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Geographic variation in flower color patterns within *Calceolaria uniflora* Lam. in Southern Patagonia

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Abstract. Infraspecific variation in flower colors was evaluated in 26 populations of *Calceolaria uniflora* Lam. in Southern Patagonia, Argentina. Computerized analysis of high-resolution photo-images was used to estimate the proportions of red, orange and yellow in surfaces of two corolla parts, ''instep'' and ''throat'', in field samples of 20–35 flowers per population. The between-populations component accounted for 48% of variance for instep colors and 24% for throat colors. Geographic differentiation was found between populations with a uniform red instep in the Andes in the west, and populations with a maculate yellow-and-red instep in the Magellanic steppe to the east. Mixed populations occurred in a transition zone. Throat colors showed a different, north-south geographic trend. Based on color pattern and distribution, two subspecies may be differentiated within *C. uniflora*. Their overall geographic distribution is related to climate and vegetation, but their detailed distribution is better explained by isolation by distance and barriers to gene flow.

Key words: Infraspecific variation, populations, image analysis, multivariate analysis, Argentina.

Introduction

The patterns of variation in characters between populations within a taxon and in related taxa are the subject matter of biosystematics (Solbrig 1970, Elkington 1986, Stace 1989). Variation between geographically separated populations within a species is of special interest as an indication of divergence and incipient speciation (Judd et al. 1999). Most studies of infraspecific variation at a geographic scale have been conducted in Europe and North America. There are few such studies on plants of temperate-cold regions in the southern hemisphere, such as Southern Patagonia.

Biosystematic studies of angiosperm taxa often focus on morphological characters of the flower (shape, color) because they usually are phenotypically more stable than vegetative characters and vary less within populations than molecular markers (e.g. Podolsky and Holtsford 1995). Variation in flower shape and color has commonly been interpreted as ''adaptive'', resulting from disruptive selection

by different pollinators (Darwin 1859, Faegri and van der Pijl 1979, Schemske and Bradshaw 1999, Clegg and Durbin 2000). However, other processes can produce floral divergence between geographically segregated populations, e.g. random drift and isolation by distance, indirect selection and genetic context (Judd et al. 1999, Schemske and Bierzychudek 2001, Armbruster 2002).

The research presented here was designed as a ***biosystematic*** study of patterns of infraspecific variation in floral colors at a geographical scale in a subantarctic species of *Calceolaria* (Scrophulariaceae). Pollination biology and selection by pollinators were not directly studied but are considered in the discussion among the possible mechanisms that may have generated the observed patterns of variation.

Reflectance spectrophotometry is commonly used to quantify flower colors, applying probes to areas of uniform color (e.g. Meléndez-Ackerman 1997). Fine-grained spatial patterns of two or more colors, as in flowers of *Calceolaria* (and many other species), are not easily quantified. In taxonomy, floriculture and plant breeding, descriptive terms (e.g. spotted, striated) are used. Semi-quantitative scores can be obtained by visual estimates of the proportion of area covered by patches of different colors. However, such estimates are inconsistent where the patches are small and fuzzy (personal observations). An objective method to measure the proportion of patches of different colors, by computerized analysis of photoimages of flowers, was developed here in the ***biosystematic*** context of infraspecific variation in *Calceolaria*. This technique does not detect patterns of colors invisible to humans but visible to some pollinators (such as UV) and therefore the results presented here do not necessarily express flower color as perceived by pollinators (Chittka et al. 1994, Vorobyev et al. 1997).

The objective of this biosystematic research was to analyze quantitatively the variation in flower colors within *Calceolaria uniflora*, in relation to geographic and ecological gradients. The following questions were addressed:

Can distinct types of floral color pattern be visually identified within the species?

What is the relative magnitude of between-populations and within-population variation in the proportion of flower areas covered by different colors?

To what extent is this variation either phenotypically stable, or plastic in response to environmental conditions?

Are colors in different parts of the flower correlated or independent?

Are there clear trends in flower colors in geographic space and in relation to climatic variables?

Are there well-defined clusters of populations in geographic and character space, so that infraspecific taxa may be identified? Or is variation between populations entirely clinal?

We will finally attempt to interpret the observed patterns of geographic variation within the species in relation to alternative evolutionary processes that may have generated them.

Materials and methods

Calceolaria uniflora Lam. The genus *Calceolaria* contains about 270 species in South America, mostly in the Andean region (Molau 1988). *C. uniflora* has a subantarctic distribution at the southernmost limit of the genus, in Southern Patagonia and Tierra del Fuego (in both Argentina and Chile) between latitudes 45° and 55°S (Descole and Borsini 1954, Moore 1983, Boelcke et al. 1985, Mascó et al. 1998, Roig 1998, Correa 1999, Ehrhart 2000). It is found in a variety of habitats from sea level on the Atlantic coast and the Magellan Strait to over 1200 m a.s.l. in the Andes Mountains.

The characteristic *Calceolaria* corolla includes a small upper lip enclosing the fertile parts, and a lower lip enlarged in the form of a shoe or slipper (Fig. 1). The surfaces of the lower lip frontally exposed to pollinators are the "throat" (the open basal part) and the "instep" (the upper surface of the saccate distal part). In most species, the proximal edge of the instep forms a lap that is folded inward, hiding the oil-secreting gland or

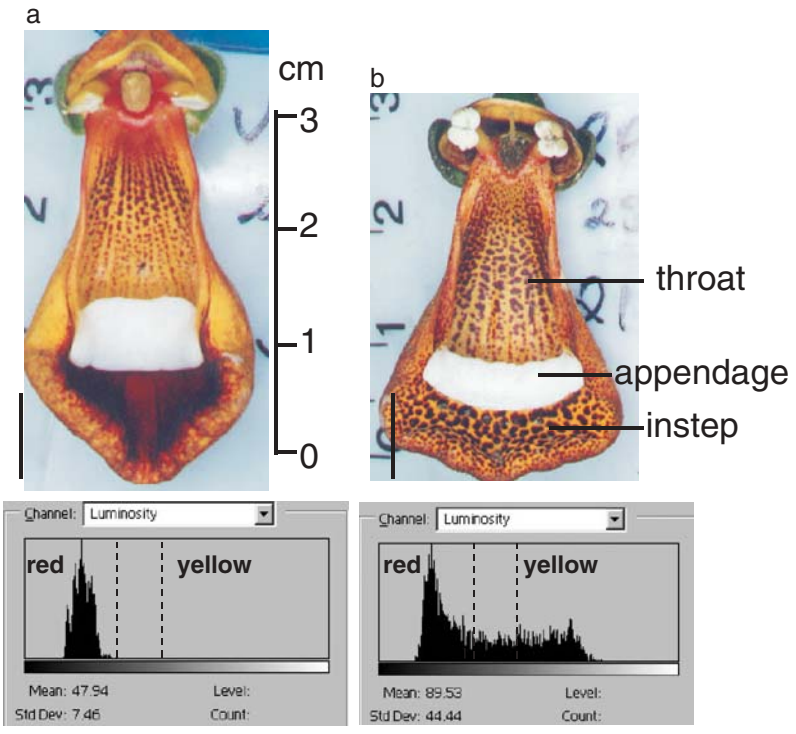

Fig. 1. Photographs of two flowers of *Calceolaria uniflora*, showing the parts of the lower corolla lip, and luminosity histograms of the instep of each flower. **a** Flower with uniform instep. **b** Flower with maculate instep

elaiophore. Most *Calceolaria* species are pollinated by oil-collecting bees (Vogel 1974, Sérsic 1994).

