

Fall 8-31-2018

# Intraspecific floral color variation as perceived by pollinators and non-pollinators: evidence for pollinator-imposed constraints?

Kellen Cedar Paine  
*University of New Mexico*

Follow this and additional works at: [https://digitalrepository.unm.edu/biol\\_etds](https://digitalrepository.unm.edu/biol_etds)

Part of the [Biology Commons](#)

---

## Recommended Citation

Paine, Kellen Cedar. "Intraspecific floral color variation as perceived by pollinators and non-pollinators: evidence for pollinator-imposed constraints?" (2018). [https://digitalrepository.unm.edu/biol\\_etds/306](https://digitalrepository.unm.edu/biol_etds/306)

This Thesis is brought to you for free and open access by the Electronic Theses and Dissertations at UNM Digital Repository. It has been accepted for inclusion in Biology ETDs by an authorized administrator of UNM Digital Repository. For more information, please contact [disc@unm.edu](mailto:disc@unm.edu).

Kellen Paine

*Candidate*

---

Biology

*Department*

---

This thesis is approved, and it is acceptable in quality and form for publication:

*Approved by the Thesis Committee:*

Kenneth Whitney , Chairperson

---

Jennifer Rudgers

---

Diane Marshall

---

---

---

---

---

---

---

---

---

---

**INTRASPECIFIC COLOR VARIATION AS PERCEIVED BY  
POLLINATORS AND NON-POLLINATORS: EVIDENCE FOR  
POLLINATOR IMPOSED CONSTRAINTS?**

**by**

**KELLEN PAINE**

B.A., Biology, Earlham College, 2012

THESIS

Submitted in Partial Fulfillment of the  
Requirements for the Degree of

**Master of Science  
Biology**

The University of New Mexico  
Albuquerque, New Mexico

**December 2018**

## **ACKNOWLEDGEMENTS**

Funding was provided by the University of New Mexico Harry Wayne Springfield Fellowship (to K.C.P.), the Society for the Study of Evolution Rosemary Grant Award (to K.C.P.), and NSF DEB 1257965 (to K.D.W.).

I would like to thank all current and former members of the Rudgers-Whitney lab for their support and advice with experimental design, data collection and analysis. Thanks to my committee (Jennifer Rudgers and Diane Marshall) for their comments and advice, and a special thank you to Ken Whitney for his endless patience and feedback. Finally, thank you to my family and partner for supporting me and making this thesis possible.

# **INTRASPECIFIC COLOR VARIATION AS PERCEIVED BY POLLINATORS AND NON-POLLINATORS: EVIDENCE FOR POLLINATOR IMPOSED CONSTRAINTS?**

by

Kellen Paine

B.A. Biology, Earlham College 2012

M.S. Biology, University of New Mexico 2018

## **ABSTRACT**

Pollinator-mediated selection is expected to constrain floral color variation within plant populations, yet populations with high color variability are common in nature. To explore this, we collected floral reflectance spectra for 34 populations of 14 plant species of New Mexico, USA, and translated them into three different visual spaces. We found evidence that the majority comparisons were indistinguishable to bees, the dominant pollinator group. We also found that floral color variation was significantly greater for two non-pollinating groups, birds and humans. Our results suggest that a portion of human-perceived floral color variation within populations persists because it is invisible to pollinators, and may evolve neutrally or via indirect selection on correlated characters. Our results suggest an explanation for the fact that many studies of floral color polymorphisms are unable to detect pollinator-mediated selection on color, yet often find evidence for non-pollinator-mediated selection.

# Table of Contents

<b>List of Figures</b> .....	<b>vi</b>
<b>List of Tables</b> .....	<b>vii</b>
<b>Chapter 1</b> .....	<b>1</b>
1.1 Introduction.....	1
1.2 Methods.....	5
1.3 Results.....	9
1.4 Discussion.....	13
<b>References</b> .....	<b>18</b>

## List of Figures and Tables

<b>Table 1</b> .....	2
<b>Table 2</b> .....	10
<b>Figure 1</b> .....	3
<b>Figure 2</b> .....	4
<b>Figure 3</b> .....	10
<b>Figure 4</b> .....	12
<b>Figure 5</b> .....	15

## List of Tables



# Chapter 1

## Introduction

Floral color is an important trait that pollinators use to select the flowers they visit (Fenster et al 2004; Schiestl and Johnson 2013; Dyer et al 2012), and thus pollinators can act as selective agents on flower color (Rausher 2008). However, because different animal clades possess different color-sensitive receptors and cognitive mechanisms, the way each pollinator group perceives color is unique (Renoult et al 2017). As a result, we often find that flowers are colored in ways that exploit the color vision of their primary pollinators (Shrestha et al 2013; Dyer et al 2012).

If a population is pollinated by a single group of pollinators, such as bees, we expect intrapopulation variation in floral color (as perceived by that group) to be low in response to pollinator-driven selection (Fenster et al 2004; Rausher 2008; Waser and Price 1983). That is, when alternate color morphs arise through mutation, they should be selected against, as pollinators tend to visit the most common color morph (Smithson 2001; Eckhart et al 2006), perhaps because animals tend to find it easier to remember more common varieties of their food (Gegear and Lavery 2001). Despite this expectation, plant species with high intrapopulation color variation (at least to humans) occur regularly in nature. Numerous studies have investigated such high variability (Table 1; reviewed in Rausher 2008; Warren and Mackenzie 2001). However, very few of these studies have actually quantified floral variability in pollinator visual spaces (for exceptions, see: Ortiz et al 2015; Campbell et al 2011), leading to questions about both the relevance and maintenance of such color variation.

Table 1: Studies investigating floral color polymorphisms (adapted from Rausher 2008, with addition of more recent research). Only two studies have investigated polymorphisms via visual modelling within the relevant pollinator visual space (noted with \*). Other symbols indicate whether a study's conclusions about pollinator visitation, selection, and trait associations were based on direct (+) or indirect (‡) evidence.

