Received: 30 September 2017 | Accepted: 5 February 2018

DOI: 10.1111/1365-2745.12948

RESEARCH ARTICLE

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

Consistent shifts in pollinator-relevant floral coloration along Rocky Mountain elevation gradients

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Funding information
Division of Environmental Biology, Grant/Award Number: 1257965

Handling Editor: Nicole Rafferty

Abstract

1. Floral colour is a fundamental signal that shapes plant–pollinator interactions. Despite theoretical reasons why floral colours might shift in representation along biotic and abiotic gradients, few studies have examined community-level shifts in colour, and even fewer significant patterns have been detected.
2. We examined floral colour on six replicated transects spanning 1,300 m in the Rocky Mountains of Colorado, USA. Along these transects, there is a hypothesized shift from bee-dominated to fly-dominated pollination with increasing elevation. The reflectance of flowers of 110 forb and shrub species was measured using a spectrophotometer, and was used to estimate three components of colour (hue, saturation and brightness) in relevant pollinator visual spaces. Percent cover data were collected from 67 sites and used to obtain community-weighted mean (cwm) estimates of floral colour.
3. We found strong patterns of elevational change in floral colour. $\text{Reflectance}_{\text{cwm}}$ of shorter wavelengths (UVB through human blue, 300–500 nm) generally decreased linearly with elevation, while $\text{reflectance}_{\text{cwm}}$ of longer wavelengths (human green through red, 500–700 nm) showed hump-shaped patterns with highest reflectance at intermediate elevations. With respect to pollinators, $\text{saturation}_{\text{cwm}}$ increased significantly with elevation in both bee and fly visual spaces, while brightness $\text{contrast}_{\text{cwm}}$ showed a hump-shaped pattern in bee space and a decline with elevation in fly visual space. For hue, cover of species perceived as bee-blue declined with elevation, while cover of bee-UV-green species showed a hump-shaped pattern. In comparison, we detected no elevational shifts in floral hues as perceived by flies.
4. *Synthesis.* Hue patterns are consistent with the hypothesis that bee pollinators have shaped the geography of floral colour. The roles of fly pollinators and of abiotic drivers are more difficult to infer, although the drop in floral brightness at high elevations is consistent with predictions that low temperatures and more intense ultraviolet radiation should favour increased pigment concentrations there. Our results indicate that floral colour can be dynamic yet predictable across the landscape, a pattern that provides opportunities to tease apart the ecological and evolutionary drivers of this important plant trait.

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KEYWORDS
bee vision, ecological filtering, fly vision, natural selection, plant–pollinator interactions, reflectance spectra, signalling, ultraviolet

1 | INTRODUCTION

Floral colour mediates plant–pollinator interactions (Reverte, Retana, Gomez, & Bosch, 2016), influencing fitness of both parties (Schaefer, Schaefer, & Levey, 2004; Waser, 1983). Despite over 150 years of interest, we are still at the beginning of quantitatively describing geographic variation in floral colour and in understanding the ecological and evolutionary drivers of that variation (Dalrymple et al., 2015). Studies have generally moved from descriptions of the geography of floral colour in exclusively human terms (e.g. a series of papers from the 1870s–1890s by Victorian naturalists, see Anderson, Lovin, Richter, & Lacey, 2013; Eidesen, Little, Muller, Dickinson, & Lord, 2017; Kevan, 1972; Weevers, 1952) to mapping quantitatively modelled colour as variously perceived by bee, fly, bird and lepidopteran pollinators (e.g. Arnold, Savolainen, & Chittka, 2009; Dalrymple et al., 2015; Shrestha, Dyer, Bhattarai, & Burd, 2014; Shrestha et al., 2016). The latter approaches take advantage of relatively well-developed visual models ("psychophysical colour spaces") that incorporate information on an organism's visual physiology, such as photoreceptor sensitivities and abundance, and opponent mechanisms that describe how signals from different photoreceptors are compared and processed (e.g. Chittka, 1992 for bees; Troje, 1993 for flies; reviewed in Renoult, Kelber, & Schaefer, 2017).

The subjective appearance of colours is commonly described by three (non-independent) components: hue, saturation and brightness (Cronin, Johnsen, Marshall, & Warrant, 2014), although brightness is technically an achromatic stimulus, as it can be perceived without a colour vision system (Lunau, 2014). Hue describes the dominant wavelength of the received light and corresponds to the lay usage of "colour," e.g. red or blue; saturation measures the perceived spectral purity (a low saturation value means that other wavelengths "wash out" the dominant wavelength); and brightness (also known as luminance) measures the perceived intensity of the signal. All three of these colour components have been shown to be relevant to choices and foraging decisions of insect pollinators. For example, bees prefer certain hues including bee-blue and bee-ultraviolet (UV)-blue (Menzel, 1967 in Giurfa, Nunez, Chittka, & Menzel, 1995; Shrestha et al., 2016), prefer more saturated colours (Lunau, 1990; Lunau, Wacht, & Chittka, 1996; Rohde, Papiorek, & Lunau, 2013), and locate flowers from a distance based on brightness (Dyer, Spaethe, & Prack, 2008; Spaethe, Tautz, & Chittka, 2001).

Geographic variation in flower colours is hypothesized to result from a suite of abiotic and biotic drivers. Hypothesized biotic drivers include pollinators, herbivores and pathogens. Evidence consistent with the idea that pollinators can shape the geography of floral colour takes several forms. For example, plant assemblages served by fly pollinators seem to have unique sets of floral colours that are typically small subsets of colours found in plant assemblages associated with a wider diversity of pollinator groups (Shrestha et al., 2016). In floras dominated by bee pollinators, evidence suggests that floral pigments have become adapted to hymenopteran visual sensitivities over evolutionary time, with steep changes in reflectance in areas of the spectrum where hymenopterans have maximal discrimination (Bischoff, Lord, Robertson, & Dyer, 2013; Chittka & Menzel, 1992; Dyer et al., 2012; Shrestha, Dyer, Boyd-Gerny, Wong, & Burd, 2013). Geographic gradients in herbivory or pathogen attack could also drive floral colour variation, given strong links between plant defensive secondary chemistry and pigment chemistry (Frey, 2004; Irwin, Strauss, Storz, Emerson, & Guibert, 2003; Simms & Bucher, 1996; Strauss & Whittall, 2006). For example, it has long been proposed that herbivore pressure varies with altitude and latitude (Dobzhansky, 1950; Garibaldi, Kitzberger, & Chaneton, 2011; Scheidel, Rohl, & Bruelheide, 2003; Schemske, Mittelbach, Cornell, Sobel, & Roy, 2009; but see Moles, Bonser, Poore, Wallis, & Foley, 2011), and such variation could drive the evolution of defensive secondary compounds (in vegetative tissue and/or floral tissue) and associated floral pigments (Arnold et al., 2009; Berardi, Fields, Abbate, & Taylor, 2016).

Among hypothesized abiotic drivers of geographic variation in flower colour, temperature and UV radiation stand out as they vary strongly in space. For example, mean annual air temperature drops 1°C with every 140 m increase in elevation in the Rocky Mountains of Colorado (Pepin & Losleben, 2002), and in mountainous areas UV-B dosages can increase 1%–2% with every 100 m increase in elevation (Berardi et al., 2016; Llorens et al., 2015). Because floral pigments including anthocyanins and other flavonoids are considered protective against these stressors, increased pigmentation is expected to be adaptive under both low temperature and high-UV conditions and is thus expected to increase with elevation (reviewed in Berardi et al., 2016; Llorens et al., 2015). Increased floral pigmentation has also been linked to improved resistance to drought stress (Schemske & Bierzychudek, 2001; Strauss & Whittall, 2006; Warren & Mackenzie, 2001) and may thus vary predictably along moisture gradients.

