

Natural hybridization between species of *Calceolaria* with different pollination syndromes in southern Patagonia, Argentina

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Abstract. *Calceolaria uniflora* and *C. polyrhiza* differ markedly in flower structure, color, and in their pollination mechanisms. Flowers of *C. polyrhiza* have a hidden glandular oil-secreting appendage that in the genus typically attracts bee pollinators, while in *C. uniflora* this is replaced by an external, white and fleshy structure that functions as food body and attracts a bird pollinator. Populations with intermediate flower morphology, presumably hybrids, were found in several sites over a large area of southern Patagonia, Argentina. The intermediate forms have so far been found always in contact or close proximity with typical populations of both presumed parent species. The distribution pattern and phenetic intermediacy in several independent flower characters strongly suggest widespread interspecific hybridization in the range of geographic overlap of the two species. The pattern of variation of intermediate forms in some sites is indicative of a hybrid swarm and possible introgression. Of particular interest is the range of intermediate structures and positions of the flower appendage, between oil gland and food tissue, that can be found in hybrid populations. The observed phenomenon raises several questions concerning the biological mechanisms generating it and its evolutionary consequences.

Key words: *Calceolaria*, Scrophulariaceae, natural hybridization, introgression, hybrid swarm, Patagonia.

Introduction

The mechanisms of reproductive isolation that normally delimit plant species are in some cases ineffective. In those cases natural breeding and hybridization between two distinct, but related and sympatric species may occur. Although interspecific hybrids are often sterile, occasional interbreeding of parent species with fertile hybrid individuals allows a certain degree of gene flow between both species. This process is responsible for the production of new gene combinations in either or both parent species, which is an important mechanism in plant evolution. Crossing between two sympatric species and backcrossing of the hybrids with one or both parental species may result in local introgression (Anderson 1953), when the gene flow is restricted to few sites where the species grow together (Judd et al. 1999). In these sites it is possible to

distinguish individuals of both parental species and a wide range of intermediate forms, the “hybrid swarm”, which show a mixture of characters of the parent species. Natural hybridization and introgression can result in the formation of a new, hybrid species, only in case that the hybrid or introgressive forms become reproductively isolated from both parent populations and then reproduce strictly within the hybrid population.

Natural hybridization is often associated with environmental disturbances (Stebbins 1959, Judd et al. 1999). Natural disturbances such as floods, fire, pests, volcanic activities can disrupt the natural ecological isolating mechanisms between two related species, creating opportunities for hybridization and suitable habitats for the development of hybrids. Human disturbance of habitats and widespread introduction of species to new regions has greatly promoted hybridization between previously isolated species (Dobzhansky et al. 1980, Judd et al. 1999). A large proportion of the published cases of natural interspecific hybridization and of hybrid speciation comes from North America and Europe, where human disturbance and introduction activities are prevalent (e.g. Anderson and Taylor 1983, Wyatt and Broyles 1992, Wolfe and Elisens 1993, Krahulcova et al. 1996, Albert et al. 1997, Gil-Ad and Reznicek 1997, Dibble et al. 1998). It has been estimated that about 30% of the 2,500 species in the well-studied British flora are of hybrid origin (Stace 1989). However, the frequency of hybridization and hybrid species in other continents is unknown. It may be lower in regions such as South America, where the modifications of habitat and biota have not been as intensive.

Calceolaria, an Andean genus of the Scrophulariaceae family, contains about 270 species (Molau 1988). Most of them are allogamic, pollinated by oil-collecting bees. About 49 species are autogamous or visited by pollen-collecting bees. Only one species, *Calceolaria uniflora* Lam. is known to be pollinated by birds, which obtain a food tissue as reward,

rather than pollen or oils (Sérsic 1994, Sérsic and Cocucci 1996). The species of this genus are reproductively isolated not only by their pollinators, but also by their geographic distribution, different phenologies, varied habitat preferences, and internal barriers (Molau 1988).

The feasibility of artificial hybridization between species of *Calceolaria* was already mentioned by Darwin (1859) in “The origin of species”. He reported that hybrids obtained from *C. integrifolia* × *C. plantaginea*, two species with very dissimilar habits, produced fertile seeds. Presently, there are many *Calceolaria* cultivars bred for ornamental purposes, known by names such as *C.* “Anytime”, *C.* Bikini series, *C.* Monarch Series, *C.* “Sunshine”, *C.* “John Innes”, *C.* “Walter Shrimpton” (Bailey 1949, Brickel 1991). Many of them were apparently obtained by artificial selection and hybridization, such as the commercially important cultivars of *C.* “*herbaeohybrida*”.

Natural hybridization in *Calceolaria* has occasionally been documented between species of the same section, mainly in the context of human disturbances in tropical latitudes (Molau 1988). Molau registered some cases of natural hybridization in marginal populations of *C. perfoliata* L.f. and *C. calycina* Benth. (Sect. *Perfoliatae*). *C. crenata* Lam. and *C. ferruginea* Cav. (Sect. *Thamnobia*), originally two allopatric species, came in contact after the construction of a road and produced hybrid swarms. *C. dilatata* Benth. and *C. lanata* H.B.K. (Sect. *Perfoliatae*), although sympatric, had been isolated by different habitat requirements. Human disturbances modified habitat features in a way that apparently favored the proliferation of a hybrid of both species. A natural hybrid between *C. glabrata* Phil. var. *glabrata* and *C. tenera* Phil. has been mentioned in south-central Chile (Hoffmann et al. 1998). Natural hybridization, although scarce, can be an important factor of speciation in the genus, as suggested by Molau (1988), with some probable cases of allopolyploidy and homogamic complexes.

