Flower colour within communities shifts from overdispersed to clustered along an alpine altitudinal gradient

Pedro Joaquim Bergamo[1*], Francismeire Jane Telles[2], Sarah E. J. Arnold[3], Vinícius Lourenço Garcia de Brito[4]

[1] Programa de Pós-Graduação em Ecologia, Instituto de Biologia, Universidade Estadual de Campinas, 13083-970, Campinas, SP, Brasil.

[2] Programa de Pós-Graduação em Ecologia e Conservação de Recursos Naturais, Universidade Federal de Uberlândia, campus Umuarama, bloco 2D, sala 26, 38400-902, Uberlândia, MG, Brasil.

[3] Natural Resources Institute, University of Greenwich, Chatham Maritime, ME4 4TB, UK.

[4] Instituto de Biologia, Universidade Federal de Uberlândia, campus Umuarama, 38400-902, Uberlândia, MG, Brasil.

We used floral colour within communities to understand the role of biotic and abiotic drivers. Patterns matched well-known shifts in pollinator fauna and UV-irradiance along altitudinal gradients.

Author Contributions

PJB, FJT and VLGB performed analyses. SEJA performed original analysis and processing of reflectance data. All authors conceived the study and contributed to the writing of the manuscript.

Summary

Altitudinal gradients are interesting models to test the effect of biotic and abiotic drivers of floral colour diversity, since an increase in UV irradiance, decrease of pollinator availability and shifts from bee- to fly-pollination in high relative to low altitudes are expected. We tested the effect of altitude and phylogeny, using several chromatic and achromatic colour properties, UV-reflectance and pollinators' discrimination capacity (*Apis mellifera*, *Bombus terrestris*, *Musca domestica* and *Eristalis tenax*), to understand the floral colour diversity in an alpine altitudinal gradient. All colour properties were weakly related to phylogeny. We found a shift from overdispersed floral colours and high chromatic contrast with the background (for bees) in the low altitude, to clustered floral colours (UV and green range for bees and flies) and clustered chromatic and achromatic properties in the high altitude. Different from flies, bees could discriminate floral colours in all altitudinal ranges. Low altitudes are likely to exhibit suitable conditions for more plant species, increasing competition for pollinators and floral colour divergence. Conversely, the increase in UV-irradiance in high altitudes may filter plants with specific floral UV-reflectance patterns. Overall, floral colour diversity suggests that both biotic (pollinator fauna) and abiotic (UV-irradiance) drivers shape floral communities, but their importance changes with altitude.

Key words

Introduction

Evolutionary history as well as biotic and abiotic factors shape current plant phenotypic diversity, leading to functional patterns within plant communities. Each pattern may be related to a specific driver (e.g. biotic factors such as plant-animal interactions) and the nature of the pattern (overdispersed – trait evenness among co-occurring species, clustered – trait similarity among co-

[Type here]

occurring species or random – no detectable pattern) may reveal how this driver acted on the assembly of the community (Webb et al. 2002, Kraft et al. 2015). Among such traits, floral colour was previously shown to structure plant communities, and thus, it is hypothesized to be subjected to the above-mentioned drivers (Gumbert et al. 1999; Briscoe Runquist et al. 2016; Shrestha et al. 2016). However, several studies have demonstrated that floral colour is an evolutionarily labile trait, and thus, often unrelated to the phylogenetic composition of plant community (McEwen and Vamosi, 2010; de Jager et al. 2011; Muchhala et al. 2014; Shrestha et al. 2014). This is in part due to local ecological pressures (*e.g.* competition) leading closely related species to diverge in floral colour through pollinator-mediated selection (Schemske and Bradshaw 1999). On the other hand, abiotic factors may also influence floral pigment production, making distantly related species under the same environmental conditions to exhibit a similar floral colour (Koski and Ashman 2016). Nevertheless, most community-level evidence is restricted to biotic drivers of floral colour diversity. Recently, some studies have incorporated a more complete framework to test the influence abiotic factors through vegetative traits, and of biotic factors through floral traits (e.g. Muchhala et al. 2014; Briscoe Runquist et al. 2016). However, since floral traits can be related to both biotic and abiotic factors (e.g. Koski and Ashman 2015), it is important to test the role of different drivers shaping floral colour diversity within communities.

Altitudinal gradients are interesting models to investigate abiotic and biotic drivers of floral colour diversity. Firstly, these gradients may present changes in plant composition over small spatial scales (Körner 2007). Moreover, an important abiotic factor, the UV-irradiation, increases with altitude (Körner 2007). The UV-irradiation imposes a selective pressure on flower colour, favouring plant defence through production of UV-absorbing flowers at high altitudes and resulting in co-occurring species with similar floral UV-reflectance patterns (Koski and Ashman 2015). In addition, biotic factors such as the pollinator fauna often varies with altitudinal gradients, with a well-known increase of dipterans and a decrease of hymenopterans with altitude (Arroyo et al. 1982; Totland 1993; Lázaro et al. 2008; Hoiss et al. 2012). Since the pollinator functional groups

[Type here]

may differ along the altitudinal gradient, as well as the preferred colours, we could expect a plant community and its floral colours to follow such changes in pollinator fauna (Renoult et al. 2015). The two above-mentioned mechanisms are not mutually exclusive, as demonstrated in *Argentina anserina,* a species presenting an altitudinal clinal variation in floral UV-reflectance in response to an increase in UV-irradiation and to a Hymenoptera-to-Diptera change in the flower visitor community (Koski and Ashman 2015). However, there is limited evidence if this species-level pattern also reflects community-level patterns of flower colour diversity.

Not only pollinator composition, but also overall pollinator abundance changes with altitude (Totland 1993; Hoiss et al. 2012). High altitudes impose restrictive conditions upon insect pollinators as well as on plants, with low pollinator availability increasing the role of plant-plant facilitative interactions (Tur et al. 2016). Thus, under this scenario, plants could be more restricted in the colour of flowers they produce, and considering facilitative interactions, floral colour similarity may enhance pollinator attractiveness for different plant species (Gumbert et al. 1999; McEwen and Vamosi 2010; Makino and Yokoyama 2015). Conversely, low altitudes provide more suitable conditions for many plant and pollinator taxa, and plant competition for pollinators is more likely to occur in these rich environments where there are more flowers relative to flower-visiting insects, leading to niche partitioning modulated by distinct floral colours (Muchhala et al. 2014; Kooi et al. 2016; Kantsa et al. 2017). An underlying assumption to infer competition for pollinators mediated by flower colour is that the pollinator fauna can discriminate among the colours present in a community. Such an assumption has rarely been tested at the community level, which may give insight on how flower colours are assembled (e.g. Chittka et al. 1997, but see Bischoff et al. 2013). Thus, to understand any possible biotic effects on the colour diversity of communities, we must consider the different pollinators' visual capacities.

In alpine systems, different pollinator groups are found, although most plant species are pollinated by bees and flies (Totland 1993; Lázaro et al. 2008). The visual system of bees is one of the most intensively studied systems, and several colour vision models have been developed on the

[Type here]

attempt to describe its visual processing capacity (Backhaus 1991; Chittka 1992; Vorobyev and Osorio 1998). In contrast to bees, fly colour vision is less understood. As an attempt to understand how the fly visual system works, Troje (1993) proposed a visual model based on behavioural responses of the blowfly *Lucilia* sp. In that study, he showed that blowflies do not discriminate continuous variations in colour hue, but rather place stimuli in spectral categories (UV, violet, blue and green) depending on the relative stimulation of two sets of opponent receptors. Consequently, while fly-pollinated flowers may only be treated as chromatically different when undergoing shifts across categories (hues), bee-pollinated flowers could be perceived as different by incremental variations within colour hues (Dyer and Chittka 2004; Dyer et al. 2008). However, it should be noted that despite its relevance, Troje's model has not being strictly tested for other fly species and the assumption that the mechanism by which blowfly visually operates might not be the same for every fly.

