GENETICS OF FLOWER COLOR IN SPIDER FLOWER, *CLEOME HASSLERANA* CHOD.

by

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This thesis is prepared in manuscript style of the Journal of the American Society for Horticultural Science.

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Genetics of Flower Color in Spider Flower, Cleome hasslerana Chod.[1]

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Abstract. Cleome hasslerana Chod., a cross-pollinated species, has five corolla colors: violet, lilac, red, pink, and white. F_1 and F_2 progenies produced from crosses among the cultivars Helen Campbell Snow Crown, Cherry Queen, Pink Queen, and Violet Queen indicate that three loci with two alleles per locus control flower color. The allele W, for colored corolla, is dominant to w, for white corolla; R, for violet color, is dominant to r for red; and I, for dilute flower color, is dominant to i, determining intense flower color.

Cleome hasslerana, a tall, summer-flowering annual, is heat, wind, and drought tolerant and has a large indeterminate inflorescence with a long blooming period. True-breeding cultivars exist for five flower colors: violet, lilac, red, pink, and white. Because there are no reports in the literature describing the inheritance of flower color or method of pollination in C. hasslerana, this study was undertaken.

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[3]Assistant Professor, and Professor, respectively. [2]Graduate Student, Present address: Dept. Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722. The authors wish to gratefully acknowledge the donation of seeds by the Burpee Seed Company, Geo. W. Park Seed Company, and W. J. Unwin, Ltd.

Materials and Methods

Seed of 'Purple Queen', 'Pink Queen', 'Cherry Queen', and 'Helen Campbell Snow Crown' (HC Snow Crown) were obtained from Geo. W. Park Seed Company, Greenwood, South Carolina; 'Rose Queen', 'Ruby Queen', and 'HC Snow Crown' were obtained from Burpee Seed Company, Warminster, Pennsylvania; and 'Pink Queen', 'Cherry Queen', and 'Violet Queen' were obtained from W. J. Unwin, Ltd., Farmingdale, New Jersey. Plants of all cultivars were grown in the greenhouse for comparison of flower colors to the Royal Horticultural Society (RHS) Color Chart, and to make the F_1 crosses. F_1 crosses made were 'HC Snow Crown' X 'Cherry Queen', 'HC Snow Crown' X 'Pink Queen', 'Pink Queen' X 'Violet Queen', 'Pink Queen' X 'Cherry Queen', and 'Cherry Queen' X 'Violet Queen'. F_1 and F_2 progenies were grown in the greenhouse in winter and additional F_1 and F_2 progenies were planted in the field in the summer. All of the progenies were scored for flower color.

Emasculation studies and field observations were made to determine the mode of pollination. Flower buds were esmasculated at different stages of development and either hand-pollinated or left unpollinated. The stigmatic surface was covered with cellophane tape, and the flower was subsequently scored for seed set. Observations on flower structure and development and on insect pollinator activity in the field were also made.

Results and Discussion

'Violet Queen' had a violet flower, hue 72A of the RHS Color Chart; 'Purple Queen' was lilac, 78A; 'Pink Queen' and 'Rose Queen' were pink, 68B; 'Cherry Queen' and 'Ruby Queen' were red, 67B; and 'HC Snow Crown' was white, 155D.

Red corolla. In the cross 'Cherry Queen' X 'Violet Queen' (*WWrrii* X *WWRRii*), all F_1 plants had violet flowers (Table 1). The F_2 progenies were

homogeneous and segregated in a ratio of 3 violet:1 red, indicating segregation for one gene pair, with R determining violet flower color and fully dominant over r for red color. Red flower color being recessive to violet flower color is in agreement with Paris' general genetic scheme for flower color (2).

Intense corolla color. In the cross 'Pink Queen' X 'Cherry Queen' (WWrrII X WWrrii) the F_1 progenies were all pink flowered (Table 1). The observed segregation of the F_2 progenies fit a ratio of 3 pink:1 red, with dilute pigmentation (I) being completely dominant over intense pigmentation (i). The same intensity factor is responsible for lilac flower color, as demonstrated by the 'Pink Queen' X 'Violet Queen' (WWrrII X WWRRii) cross. Here, the F_1 progeny all had lilac flowers and the F_2 segregated in an expected ratio of 9 lilac:3 violet:3 pink:1 red (Table 1). While it is more common for intensity factors to be dominant, there are other instances where intensity is recessive, as in common morning glory, Pharbitis purpurea (1), and China aster, Callistephus chinensis (3).

White corolla. In the cross 'HC Snow Crown' X 'Pink Queen' (wwrrII X WWrrII) all F_1 progenies had pink flowers, and the F_2 progenies segregated in a ratio of 3 pink:1 white (Table 1). This indicates that segregation was occurring only at the W locus, with w for white pigmentation fully recessive to W for coloration. The 'HC Snow Crown' X 'Cherry Queen' (wwrrII X WWrrii) cross resulted in pink-flowered F_1 plants, and the F_2 families segregated in a ratio of 9 pink:3 red:4 white (Table 1). Since the ww genotype has completely white corollas, this genotype is epistatic over the R and I alleles, resulting in four possible true-breeding white genotypes: wwRRII, wwRRii, wwrrII, and wwrrii. From our results the proposed genotype of 'HC Snow Crown' is wwrrII.