This paper is a preliminary report on intermediate floral forms between *Calceolaria uniflora* and *C. polyrhiza* Cav. in southern Patagonia, that indicate frequent natural hybridization and introgression between these two species, in sites where they co-occur.

Materials and methods

Survey. The discovery of the presumed hybrids between *Calceolaria uniflora* and *C. polyrhiza* was a result of a study of infra-specific variation of flower morphology and reproductive biology in *C. uniflora* (Mascó et al. 2000 and unpublished). This study involved an intensive survey and sampling of populations of *C. uniflora* throughout the provinces of Santa Cruz and Tierra del Fuego in Argentina for a quantitative analysis of flower morphology

(Fig. 1). A few strange flowers with characteristics intermediate between *C. uniflora* and *C. polyrhiza* were first observed and photographed in two distant sites in 1997 and in 1998, respectively. A survey of populations of *C. uniflora* in southern Santa Cruz in December 2000, following unusually abundant winter and spring precipitation, revealed several additional and in some cases abundant populations of presumed hybrids within or adjacent to populations of *C. uniflora*. Populations of the latter species were searched for and sampled along several road transects across the region, totaling about 1400 km.

Photography. At each site surveyed in 2000, a random sample of about 30 plants of *C. uniflora* was defined (Mascó unpublished). One flower of each sampled plant was removed and photographed with a scale, using a Pentax Z10 camera with annular flash and macro-lens. In sites where intermediate forms were seen, a sample of up to 30

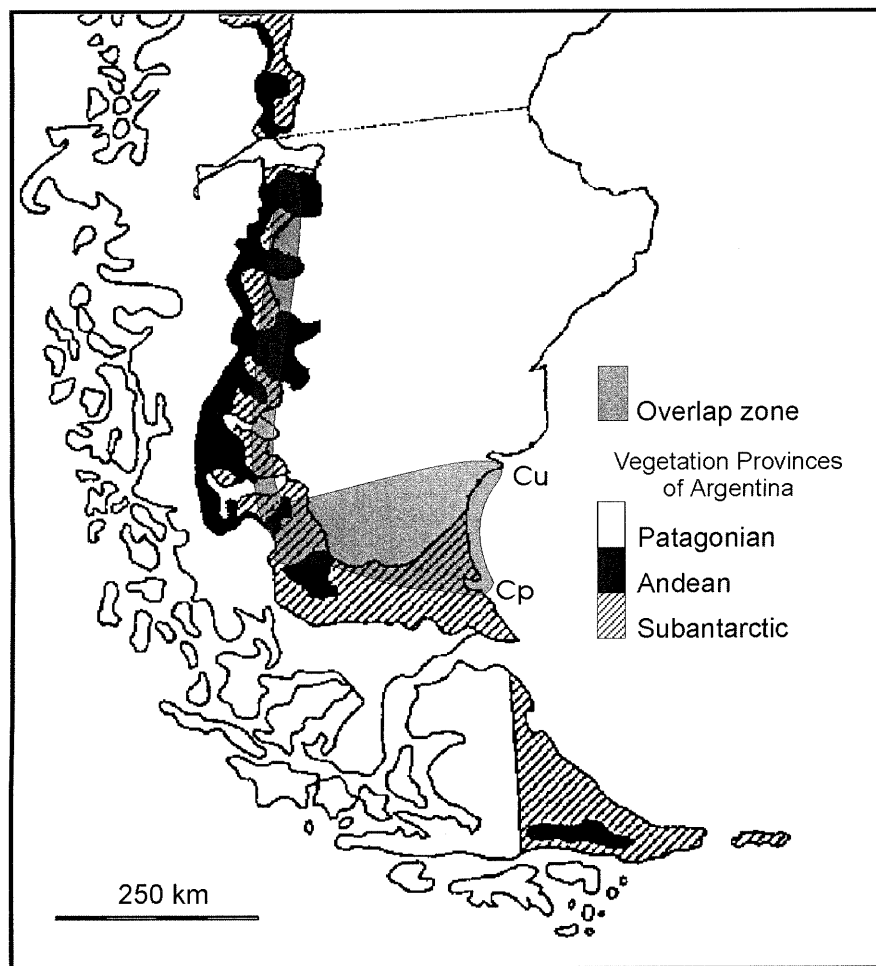


Fig. 1. Geographical distribution of the parent species, delimiting an overlap zone (gray, Cu + Cp) where the intermediate forms grow. Cu: distributional range of *Calceolaria uniflora*; Cp: distributional range of *Calceolaria polyrhiza*

intermediate flowers (or less if they were rare) was similarly taken and photographed. A few flowers of typical *C. polyrhiza* that occurred nearby were also photographed as reference.