Plant species	Color polymorphism (human hues)	Pollinators	Do pollinators impose selection?	Do different color morphs have differential visitation?	Is color associated with other traits?	Non-pollinator agent of selection	Reference
<i>Antirrhinum majus</i>	Yellow/ White	Bees		Yes <sup>†</sup>			Jones and Reithel 2001
<i>Aquilegia caerulea</i>	Blue/ White	Bees/ Moths	Yes <sup>†</sup>	Yes <sup>†</sup>			Miller 1981
<i>Bixa orellana</i>	Various	Bees/Ants	Yes <sup>†</sup>	Yes <sup>†</sup>	Yes <sup>†</sup>		Joseph and Siril 2013
<i>Clarkia gracilis</i>	Spotted / Non-Spotted	Bees	Yes <sup>†</sup>	Yes <sup>‡</sup>			Jones 1996
<i>Claytonia virginica</i>	Red, various shades	Bees	Yes <sup>‡</sup>	Yes <sup>‡</sup>	Yes <sup>‡</sup>	Herbivores <sup>†</sup>	Frey 2004
<i>Clarkia xantiana</i>	Spotted/ Non-Spotted	Bees		Yes <sup>†</sup>			Eckhart et al 2006
<i>Dactylorhiza sambucina</i>	Purple/Yellow	Bees	Yes <sup>†</sup>	Yes <sup>‡</sup>			Pellegrino et al 2005
<i>Gentiana lutea</i>	Yellow/ Orange	Mostly <i>Bombus</i>	Yes <sup>†</sup>	Yes <sup>†</sup>	Yes <sup>‡</sup>	Herbivores <sup>†</sup>	Veiga et al 2015
<i>Gentiana leucomelaena</i>	Blue/ White	Flies/Ants/ <i>Apis</i>		Yes <sup>‡</sup>	Yes <sup>†</sup>		Mu et al 2011
<i>Geranium nepalense</i>	Pink/ White	Bees/Flies	No <sup>†</sup>	Yes <sup>†</sup>			Tang et al 2016
<i>Ipomoea purpurea</i>	White/ Purple	Bees	Yes <sup>‡</sup>	Yes <sup>†</sup>			Fry and Rausher 1997
<i>Iris lutescens</i>	Yellow/ Purple	Bees	Yes <sup>‡</sup>	Yes <sup>†</sup>			Imbert et al 2014
<i>Linanthus parryae</i>	Blue/ White	Beetles	No <sup>†</sup>	No <sup>†</sup>	Yes <sup>‡</sup>	Yes <sup>†</sup>	Schemske and Bierzychudek 2001, 2007
<i>Linaria canadensis</i>	Purple/ Blue	Bees			Yes <sup>†</sup>		Wolfe and Sellers 1997
<i>Linum pubescens</i>	Yellow/ Purple	Flies	No <sup>‡</sup>	No <sup>†</sup>			Wolfe 2001
<i>Lobelia siphilitica</i>	Blue to Purple	<i>Bombus</i>	Yes <sup>†</sup>	Yes <sup>‡</sup>	Yes <sup>‡</sup>	Herbivores <sup>†</sup>	Caruso et al 2010
<i>Lobularia maritima</i>	Purple/ White	Ants/Flies	Yes <sup>†</sup>		Yes <sup>†</sup>		Gomez 2000
<i>Lysimachia arvensis</i>	Red/ Blue	Bees		Yes <sup>†</sup>			Ortiz et al 2015*
<i>Malva moschata</i>	Red/ White	Bees		Yes <sup>†</sup>	Yes <sup>†</sup>		Frey et al 2011
<i>Phlox drummondii</i>	Pink/ White	Butterflies		Yes <sup>‡</sup>			Levin 1972
<i>Phlox pilosa</i>	Pink/ White	Butterflies		Yes <sup>‡</sup>			Levin and Kerster 1967
<i>Platystemon californicus</i>	Yellow/ White,	Bees/Wind	No <sup>†</sup>	Yes <sup>†</sup>			Hannan 1981
<i>Raphanus raphanistrum</i>	Yellow/ White	Bees	Yes <sup>†</sup>	Yes <sup>†</sup>			Stanton et al 1989
<i>Raphanus sativus</i>	Yellow/ White/ Pink	Bees	Yes <sup>‡</sup>	Yes <sup>†</sup>	Yes <sup>‡</sup>	Herbivores <sup>†</sup>	Irwin and Strauss 2005
<i>Silybum marianum</i>	Purple/ White	<i>Apis</i>	No <sup>†</sup>	No <sup>‡</sup>			Keasar et al 2016
<i>Wahlenbergia albomarginata</i>	Blue/ White	Solitary bees		No <sup>†</sup>			Campbell et al 2011*

Even when analyses of spectra find that two flowers occupy different positions in a pollinator's color space, the distinction between them may not be perceptible to the pollinator (Dyer and Chittka 2004). All visual organisms have visual thresholds, defined by the minimum distances between two colors that are distinguishable (e.g., Dyer and Chittka 2004; Olsson et al 2017; Wyszecki and Stiles 1986; see Methods). The existence of these thresholds suggests that there may be effectively invisible intrapopulation color variation that escapes direct selection from pollinators. We propose that flower colors might be evolutionarily constrained such that they vary only up to a certain threshold, which remains imperceptible to their dominant pollinators (Figure 1). Thus a fundamental unanswered question is (1): Is intrapopulation variation in flower color constrained to be lower than the perceptual threshold of the dominant pollinators?

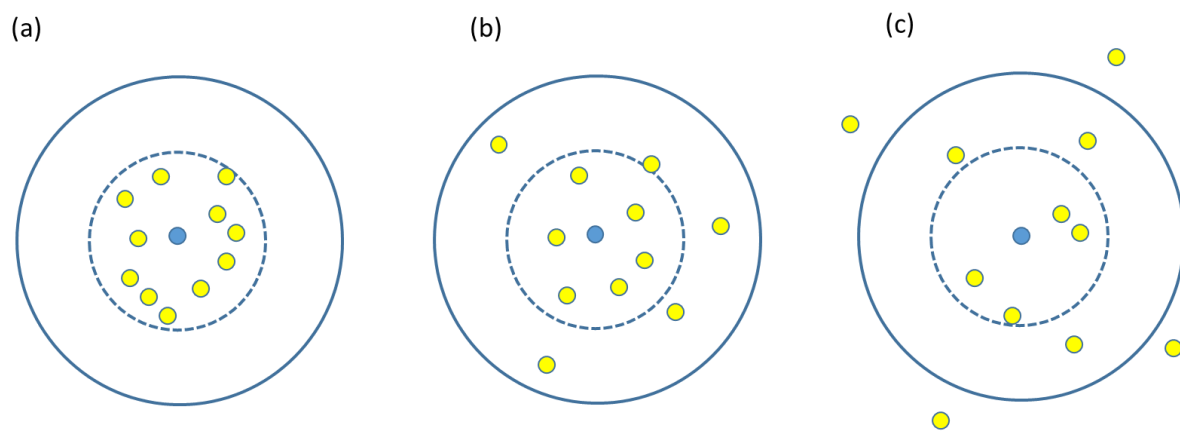


Figure 1. Hypothetical flower color variation in relation to discrimination thresholds within the bee hexagon. The yellow dots represent the color (hue and saturation) of individual flowers within a population while the blue dot is the population centroid. Circles represent the application of bee discrimination thresholds (0.11 hexagon units, Dyer 2006) to flower-flower (dashed circle) or flower-centroid (solid circle) comparisons, respectively. Three scenarios are shown reflecting different potential levels of bee-related evolutionary constraint. (a) All flower-flower pairwise distances are less than threshold (i.e. within a *diameter* of 0.11 hexagon units); consistent with strong bee-related evolutionary constraint. (b) All flower-centroid pairwise distances are less than the threshold (i.e. within a *radius* of 0.11); consistent with intermediate evolutionary constraint. (c) A large fraction of flower-centroid distances are greater than the threshold; consistent with weak or absent evolutionary constraint.

Because non-pollinators typically do not exert sexual selection on flowers, we might expect that floral color variation would be less constrained in their visual spaces. Genetic drift and/or indirect selection via genetic correlations might act to diversify the appearance of flowers to these non-pollinator species, perhaps with little counteracting selection constraining the variation. We thus ask the novel question (2): Is apparent intrapopulation variability in flower color higher for non-pollinating animals than it is for the dominant pollinator group?

To answer these questions in one geographic region, we measured the spectral reflectance of multiple individuals within one to three populations of each of 14 plant species, representing seven plant families of native bee-pollinated flowers in north-central New Mexico. We modelled these spectra in bee, human, and bird visual spaces and compared apparent floral color variability for pollinators with that perceived by the two groups of non-pollinators. Because these questions were inspired by our investigations of *Sphaeralcea polychroma* [Malvaceae] (LaDuke 1985), a highly variable species in human visual space (Figure 2), special care was taken to include several congeners of *S. polychroma* that are less variable in human visual space.



