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Significance of floral colour and scent in three *Solanum* sect. *Cyphomandropsis* species (Solanaceae) with different floral rewards

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Abstract. The role of scent and floral colour in three *Solanum* sect. *Cyphomandropsis* species with different floral rewards was studied. In the two studied species with pollen rewards, *S. glaucophyllum* Desf. and *S. stuckertii* Bitter, the principal advertisement to pollinators is the colour of the perianth and anthers, including areas that absorb UV light. In the last species, the scent emitted by the osmophores of the connective also plays a significant role in flower attraction. The floral perfume would be the only advertisement in species such as *S. adelphum* Morton, with perfume reward in which the cryptical anther colour does not contrast with that of the perianth. The internal face of the petals remains hidden. In this case no differences in the patterns of areas that absorb visible light and UV light were found. The main visitors to *Solanum* are species of *Bombus* and *Augochloropsis.* These pollinators visit *S. glaucophyllum* and *S. stuckertii,* species with pollen reward, but they do not visit flowers with perfume reward, for example *S. adelphum*.

Introduction

Floral colour and fragrance are responsible for certain patterns perceived by pollinating insects. Various of these characteristics are important in the recognition from a distance and the attraction of diurnal insects (Mulligan and Kevan 1973). The presence of pigments, intercellular spaces in the perianth, shape of the epidermal wall of the petals, and presence and kind of odour in flowers are all important parts of a floral syndrome. Sometimes these factors reinforce each other and sometimes they work independently.

The genus *Solanum* is large (Hunziker 1979) and embraces two different floral rewards. Most sections of *Solanum*—*Basarthrum*, *Cyphomandropsis* (except *S. adelphum*), *Leptostemonum*—possess pollen rewards with showy androecia and are pollinated by female bees that vibrate the anthers to obtain the reward. In section *Pachyphylla* ((Bohs and Olmstead (1999) includes *Cyphomandra* in *Solanum*, and Nee (1999) places *Cyphomandra* in sect. *Pachyphylla*), flowers are pollinated by male bees by a bellows-like mechanism (Sazima *et al*. 1993) and the perfume is obtained as reward. Floral biology and pollination in *Solanum* has been reported (Bowers 1975; Anderson and Symon 1988, 1989; Birhman 1988; Fernández Storti 1988; Sturti 1988) and the importance of floral attraction in *Solanum* pollination has been demonstrated (Sazima *et al*. 1993).

In this current study, floral features, colour and odour are studied as advertisements to insects in three species of *Solanum* (*S. adelphum* Morton, *S. glaucophyllum* Desf. and *S. stuckertii* Bitter) with different floral rewards. All these species belong to section *Cyphomandropsis* and grow in Argentina.

Materials and methods

Plant material

The flower samples were obtained either from field collections by L. Passarelli (sites are cited below) or from plants grown in the greenhouses of the University of La Plata. Vouchers of the studied species were placed in the Herbarium of the Natural Science Museum, La Plata (LP), Argentina.

- *Solanum adelphum* Morton, Argentina, Tucumán State, Passarelli 112 (LP), 22 January 1995. This species possesses perfume reward with cryptical anther colour not contrasting with the perianth (Passarelli 1998).
- *Solanum glaucophyllum* Desf., Argentina, Buenos Aires State, Passarelli 4–5 (LP), 4 December 1991. This species possesses pollen reward and flowers are pollinated by buzz-collecting female bees. The androecium exhibits optically contrasting yellow anthers as in *Solanum* and is, apparently, without perfume (Passarelli 1998).

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- *Solanum stuckertii* Bitter, Argentina, Córdoba State, Passarelli 8–9 (LP), 2 December 1992. This species possesses pollen reward and flowers are pollinated by buzz-collecting female bees. The androecium exhibits optically contrasting yellow anthers with perfume (Passarelli 1998).

Spectroscopic measurements

Solanum glaucophyllum was the only member of the studied species whose corollas exhibited colour changes during anthesis, so they were studied spectroscopically.

Fresh flowers from natural growing sites next to the laboratory were taken and floral parts were separated into three groups: (1) violet corollas from flowers of the first day of anthesis; (2) whitish corollas from flowers of the third day of anthesis; and (3) stamens. Floral parts were immersed in methanol and extraction was performed with the aid of an electromagnetic stirrer. The break-up time of the pigments was 20 min for the first group and 1 h for the others.