Considering the pollinator visual system and testing it against null models of colour composition has improved our capacity to predict flower colour diversity within communities (e.g. Gumbert et al. 1999; Muchhala et al. 2014; Shrestha et al. 2014; Makino & Yokoyama 2015; Kooi et al. 2016; Kantsa et al. 2017). A previous study considering the bumblebee (*Bombus terrestris*) and the blowfly (*Lucilia* sp.) visual systems investigated whether flower colour diversity varies along an alpine altitudinal gradient (Arnold et al. 2009). They found random trait patterns within communities for all altitudinal elevations in the bee vision, fly vision, and when testing flower colour independent of any visual system. In that study, Arnold et al. (2009) used categorical information in null models of pollinator-mediated selection of colour diversity, considering the findings of previous studies indicating that flower colours tend to cluster in perceptual categories in bee colour space (Chittka 1997). However, it is well-known that bees exhibit fine colour discrimination capacities that does not simply fall into categories (Dyer and Chittka 2004; Dyer et al. 2008). Floral colours grouped in the same category could have reduced the predictive capacity of colour niche partitioning. Indeed, besides the colour *per se* (hue), floral colour has several

[Type here]

perceptual properties including chromatic and achromatic parameters that influence pollinator foraging choices (Lunau et al. 1996; Dyer et al. 2008; Rohde et al. 2013; Brito et al. 2015; Telles & Rodríguez-Gironés 2015; Bergamo et al. 2016; Koethe et al. 2016). Adding visual perceptual information has the potential to demonstrate the existence of mechanisms by which plants partition the pollinator niche.

Here, we re-analysed the floral colour diversity of three co-flowering alpine plant communities, previously studied by Arnold et al. (2009). Considering that the flower colour diversity of communities can be a product of complex processes, we performed different sets of analyses including null models, the visual capacity of different pollinators, and phylogenetic comparative methods to evaluate whether the evolutionary history as well as biotic and/or abiotic factors had a structuring role on colour of flowers of the sampled communities. To test whether floral colour diversity within communities is predicted by biotic relevant traits, we analysed floral colours considering bee and fly colour vision systems. To test if such patterns are also predicted by abiotic relevant traits, we used a specific colour trait independent of visual system calculations (floral UV-reflectance), which was shown to be under both abiotic and biotic selective pressures (UV-irradiance, Koski and Ashman 2015). In contrast to the previous study, we used continuous colour variation. Such contrast may be more biologically relevant when analysing flower colours within communities because bees can discriminate between subtle colour differences (Dyer and Chittka 2004; Dyer et al. 2008). Specifically, we aimed to answer the following questions: (1) Does floral colour exhibit a phylogenetic signal, following local phylogenetic composition? (2) Are floral colours different among plant communities (e.g. distinct floral colours among communities)? (3) Are floral colours structured within plant communities (e.g. overdispersed or clustered patterns)? (4) Can pollinators discriminate floral colours within communities? In general, we expect a shift from overdispersed colours (i.e. widely spread across perceptual space in bee vision) to clustered colours (in UV-reflectance and fly vision) with altitude, reflecting the altitudinal gradient of UV-irradiance, pollinator fauna and changes in the pollinator availability.

[Type here]

Materials and Methods

Floral spectral reflectance and data manipulation

We used the reflectance spectra of 71 flowers belonging to 27 families (Online Resource Table S1) made online available at the FReD database (Arnold et al. 2010), and originally used in the work of Arnold et al. (2009). Details of collection methodology and original analysis are contained in Arnold et al. (2009) but in brief, the dataset originated from the Dovrefjell–Sunndalsfjella

National Park (formerly Dovrefjell National Park) in Norway were collected in 1992 along an elevational transect from 700 to 1600m asl. To evaluate the existence of differences in the colour of flowers related to the altitudinal gradient where plants were collected, together with the floral visitor assemblage, we followed the classification originally used by Arnold et al. (2009), determined as low (700m - 1000m), medium (1000m - 1300m) and high (1300m - 1600m) altitudes.

Pollinator species and colour spaces

The previous study used the bumblebee (*Bombus terrestris*) and a blowfly (*Lucilia* sp.) as models of pollinators. Here, we included the honeybee *Apis mellifera*, the muscoid fly *Musca domestica* and the hoverfly *Eristalis tenax* based on a previous study in a similar ecosystem in Norway, showing the importance of muscoid flies and hoverflies (in contrast with blowflies) as pollinators (Lázaro et al. 2008). Moreover, it is known that the two most common bee model species (*Apis mellifera* and *Bombus terrestris*) exhibit different colour discrimination abilities (Dyer et al. 2008). Since most studies investigating flower colour within communities use only one of these two bees as model, we included both to investigate if colour patterns change according to the bee species. To calculate whether the pollinator assemblage (biotic driver) was modulating the floral colour of plant species along the altitudinal gradient, we used the spectral sensitivity of *Bombus terrestris* (hereafter referred to as "bumblebee", Skorupski et al. 2007), *Apis mellifera*

[Type here]

(hereafter referred to as "honeybee", Peitsch et al. 1992), *Musca domestica* (hereafter referred to as "muscoid fly", Hardie 1986) and *Eristalis tenax* (hereafter referred to as "hoverfly", Lunau 2014). Apart from the honeybee, they are all representative pollinators from different plant communities sharing similar characteristics with the area sampled in southern Norway (Lázaro et al. 2008). Results from bumblebees and honeybees were ultimately similar, so we chose to present only the former in what follows (honeybee results can be found on Online Resource Tables S2, S3, S4 and S5).

We used the colour hexagon (Chittka 1992), and the receptor noise limited (Vorobyev and Osorio 1998) models for both honeybee and bumblebee to quantify and represent the colour of flowers as seen by these bee species, and the categorical colour vision model of Troje (1993) for the muscoid fly and the hoverfly. We decided to use the three contending colour vision models available for bees, because we are aware of the different capacities of predictions due to the models' premises (Telles and Rodríguez-Gironés 2015). In contrast to bees, the fly visual system is not fully understood for species other than *Drosophila* (Schnaitmann *et al.* 2013) and the blowfly *Lucilia*, for which a fly visual model was proposed (Troje 1993). Troje's model of fly vision was based on the capacity of wavelength discrimination of *Lucilia* sp. assuming the spectral sensitivity of *Musca domestica* (Troje 1993, Hardie & Kirschfeld 1983). According to this model, flies exhibit categorical colour vision, defined by the relative excitations of the two p-type (R7p-R8p) and y-type (R7y-R8y) receptors. The excitation values result in four possible colour categories (UV= p+ y+; Purple= p+ y−; Blue= p− y+; and Green= p− y−) (Lunau 2014). Stimuli falling within a category are indistinguishable (Troje 1993).

Using a normalized function of daylight illumination (D65, Wysecki and Stiles 1982), the reflectance of the background (green foliage, Chittka & Kevan 2005) and the spectral sensitivity of the bumblebee, honeybee, muscoid flies and hoverflies (Figs. S1-S3), we calculated the quantum catches of the different photoreceptor types, and plotted the loci of flowers according to the corresponding colour space. Despite the fact that Troje's model was based on behavioural responses

[Type here]

of *Lucilia*, and that the visual system of hoverflies and muscoid flies might differ between them and from the former, Troje's (1993) fly vision model predictions have been in agreement with some behavioural results considering different fly species (Jersáková *et al.* 2012, Moré *et al.* 2013). For the muscoid fly and hoverfly, we followed the calculation proposed by Troje (1993). Details of the models' calculations can be found in Supplementary text S1.