Figure 2. Human-perceived floral color variation in *Sphaeralcea polychroma* (Malvaceae). Each flower was collected from a different individual plant, collected within a 100m radius within a single population at the Sevilleta National Wildlife Refuge, New Mexico, USA (population SpPo6\_1, Table 1). Note that much of the variability in this species is invisible to bees as 61% of the pairwise comparisons between these flowers falls below the 0.11 hexagon unit visual threshold (Fig. 3A), even though every flower-flower comparison is distinguishable to humans.

## Methods

### Flower collection and spectrophotometry

We collected individual flowers from 14 native New Mexican species, blooming in the fall of 2017 (Table 1). For the family Asteraceae, an inflorescence is morphologically integrated to function as a single flower, and therefore we treated their inflorescences as "flowers" hereafter. Species were chosen from among those widely flowering during the field season, and included multiple *Sphaeralcea* species to act as a comparison to the distinctly variable (in human vision) *S. polychroma*. For each species, we collected a single flower from each of 15 different individual plants in each of 1-3 different populations, with the exception of one population of *Geranium caespitosum* (GeCa3\_2), for which only 14 individuals were collected. We thus sampled a total of 34 populations and 509 individuals across all species.

We used spectrophotometry to quantify spectral reflectance within three hours of collection; flowers were transported to the lab in an ice-cooled cooler to ensure freshness. Floral reflectance was measured using an AvaSpec 2048 spectrophotometer, a bifurcated coaxial fiber optic reflectance probe (Avantes FCR-7uv200-2-ME), and an AvaLight-XE xenon light source (Avantes BV, Apeldoorn, The Netherlands). Prior to reading the samples, the spectrophotometer was calibrated relative to a white standard PFTE tile (Avantes WS-2). Reflectance was measured with the probe held perpendicular to and 8.0 mm from the petal, with consistent distance enforced through a small nail connected to the probe. While there is discussion in the literature about the optimal angle (45° vs 90°) to measure floral reflectance (Chittka and Kevan 2005; White et al 2015), in practice, color components (hue, saturation or brightness) within bee visual space calculated at these two angles are highly correlated (see Gray et al 2018; Appendix S1). One petal was chosen randomly from each flower and measurements were taken either one

Table 2. Plant populations sampled in this study, New Mexico, USA. Fifteen individuals within a 100 m radius were collected from each population.

<u>Species</u>	<u>Population Code</u>	<u>Latitude</u>	<u>Longitude</u>	<u>Family</u>	<u>Human Hue</u>	<u>Bee Hue</u>	<u>Bee visitation citation</u>
<i>Baileya multiradiata</i>	BaMu_1	34.403	-106.672	Asteraceae	Yellow	Green	Cockrell 1900
	BaMu_2	35.556	-106.809				
	BaMu_3	33.774	-106.904				
<i>Fallugia paradoxa</i>	FaPa_1	35.556	-106.809	Rosaceae	White	Blue-Green	Buchmann 1985
<i>Geranium caespitosum</i>	GeCa3_1	35.180	-106.391	Geraniaceae	Reddish Purple	Blue	Hessing 1988
	GeCa3_2	35.256	-106.406				
	GeCa3_3	35.162	-106.295				
<i>Glandularia bipinnatifida</i>	GlBi2_1	34.269	-106.670	Verbenaceae	Pink to Purple	Blue-Green to Blue	Cockrell 1906
	GlBi2_2	35.034	-106.354				
	GlBi2_3	35.162	-106.295				
<i>Helianthus petiolaris</i>	HePe_1	33.774	-106.904	Asteraceae	Yellow	Green to UV-Green	Heiser et al 1969
	HePe_2	35.034	-106.354				
	HePe_3	33.734	-106.977				
<i>Macranthera tanecitifolia</i>	MaTa2_1	34.403	-106.672	Asteraceae	Light purple	Blue to Blue-Green	KCP, pers. obs.
	MaTa2_2	35.034	-106.354				
	MaTa2_3	33.774	-106.904				
<i>Nama hispidum</i>	NaHi_1	34.403	-106.673	Boraginaceae	Light Purple	Blue-UV	Tyrl et al 1984
<i>Penstemon ambiguus</i>	PeAm_1	34.404	-106.673	Plantaginaceae	White	Blue-Green	KCP pers. obs.
	PeAm_2	35.034	-106.354				
<i>Psoralea scoparius</i>	PsSc6_1	33.163	-107.220	Fabaceae	Dark Purple	UV-Blue to Blue	Rozen and Rozen 1986
	PsSc6_2	35.556	-106.809				
	PsSc6_3	33.774	-106.904				
<i>Schoenocrambe linearifolia</i>	SclI2_1	35.034	-106.354	Brassicaceae	Light Purple	Blue-Green	Lewis and Schupp 2014
<i>Sphaeralcea angustifolia</i>	SpAn_1	35.142	-106.683	Malvaceae	Orange	UV-Blue to UV	LaDuke 1985
	SpAn_2	35.034	-106.354				
<i>Sphaeralcea hastulata</i>	SpHa_1	34.403	-106.672		Orange	UV-Blue to UV	LaDuke 1985
	SpHa_2	33.774	-106.904				
	SpHa_3	33.774	-106.904				
<i>Sphaeralcea incana</i>	SpIn2_1	35.556	-106.809		Orange	UV-Blue to UV	LaDuke 1985
	SpIn2_2	33.163	-107.220				
	SpIn2_3	33.774	-106.904				
<i>Sphaeralcea polychroma</i>	SpPo6_1	34.403	-106.672		White, pink, red, to violet	UV-Blue to UV	LaDuke 1985
	SpPo6_2	33.734	-106.977				
	SpPo6_3	33.163	-107.220				

centimeter from the base for longer petals (e.g. *Helianthus petiolaris*), or 1/3 of the distance from the base to the tip for smaller petals (e.g. *Macaranthera tanecitifolia*). Spectral processing and visual modelling was carried out using the R package ‘pavo’ (Maia et al 2013). We first

trimmed the spectra to 300-700 nm, and then set spurious negative reflectance values to zero using the *prospec* command.

