ULTRAVIOLET LIGHT REFLECTION AND ABSORPTION PATTERNS IN POPULATIONS OF RUDBECKIA (COMPOSITAE)

WARREN G. ABRAHAMSON AND KENNETH D. MCCREA

Coevolution has produced intricate relationships between entomophilous flowers and their insect pollinators. These relationships are established by means of one or more floral attractants (visual phenomena, odor, nectar, pollen, etc.). Visual attraction of pollinators involves not only the shape and size of the flower but also its color(s) and the contrast of the flower with its surroundings. In addition, patterns of contrasting color are present in many flowers (nectar guides) and may take a variety of forms such as concentric circles, lines which radiate from the center, or blotches of color. Daumer (1956, 1958) showed that many nectar guides consist of patterns of differential ultraviolet (UV) reflection which are visible to most pollinating insects.

This study examines intraspecific and interspecific variation in the ultraviolet nectar guide patterns in populations of three species of *Rudbeckia*.

MATERIALS AND METHODS

Seven populations of *Rudbeckia hirta* (sensu most auth., not L.; *R. serotina* Nuttall), five populations of *R. triloba* L., and five populations of *R. laciniata* L. (Fernald, 1950) were sampled during their respective blooming periods in 1974. Initially, all ray florets (102) from all capitula (10) of one plant of *R. hirta* were sampled to determine the degree of variability in the percentage of the length of the ray floret reflecting ultraviolet light. This variability was found to be reasonably low (0.483 ± 0.0212 S.D. of all ray florets of 1 plant) making it possible to sample 1 ray floret/capitulum. These florets were collected and placed into glassine envelopes in the field, pressed, and dried. We sampled each population by collecting from 12 to 15 plants and collecting 1 ray floret/capitulum from 1 to 13 capitula. Thus, for each population a mean percent reflecting length was calculated for each plant and these means were then used to calculate a mean percent reflecting length for the local population.

The pattern of ultraviolet reflectance of the ray florets was determined by photographing the florets on 35 mm Kodak Plus-X film using a Kodak 18A filter (UV transmitting). Time exposures were used with an UV light source making it possible to use a regular glass camera lens. The exposed film was developed in Kodak D–19 developer (for high contrast) for 5 minutes at 20° C. The negatives were projected to twice life size and the total length and UV absorbing length of the ray floret were measured to the nearest mm. Because the border between the UV absorbing and UV reflecting portions of the florets is not a straight line, the center of the border was approximated for measurement. This UV reflectance pattern could also be determined using the simpler technique of Eisner *et al.* (1973) but with less precision than our method.

RESULTS

The mean fraction reflecting length of ray florets for each population is given in Table 1 and is shown graphically in Figure 1. *Rudbeckia triloba* had the smallest percentage of ultraviolet reflecting length (33%). *Rudbeckia hirta* was intermediate with 51%. *Rudbeckia laciniata* had the greatest percentage (83%) (see Figure 2). These differences are significant at the 0.001 level as tested by the Mann-Whitney one-tailed *U*-test. The intra-population variation was low in both *R. triloba* and *R. laciniata* but relatively high in *R. hirta*. The inter-population variation was low in *R. triloba* as well as in the Pennsylvania populations of *R. laciniata*. However, there was a marked difference between the Pennsylvania and Connecticut populations of *R. laciniata* ($p < 0.001$, Mann-Whitney one-tailed *U*-test) which increased the standard deviation for the species as a whole. We found more inter-population variation in the Connecticut populations (0.52 ± 0.054 S.D.) of *R. hirta* than in the Pennsylvania populations (0.506 ± 0.0253 S.D.). However, there was no significant difference between the mean of the Connecticut populations and the mean of the Pennsylvania populations.