Despite altitudinal variation in all of the above-described factors, there is remarkably little empirical evidence supporting community-level shifts in pollinator-relevant floral colour with elevation. Arnold et al. (2009) examined an alpine elevation gradient spanning 900 m in Norway, grouping 74 plant species into three elevation bands. They found no significant changes in floral hues in bee, fly or human visual spaces, nor did they detect elevation patterns in the raw spectra. Shrestha et al. (2014) examined

TABLE 1 Predictions for community-level shifts in floral colour with elevation for our study area in the Colorado Rocky Mountains, USA. Underlying assumptions: (1) Bees decrease (and flies increase) in pollination importance with increasing elevation (see Section 1). (2) Abiotic drivers are expected to have their most direct effects on pigment concentrations along elevation gradients (see Section 1), with only indirect effects on pollinator signalling. But, because relationships between pigment concentrations and hue and saturation within pollinator visual spaces are not simple to infer (Renoult et al., 2017), we make brightness predictions only for abiotic drivers. Because pigments absorb light, pigment concentration and brightness should be negatively correlated

Driver Colour component	Predicted change with ↑ elevation	Rationale
Bees		
Bee hue	Bee-blue spp. ↓ Bee-UV-blue spp. ↓	Bees' innate preferences (Giurfa et al., 1995)
Bee saturation	↓	Bees' innate preferences (Lunau, 1990; Lunau et al., 1996; Rohde et al., 2013)
Bee brightness	No prediction	While bees use brightness (specifically, green receptor contrast) to detect flowers at longer distances (Dyer et al., 2008; Spaethe et al., 2001), their reliance on chromatic features of floral signals at short distances (Giurfa, Vorobyev, Brandt, Posner, & Menzel, 1997), plus a typically negative correlation between saturation and brightness in pigment-based colours (Johnsen, 2012), make it unclear whether bees would select for ↓or ↑ brightness
Flies		
Fly hue	Fly-yellow spp. ↑	Predominance of fly-yellow species in fly-dominated floras (e.g. Shrestha et al., 2016)
Fly saturation	No prediction	Lack of behavioural studies of fly preferences for saturation
Fly brightness	↑	Flies' innate preferences (e.g. in the flower-visiting blowfly *Lucilia* sp. (Troje, 1993)
UV + temperature		
Bee brightness	↓	Both ↑ UV and ↓ temperature favour ↑ pigment concentration with elevation (Berardi et al., 2016; Llorens et al., 2015)
Fly brightness	↓	
Soil moisture		
Bee brightness	↑	Soil moisture ↑ with elevation in the Rocky Mountain Biological Laboratory area (e.g. Dunne, Harte, & Taylor, 2003); ↓ drought stress should favour ↓ pigment concentrations (Schemske & Bierzychudek, 2001; Strauss & Whittall, 2006; Warren & Mackenzie, 2001)
Fly brightness	↑	

hues in bee visual space for 107 species occurring in two elevation bands in the Himalayas, but did not test the relationship between hue prevalence and elevation. To our knowledge, all other studies recording flower colours along elevation gradients report only human-subjective judgments of hue (and ignore saturation and brightness), and thus are difficult to interpret in terms of relevance to pollinators.

Here, we examine how community-level floral colour shifts with elevation in open meadow habitats spanning 1,300 m in the Colorado Rocky Mountains (USA). As in many other mountain regions, there is a hypothesized shift from bee-dominated to fly-dominated pollination with increasing elevation (Kearns, 1992; Kearns & Inouye, 1994; Moldenke & Lincoln, 1979). We thus examine colour shifts in these two pollinator-relevant visual spaces as well as in a visual system-independent spectral analysis. We summarize our predictions for elevation-floral colour relationships in Table 1. We note that fly colour perception and colour preferences are less well understood than those of bees (De Ibarra, Vorobyev, & Menzel, 2014; Lunau, 2014), and thus our predictions based on fly preferences (Table 1) are more tentative. An alternative (and more conservative) prediction would simply be that flower colours at high elevations do not retain the bee-favoured characteristics from lower elevations.

Our study includes several novel approaches to the study of flower colour along elevation gradients. In addition to hue, we examine how other important components of colour (saturation, brightness) change with elevation. We treat elevation as a continuous variable rather than comparing a small number of arbitrary elevation zones, and we achieve replication by examining six independent valley-peak systems. Because multiple and potentially conflicting drivers (see Table 1) may combine to generate nonlinear elevation–colour relationships, we test for both linear and nonlinear effects. Finally, we go beyond presence/absence to consider plant species abundance when estimating how colour shifts with elevation. This latter approach should help to limit the influence of relatively rare plant species on the perceived distribution of colours, and should better represent the floral colour landscape experienced by pollinators and other flower visitors.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in the West Elk Mountains surrounding the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (N 38.95807°, W 106.98853°; elev. 2,889 m). The annual average maximum and minimum temperatures are 10.8 and −7.8°C, respectively. Average total precipitation is 59.9 cm/year, and average snowfall is 502.7 cm/year, with an average snow event depth of 25.4 cm (NOAA, 2014). The area is topographically and biotically diverse, with over 700 species of vascular plants reported from a 10 km radius of RMBL (SEINet, 2017). There are four major vegetation community types: sagebrush, spruce-fir, upland-herbaceous and alpine (Langenheim, 1962; Zorio, Williams, & Aho, 2016). We focused on the three open, forb-dominated communities (all but the spruce-fir type), combining vegetation surveys with measurements of floral spectral reflectance to estimate how community-level floral colour changes with elevation.

Bees and flies are the major pollinators in this ecosystem, with a hypothesized shift from bee-dominated to fly-dominated pollination with increasing elevation (Kearns, 1992; Kearns & Inouye, 1994; Moldenke & Lincoln, 1979; but see Galen, 1995 for a counterexample). Other pollinators include Lepidoptera and hummingbirds (Trochilidae), but these are relatively infrequent. In a three-year study of 24 forb species from 19 families near RMBL (within the lower-middle range of our elevation gradient), only 3% of 5,568 flower visits by insects were made by butterflies and moths (bees made 43% and flies 40%; K. D. Whitney, unpubl. data); Moldenke and Lincoln (1979) report similar percentages. Hummingbirds visit only a small subset of the forb species, e.g. only 8.2% of the animal-pollinated species representing only 2.4% of the cover in our plots are known to be visited by the Broad-tailed hummingbird *Selasphorus platycercus* (Waser, 1983; N. Alexandre, pers. comm.).

2.2 | Vegetation community composition

Six elevation transects were sampled for vegetation community composition in the late summer of, 2014 (July 28 to September 24). The peaks surveyed were Avery, Cinnamon, Hunter's Hill, Ruby, Teocalli and Treasury (Figure 1 and Table S2). The average linear distance between these peaks was 15.9 km, with a minimum and maximum distance of 8.0 and 29.9 km, respectively. From the highest point (peak) of each transect, sites were established at *c*. 100 m increments of elevation dropping towards the valley floor. Between 9 and 13 sites per transect were established, for a total of 67 sites across the six transects and an overall sampling range of 2,700–4,000 m a.s.l. At each site, 33 quadrats, each 20 × 20 cm and spaced 2.5 m apart, were established within a 20 × 20 m sampling grid. Thus, there were a total of 2,211 quadrats along the six transects. In each quadrat, percent cover of each plant species as well as bare ground was visually estimated such that cover summed to 100%. We note that while sampling occurred in the late summer, we were careful to record cover of all individuals present, including those that were post-flowering or senescent. Thus, the cover estimates include early-flowering species and should capture a representative sample of the flora at our sites. Plant identifications were made following Weber and Wittmann (2012) and with reference to collections in the RMBL herbarium.