### **Conversion of floral spectral data into visual spaces**

We estimated the subjective perception of floral signals using models of color vision appropriate for the viewers of interest. Namely, we used the color hexagon for bees (Chittka 1992), the receptor-noise limited model for birds (Vorobyev and Osorio 1998), and the CIELab model for humans (Wyszecki and Stiles 1986). Though they vary in their underlying assumptions, each of these models allows colors to be represented as points in a space delimited by the number and sensitivity of photoreceptors, while accounting for factors such as the structure of viewing backgrounds and signals, veiling and incident light, and more species-specific features of visual processing and perception (Maia and White 2018; Kemp et al 2015). Crucially for the questions at hand, the distances between points in these spaces can be interpreted as measures of the subjective difference between colors, with values less than a behaviorally-validated ‘threshold’ of discrimination likely to be indistinguishable to a given viewer. In the color hexagon, psychophysical testing of bumblebees and honeybees suggests colors separated by a Euclidean distance of 0.11 hexagon units are unlikely to be distinguishable without differential conditioning, which is unlikely in natural settings (Dyer 2006; Dyer and Neumeyer 2005; Dyer and Chittka 2004). In the receptor-noise limited and CIELab models, color distances are expressed as weighted Euclidean distances ( $\Delta S$  and  $\Delta E$ , respectively), with values of 1.0 for diurnal birds, and 2.3 for humans, taken to delimit the threshold below which colors are expected to be indistinguishable under ecologically relevant conditions (reviewed in Olsson et al 2017; Wyszecki and Stiles 1986). With respect to model parameters, we drew on the receptor sensitivities of *Apis mellifera*, modelled using a vitamin A1 visual template (Chittka

1992). For birds, we used the visual phenotype of an average violet-sensitive avian viewer for receptor-noise modelling (Bennet and Théry 2007). We specified a relative receptor density of 1:2:2:4 (ultraviolet:short:medium:long wavelength receptors), used a signal-to-noise ratio yielding a Weber fraction of 0.1, and assumed that noise is proportional to the Weber fraction and independent of the magnitude of receptor stimulation (Vorobyev and Osorio 1998). Finally, we used the CIE 10-degree color matching functions for CIELab modelling. In all cases we normalized receptor stimulation against a leaf-green visual background, and assumed a D65 ‘standard daylight’ illuminant.

### **Statistical Analysis**

*Is intrapopulation variation in flower color constrained to be lower than the perceptual threshold of the dominant pollinators (bees)?*

If a population of flowers is constrained such that all variation is lower than a bees’ visual threshold, then a population should have no flower-flower pairs separated by more than 0.11 hexagon units (Figure 1A). We thus compared all pairwise distances between flowers in each population sampled.

However, analyses of average pairwise distance between flowers might exaggerate the variation perceived by pollinators, if pollinators instead evaluate a given flower based on its similarity to an average flower (i.e., a search image) as opposed to all flowers in the population, including extremes (Figure 1B). Thus we conducted an alternative analysis of pairwise distances between individual flowers and the population centroid, with each population’s centroid calculated by averaging the XY coordinates of all 15 members of the population in the bee hexagon.



We then evaluated whether the data are consistent with strong constraints (Fig 1A), moderate constraints (Fig 1B), or weak or no constraints (Fig 1C) by assessing whether— analogous to the conventional  $P = 0.05$ —95% of the observed distances fall within a given discrimination threshold. Thus, for example, the data for a given population would be judged consistent with moderate constraints imposed by bees if  $< 5\%$  of flower-centroid distances were  $> 0.11$  hexagon units (corresponding to the model depicted in Fig 1B).

*Is apparent intrapopulation variability in flower color greater for non-pollinating animals (humans and birds) than it is for the dominant pollinator group (bees)?*

We chose humans and birds as our representative non-pollinator groups because visual models with behaviorally-validated discrimination thresholds exist for both, and neither are known to pollinate any of the plant species in our dataset (see references cited in Table 2). To test whether apparent floral color variability is higher for non-pollinators than for bees, we first performed calculations of flower-flower and flower-centroid distances using human and avian visual models, as detailed above (note that centroids in receptor-noise limited space were calculated via the population-wise averaging of floral reflectance spectra prior to modelling, for convenience). We then calculated the fraction of comparisons (flower-flower, or flower-centroid) within a population that exceeded the respective discrimination threshold in each of the models and then compared them via Wilcoxon signed-rank tests, implemented in R (R Core Team 2017).

## **Results**

### **Floral color variation in relation to bee discrimination thresholds**

Across all intrapopulation flower-flower comparisons, 89.8% were estimated to be indistinguishable to bees (<0.11 hexagon units), and only 10.2% were discriminable without conditioning (>0.11 units, Figure 3A). For flower-centroid comparisons, 96.9% of all comparisons were estimated to be indistinguishable (< 0.11 hexagon units), with only 3.1% discriminable (>0.11 units, Figure 3B).

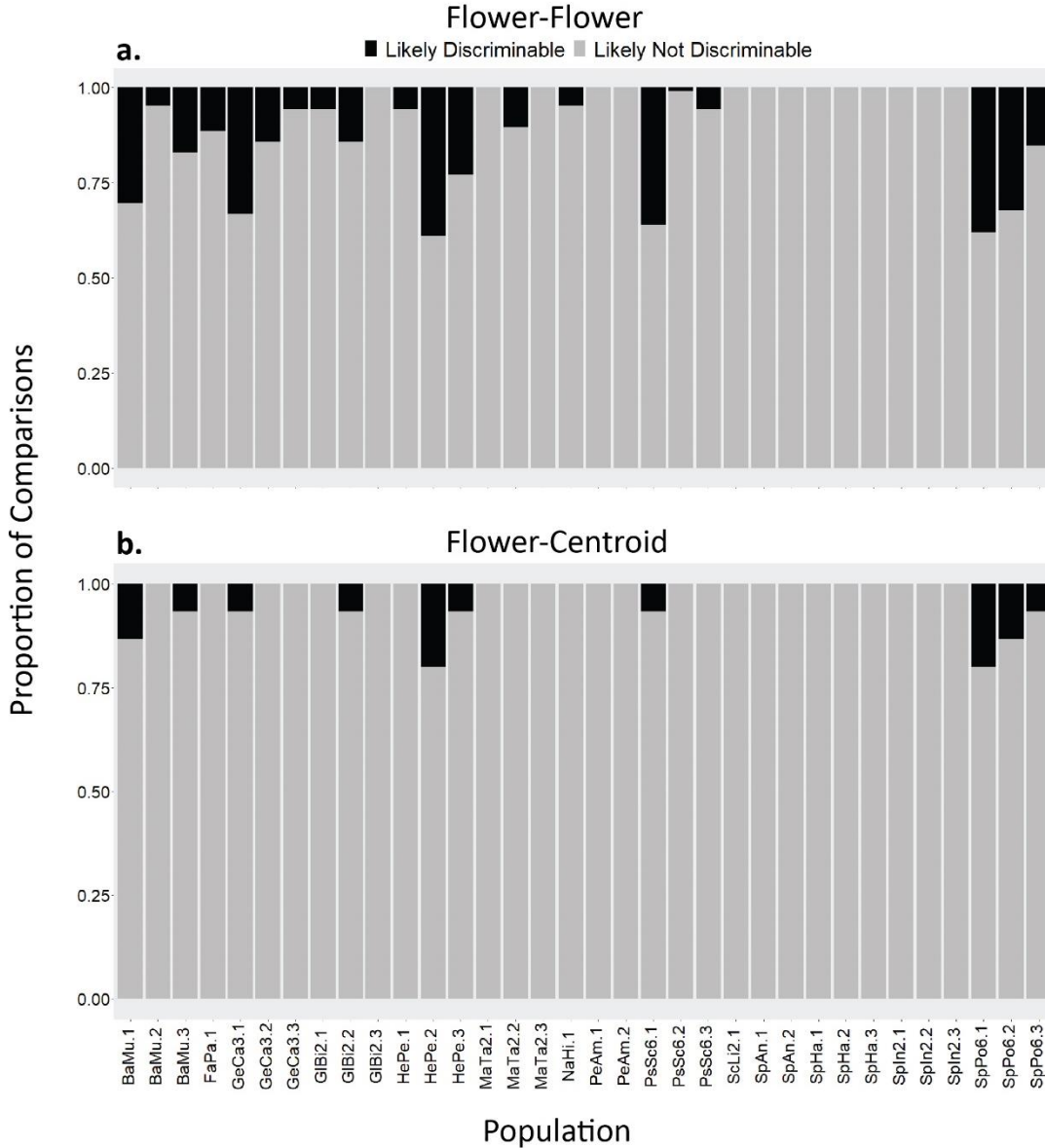


Figure 3. Bee visual space: the proportions of a) distances between flower-flower pairs or b) distances between flowers and their population centroid, in relation to bee discrimination thresholds. Grey represents pairwise comparisons that are likely indistinguishable (<0.11 hexagon units), while black represents pairwise comparisons that are likely distinguishable (>0.11 hexagon units). Data represent 34 populations of 14 New Mexico plant species.

