

The effect of surrounding bloom color on pan trap success in sampling Rocky Mountain bees

By Matt Turnley

Mentored by: Michael Stemkovski and Rebecca Irwin
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Abstract

On a global scale, bees are important pollinators but face declining populations. In order to monitor the health of bee populations and communities, accurate sampling methods are needed. One common sampling protocol utilizes a combination of pan-trapping and sweep netting. While pan-trapping has its benefits in removing observation bias, there is limited information on how surrounding flower color impacts the success of similarly colored pan traps. We analyzed a long-term data set from the Rocky Mountains (Colorado, USA) to gain a better understanding of the degree to which flower color biases pan trap success. Bees were sampled in 16 sites every two weeks over a nine-year period. Calculating the proportions of bees caught in certain colored pan traps as well as the proportions of certain colored flowers available allowed us to model the relationship between the two factors. We found that similarly colored surrounding bloom color had a small but statistically significant impact on pan trap success in sampling bees, with similar color reducing trap success. Considering this bias, we recommend recording flowering species abundance, richness, and evenness when bees are sampled so that the effects of flower color bias on pan trap success can be controlled statistically in future research.

Introduction

Plant-pollinator interactions provide important ecosystem services. Approximately 90% of angiosperms depend on animals for pollination (Ollerton et al., 2011) and over one-third of global food production is dependent on animal-aided pollination (Klein et al., 2007). Bees, major pollinators in both natural and agricultural systems (Kearns and Inouye, 1997), have experienced considerable declines in population size, particularly in the genus *Bombus* (Cameron et al., 2011), and climate changes are expected to create even greater declines (Goulsen et al., 2015). Little is known about the population trends of solitary bees, which represent the vast majority of bee species, a reality that demonstrates the need for comprehensive bee population studies.

Sufficient sampling methods are necessary to observe and quantify any changes in bee populations. One standardized sampling protocol utilizes a combination of sweep netting and pan traps (The Bee Plot Protocol; LeBuhn et al., 2003), and does not affect bee abundance or community structure when implemented once every two weeks (Gezon et al., 2015). Pan traps, colored plastic bowls filled with soapy water, passively collect insects who drown due to the reduced surface tension. Pan traps are particularly efficient at collecting smaller bodied bees, eliminating observation bias, and do not rely on collector skill (Westphal et al., 2008). However, they often fail to accurately sample larger bees, and may underestimate species richness and abundance when floral resources are high (Roulston et al., 2007, Baum and Wallen, 2011). Little research has been done on how floral color, rather than floral abundance, affects pan trap success when sampling bees. One five-week study in western Utah found no evidence that background bloom color impacted pan trap catch rate (Toler et al., 2005). The same study did observe that some blue trap-preferring bee species (e.g., *Eucera lutziana* and *Anthophora affabilis*) were primarily sampled in areas without surrounding blue flowers. This finding left open the possibility that bees may visit certain traps more frequently when similarly colored flowers are not available, and visit traps less frequently when similarly colored flowers are available.

We performed a similar study using a long-term data set covering a span of years rather than weeks to try and assess the effect of surrounding bloom color on pan trap success in sampling bees. Individual bees often focus their foraging efforts on flower types that have been previously

highly rewarding (Chittka, 2017). Bees may prefer the surrounding, known blooms over a similarly colored but novel pan trap. Anecdotal observations from field workers associated with the long-term data set have also supported that yellow pan traps may have lower success rates when there are higher numbers of yellow flowers. We hypothesized that as the proportion of a certain colored flower rises, the proportion of total pan trap catch for the similarly colored pan traps will fall. Through this research, we hope to reduce pan trap biases, improve interpretations of current sampling methods, and allow for more accurate sampling methods to develop - increasingly important practices as the concerns over pollinator health continue to rise.

Materials and Methods

Data Set and Study Area

As detailed by Gezon et al. (2015), we used a long-term data set monitoring bee and plant phenology created by Dr. Rebecca Irwin (2009-present; all data housed online at <http://irwinbees.com>). Sampling dates ranged from 2009-2017. All sites were located in the Elk Mountains near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado (Fig. 1). The sites ranged from 2496 m – 3428 m in elevation, and were home to at least 190 species/morphospecies of native bees from 33 genera.