The mean length of ray florets for each population is given in Table 1. *Rudbeckia triloba* had the shortest ray florets ($\bar{x} = 16.2$ mm) with both low intra-population and inter-population variation. *Rudbeckia hirta* had intermediate length ray florets ($\bar{x} = 25$ mm) with both relatively high intra-population and inter-popu-

Table 1. Means and standard deviations for fraction of UV reflecting length and total length of ray florets for populations of *Rudbeckia*.

Site	Locality (see Collection Data)	Species	N (plants)	*Reflecting length* / Total length (± S.D. -plants)	Total length (mm)
1	PA	*R. hirta*	15	0.50 ± 0.067	27. ± 5.4
2	PA	*R. hirta*	14	0.54 ± 0.079	28. ± 4.3
3	PA	*R. hirta*	12	0.50 ± 0.054	23. ± 3.5
4	PA	*R. hirta*	12	0.48 ± 0.059	27. ± 3.1
5	CT	*R. hirta*	12	0.53 ± 0.073	20.0 ± 2.86
6	CT	*R. hirta*	12	0.47 ± 0.043	22. ± 3.1
7	CT	*R.hirta*	12	0.57 ± 0.050	29. ± 4.6
	R. hirta			0.51 ± 0.037 (± S.D. -populations)	25. ± 3.4
8	PA	*R. triloba*	12	0.314 ± 0.0166	14.7 ± 1.46
9	PA	*R. triloba*	12	0.37 ± 0.043	15.6 ± 1.96
10	PA	*R. triloba*	12	0.330 ± 0.0118	16.3 ± 0.56
11	PA	*R. triloba*	12	0.307 ± 0.0087	17.1 ± 1.49
12	PA	*R. triloba*	12	0.319 ± 0.0122	17.2 ± 0.99
	R. triloba			0.327 ± 0.0228	16.2 ± 1.05
13	PA	*R. laciniata*	12	0.829 ± 0.0256	39.3 ± 2.82
14	PA	*R. laciniata*	12	0.850 ± 0.0170	36. ± 4.2
15	PA	*R. laciniata*	12	0.880 ± 0.0159	34. ± 4.2
16	PA	*R. laciniata*	12	0.840 ± 0.0128	34. ± 4.4
17	CT	*R. laciniata*	12	0.725 ± 0.0228	31.1 ± 0.70
	R. laciniata			0.83 ± 0.059	35. ± 3.0