On a population basis, 50.0% of populations (17 of 34) examined had >95% of flower-flower distances fall below 0.11 hexagon units (Fig. 3A), thus meeting our criterion for "strong constraints" (Fig 1A). Another 20.6% of populations (7 of 34) had >95% of measured flowers within 0.11 hexagon units of the centroid (Fig. 3B), thus meeting our criterion for "moderate constraints" (Figure 1B). The remaining 29.4% of populations (10 of 34) had  $\geq 5\%$  of measured flowers at least 0.11 or more hexagon units from the centroid (Fig. 3B), suggesting that constraints were weak or absent (Fig 1C).

### **Floral color variation in pollinator and non-pollinator visual spaces**

In avian visual space, 37.0% of all intrapopulation flower-flower comparisons were estimated to be indistinguishable, while the remaining 63.0% were likely discriminable (Fig 4C). For flower-centroid comparisons, 51.9% were estimated to be indistinguishable, while the remaining 48.1% were likely discriminable (Fig 4D.)

In human visual space, 3.4% of all intrapopulation flower-flower comparisons were estimated to be indistinguishable (<2.3 CIELab units), while 96.6% were likely discriminable (Fig 4E). For flower-centroid comparisons, 5.9% of comparisons were indistinguishable, while the remaining 94.1% were likely discriminable (Fig. 4F).

Intrapopulation floral color variation was significantly more likely to be discriminable for humans than bees in both flower-flower ( $P < 0.0001$ ,  $W = 1156$ ;  $N = 34$  populations) and flower-centroid ( $P < 0.0001$ ;  $W = 1156$ ;  $N = 34$  populations) comparisons. Similarly, intrapopulation floral color variation was significantly more likely to be discriminable for birds than bees (flower-flower comparisons,  $P < 0.0001$ ,  $W = 1082.5$ ,  $N = 34$  populations; flower-centroid comparisons,  $P < 0.0001$ ,  $W = 1080$ ,  $N = 34$  populations).

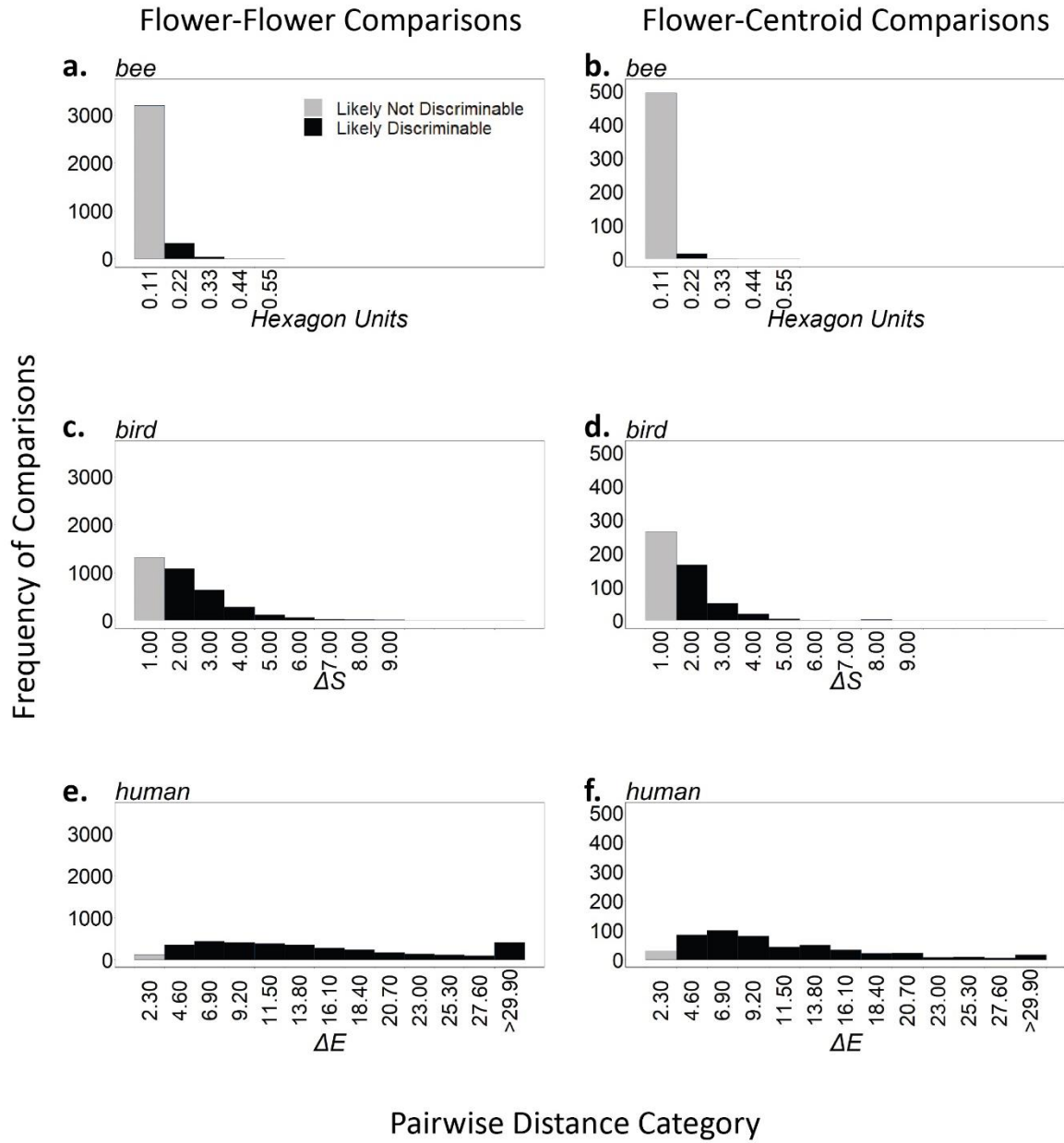


Figure 4. Comparisons of visually-modelled color variation in pollinator and non-pollinator visual spaces for 34 populations of 14 New Mexico plant species. Histograms show the distributions of within-population flower-flower (a,c,e) and flower-centroid (b,d,f) distances. Grey bars represent the counts of pairwise comparisons that are likely indistinguishable. Black bars represent the counts of comparisons that are likely distinguishable, in increasing multiples of units that we best understand to be the minimum discriminable distance for each species. For bees (a,b), the majority of comparisons are indistinguishable, while for birds (c,d) and humans (e,f) the majority of comparisons are distinguishable.