Study Sites

Most sites have been sampled since 2009 using the standard Bee Plot Protocol (LeBuhn et al. 2003). 16 sites were used for this research. All sites included two 42 meter transects running approximately perpendicular to each other and six 2x2 meter plots. Each site was sampled once every two weeks. The sites included three common habitat types (dry meadow, wet meadow-willow, and wet meadow-*Veratrum*) around the RMBL, as well as dry meadow sites across an elevation gradient from Almont to Mexican Cut.

Sampling

Pan-trapping: We placed 15 plastic pan traps (6 oz. each) on each transect in the morning. We set one trap every 3 meters following the color pattern of white, fluorescent yellow, fluorescent blue (repeat). Each trap was filled with soapy water (water with a small amount of Dawn Ultra dishwashing soap, original scent) and left at the site for approximately 6 hours per sampling day. Bees remaining in the traps at the end of the sampling day were placed into vials of 70% alcohol aligning with the color of the trap until pinning and identification.

Flower surveys: Within the six 2x2 meter plots at each site, each species of flowering plant in bloom, number of plants per species, and number of flowers per plant of each species were recorded. All blooming flowering species in the site that were not in the 2x2 meter plots were also recorded.

Data Analysis

We calculated the ratio of bees collected in a particularly colored pan trap (e.g., white) relative to the other two pan trap colors (e.g., yellow and blue) for each unique sampling period (unique site/date combination). Using the pan trap colors as a reference, we also calculated the ratio of yellow, blue, and white colored flowers relative to total flowers. Flower colors were decided subjectively, and confirmed using the USDA PLANTS Database when applicable (<https://plants.sc.egov.usda.gov>). In order to analyze the effect of surrounding bloom color on pan trap success, we then compared the two ratios (i.e., trap color catch ratios and flower color ratios) with simple linear regression models. Points were weighted by the total number of bees to account for discrepancies in daily catch success. Two-tailed ANOVA (Analysis of Variance) and Tukey's Honest Significant Difference tests were performed to further interpret the results. We tested mixed effects models with site and date as factors, but the simple linear regression models had the best overall fit (based on AIC values). All data were analyzed using R version 3.5.0 (packages tidyverse_1.2.1 and ggplot2_3.0.0).

Results

We analyzed data from 652 unique sampling periods across 16 sites over a period of nine years. A total of 29,738 bees were caught in pan traps (9,980 in white traps, 12,592 in yellow traps, and 7,166 in blue traps). A total of 274,786 flowers were counted (105,539 white flowers, 118,141 yellow flowers, and 51,106 blue flowers).

We first calculated the proportions of total bee catch in pan traps for the white, yellow, and blue pan traps during each unique sampling period (Fig. 2). On average, white traps accounted for 32.86% of bees caught, yellow traps accounted for 41.90% of bees caught, and blue traps accounted for 25.25% of bees caught. A two-tailed ANOVA test was used to compare pan trap catch proportions by color. A significant difference was found ($F_{2, 1953} = 139.44$, $P = 2.22e-16$), with yellow catching significantly more bees than white and white catching significantly more bees than blue (Tukey's HSD tests: White-Blue; $P < 0.0001$, White-Yellow; $P < 0.0001$, Yellow-Blue; $P < 0.0001$).

We then calculated the proportions of total white, yellow, and blue flowers in the plots for each unique sampling period (Fig. 3). On average, white flowers accounted for 35.50% of flowers in plots, yellow flowers accounted for 42.38% of flowers in plots, and blue flowers accounted for 18.15% of flowers in plots (all other colored flowers accounted for 3.99% of flowers in plots). A two-tailed ANOVA test was used to compare flower color proportions. A significant difference was found ($F_{2, 1953} = 116.98$, $P = 2.22e-16$), with yellow flowers being significantly more common than white and white significantly more common than blue (Tukey HSD tests: White-Blue; $P < 0.0001$, White-Yellow; $P < 0.0001$, Yellow-Blue; $P < 0.0001$).

We also performed a simple linear regression model for each color grouping. Points were weighted by the total number of bees to account for discrepancies in daily catch success (Fig. 4). The proportion of total flowers in plots for each model had a slightly negative and statistically significant impact on the proportion of total pan trap catch (White; Adjusted $R^2 = 0.03$, $F_{1, 650} = 18.23$, $P = 2.25e-05$, Yellow; Adjusted $R^2 = 0.04$, $F_{1, 650} = 25.20$, $P = 6.70e-07$, Blue; Adjusted $R^2 = 0.03$, $F_{1, 650} = 21.12$, $P = 5.18e-06$).

