

Chapter III

Studies on floral colouration and reward properties in Argentinean plant-pollinators communities.

Abstract Floral features influence foraging strategies of pollinators. Several studies have shown that characteristics like reward properties, display size and colour as well as floral shapes have an impact on learning speed and flower constancy of bee pollinators in laboratory as well as in natural conditions. Rather controversial is the question whether choice preferences of bees during foraging have influenced the distribution of colour-reward pairings in natural flowering plant communities. Results in a recent study in European plant-pollinator communities did not support the idea that the bees' innate colour preferences for blue-violet colours have developed because of the high reward properties of plants presenting these colours. However, results also indicated that the plant-pollinator system can be characterised by various changing parameters which indicate that plants exploit sensory and cognitive abilities of their pollinators. In the present study, we compare these results with new data obtained from Argentinean plant-pollinator communities. In four undisturbed communities, we measured flower colours and reward properties of co-flowering plants as well as floral abundance and pollinator activity. We related the data to groups of functional flower shapes and applied the Receptor Noise Limited Model of honeybee colour vision to approach the question from the pollinator's point of view.

Contrarily to European sites, plant species that were highly rewarding and commonly visited by bees, mostly displayed yellow colours (with and without UV). Colours of highly rewarding or highly visited species tended to differ visibly from those of less rewarding or less commonly visited species. The investigation of reward properties in relation to chromatic properties indicated a clustered distribution of rewards. Further, plant species with hidden nectar were usually more rewarding than those with open access to their nectar, and visits of Apoidea and non-Apoidea occurred equally often in all groups of different functional flower shapes. Apoidea were not the main pollinators of plants with hidden nectar as observed in European sites. Thus, honeybees and bumblebees, which have close phylogenetic origins to the European species and which populated the New World much later, behaved differently in their new environment. We concluded further, that the composition of the pollinator community in this environment was a selective factor that led to different adaptations in colour-reward distributions of plants.

Introduction

Bees are very efficient foragers (von Frisch 1965, Heinrich 1975). Flowering plant species, in turn, require efficient pollination and have evolved a number of different strategies to assure reproduction and population growth (Faegri and van der Pijl 1979, Jones and Little 1983, Paulus 1988, Cohen and Shmida 1993, Dafni *et al.* 1997, Chittka *et al.* 2001, Waser and Ollerton 2006). In a recent study, we were able to identify a number of floral features and rewards that foraging bees preferred during foraging in a natural flower population (Wertlen *et al.*, in prep.). They often visited plant species with a significantly higher reward rate – taking into account the maximum (max.) sugar crop of the flower and the floral abundance. This result was explained by the honeybees' and bumblebees' ability to estimate reward quality and quantity in relation to the visual cues of the flower, in laboratory as well as in field conditions (Núñez 1970, 1977, Varjú and Núñez 1991, Greggers and Menzel 1993, Klinkhamer *et al.* 2001). These abilities were an important requirement for the development of flower constancy, which honeybees and bumblebees exhibit depending on the amount and frequency of available resources (Grant 1950, von Frisch 1965, Heinrich 1975, Kevan and Baker 1983, Menzel 1985, Chittka *et al.* 1999). Further, we found that bee visitation rates were also influenced by the functional flower shape, i.e. according to the degree of difficulty with which the flower visitor could access the nectar. The group of plant species that had flowers with open nectar was usually higher rewarding and attracted most visitors, of which most were non-Apoidea. Plant species with less easily accessible nectar were usually less rewarding and attracted less visitors – in this case mostly Apoidea. This result was surprising, because it is commonly believed that flowers with hidden nectar offer on average a higher reward, which is preferred by bees (Kugler 1970, Faegri and van der Pijl 1979). The main question of the European study – how strong choice strategies of pollinators influence the relation of floral colouration and reward properties and *vice-versa* – did not have a straightforward answer. Instead, we revealed a rather complex relationship between plant species' strategies for the attraction of pollinators and the pollinators' foraging decisions that were in turn based on their prior experience of the reward qualities of flowers with various colours. In the present study, we investigated whether these results are also valid for Argentinean habitats, which have a different plant-pollinator composition, although honeybees and bumblebees are present in both.

The acquired memory for floral features in combination with reward properties can influence the bees' choices and their degree of flower constancy temporarily (Fülöp and Menzel 2000, Keasar *et al.* 2002). Highly profitable food sources or plant species with hidden nectar (both frequented by bees) were often blue-violet in colour (including UV or not). However, these colours did not reflect the honeybees' and bumblebees' innate colour preferences which contradicted suggestions made by Giurfa *et al.* (1995) and Chittka *et al.* (2004). They hypothesised that bees developed innate colour preferences to ensure that young foragers quickly find the best rewarding plant species on their first foraging flights. Hence, it might be that the flower-pollinator system is too complex to be reduced to such a generalisation. The bee's ability to quickly react to a changing environment with blossoming and withering plant species on the basis of their cognitive abilities rather seems to be an adaptive strategy (Menzel 1985, Greggers and Menzel 1993).

On the other hand, investigations were only carried out at four different sites, which is a rather low sample size. Now, we add another four sites to the analysis, including habitats with new plant and pollinator communities in the low-mountain and in the high-mountain range in Argentina. The approach was the same as in our former studies. Investigation sites were selected considering the occurrence of authentic, well defined plant communities and undisturbed plant-pollinator interactions. We measured reward properties, floral abundance and visitation frequencies of plant species and we divided plant species into groups of different functional shape of flowers. We focused on bees, because they form the main pollinator group in European and Argentinean plant-pollinator communities (Goulson 1999, Cocucci, personal observations) and their foraging behaviour and visual perception has been studied in detail (von Frisch 1965, Heinrich 1975, 2001, Menzel and Backhaus 1991, Varjú and Núñez 1991, Peitsch *et al.* 1992, Goulson 1999). They possess an excellent trichromatic colour vision which is based on three types of photoreceptors, which are sensitive in the short (S) or ultra-violet (UV), medium (M) or blue, and long (L) or green part of the wavelength spectrum. Bees' photoreceptors are well-adjusted for the discrimination of their natural coloured targets, the flowers (Chittka *et al.* 1994, Vorobyev and Menzel 1999), although there are many additional colours which can be perceived by the bees (Vorobyev and Menzel 1999). They learn colours of food sources very quickly and may generalise them (Menzel 1968, Backhaus *et al.* 1987a, Giurfa 1991). During foraging, bees develop an expectation of reward based on the food source's signals (Greggers and Menzel 1993).

Based on our measurements, we tested whether highly rewarding plant species have different colours than less rewarding species, which would enhance the bees' detection speed and

association strength. Further, we investigated how reliable reward properties would be if the pollinator were to categorise colours in fine or broad categories, i.e. whether reward distributions were clustered. Results are discussed to elucidate evolutionary trends in different plant-pollinator communities.

