Laboratory Exercise on the Segregation of Flower Color and Related Genes Using Velvet Flower (*Salpiglossis sinuata* Ruiz et Pavon)

Chiwon W. Lee

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SUMMARY. Velvet flower (*Salpiglossis sinuata*, Solanaceae) can be used as an excellent demonstration plant for horticultural crop breeding classes. Salpiglossis produces large trumpetlike flowers exhibiting an assortment of corolla colors and pigmentation patterns. The pistil is large (3 to 4 cm or 1.2 to 1.6 inches long) with a sticky stigmatal tip and flowers can be easily emasculated prior to anthesis. The large pollen grains are shed in tetrads which can be separated and placed on the stigmatal surface. It takes eight to nine weeks from seeding to blooming, with a prolific flowering cycle that comes in flushes. Numerous seeds (about 750 per capsule) are obtained in three weeks after self- or cross-pollination. The influences of three genes that control flower color and pigmentation pattern can be conveniently demonstrated with their dominant and recessive alleles. The *R* gene controls flower color with red (*RR* or *Rr*) being dominant over yellow (*rr*). The *D* gene controls the density of pigmentation with solid (*DD* or *Dd*) color being dominant over dilute (dd) color. Corolla color striping is controlled by the *St* gene with striped (*stst*) being recessive to nonstriped (*StSt* or *Stst*) pattern. By using diploid lines of genotypes *RRDD* (red, solid), *RRdd* (red, dilute), or *rrdd* (yellow, dilute) and their crosses, students can easily observe a dominant phenotypic expression in the F_1 hybrid and the digenic 9:3:3:1 segregation ratio in the F_2 progeny. Another gene (*C*) that controls flower opening can also be used to show its influence on cleistogamous (closed, self-pollinated, *CC* or *Cc*) versus normal chasmogamous (open-pollinated, *cc*) corolla development. In addition, the induction and use of polyploid (4x) plants in plant breeding can also be demonstrated using this species.

A number of annual flowering plants can be used as demonstration materials for plant breeding classes if certain genes and their expressions are readily identifiable. Requirements for such model plants include easily recognizable genetic traits, consistency in phenotypic expression, ease of plant culture and pollination, a clear pattern of genetic segregation in the progeny, abundant seed production, and a short life cycle. One of the most excellent demonstration plants is the velvet flower. Native to Chile, *Salpiglossis sinuata* is a member of Solanaceae, closely related to petunia, and used as an annual flowering garden plant. This plant has many desirable characteristics such as large pistils and anthers, rich corolla color variation, and numerous seeds formed from one pollination.

Department of Plant Sciences, North Dakota State University, Fargo, ND 58105; e-mail: *chlee@badlands.nodak.edu*.

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Table 1. Chasmogamous breeding lines of *Salpiglossis sinuata* available for use in genetic studies (Erickson et al., 1982).

Line	Flower color	Ploidy	Genotype
P-1	Solid red	2x	*ccRRDD*
P-2	Dilute red	2x	*ccRRdd*
P-3	Blue stripes	2x	*ccYYstst*
P-4	Solid yellow	2x	*ccrrDD*
P-5	Dilute yellow	2x	*ccrrdd*
P-6	Solid red	4x	*ccccRRRRDDDD*
P-7	Dilute red	4x	*ccccRRRRdddd*
P-8	Solid yellow	4x	*ccccrrrrDDDD*

This paper describes how salpiglossis can effectively be used in classroom demonstration for the study of reproductive biology and the inheritance of flower color and related traits.