Discussion

Our results showed a significant difference in total pan trap catch proportions between each of the three bowl colors. Yellow pan traps had the highest proportion of total bees caught across the nine years of data, followed by white pan traps, and blue pan traps caught the lowest proportion of total bees caught. These results differ from previous studies analyzing bee trap preference, which showed no significant difference between total bee abundance in pan traps by color (Toler et al., 2005; Wilson et al., 2008). Individual species have been shown to exhibit a preference for certain colored pan traps, which may have influenced our results (Toler et al., 2005; Wilson et al., 2008).

Significant differences were also found between the flower color proportions. Interestingly, flower color proportions followed the same pattern as pan trap catch proportions - yellow flowers had the highest proportion of total flowers in the plots, followed by white flowers, and blue flowers had the lowest proportion of total flowers.

The results of the linear regression models helped to assess the effect of surrounding bloom color on pan trap success in sampling bees. We hypothesized that as the proportion of a certain colored flower rises, the proportion of total pan trap catch for the similarly colored pan traps would fall. The proportion of total flowers in plots for the white, yellow, and blue regressions all had a slightly negative and statistically significant impact on the proportion of total pan trap catch. These results support our hypothesis, and show that surrounding bloom color has a small, but statistically significant impact on the catch rate of similarly colored pan traps.

Individual bees often focus their foraging efforts on flower types that have been previously highly rewarding (Chittka, 2017), so it is possible bees are less attracted to the novel pan traps when similarly colored, known flowers are available. Bees acquire a preferred search image while foraging, which is refined if flowers are distinctive and if the identifying features are uniform (Heinrich, 1974). An increased proportion of similarly colored flowers may help to improve this search image, reducing pan trap success.

Toler et al. (2005) discussed the main conclusions from previous pan trap research, as well as their own findings, with a disparity of results: (1) pan traps may outcompete similar colored flowers (Leong and Thorp, 1999), (2) flowers may outcompete similar colored pan traps (Cane et al., 2000), and (3) surrounding flower color has no impact on pan trap success (Toler et al., 2005). Our results support the findings of Cane et al. (2000), a study which only analyzed *Larrea tridentata*, a desert shrub with yellow flowers. Notably, our yellow color grouping had a higher correlation and more statistically significant results than our white and blue color groupings.

A variety of factors may be leading to the continued discrepancies in results. Height of pan traps, visibility, reflectance, variance in species richness and dominance between different study areas, and other factors may all influence pan trap success, and could all be confounding variables leading to different findings in different studies. Using floral reflectance data to objectively designate flower colors, rather than a subjective determination, may also increase accuracy. More work remains to be done to fully comprehend a question which has drawn such different results. Our work shows that pan traps do appear to be affected by surrounding bloom color, so we recommend the continued use of standard sampling protocols which utilize both sweep netting and pan traps in any further research. Moreover, we recommend recording flowering species abundance, richness, and evenness when bees are sampled so that the effects of flower color bias on pan trap success can be controlled statistically in future research.

Acknowledgements

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Figure Captions

Fig. 1. Locations of the 16 sites sampled from 2009-2017. All sites located in the Elk Mountains near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. Each site included two 42 meter transects running approximately perpendicular to each other and six 2x2

meter plots. The sites included three common habitat types (dry meadow, wet meadow-willow, and wet meadow-*Veratrum*) and ranged from 2496 m – 3428 m in elevation.