Materials and methods

We analysed the spectral properties as well as reward properties of co-flowering plant species in four different plant communities located in preserved natural environments, from the pollinator's point of view, as we had done in former studies in Germany and Austria (Wertlen *et al.*, in prep.). The anthropogenic influence on the sites was about equal to that on the sites in Europe. In Cuesta Blanca (31° 24' 21.6'' S, 64° 34' 0.5'' W, 850 msm; henceforth: CB site), measurements were performed on an unused pasture, located in the intermountain valley at the foot of the Sierras Grandes, Córdoba Province. Such pastures appear in clearings of the original xerophyllous savannah-like Chaco woodland (Cabido and Zak 1999). The pasture in El Durazno, located in the Sierras Grandes, Córdoba Province (31° 22' 3.9'' S, 64° 37' 41.88'' W, 1117 msm; ED site) was occasionally grazed by cows. It belongs to a scrub vegetation belt immediately above the timber line. It is dominated by tussock grasses of the genera *Stipa* and *Festuca* (Cabido and Zak 1999). The pasture in Tafi del Valle, Tucumán Province (26° 44' 2.4'' S, 65° 47' 12.8'' W, 2100 msm; TV site) was grazed by sheep. It is subalpine grassland, dominated by soft grasses, immediately above the Yungas rainforest on the moist east side of the Aconquija mountain range (Cabrera 1971, 1976). And the investigation site in Vallecitos, Mendoza Province (32° 58' 48.3'' S, 69° 21' 19.8'' W, 2826 msm; VA site) was subject to moderate recreational usage. It is an alpine-like meadow located above the Monte scrub desert and below the high mountain Puna desert. It is dominated by tussock grasses forming cushions or rings (Cabrera 1971, 1976). About 14 % of the total number of plant species and 4,5 % of the plant species that were included in the nectar measurements were introduced (see App. 1 and 2 for complete species list). The measurements took place from 25.11.-05.12.2002 (CB site), 07.-16.12.2002 (ED site), 08.-18.01.2003 (VA site) and 04.-12.12.2003 (TV site).

We used identical methods to measure spectral reflectance of the flower petals and to determine the colour loci of the flowers in the honeybee colour space with the Receptor Noise Limited (RNL)-Model of honeybee colour vision (Vorobyev *et al.* 2001). Spectral reflectances were measured in the four investigation sites and in 17 additional sites to

approximate an authentic survey of the flower colour distribution in North-East Argentina (N = 508, App. 1, 2). We used human colour names and UV for illustrative purposes, derived from categorised colour spectra (Fig. 1A-D), although not accurately applicable to bee vision (Daumer 1956, Backhaus and Menzel 1987). We quantified the abundance of plant species by the occurrence of their open flowers in the site as perceived by the pollinator as the ratio of open flowers of a specific species to the total number of open flowers per m² at the site in percent. Pollinators were classified into groups of *Apis*, *Bombus*, other Apoidea, other Hymenoptera, Coleoptera, Diptera and Lepidoptera. Their flower visits were counted during random single flower observations (henceforth: SFO) and activity walks (AW) that included the uniform observation during a period of 30 minutes each, of all plant species (see App. 3 for frequency of visits/visitor category/plant species). Nectar volume was subsequently measured as the full crop, accumulated during 24h, at the observed individuals. Sugar concentration of the nectar was measured using a refractometer (0-80 % Brix scale, Optech, Canada). Reward properties were estimated taking account of maximum (max.) sugar crop and abundance.

Observations and nectar measurements were completed for 10-12 plant species per investigation site (N = 44) and on average 16 ± 6 (SD) individuals per plant species (App. 1, 2). They took place randomly and in parallel during 8-10 consecutive days. The flowers of all measured plant species were divided into three groups of functional shapes, i.e. the degree of difficulty with which the flower visitor was able to access the nectar. The first group contained flowers that required only a minor effort on the part of the pollinator to obtain the openly, easily accessible nectar. Flowers in the second group required a medium effort, e.g. crawling into the flower to obtain the hidden nectar or robbing the nectar. And the third group required a high effort of the pollinator, who had to move parts of the flower to obtain the hidden nectar. Flowers whose nectar was not obtainable by bees, e.g. because the nectar was hidden too deeply, were not included in the analysis. This was the case for *Arjona patagonica*, *Dyckia* spec., *Glandularia mycrophylla*, *Glandularia peruviana*, *Lantana camara*, *Oenothera* spec., *Pfaffia gnaphaloides*, *Stenandrium dulce* and *Stevia satureifolia* (App. 1).