Plant culture

Seeds obtained from self- or cross-pollination can easily be germinated and grown in soil-containing or peat-lite media that is well drained. Usually seeds are sown in soil flats or plug trays. Germination completes in 7 to 10 d. At the two true-leaf stage, seedlings are

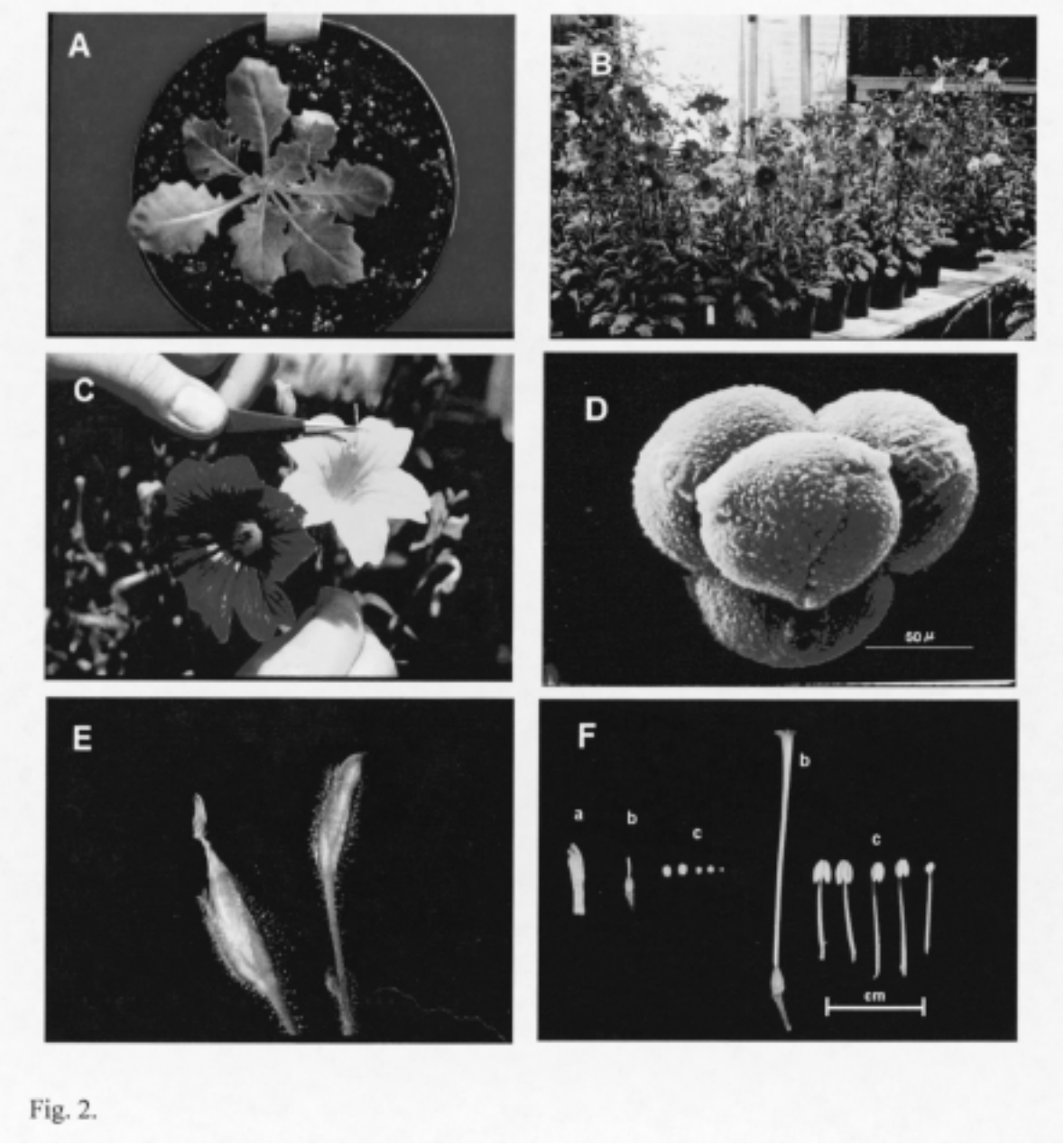

Fig. 1. Growth and flowering in *Salpiglossis sinuata*: (A) a seedling plant (30 d old); (B) flowering plants (60 d old); (C) hand pollination on chasmogamous flowers; (D) four pollen grains shed as a tetrad; (E) cleistogamous flowers at pollination stage (right) and 1 week later (left); (F) reproductive parts of cleistogamous (left) and chasmogamous flowers (right) at pollination stage (*a*-corolla of the cleistogamous flower bud, *b*-pistil, *c*-anthers, corolla of the chasmogamous flower not shown).