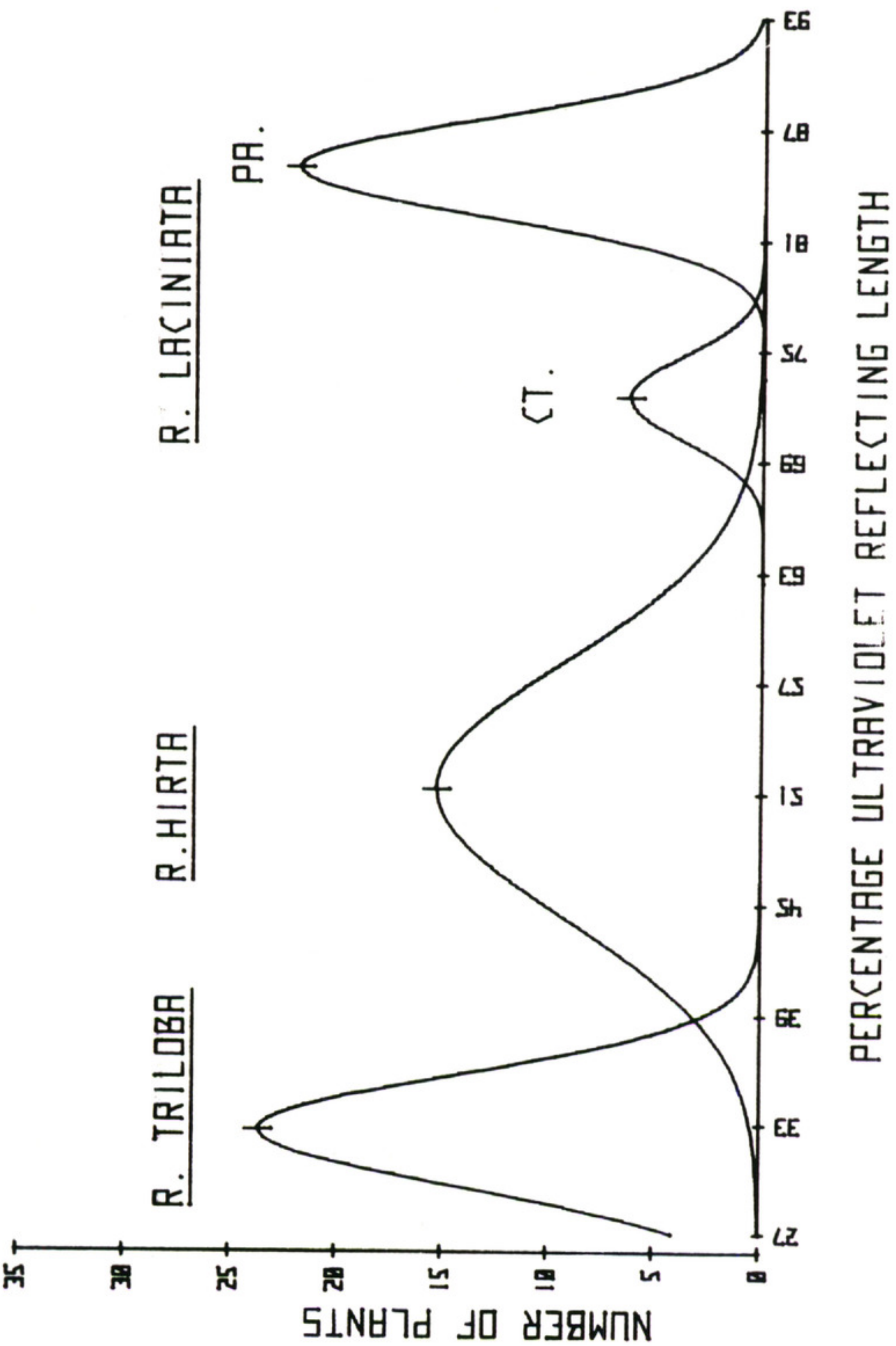

Figure 1. Number of plants plotted on the ordinate as a function of percentage UV reflecting length (plotted on the abscissa) for populations of *Rudbeckia triloba*, *R. hirta*, and *R. laciniata* from Connecticut, and *R. laciniata* from Pennsylvania.

Figure 2. Visible and ultraviolet light reflection and absorption patterns in *Rudbeckia*. Top row, *R. laciniata:* left, visible light, right, UV light. Middle row, *R. hirta:* left, visible light, right, UV light. Bottom row, *R. triloba:* left, visible light, right, UV light. All shown at approximately the same scale.

lation variation. We found more inter-population variation in the Connecticut populations (24. ± 4.7 S.D.) of *R. hirta* than in the Pennsylvania populations (26.3 ± 2.22 S.D.). However, there was no significant difference between the mean of the Connecticut populations and the mean of the Pennsylvania populations. *Rudbeckia laciniata* had the largest ray florets ($\bar{x}$ = 35 mm) with both relatively high intra-population and inter-population variation. The Connecticut population was appreciably different from the Pennsylvania populations for this character.

We tested each population to see if the percentage of ultraviolet reflecting length was related to total length of the ray florets. No correlation was found within the populations. However, we did note a high correlation ($Y = 36.41X + 5.34$, $r = 0.94$, $N = 17$) between the percent reflecting length and total length of ray florets when using the means of all 17 *Rudbeckia* populations. It is evident that the lesser the population UV mean reflecting percentage, the shorter the population mean ray floret length.