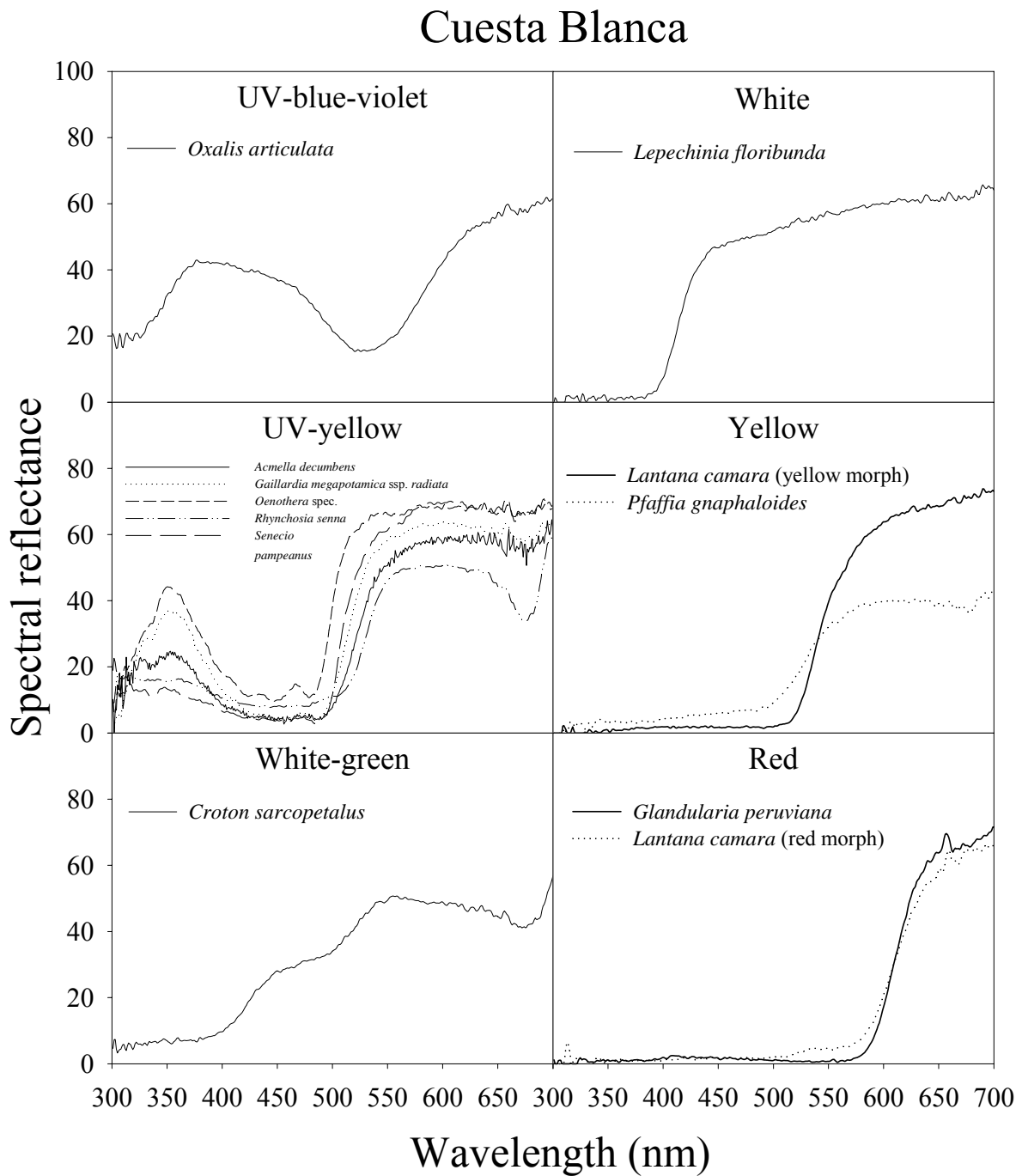
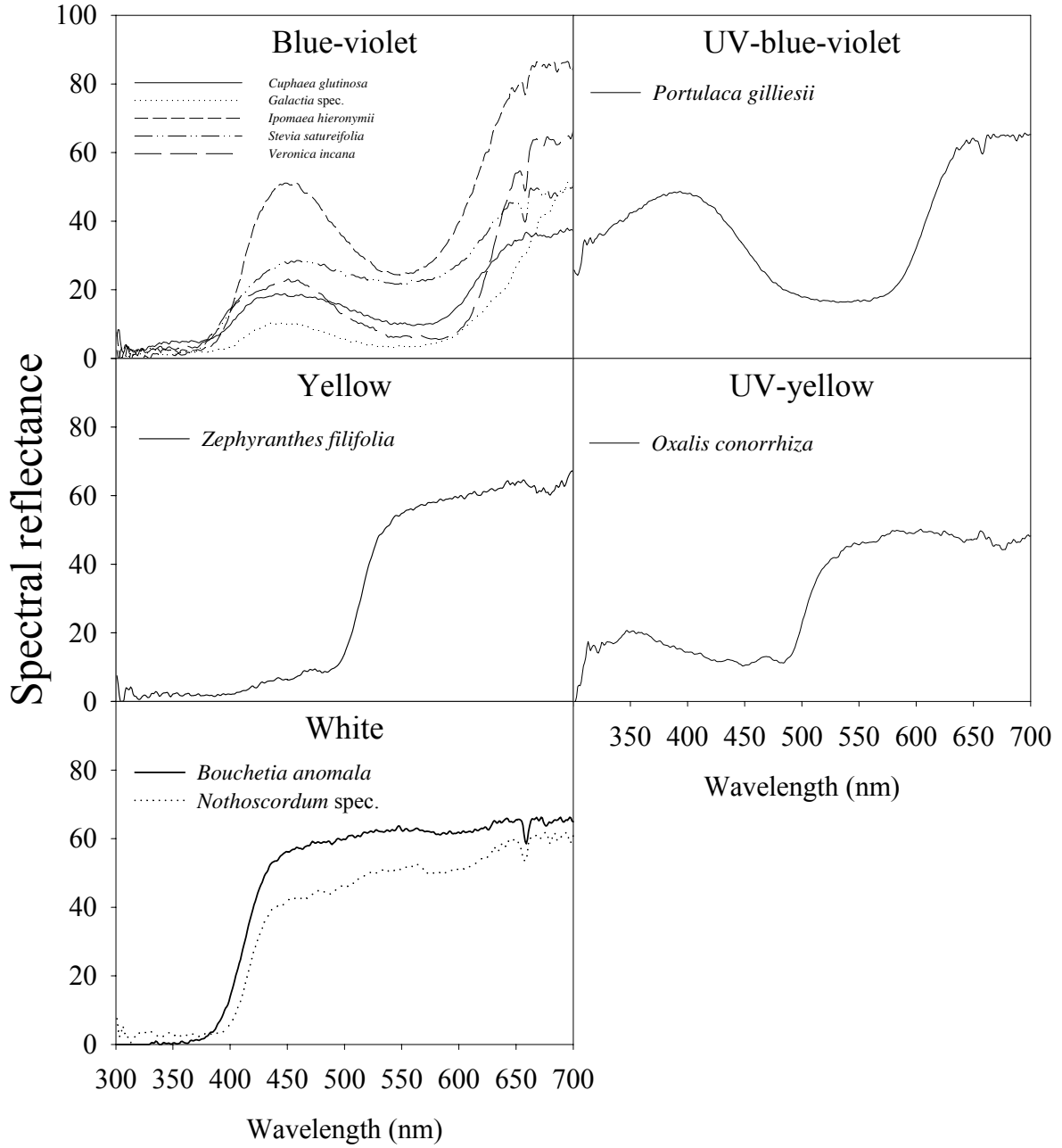
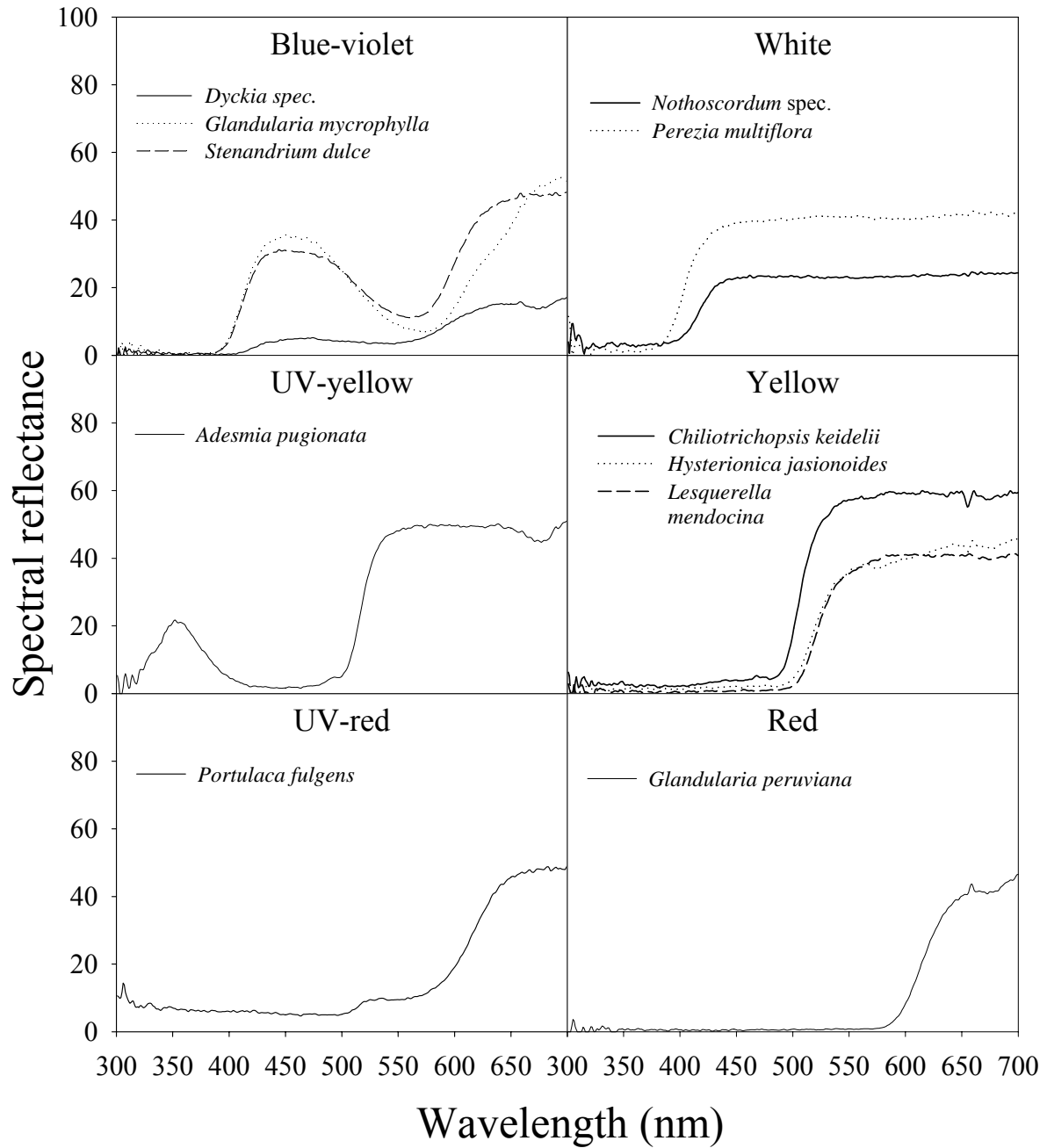


Fig. 1 Colour classifications of flower colours according to their reflectance spectra, separately for the sites **A** Cuesta Blanca, **B** El Durazno, **C** Tafi del Valle and **D** Vallecitos.