Angiosperm species were classified as wind-pollinated (here defined as all species in families Betulaceae, Cyperaceae, Juncaceae, Poaceae, Typhaceae, Juncaginaceae and Salicaceae) or animal-pollinated (all others). Across all sites, average cover of identified animal-pollinated species was 29%. The remaining cover consisted of wind-pollinated angiosperm species (mostly grasses and sedges) and non-angiosperms (e.g. mosses), 26%; unidentified species, 6%; and bare ground, 39%.

2.3 | Floral spectral database

We compiled a floral spectral database consisting of reflectance measurements of the animal-pollinated species found in the vegetation community composition surveys (Table S3). These spectra were

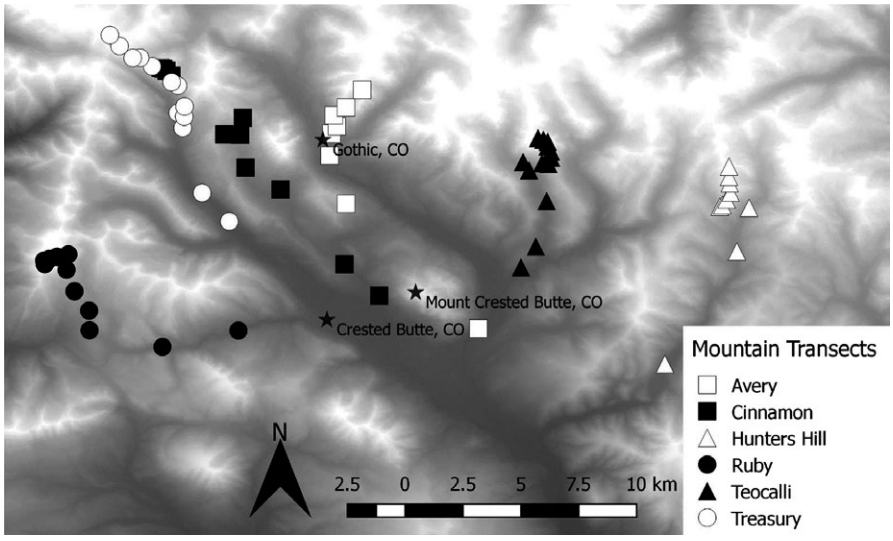


FIGURE 1 Map of the study area in the West Elk Mountains of Colorado, USA, showing locations of vegetation sampling sites along the six transects. On the base map, the darkest shading represents <2,700 m elevation, while the lightest represents >4,000 m

collected from the general RMBL area in 2009, 2013 and, 2016, as well as from sites specifically along the elevation transects in 2016. While we were unable to obtain spectra for 22 of the 132 species, most of the missing spectra were for locally rare species, as the spectra we did obtain represented 96.6% of the identified animal-pollinated cover.

For each species whose spectra were analysed, a full voucher specimen was deposited in the RMBL herbarium, and three individual flowers (each from a different plant) were collected for analysis. For the family Asteraceae, an inflorescence is morphologically integrated to function as a single flower, and therefore we treated their inflorescences as "flowers" hereafter. Within 12 hr of collection, spectrometer readings were taken on each flower using an Avantes model 2048 spectrometer, a bifurcated coaxial fibre optic reflectance probe (Avantes FCR-7uv200-2-ME) and an AvaLight-XE xenon light source (Avantes BV, Apeldoorn, The Netherlands). Calibration was first made relative to a diffuse white PFTE tile (Avantes WS-2). Integration time was 10 ms. Measurements were taken with the fibre optic probe held perpendicularly to, and at 8.0 mm from, the flower surface. We acknowledge that there has been discussion in the literature about the importance of diffuse vs. specular reflectance (Whitney et al., 2009) and the optimal angle (45° or 90°) at which to measure floral reflectance (Chittka & Kevan, 2005). We simply note here that probe angle seems to have little empirical effect on estimated colour components in either bee or fly visual spaces: values of hue, saturation or brightness measured at 45° vs. 90° are highly correlated (see Appendix S1). Three spectral readings (spanning 300–700 nm) were taken per flower, one per "zone." For actinomorphic (radially symmetric) species, zone one was at the base of the petal (near the flower centre), zone two was at the middle of the petal and zone three was at the tip of the petal. For zygomorphic (bilaterally symmetric) species, the zones were chosen to capture major variation in colour (as perceived by the human eye). For each species, we averaged reflectance measurements across the three individuals to calculate a single species-specific value for each wavelength in each zone. For the analyses presented here, we used data only from the dominant zone, identified as the zone representing the greatest surface area of the flower.

2.4 | Community-weighted mean reflectance: Raw spectra

To examine colour patterns independently of a particular animal visual system, we calculated community-weighted mean (CWM) reflectance for raw spectra binned arbitrarily into eight spectral bands, with those >400 nm here named for how they appear in the human visual system: UVB (300–315 nm), UVA (315–400 nm), Violet (400–440 nm), Blue (440–500 nm), Green (500–565 nm), Yellow (565–590 nm), Orange (590–625 nm) and Red (625–700 nm). First, we calculated the absolute percent cover for each species at each site (averaged across the 33 quadrats). We then calculated absolute percent cover of identified animal-pollinated species at each site. This allowed the calculation of the relative percent cover of each species (relative to total animal-pollinated cover). Relative percent cover was then used to weight the average reflectance of each species, resulting in a CWM reflectance at each site for each of the eight spectral bands. As a simple heuristic example, if only two animal-pollinated species existed at a site and they represented 25% and 75% of animal-pollinated cover, and further if they reflected at, 20% and 80% within blue wavelengths (440–500 nm), respectively, the $\text{reflectance}_{\text{cwm}}$ of blue at this site would be (25%*20% + 75%*80%) or 65%. We thus made the simplifying assumption that percent vegetative cover of a given species is indicative of its flower production (and the encounter rate of its flowers with pollinators) and did not attempt to correct for species-level differences in the size or number of flowers per unit vegetative cover in calculating CWM reflectances.

2.5 | Community-weighted mean hue, saturation and brightness in pollinator visual spaces

For each plant species in the spectral database, we calculated components of colour in both bee and fly visual spaces using the R package *pavo* v. 1.0 (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013). Bee and fly visual spaces used parameter values for *Bombus* and *Musca*, respectively.

In Chittka's (1992) hexagon model of bee vision, hue is represented by the radial angle within the hexagon, and saturation is distance from the (0,0) origin of the hexagon. These are output by *pavo* as variables "h.theta" and "r.vec." In addition, bee hue is also reported categorically via the variable "sec.coarse" by dividing the hexagon into six arbitrary (but traditionally recognized) sectors: bee-blue, bee-blue-green, bee-green, bee-UV-green, bee-UV and bee-UV-blue. In our analyses (below), we focused on the categorical rather than the continuous classification of hue in bee space for two reasons. First, there is a "wrap-around effect" (i.e. circularity) in the continuous version, where hues of e.g. 359° and 0° are perceived as similar by the bee but have widely divergent numeric values; this presents analytical difficulties when trying to interpret linear relationships between hue and a predictor variable (e.g. elevation). Second, by using the categorical version we can analyse hue–elevation relationships in bee and fly visual spaces in a comparable manner (see below). The third colour variable, brightness (*pavo* output "lum"), is calculated without reference to the hexagonal space. For all subsequent analyses, brightness in units $\mu\text{mol}\ s^{-1}\ m^{-2}$ was converted to a brightness contrast value (i.e. how bright are flowers relative to the vegetation background) by dividing by the bee's achromatic "green" receptor stimulation when viewing a green vegetation background. As a ratio of brightnesses, this contrast is unitless.

