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Using the Unispec to Test the Difference in Reflectance of the Yellow Petals of Encelia californica, Encelia farinosa, and Spartium junceum

Mariam E. Fam
Pepperdine University

Valerie E. Espinoza
Pepperdine University

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Mariam E. Fam & Valerie E. Espinoza

Division of Natural Science, Pepperdine University, Malibu 90263

Abstract

The reflectance spectrum of three yellow flowered species (*Encelia californica, Encelia farinosa, and Spartium junceum*), all grown on the coast of California, was tested to determine if reflectance among the yellow flowers was significantly different. It was hypothesized that there would be a significant difference in reflectance in the 400-700 nm wavebands for the three different species of flowers which all possess yellow petals. Using a Unispec Spectral Analysis System to obtain reflectance spectra for 12 yellow petals of three flowered species, a custom index was created in order to observe differences in the yellow color of the petals, which may account for pollinator attraction and possible natural selection based evolution (N. M. Waser et. al. 1983). The index accounted for a specific range of visible light from approximately 600-700 nm, where the largest reflectance differences occurred among the three tested species. Based on the obtained measurements of the 36 different flower reflection spectra, it was observed that the reflectance for the three different species of yellow flowers was significantly different for not only the created Mariam-Valerie Index, MVI, but also for the Normalized Difference Vegetation Index, NDVI, as well. This difference among similar species may have biological importance, as this significance may account for natural selection based evolution of the flowers which could include specific pollinator selection and increased survival of these particular plants among other yellow flowered species.

Introduction

The mere fact that bees, butterflies, and other insects see something the human eye cannot see has created a sensation around reflectance in certain wavelengths as being particularly meaningful for the receiver. Flower colors compete for niches in the color memory of pollinators, so as to increase the probability of co specific flowers being visited (Chittka & Menzel et al. 1992). For pollen transfer to be discovered from one flower to another of the same species, flowers must be distinguishable from competitors. In order to favor pollinator discrimination, the colors of flowers of different species in a given habitat should theoretically be maximally and equally different from each other. That raised the question of how do flowers which all have yellow petals make themselves distinguishable from competitors? It was hypothesized that there would be a significant difference in reflectance in the 400-700 nm wavebands for the three different species of flowers which all possess yellow petals.

To predict flower color appearance to pollinators requires measurement of spectral reflectance of petals from different flower species, quantifying the proportion of light reflected by the flower at different wavelengths. By testing the spectral reflectance of three yellow colored flowers using a Unispec, differences in reflectance may be used to demonstrate a significant difference, which may account for the relationship between a flower and its pollinator. This produces a reflectance spectrum (Fig. 1), which can provide information on the flower's appearance to an insect and possible pollinator.

The selected species of study for this particular experiment included *Encelia californica, Encelia farinosa, and Spartium junceum*, which all obtain yellow colored petals, and which exhibit an indistinguishable color difference to the human eye. A significant difference in reflectance in the 400-700 nm waveband for the three species of flower may account for the difference seen by pollinators. Any difference in the reflectance spectra for the three yellow flowers may also account for possible changes caused by natural selection in order to enhance the plant's chances for pollination and survival among other yellow colored plants.

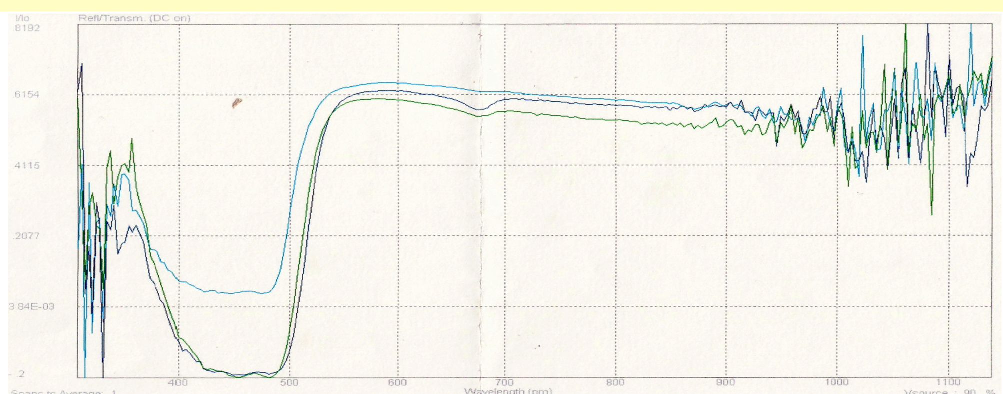

Figure 1. The reflectance spectrum of the three yellow flowers is given as a function of wavelength (colors of lines corresponding to species). The reflectance is the proportion of light at each wavelength reflected by the sample. Spectra were recorded by using a Field Portable Spectrophotometer (Unispec).