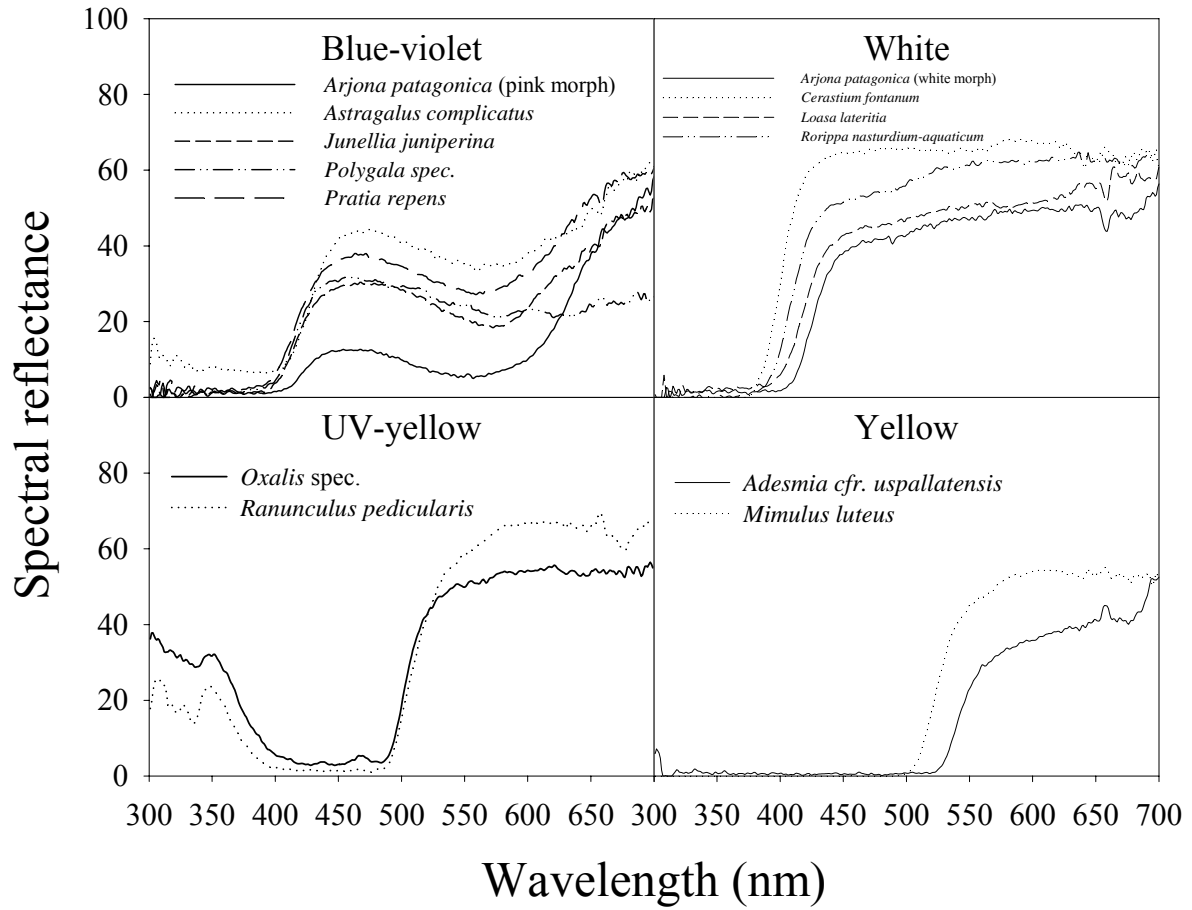
El Durazno



Tafi del Valle



Vallecitos



Results

Flower colours and reward properties

Max. nectar crop and max. sugar crop correlated positively ($R = 0.999$, $P < 0.0001$, Pearson Product Moment Correlation). We found between 2 and 4 species per site presenting flowers with a significantly higher max. sugar crop than most of the other species (Fig. 2A, App. 1; $P < 0.05$, Kruskal-Wallis-Test). Species varied widely in their abundance, but did not show any statistical differences (Fig. 2B; $P > 0.05$, Kruskal-Wallis-Test). However, half of the plant species with the highest max. sugar crop also had the highest reward rate (i.e. the product of max. sugar crop and floral abundance) (Fig. 2A, $P < 0.05$, Kruskal-Wallis-Test). In the CB site, *Lepechinia floribunda* (white, in human terms) and *Acmella decumbens*, *Gaillardia megapotamica* and *Oenothera* spec. (all UV-yellow, the latter only moth pollinated), had the highest max. sugar crop, but only *Lepechinia floribunda* also had the highest reward rate. In the ED site, *Cuphea glutinosa* and *Ipomoea hieronymii* (both blue-violet), *Nothoscordum* spec. (white) and *Oxalis conorrhiza* (UV-yellow) had both highest max. sugar crop and highest reward rate. In the TV site, *Adesmia pugionata* (UV-yellow) and *Dyckia* spec. (blue-violet) also combined both properties. Here, *Glandularia peruviana* (red, butterfly pollinated) also had one of the highest reward rates. And in the VA site, *Adesmia* cfr. *uspallatensis* and *Mimulus luteus* (both yellow) and *Loasa lateritia* (white) were further species with both the highest max. sugar crop and highest reward rate and were all pollinated by bees. To summarise, 50 % of these highly rewarding species displayed yellow colours with or without UV, 21.4 % displayed blue-violet colours, another 21.4 % displayed white colours and 7.1 % displayed a red colour (Fig. 1A-D, 2A). However, none of these plant species presented flowers with colours that lay within the region of the bee colour space that was reported to be preferentially selected by colour-naïve bees (Giurfa *et al.* 1995).