Only the five flower colors observed in the cultivars we obtained occurred in the progenies; no intermediate or other shades were seen (Table 1). Although 'Purple Queen' was not used in this series of crosses, its flower color corresponds precisely to that of the W _ R _ I _ genotypes in the F_2 progenies and can be presumed to be of the genotype WWRRII.

Pollination. We determined that C. hasslerana is a predominantly cross-pollinated species on the bases of examination of flower structure and development, emasculation studies, and observation of insect pollinator activity. The anthers do not dehisce until after the flower bud opens, and at no time are the anthers above the stigmatic surface when the flower is in bud (Figure 1). Emasculation of flower buds at different stages of development (Figure 2) resulted in no seed set in the unpollinated flowers and ample seed set in the hand-pollinated flowers.

Sphinx moth [Celerio lineata (Fabricius)] activity was observed at dusk on C. hasslerana grown in the field. Bumblebee [Megabombus pennsylvanicus (De Geer)] and halictid bee [Augochloropsis metallica (Fabricius)] activity was observed during the morning on field-grown C. hasslerana. The flower structure also makes wind pollination possible. Anthers producing abundant pollen are borne on elongated filaments and the pistil is borne on an elongated stipe. In the greenhouse numerous pods containing seed developed despite minimal insect activity.

Determining the inheritance of flower color provides a first step towards development of improved cultivars in Cleome hasslerana. Studies of variations we have observed in plant height, earliness to branch, and earliness to flower are needed to expand the genetic knowledge available to plant breeders who wish to work with this species.

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Table 1. Phenotypes in F_1 and F_2 generations resulting from crosses of four parents of Cleome hasslerana.

Generations (♀ X ♂)	No. of[z] Families	Phenotypes[y] Lilac	Violet	Pink	Red	White	Frequency Expected	χ^2[x]	P
P_1(Helen Campbell Snow Crown)						11			
P_2(Cherry Queen)					6				
$F_1(P_1XP_2)$	8			154					
$F_2(P_1XP_2)$	6			498	167	217	9:3:4	0.08	>.95
P_3(Pink Queen)				12					
$F_1(P_1XP_3)$	9			110					
$F_2(P_1XP_3)$	2			82		26	3:1	0.01	>.90
P_4(Violet Queen)			6						
$F_1(P_3XP_4)$	6	131							
$F_2(P_3XP_4)$	2	52	14	14	6		9:3:3:1	0.90	>.75
$F_1(P_3XP_2)$	6			130					
$F_2(P_3XP_2)$	6			195	69		3:1	0.13	>.50
$F_1(P_2XP_4)$	6		127						
$F_2(P_2XP_4)$	6		147		54		3:1	0.28	>.50

[z]All F_2 families were homogeneous to the expected frequencies.

[y]Lilac = 78A, Royal Horticultural Society color chart; violet = 72A; pink = 68B; red = 67B; white = 155D. Data represents field and greenhouse totals.

[x]Pooled χ^2 values for the expected frequencies.

Figure 1. Anthers are below the stigmatic surface while in the bud. Flower buds at different stages of development with petals removed are: Left, 2 days before flower bud opens; Center, 1 day before opening; and Right, just prior to opening.

THIS BOOK CONTAINS NUMEROUS PAGES WITH PICTURES THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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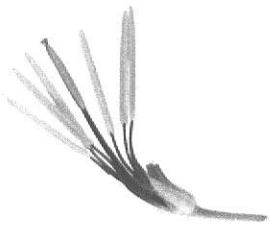
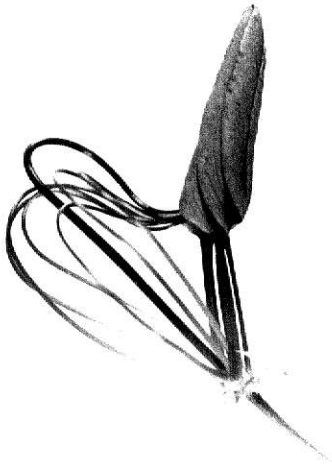


Figure 2. Different stages of flower bud development upon which emasculation studies were performed: Left, 2 days before flower bud opens; Center, 1 day before opening; and Right, just prior to opening.



APPENDIX

Observations Pertaining to Growing *Cleome hasslerana*

Cleome hasslerana is difficult and slow to germinate. Best germination is achieved with diurnal temperatures of 30°C day and 20°C night (1). At these temperatures most fresh seed will germinate in 7-10 days with a small percentage taking 2-3 weeks.

Koevenig (2) reported that *C. hasslerana* is a facultative long day plant which flowers under short days (> 16 hr. darkness) with a mean of 30.6 palmately compound leaves, and flowers under long days (< 10 hr. darkness) with a mean of 18.4 compound leaves. Attempts to grow the F_2 generation during fall 1982 and winter 1983 with incandescent lights providing long days (18:00 - 24:00 hr.) at approximately 10fc of light at plant height failed to induce early flowering. Apparently light intensity was too low.