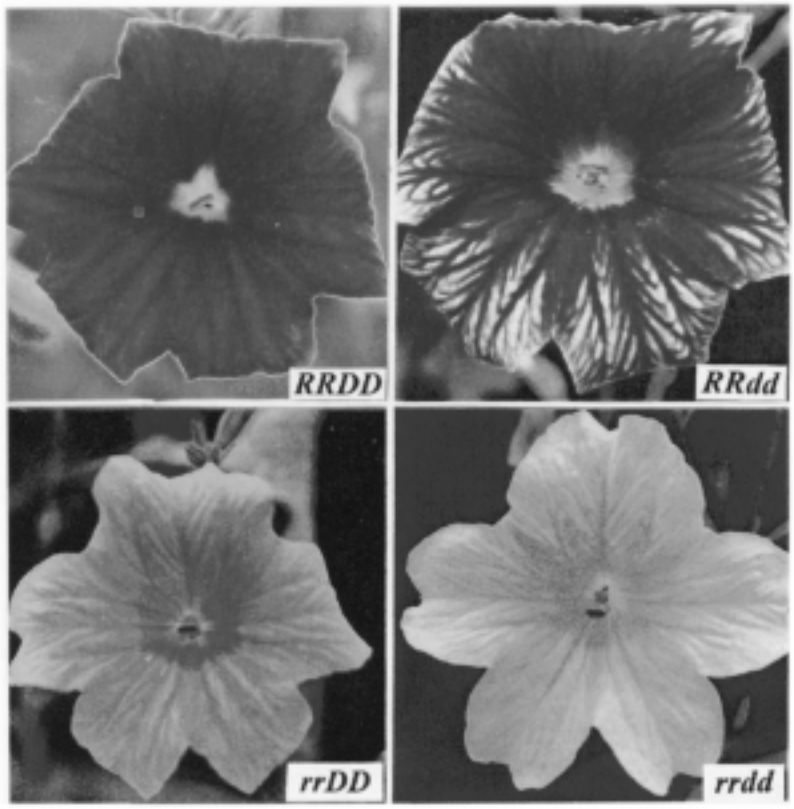

Fig. 2. Four different phenotypes of salpiglossis flowers that can be used in the classroom demonstration.

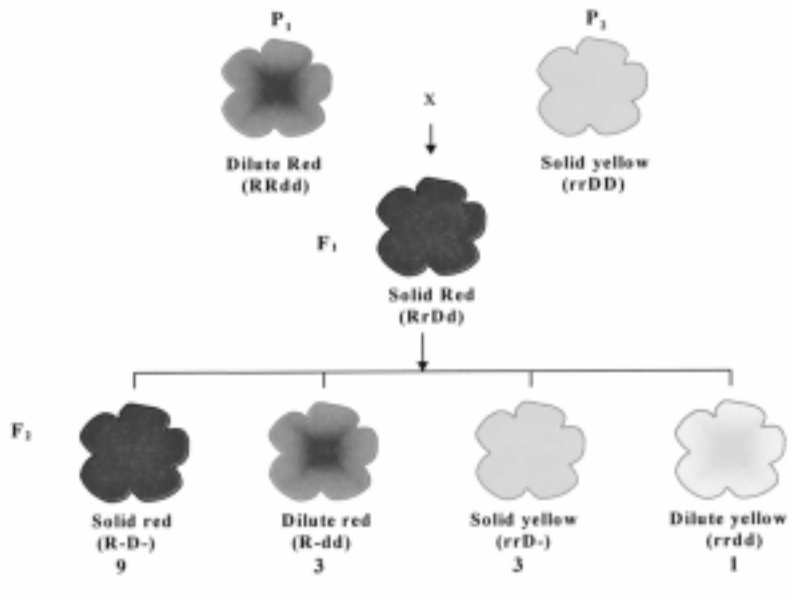

Fig. 4. Inheritance of flower color and color pattern in *Salpiglossis sinuata* controlled by two pairs of genes.

transplanted into a larger container such as 5-cm (6-inch) diameter standard plastic pot. It is recommended that plants be individually staked with bamboo sticks to prevent lodging during the heavy blooming period. Plant height control for salpiglossis can be achieved by using such growth regulators as chlormequat chloride (Cycocel), daminozide (B-Nine SP) and uniconazole (Sumagic) (Needham and Hammer, 1990). At an early stage of growth, plants tend to become rossetted especially under short day conditions. Depending on light intensity, plants start blooming in two months and continue to flower profusely for about one month followed by a cycle of flushes.