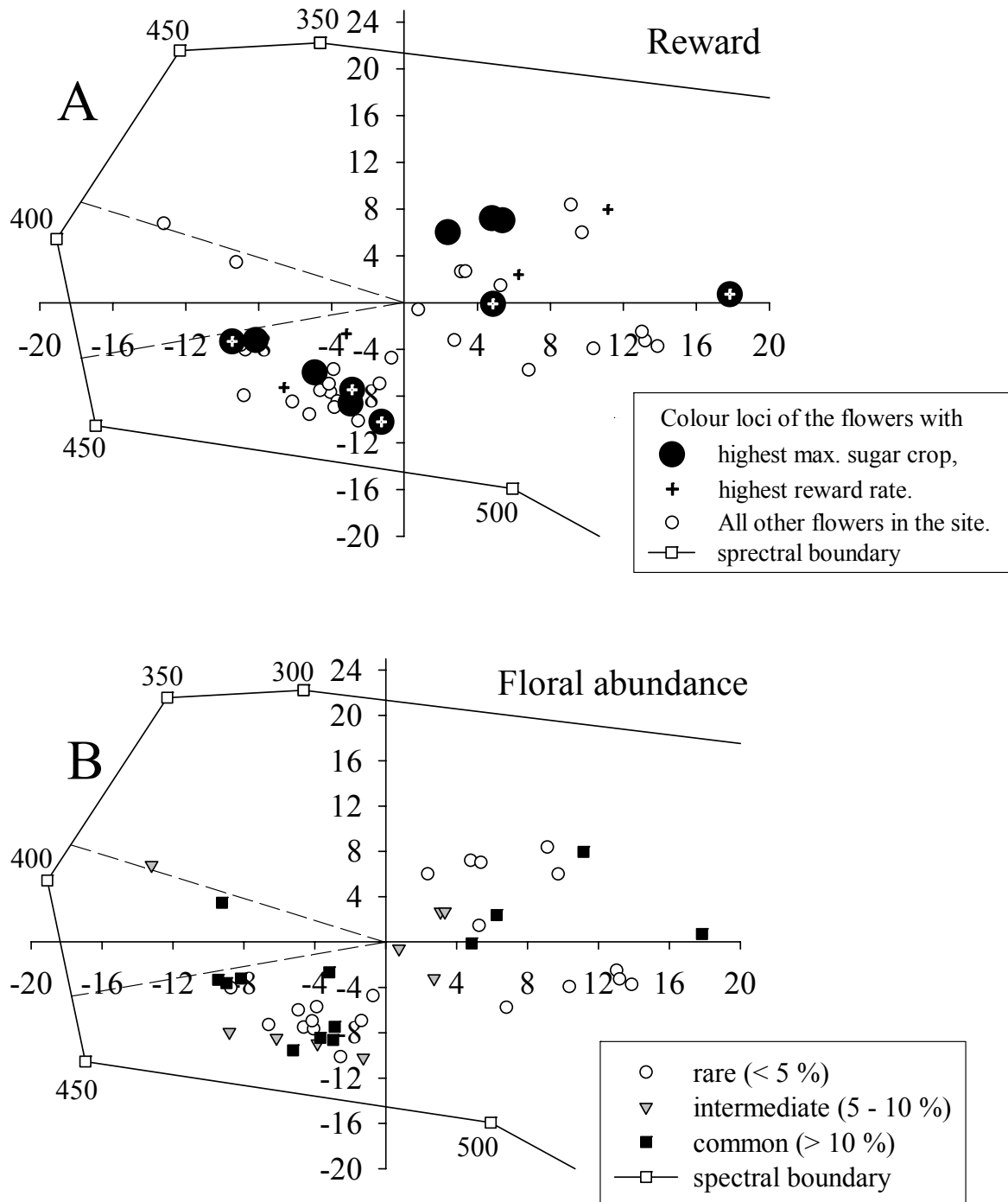
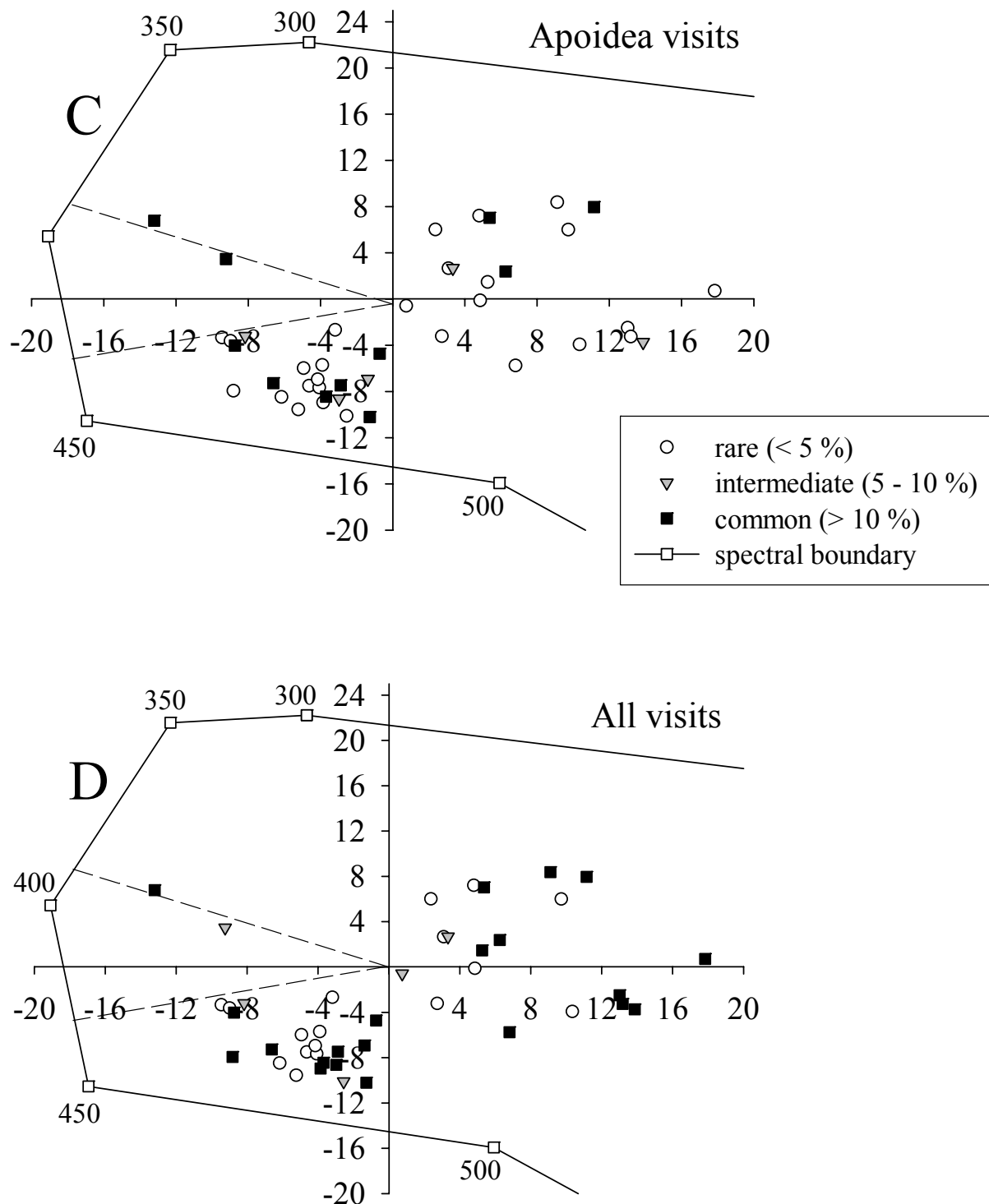


Fig. 2 Colour loci of the flowers of all measured plant species in the honeybee colour space, according to **A** their reward probabilities, **B** to their floral abundance (percentage of open flowers of the total number of open flowers), **C** to the frequency of bee visits (percentage of visits of the total number of visits) and **D** to the frequency of all pollinator visits. The chromatic coordinates and distances to background have been calculated for each flower using the relative quantum catch of each photoreceptor according to the RNL-Model of honeybee colour vision (Vorobyev *et al.* 2001). The unity distance corresponds to one standard deviation of noise. The colour locus of the background (leaf green) is per definition at the cross-point of the axes of the colour diagram. The spectral boundaries of the bee colour space are indicated by the line with open squares.



Continuation Fig. 2 A Max. sugar crop (mg) and reward rate (the product of max. sugar crop and abundance) were measured. Some species combined both properties, symbolised by a white cross on a black circle. Colours of the best rewarding or largest flowers occurred in various areas of the colour space and were not located in the approximate area preferred by inexperienced bees under laboratory conditions, indicated by the dashed line (Giurfa *et al.* 1995). **B** Colours of plant species with common flowers (> 10 % of the flowers) appeared in various areas of the honeybee colour space. Also, visits of **C** bees and **D** all pollinators commonly (> 10 % of visits) occurred on flowers with colour loci of various different colours.