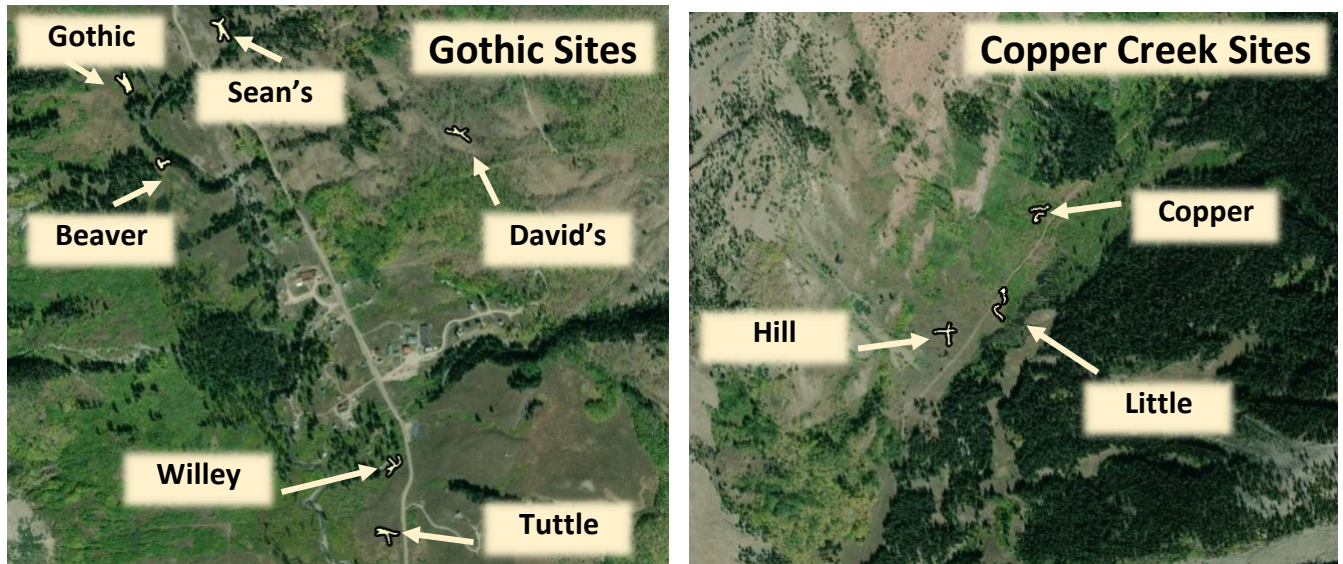
Fig. 2. Proportion distributions of total pan trap bee catch for each color grouping from each unique sampling period across nine years of sampling ($N = 652$). Dashed line represents mean proportion of total pan trap catch. Yellow pan traps had the highest proportion of total bees caught (B), followed by white pan traps (A), and blue pan traps caught the lowest proportion of total bees caught (C).

Fig. 3. Proportion distributions of total flowers in plots for each color grouping from each unique sampling period across nine years of sampling ($N = 652$). Dashed line represents mean proportion of total flowers. Yellow flowers had the highest proportion of total flowers (B), followed by white flowers (A), and blue flowers had the lowest proportion of total flowers (C).

Fig. 4. Simple linear regression model displaying the relationship between surrounding bloom color and pan trap success from each unique sampling period across nine years of sampling ($N = 652$). Points weighted by total catch, as visualized by the model (larger point=higher catch). The proportion of total flowers in plots for the white (A), yellow (B), and blue (C) regressions all had a statistically significant impact on the proportion of total pan trap catch and a slightly negative relationship (White; $R^2 = 0.03$, $F_{1, 650} = 18.23$, $P = 2.25e-05$, Yellow; $R^2 = 0.04$, $F_{1, 650} = 25.20$, $P = 6.70e-07$, Blue; $R^2 = 0.03$, $F_{1, 650} = 21.12$, $P = 5.18e-06$).

Figures

Fig. 1.