The UV spectra of the extracts were examined with a Shimadzu UV 240 recording spectrophotometer. Fluorescence spectra of the petal and stamen extracts were obtained on an Aminco-Bowman spectrofluorometer equipped with an off-axis ellipsoidal mirror condensing system and an Aminco ratio photometer with excitation at the wavelength of maximum absorption (350 nm for the petals and 325 nm for the stamens).

Solanum glaucophyllum whole petals were placed in a quartz plate and the absorption spectra were registered in a SuperScan Varian spectrophotometer equipped with a reflectance accessory in the 400–700-nm range.

Floral colour

The existence of flavonols was detected by the procedure of Vogel (1949). Open flowers were immersed in a ferric chloride solution (10 : 100 w/v in distilled water). Dark stains in some floral parts indicated the presence of these pigments, which absorb UV light.

Anthocyanin presence in the coloured petals of *S. adelphum* and *S. glaucophyllum* was determined by observing these pigments turning red under acidic conditions; this is due to their anphoteric properties. Flowers were placed in Petri dishes next to one containing concentrated acetic acid. All were put in a closed receptacle and after 2 h petals changed colour from blue to pink.

In order to determine whether the whiteness of the petals (as observed by human eye) was caused by light diffraction in the air spaces of the mesophyll, the perianth of *S. stuckertii* was saturated with water; white petals should turn transparent and colourless. Optical microscopic studies of the petals to locate air spaces were carried out by fixing the petals in FAA (formalin–acetic acid–ethanol), dehydrating in an ethanol series, and embedding in paraplast. Semithin sections of the petals (10 µm) were cut with a microtome to locate air spaces in the mesophyll.

Photographs

Ultraviolet and human visible-light photographs of the three species were taken on black and white Kodak Plus X film, 125 ASA, with an Asahi Pentax PZ10 camera. UV reflective and absorptive patterns on the flowers were obtained with a UG Schott 2 ultraviolet-passing filter.

Floral odour

Osmophores were located by the method of Vogel (1990). Living flowers were immersed in neutral red solution (1 : 10 000 w/v in tap water) and left overnight. Flower organs with secretor activity concentrated the red dye.

Olfaction tests were carried out by placing different floral parts (stamens, petals and sepals) in clean and tightly closed flasks. After several hours, the flasks were opened and smelled by different people.

Floral parts and visitor attraction

The effect of the different flower parts on visitor attraction was considered by determining pollinator visitation frequency per inflorescence subjected to (1) flowers without modification, (2) flowers without perianth, (3) flowers without androecium, or (4) mixed group (complete flowers, flowers without perianth and flowers without androecium). Sample size was $n = 8$ inflorescences. Visits per inflorescence were recorded during a 3-h period. Observations were carried out simultaneously on nearby plants belonging to the same natural population to avoid climatic variation.

Field observations were carried out to observe anthesis, floral colour change and the presence and activity of pollinators.

Results

Floral coloration

Corollas of *S. adelphum* and *S. glaucophyllum* changed from purple to pink in acetic acid, indicating the presence of anthocyanins. In *S. stuckertii,* the white petals became colourless and transparent when the flower was saturated with water, indicating light diffraction in the air spaces between the cells of the corolla. In a transverse section, a wide intercellular air-space system was present (Fig. 1*B*) except in the region near the main vein (Fig. 1*A*).

Both species with pollen reward, *S. stuckertii* and *S. glaucophyllum*, had modified areas of the corolla at the petal base, in the region surrounding the anther cone and along the principal vein, on the adaxial face (Figs 2*C*, *E*, 3*I*, *K*). These brilliant-green areas had a silky appearance. Their cells were long, narrow and without papillae. They were arranged in parallel rows, oriented radially. Their presence probably contributed to reward location because these were areas of UV light absorption (Fig. 2*D*, *F*).

The anthers, the half sector of each petal and the central design of the corolla absorbed UV light, appearing as dark areas in the photographs (Figs 2*D*, *F*, 3*J*, *L*). These areas of the petals had green pigmentation in fresh flowers. On the other hand, in *S. adelphum*, the only species with perfume rewards, no differences between visible light and UV-light patterns were found (Figs 2*B*, 3*H*).