Chromatic and achromatic properties

For both bumblebees and honeybees, we calculated the relative spectral purity (SP), the colour contrast against the background (CCB) and the contrast produced in the green photoreceptor (GC) for each sampled flower. Colour contrast was determined as the perceptual distance between the stimulus and background, according to the assumptions of each bee colour vision model (Supplementary text S1). The relative spectral purity in the hexagon model was calculated as the distance of the flower colour locus from the centre of the hexagon relative to the distance of the maximal spectral purity locus from the centre considering the same flower locus (Lunau et al. 1996). Green contrast was calculated as the specific excitation of the green photoreceptor produced between flowers and background according to the colour hexagon model (Chittka 1992). Assuming a categorical visual system of flies (Troje 1993) we did not calculate achromatic and chromatic properties of flowers.

Does floral colour exhibit a phylogenetic signal?

We used the phylogeny assembled in Arnold et al. (2009) to assess phylogenetic relationships. In the previous study, there was no phylogenetic structure regarding elevation, and raw floral reflectance data was not related to phylogenetic relatedness (Arnold. et al. 2009). However, phylogeny could also constrain perceived floral colour and colour properties, e.g. if closely related plant species with shared pollinators exhibit similar colour properties. For colour differences, we calculated a perceptual distance matrix based on the outcome of vision models for

[Type here]

Bombus terrestris and *Apis mellifera*. Then, we assessed the phylogenetic signal as the correlation between the perceptual distance matrix and phylogenetic distances using a Mantel test following Shrestha et al. (2014). Since we had absolute values for each plant species for the achromatic and chromatic properties (SP, CCB and GC), we calculated phylogenetic signal with Blomberg's K (Blomberg et al. 2003). For this, we evaluated whether plant species presented colour properties different from what would have been expected under a Brownian motion of evolution model. When K > 1, closely related species are more similar on their colour properties than expected by Brownian motion, while K < 1 indicates that closely related species are less similar than expected by Brownian motion (Blomberg et al. 2003). We assessed the significance of the K statistic by calculating phylogenetic independent contrasts (PIC, Felsensetein 1985) for the achromatic and chromatic properties (SP, CCB and GC). For this, we compared the PIC of each colour property with a random distribution of 10,000 phylogenies. This distribution was generated by reshuffling the original species relationships into null phylogenies.

Are floral colours different among plant communities?

Because colour properties can differ among communities, we performed phylogenetic ANOVA to test for the existence of differences in the UV-reflectance and each achromatic and chromatic properties (SP, CCB and GC) for both bumblebee and honeybee among the elevations (Garland Jr et al. 1993). This analysis compares the values of colour properties among altitudinal ranges correcting for the phylogenetic dependence among species due to common ancestry. In this test, 1,000 new sets of colour properties were simulated based on the phylogenetic information and a Brownian motion evolution model. After each one of this calculation, simple ANOVA is performed using the simulated values and a null distribution of F is obtained against with the real F is tested considering a 95% confidence interval. Considering the categorical colour vision of flies (Troje 1993; Lunau 2014) we could not perform this analysis for the model species. Analyses were performed using the function *phylANOVA* from the R-package *phytools* (Revell 2012).

[Type here]

Are floral colours structured within plant communities?

We tested if colour diversity within communities supported the effect of biotic drivers (pollinator fauna) within each altitudinal range. This was tested using bee- and fly-subjective view of flower colours. To do so, we calculated the Euclidian pairwise distances between all species of each elevation and used the mean value as a descriptor of functional structure of each community (Webb et al. 2002). For the calculation of the mean pairwise distances (MPD), we used bees perceptual distances calculated as Euclidian distances for the CH and RN models, as well as Euclidian distances for the colour properties (SP, CCB and GC). Considering the categorical vision of flies (Troje 1993; Lunau 2014), we calculated perceptual distances as Manhattan distances for the muscoid fly and hoverfly. The mean pairwise distances (MPD) of each community was then compared with a null distribution of 10,000 random communities. The species pool to generate random communities was formed by all plant species sampled along the altitudinal gradient. This null distribution was generated using the independent-swap algorithm, which preserves the original species richness within the community (Gotelli 2000). MPD values higher than the null expectation indicate an overdispersed pattern, and thus, divergent colours and/or colour properties. On the other hand, MPD values lower than the null expectation indicate a clustered pattern, and thus, similar colours and/or colour properties within the community. These analyses were performed using the *ses.mpd* function of the R-package picante (Kembel et al. 2010).

We also tested whether colour diversity within communities were consistent with pressure from an abiotic driver (UV-irradiance) on floral colour within each altitudinal range. This was tested by using the floral UV-reflectance of each plant species without assuming any visual system. To do so, we first summed the reflectance in the ultraviolet region (300nm-400nm) of each sampled flower species. Then, we performed the same analysis as for colour and colour properties in bees and fly visual systems to test for the effect of the considered abiotic driver on floral colour diversity within altitudinal gradients. To ensure that UV-reflectance is the factor shaping flower colour

[Type here]

diversity, we also performed analyses using the summed reflectance in the blue (400nm-500nm), green (500nm-600nm) and red (600nm-700nm) regions of the spectral reflectance data. Significance was tested using a similar quantitative null model as in the previous analysis, with a similar interpretation. Supplementary figures S4-S11 show the observed MPD value in relation to the frequency of null MPD values for all colour traits.

Do pollinators perceive floral colours as different within communities?

We calculated pollinators' capacity for discriminating between floral colours belonging to the same community to determine the potential of pollinator niche partitioning through specialisation on distinct colours. We fixed thresholds of colour discriminability based on studies using behavioural experiments. For bumblebees and honeybees, we used a minimum distance of 0.09 hexagon units in the CH model (Dyer et al. 2008), and 0.27 JNDs in the RN model (Telles and Rodríguez-Gironés 2015). From the matrix of colour distances (calculated using the CH and RN models), we subtracted the discrimination value between two species from the same plant community by the set threshold according to the correspondent visual model. After this, we compared if these values were different from zero (and thus, distinguishable in the pollinator vision) using a one sample t-test (Bergamo et al. 2016). For the muscoid fly and hoverfly, we assumed that colours falling in different sections of Troje's model would be distinguishable (Troje 1993). Then, we performed a chi-test to assess if floral colours were evenly represented in the model sections for each community, i.e., the scenario of maximum possibility for pollinator niche partitioning through distinct colours.

Results

Does floral colour exhibit a phylogenetic signal?

Floral colours were weakly related to phylogenetic distances when considering the bumblebee vision model (Mantel test: CH - r = 0.12, p = 0.012; RN - r = 0.13, p = 0.010), did not

[Type here]

present any relationship in the muscoid fly vision model (Mantel test: r = 0.01, p = 0.467) and a weak relationship with the hoverfly vision model (Mantel test: r = 0.06, p = 0.026). For bumblebees, achromatic and chromatic properties (SP, CCB and GC) exhibited K < 1 (i.e. low phylogenetic signal under a Brownian motion evolution), with no relation to phylogeny (K = 0.21 - 0.28, p = 0.061 - 0.579, Table 1). UV-reflectance was not related to phylogeny (K = 0.25, p = 0.478, Table 1). Patterns were qualitatively similar for the honeybee (Online Resource Table S2).

Are floral colours different among plant communities?

We found no differences in floral UV-reflectance and perceptual colour properties in the outcome of visual models considering both bee species between altitudinal ranges (phylogenetic ANOVA: F = 0.01 - 1.98, p = 0.229 - 0.752, Table 2; honeybee data = Online Resource Table S3).

Are floral colours structured within plant communities?