Flower colours and visitations

Flower visits were not distributed equally over all plant species (Fig. 2C, D, App. 3). Species with very commonly visited flowers (i.e. flowers that received more than 25 % of the total number of visits/site) sometimes had the highest max. sugar crop and/or reward rate in the site, e.g. *Gaillardia megapotamica* (CB site, mainly visited by (m.v.b.) *Apis*), *Lepechinia floribunda* (CB site, m.v.b. other Hymenoptera) and *Adesmia pugionata* (TV site, m.v.b. other Hymenoptera and Diptera). Sometimes species with very commonly visited flowers had a low max. sugar crop and/or reward rate, e.g. *Zephyranthes filifolia* (CB site, yellow, m.v.b. Lepidoptera), *Oxalis* spec. (VA site, UV-yellow, m.v.b. Diptera) and *Ranunculus pedicularis* (VA site, UV-yellow, m.v.b. Diptera). *Apis* and *Bombus* were generally very rare in the ED, TV and VA sites, which is not unusual (Cocucci, personal observation). Their visits occurred on *Croton sarcopetalus* (green), *Lepechinia floribunda* (white), *Oxalis articulata* (UV-blue-violet), *Gaillardia megapotamica*, *Rhynchosia senna* and *Senecio pampeanus* (all UV-yellow) in the CB site (Fig. 2C, App. 3). Additionally, we could observe *Apis* on *Lesquerella mendocina* (TV site, yellow) and *Loasa lateritia* (VA site, white). But numerous visits of mostly different plants by wild, native bees occurred in all investigation sites. They were common (> 10 % of the total number of visits/site; App. 3) on *Croton sarcopetalus* (CB site, green), *Nothoscordum* spec. (ED site, white), *Cerastium fontanum* ssp. *vulgare* and *Loasa lateritia* (both VA site, white), *Galactia* spec., *Ipomoea hieronymii* and *Portulaca gilliesii* (all three ED site, blue-violet), *Adesmia pugionata* (TV site) and *Oxalis conorrhiza* (ED site, both UV-yellow). To sum up, 83.3 % of the very commonly visited plant species displayed yellow colours with or without UV and 16.7 % displayed white colours (Fig. 1, 2D). Bee visits occurred mainly on plant species with UV-yellow or yellow colours (40 %), but also on species presenting blue-violet colours with or without UV (26.7 %), white colours (26.7 %) or green colours (6.7 %; Fig. 1, 2C).

Functional flower shape

Plant species were grouped according to their functional flower shape. 16 species belonged to the group with the most easily accessible nectar for bees (group 1), 12 species belonged to the group with intermediately accessible nectar (group 2) and 6 species belonged to the group with complicatedly accessible nectar (group 3). Group 1 had a significantly lower mean max. sugar crop (0.43 ± 0.08 mg \pm SE; $P < 0.05$, Kruskal-Wallis-Test) than the other two groups,

which did not differ from each other (group 2: 0.82 ± 0.14 mg, group 3: 0.55 ± 0.06 mg; $P > 0.05$, Kruskal-Wallis-Test) (Fig. 3). The same picture arose from the comparison of reward rates (the product of max. sugar crop and including abundance), where group 1 also had significantly lower mean value (3.99 ± 0.74 mg sugar; $P > 0.05$, Kruskal-Wallis-Test) than the other two groups, which did not differ from each other (group 1: 0.40 ± 0.05 mg, group 2: 1.72 ± 0.19 mg; $P > 0.05$, Kruskal-Wallis-Test). The number of flower visits per 30 min did not differ between the groups (group 1: 11.9 ± 2.6 , group 2: 14.4 ± 3.0 , group 3: 14.6 ± 9.6 ; $P = 0.867$; One Way ANOVA). An equal number of plant species was either mainly visited by Apoidea or non-Apoidea in all three groups, a minor number of species was equally often visited by Apoidea and non-Apoidea and only on a few species could we observe no visitors at all (App. 3). Hence, the group of plant species that have flowers with easily accessible nectar had the lowest mean max. sugar crop and reward rate. The groups of plant species with intermediately or complicatedly accessible nectar did not differ from each other in that respect. Visits of Apoidea and non-Apoidea were distributed equally in all three groups of floral functional shape.

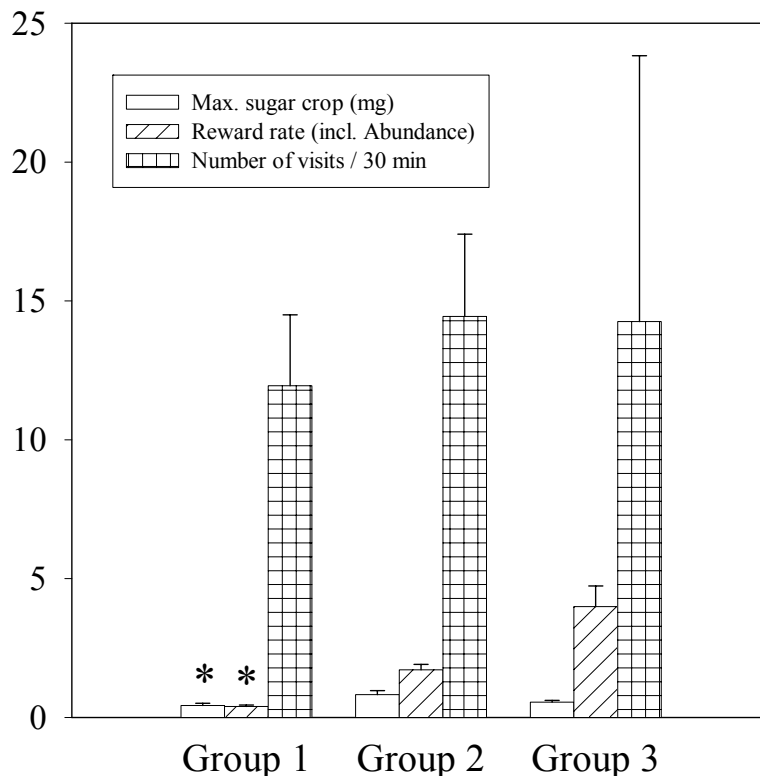


Fig. 3 The mean max. sugar crop (mg), mean reward rate (the product of max. sugar crop and abundance) and mean number of visitors per 30 min according to the three types of functional flower shapes. Plant species presenting flowers with easily accessible nectar (group 1, $N = 16$) offer a significantly lower max. sugar crop and reward rate ($P < 0.05$; Kruskal-Wallis Test, Dunn's Method) than species with hidden nectar, which do not differ from each other ($P > 0.05$, group 2, $N = 12$ and group 3, $N = 6$). Visitation rates were similar in all groups ($P > 0.05$).

After comparison of the percentages of different flower colours, achieved through the grouping of their colour spectra (Fig. 1), between the groups of plant species of different nectar accessibilities, we can also state that plant species with complicatedly accessible nectar presented a fewer number of different colours (Fig. 4). However, the number of species in this group was also much lower – between 1 and 3 per site – which probably explains the lower variability.