The UV absorption bands observed in stamen and perianth extracts indicated the presence of flavonols (Fig. 4). The spectrum of the whole petals showed a maximum absorbance peak at 550 nm (Fig. 5). There was very little difference between purple (first-day anthesis) and whitish (third-day anthesis) flowers.

Fluorescence spectra of the extracts were determined with excitation at the maximum wavelength of absorption (350 nm for the petals and 325 nm for the stamens). A high intensity of fluorescence emission at 444 nm was observed for the

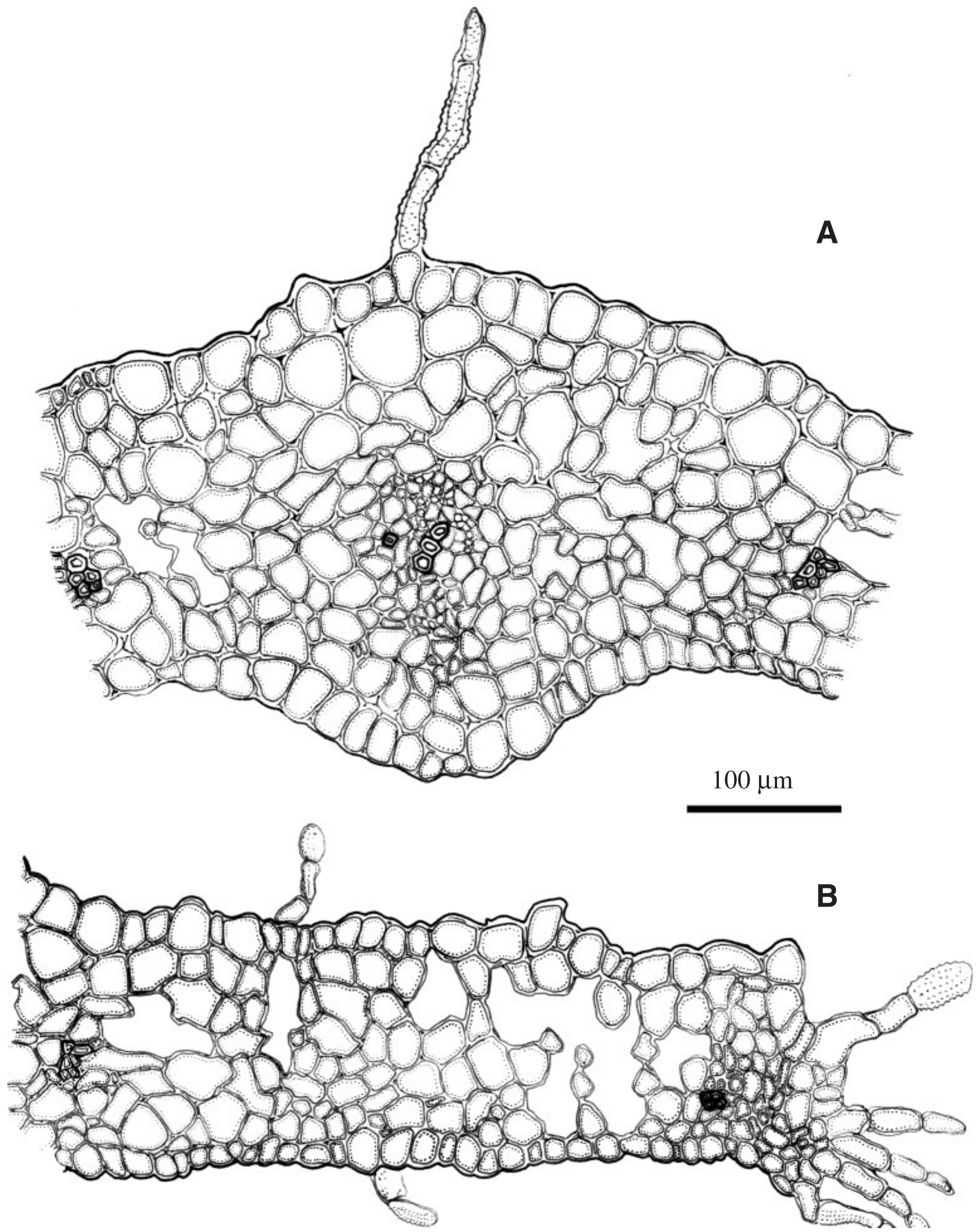

Fig. 1. *Solanum stuckerti*, transverse sections of the petals. Floral coloration: the white colour of petals is caused by light diffraction in the air spaces between the cells of the corolla. (*A*) Area without air spaces, which corresponds to the main vein. (*B*) Mesophyll with air spaces.

petals. On the other hand, the stamens exhibited little or no fluorescence (Fig. 6).