Floral colour diversity within communities changed with altitudinal range. In the low elevation, we found an overdispersed pattern for floral colour as discriminated by bumblebees in the CH and RN models (CH: MPD = 0.38, p = 0.026; RN: MPD = 1.71, p = 0.033, Figs 1-2, Table 3). Floral colour discrimination showed a random pattern when calculated using the Troje's model for the muscoid fly and hoverfly, and for all other achromatic and chromatic properties (SP, CCB and GC; MPD = 0.14 − 1.71, p = 0.071 - 0.940, Figure 1D-F, Figs 1-2, Table 3). For the medium elevation, floral colours as discriminated by bumblebees, muscoid flies and hoverflies in all visual models and for all achromatic and chromatic properties exhibited a random structure (MPD = 0.14 − 1.71, p = 0.259 - 0.720, Figs 1-2, Table 3). In the high elevation, floral colours as discriminated by bumblebees, muscoid flies and hoverflies in all visual models exhibited a clustered pattern (MPD = 0.32 − 1.57; p = 0.006 − 0.020, Figs 1-2, Table 3). Spectral purity and green contrast also exhibited a clustered pattern (MPD = 0.10 - 0.12, p = 0.014 - 0.042, Table 3). Chromatic contras against the background exhibited a random pattern (MPD = 0.09 − 0.55, p = 0.074 - 0.107, Table 3).

[Type here]

Results were qualitatively similar for the honeybee at medium elevation, overdispersed in the low elevation, and clustered in the high elevation when considering CCB in the CH and RN models (Online Resource Table S4).

Floral UV-reflectance similarity among plant species within communities also changed with altitude. The low and medium elevations exhibited a random structure for UV-reflectance (MPD = 0.04, p = 0.111 and MPD = 0.05, p = 0.343, respectively, Fig. 2, Table 3). Conversely, the high elevation exhibited a clustered structure, meaning that species in this community were more similar in floral UV-reflectance than expected by chance (MPD = 0.03, p < 0.001, Fig. 2, Table 3). We found random structure for the blue and red colour regions in the three communities (MPD = 0.12 – 0.14, p = 0.102 – 0.891, Supporting Information Table S4) and a clustered structure for green-reflectance at the high elevation (MPD = 0.10, p = 0.029, Online Resource Table S4).

Do pollinators perceive distinct floral colours within communities?

For all communities, colour differences between flowers were above the discrimination criteria adopted for bumblebees (Table 4). Results were qualitatively similar for honeybees (Online Resource Table S5). On the other hand, for all communities, floral colours were not sufficiently divergent to be perceived as distinct in the muscoid and in the hoverfly visual system (Table 4).

Discussion

Floral colour diversity within communities changed with altitude, with a predominance of overdispersed patterns in the low elevation, followed by a shift to clustered patterns in the high elevation. Floral colours (together with spectral purity, colour contrast against the background and green contrast) as discriminated by bees and flies were weakly related to evolutionary relatedness, which is expected when considering a highly labile trait as flower colour (Rausher 2008). This result agrees with previous reports showing that flower colour diversity within communities are not constrained by shared ancestry (McEwen and Vamosi 2010; Shrestha et al. 2014). We also found no

[Type here]

difference in floral colours among communities, suggesting that the observed variation in colour diversity is shaped mainly within communities. This could be explained by the fact that these communities share plant species between them (Arnold et al. 2009). Change in conditions along altitudinal gradients may impose limits to the persistence of plant species with certain flower colours in high altitudes, rather than directly promoting distinctive floral colours among the communities (but see Gray et al. 2018). For the UV-reflectance, we did not find a reduction in the UV-reflective pattern of flowers in the high elevation, as initially predicted. However, the clustered UV-reflectance on this elevation suggests that UV-irradiance might filter species with similar floral UV-traits, considering the species pool, which is in accordance with the observed distinctive patterns only within communities.

The new analyses revealed floral colour patterns within communities unnoticed in the previous assessment of the same data in Arnold et al. (2009). We attribute the difference between studies to the use of continuous colour variation (instead of categorical), which probably increased the power of the analyses in detecting more subtle colour patterns within co-occurring plant species. Furthermore, continuous colour variation also allows to investigate how other parameters derived from the visual model calculations (e.g. spectral purity) are structured within communities. Such parameters were recently shown to vary among floral communities along elevational bands (Gray et al. 2018). Here, we extend it to variation within communities.

Floral colours were less similar than expected within the low elevation according to both bees' visual systems. This result agrees with the importance of bee pollination in low altitudes (Arroyo et al. 1982; Hoiss et al. 2012). Plants with distinctive floral colours are expected to be more easily differentiated by their visitors, which may promote floral constancy and increase conspecific pollen deposition (Chittka et al. 1999). Such a scenario is expected in communities with a high density/diversity of plants and pollinators, promoting pollinator partitioning based on distinct floral traits (Rathke 1983; Muchhala et al. 2010; Seifan et al. 2014). Therefore, pollinators' preferences and visual capacities can strongly reinforce the overdispersed colour pattern. On the other hand, we

[Type here]

found random patterns within the medium elevation. One explanation for this finding is that the mid-elevation community possesses intermediate biotic and abiotic conditions relative to the lower and higher altitudinal ranges (Lázaro et al. 2008). Consequently, abiotic and biotic filters would be diffused, leading to the absence of floral colour patterns within the community.

Floral colours were more similar among species than expected in the high elevation regardless of whether the floral colour was scored using the visual system of bees or flies. This may reflect selection of floral colours better adjusted to fly pollinators, following an increase in fly over bee pollination with altitude (Kevan 1972, Lázaro et al. 2008). Another possibility is an overall decrease in pollinator and plant densities in high altitudes (Tur et al. 2016). At low densities, plants sharing pollinators may benefit from facilitative interactions (Rathcke 1983; Lázaro et al. 2014; Tur et al. 2016; Wolowski et al. 2017), and species with similar floral colours may benefit from joint attraction of pollinators (Gumbert et al. 1999; McEwen and Vamosi 2010). The latter is more expected for a fly pollinator, since flies are likely to not discriminate between some of the floral colours in the high elevation and thus, could be predicted to visit multiple species with similar colours. Flies visual limitation could result in considerable deposition of heterospecific pollen among plants sharing fly pollinators. However, when flowers are in low density, this may be a more reliable strategy than being a unique, rare or hard to discriminate colour, consequently receiving few visits (Tur et al. 2016; Kantsa et al. 2017). Nevertheless, our results do not allow us to distinguish between the possible processes operating in the high altitudinal ranges. Detailed information on pollinator identity and plant reproductive success would be necessary to discriminate between the two possible strategies.

Floral UV-reflectance was also structured within communities in a similar fashion. A pattern that fits the prediction based on the increase of UV-irradiance commonly found in altitudinal gradients (Koski and Ashman 2016). Most of the flowers in the high community were UV-absorbent, with exception of *Viola biflora* and *Ranunculus acris*, both species occurring at the mid- and low-elevation communities. The presence of UV-absorbent colours can enhance plant fitness

[Type here]

through protection of flower structures against UV-irradiance (Koski and Ashman 2015). We also found a shift from random to clustered in the green reflectance region, which may be related to a predominance of yellow and white UV-absorbing colours in high altitudes (Kevan 1972). Indeed, several plant species found in our high community have shown these colour patterns (e.g. *Pedicularis oederi*, *Potentilla crantzii*, *Ranunculus acris*, etc.), which demonstrate how abiotic conditions can also shape the establishment of plants in high elevations through a constraint on floral colours. It also raises the possibility of a trade-off, in which abiotic and biotic factors may exert together selective forces, promoting the existence of different colours across the community. Other abiotic factors such as temperature, pH, illumination and background characteristics also influence flower colour (e.g. Bukovac et al. 2017). A next step would be to explore the role of these drivers on floral colour diversity within communities.