Data analysis. A quantitative analysis of flower dimensions from scaled photos of individual flowers was carried out in four sites where large populations of both intermediate forms and *C. uniflora* occurred, as well as some *C. polyrhiza*. The trends were similar in the four sites, hence the results are presented for the pooled data from the four sites. Non-parametric analysis of variance (Kruskal–Wallis test) was used to test for significant differences between the three groups in several dimensions of the corolla.

Results

Description of parent species. The flowers of all species of *Calceolaria* are built on the same pattern. They have a tetramerous calyx and a two lipped zygomorphic corolla. The upper lip, commonly smaller than the lower one, normally encloses the fertile parts. The androecium includes two stamens and the gynoecium two carpels. The lower lip always shows a slipper-like structure. The basal part of the lip, near to the insertion, is not inflated and is called the throat. The distal end of the throat continues forming the bubble or saccate portion of the lip. This portion shows a sole, which is the lower surface of the slipper; an instep, which is the upper surface of the slipper and a point, which corresponds to the most distal margin of the slipper, between the sole and the instep. The proximal end of the instep is prolonged into a lap or an appendage, which is normally folded to the inside of the bubble hiding in that way the oil-secreting gland or elaiophore, which most of the species develop on this structure. Depending on the species, there is a considerable variation in the position of the lap, reaching different angles of inflection, which vary from 0 to 360 degrees. The lower lip can experiment an inflection upwards, building on the throat an inflection line called the heel.

***Calceolaria uniflora* Lam.** Rosulate plants, forming small patches of ca. 8 to 15 cm height. Leaves spatulate and glandular, attenuate at

the petiole, margin entire or irregularly toothed. Flowers are solitary, large and notable colored, of about 2–3.5 cm in length (Fig. 2A). The yellow or sometimes reddish upper lip is wide and hooded not covering the stamens. These are relatively long and extend diverging from the median line; the thecae are versatile. The lower lip is long and straight (1.8–3.3 cm), the opening is large, the throat is normally yellow with red flecks; the saccate portion shows the appendage flexed outwards in an angle of 360 degrees, as a notable transversal white and fleshy band (Sérsic 1994, Sérsic and Cocucci 1996). The white color contrasts with the yellow and red colors of the instep and throat. The elaiophore is very weakly developed and stays hidden inside the fold, between the instep and the thickened lap. The glandular hairs are not functional. Seeds are ellipsoidal.

***Calceolaria polyrhiza* Cav.** Rosulate to subrosulate plants of ca. 10 to 20 cm height. Leaves ovate to lanceolate attenuate at the petiole, margin entire to crenulate. Flowers in few flowered, cymose inflorescences. Upper lip small, hooded and partially covering both stamens, these are not divergent, anthers are not versatile, but have dorso-ventral movements. Lower lip is curved upwards with a total length of 1–2 cm, the opening is as long as the instep (Fig. 2B). The appendage is inflected to the inside of the saccate portion and has a well developed and functional elaiophore. Characteristic of this species in contrast with *C. uniflora* is the development of a lateral constriction at the beginning of the instep. Seeds are oblong. The color of the flower is mainly yellow sometimes with some sparse red spots on the throat and the instep.

Comparison of flower morphology between parent species and intermediate forms. The presumed hybrids are intermediate between *Calceolaria uniflora* and *C. polyrhiza* in a combination of several quantitative and qualitative characters of flower morphology (Table 1). At any site, the hybrid flowers vary in size (length and width) over a range that spans the typical difference between the larger

C. uniflora and the smaller *C. polyrhiza*. However, since there is also considerable variation in size within each parent species, size alone is not an absolute diagnostic for the identification of hybrids. Neither is the number of flower per inflorescence, although *C. uniflora*

always has single flowers and hybrids can be found with inflorescences of two flowers.

In the statistical analysis of the floral dimensions, significant differences between the means of *C. uniflora*, presumed hybrids and *C. polyrhiza* were found for six of the