Breeding lines

Several inbred lines showing selected flower colors and color patterns are available. Homer T. Erickson and Jules Janick at Purdue University and their graduate students developed several homozygous inbred lines showing distinctive color and color pattern variation for use in genetic studies (Conner and Erickson, 1991; Erickson et al., 1982; Lee et al., 1976, Needham and Erickson, 1992). Both homozygous diploid as well as tetraploid inbred lines that are completely chasmogamous are available (Table 1). In addition, lines that carry a dominant allele for cleistogamy (*CC*, *Cc*) are also available. Fig. 1 shows four different types of flower colors and color patterns.

Classroom demonstration

OBSERVATION ON FLORAL MORPHOLOGY. At the time of flowering, which takes about two months from seed planting (Fig. 2B), students can observe and learn about many different structural parts of the flower and their functions. The chasmogamous flower is pentamerous and the large trumpetlike corolla is bilaterally symmetrical (Fig. 1). The calyx is five-lobed and green in color. Students can easily identify the structure of gynoecium and androecium inside an open corolla (Fig. 1). The pistil is long (3 to 3.5 cm, 1.2 to 1.4 inches) with a gradually flattened stigma which becomes moistened with watery exudate at the time of anthesis. Five two-lobed anthers are in three different sizes (two large, two intermediate, one small) and are borne versatilely on the long (0.8 to 1.5 cm, 0.3 to 0.6 inches) filaments that are attached epipetalously to throat of the corolla. Anthers are easily removed before anthesis. Students can easily separate the structural parts of the flower individually.

Students will learn two different types of flowers: chasmogamous (open-pollinated) and cleistogamous (self-pollinated). The cleistogamous flowers of salpiglossis are tightly closed at the time of pollination which results in autogamy (Fig. 2E). Depending on the genotype, some cleistogamous plants produce chasmogamous flowers at the early

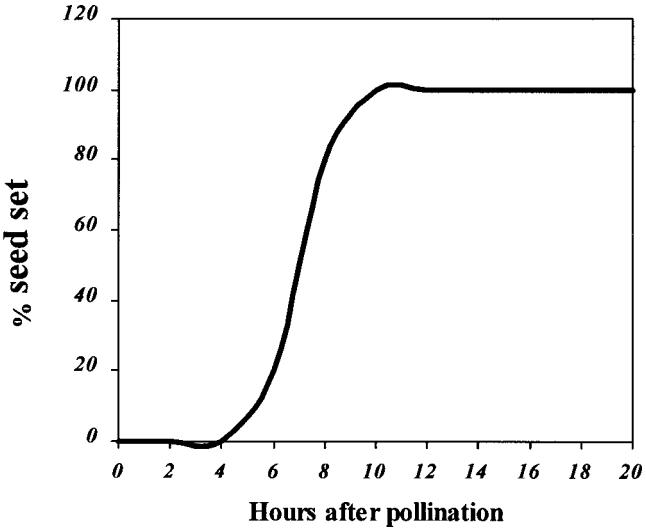

Fig. 4. Percent flowers of salpiglossis producing seed from the pistils decapitated at various time period after pollination.

Table 2. Gene symbols used for flower color and cleistogamy in *Salpiglossis sinuata*.

Symbol	Phenotype
CC or *Cc*	Cleistogamous pollination
cc	Chasmogamous pollination
RR or *Rr*	Red flower color
rr	Yellow flower color
DD or *Dd*	Solid flower color pattern
dd	Dilute flower color pattern

stage of blooming and become cleistogamous later. The morphology and sizes of the structural parts of cleistogamous flowers are drastically different from those of chasmogamous flowers (Fig. 3F).