Scent attractive osmophores

Some areas in the base and apex of the petals, dispersed areas of the corolla and particularly in the dorsal part of the connective in the two species with odour *S. adelphum* (perfume reward) and *S. stuckertii* (pollen reward) possessed osmophores. These areas were coloured intensely by neutral red (Fig. 7*A*, *B*, *E*, *F*). The adaxial region of the anthers did not show significant coloration (Fig. 7*A*, *B*). Only anther walls were coloured in *S. glaucophyllum* (without apparent odour and with pollen reward) (Fig. 7*C*, *D*).

Results from olfaction tests agreed with the results cited above. In the two species that produce odours (*S. adelphum* and *S. stuckertii*), the jars with the stamens possessed stronger scent than those containing only the petals. Floral parts of *S. glaucophyllum* did not emit different aromas from those associated with vegetative parts.

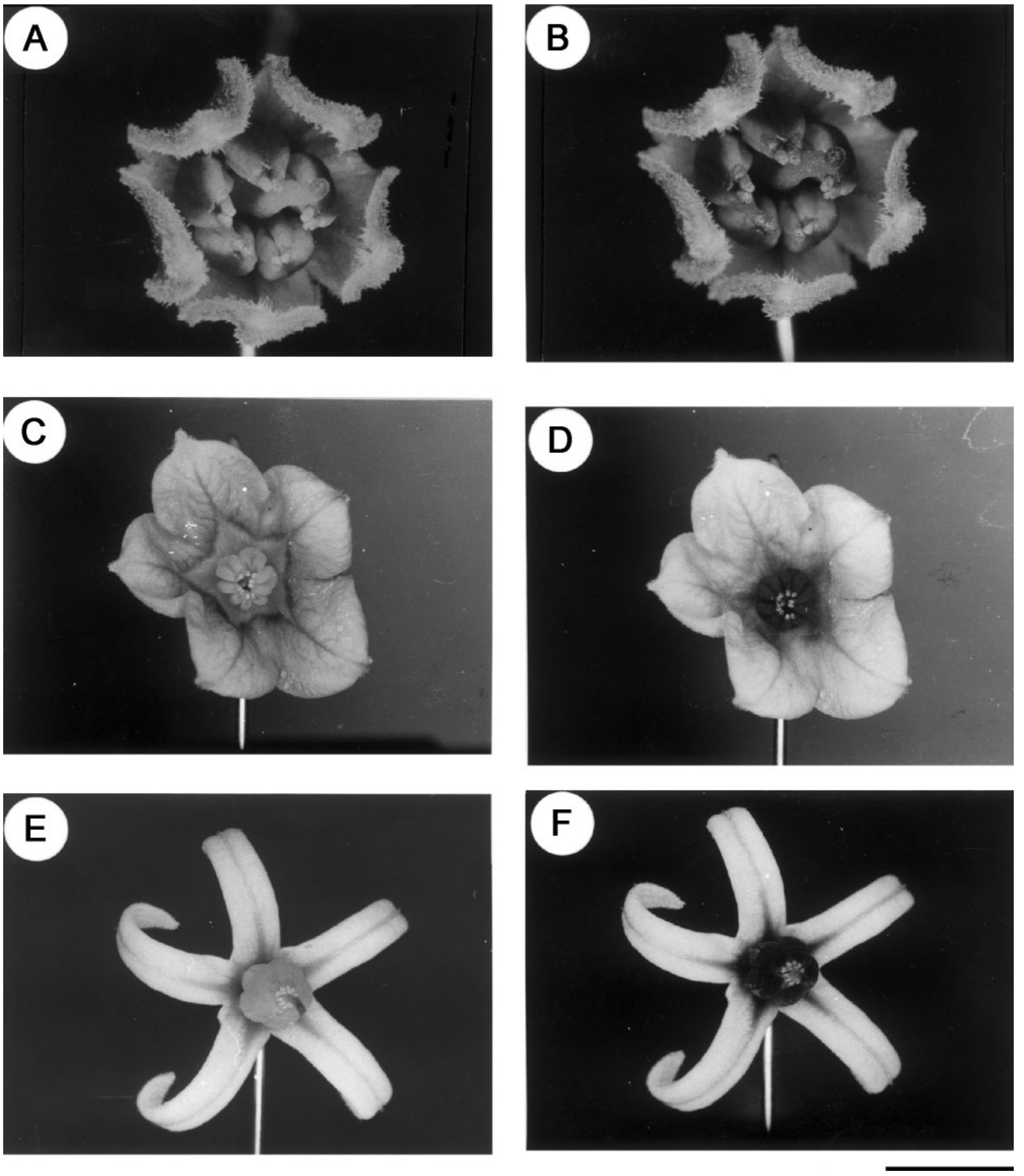

Fig. 2. UV-light flower patterns, frontal view. (*A*, *B*) *Solanum adelphum*: no differences between visible-light and UV-light patterns are observed. (*C*, *D*) *Solanum glaucophyllum*. (*E*, *F*) *Solanum stuckertii.* (*D*, *F*) The half sector of each petal and the central design of the corolla absorb UV-light. The absorbant portions of the corolla (dark zone) contrast with the rest. *A*, *C*, *E*, photographed in visible light; *B*, *D*, *F*, photographed in UV-light. Scale bar = 5 mm.