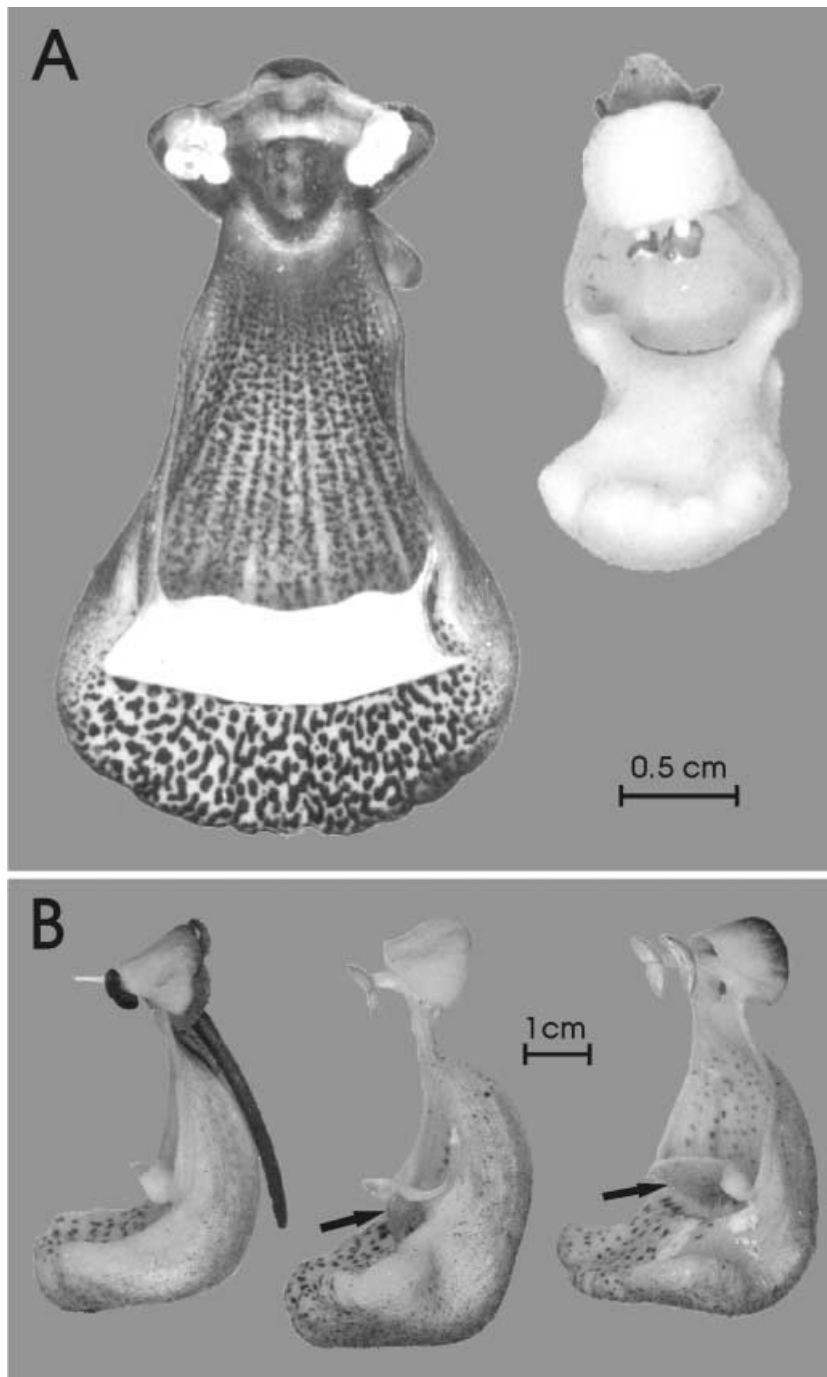


Fig. 2. **A** Parent species. Left: *Calceolaria uniflora*, right: *Calceolaria polyrhiza*. **B** Intermediate forms. Note the different inflection angles of the appendage. Left and middle flowers show an horizontal appendage and right flower shows it almost in a vertical position. The middle and right flowers also show the elaiophore (arrows)

Table 1. Comparison of flower morphology of intermediate forms with *Calceolaria uniflora* and *C. polyrhiza* observed at the same sites

Character	<i>C. uniflora</i>		<i>C. polyrhiza</i>	
	<i>C. uniflora</i>	Intermediate forms	Intermediate forms	<i>C. polyrhiza</i>
Inflorescence	1 flower	1–2 flowers	1–2 flowers	1–4 flowers
Stamens	divergent	Not divergent	Not divergent	Not divergent
Length of lower corolla lip	2.5–3.3 cm	1.9–2.9 cm	1.6–2.2 cm	1.6–2.2 cm
Length of opening of lower lip	1.5–2.0 cm	0.7–1.6 cm	0.7–1.0 cm	0.7–1.0 cm
Width of instep of lower lip	1.6–2.3 cm	1.3–2.0 cm	1.1–1.6 cm	1.1–1.6 cm
Inflection of lower lip with respect to peduncle (lateral view)	Slightly inflected	Intermediate inflection	Intermediate inflection	Strongly folded
Constriction of lower lip at base of instep (frontal view)	Not constricted	Usually slightly constricted	Usually slightly constricted	Constricted
Position of the appendage	Entirely folded outward	Intermediate positions, usually? erect	Intermediate positions, usually? erect	Entirely folded inward
Presence of elaiophore and/or food tissue	Food tissue as a broad white band	Food tissue as a broad white band	Food tissue: narrow yellowish-white band above an elaiophore	Elaiophore, not visible frontally
Colour of instep	Dense red-brown patches on yellow background; or reticulate red-brown pattern on yellow background; or uniform red-brown	Dense red-brown patches on yellow background; or reticulate red-brown pattern on yellow background; or uniform red-brown	Yellow with red speckles of varying density; sometimes reticulate red pattern on yellow background; rarely uniform red or yellow	Yellow, sometimes with few small red speckles
Colour of throat	Yellow background with usually dense or red-brown speckles, usually aligned in vertical lines	Yellow background with usually dense or red-brown speckles, usually aligned in vertical lines	Yellow, usually with red speckles of varying density	Yellow, often with sparse small red speckles