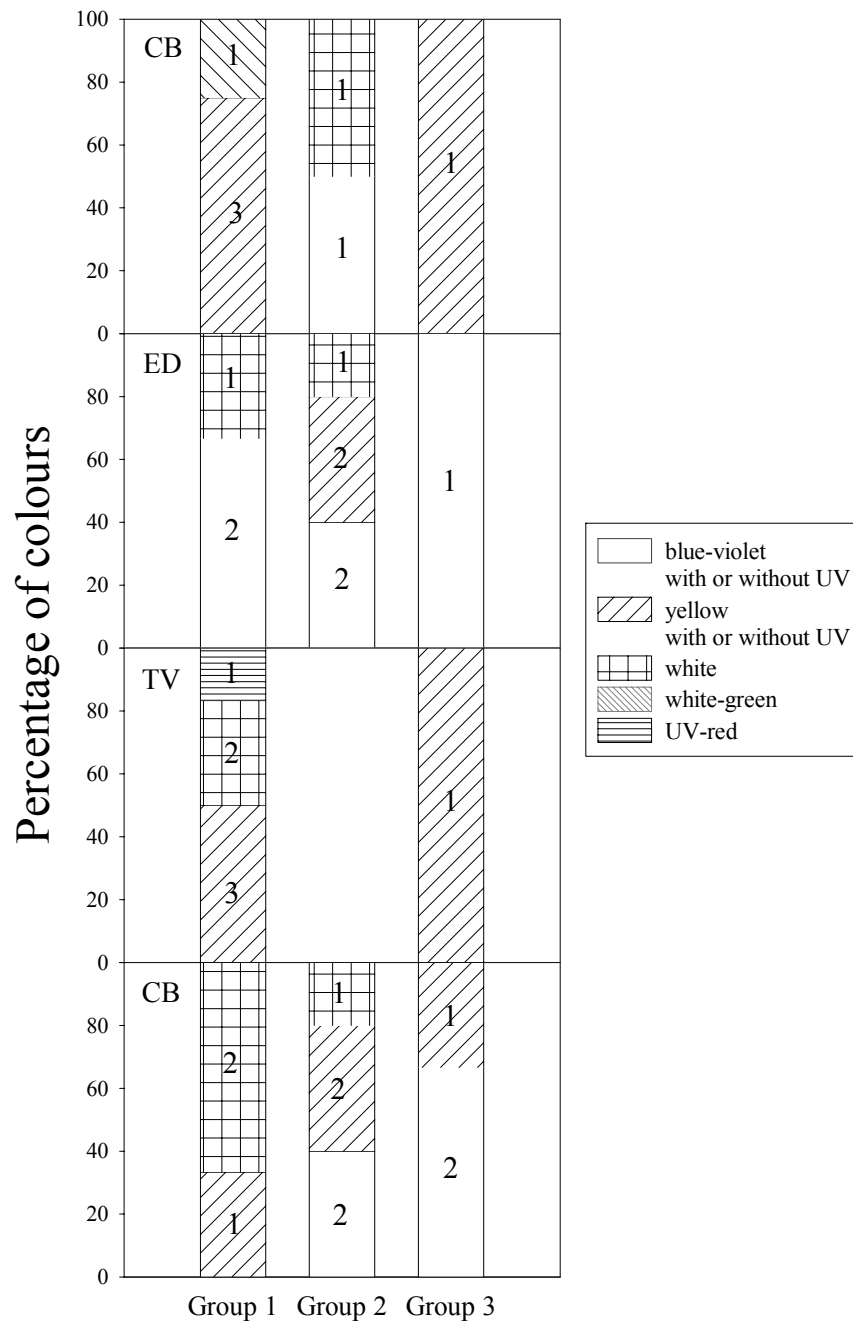


Fig. 4 The percentage of flower colours in the investigation sites, according to functional flower shape. Plant species are grouped according to their flowers' colour spectra as organised in Fig. 1. The occurrence of a higher number of colours in group 1 is due to lower sample sizes in group 2 and 3. Numbers in the bars depict the number of plant species.

Flower colour similarities and colour generalisation by bees

To assess colour similarity from the bee's perceptual point of view, we calculated the chromatic distances between the colour loci of all measured species' flowers in the honeybee colour space (Vorobyev *et al.* 2001). By comparing values in our analysis of mean chromatic distances, we answered the following questions for each investigation site: Did highly rewarding species or commonly visited species differ strongly in colour from other co-flowering species? What was the colour spread within the group of low rewarding species? Did low rewarding species differ strongly in colour from highly rewarding species? (Fig. 5A) The same comparisons were applied to the species, which we identified to be very commonly (> 25 % of the total number of visits/site; App. 3) and less commonly (< 25 %) visited (Fig. 5B). Colours of flowers of highly rewarding or very commonly visited species were widespread in the honeybee colour space (Fig. 2A, b). But their loci were often well distinguishable (Fig. 5A, B), i.e. the mean chromatic distance of highly rewarding to low rewarding species was often close to 2.3, the threshold of colour discrimination in honeybees (Vorobyev *et al.* 2001). Only 28.9 % of all investigated plant species in our sites displayed colours that were indistinguishable from at least one other species' flower colour. Therefore, we were interested if plant species with similar colours offered a similar reward to the pollinator, i.e. if colour generalisation by the pollinator is helpful for successful foraging. We compared median max. sugar crop and reward rate for species with chromatic distances of less than 2.3 RNL units (same colour), less than 3.5 and less than 5 RNL units, respectively, and between colours of all species' flowers. We detected a tendency of decreasing reward values in relation to increasing colour distance within plant species of one community (Tab. 1). Hence, exact colour discrimination can be more useful for the pollinator than broader colour generalisation.