PRACTICE ON POLLINATION AND FERTILIZATION. Students will use only chasmogamous flowers for pollination and fertilization studies. When the corolla begins to open, the large anthers look puffy and can easily be removed by a pair of tweezers or even fingers. Pollen grains are large (63 to 72 μm in diameter) and shed in tetrads (Fig. 2D). The pollen tetrads are sticky and stay on the top surface of the open anther lobes without dispersal. When grown in the greenhouse, salpiglossis does not normally set seed mainly because of lack of insect vectors. At the time of anthesis, the large stigmatal surface is covered with viscous watery exudate that makes pollen tetrads adhere. Pollen tetrads can be picked up with a camel hair brush and placed on the stigmatal tip during hand pollination. Students can also pick individual anthers with a pair of tweezers or fingers and directly apply pollen on the stigma.

Using a magnifying glass or dissecting microscope, students can easily separate and pick up individual pollen tetrads on the tip of a dissecting needle and place them on the stigma. The number of seeds obtained is directly proportional to the number of pollen tetrads placed on the stigma. However, the minimum number of pollen tetrads that induced seed set was three (Lee et al., 1978). If pollination with one pollen tetrad results in seed set, use of salpiglossis for tetrad analysis in genetic studies may well be possible. Students may experiment to see if a large quantity of sonicated (inactivated) pollen applied to the stigma would help induce seed set from pollination with only one nonsonicated pollen tetrad.

The phenomenon of postpollination ethylene production can be demonstrated by using the pistils after pollination. Ethylene is produced as a result of interaction between the pollen tube and the stylar tissue. Pistils pollinated with increasing numbers of pollen tetrads are harvested and placed inside a sealed beaker. The amount of ethylene evolved from the pistils inside the sealed beaker, when determined by gas chromatography, is directly proportional to amount of pollen tetrads used for pollination.

Students can determine the time needed from pollination to the completion of fertilization in salpiglossis. They can pollinate ten flowers every 2 h for a period of 12 h and then decapitate the stigmatal tips using a razor blade. Seed set from these decapitated pistils is recorded. It usually takes 8 to 10 h for the pollen tubes to reach the ovules and complete double fertilization under normal greenhouse conditions (Fig. 3). The presence of numerous pollen tubes growing through the style can also be visualized by light microscopy after staining the tissue in lacmoid-martius-yellow solution (Nebel, 1931). Pollen tubes appear blue in this stain. Ripened seeds can be harvested 3 weeks after pollination. The average seed set is around 750 per capsule. Seeds stored inside a desiccation jar at 4 °C (39 °F) maintained viability for 3 years.

DEMONSTRATION ON INHERITANCE OF FLOWER COLOR. Using inbred lines of salpiglossis (Table 1), students can learn about the genetics and inheritance of flower color and pigmentation pattern. Three genes that control flower color and pigmentation pattern in salpiglossis have been studied (Table 2). The *R* gene controls flower color with red (*RR* or *Rr*) dominant over yellow (*rr*). The *D* gene affects the corolla pigmentation pattern with solid (*DD* or *Dd*) color dominant over dilute (*dd*) color pattern (Figs. 1 and 4). Although not discussed in this paper, the striping of corolla color is controlled by the *St* gene with striped (*stst*) being recessive to nonstriped (*StSt* or *Stst*) pattern (Conner and Erickson, 1991).

At the beginning of a new semester, the instructor will provide students with seeds obtained from the previous class. If possible, obtain a complete set of seeds for two parental lines (P_1, P_2), their cross (F_1), and its selfed seed (F_2) to make the flower color inheritance study more comprehensive and enjoyable. For example, using the two different parental lines of *RRdd* (red, dilute) and *rrDD* (yellow, solid) and their crosses, students will be able to determine the dominance of *R* and *D* alleles over *r* and *d* in the F_1 hybrid and a digenic segregation ratio of 9:3:3:1 in the F_2 progeny (Fig. 4). The number of plants to be used to warrant the detection of homozygous recessive traits will vary with the number of genes involved. The instructor can cover the topic of minimum population size needed for 0^2 test and genetic analysis for various traits being investigated.