Table 2. Means, lower (Q10%) and upper (Q90%) deciles of distributions of flower dimensions of *C. uniflora*, intermediate forms and *C. polyrhiza*. Data pooled from four sites sampled in December 2000. Significance of differences between groups by Kruskal–Wallis non-parametric analysis of variance: *** $P < 0.001$, NS $P > 0.05$

Dimensions (cm)	<i>C. uniflora</i> N = 131		Intermediate forms N = 83		<i>C. polyrhiza</i> N = 20		Significance (Kruskal–Wallis)
	Mean	Q (10%)	Q (90%)	Mean	Q (10%)	Q (90%)	
Lower lip total length	2.89	2.47	3.31	2.34	1.92	2.86	***
Instep length (inclusive appendage)	1.12	0.89	1.38	1.18	0.86	1.45	NS
Instep length (exclusive appendage)	0.67	0.49	0.88	0.93	0.66	1.21	***
Visible appendage length	0.45	0.30	0.58	0.25	0.15	0.32	***
Opening length	1.76	1.48	2.01	1.16	0.74	1.65	***
Lower lip maximum width	1.93	1.59	2.27	1.71	1.32	2.02	***
Upper lip width	1.03	0.77	1.29	0.90	0.72	1.15	***
				Mean	Q (10%)	Q (90%)	
				1.95	1.60	2.16	
				1.12	0.82	1.43	
				1.12	0.82	1.43	
				0.00	0.00	0.00	
				0.82	0.67	0.97	
				1.37	1.13	1.57	
				0.78	0.62	0.88	

seven variables measured (Table 2). For all these variables, the hybrid group showed mean values intermediate between the two parent species and distributions that overlapped with one or both of them at the extreme deciles. Only the mean length of the instep including the appendage was similar between the groups. As the length of the appendage visible in frontal view decreases from *C. uniflora* to hybrids and to *C. polyrhiza*, the length of the exposed part of the instep below the appendage increases. All other floral dimensions decreased from *C. uniflora* to *C. polyrhiza*.

The shape of the flowers of the two parent species is quite different. This can be observed, for instance, in the degree of curvature of the lower lip in a lateral view and in the presence or absence of a constriction in the middle of the lower lip viewed frontally (Fig. 2A, B). Hybrid flowers are usually intermediate in both characters, although there is a certain amount of variation (Table 1, Fig. 3).

The flowers of *Calceolaria uniflora* and *C. polyrhiza* are unmistakably distinct in the colors and color patterns of the lower lip, although within *C. uniflora* there is considerable variation (Mascó et al. 2000 and unpublished). The coloration of presumed hybrid flowers is typically intermediate between the predominant yellow of *C. polyrhiza* and the more or less intense speckling with dark red to brown in *C. uniflora* (Table 1). However, there is considerable diversity in color patterns between individual plants. A few are almost pure yellow, or conversely have large areas of the darker colors.

The most striking contrast between *C. uniflora* and *C. polyrhiza* is in the location and shape of the appendage of the lower lip, that in turn is associated with the pollination biology of the two species. In *C. polyrhiza*, like in many other species of the genus, the appendage is curved inward against the inside of the instep and covered with a patch of oil secreting glandular hairs or elaiophore (Sérsic 1994, Sérsic and Cocucci 1996, Vogel 1974). The oils secreted by this structure are collected by