By contrast, in *C. uniflora* the upper edge of the instep is folded outward as a transversal white and fleshy appendage (Fig. 1), while the elaiophore is weakly developed and non-functional (Sérsic and Cocucci 1996, Roitman et al. 2002). This structure appears only in one additional, closely related species, *C. fothergillii* Ait. of the Falkland Islands and is the main diagnostic character of the section *Kremastocheilos* (Witasek 1905, Ehrhart 2000). The appendage of *C. uniflora* is fleshy and sweet (~2% sugar in expressed juice) and is consumed by birds (*Thinocorus rumicivorus*) that in the process pollinate the flower (Sérsic and Cocucci 1996). The species is largely allogamous, though a few fruits and seeds can be produced by autogamy (Arroyo and Squeo 1990, Mascó et al. 2000, Mascó 2003).

Population sampling. Populations of *C. uniflora* throughout its range in Argentina were sampled in the flowering season in December 1998, 1999 and 2000. Extensive exploration trips, covering over 4000 km of roads and tracks in three years, were directed to areas where populations of the species were known or suspected to exist. In total, 26 populations were sampled: 4 in 1998, 11 in 1999 and 11 in 2000. One population was repeatedly sampled in both 1999 and 2000. The geographic coordinates of each population were measured using a Garmin 75 GPS. Topographic and vegetation data were recorded at each site. Normal mean precipitation and temperature data were interpolated from climatic maps (Conti 1998, Oliva et al. 2001) and meteorological station records (De Fina et al. 1968). Monthly precipitation data for 1998 to 2000 were obtained from Mr. J. Aldridge, Río Gallegos.

In every population, between 20 (in 1998) and 30–35 (in 1999 and 2000) flowers from different plants were sampled at intervals of at least 5 m. The total sample included 777 flowers for which complete data were obtained.

Flower color photography. Each flower was photographed frontally from a distance of 6.5 cm using a Nikon FM10 camera, with a Nikon AF Nikkor 60 mm lens. A Starblitz 1000 Auto Macro-Lite annular flash was used to ensure standard and uniform illumination.

The colors of the two frontal parts of the flower were estimated separately:

– the ***instep***, the external surface of the inflated distal part of the lower corolla lip.

– the ***throat***, the internal surface of the lower lip, visible above the instep (Fig. 1).

Objective color measurements on photo images. An original objective quantitative method was developed to estimate the proportions of surface covered by different colors in the flower parts of interest (Fig. 1). The photograph of each flower was scanned at a resolution of 150 ppi and standard settings and the image was analyzed using Corel Photo Paint 8. The area of a flower part (instep or throat) in the image was marked by a polygon. The Corel Histogram function for RGB total luminosity (on a scale from 0 = black to 255 = white) was applied to the marked area, to read the proportion of area (pixels) in three ranges of luminosity: <80 (dark), 80–120 (intermediate) and >120 (light) (Fig. 1). In flowers of *C. uniflora*, these ranges correspond approximately to the human-perceived colors "red" (including red-brown), "orange" and "yellow", respectively, as was established by marking small areas of uniform hue in the flower image and reading their luminosity.

The yellow color of *Calceolaria* flowers is associated with carotenoid pigments in the chromoplasts (Wrischer and Ljubesic 1984). The darker red and brown patches indicate anthocyanins in the vacuoles**.** The luminosity values we measured are probably negatively correlated with anthocyanin concentrations in a patch.

Statistical analysis. Discriminant analysis was used to evaluate the coincidence between visually identified types of color patterns and objectively measured color variables. Analysis of variance within and between populations was performed on the arcsine transformed area proportions of red, orange and yellow in the instep and in the throat. The stability (vs. plasticity) of these color variables was tested by the significance of differences between samples obtained in different years, over all populations and within a single population. Where non-linear relationships were indicated, e.g. between color variables and geographic and climatic variables, the Spearman rank correlation coefficient was used to test for monotonic trends. Multivariate analysis of populations by cluster analysis (Ward's minimum variance method, Di Rienzo et al. 2000) and principal component analysis was performed with a set of 7 color variables: the mean proportions of the three colors in the instep and in the throat, and the proportion of flowers with uniform instep. Phenetic Euclidean distances between all pairs of populations were calculated from the 6 color variables (proportions of red, orange and yellow in instep and throat) and compared with geographic distances by various tests. All statistical analysis and most graphs were performed with INFOSTAT software (Di Rienzo et al. 2000).

Results

Visual identification of color patterns. Even before the quantitative analysis, we identified two visually distinct types of color pattern in the instep area:

– ***uniform*:** the surface of the instep is uniform red, in hues that range from red-orange to wine red, red-brown and dark brown (Fig. 1a);
– ***maculate:*** the surface of the instep shows a pattern of darker patches of red or red-brown and lighter patches of yellow grading to orange (Fig. 1b).

The proportion of the surface covered by red and yellow patches in maculate flowers is highly variable. The darker patches are in the form of irregular spots (maculae) on the yellow background, when they cover less than half of the surface. When they cover more, they merge into a reticulate pattern, forming a labyrinth of contrasting red-brown and yellow corridors.

The two types of instep color pattern were easily identifiable in the field and in photographs. Image analysis differentiated clearly between the two types (Fig. 1). In flowers with uniform instep, the histogram of luminosity showed a single sharp peak with the mean in the darker (<80) range, with a standard deviation of 5 to 10 units. In flowers with maculate instep, the histogram was bimodal or strongly skewed, with a standard deviation of 15 to 50 units. In a discriminant analysis based on objectively measured proportions of three colors in the instep over all flowers (N = 777) in relation to the visual "instep type" classification, there was 87% coincidence between visual and discriminant classifications. The histogram of positions of individual flowers along the axis of the discriminant function

showed a strongly bimodal distribution (Fig. 2).

In contrast to the instep, the inner surface of the throat was always maculate, with small spots of dark orange, red or brown color aligned on vertical lines on a yellow to light orange background (Fig. 1). The proportions and hues of the darker and lighter patches were variable, but no discrete types of color pattern could be distinguished in the throat.