Let students make self- and cross-pollinations and harvest sufficient number of seeds to be used by each class the following year. Students can also make reciprocal backcrosses involving the F_1 and each of the parental lines for use in genetic analysis.

INHERITANCE OF CLEISTOGAMY. The *C* gene in salpiglossis is responsible for the development of cleistogamy (closed pollination or autogamy). In contrast to chasmogamous (*cc*) lines, plants with *CC* or *Cc* exhibit a series of altered developmental conditions that lead to cleistogamous pollination and seed set in a tightly closed flower bud (Lee et al., 1978; Lee et al., 1979). In cleistoga-

Table 3. A sample data showing the segregation of flower color and pattern and cleistogamy in the cross between two homozygous *Salpiglossis sinuata* lines (*CCrrDD* (*ccRRdd*) as demonstrated by Lee et al. (1976).

	Cleistogamous (*C*-)				Chasmogamous (*cc*)							
	Red (*R*-)		Yellow (*rr*)		Red (*R*-)		Yellow (*rr*)		Total			
Generation	Solid (*D*-)	Dilute (*dd*)	Solid (*D*-)	Dilute (*dd*)	Solid (*D*-)	Dilute (*dd*)	Solid (*D*-)	Dilute (*dd*)	plants (no.)	Expected ratio	χ^2	*P*
P_1			105							105		
P_2							105			105		
F_1	108									108		
F_2	252	77	83	30	84	33	22	11	592	27:9:9:3:9:3:3:1	3.20	0.87
$F_1 \times P_1$	168		159							3271:1	0.24	0.65
$F_1 \times P_2$	70	77			70	78				2951:1:1:1	0.75	0.86

mous plants, massive pollen germination takes place inside the anther at the early stage of flower bed development. Pollen tubes grow out of the anther wall, penetrate the stigma and stylar tissues, and quickly complete double fertilization. Cleistogamous plants massively produce seeds under normal greenhouse conditions where pollination vectors are not present.

Since the cleistogamous plants (*CC*, *Cc*) also produce chasmogamous flowers at early stages of flowering, crosses between normal and cleistogamous plants have been possible. Plants possessing the *CC* genotype produce more seeds than those with the *Cc* genotype. The action of the *C* gene is independent of any of the genes involved in flower color determination (Table 4). When cleistogamous plants with solid yellow flower color (*CCrrDD*) and the chasmogamous plants with dilute red flower color (*ccRRdd*) were crossed, the F_1 hybrid plants became cleistogamous with solid red flower color (*CcRrDd*). The F_2 progeny showed a typical trigenic segregation ratio of 27:9:9:3:9:3:3:1 for *C-R-D-*:*C-R-dd*:*C-rrD-*:*C-rrdd*:*ccR-D-*:*ccR-dd* :*ccrrD-*:*ccrrdd* phenotypes (Table 3). The use of these three independent genes for classroom may be too cumbersome since the demonstration requires a large number (>200) of plants in the F_2 population to obtain statistically significant segregation data. However, seeds of these lines have been kept for future use and distribution. The use of the *C* gene in the study of floral morphogenesis may well be pursued by future graduate students.

Discussion

Several well-documented genes, unique floral morphology, and a relatively short growing cycle make salpiglossis an excellent plant for use in plant breeding classes. Several breeding lines of this species have been developed to facilitate genetic studies. There are some drawbacks in growing salpiglossis in the greenhouse, mainly its vulnerability to whitefly (*Trialeurodes* sp.) and glandular secretions that make the plants somewhat sticky. Although not discussed in this paper, salpiglossis can also be used for demonstration in teaching plant regeneration (Lee et al., 1977) and transformation (C.W. Lee, unpublished). Instructors of general botany, introductory horticulture, and plant breeding classes may well take the full advantage of using salpiglossis as a demonstration plant.

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