Study Sites

The tested flowers of both *Encelia californica* and *Encelia farinosa* were obtained from Solstice Canyon in Malibu, California. The tested flowers of *Spartium junceum* were obtained from the Dana Martel Trail on the Malibu campus of Pepperdine University. Both vicinities were approximately two and a half miles from one another.

Data & Results

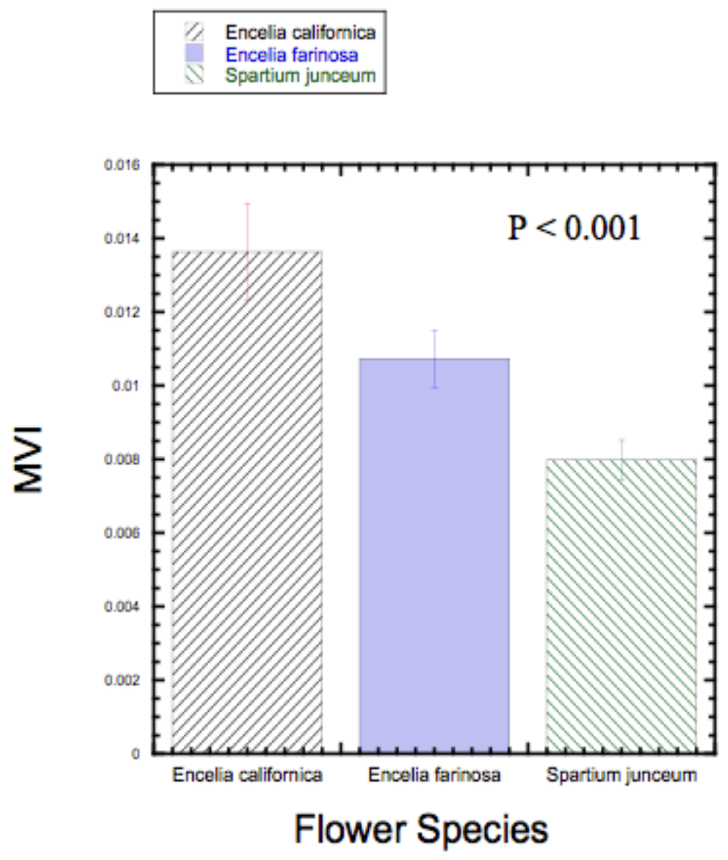

Figure 2. For our created index (MVI) over the indices of 600-620 nm and 660-680 nm, it was determined using a one-way Anova test that the reflectance spectra of all three species were significantly different **(P < 0.001)**.