In the total sample there were 217 (28%) flowers with uniform and 560 (72%) with maculate instep. The frequency of flowers with uniform vs. maculate instep varied greatly between populations (Fig. 3). In two populations all flowers were uniform and in three additional populations more than 85% of flowers were uniform. These 5 populations were labeled ***uniform*** populations. In 11 populations all flowers were maculate and in 5 additional populations more than 90% of

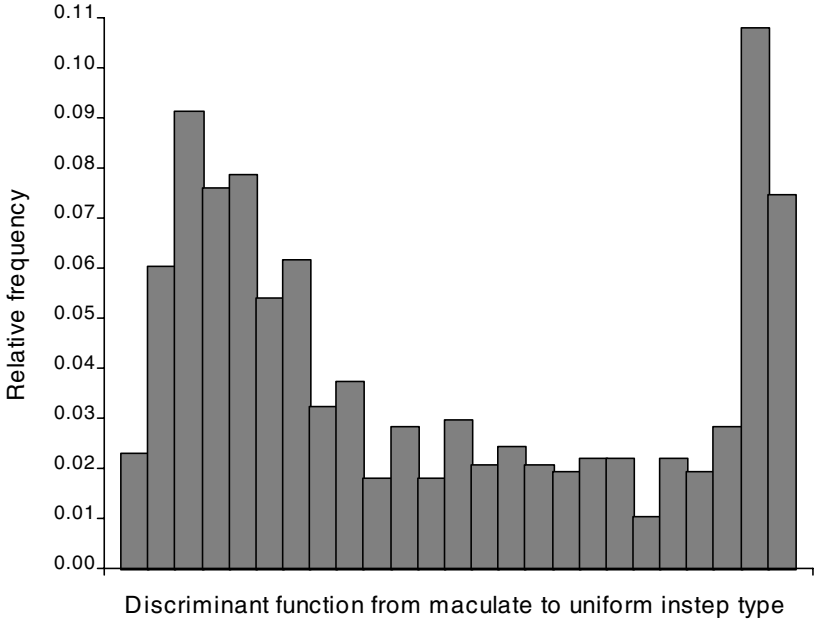

Fig. 2. Frequency distribution of positions of individual flowers (N = 777) along discriminant axis with instep color pattern (uniform/maculate) as class and three objective instep colors as variables

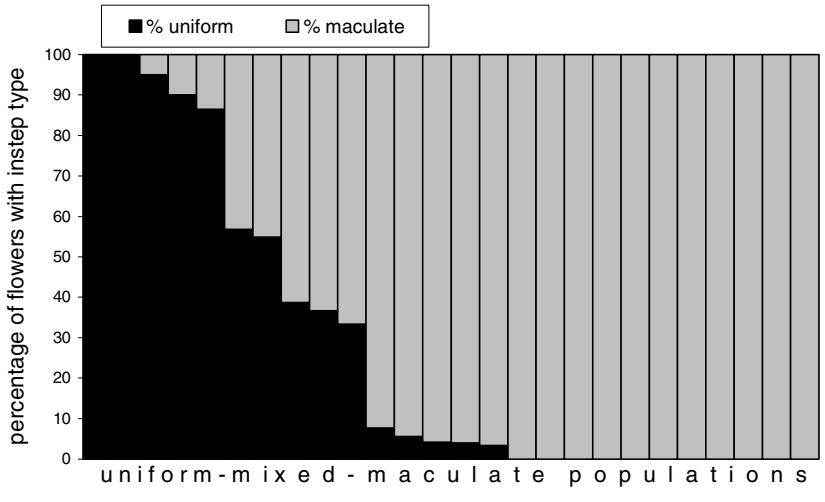

Fig. 3. Distribution of the frequencies of uniform and maculate instep flowers over *C. uniflora* populations, ranked in decreasing frequency of uniform flowers

flowers were maculate; these were labeled ***maculate*** populations. There were 5 ***mixed*** populations where plants with flowers of both instep types occurred in frequencies between 30 and 70%.

Character stability. The objectively measured proportions of red, orange and yellow in the instep were not significantly different ($P > 0.15$) between populations sampled in years with drought periods in spring (1998–99) and in the year 2000, when spring precipitation was evenly distributed and flowers were abundant. In the Río Rubens population, sampled in both 1999 and 2000, the frequency of uniform flowers remained constant (61% and 58% respectively, $P = 0.80$ with Fisher's test for independence, $N = 62$) and there were no significant differences between years in the proportions of the three instep colors.

As to throat colors, populations sampled in 2000 showed a slightly lower proportion of orange and greater proportion of yellow than those sampled in 1998–99. The difference was about 7% and was significant ($P = 0.0182$) only for orange. A similar minor shift was observed in the Río Rubens population between 1999 and 2000 ($P = 0.0379$).

Variance within and between populations. The between-populations component accounted for over 50% of total variance in the proportions of red and yellow in the instep; the mean over the three instep colors was 48% (Table 1). Throat colors had relatively more within-population and less between-populations variance (mean 24%).

Correlation between instep and throat colors. The proportion of different colors in the throat did not differ significantly between instep color types ($N = 777$ flowers). All Spearman correlation coefficients between the proportions of the three main instep colors and the three main throat colors were not significant over all populations ($P > 0.10$, $N = 26$), indicating overall independent color variation in the two flower parts (Fig. 4).

However, within the set of maculate populations ($N = 16$) there was a tendency for coincident variation in color proportions in the throat and the instep (Fig. 4). Yellow in the throat was positively correlated (rho = +0.61) with yellow in the instep, and orange in the throat was positively correlated (+0.66) with red in the instep ($P < 0.05$).

Geographic trends in flower colors. Instep color pattern types showed a strong longitudinal pattern (Fig. 5): populations with uniform instep occurred at or west of 72° W, mixed populations occurred between 71 and 72° W, and only maculate populations were found east of 71° W. The frequency of flowers with uniform instep was strongly but not linearly correlated with longitude west (Spearman

Table 1. Components of variance between and within 26 populations in the objectively measured area proportions of colors in the instep and throat of *Calceolaria uniflora* flowers ($N = 777$). Angular transformation was applied to the proportions. The between population variance was highly significant ($P < 0.0001$) in all cases

Color variable	Luminosity range	% variance between	% variance within
Instep			
Red	<=80	52.2	47.8
Orange	80–120	34.8	65.2
Yellow	>120	56.8	43.2
Mean		**47.9**	52.1
Throat			
Red	<=80	21.0	79.0
Orange	80–120	26.9	73.1
Yellow	>120	25.0	75.0
Mean		**24.3**	75.7