Not all achromatic and chromatic properties occurred with the same distribution pattern within communities when considering colours in the bees' visual systems. In the low elevation, only the chromatic contrast with the background was overdispersed considering the honeybee data calculated with visual models, indicating that this may be an important cue for bees when detecting the selected flower from other co-occurring species. However, spectral purity and green contrast were random within this community. These properties are considered important cues for long distance detection of flowers (Giurfa et al. 1996; Hempel de Ibarra et al. 2002; Vasas et al. 2017) and could act as effective cues to foragers from some distance. Conversely, all measured colour properties were clustered in the high community (SP, CCB, and GC). This is likely to be a by-product of the similarity between the colour of flowers belonging to this altitudinal range. These contrasting results among colour properties offer the possibility to evaluate the most likely mechanisms structuring the floral colours within communities.

We also found contrasting results produced by the different colour vision models. The CH and RN differed in their ability to predict the chromatic contrast against the background within the high community in the honeybee vision (clustered for CH and random for RN). This reinforces the

[Type here]

idea that the RN model is more conservative when predicting the limits of colour discrimination (Vorobyev and Osorio 1998). Even when considering the same vision model, results of the chromatic contrast against the background were different depending on the bee species (random for bumblebees in the CH and RN models and overdispersed/clustered for honeybees). Honeybees and bumblebees have distinct colour discrimination capacities, with the former exhibiting higher discrimination abilities than the latter (Dyer et al. 2008, Garcia et al. 2017). Thus, it is easier to find colour differences among plant species using the honeybee vision, leading to detection of more distinctive patterns than for bumblebees (Garcia et al. 2017). Our contrasting results indicate the importance of testing different vision models and the most representative pollinator species to better interpret floral colour diversity, as that found within our communities.

In summary, we found a shift from overdispersed to clustered floral colour patterns across low and high communities, which is likely to represent changes in pollinator density and abiotic conditions along this altitudinal gradient. Our results suggest that the effect and importance of these drivers change with altitude, leading to a unique floral colour composition on each community. The incorporation of quantitative variation in floral colour, different visual systems and relevant chromatic and achromatic properties for floral visitors, improved the capacity to predict floral colour patterns at the community level. In an altitudinal gradient from Nepal, pollinators and floral colours were similar between altitudinal ranges, reinforcing the resilience of these interactions to climate disruption (Shrestha et al. 2014). In our studied system, however, all communities showed a unique floral colour composition, similarly to the communities along an altitudinal gradient of the Rocky Mountains (Gray et al. 2018). Thus, the studied system in Norway may be more sensitive to the displacement of plants and pollinators, leading to the extinction of unique interactions and with unpredictable consequences for the plants (Valiente-Banuet et al. 2015). Since altitudinal gradients are models to predict the influence of climate change in disrupting plant-pollinator interactions (Hegland et al. 2009), a more complete framework is essential. Detailed assessments of the

[Type here]

assembly of floral colours is one step towards this objective, providing evidence of which biotic and abiotic drivers are acting on these communities.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

[Type here]

References

Arnold SEJ, Savolainen V, Chittka L (2009) Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators. Arthropod-Plant Interactions 3:27-43. https://doi.org/10.1007/s11829-009-9056-9

Arnold SEJ, Faruq S, Savolainen V, McOwan PW, Chittka L (2010) FReD: the floral reflectance database-a web portal for analysis of flower colour. PLoS One 5:e14287. htps://doi.org/10.1371/journal.pone.0014287

Arroyo MTK, Primack R, Armesto J (1982) Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. American Journal of Botany 69:82-97. htps://doi.org/10.2307/2442833

Backhaus W (1991) Color opponent coding in the visual system of the honeybee. Vision Research 31:1381-1397. https://doi.org/10.106/0042-6989(91)90059-E

Bergamo PJ, Rech AR, Brito VLG, Sazima M (2016) Flower colour and visitation rates of *Costus arabicus* support the "bee avoidance" hypothesis for red-reflecting hummingbird-pollinated flowers. Functional Ecology 30:710-720. https://doi.org/10.1111/1365-2435.12537

Bischoff M, Lord JM, Robertson AW, Dyer AG (2013) Hymenopteran pollinators as agents of selection on flower colour in the New Zealand mountains: salient chromatic signals enhance flower discrimination. New Zealand Journal of Botany 51:181-193. https://doi.org/10.1080/0028825X.2013.806933

Blomberg SP, Garland Jr T, Ives AR (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717-745. https://doi.org/10.1554/0014-3820(2003)057[0717:TFPSIC]2.0.CO;2

[Type here]

Briscoe Runquist R, Grossenbacher D, Porter S, Kay K, Smith J (2016) Pollinator-mediated assemblage processes in California wildflowers. Journal of Evolutionary Biology 29:1045-1058. https://doi.org/10.1111/jeb.12845

Brito VLG, Weynans K, Sazima M, Lunau K (2015) Trees as huge flowers and flowers as oversized floral guides: the role of floral color change and retention of old flowers in *Tibouchina pulchra*. Frontiers in Plant Science 6:362. https://doi.org/10.3389/fpls.2015.00362

Bukovac Z, Shrestha M, Garcia JE, Burd M, Dorin A, Dyer AG (2017) Why background colour matters to bees and flowers. Journal of Comparative Physiology A 203:369-380. https://doi.org/10.1007/s00359-017-1175-7

Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. Journal of Comparative Physiology A 170:533-543. https://doi.org/10.1007/BF00199331

Chittka L (1997) Bee color vision is optimal for coding flower color, but flower colors are not optimal for being coded—why? Israel Journal of Plant Sciences 45:115-127. https://doi.org/10.1080/07929978.1997.10676678

Chittka L, Gumbert A, Kunze J (1997) Foraging dynamics of bumble bees: correlates of movement within and between plant species. Behavioral Ecology 8:239-249. https://doi.org/10.1093/beheco/8.3.239

Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86:361-377. https://doi.org/10.1007/s001140050636

Chittka L, Kevan PG (2005) Flower colour as advertisement. In: Dafni A, Kevan PG, Husband BC (eds) Practical Pollination Biology. Environquest Ltd., Cambridge, Canada, pp 157-196.

[Type here]

de Jager ML, Dreyer LL, Ellis AG (2011) Do pollinators influence the assembly of flower colours within plant communities? Oecologia 166:543-553. https://doi.org/10.1007/s00442-010-1879-7

Dyer AG, Chittka L (2004) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. Journal of Comparative Physiology A 190:105-114. https://doi.org/10.1007/s00359-003-0475-2

Dyer AG, Spaethe J, Prack S (2008) Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. Journal of Comparative Physiology A 194:617-627. https://doi.org/10.1007/s00359-008-0335-1

Felsenstein J (1985) Phylogenies and the comparative method. American Naturalist 125:1-15. https://doi.org/10.1086/284325

Garland Jr T, Dickerman AW, Janis CM, Jones JA (1993) Phylogenetic analysis of covariance by computer simulation. Systematic Biology 42:265-292. https://doi.org/10.1093/sysbio/42.3.265

Garcia JE, Spaethe J, Dyer AG (2017) The path to colour discrimination is S-shaped: behavior determines the interpretation of colour models. Journal of Comparative Physiology A 203:983-997. https://doi.org/10.1007/s00359-017-1208-2

Gray M, Stansberry MJ, Lynn JS, Williams CF, White TF, Whitney KD (2018) Consistent shifts in pollinator-relevant floral coloration along Rocky Mountain elevation gradients. Journal of Ecology 00:1-15. https://doi.org/10.1111/1365-2745.12948