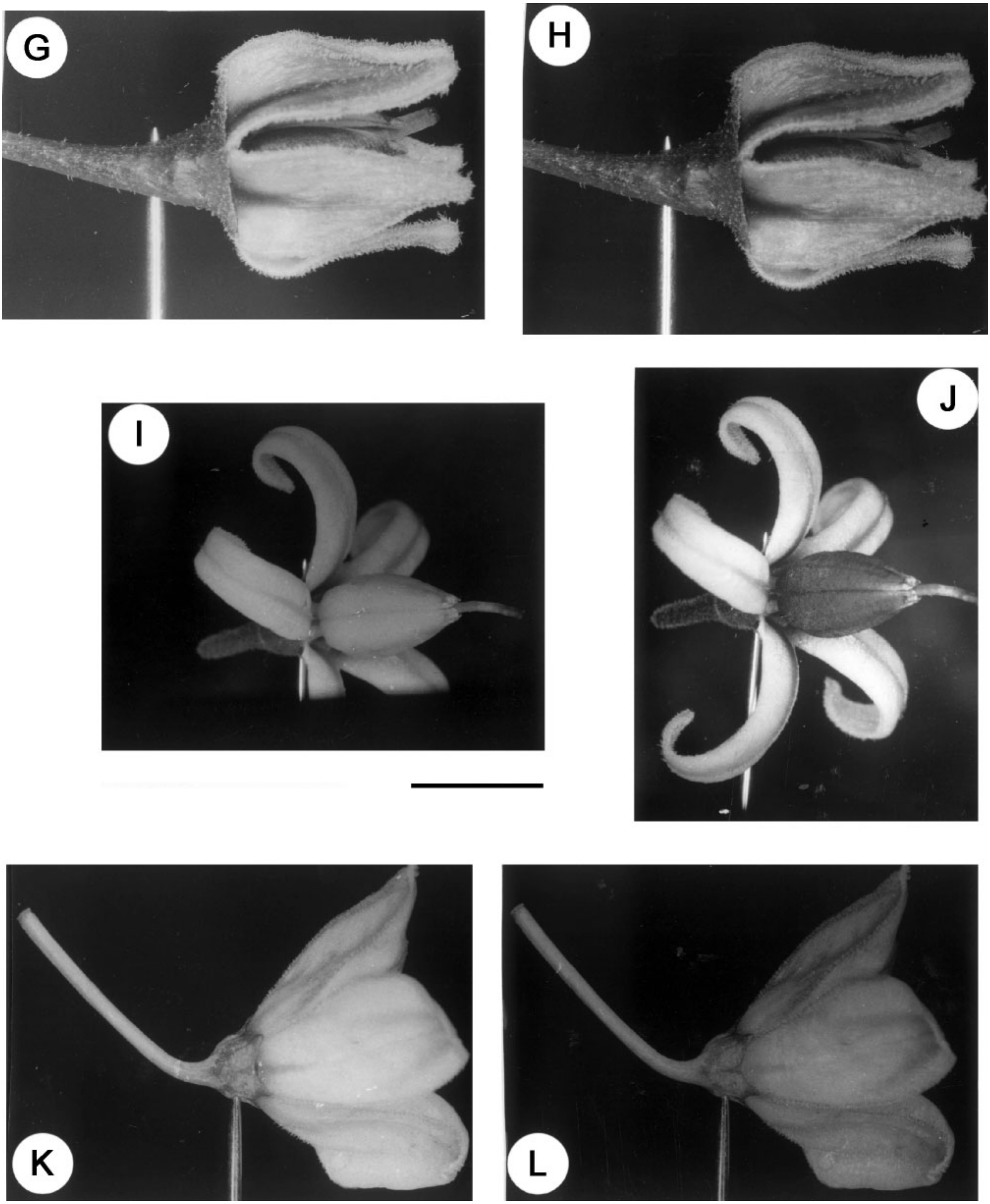

Fig. 3. UV-light patterns, lateral view. (*G*, *H*) *Solanum adelphum*. (*I*, *J*) *Solanum glaucophyllum*. (*K*, *L*) *Solanum stuckertii*. *G*, *I*, *K*, photographed in visible