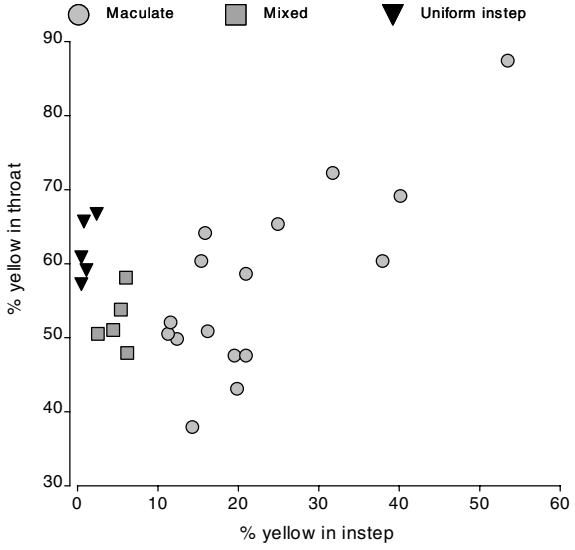

Fig. 4. Mean % of yellow in throat vs. mean % of yellow in instep in populations of *C. uniflora* (N = 26) by instep color type: black triangles – uniform, light gray circles – maculate, dark gray squares – mixed populations

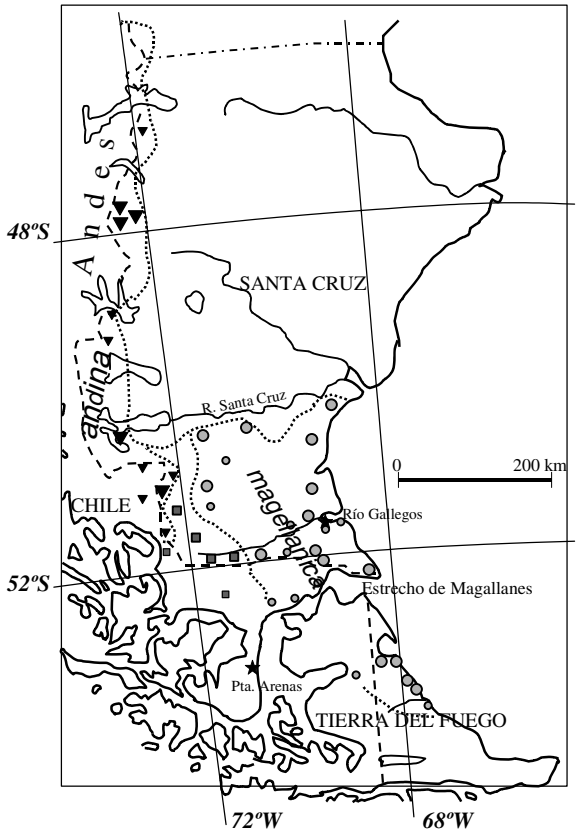

Fig. 5. Map of study area and populations of *C. uniflora* by instep type: black triangles – uniform, light gray circles – maculate, dark gray squares – mixed populations. Large symbols – populations sampled, small symbols – other populations. Dotted lines: northern and eastern limit of *C. uniflora*, and approximate limits of uniform, maculate and mixed populations

rho = +0.78, $P < 0.0001$, Table 2, Fig. 6). This longitudinal limit between uniform and maculate instep populations corresponded to the biogeographic limit between Andean-subandean forest/grassland formations and Magellanic steppe formations (Oliva et al. 2001). Mixed populations occurred in the southern Andean-subandean region, where Subandean grassland grades into Magellanic steppe. The frequency of flowers with uniform instep was not significantly correlated with latitude and only weakly with altitude (Table 2).

The proportions of red, orange and yellow in the instep were strongly (|rho| = 0.65 to 0.70) and significantly ($P < 0.01$, $P < 0.001$) correlated with longitude (Table 2). The trend was stepwise rather than linear: between 71 and 72° W the proportion of red increased sharply towards the west, while the proportions of yellow and orange increased towards the east. Instep colors did not show any trend with either latitude or altitude over the entire sample (Table 2). However, within the subsample of maculate populations (N = 16), red in the instep increased and yellow decreased significantly with latitude south (|rho| = 0.66, $P < 0.05$).

Throat colors showed no significant longitudinal trend but did vary with latitude. The proportion of orange in the throat increased significantly from north to south, while yellow decreased in the same direction (Table 2). This latitudinal trend in throat colors was manifested mainly within maculate populations.

Correlation of flower colors with climatic variables. The proportion of uniform instep flowers in the population was not significantly correlated with temperature ($P > 0.20$) and

Table 2. Spearman rank correlation coefficients (rho) between site geographic variables (latitude, longitude and altitude) and site (population) means of color variables in the instep and throat of *Calceolaria uniflora* flowers (N = 26 sites). Significance levels: NS – $P > 0.05$; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$. Significant ($P < 0.05$) values highlighted in bold type

	Latitude		Longitude		Altitude	
Instep type						
% of uniform flowers	−0.30	NS	**0.78**	***	0.48	*
Instep colors						
Red (<80)	−0.05	NS	**0.65**	**	0.21	NS
Orange (80–120)	0.18	NS	**−0.70**	***	−0.38	NS
Yellow (>120)	0.16	NS	**−0.67**	***	−0.27	NS
Throat colors						
Red (<80)	0.16	NS	0.18	NS	−0.00	NS
Orange (80–120)	**0.57**	**	−0.25	**NS**	−0.44	*
Yellow (>120)	**−0.44**	*	0.17	NS	0.29	NS

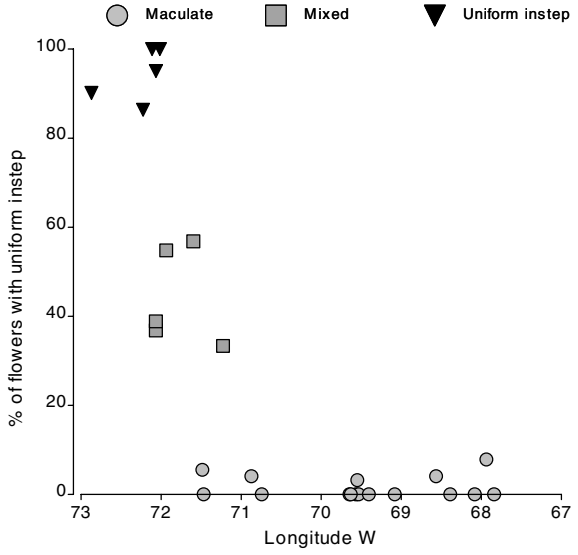

Fig. 6. Percentage of uniform-instep flowers in *C. uniflora* populations vs. longitude West (scale inverted to fit map vision), by instep color type. Symbols as in Fig. 4

only weakly correlated with precipitation (rho = +0.46, $P = 0.0222$). However, there were significant correlations between temperature and increased proportions of yellow and orange in the instep and of yellow in the throat (Table 3). Conversely, the proportions of red in the instep and of orange in the throat increased in colder sites. Increased precipitation was significantly correlated with increasing red in instep and orange in throat and with decreasing yellow in instep.

In general, color variables were not as strongly correlated with climatic variables ($|rho| = 0.5 - 0.6$) as they were with longitude and latitude ($|rho| > 0.6$; Table 2). Though only maculate populations occurred in the driest and warmest parts of the species range, there was a range of temperature (10–11 °C) and precipitation (250–350 mm) where uniform, maculate and mixed populations overlapped.