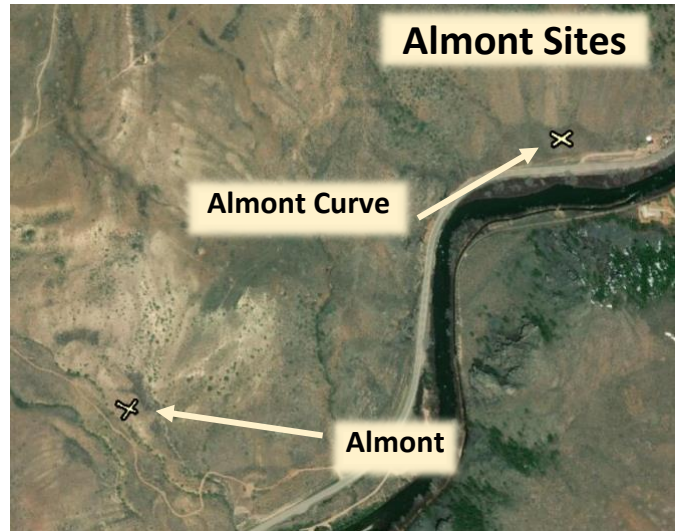
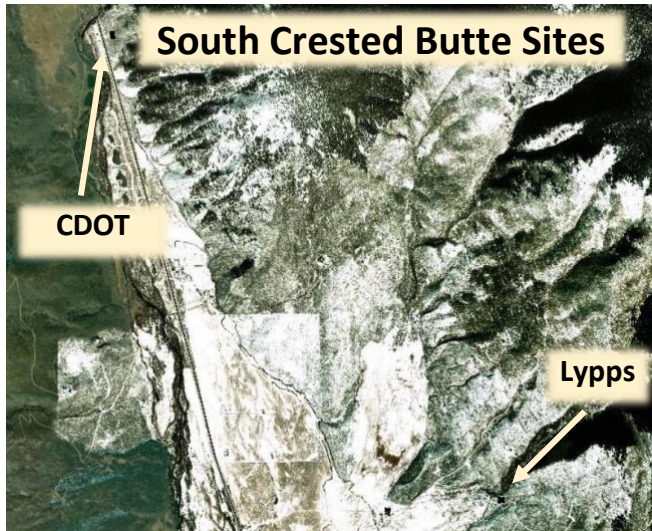
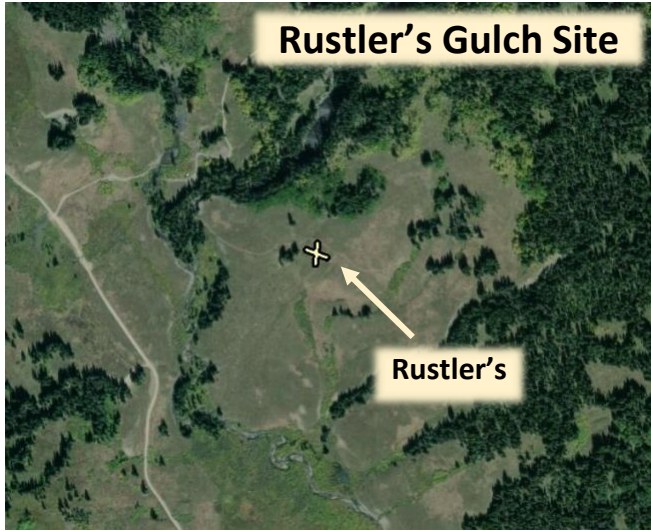


Fig. 2.