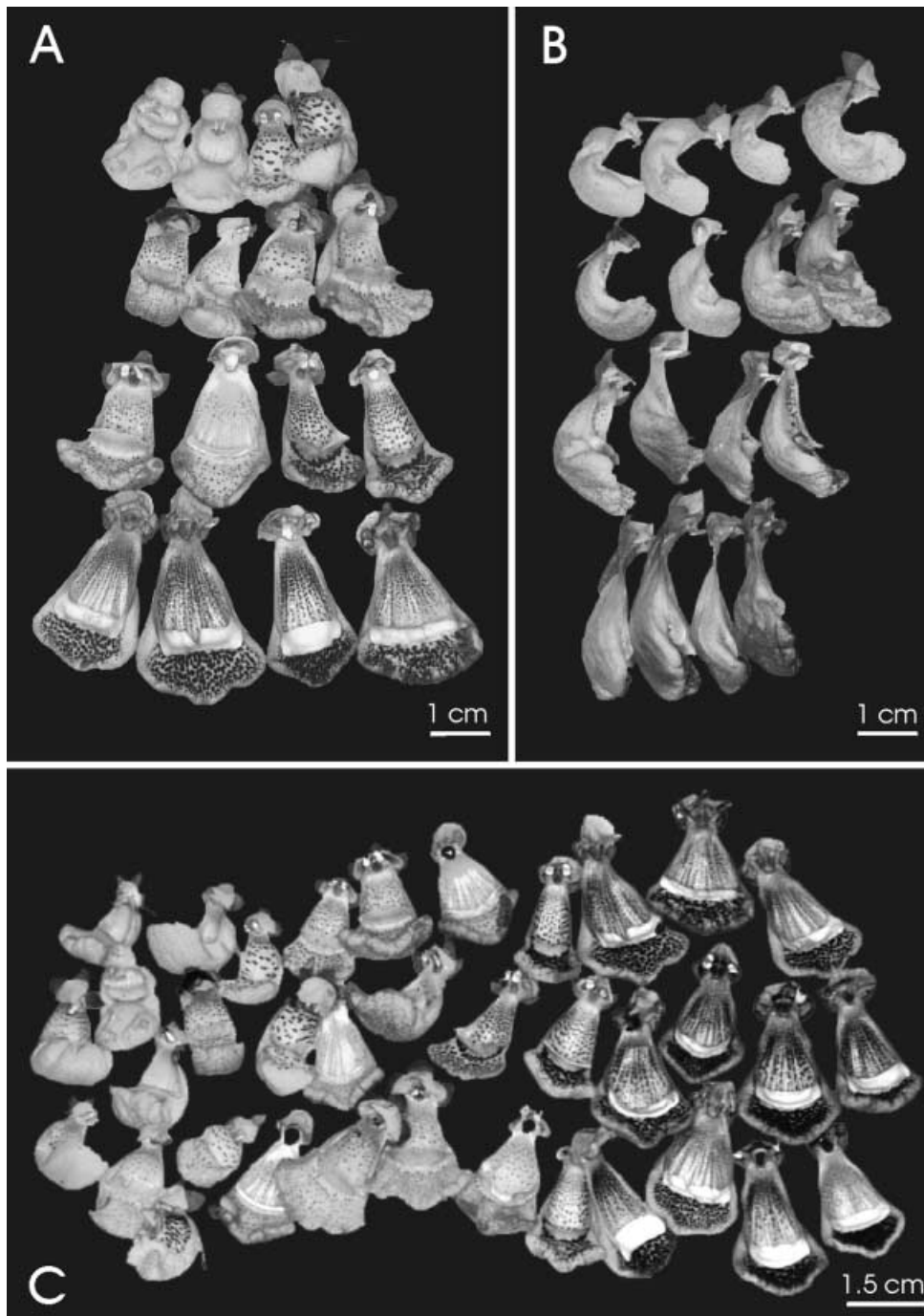


Fig. 3. Variation of flower forms and colors from *C. polyrhiza* (upper row) to *Calceolaria uniflora* (lower row) with intermediate forms between them, all from one site (GAC21). **A** Frontal view. **B** Lateral view. **C** Full spectrum of variation at the site, with *C. polyrhiza* at left and *C. uniflora* at right

specialized oil-collecting bees of the tribes Centridini and Tapinotaspidini, resulting the main pollinators in most species of *Calceolaria*. In *C. uniflora* (and in the similar

C. fothergillii), by contrast, the appendage is entirely folded outward and adheres tightly to the surface of the instep. It has the form of a broad, thick, sharply bounded white band

across the lower lip. The hairs are small and hidden in the fold of the appendage and do not secrete oil (Sérsic and Cocucci 1996). The tissue of the appendage contains sugars in a concentration similar to fruits. It is avidly consumed in nature by a bird, *Thinocorus rumicivorus*, which in the process acts as the main pollinator of *C. uniflora* (Sérsic and Cocucci 1996).

This sharp discontinuity between the two parent species in the structure of the appendage is bridged in the structure of the presumed hybrid intermediate flowers (Table 1). The position of the appendage is usually neither curved inward (like in *C. polyrhiza*) nor totally folded outward (like in *C. uniflora*). A narrow white-yellowish band is often seen bending slightly outward, to form a more or less right angle with the surface of the instep (Fig. 2C). In many intermediate flowers, a yellow glandular surface is exposed on the outer surface of the instep, below this band (Fig. 3A, C). In some intermediate flowers the band is folded outward to the extent that it entirely covers the glandular surface in frontal view, like in a typical *C. uniflora* flower. In some intermediate flowers, only the yellow glandular surface and no white band is visible in the frontal view. Presence of oils over the elaiophore was confirmed in some of these flowers. In a few intermediate flowers, even the glandular surface is partially hidden on the inside, like in *C. polyrhiza*.

In intermediate populations, the different characters shown in Table 1 are found in diverse combinations, and are not always tightly linked. A combination of characters that is “typical” of the presumed hybrids may be identified, but occasionally flowers are found that resemble one of the parent species in most characters and differ from it only in one trait, e.g. in color. This almost continuous pattern of multi-character variation between the presumed hybrids and both parent species suggest the phenomenon of “hybrid swarm” (Fig. 3C).

Distribution and characterization of intermediate populations. The seven sites where

intermediate forms have been recorded so far, are all in the geographic range common to *C. uniflora* and *C. polyrhiza* (see map, Fig. 1). They have not been found in the survey in the southernmost part of Santa Cruz and in northern Tierra del Fuego, i.e. on both sides of the straits of Magallanes, where *C. uniflora* is common but *C. polyrhiza* is absent. Nor have they been found in the arid central part of Santa Cruz, where only *C. polyrhiza* occurs.