## **Discussion**

### **Implications for the evolution of floral color variation**

Our data suggest that the majority (70.6%) of populations surveyed have levels of intrapopulation color variation that are consistent with moderate or strong constraints imposed by their main pollinators, bees. This is likely because bees reward flowers that are not visually distinct through higher visitation rates (Smithson 2001), with correspondingly lower visitation rates to visually distinct individuals. Such pollinator-generated selection might apply to all populations, even those that are not pollen limited, as increased visitation rates should increase male fitness even when female fitness is unaffected (Stanton et al 1989).

Many populations, however, did contain significant outliers that were estimated to be visually distinct to bees (Figure 3). Further work is required to determine the roles that bees play in possibly selecting against these outliers. Bees may not notice some color variants (Dyer and Chittka 2004; Smithson 2001; Papiorek et al 2013), but do variants that are distinct from average colors actually have lower fecundity because of their color, and if so how do they persist in these populations?

The presence of exceptional individuals suggests that if pollinators do generate constraints on floral color variation (Dyer et al 2012), other factors may moderate the level of constraint. Because pollinator preferences tend to be context-dependent (Hersch and Roy 2007), color variants could persist as pollinator preferences change over the course of years or during the season. It is also possible that we overestimate the importance of pollinators in constraining variability in some environments. Receiving fewer visits may have no effects on female fitness if visitation rates are high (Smithson 2001), which we might expect in resource-poor environments

like the southwestern US deserts. Finally, floral color variation that is detectable to pollinators may ultimately be maintained by factors other than pollinators. Floral color is often pleiotropic (Schoen et al 1984; Rausher 2008) and can covary with traits such as herbivore resistance (Irwin and Strauss 2006) and drought resistance (Vaidya et al 2018; Schemske and Bierzychudek 2001; Warren and Mackenzie 2001), which are expected to be under selection in many populations.

Perhaps stronger evidence consistent with the hypothesis that pollinators constrain floral color variation is our finding that, for the plant species studied, little variability is perceived by bees relative to the extreme variability perceived by animals that play no role in pollination (Fig 4). This finding is consistent with the idea that flowers adapt to the vision of their pollinators (Schiestel and Johnson 2013), as none of the plant species in our study are pollinated by birds or humans. We note that human vision may be useful in a wide variety of contexts as a "non-pollinator" visual system, as primates have rarely been responsible for the direct pollination of flowers (Heymann 2011), except in recent cases of domesticated plants.

### **Implications for the study of flower color**

Our results highlight that large differences exist in perceived floral color variability across different visual systems. While the human visual system has frequently been used to identify species of flowers to investigate for maintenance-of-variation questions (Table 1), it is not a reliable guide to what species appear variable to relevant selective agents (Renoult et al 2017). There are scenarios where our perception of flowers can inform how bees see flowers; for example, *Sphaeralcea polychroma* is variable in both bee and human vision (though less so in the former than the latter, see Figure 5). However, we can also encounter false positives. For instance, *Glandularia bipinnatifida* appears variable in human vision, but bees can see very little of that variability, with 92.6% of flower-flower pairs and 97.7% of flower-centroid pairs

effectively indistinguishable to bees. Perhaps most importantly, we may not recognize relevant variation, e.g. *Helianthus petiolaris* and *Baileya multiradiata* were distinctly variable in bee visual space but relatively invariant to humans. The mismatch between human and other visual systems affects other research areas, and has been highlighted especially in studies of plumage-based avian sexual signalling (Cuthill et al 1999; Eaton 2005; Endler and Mielke 2005).

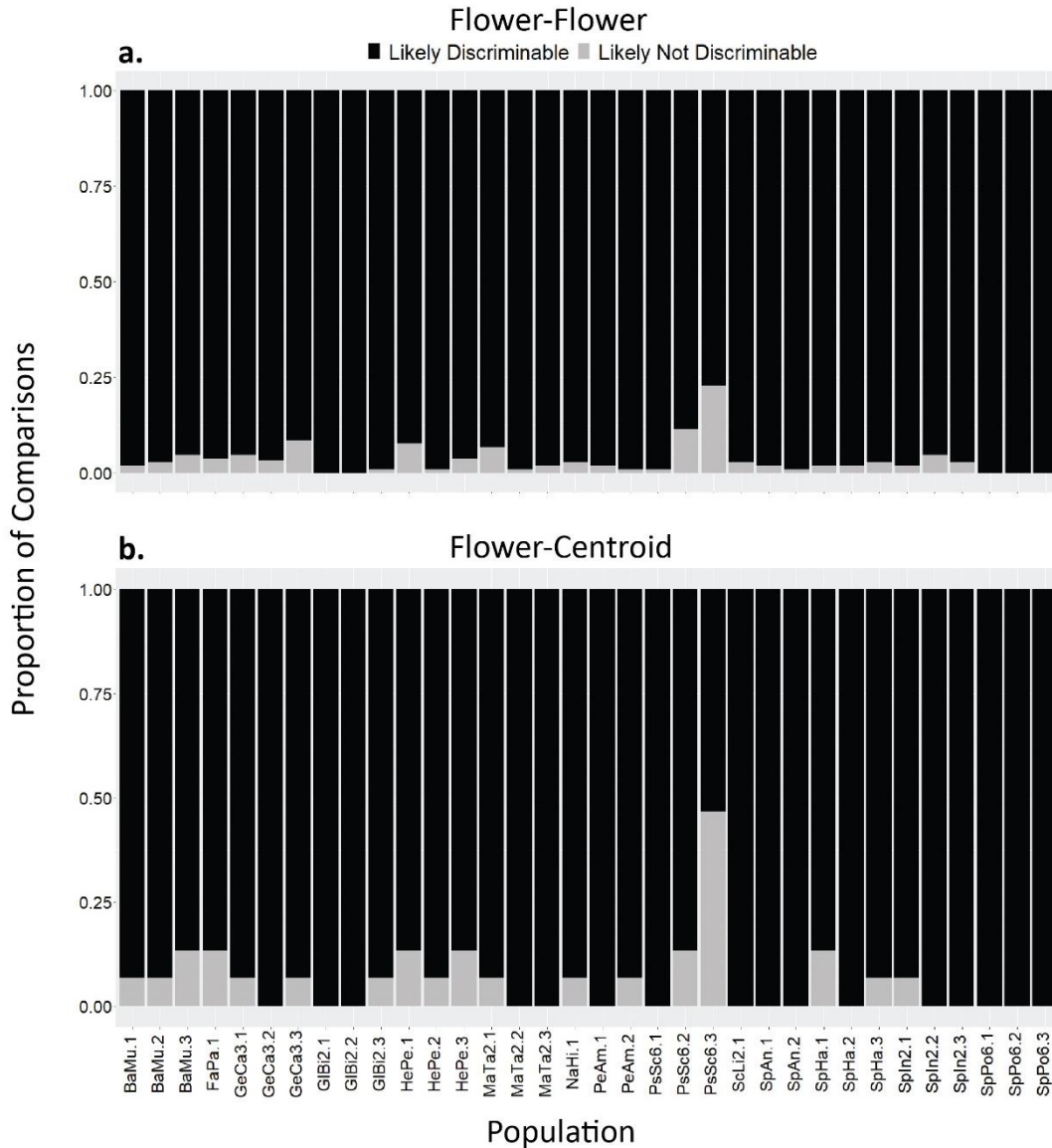


Figure 5. Human visual space: the proportions of a) distance between flower-flower pairs or b) distances between flowers and their population centroid, in relation to human discrimination thresholds. Grey represents pairwise comparisons that are likely indistinguishable ( $<2.3 \Delta E$ ), while black represents pairwise comparisons that are likely distinguishable ( $>2.3 \Delta E$ ). Data represents 34 populations of 14 New Mexico plant species.