DISCUSSION

Color vision in honeybees was demonstrated over sixty years ago (von Frisch, 1915) and the ability of honeybees to perceive ultraviolet light was shown by Hertz (1937a, b, c, 1939). A number of studies has now been made examining UV and color patterns of flowers (e.g. Richtmyer, 1923; Lutz, 1924, 1933; Lotmar, 1933; Mazokhin-Porshnyakov, 1959; Kevan, 1972; Horovitz & Cohen, 1972; Carter, 1974; Guldberg & Atsatt, 1975). We can assume that the various patterns are the result of coevolution of flower and pollinator. However, detailed studies of specific pollinator and flower UV pattern relationships are lacking. Thus it is only possible for us to state that we believe the differences between the three species of *Rudbeckia* are sufficient so as to allow pollinator discrimination, which is based in part on UV pattern.

Our finding of a significant correlation between population mean ultraviolet reflecting percentage and population mean ray floret length suggests a possible evolutionary trend to keep the "bull's-eye" of the capitulum at a relatively small UV absorbing size in the three species examined. Thus, even though the heads of *Rudbeckia laciniata* are large relative to the other two species examined, their UV absorbing "bull's-eye" remains small. However, this finding is not conclusive as many more species of *Rud-*

beckia would need to be examined for this character.

The basis of ultraviolet absorption in *Rudbeckia hirta* has been shown to be due to the presence of flavonol glucosides (Thompson *et al.*, 1972). Based on the similarity of colors and intensity of UV absorption of heads of *R. laciniata* and *R. triloba* to *R. hirta*, it is likely that similar flavonol glucosides are involved in these species as well.

This study further indicates the considerable taxonomic value of ultraviolet floral patterns (e.g., Carter, 1974). Correlation of breeding system with UV reflection should provide interesting results. For example, the limited intra-population variation in the UV nectar guide pattern in *Rudbeckia triloba* and *R. lanciniata* correlates with the apomictic breeding system of these two species (Gustafsson, 1947) and suggests a clonal origin of populations. The greater variation in the UV nectar guide pattern within populations of *Rudbeckia hirta*, an obligate outbreeder, probably indicates that there is only relatively weak stabilizing selection acting on the UV nectar guide pattern.

ACKNOWLEDGMENTS

W. G. A. gratefully acknowledges a Bucknell University scholarly development research grant and K. D. M. gratefully acknowledges a National Science Foundation Undergraduate Research Participation stipend. We both wish to thank Robert Stevens and Morris Levy for their assistance and advice.

LITERATURE CITED

CARTER, A. 1974. Evidence for hybrid origin of *Cercibium sonorae* (Leguminosae: Caesalpinioideae) of Northwestern Mexico. Madroño **22:** 266–272.

DAUMER, K. 1956. Reizmetrische Untersuchung des Farbensehens der Bienen. Zeitschrift für Vergleichende Physiologie **38:** 413–478.

______. 1958. Blumenfarben: wie sie die Bienen sehen. Zeitschrift für Vergleichende Physiologie **41:** 49–110.

EISNER, T., M. EISNER, P. A. HYYPIO, D. ANESHANSLEY, & R. E. SILBERGLIED. 1973. Plant taxonomy: Ultraviolet patterns of flowers visible as fluorescent patterns in pressed herbarium specimens. Science **179:** 486–487.

FERNALD, M. L. 1950. Gray's manual of botany. Van Nostrand Reinhold Company, N. Y. 1632 pp.

FRISCH, K. VON. 1915. Der Farbensinn und Formensinn der Biene. Zoologische Jahrbucher, Abteilung für allgemeine Zoologie und Physiologie **35:** 1–182.

GULDBERG, L. D., & P. R. ATSATT. 1975. Frequency of reflection and absorption of ultraviolet light in flowering plants. Am. Midl. Nat. **93:** 35–43.

Gustafsson, A. 1947. Apomixis in higher plants. Part III. Biotype and species formation. Lunds Universitets Arsskrift N.F. Aud. 2, **43**(12): 183–370.