Giurfa M, Vorobyev M, Kevan P, Menzel R (1996) Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. Journal of Comparative Physiology A 178: 699-709. https://doi.org/10.1007/BF00227381

Gotelli NJ (2000) Null model analysis of species co-occurrence patterns. Ecology 81:2606-2621. https://doi.org/10.1890/0012-9658(2000)081[2606:NMAOSC]2.0.CO;2

[Type here]

Gumbert A, Kunze J, Chittka L (1999) Floral colour diversity in plant communities, bee colour space and a null model. Proceedings of the Royal Society B 266:1711-1716. http://doi.org/10.1098/rspb.1999.0836

Hardie RC, Kirschfeld K (1983) Ultraviolet sensitivity of fly photoreceptors R7 and R8: Evidence for a sensitising function. Biophysics and Structural Mechanics 9:171–180. https://doi.org/10.1007/BF00537814

Hegland SJ, Nielsen A, Lázaro A, Bjerknes AL, Totland Ø (2009) How does climate warming affect plant-pollinator interactions? Ecology Letters 12:184-195. https://doi.org/10.1111/j.1461-0248.2008.01269.x

Hempel de Ibarra N, Vorobyev M, Brandt R, Giurfa M (2000) Detection of bright and dim colours by honeybees. Journal of Experimental Biology 203:3289-3298.

Hempel de Ibarra N, Giurfa M, Vorobyev M (2002) Discrimination of coloured patterns by honeybees through chromatic and achromatic cues. Journal of Comparative Physiology A 188:503-512. https://doi.org/10.1007/s00359-002-0322-x

Hoiss B, Krauss J, Potts SG, Roberts S, Steffan-Dewenter I (2012) Altitude acts as an environmental filter on phylogenetic composition, traits and diversity in bee communities. Proceedings of the Royal Society B 279. https://doi:10.1098/rspb.2012.1581.

Jersáková J, Jürgens A, Šmilauer P, Johnson SD (2012) The evolution of floral mimicry: Identifying traits that visually attract pollinators. Functional Ecology 26:1381–1389. https://doi.org/10.1111/j.1365-2435.2012.02059.x

Kantsa A, Raguso RA, Dyer AG, Sgardelis SP, Olesen JM, Petanidou T (2017) Community-wide integration of floral colour and scent in a Mediterranean scrubland. Nature Ecology & Evolution 1:1502-1510. https://doi.org/10.1038/s41559-017-0298-0

[Type here]

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb
    CO (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics
    26:1463-1464. https://doi.org/10.1093/bioinformatics/btq166

Kevan PG (1972) Floral colors in the high arctic with reference to insect-flower relations and
    pollination. Canadian Journal of Botany 50:2289-2316. https://doi.org/10.1139/b72-298

Koethe S, Bossems J, Dyer AG, Lunau K (2016) Colour is more than hue: preferences for compiled
    colour traits in the stingless bees *Melipona mondury* and *M. quadrifasciata*. Journal of
    Comparative Physiology A 202:615-627. https://doi.org/10.1007/s00359-016-1115-y

Kooi CJ, Pen I, Staal M, Stavenga DG, Elzenga JT (2016) Competition for pollinators and intra-
    communal spectral dissimilarity of flowers. Plant Biology 18:56-62.
    https://doi.org/10.1111/plb.12328

Körner C (2007) The use of 'altitude' in ecological research. Trends in Ecology and Evolution 22
    569-574. https://doi.org/10.1016/j.tree.2007.09.006

Koski MH, Ashman TL (2015) An altitudinal cline in UV floral pattern corresponds with a
    behavioral change of a generalist pollinator assemblage. Ecology 96:3343-3353.
    https://doi.org/10.1890/15-0242.1

Koski MH, Ashman TL (2016) Reproductive character displacement and environmental filtering
    shape floral variation between sympatric sister taxa. Evolution 70:2616-2622.
    https://doi.org/10.1111/evo.13042

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM (2015) Community assembly,
    coexistence and the environmental filtering metaphor. Functional Ecology 29:592-599.
    https://doi.org/10.1111/1365-2435.12345

Lázaro A, Hegland SJ, Totland Ø (2008) The relationships between floral traits and specificity of
    pollination systems in three Scandinavian plant communities. Oecologia 157:249-257.
    https://doi.org/10.1007/s00442-008-1066-2

[Type here]

Lázaro A, Lundgren R, Totland Ø (2014) Experimental reduction of pollinator visitation modifies plant-plant interactions for pollination. Oikos 123:1037-1048. https://doi.org/10.1111/oik.01268

McEwen JR, Vamosi JC (2010) Floral colour versus phylogeny in structuring subalpine flowering communities. Proceedings of the Royal Society B 277. https://doi.org/10.1098/rspb.2010.0501

Moré M, Cocucci AA, Raguso RA (2013) The importance of oligosulfides in the attraction of fly pollinators to the brood-site deceptive species *Jaborosa rotacea* (Solanaceae). International Journal of Plant Sciences 174:1–15. https://doi.org/10.1086/670367

Lunau K (2014) Visual ecology of flies with particular reference to colour vision and colour preferences. Journal of Comparative Physiology 200:497-512. https://doi/org/10.1007/s00359-014-0895-1

Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. Journal of Comparative Physiology A 178:477-489. https://doi.org/10.1007/BF00190178

Makino TT, Yokoyama J (2015) Nonrandom composition of flower colors in a plant community: mutually different co-flowering natives and disturbance by aliens. PLoS One 10:e0143443. https://doi.org/10.1371/journal.pone.0143443

Muchhala N, Brown Z, Armbruster WS, Potts MD (2010) Competition drives specialization in pollination systems through costs to male fitness. The American Naturalist 176:732-743. https://doi.org/10.1086/657049

Muchhala N, Johnsen S, Smith SD (2014) Competition for hummingbird pollination shapes flower color variation among in Andean Solanaceae. Evolution 68:2275-2286. https://doi.org/10.1111/evo.12441

[Type here]

Peitsch D, Fietz A, Hertel H, Souza J, Ventura DF, Menzel R (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. Journal of Comparative Physiology A 170:23-40. https://doi.org/10.1007/BF00190398

Ratchke B (1983) Competition and facilitation among plants for pollinators. In: Real L (ed) Pollination Biology. Academic Press, New York, USA, pp 309-329

Rausher MD (2008) Evolutionary transitions in floral color. International Journal of Plant Sciences 169:7-21. https://doi.org/10.1086/523358

Renoult JP, Blüthgen N, Binkenstein J, Weiner CN, Werner M, Schaefer HM (2015) The relative importance of color signaling for plant generalization in pollination networks. Oikos 124:347-354. https://doi.org/10.1111/oik.01361

Revell LJ (2012) phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology Evolution 3:217-223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rohde K, Papiorek S, Lunau K (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. Journal of Comparative Physiology A 199:197-210. https://doi.org/10.1007/s00359-012-0783-5

Seifan M, Hoch EM, Hanoteaux S, Tielbörger K (2014) The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbor. Journal of Ecology 102:953-962. https://doi.org/10.1111/1365-2745.122256

Schemske DW, Bradshaw HD (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (Mimulus). Proceedings of the National Academy of Sciences 96:11910-11915. https://doi.org/10.1073/pnas.96.21.11910

Schnaitmann C, Garbers C, Wachtler T, Tanimoto H (2013) Color discrimination with broadband photoreceptors. Current Biology 23:2375-2383. https://doi.org/10/1016/j.cub.2013.10.037

[Type here]

Shrestha M, Dyer AG, Bhattarai P, Burd M (2014) Flower colour and phylogeny along an altitudinal gradient in the Himalayas of Nepal. Journal of Ecology 102:126-135. https://doi.org/10.1111/1365-2645.12185