In Troje's (1993) model of fly vision, hue is categorical and takes on four values corresponding to the four quadrants of the square visual space: fly-UV (p+y+), fly-blue (p−y+), fly-yellow (p−y−) and fly-purple (p+y−). Saturation is the distance from the origin and brightness is again calculated separately, without reference to the two-dimensional visual space. These are output by *pavo* as variables "category," "r.vec" and "lum." As with bee visual space, brightness in units $\mu\text{mol}\ s^{-1}\ m^{-2}$ was subsequently converted to a unitless brightness contrast value, in this case by dividing by the fly's R1-6 receptor stimulation when viewing a green vegetation background.

Based on this output, we then calculated CWM values of saturation and brightness contrast in both bee and fly visual spaces for each site, using relative percent cover of each plant species, as described above for the raw spectra. For hue, we calculated the percent cover of each site consisting of plant species perceived within each of the six bee hues and four fly hues (bee-blue$_{\%cover}$, fly-yellow$_{\%cover}$, etc.). However, in subsequent analyses, we examined only four bee hues and two fly hues, for the following reasons. First, because percent cover sums to 100%, the hue measurements are not independent, necessitating dropping at least one hue variable within each visual system. Thus, we identified the most highly negatively correlated pair of hues within each system and dropped one of them: we dropped bee-blue-green because its cover is negatively correlated with that of bee-green across the 67 sites (Spearman $r = -.50$, $p < .001$); we dropped fly-blue because its cover is negatively correlated with that of fly-yellow (Spearman $r = -.87$, $p < .0001$). Finally, certain hues were either completely absent (fly-UV) or at very low percent cover (bee-UV, 0.1% cover) in the dataset and so were not examined further.

2.6 | Statistical analysis: Changes in colour components with elevation

All statistical analyses were performed in R (R Core Team, 2016). We used the *nlme* package (Pinheiro, Bates, DebRoy, & Sarkar, 2017) to perform separate linear regressions for each of 18 response variables. These included Reflectance$_{cwm}$ in each of the eight raw spectral bands; Saturation$_{cwm}$ and Brightness$_{cwm}$ in both bee and fly visual spaces; and six hue$_{\%cover}$ variables (four in bee visual space and two in fly visual space, as described above). Predictor variables were elevation and elevation^2 (both continuous) and transect (random categorical with six values: Avery, Cinnamon, Hunter's Hill, Ruby, Teocalli and Treasury). For each colour component, we first used a likelihood ratio test to compare a null model with a model including only transect (random) as a predictor. If transect explained significant variation, subsequent models examining elevation effects for that colour component all included transect as a predictor (otherwise, they did not). We then fit three models: a null, a model in which a linear elevation term was included, and a model in which both linear and quadratic elevation terms were included. A likelihood ratio test was then used to determine if the model with the lowest AIC$_c$ significantly outperformed the null. When transect was included in the final model, results are plotted as partial leverage plots, in which the relationship between elevation and colour component is plotted after accounting for the random effect of transect.

A major difference between our study and previous elevation-colour studies is that we weighted colour observations by percent cover, thus reducing the influence of rarer flowering species on the perceived colour patterns. To provide comparability, we re-ran the above analyses, substituting for the actual cover matrix a dummy matrix in which all species within a site were constrained to have equal cover. This effectively created a "presence–absence" analysis.

We note that we do not include analyses accounting for plant phylogeny for two reasons. First, we are primarily interested in describing the pattern of floral colour shifts with elevation, with less interest (at present) in whether that pattern is influenced by phylogenetic inertia. More importantly, the data points in our analyses do not correspond to species, but instead are weighted averages of trait values across species assemblages; techniques for accounting for phylogeny in such data do not currently exist.

3 | RESULTS

3.1 | Change in colour with elevation: Raw spectra (visual system-independent analysis)

Reflectance$_{cwm}$ in seven of the eight spectral bands changed significantly with elevation (Table 2, Figure 2). Shorter wavelengths—UVB, UVA, human violet, human blue—decreased linearly with elevation (Figure 2), although the relationship was nonsignificant for UVA. Longer wavelengths—human green through red—had a very different pattern (Figure 2). There was a significant linear component that was positive in each case (Table 2), indicating some increase with elevation, but also a strong negative quadratic component, indicating hump-shaped distributions with the highest reflectances at middle elevations of *c*. 3,400–3,600 m (Figure 2).

3.2 | Change in colour with elevation: Bee visual space

Flowers of plant species in the study area showed a range of values for saturation, brightness contrast, and floral hues as modelled in bee visual space (Table 2; Figures 3–5). With increasing elevation, floral communities appeared more strongly saturated in bee visual space, although saturation$_{cwm}$ plateaued around 3,400 m (Figure 3a). In contrast, brightness contrast$_{cwm}$ showed a strongly hump-shaped pattern with the brightest floral communities at around 3,400 m (Figure 3c; linear elevation term N.S., quadratic term strongly negative, Table 2).

With respect to hue, when considering a division of the space into the six sectors (Figure 4), bee-blue-green was the most common hue (44.2% of the cover across all sites), followed by bee-green (24.5%), bee-blue (17.4%) and bee-UV-green (12.2%). Bee-UV-blue and bee-UV were both relatively rare (1.6% and 0.1%, respectively). The percent cover of plant species with flowers perceived as bee-blue decreased linearly with elevation (Table 2; Figure 5a), while cover of bee-UV-green species showed a hump-shaped pattern with peak cover at 3,400–3,600 m (Figure 5c). Cover of bee-green and bee-UV-blue species showed positive and negative trends with elevation, respectively, that did not reach statistical significance (Table 2; Figures 5b,d).

3.3 | Change in colour with elevation: Fly visual space

Saturation$_{cwm}$ in fly visual space increased linearly with elevation (Table 2, Figure 3b). Brightness contrast$_{cwm}$ showed a more complex pattern, with little change across lower elevation communities

TABLE 2 Relationships between community-weighted mean floral colour and elevation in the Colorado Rocky Mountains. Transect *p*, when significant, indicates models including transect outperformed those without, and that transect was included as a random effect in subsequent models. For each colour component, three regression models were then tested: a null, a linear effect of elevation, both linear and quadratic effects of elevation. A more complex model was retained if a likelihood ratio test indicated a significantly better fit than the null. Elevation was mean-centred prior to analysis. Raw spectral bands above 400 nm are named based on how they appear in human visual space. Bolded *p*-values are significant at the $\alpha<0.05$ level