Explicit modeling of the distance between flowers in pollinator visual spaces is critical as investigators think about which questions to ask about floral color and in which systems to pursue them. For instance, in cases where a population appears variable to human observers, but much of that variability exists below discrimination thresholds for pollinators, questions about how pollinators shape floral color would be less fruitful research avenues than investigations of alternative biotic (e.g., herbivores, Karageorgou and Manetas 2006) or abiotic factors (e.g., drought, Vaidya et al 2018) as agents of selection on pigmentation. In contrast, studies on variability in cryptically colorful species (to humans) like *Helianthus petiolaris* might reasonably focus on pollinator visitation as a selective force, as individual-level color variation is clearly visible to bees. By measuring floral color variability in pollinator spaces, we can ask better questions about the origin and maintenance of intraspecific variation in plant traits.

A focus on the degree of pollinator-relevant intraspecific floral color variability might also help to explain some patterns from the literature. For instance, both Mu et al (2011) and Gomez (2000) found fitness differences associated with human-perceived color variation (in *Gentiana leucomelaena* and *Lobularia maritima*, respectively), but in those species color variation was also associated with variation in a physical character of floral displays (display size and flower size, respectively). Without knowing whether or not this color variation is visible to pollinators in the first place, it is impossible to determine whether or not differences in pollinator behavior are driven by color or simply by size. Understanding how distinct color differences are to pollinators could help us ask clearer questions about these systems in the future. Further, our results suggest an explanation for the curious fact that many studies of floral color polymorphisms are unable to detect pollinator-mediated selection on color (e.g., Keasar et al 2016; Tang et al 2016), yet often find evidence for non-pollinator-mediated selection (e.g.,



Caruso et al 2010;). This pattern may simply be driven by the fact that the species studied (e.g., Table 1) are a biased subset from nature, emphasizing color variation that is striking to human investigators but is potentially imperceptible to pollinators.

### **Caveats**

Both visual spaces and discrimination thresholds may vary within groups, and may also vary with environmental conditions and context (De Ibarra et al 2014; Dyer 2012; Olsson et al 2017). Given that little of this variation has been explored, our approach necessarily treats groups as monolithic with regard to their color perception. We thus consider our results on pollinator-imposed constraints to be preliminary. However, we note that visual models for bees, birds and humans are among the most well-developed and rigorously tested in existence (reviewed in De Ibarra et al 2014; Fairchild 2013; Olsson et al 2017; Kelber et al 2003), and we argue that for our dataset, the observed stark contrasts in levels of floral color variation perceived by pollinators vs. non-pollinators are unlikely to disappear with future refinements of the visual models.

### **Conclusion**

As has been often hypothesized (Fenster et al 2014; Dyer et al 2012; Papiorek et al 2013), our data are consistent with a scenario in which bees play a role in constraining color variation in species they pollinate. However, not all individual plants fit neatly into these constraints, and it appears that outliers are common. The persistence of these individuals suggests that non-pollinator factors such as drought (Vaidya et al 2018) or herbivory (Irwin and Strauss 2008) may play a major role in the persistence of human-perceived intraspecific floral color variation.

## References Cited

- Bennett ATD, Théry M (2007) Avian color vision and coloration: multidisciplinary evolutionary biology. *Am Nat* 169:S1–S6
- Buchmann SL (1985) Bees use vibration to aid pollen collection from non-poricidal flowers. *J Kansas Entomol Soc* 58:517–525
- Campbell DR, Bischoff M, Lord JM, Robertson AW (2012) Where have all the blue flowers gone: pollinator responses and selection on flower colour in New Zealand *Wahlenbergia albomarginata*. *J Evolution Biol* 25:352–364
- Caruso CM, Scott SL, Wray JC, Walsh CA (2010) Pollinators, herbivores, and the maintenance of flower color variation: a case study with *Lobelia siphilitica*. *Int J Plant Sci* 171:1020–1028
- Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *J Comp Physiol A* 170:533–534
- Cockerell TDA (1900) The New Mexico bees of the genus *Megachile* and a new *Andrena*. *Ann Mag Nat Hist* 6:7–20
- Cockerell TDA (1906) The bees of New Mexico. *T Am Entomol Soc* 32:289–314
- Cuthill IC, Bennett ATD, Partridge JC, Maier EJ (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *Am Nat* 153:183–200
- De Ibarra NH, Vorobyev M, Menzel R (2014) Mechanisms, functions and ecology of colour vision in the honeybee. *J Comp Physiol A* 200:411–433
- Dyer AG (2006) Discrimination of flower colours in natural settings by the bumblebee species *Bombus terrestris* (Hymenoptera: Apidae). *Entomol Gen* 28:257–268
- Dyer AG (2012) The mysterious cognitive abilities of bees: why models of visual processing need to consider experience and individual differences in animal performance. *J Exp Biol* 215:387–395
- Dyer AG, Chittka L (2004) Fine colour discrimination requires differential conditioning in bumblebees. *Naturwissenschaften* 91:224–227
- Dyer AG, Neumeyer C (2005) Simultaneous and successive colour discrimination in the honeybee (*Apis mellifera*). *J Comp Physiol A* 191:547–557
- Dyer AG, Boyd-Gerny S, McLoughlin S, et al (2012) Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision. *P Roy Soc B-Biol Sci* 279:3606–3615
- Eaton MD (2005) Human vision fails to distinguish widespread sexual dichromatism among sexually “monochromatic” birds. *P Natl Acad Sci* 102:10942–10946

- Eckhart VM, Rushing NS, Hart GM, Hansen JD (2006) Frequency-dependent pollinator foraging in polymorphic *Clarkia xantiana* ssp. *xantiana* populations: implications for flower colour evolution and pollinator interactions. *Oikos* 112:412–421
- Endler JA, Mielke PW (2005) Comparing entire colour patterns as birds see them. *Biol J Linn Soc* 86:405–431
- Fairchild MD (2013) Color appearance models, Third edition. Wiley, Chichester, West Sussex
- Fenster CB, Armbruster W, Wilson P, et al (2004) Pollination syndromes and floral specialization. *Annu Rev Ecol Evol Syst* 35:375–403
- Frey FM (2004) Opposing natural selection from herbivores and pathogens may maintain floral-color variation in *Claytonia virginica* (Portulacaceae). *Evolution* 58:2426–2437
- Frey FM, Dunton J, Garland K (2011) Floral color variation and associations with fitness-related traits in *Malva moschata* (Malvaceae). *Plant Spec Biol* 26:235–243
- Fry JD, Rausher MD (1997) Selection on a floral color polymorphism in the tall morning glory (*Ipomoea purpurea*): transmission success of the alleles through pollen. *Evolution* 51:66–78
- Gegeer RJ, Lavery TM (2001) The effect of variation among floral traits on the flower constancy of pollinators. In: Chittka L, Thomson JD (eds) *Cognitive Ecology of Pollination*. Cambridge University Press, Cambridge, pp 1–20
- Gray M, Stansberry MJ, Lynn JS, et al (2018) Consistent shifts in pollinator-relevant floral coloration along Rocky Mountain elevation gradients. *J Ecol* 106:1910–1924
- Gomez JM (2000) Phenotypic selection and response to selection in *Lobularia maritima*: importance of direct and correlational components of natural selection. *J Evol Biol* 13:689–699
- Hannan GL (1981) Flower color polymorphism and pollination biology of *Platystemon californicus* Benth. (Papaveraceae). *Am J of Bot* 68:233
- Heiser CB, Smith DM, Clevanger SB, Martin WC (1969) The North American sunflowers (*Helianthus*). *Mem Torrey Bot Club* 22:1–218
- Hersch EI, Roy BA (2007) Context-dependent pollinator behavior: an explanation for patterns of hybridization among three species of indian paintbrush. *Evolution* 61:111–124
- Hessing MB (1988) Geitonogamous pollination and its consequences in *Geranium caespitosum*. *Am J Bot* 75:1324–1333
- Imbert E, Wang H, Anderson B, et al (2014) Reproductive biology and colour polymorphism in the food-deceptive *Iris lutescens* (Iridaceae). *Acta Botanica Gallica* 161:117–127
- Irwin RE, Strauss SY (2005) Flower color microevolution in wild radish: evolutionary response to pollinator-mediated selection. *Am Nat* 165:225–237