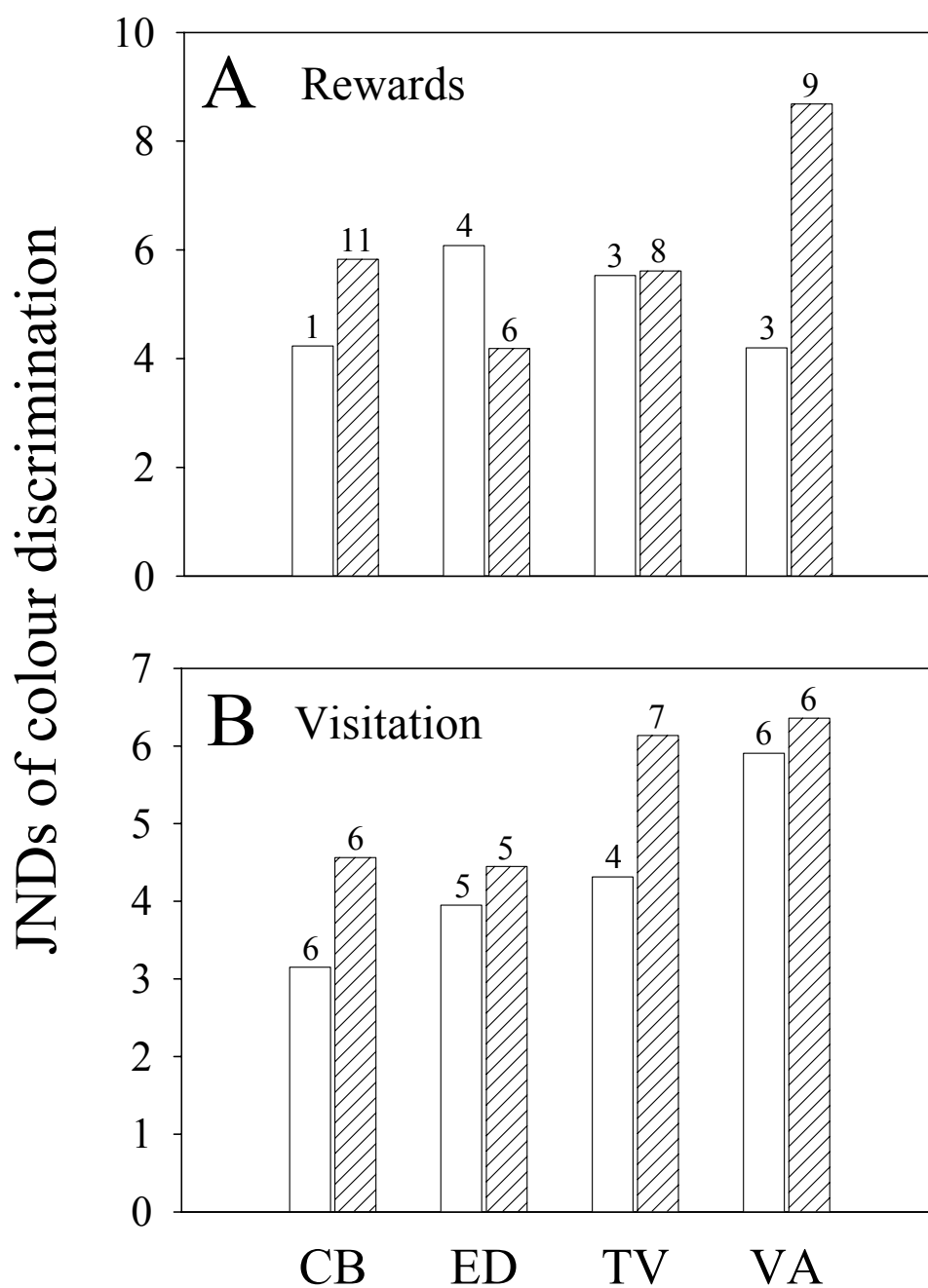


Fig. 5 Mean chromatic distance **A** within the group of species with low reward rates (open bars) and between the group of species with low reward rates and the group of species with high reward rates (hatched bars) and **B** within the group of species with low visitation rates (open bars) and between the group of species with high and the group of species with low visitation rates (hatched bars). One JND (just noticeable differences) corresponds to 2.3 RNL model units (Vorobyev *et al.* 2001); Numbers indicate the N of highly rewarding species (above open bars) and of low rewarding species (above hatched bars).

Tab. 1 Median max. sugar crop (mg) and reward rate (the product of max. sugar crop and floral abundance) of the flower species of the four investigation sites, categorised into groups of flowers with different chromatic distances (ΔS) in the honeybee colour space (Vorobyev *et al.* 2001).

Site	Chromatic distance	ΔS			
		< 2.3	< 3.5	< 5	All
CB	sugar crop	1,19	1,38	0,90	0,31
	reward rate	0,47	0,55	0,37	0,29
ED	sugar crop	0,47	0,14	0,12	0,14
	reward rate	0,40	0,29	0,25	0,16
TV	sugar crop	0,12	0,10	0,08	0,12
	reward rate	0,10	0,10	0,10	0,13
VA	sugar crop	0,07	0,08	0,08	0,39
	reward rate	0,70	0,39	0,35	1,22
All	sugar crop	0,29	0,12	0,10	0,23
	reward rate	0,44	0,34	0,30	0,22

Discussion

Analogue to former studies on the distribution of floral colours and sugar rewards in the bee's natural environment (Wertlen *et al.*, in prep.) the present study investigated the interrelation of floral colouration and foraging decisions of pollinators. We focused our measurements on reward and colour distributions in natural plant communities in four new sites with different pollinator compositions to compare the data with results from European plant-pollinator communities. We integrated observations of pollinator activities as well as determinations of floral abundances and functional flower shapes to obtain authentic values for reward properties as encountered by bee pollinators. Further, we applied the RNL Model of honeybee colour vision (Vorobyev *et al.* 2001) to quantify spectral data and to analyse colour differences and similarities of flowers of the occurring plant species according to the honeybee's perception. In our approach to understanding the interrelation between the determined reward and colour properties we focused on i) the analysis of flower colour distributions within highly rewarding bee pollinated plant species in relation to the colour distributions of less rewarding species to find out if differences are perceived by bees and ii) the analysis of reward properties of flowers with very similar or very different colours to determine whether reward distribution in the site is clustered which would allow the bees to select adaptive choice strategies based on different degrees of colour generalisation.

In our European studies we found that *very rare* species (presenting less than 1 % of the flowers of the total number of flowers in the site) often try to be as similar as possible in

colour to more common species. In Argentinean sites, we found only one *very rare* species (*Senecio pampeanus*, CB site). This species displayed a colour distinct from other flowers' colours. Further, only 38,1 % of the *rare* plants (presenting less than 5 % flowers) displayed colours similar to flowers of other plant species. In two sites, the similarly coloured flowers belonged to other rare species (*Acmella decumbens* and *Gaillardia megapotamica* (both CB site), *Nothoscordum* spec. and *Perezia multiflora* (both TV site); App. 1, Fig. 2B). *Galactia* spec. and *Cuphea glutinosa* (both ED site) formed a group of a rare and a common (10-25 %) species, respectively. And in the VA site a large cluster displaying similar blue-violet and whitish colours consisting of *Arjona patagonica*, *Polygala* spec., *Ranunculus pedicularis* and *Rorippa nasturtium-aquaticum* (all rare) and *Cerastium arvense* (common), *Junellia juniperina* (very common: > 25 %), *Loasa lateritia* and *Pratia repens* (both intermediate: 5 - 10 %) was found (App. 1, Fig. 2B). These results differed from the European results, where 46,2 % of the species with *very rare* flowers displayed indistinguishable colours to flowers of other more common plant species (App. 1). Thus, in Argentinean sites, rare species more seldom displayed similar colours to other species. These, in turn, were often also rare.

Our hypothesis for European sites that *very rare* plant species may have similar flowers to profit from pollinators, which are attracted through the more common co-flowering species presenting a similar colour (see also: Gumbert and Kunze 2001, Johnson *et al.* 2003a, 2003b, Benitez-Vieyra *et al.* 2006, for a review see Dafni 1984) seems to be invalid for Argentinean sites. Here, rare plant species seem to compete for pollinators by implementing the strategy of colour diversification, independently of the abundance of neighbours (Fig. 5A). We did not find strong specialisation patterns demonstrating the adaptation of plant species in terms of their functional flower shape and floral colouration, to the needs of particular pollinators. The number of species with more complicatedly accessible nectar was relatively low and they presented a great variety of colours (Fig. 4). We suggest that they developed to be as different as possible in colour from other co-flowering plants. The fact that they offer a higher reward (Fig. 3) is in line with suggestions by Waser (1983), who hypothesised that plant species that require a higher energy investment by pollinators to obtain the nectar (through their low abundance), have to be more rewarding, because bees will prefer to specialise in visiting such flowers only when they are more profitable. One can extend this hypothesis to include complicated access as a factor requiring additional energy investment, as it is described in various studies (Kugler 1970, Faegri and van der Pijl 1979). Contrarily to our European studies, this hypothesis is supported by our findings in the Argentinean sites. Another

difference to the European sites was that flower visits of Apoidea and non-Apoidea occurred to an equal amount in all three groups of plant species with different accessibilities of nectar.

We conclude that the composition of the pollinator community with a lower representation of bees – as compared to European sites – is a selective factor which leads to different adaptations in colour-reward distributions of plants in natural habitats.

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AAC *et al.* The vouchers of the determined plant species are deposited in the following museums: Museo Botánico de la Universidad Nacional de Córdoba (CORD), Instituto de Botánica Darwinion, San Isidro (SI) and Instituto de Botánica del Noroeste, Corrientes (CTES). Details on most these vouchers are given in the data base of the Missouri Botanical Garden (USA): <http://mobot.mobot.org/W3T/Search/vast.html>.