Colour component	Transect *p*	Best model	ΔAIC_c	Linear coefficient	*p*	Quadratic coefficient	*p*
Raw spectral bands							
UVB_{cwm}	.960	Linear	−2.31	−0.00085	**.037**		
UVA_{cwm}	.723	Null	0.00				
$Violet_{cwm}$	**<.001**	Linear	−6.20	−0.00781	**<.001**		
$Blue_{cwm}$	**<.001**	Linear	−6.89	−0.01060	**<.001**		
$Green_{cwm}$	.052	Quadratic	−11.89	0.00584	**.007**	−0.0000204	**.001**
$Yellow_{cwm}$	**.005**	Quadratic	−9.59	0.01319	**.004**	−0.0000258	**.004**
$Orange_{cwm}$	**.007**	Quadratic	−8.26	0.01243	**.008**	−0.0000245	**.006**
Red_{cwm}	**.025**	Quadratic	−5.34	0.00940	**.050**	−0.0000229	**.010**
Bee visual space							
$Saturation_{cwm}$	**.006**	Quadratic	−7.47	0.00012	**.001**	−0.0000002	**.024**
$Brightness_{cwm}$	.459	Quadratic	−4.89	0.00005	.286	−0.0000004	**.004**
$Bee\text{-}blue_{\%cover}$	.571	Linear	−6.40	−0.00018	**.004**		
$Bee\text{-}green_{\%cover}$	**.003**	Null	0.00				
$Bee\text{-}UV\text{-}green_{\%cover}$	.733	Quadratic	−8.82	0.00011	**.019**	−0.0000004	**.004**
$Bee\text{-}UV\text{-}blue_{\%cover}$	**.012**	Null	0.00				
Fly visual space							
$Saturation_{cwm}$	**<.001**	Linear	−3.49	0.00011	**.004**		
$Brightness_{cwm}$	.311	Quadratic	−12.75	−0.00047	**.001**	−0.0000010	**.015**
$Fly\text{-}yellow_{\%cover}$	.230	Null	0.00				
$Fly\text{-}purple_{\%cover}$	.703	Null	0.00				

from 2,700 to 3,200 m and a relatively steep drop starting around 3,200 m (Figure 3d).

With respect to hue, the floral landscape is dominated by fly-yellow (87.7% of cover), followed by fly-blue (7.7%) and fly-purple (4.6%). Flowers registering as fly-UV were not present in our sample. We detected no significant relationships between cover of plant species of particular fly hues and elevation (Table 2, Figure 6).

3.4 | The influence of accounting for plant species abundance in assessing elevation–colour relationships

Relative to the above-reported analysis, an alternative analysis in which all species within a site were constrained to have equal cover (Table S4) had the effect of weakening the perceived strength of many elevation–colour relationships. Focusing on the linear components of the models, seven relationships that were previously significant became nonsignificant (elevation vs. UVB, Blue, Green, Yellow, Orange, Red, fly brightness; compare Table 2 vs. Table S4). Those relationships that remained significant tended to have shallower slopes, and no previously nonsignificant relationships became significant.

4 | DISCUSSION

We have demonstrated the existence of a robust elevation gradient in floral colours. The gradient exists both in visual system-independent analysis and in the visual spaces of the dominant pollinators in this system, bees and flies. It is characterized by linear decreases in reflectance of shorter wavelengths with elevation, and hump-shaped patterns for longer wavelengths. In pollinator visual spaces, saturation increases with elevation, while brightness shows a hump-shaped pattern (bees) or modest increase followed by a steep decline (flies). There were significant shifts with elevation in representation of floral hues as perceived by bees, but not flies.

4.1 | Proximal causes of elevation–floral colour relationships

While our main focus is on potential ecological and evolutionary drivers of elevation-floral colour relationships (see below), it is important to note there are three potential (and non-mutually exclusive) proximal causes of any elevation–floral colour relationship. These are turnover of plant species varying in colour;

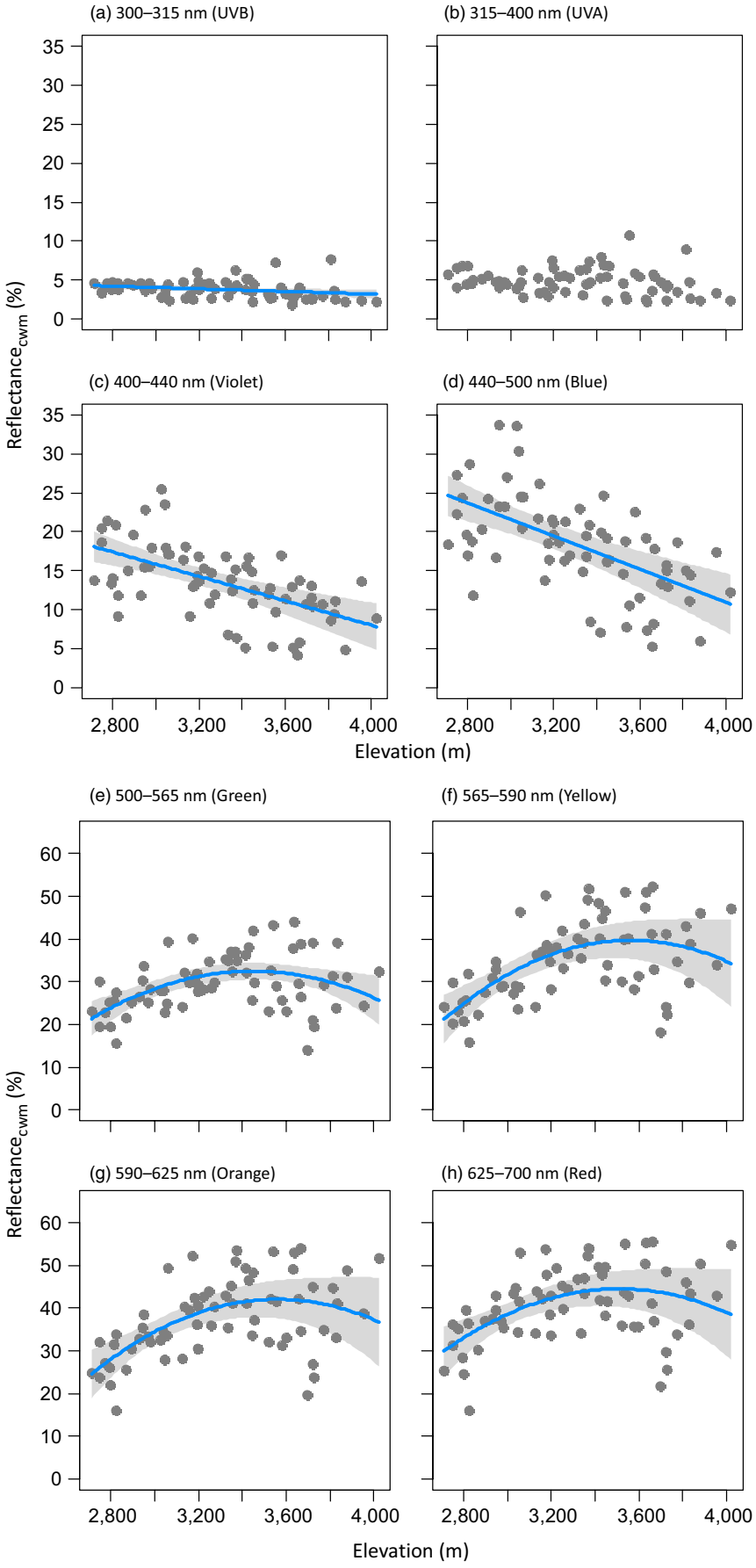


FIGURE 2 Raw spectra: relationships between elevation and floral reflectance$_{cwm}$. The visible portion of the spectrum has been arbitrarily divided into eight spectral bands, each labeled by the hue as it appears in human vision (panels a-h). Each point represents the community-weighted mean reflectance in that band for a site. Conventions: lines and 95% confidence bands are plotted only when the best model included elevation. When the best model included transect, the plot is a partial leverage plot showing the effect of elevation after accounting for the random effect of transect. [Colour figure can be viewed at wileyonlinelibrary.com]