Moreover, the intermediate forms occurred in close spatial association with populations, or at least plants, of both parent species (Table 3). In some cases the presumed hybrids occurred abundantly within a large dense population of *C. uniflora* that was near a large population of *C. polyrhiza* (sites 5, 7). Where sparser populations of both species overlapped, the hybrids were also sparse (sites 2, 3). In one case (site 1) the hybrids occurred within and near a small patch of *C. uniflora* plants, at the edge of an extensive population of *C. polyrhiza*. In site 6, a single plant with intermediate flowers was found at the edge of a pure *C. uniflora* population; however, a search revealed some *C. polyrhiza* nearby.

The presumed hybrid forms were found over a wide range of geographic, ecological and vegetation zones in Santa Cruz province (Table 3), from the Patagonian steppe plains near the Atlantic Ocean in the east to slopes near the foot of the Andes in the west. Within the same region and in similar communities, we observed also several pure populations of *C. uniflora* and *C. polyrhiza* that were spatially isolated from each other. In those sites, no intermediate flowers were found.

Discussion

Are the intermediate forms interspecific hybrids? One of the main criteria for identifying hybrids (Stace 1989, Judd et al. 1999) is phenetic intermediacy between the supposed parents. The individuals of *Calceolaria* described here are phenetically intermediate

Table 3. Characteristics of populations of intermediate forms

Site No.	Site code, Year observed	Habitat and vegetation	Abundance of intermediate forms	Abundance and proximity of <i>C. uniflora</i>	Abundance and proximity of <i>C. polyrhiza</i>
1	GAC21 1997–2000	Slope, Grass steppe of <i>Festuca gracillima</i> + dwarf shrubs	Locally dense	Locally dense	Dense extensive population
2	LM22 2000	Plain, Open shrub steppe of <i>Junellia tridens</i>	Sparse	Common	Common
3	ML23 2000	Plain, Shrub steppe of <i>Junellia tridens</i>	Very sparse	Sparse	Sparse
4	R10K5 2000	Plain, Shrub steppe of <i>Junellia tridens</i>	Common	Common	Common
5	RP25 2000	Undulating plain, Grass steppe of <i>Festuca pallescens</i>	Common, extensive	Very dense extensive population	Abundant at 1 km, contiguous
6	PMLO7 1998	Rocky slope, shrubland	One plant found	Locally common	A few plants at 0.2 km distance
7	VA26 2000	Rocky slope, Grass steppe <i>Festuca</i> spp.	Fairly common	Dense extensive population	Common at foot of slope, contiguous

between *C. uniflora* and *C. polyrhiza* in several distinct floral characters, both quantitative and qualitative: flower dimensions, the shape of the lower lip, the color of the instep and of the throat. The intermediate form and position of the appendage, that in the two parent species has entirely different characteristic structure and function, is possibly the strongest morphological evidence for the hybrid origin of the intermediate forms.

A second line of evidence is distributional (Stace 1989). The intermediate forms occur only in the geographic region where *C. uniflora* and *C. polyrhiza* are sympatric. Moreover, all intermediate individuals and populations recorded so far occurred in contact or in close proximity to populations of both species, where at least one (often both) of them was a large population.

We have no direct evidence on F2 segregation or artificial resynthesis that would be necessary to prove definitively the hybrid origin of the intermediate forms. However, in a preliminary experiment (N = 6) of artificial cross-pollination between the two parent species, 83% of flowers produced fertile fruits, with a mean of 368 seeds per fertile fruit and a mean germination percentage of 32% (Mascó, Sérsic and Noy-Meir, unpublished). These substantial levels of reproductive success, similar or somewhat lower than those obtained from artificial intraspecific cross-fertilization of *Calceolaria uniflora* in the same experiment (67%, 687 seeds/fruit and 59%, respectively; N = 18), indicate that there is a certain degree of compatibility between the two species that is a prerequisite for hybridization.

Chromosome numbers of both the parental species have been reported as diploid, $2n = 18$ (Moore 1983; Ehrhart 1997, 2000). However, one count made by Ehrhart 1997 in a cultivated *C. uniflora* resulted tetraploid ($2n = 36$). We have started cytological studies on the parental species and the hybrid, but they have not yet been completed.

How does hybridization occur? The apparently relatively common occurrence of natural interspecific hybridization between *C. uniflora*

and *C. polyrhiza* where they are sympatric raises several questions. First, how does cross-pollination and hybridization occur between two species which, as far as we know, have different reproductive systems? The species occur together in the same region, in adjacent and similar though not necessarily identical habitats, their flowering periods overlap, and we have evidence that artificial cross-pollination produces viable seeds. The isolating mechanism between them appears to be different pollination agents. Flower structure and field observations indicate that *C. uniflora* is mainly cross-pollinated by a bird attracted by the sweet white external appendage (Sérsic and Cocucci 1996). In *C. polyrhiza* this food body is lacking and instead there is an internal glandular elaiophore that indicates pollination by bees, although oil-collecting bees have not been registered at these latitudes. The constriction present on the lower lip of this species seems to be also an adaptation of these flowers to bee pollination. A similar constriction was already described for other species of *Calceolaria* (Sérsic 1994) where it functioned as a grasping place for the bees. Wind pollination is unknown in Scrophulariaceae and is unlikely in *Calceolaria* because of the type of pollen and flower structure. It is not clear then how does pollen get from flowers of *C. uniflora* to those of *C. polyrhiza* or vice versa to produce hybrid offspring, even when plants grow at a short distances from each other. Does the bird pollinator of *C. uniflora* occasionally consume also parts of flowers of *C. polyrhiza* and thus transfers pollen?