Multivariate analysis. Cluster analysis (with 7 variables: 3 instep, 3 throat colors, % of uniform instep) separated at the highest level all Andean-subandean populations of uniform or mixed instep type (N = 10) from all Magellanic populations with maculate instep (N = 16) (Fig. 7). The second division of the first group segregated uniform from mixed populations. The second division of the maculate group separated populations with lighter colors in both throat and instep (mostly in the north) from populations with darker colors (mostly in Tierra del Fuego and in the south). One population with a very high proportion of yellow was set apart as an outlier group and was excluded from the principal components analysis.

In PCA of the 25 populations using the same 7 variables, the first principal component

Table 3. Spearman rank correlation coefficients (rho) between normal climatic variables (mean January and mean annual temperature, annual precipitation) and site means of area proportions of colors in the instep and throat of *Calceolaria uniflora* flowers (N = 26 sites). Significance levels: NS – $P > 0.05$; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$. Significant ($P < 0.05$) values highlighted in bold type

	Mean January temperature		Mean annual temperature		Annual precipitation	
Instep colors						
Red (<80)	**−0.50**	*	**−0.51**	*	**0.58**	**
Orange (80–120)	**0.49**	*	**0.63**	**	−0.36	NS
Yellow (>120)	**0.57**	**	**0.58**	**	**−0.61**	**
Throat colors						
Red (<80)	−0.24	NS	−0.36	NS	0.01	NS
Orange (80–120)	**−0.57**	**	**−0.51**	*	**0.46**	*
Yellow (>120)	**0.57**	**	**0.61**	**	−0.20	NS

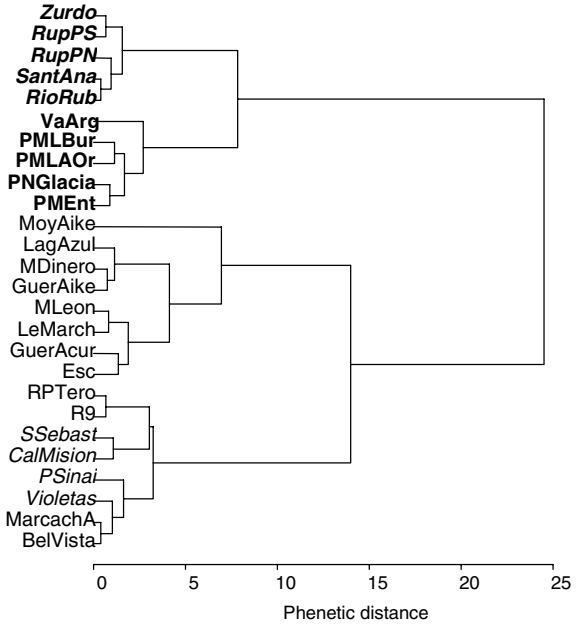

Fig. 7. Dendrogram of cluster analysis of *C. uniflora* populations (N = 26) by Ward's minimum variance method with seven color variables. Bold type – uniform instep populations, bold italic – mixed populations, regular type – maculate populations on the continent, italic – maculate populations on Tierra del Fuego

accounted for 51% of variance and the second one for 36%. The first axis expressed mainly variation in instep color, contrasting uniform instep and high proportion of red at the left extreme with maculate instep and high proportions of yellow and orange at the right extreme (Fig. 8). The second axis expressed mainly variation in throat color, with more of red and orange at the lower extreme and more of yellow at the upper extreme. The three groups of populations defined by instep type, uniform, maculate and mixed, were clearly clustered in ordination space.

The first axis showed a strong correlation with longitude ($rho = -0.70$, $P = 0.0006$) and a weaker correlation with temperature ($rho = +0.51$, $P = 0.0126$). The populations clustered on the left end of the first axis (instep colors) were mostly in Andean-subandean vegetation, while those at the right end were in Magellanic steppe. The second axis (throat colors) showed a weak trend with latitude ($rho = -0.44$, $P = 0.0302$), and a stronger correlation with temperature ($rho = +0.61$, $P = 0.0027$).

Geographic distance and phenetic distance. Only 17% of population pairs within less than 50 km distance were of different instep color type, compared to 43% of pairs at distances of 50 to 150 km, and 61% of pairs that were more than 150 km distant (Chi-square for independence = 16.3, df = 2, $P = 0.0003$, N = 276). The correlation between the phenetic distance calculated from 6 color variables and the geographic distance was positive ($rho = +0.405$) and highly significant ($P = 0.0010$; distances < 150 km;

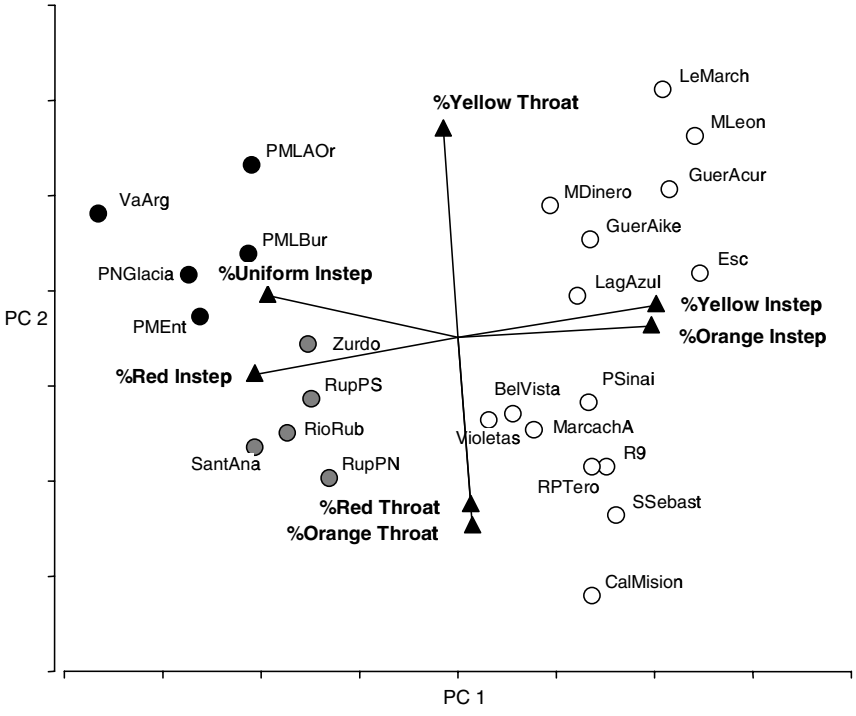

Fig. 8. Biplot ordination of color variables (vectors with black triangles) and populations of *C. uniflora* (circles; N = 25) by first (PC1) and second (PC2) principal components from correlations between seven color variables. Black circles – uniform instep, dark gray – mixed, light gray – maculate populations

N = 76). The mean phenetic distance between populations within 25 km of each other was only 0.17, compared to >0.50 between populations at 100 to 150 km distance (Fig. 9; ANOVA: $P = 0.0324$, $R^2 = 0.10$, N = 76).