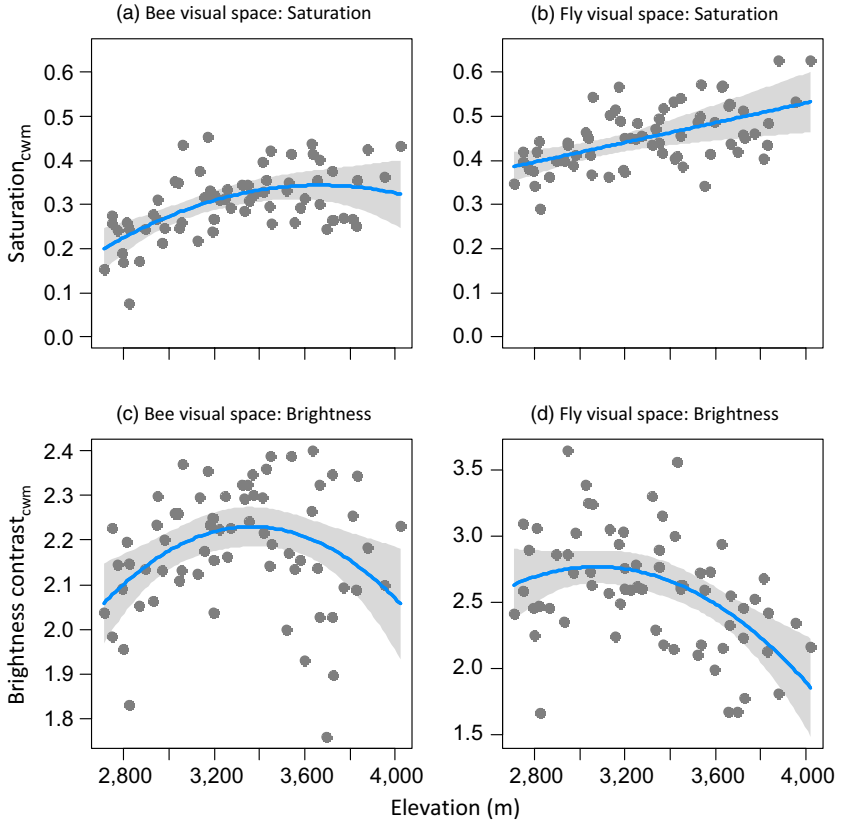


FIGURE 3 Relationships between elevation and floral saturation$_{cwm}$ (panels a,b) and brightness contrast$_{cwm}$ (panels c,d) in pollinator visual spaces. Saturation is in units of Euclidean distance in the hexagonal and square visual spaces of bees and flies, respectively. Brightness contrast is relative to the brightness of green background vegetation and is unitless. Each point represents the community-weighted mean value for a site. Other plotting conventions as in Figure 2 [Colour figure can be viewed at wileyonlinelibrary.com]

intraspecific plasticity in colour (e.g. Anderson et al., 2013; Ben-Tal & King, 1997); and intraspecific genetic variation in colour (e.g. Schemske & Bierzychudek, 2001; Warren & Mackenzie, 2001). Here, we have focused on species turnover as we expect it to be the major driver of elevation–colour patterns. For example, in our dataset, there are 34 plant species present at the lowest sites (2,711–2,868 m) and 33 at the highest sites (3,815–4,023 m), but only eight species are shared between these two elevation bands, indicating substantial turnover. In using a single average reflectance curve per species, we are assuming that interspecific colour variation is much larger than intraspecific variation. However, we note that the contributions of intraspecific variation in coloration (both plastic and fixed) to elevation–floral colour patterns would be interesting to explore in the future. Such variation could either enhance or temper the patterns described here. For example, if individual species varied such that higher elevation populations showed greater saturation in floral colour, the overall increase in saturation with elevation (here generated solely by species turnover) would be enhanced.

4.2 | Potential biotic drivers of elevation–floral colour relationships

With respect to bee visual space, and following Arnold et al. (2009), we predicted that species perceived as bee-blue and bee-UV-blue should generally show a decrease with elevation (Table 1). The prediction was supported for bee-blue species (Figure 5, Table 2) but the negative trend for bee-UV-blue species did not reach statistical significance, perhaps because the relative rarity of these species in our study area (only 1.6% of total cover) left insufficient room for variation across elevations. Our second prediction that colour saturation in bee space should be highest at lower elevations was not met, as saturation increased with elevation (up to a plateau at roughly 3,400 m; Figure 3a). We note that this prediction included an unstated assumption that saturation in bee and fly visual spaces is uncorrelated, such that bee-imposed selection or filtering would be free to shape saturation patterns. Instead, in our dataset, bee and fly-perceived saturation was highly correlated across plant species (R^2 = .84), perhaps explaining why the simple prediction was not met.

Our results are thus consistent with the idea that bees have shaped elevation-hue (but not elevation–saturation) relationships. This shaping could have occurred through in situ natural selection for particular hues, ecological filtering (in which plant species with "inappropriate" floral hues failed to colonize lower elevation habitats because of insufficient pollination), or both. The ecological filtering argument has recently been advanced to explain why human-blue-purple flowers (traditionally considered attractive to bees) are rare in the Arctic (Eidesen et al., 2017). The frequency of human-blue-purple species in the Arctic is positively correlated with bumblebee species richness and negatively correlated with the extent of past glaciation, suggesting that low abundances of bee pollinators in previously glaciated areas have limited recolonization by these plant species (Eidesen et al., 2017).