Shrestha M, Lunau K, Dorin A, Schulze B, Bischoff M, Burd M, Dyer AG (2016) Floral colours in a world without birds and bees: the plants of Macquarie Island. Plant Biology 18:842-850. https://doi.org/10.1111/plb/12456

Skorupski P, Döring TF, Chittka L (2007) Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. Journal of Comparative Physiology A 193:485-494. https://doi.org/10.1007/s00359-006-0206-6

Telles FJ, Rodríguez-Gironés MA (2015) Insect vision models under scrutiny: what bumblebees (*Bombus terrestris terrestris* L.) can still tell us. The Science of Nature 102:1-13. https://doi.org/10.1007/s00114-014-1256-1

Totland Ø (1993) Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. Canadian Journal of Botany 71:1072-1079. https://doi.org/10.1139/b93-124

Troje N (1993) Spectral categories in the learning behaviour of blowflies. Zeitschrift für Naturforschung C 48:96-104. https://doi.org/10.1515/znc-1993-1-218

Tur C, Sáez A, Traveset A, Aizen MA (2016) Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. Ecology Letters 19:576-586. https://doi.org/10.1111/ele.12594

Valiente-Banuet A, Aizen MA, Alcántara JM, Arroyo J, Cocucci A, Galetti M, García MB, García D, Gómez JM, Jordano P, Medel R, Navarro L, Obeso JR, Oviedo R, Ramírez N, Rey PJ, Traveset A, Verdú M, Zamora R (2015) Beyond species loss: the extinction of ecological interactions in a changing world. Functional Ecology 29:299-307. https://doi.org/10.1111/1365-2435.12356

[Type here]

Vasas V, Hanley D, Kevan PG, Chittka L (2017) Multispectral images of flowers reveal the adaptive significance of using long-wavelength-sensitive receptors for edge detection in bees. Journal of Comparative Physiology A 203:301-311. https://doi.org/10.1007/s00359-017-1156-x

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society B 265:351-358. https://doi.org/10.1098/rspb.1998.0302

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475-505. https://doi.org/10.1146/annurev.ecolsys.33.010802.150448

Wolowski M, Carvalheiro LG, Freitas L (2017) Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. Journal of Ecology 105:332-344. https://doi.org/10.1111/1365.2745.12684

Wyszecki G, Stiles WS (1982) Color science. Wiley, New York

[Type here]

Table 1. Phylogenetic signal (calculated with the K statistic, Blomberg *et al.*, 2003) of floral UV-reflectance and colour perceptual properties of species in the altitudinal gradient of Dovrefjell–Sunndalsfjella National Park, Norway. CH = Colour hexagon model for *Bombus terrestris* (Chittka 1992), RN = Receptor noise-limited model for *Bombus terrestris* (Vorobyev & Osorio 1998). Significance was tested using phylogenetic independent contrasts (Felsenstein 1981)

Floral colour trait	K	p
UV-reflectance	0.25	0.478
Green contrast (CH)	0.28	0.061
Spectral purity (CH)	0.21	0.579
Background contrast (CH)	0.25	0.201
Background contrast (RN)	0.24	0.221

[Type here]

Table 2. Variation of floral colour traits among communities from the altitudinal gradient in the Dovrefjell–Sunndalsfjella National Park, Norway. CH = Colour hexagon model (Chittka 1992), RN = Receptor noise-limited model (Vorobyev & Osorio 1998). All models used *Bombus terrestris* visual system

Floral colour trait	*F*	*P*
UV-reflectance	1.70	0.262
Green contrast (CH)	1.98	0.229
Spectral purity (CH)	0.38	0.752
Background contrast (CH)	1.54	0.324
Background contrast (RN)	1.08	0.449

[Type here]

Table 3. Floral colour diversity of three sampled communities in Dovrefjell–Sunndalsfjella National Park, Norway. Communities were divided in altitudinal gradients: low (700-1000m), medium or mid-elevation (1000-1300m) and high (1300-1600m). MPDobs = Mean pairwise distance of the observed community. MPDnull = Mean pair wise distance of the 10,000 null random assembled communities for the factor evaluated (values are mean ± SD). CH = Colour hexagon model for *Bombus terrestris* (Chittka 1992), RN = Receptor noise-limited model for *Bombus terrestris* (Vorobyev & Osorio 1998). Troje refers to a fly visual model applied for the muscoid fly *Musca domestica* and the hoverfly *Eristalis tenax* (Troje 1993)

Floral colour trait	Community	MPDobs	MPDnull	*P*
UV-reflectance	Low	0.04	0.04 ± 0.003	0.111
	Medium	0.05	0.05 ± 0.005	0.343
	High	0.03	0.05 ± 0.008	0.009
Colour discriminated by muscoid flies (Troje)	Low	0.65	0.63 ± 0.01	0.940
	Medium	0.69	0.67 ± 0.02	0.789
	High	0.59	0.68 ± 0.04	0.008
Colour discriminated by hoverflies (Troje)	Low	0.64	0.63 ± 0.01	0.782
	Medium	0.67	0.66 ± 0.02	0.720
	High	0.56	0.65 ± 0.05	0.020
Colour discriminated by bees (CH)	Low	0.38	0.36 ± 0.01	0.026

[Type here]

	Medium	0.39	0.39 ± 0.02	0.473
	High	0.35	0.42 ± 0.03	0.006
Colour discriminated by bees (RN)	Low	1.71	1.62 ± 0.05	0.033
	Medium	1.71	1.72 ± 0.09	0.431
	High	1.57	1.86 ± 0.13	0.008
Green contrast (CH)	Low	0.16	0.16 ± 0.01	0.240
	Medium	0.17	0.16 ± 0.01	0.259
	High	0.10	0.15 ± 0.02	0.014
Spectral purity (CH)	Low	0.25	0.18 ± 0.01	0.071
	Medium	0.20	0.19 ± 0.02	0.464
	High	0.12	0.16 ± 0.02	0.042
Background contrast (CH)	Low	0.14	0.13 ± 0.01	0.161
	Medium	0.14	0.14 ± 0.01	0.540
	High	0.09	0.13 ± 0.02	0.074
Background contrast (RN)	Low	0.76	0.73 ± 0.03	0.200
	Medium	0.78	0.76 ± 0.06	0.586
	High	0.55	0.71 ± 0.12	0.107

[Type here]

Table 4. Pollinator capacity for discrimination of floral colours from the same altitude in the Dovrefjell–Sunndalsfjella National Park, Norway. Colour distances in the colour hexagon (CH) and receptor noise-limited (RN) model were calculated for *Bombus terrestris*. Significant values indicate that colour distances were above the discrimination criteria (t test, $p < 0.05$). For flies, we tested if colours were overrepresented in one of the four categories perceived by muscoid flies (*Musca domestica*) and hoverflies (*Eristalis tenax*). Significant values indicate overrepresentation and thus, no discrimination (chi test, $p < 0.05$). Distances indicate average colour distance above the discrimination criteria, in hexagon units for CH and JNDs for RN