- Jones KN (1996) Fertility selection on a discrete floral polymorphism in *Clarkia* (Onagraceae). *Evolution* 50:71-79
- Jones KN, Reithel JS (2001) Pollinator-mediated selection on a flower color polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). *Am J Bot* 88:447-454
- Joseph N, Siril EA (2013) Floral color polymorphism and reproductive success in annatto (*Bixa orellana* L.). *Trop Plant Biol* 6:217-227
- Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. *Tree Physiol* 26:613-621
- Keasar T, Gerchman Y, Lev-Yadun S (2016) A seven-year study of flower-color polymorphism in a Mediterranean annual plant. *Basic Appl Ecol* 17:741-750
- Kelber A, Vorobyev M, Osorio D (2003) Animal colour vision – behavioural tests and physiological concepts. *Biological Rev Camb Philos* 78:81-118
- Kemp DJ, Herberstein ME, Fleishman LJ, et al (2015) An integrative framework for the appraisal of coloration in nature. *Am Nat* 185:705-724
- LaDuke JC (1985) A new species of *Sphaeralcea* (Malvaceae). *Southwest Nat* 30:433
- Levin DA (1972) Low frequency disadvantage in the exploitation of pollinators by corolla variants in *Phlox*. *Am Nat* 106:453-460
- Levin DA, Kerster HW (1967) Natural selection for reproductive isolation in *Phlox*. *Evolution* 21:679-687
- Lewis MB, Schupp EW (2014) Reproductive ecology of the endangered Utah endemic *Hesperidanthus suffrutescens* with implications for conservation. *Am Midl Nat* 172:236-251
- Maia R, Eliason CM, Bitton P-P, et al (2013) pavo : an R package for the analysis, visualization and organization of spectral data. *Methods Ecol and Evol* 4:906-913
- Maia R, White TE (2018) Comparing colors using visual models. *Behav Ecol* 29:649-659
- Miller RB (1981) Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* 35:763-774
- Mu J, Li G, Niklas KJ, Sun S (2011) Difference in floral traits, pollination, and reproductive success between white and blue flowers of *Gentiana leucomelaena* (Gentianaceae) in an alpine meadow. *Arct Antarct Alp Res* 43:410-416
- Olsson P, Lind O, Kelber A (2017) Chromatic and achromatic vision: parameter choice and limitations for reliable model predictions. *Behav Ecol* 29:273-282

- Ortiz PL, Berjano R, Talavera M, et al (2015) Flower colour polymorphism in *Lysimachia arvensis*: How is the red morph maintained in Mediterranean environments? *Perspect Plant Ecol* 17:142–150
- Papiorek S, Rohde K, Lunau K (2013) Bees' subtle colour preferences: how bees respond to small changes in pigment concentration. *Naturwissenschaften* 100:633–643
- Pellegrino G, Caimi D, Noce ME, Musacchio A (2005) Effects of local density and flower colour polymorphism on pollination and reproduction in the rewardless orchid *Dactylorhiza sambucina* (L.). *Plant Syst Evol* 251:119–129
- R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>
- Rausher MD (2008) Evolutionary transitions in floral color. *Int J Plant Sci* 169:7–21
- Renoult JP, Kelber A, Schaefer HM (2017) Colour spaces in ecology and evolutionary biology. *Biol Rev* 92:292–315
- Schemske DW, Bierzychudek P (2001) Perspective: evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. *Evolution* 55:1269–1282
- Schemske DW, Bierzychudek P (2007) Spatial differentiation for flower color in the desert annual *Linanthus parryae*: was Wright right? *Evolution* 61:2528–2543
- Schiestl FP, Johnson SD (2013) Pollinator-mediated evolution of floral signals. *Trends Ecol Evol* 28:307–315
- Schoen DJ, Giannasi DE, Ennos RA, Clegg MT (1984) Stem color and pleiotropy of genes determining flower color in the common morning glory. *J Hered* 75:113–116
- Shrestha M, Dyer AG, Boyd-Gerny S, et al (2013) Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytol* 198:301–310
- Smithson A (2001) Pollinator preference, frequency dependence, and floral evolution. In: *Cognitive ecology of pollination: animal behavior and floral evolution*
- Stanton ML, Snow AA, Handel SN, Berezky J (1989) The impact of a flower-color polymorphism on mating patterns in experimental populations of wild radish (*Raphanus raphanistrum* L.). *Evolution* 43:335–346
- Tang XX, Liu HL, Zhang C, et al (2016) Flower colour polymorphism in *Geranium nepalense* (Geraniaceae): adaptation to non-pollinator agents. *Pol J Ecol* 64:526–533
- Tyrl RJ, Erteeb FB, Bruner JL, et al (1984) A biosystematic study of the relationship of *Nama hispidum* and *Nama stevensii* (Hydrophyllaceae). *Southwest Nat* 29:367
- Vaidya P, McDurmon A, Mattoon E, et al (2018) Ecological causes and consequences of flower color polymorphism in a self-pollinating plant (*Boechera stricta*). *New Phytol* 218:380–392

- Veiga T, Guitián J, Guitián P, et al (2015) Are pollinators and seed predators selective agents on flower color in *Gentiana lutea*? *Evol Ecol* 29:451–464
- Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. *P Roy Soc B-Biol Sci* 265:351–358
- Warren J, Mackenzie S (2001) Why are all colour combinations not equally represented as flower-colour polymorphisms? *New Phytol* 151:237–241
- Waser NM, Price MV (1983) Pollinator behaviour and natural selection for flower colour in *Delphinium nelsonii*. *Nature* 302:422–424
- White TE, Dalrymple RL, Noble DWA, et al (2015) Reproducible research in the study of biological coloration. *Anim Behav* 106:51–57
- Wolfe LM (2001) Associations among multiple floral polymorphisms in *Linum pubescens* (Linaceae), a heterostylous plant. *Int J Plant Sci* 162:335–342
- Wolfe LM, Sellers SE (1997) Polymorphic floral traits in *Linaria canadensis* (Scrophulariaceae). *The Am Midl Nat* 138:134–139
- Wyszecki G, Stiles WS (2000) Color science: concepts and methods, quantitative data, and formulae, Wiley classics library ed. John Wiley & Sons, New York