light; *H*, *J*, *L*, photographed in UV-light. Scale bar = 5 mm.

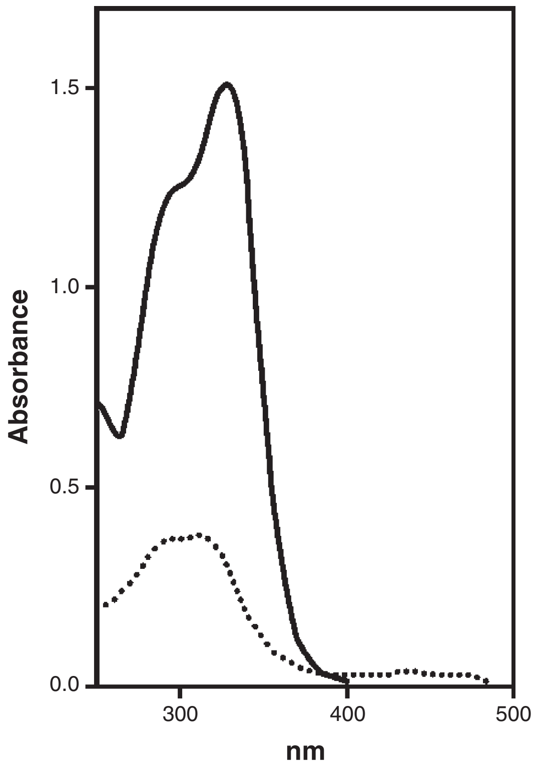

Fig. 4. *Solanum glaucophyllum.* Absorption spectra of methanol extracts of stamen (—) and petals (· · · ·).