Previous observations made in *Calceolaria brunellifolia* Phil. in the Province Mendoza (Argentina) confirm that the ingestion of the corolla by birds (*Muscisaxicola alpina argentina* Hellmayr, Tyrannidae) was not rare in this species (Sérsic and Cocucci 1996). Such occasional foraging behavior of birds may initiate the evolution of a specialized relationship between birds and flowers, as was found between *C. uniflora* and *Thinocorus*.

How are interspecific barriers maintained? If there is natural hybridization and

it is not a rare event, what limits gene flow between neighboring populations of the two species and keeps them genetically distinct? As mentioned above, there are no strong geographic, ecological, phenological or compatibility barriers (either pre- or post-zygotic) between the two species, and the supposed pollination barriers appear to be leaky when the species co-occur in large populations. Most of the remaining potential isolation mechanisms (Stace 1989, Judd et al. 1999) have to do with reduced viability, fertility or fitness of hybrids in the F1, F2 or subsequent generations. In the field, hybrid plants appeared to be as vigorous as parent plants, and their flowers appeared to be fertile. At least in one site, we have found an intermediate flower with the lower lip removed in a way characteristic of pecking by *Thinocorus rumicivorus*, indicating possible cross-pollination of hybrids mediated by this bird (Fig. 4). We have as yet insufficient information on hybrid fitness in further stages of the life cycle. The distribution of morphologically intermediate forms in some populations is consistent with a certain degree of backcrossing with parent species and the formation of a hybrid swarm. However, since we do not know the genetic base of the relevant floral traits, there are other possible explanations, such as pollination within or between hybrid flowers and segregation in F2 and subsequent generations.

One possibility is that pollen flow between the two species, and between them and hybrids, is quantitatively much less efficient than within each species. This is a likely hypothesis given the distinct structure of the floral appendage and its attraction to pollinators of different kinds. In this case, limited interspecific pollen flow can produce a certain proportion of hybrid forms, and a certain amount of introgressive gene flow from one species to the other. However, most flowers of the parent species would still be pollinated by pollen of their own kind. Selection at this stage allows large populations of the two species to remain distinct even where they come in contact and hybridize. Only when one or a few plants of



Fig. 4. Lower lip of one hybrid flower possibly pecked by a bird

one parent species occur within a large population of the other, they may be genetically swamped and absorbed. The outcome of hybridization in later generations is also expected to depend on the relative abundance of the pollinators of the two parent species and their relative efficiency in pollinating hybrid flowers (Grant 1993).

What is the systematic and evolutionary status of the intermediate forms? The structure of intermediate flowers is quite distinct from that of both *C. uniflora* and *C. polyrhiza*. A herbarium specimen seen in isolation from the field context could have easily been identified as a third species. We have no evidence of the existence of such specimens, but it would be interesting to search for them. In any case, the field evidence we have so far indicates that the presumed hybrid forms, though not uncommon, occurred always in contact with both parent species. In the region we surveyed, at least, we found no independent populations composed only of intermediate forms. This indicates that the hybrids have not stabilized and are not in the evolutionary process of forming a new species. This can be explained

by ecological overlap with both parent species and by low rates of pollen flow between hybrid forms, probably associated with the intermediate structure of the appendage that is suboptimal for attracting either birds or bees. If this is the case, there is obviously no base for recognizing the intermediate forms as a new species. At most, they can be systematically labeled as *C. uniflora* × *C. polyrhiza* forms.

Potential evolutionary significance of introgression in *Calceolaria*. Apparently, there are no absolute reproductive barriers between *C. uniflora* and *C. polyrhiza*. Gene flow between the two species can occur where their populations come in contact, leading to hybridization and introgression. The same may occur between other pairs of Patagonian *Calceolaria* species. Certain characters thus transferred from one species could have been absorbed and stabilized within the genome of the other species if they increased its fitness, or even if they did not reduce it, and particularly if they did not interfere with its basic reproductive mechanisms. It is interesting in this context to speculate on the evolution of flower color in Patagonian species of the genus. The most common flower color in *Calceolaria* is yellow. *C. uniflora* has strong red to brown pigmentation in both the instep and the throat of the lower corolla lip (Table 1), though the proportion and intensity of this color vary between populations (Mascó et al. 2000 and unpublished). In populations in the Andean and subandean (northern and western) part of the species range, the instep typically has a uniform red to dark-brown color. In southern and eastern populations, the instep usually shows a pattern of reticulate brown maculae on a yellow background. In *C. polyrhiza*, the instep is often pure yellow, but small red maculae on the yellow background occur in varying amounts in the throat and the exterior of the lower lip. Possibly, genes affecting flower colors have been interchanged between the two species in their evolutionary history.

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Note added in proof: Populations of *C. uniflora* × *polyrhiza* hybrids with both parent species have been found also in the adjacent páine region of Chile (E. Dominguez, personal communication; A. Elvebakk, Punta Arenas Herbarium).

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