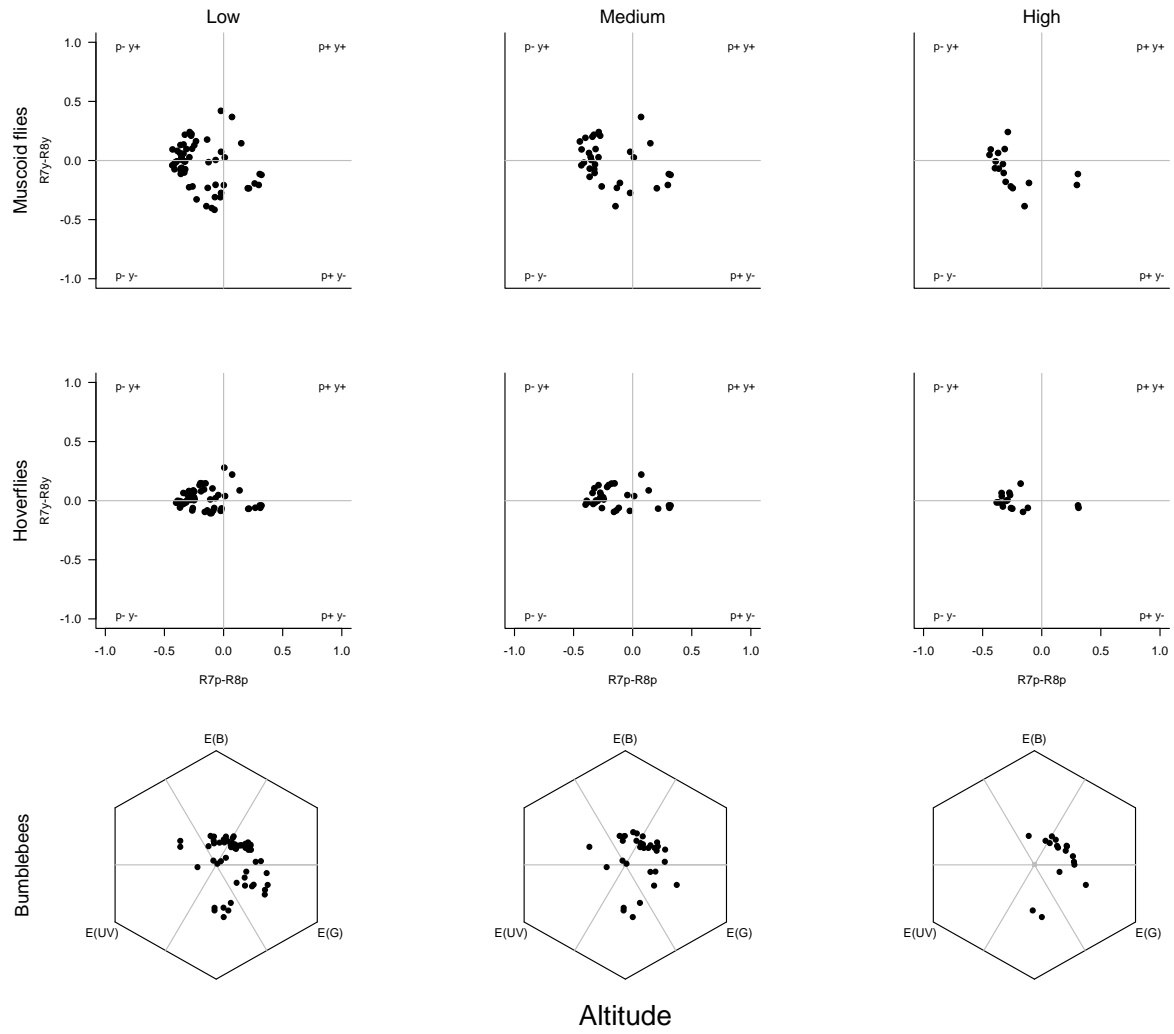| Pollinator	Community	Distances	Statistic	p
Bees (CH)	Low	0.38	59.56	<0.001
	Medium	0.39	31.69	<0.001
	High	0.35	13.01	<0.001
Bees (RN)	Low	1.71	27.94	<0.001
	Medium	1.71	6.15	<0.001
	High	1.57	14.01	<0.001
Muscoid flies	Low	-	26.00	<0.001
	Medium	-	10.25	<0.050
	High	-	11.47	<0.010
Hoverflies	Low	-	26.28	<0.001
	Medium	-	10.75	<0.050
	High	-	11.47	<0.010

[Type here]

Figure legends

Figure 1.

Floral colours of the plant species within each elevational community in the Dovrefjell–Sunndalsfjella National Park, Norway. Categorical model for *Musca domestica* (top row), *Eristalis tenax* vision (middle row) and hexagon model for *Bombus terrestris* vision (bottom row. The black points represent loci for each flower for the species occurring in the low, medium and high elevation communities. In the categorical model, locus fall in one of the four categories proposed for colour vision in flies: UV = p+y+; Purple = p+y-; Blue = p-y+ and green = p-y-. In the hexagon model, the grey point represents achromatic centre. The excitation of ultraviolet - E(UV), blue E(B) and green E(G) photoreceptor types are indicated in its respective region in the hexagon boards.

Figure 2.

Floral colour diversity of the altitudinal gradient in the Dovrefjell–Sunndalsfjella National Park, Norway. Floral colour diversity structure was evaluated with a regional species pool comprising all sampled communities (low = 700-1000m; medium = 1000-1300m; high = 1300-1600m). Grey triangle = Mean pairwise distance (MPD) observed for each community. Empty circle = mean MPD of 10,000 null assembled communities with independent swap algorithm (Gotelli 2000), with bars representing 95% confidence interval. a) Mean perceptual distance in the fly visual model using the hoverly *Eristalis tenax* (Troje 1993), b) mean perceptual distance in the colour hexagon model (CH) for *Bombus terrestris* (Chittka 1992) and c) Mean distance in UV-reflectance. Letter "C" represents a clustered structure and letter "O" represents overdispersed structure at $p < 0.05$ level.
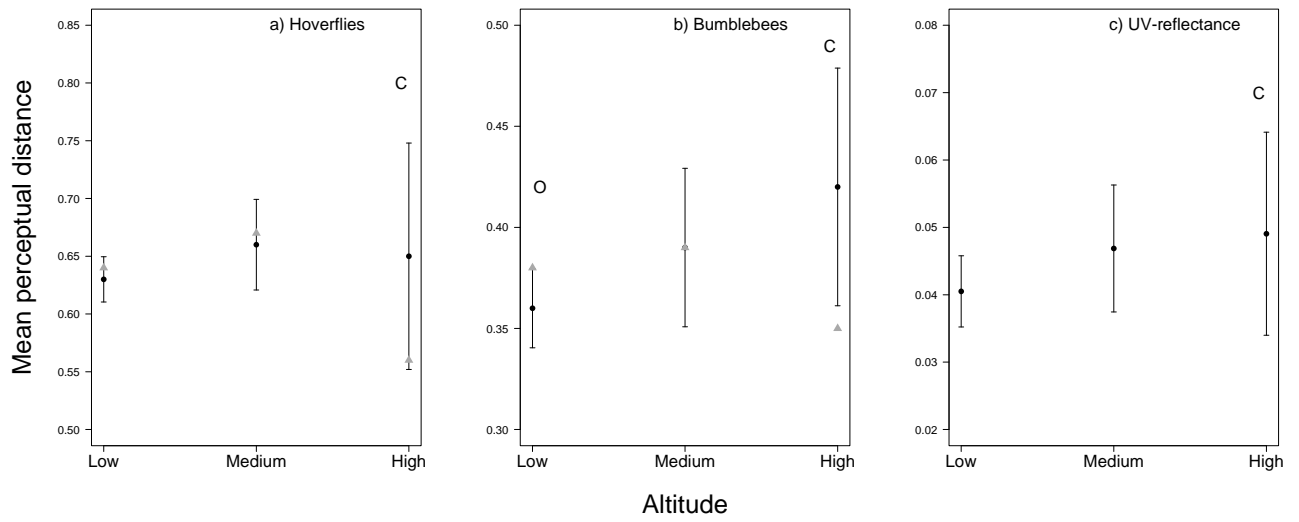
[Type here]

Figure 1.

Figure 2.