C. hasslerana can be grown in the greenhouse in a 15 cm pot. This provides a sufficient soil mass to obtain a healthy plant. However, for holding the plant over an extended period of time for breeding purposes, an 11 liter container is better. In the greenhouse *C. hasslerana* is very susceptible to powdery mildew. This is not a serious problem in the field.

Each day a new whorl of flowers opens at dusk. The new flowers rapidly fade in the heat of the next day. The best time to observe flower color and to perform hand pollinations is in the early morning when the flowers and pollen are still fresh. *C. hasslerana* has zones of male-sterile flowers,

female-sterile flowers, and perfect flowers (2,3). When making hand pollinations a female-sterile flower can easily be identified because the stipe of the pistil fails to elongate (Figure 1A). No seed set occurs in pollinated short stipe pistils. Hand pollinations were made by unrolling the unopened flower bud, removing the anthers, applying pollen, and protecting the stigmatic surface with cellophane tape to prevent random pollination (Figure 2A). It takes 5 weeks for seed to mature and be harvested.

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Figure IA. A normal, female-fertile flower (left) of Cleome hasslerana and a female-sterile flower (right) with anthers and petals removed.

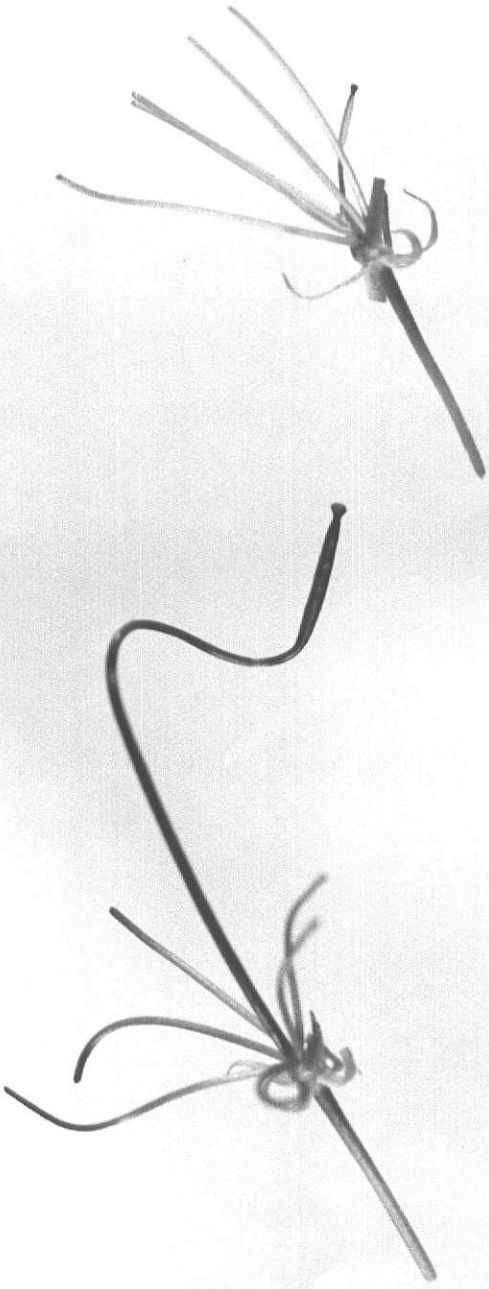
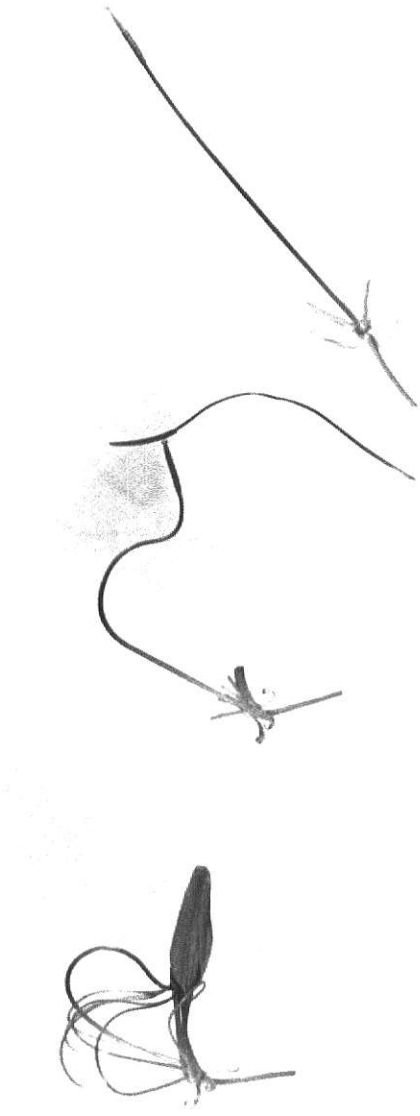


Figure 2A. Hand-pollination sequence of Cleome hasslerana. Stage of flower bud development when hand-pollinated (left); petals removed, anthers removed and pollen applied (center); and stigmatic surface covered with cellophane tape (right).



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