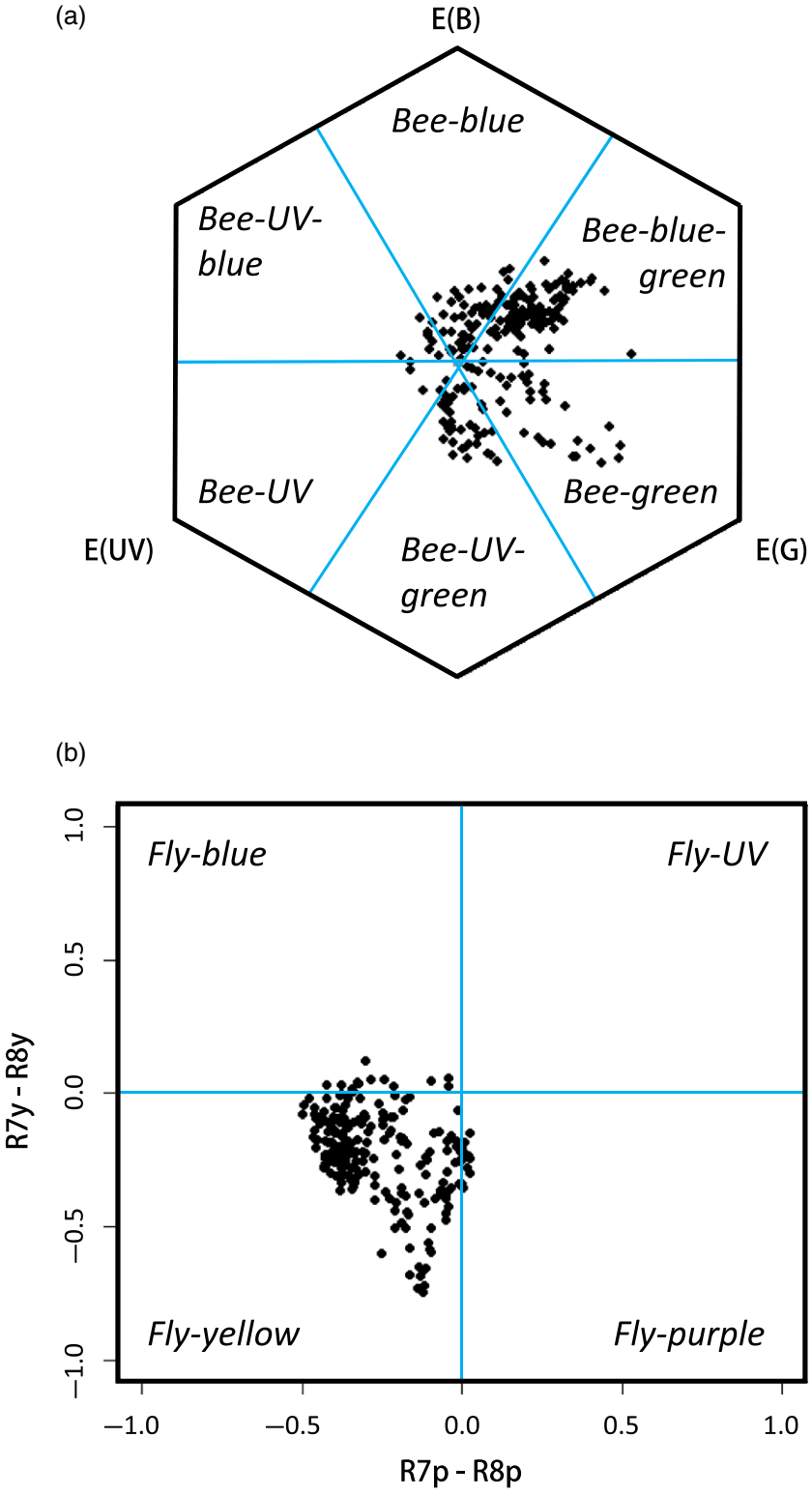


FIGURE 4 Floral hue and saturation for 110 Colorado Rocky Mountain plant species in bee and fly visual spaces. Bee visual space (a) has been arbitrarily divided into the six traditional sectors, but because bees perceive hue continuously, they are understood to be able to distinguish the hues of species at different radial angles within a sector. In fly visual space (b), hue is perceived categorically, so species within a quadrant are not distinguishable by hue. In both visual spaces, saturation is represented by distance from the origin, such that species towards the outer edges of the space (hexagon or square) are perceived as highly saturated [Colour figure can be viewed at wileyonlinelibrary.com]

Evidence that floral hue in bee space shifts predictably with elevation complements shifts seen in other aspects of floral morphology in other mountainous areas. For example, several studies have documented shifts in floral complexity, from more specialized (e.g. bilabiate) flowers at lower elevations to more generalized (e.g. open disk) flowers at high elevations (reviewed in Pellissier, Alvarez, & Guisan, 2012). As with hues in bee space in our study, these gradients in floral complexity appear to match elevational shifts from bee-dominated to fly-dominated pollinator assemblages, as bees are more capable of pollinating complex flowers than are flies (Pellissier et al., 2012).

With respect to fly visual space, we predicted that the cover of fly-yellow species would increase with elevation, as would fly-perceived

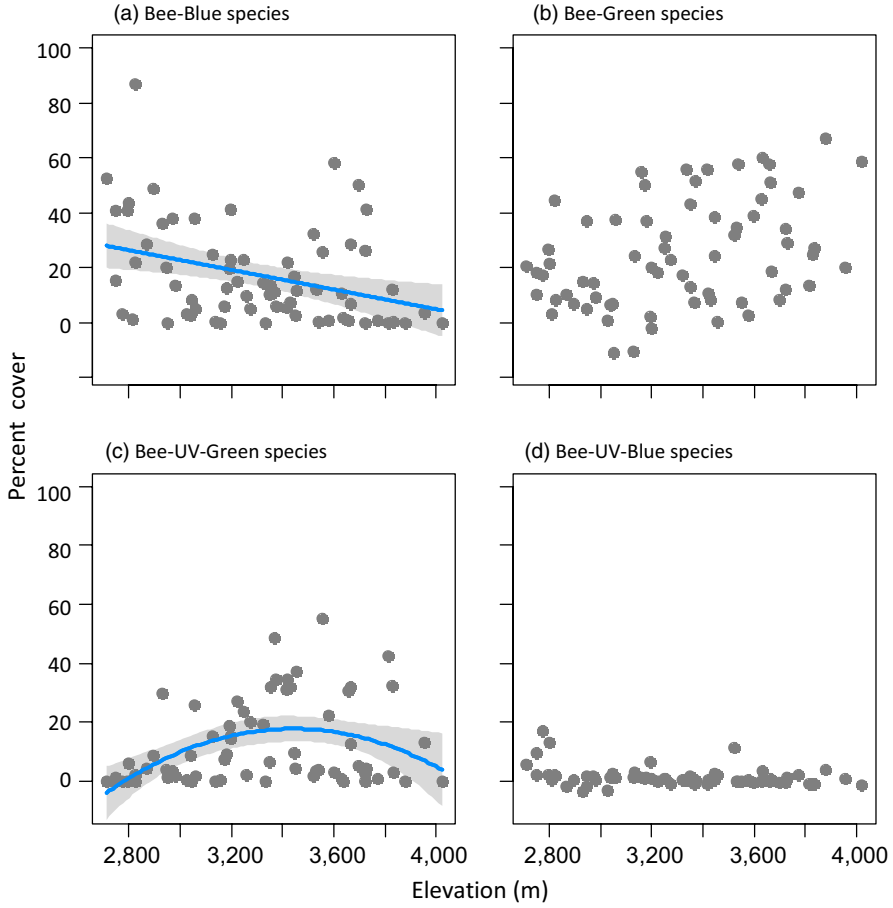


FIGURE 5 Relationships between elevation and $hue_{\%cover}$ in bee visual space. Each point represents the percent cover at a site reflecting a particular bee hue. (a) bee-blue; (b) bee-green; (c) bee-UV-green; (d) bee-UV-blue. Bee-UV and bee-blue-green were not examined to increase statistical independence of the response variables (see Section 2). Other plotting conventions as in Figure 2 [Colour figure can be viewed at wileyonlinelibrary.com]

brightness. Instead, we saw no elevation trends in any fly hues (Figure 6) and brightness showed only a modest increase across lower elevation communities from 2,700 to 3,200 m and a relatively steep drop starting around 3,200 m (Figure 3d). The general failure to meet these predictions may simply reflect that fly visual systems and preferences are not sufficiently understood to make informed predictions, including the idea that the *Musca* model we employ is not representative of the visual systems of the diverse pollinating flies in our study area. Alternately, it could be that flies do not exert strong selection or ecological filtering on floral traits, so that higher elevation communities are characterized by traits reflecting the "release" of bee-specific pressures but not the imposition of new fly-specific ones. This hypothesis may be unlikely, as there is strong fly discrimination among flower colours in the New Zealand alpine, a region previously hypothesized to be relatively free from pollinator-mediated selection (Campbell, Bischoff, Lord, & Robertson, 2010). Finally, it could be that abiotic drivers of floral coloration may supersede biotic drivers at higher elevations (see below).

