species and evolution. – Cambridge, Mass.: Harvard University Press.

OLESEN, J. M., 1985: The Macaronesian bird-flower element and its relation to bird and bee opportunists. – Bot. J. Linn. Soc. **91**: 395–414.

– 1988: Floral biology of the Canarian *Echium wildpretii*: bird-flower or a water resource to desert bees: – Acta Bot. Neerl. **37**: 509–513.

– 1992: How do plants reproduce on their range margin? – In THASOS, C. A., (Ed.): Plant-animal interactions in mediterranean-type ecosystems, pp. 217–222. – Athens: University of Athens.

– 1997: From naïveté to experience: bumblebee queens (*Bombus terrestris*) foraging on *Corydalis cava* (*Fumariaceae*). – J. Kansas Entomol. Soc. **69** (Suppl.): 274–286.

– WARNCKE, E., 1989a: Predation and potential transfer of pollen in a population of *Saxifraga hirculus* L. – Holarc. Ecol. **12**: 87–95.

– – 1989b: Temporal changes in pollen flow and neighbourhood structure in a population of *Saxifraga hirculus* L. – Oecologia **79**: 205–211.

PÉREZ DE PAZ, P. L., PÉREZ ALONSO, M. J., VELASCO NEGUERUELA, A., GIL PINILLA, M., GARCÍA VALLEJO C., ESTEBAN, J. L., 1996: Variación morfológica y aceites esenciales de *Cedronella canariensis* (L.) WEBB & BERTHEL. (*Labiatae*). – Anales Jard. Bot. Madrid **54**: 303–307.

SCHMITT, J., 1980: Pollinator foraging behaviour and gene dispersal in *Senecio* (*Compositae*). – Evolution **34**: 934–943.

SIEGEL, S., CASTELLAN, N. J., 1988: Nonparametric statistics for the behavioral sciences. – New York: McGraw-Hill.

SUNDING, P., 1970: Elementer i Kanariøyenes flora, og teorier til forklaring av floraens opprinnelse. – Blyttia **28**: 229–259.

VOGEL, S., WESTERKAMP, C., THIEL, B., GESSNER, K., 1984: Ornithophilie auf den Canarischen Inseln. – Pl. Syst. Evol. **146**: 225–248.

WEBB, P. B., BERTHELOT, S., 1890: La histoire naturelle des ïles Canaries, III. Botanique. – Paris: Phytographia canariensis.
WESTERKAMP, C., 1990: Blumenvögel auf den Islas Canarias. – Trochilus **11**: 79–81.

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