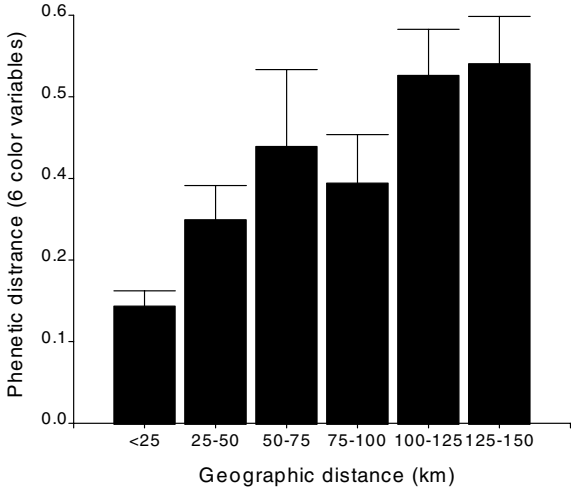

Fig. 9. Mean and standard error of phenetic distances, calculated as Euclidean distances from six color variables, between *C. uniflora* populations vs. classes of geographic distances between them, up to 150 km distance

Discussion

Patterns of variation in flower colors. Differences in morphological characters between geographically distant populations are ideally evaluated in common garden experiments. However, this is not always feasible with perennial species that are difficult to grow and maintain in culture. Many biosystematic studies are based on *in situ* measurements in wild populations (e.g. Domínguez et al. 1998) or on herbarium specimens (e.g. Lefebvre and Vekemans 1995). This creates a problem in interpreting observed differences between populations. To what extent do the latter reflect genetic differences and to what extent – plastic phenotypic responses to environmental conditions at the site and time the population was sampled? Differences between *in situ* populations are reliable indicators of genetic differences only for characters that are phenotypically stable under normal variations in environmental conditions. In this study, comparison between samples taken in years with different precipitation indicated that flower

colors and color patterns in *C. uniflora* are relatively stable. In contrast, some of the quantitative morphometric variables, such as flower dimensions, showed significant plastic responses to year and site conditions (Mascó 2003). Evidence from other species suggests that the ***presence*** of a flower color pattern (e.g. spots) is often phenotypically stable and is determined by a single major gene (e.g. Jones 1996, Mol et al. 1998). However, the expression of color and its ***intensity*** can be influenced by current environmental conditions (e.g. Weiss 2000).

C. uniflora is an outcrossing species (Mascó 2003), in which high variation between individuals within local populations can be expected. In the outcrossing *Ipomopsis aggregata,* 70–80% of the variance in different flower morphometric variables was within populations and only 20–30% between populations (Wolf and Campbell 1995). In *C. uniflora* flowers, between-populations variance in the proportions of red and yellow in the instep was relatively high (>50%, Table 1). The stable and large between-populations variation suggests that instep colors and color patterns are diagnostic characters for geographic variation within *C. uniflora.*

Throat colors showed lower between-populations variance (20–25%). Over all populations, throat colors varied independently of instep colors (Figs. 4, 8), indicating that different genes may be involved. In flowers with radial symmetry, throat and limb color often vary independently (e.g. Clegg and Durbin 2000, Wolfe 2001).

Infraspecific taxa? To what extent is variation within a species either discontinuous or continuous in character space and in geographic space (Prentice 1986)? Where it is discontinuous or strongly clustered, there may be justification for distinguishing infraspecific taxa. Where it is largely continuous, any subclassification will be arbitrary and it is more meaningful to define clines of variation. Within *C. uniflora,* uniform and maculate instep populations formed distinct clusters in character space (Fig. 8) and were clearly segregated in geographic space (Fig. 5). Additional qualitative information on instep color pattern from another 14 sites in Argentina and Chile and from herbarium specimens confirmed this pattern. No overlap between the geographic distributions of pure uniform and maculate populations was found, though mixed populations occurred in an intermediate zone. Uniform populations differed significantly from maculate populations in four phenotypically stable morphometric characters of flower size and shape (Mascó 2003). According to the criteria of Du Rietz (1930; cited by Stace 1989), "subspecies" are morphological races differentiated at a regional scale. Thus, there is preliminary evidence for defining two "subspecies" within *C. uniflora*: 1) populations with uniform red corolla instep, distributed along the Andes (informally labeled "*andina*"); 2) populations with maculate yellow-red instep, distributed in the steppe on both sides of the Strait of Magellan ("*magellanica*"). Between the core areas of the two subspecies there is a phenocline of mixed populations. The phenocline could indicate a focus of recent divergence of the two subspecies, or it may be the result of a secondary contact and hybridization between taxa that diverged long ago.

A closely related taxon is *C. fothergillii* Ait., that shares with *C. uniflora* the white transversal appendage on the lower corolla lip. This species is reliably documented only from the Falkland Islands, where it is isolated by 500 km of ocean from the nearest populations of *C. uniflora* on the continent. The color pattern on the instep of *C. fothergillii*, as seen in flower images (Curtis 1796, Vallentin and Cotton 1921, Davies and McAdam 1989, Woods 2000) is quite distinct from that of both subspecies of *C. uniflora*.

Mechanisms of differentiation: pollinators, climate or distance? What evolutionary mechanisms generated and maintained the geographic differentiation in floral color patterns within *C. uniflora*? A prevalent hypothesis (since Darwin 1859) is that selection by different pollinators in different regions can

trigger and stabilize "adaptive" differentiation in flower colors. Experimental evidence supporting this mechanism has been frequently reported (e.g. Vickery 1992, Schemske and Bradshaw 1999, Medel et al., in press). The only known pollinator of *C. uniflora* is the seedsnipe *Thinocorus rumicivorus* (Sérsic and Cocucci 1996) that consumes the appendage in a large proportion of flowers and apparently induces high fruit set observed in natural populations (Mascó 2003). The large (~1,1 X 0,5 cm) and brilliant white appendage is the reward as well as a specific visual signal for the pollinator. The red, orange and yellow surfaces of the lower lip produce with the white band a striking color combination, that in the open Patagonian landscape is highly visible to the human eye, as it probably is to birds. The high proportion of red (anthocyanin) surfaces in the flowers of *C. uniflora*, compared to sympatric species (most *Calceolaria* species are yellow with at most small reddish spots) could have evolved as an "advertisement" that increased the visibility of the flower to birds foraging on the ground for seeds and berries. But there is no evidence to attribute to pollinator selection the differentiation between the uniform red instep Andean populations and the maculate yellow-red Magellanic populations. *Thinocorus rumicivorus* is a migratory bird that in spring and summer is common throughout the Magellanic steppe. It is less common in the Andean region, but there it is replaced by other seedsnipes (*Thinocorus orbignyanus, Attagis gayi, A. malouinus*; A. Manero, pers. comm.). Flowers with damaged appendage were observed also in some Andean populations (Sérsic and Cocucci 1996 and personal observations). The Andean seedsnipes probably function as pollinators in much the same way as *T. rumicivorus* in the steppe. It seems unlikely that closely related bird taxa select for different color patterns in the two regions. The hypothesis that a larger amount of yellow surfaces in Magellanic populations is maintained through selection by alternative pollinators (pollen-collecting bees?) cannot be rejected but is speculative in the absence of supporting evidence.