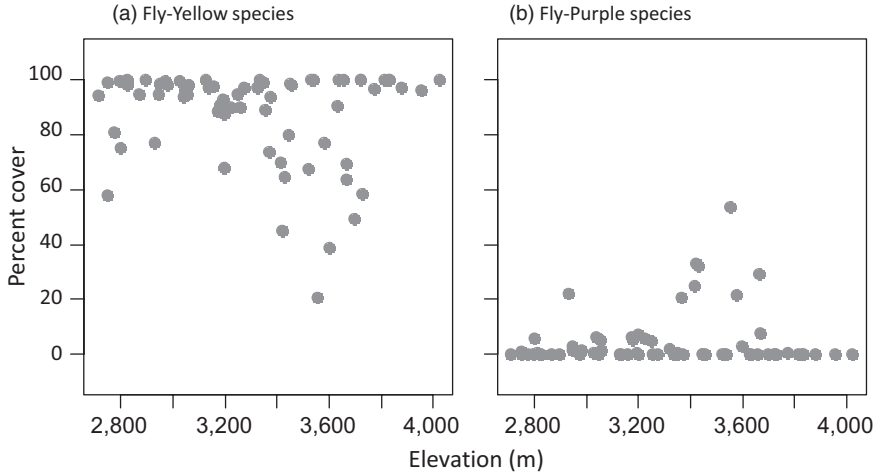


FIGURE 6 Relationships between elevation and $hue_{\%cover}$ in fly visual space. Each point represents the percent cover at a site reflecting a particular fly hue. (a) fly-yellow; (b) fly-purple. Cover of species reflecting fly-blue was not examined to increase statistical independence of the response variables (see Section 2); species reflecting fly-UV are not present in the dataset. Other plotting conventions as in Figure 2 [Colour figure can be viewed at wileyonlinelibrary.com]

4.3 | Potential abiotic drivers of elevation–floral colour relationships

It is harder to evaluate the possible match between specific abiotic drivers such as temperature, UV and drought and the elevation–floral colour relationships seen in the current study. First, these drivers are expected to be highly correlated, making it difficult to disentangle their effects. Next, as mentioned previously, most predictions regarding abiotic drivers reference underlying pigment concentrations rather than the emergent pollinator-relevant signals. It is not straightforward to convert pigment concentrations to colour components (Renoult et al., 2017): increased concentrations can influence both hue and saturation or sometimes, just hue alone (Andersson & Prager, 2006; Lancaster, Lister, Reay, & Triggs, 1997). Further, different abiotic drivers predict opposing elevation–colour trends (Table 1). Keeping in mind these issues, we note that floral brightness did not exhibit strict linear relationships with elevation (Figure 3c,d). Thus, there was no evidence that any single abiotic driver dominated the pigmentation patterns. However, we note that the least-bright flowers were at the highest elevations in both visual spaces (Figure 3c,d), consistent with the hypothesis that low temperature and/or high UV favour increased pigmentation at high elevations.

We note the large number of hump-shaped relationships between elevation and colour components in our study (e.g. Figures 2, 3 and 5). One possibility is that multiple drivers may shift in importance and combine in such a way as to generate nonlinear relationships between colour and elevation. To illustrate a hypothetical scenario, the ascending (left-hand) part of the curve for brightness in fly space (Figure 3d) could be driven by increasing dominance of flies with elevation and higher fitness for flowers that are conspicuous to them. But by mid-elevation, abiotic drivers could take over in importance and shape a different response. Again hypothetically, UV stress could increasingly select for higher pigment concentrations with elevation; since pigment absorbs light and reduces brightness, the descending limb of the brightness curve (Figure 3d) could be generated in this manner.

4.4 | The importance of accounting for plant species abundance in assessing elevation–colour relationships

In contrast to our results, Arnold et al. (2009) found no significant changes in floral hues in bee, fly or human visual spaces, or in the raw spectra, along an alpine elevation gradient in Norway. Does this discrepancy represent true geographic variation in outcomes, or might it reflect different methodological approaches? A major difference between that study and ours is that we weighted colour observations by percent cover, thus reducing the influence of rarer species on the perceived colour patterns. Reanalysis of our data in a "presence–absence" framework similar to that in Arnold et al. (2009) (Table S4), by means of artificially constraining all species within a site to have equal cover, showed weakened elevation–colour relationships. Thus, accounting for variation in plant species abundance within an elevation zone may be key to detecting signal in elevation–floral colour relationships.

4.5 | Geography of floral colour: elevation vs. latitude

Climates vary in parallel along latitudinal and elevational gradients, e.g. the classic textbook observation that climates at the tops of tropical mountains resemble those of distant high latitudes more than those of the tropical lowlands at their bases. Biological responses to climate can also vary in this way, leading to the question of whether there is any similarity in latitudinal and elevational gradients in floral colour. To our knowledge, the only relevant analysis of latitudinal variation is in Dalrymple et al. (2015), who examine floral coloration across 339 Australian angiosperm taxa spanning roughly 30° of latitude. While these authors do not employ visual spaces for specific pollinator groups, they do present an analysis of colour saturation in a generalized visual space characterized by opponency-based processing. They find that average saturation increases with latitude, which parallel our findings that saturation increases with elevation in both bee or fly visual spaces. These patterns suggest that the drivers of floral colour variation may be similar along latitudinal and elevational gradients.

4.6 | Future directions

Teasing apart the influence of pollinators on elevational shifts in floral colour from that of abiotic drivers is an important area of future work. One approach would be to specifically examine patterns of elevation change in reflectance in narrow spectral regions to which certain pollinators have high sensitivity (marker-point analysis; see Bischoff et al., 2013; Chittka & Menzel, 1992; Dyer et al., 2012; Shrestha et al., 2013). For example, a pattern in which spectra of low-elevation species more closely match regions of hymenopteran visual sensitivity than do high-elevation species would be additional evidence consistent with an influence of pollinators on colour distributions. Another approach could use reciprocal transplant/common garden studies to ask: are high-elevation plants more attractive to flies? Are low-elevation plants more attractive to bees? Are plant species with atypical coloration for their elevation (e.g. bee-blue species at high elevation) less likely to be visited by the dominant pollinator for that elevation, and do they achieve lower fitness as a result?

With respect to abiotic drivers, we suggest two approaches that may be fruitful. First, more empirical investigation is needed to understand how pigment concentration variation translates into variation in the subjective visual appearance of flowers. Do different classes of pigments (e.g. anthocyanins, carotenoids) have predictable concentration-saturation or concentration-hue relationships? Second, the effect of abiotic drivers could be isolated experimentally. One possible design involves planting plant populations across an elevation gradient. Each population would contain plants with a range of pigment concentrations; this range could be enhanced by including genetic mutants for high or low pigment production. Plants would then be shielded from variation in biotic agents: hand pollination could be used to remove pollination limitation and the influence

of elevation-specific pollinators, and caging or pesticides could eliminate elevation-specific herbivores and pathogens. Individual plant fitness would then be measured. If the pigment concentration conferring highest fitness varied consistently with elevation, this would be evidence that abiotic drivers are important and contribute to floral colour variation along elevation gradients. We note that an alternative design to isolate the effects of biotic agents such as pollinators or herbivores (that is, standardizing abiotic variables such as temperature and UV while letting elevation-specific biotic agents act on the plants) would be much more logistically difficult to implement.

ACKNOWLEDGEMENTS

Many thanks to Jennifer Reithel for facilitating this work, to Shannon Sprott for assistance with GIS, and to the RMBL community (especially Nick Waser, Mary Price, Diane Campbell, and Jill Anderson) for helpful feedback along the way. Kellen Paine helped with data collection, and Jennifer Rudgers provided statistical advice. K.D.W. was supported by NSF DEB 1257965.

AUTHORS' CONTRIBUTIONS

K.D.W. conceived the idea and led the writing of the manuscript; M.G. and M.J.S. collected the plant samples and the spectral data; J.S.L. collected the vegetation composition data; C.F.W. performed the plant species identifications and specimen curation; K.D.W. and T.E.W. analysed the data. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Datasets associated with this project are available in the Supporting Information and can also be downloaded from the Open Science Framework Repository https://doi.org/10.17605/osf.io/6y8ke (Whitney, 2018).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Gray M, Stansberry MJ, Lynn JS, Williams CF, White TE, Whitney KD. Consistent shifts in pollinator-relevant floral coloration along Rocky Mountain elevation gradients. *J Ecol*. 2018;106:1910–1924. https://doi.org/10.1111/1365-2745.12948