Hertz, M. 1937a. Beitrag zum Farbensinn und Formensinn der Biene. Zeitschrift für Vergleichende Physiologie **24:** 413–421.

______. 1937b. Verusche über das Farbensystem der Bienen. Die Naturwissenschaften **25:** 492–493.

______. 1937c. Zur Technik und Methode der Bienenverusche mit Farbpapieren und Glasfiltern. Zietschrift für Vergleichende Physiologie **25:** 239–250.

______. 1939. New experiments on colour vision in bees. Jour. Exper. Biol. **16:** 1–8.

Horovitz, A., & Y. Cohen. 1972. Ultraviolet reflectance characteristics in flowers of crucifers. Am. Jour. Bot. **59:** 706–713.

Kevan, P. G. 1972. Floral colors in the high arctic with reference to insect-flower relations and pollination. Can. Jour. Bot. **50:** 2289–2316.

Lotmar, R. 1933. Neue Untersuchungen über den Farbensinn der Bienen, mit besonderer Berucksichtigung des Ultravioletts. Zeitschrift für Vergleichende Physiologie **19:** 673–723.

Lutz, F. E. 1924. Apparently non-selective characters and combinations of characters including a study of ultraviolet in relation to the flower-visiting insects. Ann. N. Y. Acad. Sci. **29:** 181–283.

______. 1933. "Invisible" colors of flowers and butterflies. Nat. Hist. **33:** 565–576.

Mazokhin-Porshnyakov, G. A. 1959. Reflection of ultraviolet rays by flowers and insect vision. Entomol. Rev. **38:** 285–296.

Richtmyer, F. K. 1923. The reflection of ultraviolet by flowers. Jour. Opt. Soc. Am. **7:** 151–168.

Thompson, W. R., J. Meinwald, D. Aneshansley, & T. Eisner. 1972. Flavonols: pigments responsible for ultraviolet absorption in nectar guide of flower. Science **171:** 528–530.

DEPARTMENT OF BIOLOGY
BUCKNELL UNIVERSITY
LEWISBURG, PENNSYLVANIA 17837

Present address, K. D. M.
DEPARTMENT OF BIOLOGICAL SCIENCES
PURDUE UNIVERSITY
WEST LAFAYETTE, INDIANA 47907

APPENDIX: COLLECTION DATA

Rudbeckia hirta

Sites

1 Pennsylvania, on Rte. 44, 2 miles W. of Oval, open field
2 Pennsylvania, Dale's Ridge Area near Lewisburg, open field

3 Pennsylvania, Dale's Ridge Area near Lewisburg, open field
4 Pennsylvania, Dale's Ridge Area near Lewisburg, open field
5 Connecticut, Mt. Carmel Ave., 0.3 mile E. of Rte. 10, Hamden, shaded roadside
6 Connecticut, Brewster Lane, No. Haven, open field
7 Connecticut, Spruce Bank Road, Hamden, open field

Rudbeckia triloba

Sites

8 Pennsylvania, on Rte. 45, 0.5 mile E. of Montandon, open field
9 Pennsylvania, Covered Bridge at Chillisquaque Creek, 2 miles E. of Montandon, partly shaded
10 Pennsylvania, 1 mile SE. of Montandon, open roadside
11 Pennsylvania, 0.1 mile N. of Rte. 192 in Cowan, partial shade
12 Pennsylvania, 0.5 mile N. of Cowan on Shinbone Road, open field

Rudbeckia laciniata

Sites

13 Pennsylvania, River Road, 0.8 mile south of Lewisburg, open
14 Pennsylvania, Covered Bridge at Chillisquaque Creek, 2 miles E. of Montandon, shaded
15 Pennsylvania, 0.1 mile N. of Rte. 192 in Cowan, shaded
16 Pennsylvania, 0.6 mile W. of Cowan on Rte. 192, partial shade
17 Connecticut, Mansfield Road, No. Haven, open