An alternative "adaptive" hypothesis is that geographic differentiation in *C. uniflora* flower colors resulted from selection by factors other than pollinators, in particular climate. The presence and concentration of anthocyanins in leaves and stems are often correlated with drought, cold or UV-radiation stress, leading to the hypothesis that the primary adaptive role of these pigments may have been protection from stress (Warren and Mackenzie 2001). In some taxa, their concentration in flowers may have resulted from indirect selection by climatic stress rather than selection by pollinators (Armbruster 2002). In this study we found a significant correlation between increase in red (anthocyanin) areas in both parts of the corolla and colder temperatures in the south (Table 3). UV radiation probably increases along the same gradient. The increase in anthocyanin may protect flowers and leaves from cold and radiation damage.

Another possible mechanism for this latitudinal trend could be introgression from the yellow-flowered *Calceolaria polyrhiza* Cav. This species, although from a different section than *C. uniflora* and lacking the white appendage, hybridizes with it naturally throughout the northern Magellanic region (Sérsic et al. 2001) but was not found south of the R. Gallegos nor in Tierra del Fuego.

The longitudinal differentiation in instep type between Andean and Magellanic populations is not easily explained by climatic or pollinator factors alone. The proportion of flowers with uniform instep was not significantly correlated with temperature and only weakly with precipitation. The climatic ranges of populations with different instep types overlapped much more than their geographic ranges. The distribution of instep types in the multivariate space defined by color variables was remarkably similar to the geographic distribution (Figs. 8 vs. 5). A weak but significant correlation was found between phenetic and geographic distances between populations up to 150 km (Fig. 9). The color characters of

some populations seem to reflect geographic proximity and continuity rather than site conditions. For instance, the northeastern coast of Tierra del Fuego is as cold and rainy as some of the Andean sites but harbors maculate populations very similar to those in the continental steppe (Figs. 5, 7). At the northwestern extreme of a large steppe region where "*magellanica*" populations are nearly continuously distributed, a typically maculate population inhabits one of the highest and coldest sites in our sample (Los Escarchados). It resembles "*magellanica*" populations to the east and south rather than "*andina*" populations to the west, across the dry Santa Cruz Valley where *C. uniflora* is absent. On the other hand, uniform "*andina*" populations with little color variation inhabit various microclimates along the Andes, from high mountain semi-desert to Subandean grassland and clearings in lowland *Nothofagus* forest.

These observations suggest that gene flow, isolation by distance and by geographic barriers have had a role in generating the present geographic variation in flower colors in *C. uniflora*. The original divergence between "*andina*" and "*magellanica*", whether ancient or recent, may have involved selection by the colder and wetter Andean climate vs. the drier climate of the Magellanic steppe, or by different pollinators. These factors may still be operating in the phenocline, where mixed populations can serve as stepping-stones for gene flow between uniform and maculate populations. However, where geographic distance and discontinuities in distribution restrict gene flow, populations tend to be most similar to the nearest populations from which they originated and with which they maintain gene flow.

There is no direct information on gene flow in this species. Movements of the pollinating birds (*Thinocorus* spp.) are mostly local in the flowering season, but the seedsnipe is capable of occasionally transporting pollen between populations over distances of a few kilometers. Another mechanism of gene flow over medium distances can be the dispersal of the tiny seeds (~0.05 mg; Mascó 2003) of *C. uniflora* by the extremely strong winds, mainly from the west, that characterize the Southern Patagonian summer. The similarity between Fuegian and continental populations on opposite sides of the Strait of Magellan suggests that the latter is not an effective barrier to gene flow. The Strait is about 9,000 years old and has an average width of about 30 km but narrows to 4–7 km at two points. By contrast, an arid salient about 80 km wide seems to effectively isolate the northernmost maculate populations from uniform Andean populations. These two observations indicate a scale for gene flow in this species.

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References

Armbruster W. S. (2002) Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. J. Evol. Biol. 15: 468–486.

Arroyo M. T. K., Squeo F. (1990) Relationship between plant breeding systems and pollination. In: Kawano S. (ed.) Biological approaches and evolutionary trends in plants. Academic Press, London, pp. 205–227.

Boelcke O., Correa M. N., Moore D., Roig F. (1985) Catálogo de plantas Vasculares. In: Boelcke O., Moore D., Roig F. (eds.) Transecta botánica de la Patagonia Austral. CONICET, Instituto de la Patagonia & Royal Society, Buenos Aires, pp. 129–255.

Chittka L., Shmida A., Troje N., Menzel R. (1994) Ultraviolet as a component of flower reflections, and the color perception of Hymenoptera. Vision Res. 34: 1489–1508.

Clegg M. T., Durbin M. L. (2000) Flower color variation: A model for the experimental study of evolution. Proc. Natl. Acad. Sci. USA 97: 7016–7023.

Conti H. A. (1998) Características climáticas de la Patagonia. In: Correa M. N. (ed.) Flora Patagónica, Pt. I. Colección Científica, INTA, Buenos Aires, pp. 31–47.

Correa, M. N. (1999) Flora Patagónica. Pt. VI. Dicotyledones Gamopetalas (Ericaceae a Calyceraceae). Colección Científica, INTA, Buenos Aires, pp. 1–536.

Curtis W. (1796) Curtis' flower prints. Bot. Mag., London 10: 348.

Darwin C. (1859) The origin of species. Reprint of Oxford University Press, Oxford, pp. 1–437.

Davies T. H., Mc Adam J. H. (1989) Wild flowers of the Falkland Islands. Falkland Islands Trust, Stanley, Falkland Islands, pp. 1–48.

Descole H., Borsini O. (1954) Scrophulariaceae. In: Descole H. (ed.) Genera et species plantarum Argentinarum. Guillermo Kraft, Buenos Aires, Vol. 5., pp. 1–92.

De Fina A., Garbosky A., Giaunetto F., Sabella L. (1968) Difusión geográfica de los cultivos índices en la provincia de Santa Cruz y sus causas. Publicaciones Técnicas, INTA, Buenos Aires, pp. 1–30.

Di Rienzo J., Robledo W., Casanoves F., Balzarini M. (2000) InfoStat, Software estadístico. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina.

Domínguez C. A., Eguiarte L. E., Núñez-Farfán J., Dirzo R. (1998) Flower morphometry of *Rhizophora mangle* (Rhizophoraceae): geographic variation in Mexican populations. Amer J. Bot. 85: 637–643.