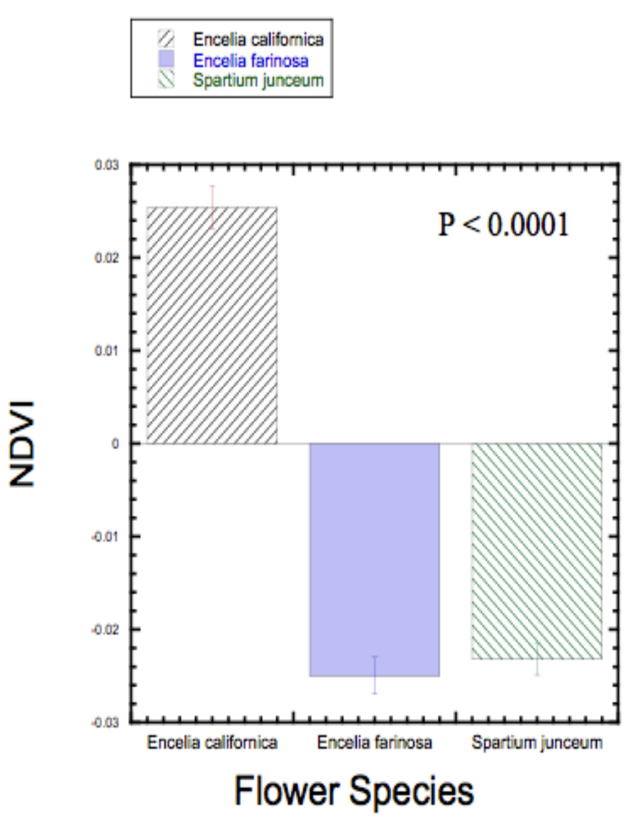

Figure 3. For the NDVI data, it was determined using a one-way Anova test that the reflectance spectra of all three species were significantly different **(P< 0.0001) .**

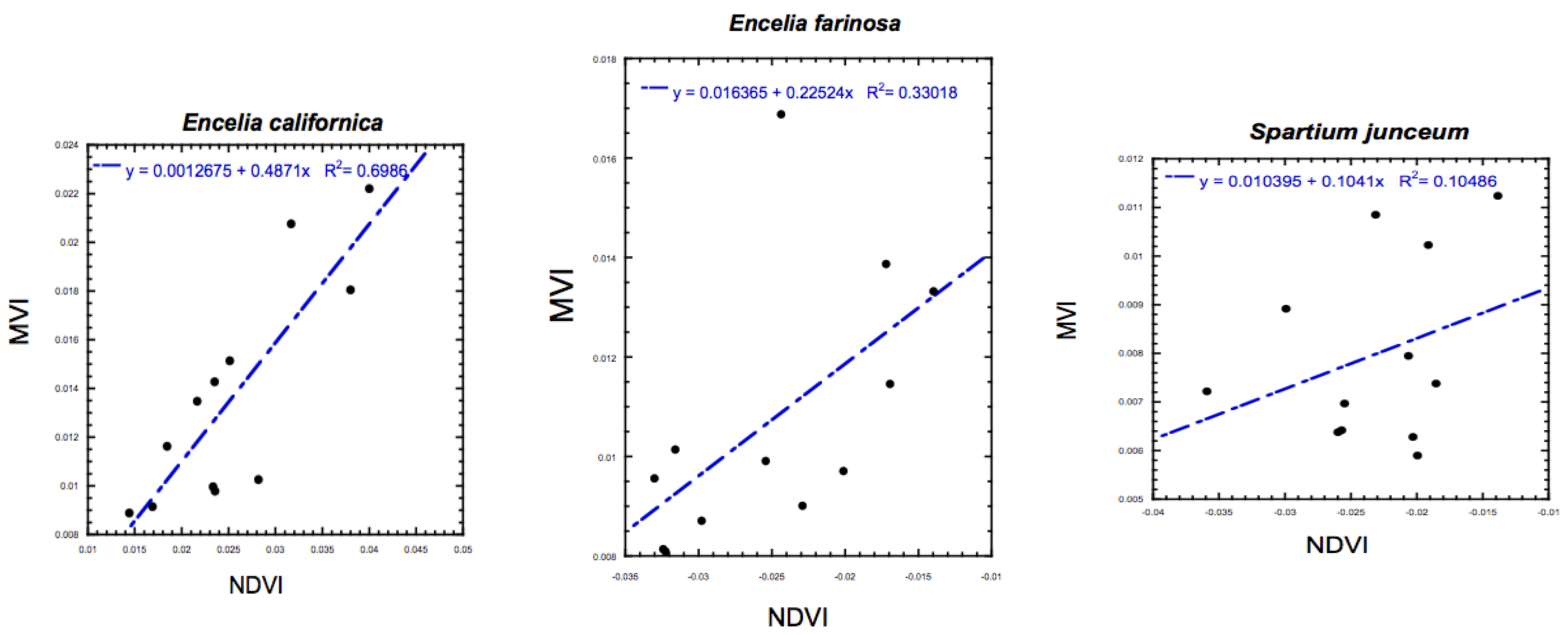

Figure 4, 5, 6 respectively. When testing MVI and NDVI, both the Analysis of Variance Results and the Fisher's Least Significant Difference Comparison indicated that the three species' reflectance spectrum were significantly different.