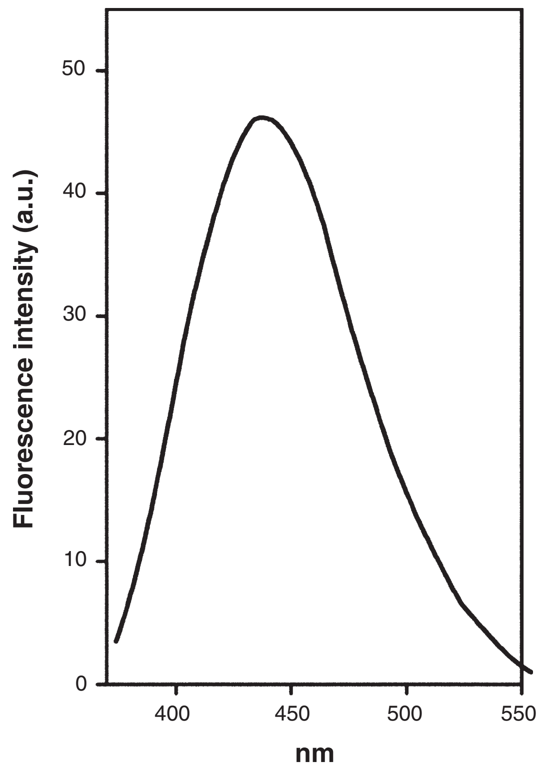

Fig. 6. *Solanum glaucophyllum.* Fluorescence spectrum of methanol extracts of petals ($\lambda_{ex} = 350$ nm).

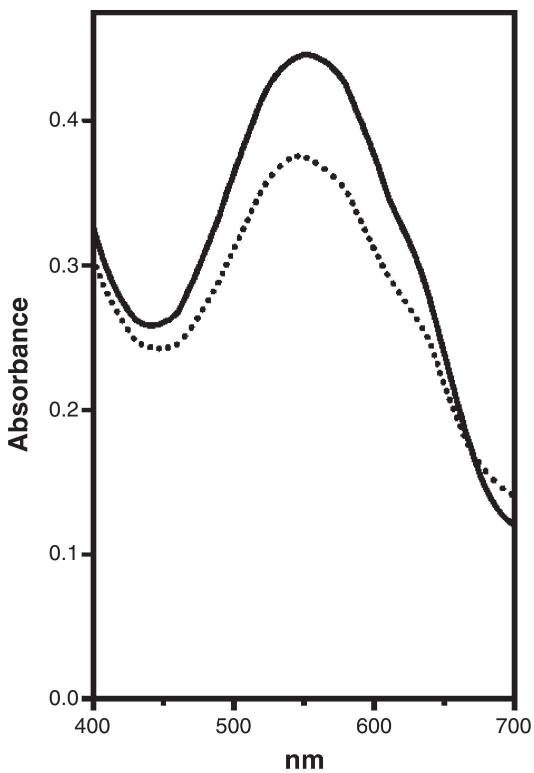

Fig. 5. *Solanum glaucophyllum.* Absorption spectra of whole petals: purple (· · · ·) and whitish (—) flowers.

Attraction effect of the different parts of the flower in Solanum glaucophyllum *(floral colour change during anthesis)*

Anthers were showy and yellow during the first days of anthesis. After the flowers had been visited several times, the anthers withered because of the damage caused by the jaws and legs of the insects, and became brown. At this stage, visual attraction at a short distance disappeared and the visitors avoided the flowers in all the cases. Nevertheless, the presence of these flowers may contribute to the attraction of the inflorescence as a whole.

In experiments carried out *in situ*, the contribution of the perianth and the androecium to *S. glaucophyllum* attraction differed according to the pollinator (Table 1). In the case of *Augochloropsis* (Hymenoptera, Halictidae, Halictinae), the group of flowers without petals was more attractive than the control group. Also, in the mixed group (whole flowers, flowers without petals and flowers without androecium) those flowers without petals were the most visited. On the other hand, *Bombus atratus* (Hymenoptera, Apidae, Apinae) did not visit incomplete flowers (without androecium or corolla). They visited control groups and whole flowers mainly of the mixed groups. Only once did this bee alight on the anthers of a flower without petals in the mixed group, but even then it flew off without vibrating the flower.

Fig. 7. Scent attractive osmophores coloured by neutral red. (*A*, *B*) *Solanum adelphum.* (*C*, *D*) *Solanum glaucophyllum.* (*E*, *F*) *Solanum stuckertii.* Scale bar = 5 mm (*A*, *B*), 4 mm (*C*, *D*), 6 mm (*E*, *F*).

Table 1. Attractiveness of the different parts of *Solanum glaucophyllum* flowers as determined by frequency of pollinator visits
$n = 8$ inflorescences

Pollinator species	Control (n/%)	Flowers without perianth (n/%)	Flowers without androecium (n/%)	Mixed group (n/%)
Augochloropsis sp. ($n = 38$)	8/21.05	15/39.47	5/13.15	10/26.31[A]
Bombus atratus ($n = 54$)	18/33.33			36/66.66[B]

[A] The 60% of the visited flowers were without perianth.
[B] All the visited flowers were complete. Visits were registered from 0800 to 1100 hours (period of most frequent visitation).