Du Rietz G. E. (1930) The fundamental units of biological taxonomy. Svensk. Bot. Tidskr. 24: 333–428.

Ehrhart C. (2000) Die Gattung *Calceolaria* (Scrophulariaceae) in Chile. Bibliotheca Botanica 153: 1–283.

Elkington T. T. (1986) Patterns of variation in wild plants in relation to selection pressures and infraspecific categories. In: Styles B. T. (ed.) Infraspecific classification of wild and cultivated plants. The Systematics Association, Special Vol. 29: 33–52. Clarendon Press, Oxford.

Faegri K., van der Pijl I. (1979) The principles of pollination ecology, 3rd edn. Pergamon, Oxford, pp. 1–244.

Jones K. N. (1996) Fertility selection on a discrete floral polymorphism in *Clarkia* (Onagraceae). Evolution 50: 71–79.

Judd W., Campbell C., Kellogg E., Stevens P. (1999) Plant systematics. A phylogenetic approach. Sinauer Associates, Inc. Sunderland, pp. 1–464.

Lefebvre C., Vekemans X. (1995) A numerical taxonomic study of *Armeria maritima* (Plumbaginaceae) in North America and Greenland. Canad. J. Bot. 73: 1583–1595.

Mascó M. (2003) Sistema reproductivo y variabilidad floral infraespecífica en *Calceolaria uniflora* Lam., una especie ornamental endémica de la Patagonia austral. M. Sc. Thesis. Universidad Nacional de Córdoba, Argentina, pp. 1–108.

Mascó M., Oliva G., Kofalt R., Humano G. (1998) Flores Nativas de la Patagonia Austral. Una selección de especies silvestres con potencial ornamental de Santa Cruz y Tierra del Fuego. Montes, L. (ed). EEA-INTA Santa Cruz, pp. 1–48.

Mascó M., Sérsic A. N., Noy-Meir I. (2000) Observaciones sobre la biología reproductiva y diversidad infra-específica de *Calceolaria uniflora* (Scrophulariaceae). Gayana Botanica 57: 93.

Medel R., Botto-Mahan C., Arroyo M. T. K. (2003) Pollinator-mediated selection on the nectar guide phenotype in the Andean monkey flower, *Mimulus luteus*. Ecology (in press).

Meléndez-Ackerman E. J. (1997) Pattern of color and nectar variation across an *Ipomopsis* (Polemoniaceae) hybrid zone. Amer. J. Bot. 84: 41–47.

Moore D. (1983) Flora of Tierra del Fuego. Anthony Nelson, London, pp. 1–369.

Mol J., Grotewold E., Koes R. (1998) How genes paint flowers and seeds. Trends in Plant Science 3: 212–217.

Molau U. (1988) Scrophulariaceae. Part I. Calceolarieae. Flora Neotropica. The New York Botanical Garden, Bronx, New York, pp. 1–326.

Oliva G., Gonzalez L., Rial P., Livraghi E. (2001) El ambiente en la Patagonia Austral. In: Borrelli P., Oliva G. (eds.) Ganadería sustentable en la Patagonia Austral. EEA Santa Cruz, INTA, Río Gallegos, pp. 41–82.

Podolsky R. H., Holtsford T. P. (1995) Population structure of morphological traits in *Clarkia*

dudleyana. I. Comparison of F_{st} between allozymes and morphological traits. Genetica 140: 733–744.

Prentice H. C. (1986) Continuous variation and classification. In: Styles B. T. (ed.) Infraspecific classification of wild and cultivated plants. The Systematics Association, Special Vol. 29: 21–32. Clarendon Press. Oxford.

Roig F. A. O. (1998) La vegetación de la Patagonia. In: Correa M. N. (ed.) Flora Patagónica, Pt. I. Colección Científica, INTA, Buenos Aires, pp. 48–166.

Roitman G. G., Sérsic A. N., Cocucci A. A., Montaldo N. H. (2002) Flower food tissue as reward for pollinating birds. Bol. Soc. Argent. Bot. 37: 71–77.

Schemske D. W., Bierzychudek P. (2001) Perspective: evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. Evolution 55: 1269–1282.

Schemske D. W., Bradshaw H. D. Jr. (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. USA 96: 11910–11915.

Sérsic A. (1994) Estudios de la biología floral del género *Calceolaria* L. Ph. D. Thesis, Universidad Nacional de Córdoba, Argentina.

Sérsic A., Cocucci A. A. (1996) A remarkable case of ornithophily in *Calceolaria*: food bodies as rewards for a non-nectarivorous bird. Botanica Acta 109: 172–176.

Sérsic A., Mascó M., Noy-Meir I. (2001) Natural hybridization between species of *Calceolaria* with different pollination syndromes in southern Patagonia, Argentina. Plant Syst. Evol. 230: 111–124

Solbrig O. (1970) Principles and methods of plant biosystematics. MacMillan, New York, pp. 1–226.

Stace C. A. (1989) Plant taxonomy and biosystematics, 2nd edn. Edward Arnold, London, pp. 1–279.

Vallentin E. F., Cotton E. M. (1921) Illustrations of the flowering plants and ferns of the Falkland Islands. L. Reeve, London, pp. 1–130.

Vickery R. K. (1992) Pollinator preferences for yellow, orange and red flowers of *Mimulus verbenaceus* and *M. cardinalis*. Great Basin Naturalist 52: 145–148.

Vogel S. (1974) Ölblumen und ölsammelnde Bienen. Abh. Akad. Wiss. Lit., Math. Naturwiss. Kl. 7: 285–547.

Vorobyev M., Gumbert A., Kunze J., Giurfa M., Menzel R. (1997) Flowers through insect eyes. Israel J. Plant Sci. 45: 93–101.

Warren J., Mackenzie S. (2001) Why are all colour combinations not equally represented as flower-colour polymorphisms? New Phytol. 151: 237–241.

Weiss D. (2000) Regulation of flower pigmentation and growth: Multiple signaling pathways control anthocyanin synthesis in expanding petals. Physiol. Pl. 10: 152–157.

Witasek J. (1905) Die chilenische Arten der Gattung *Calceolaria*. Öst. Bot. Zeitschrift 55: 449–456.

Wolf P. G., Campbell D. R. (1995) Hierarchical analysis of allozymic and morphometric variation in a montane herb, *Ipomopsis aggregata* (Polemoniaceae). J. Heredity 86: 386–394.

Wolfe L. M. (2001) Associations among multiple floral polymorphisms in *Linum pubescens* (Linaceae), a heterostylous plant. Int. J. Plant Sci. 162: 335–342.

Woods R. (2000) Flowering plants of the Falkland Islands. Falklands Conservation, Stanley, pp. 1–108.

Wrischer M., Ljubesic N. (1984) Plastid differentiation in *Calceolaria* petals. Acta Bot. Croat. 43: 19–24.

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