Acknowledgements

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Materials & Methods

In order to assess the significance of the visible light (400-700 nm) signals in flowers, a large survey of measurements of floral spectral reflectance was taken from the three different species in order to obtain 12 samples of each species. The three different species collected from the study sites to be tested were *Encelia californica*, *Encelia farinosa*, and *Spartium junceum*. The 36 flowers chosen for testing were obtained from plants in the same vicinity of one another and under similar lighting and soil conditions. The flowers were cut and immediately placed in a plastic sample bag and then transferred to a cooler containing ice in order to ensure freshness and vitality for optimal Unispec testing. In a timely fashion, the collected flower samples were brought into the lab and similar petals were chosen and tested using a field portable Unispec Spectral Analysis System to obtain their floral reflectance spectrum. By analyzing the reflectance curves of 12 different petals (n=12) from each species, the properties of the floral spectral reflectance was observed to determine if the spectrum were randomly distributed or if there were distinct types of curves and repeating features in all three species that might indicate some type of function or purpose. From the spectrum, a custom index, the Mariam-Valerie Index, was created after observing distinctively different features in the curves of the three species. Thereafter, the NDVI and MVI was tested and recorded for each of the 36 petals, along with obtaining the reflectance spectrum. The Unispec and computer were recalibrated for all trials and all conditions were kept constant throughout the experiment for the 36 petals in order to ensure no discrepancies. Lastly, the data was graphically analyzed to assess whether there was a significant difference in the yellow petals of the three species.

Discussion

In order to address perceptual niches of pollinators, flowers may have evolved several strategies to be inconspicuous and to stand out from competitors (E. H. Erikson et. al. 1983). The selected species all have similar pollinators from solely bees (*Spartium junceum*), to bees and butterflies (*Encelia californica*), to a variety of pollinators including butterflies, moths, solitary bees, honeybees, and beetles (*Encelia farinosa*) (A. G. Dyer et. al. 2007).

Although all three species' yellow petals are indistinguishable in color to the human eye, it was demonstrated that the three yellow petals are, in actuality, significantly different. Several recent studies, some using hybridization to manipulate flower color in human visible wavelengths (400–700 nm), have provided evidence for the classical idea that there is pollinator preference for certain colors dominated by long wavelengths (e.g., orange and red) (Chittka et al. 2001). Both the orange and red wavelengths exhibited a strong difference throughout the spectrum for the three species tested and were solely focused on during the study with the creation of a custom index. For our created index (MVI) over the indices of 600 nm-620 nm and 660 nm- 680 nm, it was determined using a one-way Anova test (Fig. 2) that the reflectance spectra of all three species (*Encelia californica, Encelia farinosa, Spartium junceum*) were significantly different (P < 0.001). For the NDVI data, also, it was determined using a one-way Anova test (Fig. 3) that the reflectance spectra of all three species were significantly different (P< 0.0001). When testing MVI and NDVI using, both the Analysis of Variance Results and the Fisher's Least Significant Difference Comparison indicated that the three species' reflectance spectrum were significantly different (Fig. 4, 5, 6).

The data implies that since the reflectance spectra of the three species are significantly different from one another there may be a significant correlation to pollinator selection and discrimination. This significant difference among similar species may have a biological significance, as this significance may account for the success of pollination, and therefore overall survival of the plant. It has also been suggested that alternative explanations of evolution of flower color may be accounted for by pleiotropy, phylogenetic constaints, and genetic drift (Chittka et al. 2001).

Conclusion

The reflectance for *Encelia californica, Encelia farinosa,* and *Spartium junceum* was observed to be significantly different from one another even though all petals of the three different yellow flowers appear indistinguishable in color to the human eye. The reflectance spectrum for the three species was significantly different for both the Mariam-Valerie Index and the Normalized Difference Vegetation Index. The data implies that since the reflectance spectra of the three species are significantly different there may be a significant correlation to pollinator selection and discrimination. The observance differences may account for natural selection based evolution of the flowers, which could possibly include specific pollinator selection, and increased survival of these particular plants among other yellow flowered species. This could open a further study that could test the correlation between pollinator perception and reflectance of floral parts, along with evolved genetics.

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