Discussion

This study has demonstrated that floral advertisement varies with the pollination reward. It seems that divergent evolution within a section and convergent evolution between *Pachyphylla* and *Cyphomandropsis* sections has occurred.

Different suites of floral characteristics have been assigned to different groups of bees (Faegri and Van der Pijl 1979; Roubik 1989; Dafni and Kevan 1995). Herrera (1996) has suggested that ecological factors might constrain the occurrence of, and the response to, selection by pollinators.

Pollen flowers of *Solanum s.l.* are relatively constant in their morphology, with the prevailing blue purplish or white corollas contrasting with the brilliant yellow cone of the anthers. Some authors argue that this morphologic pattern is characteristic of the genus (Buchmann 1983; Cocucci 1988). Faegri (1986) refers to this combination of characteristics as the 'solanoid feature', and Symon (1979) considers this floral uniformity to be the result of specialisation in pollination. The two species with pollen rewards observed here (*S. glaucophyllum* and *S. stuckertii*) possess similar characteristics to those mentioned. The colour and the presence of UV patterns are the main attractive features of the flowers. First, at short distances, attractions are due to the perianth and anthers. When the anthers have been emptied, their attraction persists, because their colour does not change, thus deceiving floral visitors (Vogel 1978). The reflection in *S. glaucophyllum* corollas is in the blue-purple wavelength (complementary colour to the green-yellow corresponding to the 550 nm absorption). This supports the conclusions of Kevan (1978), who suggested that corollas that reflect blue wavelengths would receive more attention from bumblebees (*Bombus*) than from other insects.

Species of *Bombus* (e.g. *B. attratus, B. opifex*) are the main visitors to this genus, according to our personal observations and several reports of *Solanum* pollination (Linsley and Cazier 1963; Bowers 1975; Buchmann 1983; Cocucci 1988; Fernández Storti 1988; King and Buchmann 1996). The greenish areas at the base of the petals of *Solanum* flowers, known as pseudonectaries, are assigned the function of pollen and/or nectar guides (Buchmann 1983). These modifications of the perianth epidermis, present in *S. glaucophyllum* and *S. stuckertii,* are UV-light-absorbing areas, which contrasts with the rest of the petals. However, they should not really be considered pseudonectaries because no insects have been observed actively searching for nectar.

Solanum adelphum has perfume reward, which is very different from most congeners. The flowers are not particularly showy, and possess a campanulate corolla with cryptic anthers covered by the perianth. These characteristics are indeed of sect. *Pachyphylla.* The odour emitted by the connective is the only attractive feature in species with perfume reward, due to the absence of contrasting areas between the perianth and anthers, and UV-light patterns. The presence of osmophores is also evidenced in *S. stuckertii*; both species possess well-developed connective. D'Arcy (1990) described scented anthers in Solanaceae, recognising the presence of anthers with sweet fragrances in some members of *Solanum* subgenus *Leptostemonum* as an important specialisation. D'Arcy attributed great significance to this characteristic and suggested that the pollinators of fragrant species may be different from those for species without perfume. The species of sec. *Cyphomandropsis* (included in subgenus *Leptostemonum*) with osmophores have a pleasant fragrance. However, *S. glaucophyllum* (species without detectable odour) and *S. stuckertii* (with odour) share the same pollinators (*B. attratus, B. belicosus* and *Augochloropsis*), as observed in our experimental areas. These insects have ignored *S. adelphum* flowers (cultivated in the same place).

The visitation rate of pollinators, such as bumblebees, was reduced when some parts of flower petals were separated. Bumblebees are selective in flower visits on the basis of floral symmetry (Chittka *et al*. 1994; Waser *et al*. 1996). This was not the case for *Augocchlora* sp. The study of innate preferences of all pollinator groups as well as their relative qualities as pollinator reward should be continued.

Conclusions

The significance of floral colour and scent in three *Solanum* species is related to floral reward. Colour contrast between anthers and perianth and the presence of UV patterns are the principal attraction to pollinators in species with pollen reward. The flowers of *S. adelphum*, a species with perfume reward, are not showy and the odour is the main feature recognised by pollinators.

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