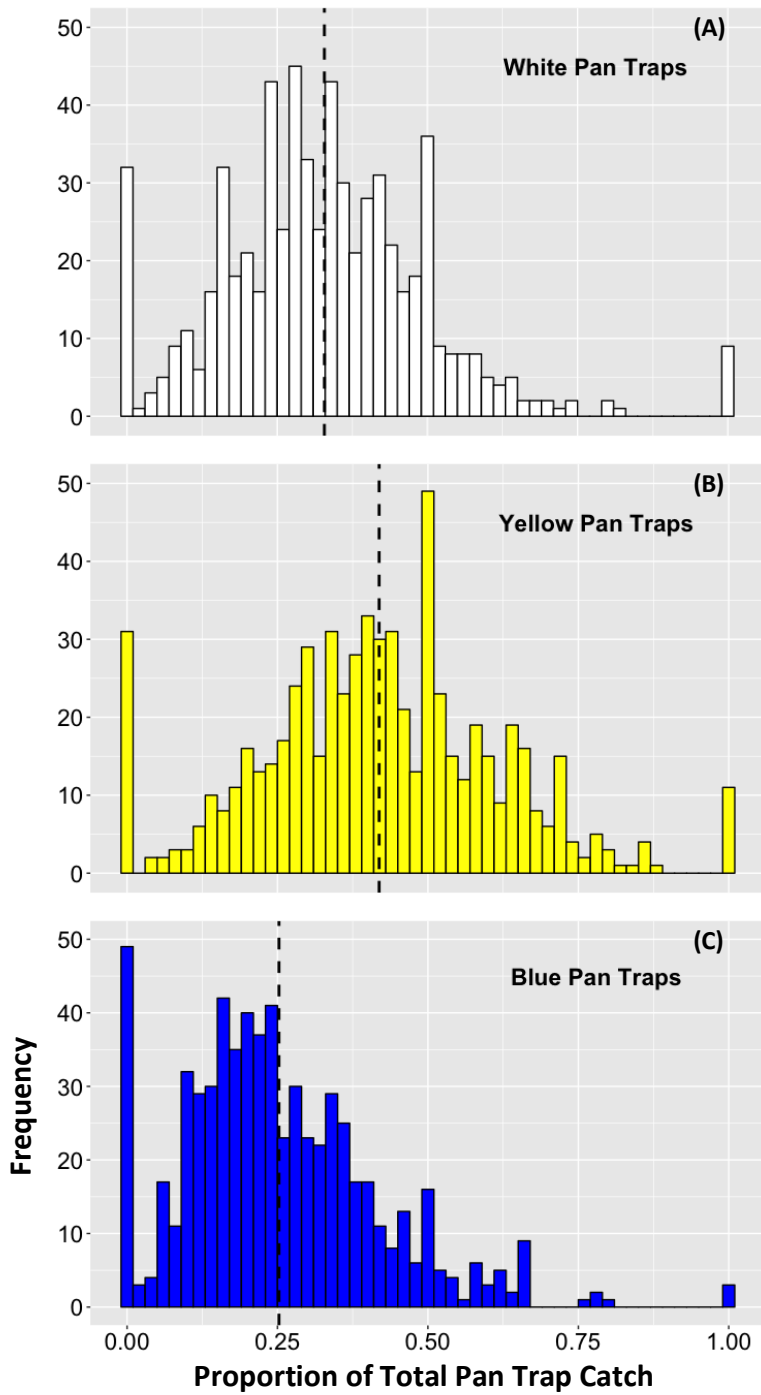


Fig.3

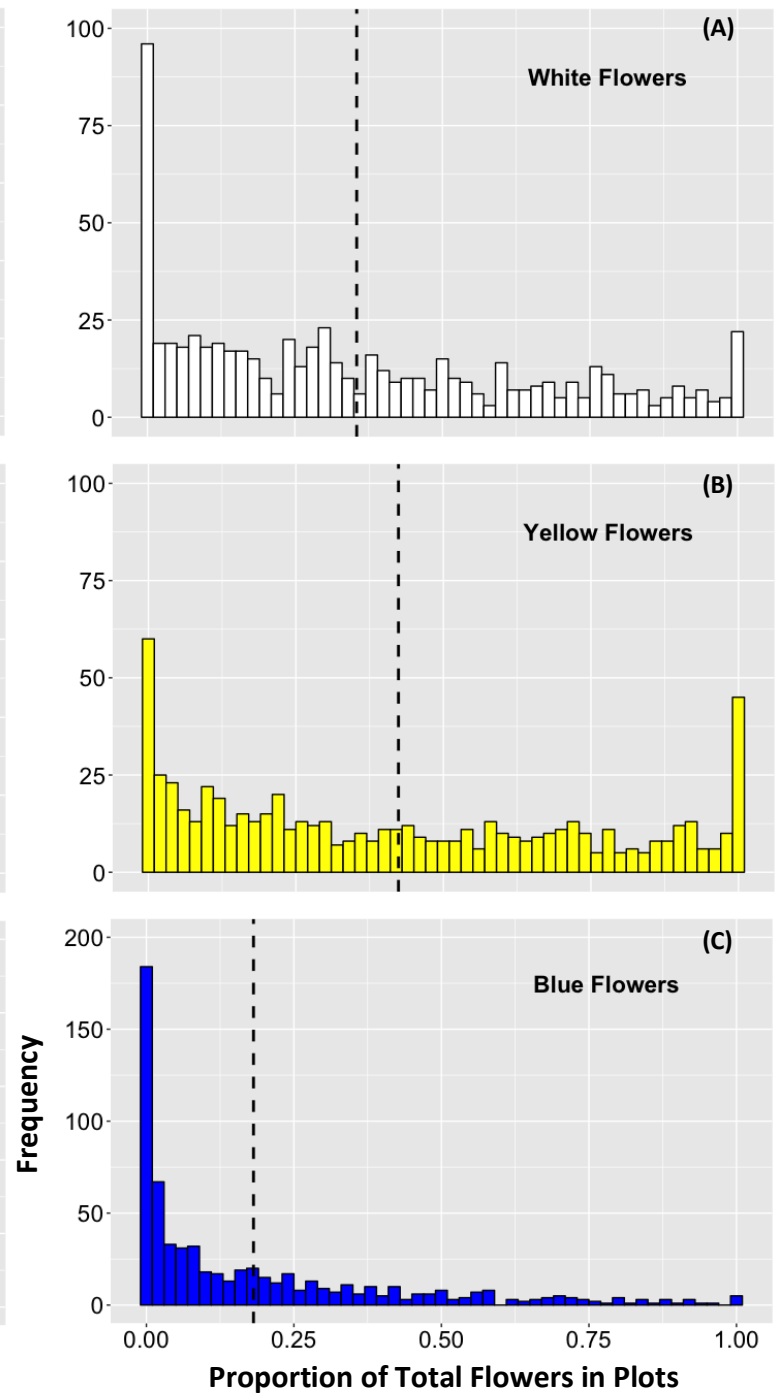


Fig. 4.

