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Hereditary Variation in *Collinsia concolor*

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ABSTRACT

Five gene loci of *Collinsia concolor* Greene have been identified. (1) The *F* locus, controlling the formation of white markings of cotyledons and leaves, has multiple alleles: *f*, produces no markings; F^p, produces few small dots; F^s, produces numerous large spots; F^pR and F^sR produce small and large red-tinged and/or red-rimmed white dots respectively. (2) Dominant gene *Ld* controls the formation of maroon dots on cotyledons and leaves. (3) Dominant gene *R* controls the development of the reddish-marron midrib of leaves. (4) Semidominant gene *D* controls the dissection of leaf blade. (5) Dominant gene *Ch* controls the formation of hairs on the outer surface of calyx. Floral variants, whose mode of inheritance has not been fully established, include (1) maroon markings (ring, dots, crossline) of the upper lip, (2) violet tipped upper lip, (3) folded upper lip, (4) hairy keel, (5) restricted gland distribution, and (6) glandless.

INTRODUCTION

Rasmuson (1920) was the first investigator of genetic variability and interspecific crossability of *Collinsia* Nutt. (Scrophulariaceae), a native North American genus of 21 species (Abrams 1951). Hiorth (1930, 1934a, b) established the genetic basis for 12 characters of *C. bicolor* (synonym for *C. heterophylla*), and analyzed interspecific hybrids between *C. bicolor* and *C. bartsiaefolia*. Garber (1956) reported the diploid chromosome number for *C. concolor* to be 14. The first interspecific hybridizations involving *C. concolor* were performed by Garber and Gorsic (1956).

The work, reported in this article, was done at the University of Chicago, and was undertaken with an intention to acquire data for the studies of gene homologies and speciation patterns in the genus *Collinsia*.

MATERIALS AND METHODS

The center of natural distribution of *C. concolor*, an insect pollinated species, is the coastal mountains between San Bernardino County and the northern Lower California. Seed of *C. concolor*, used in this investigation, was collected in the vicinity of Sage, south-western Riverside County, California.

Seeds were germinated on moist filter paper in Petri dishes, kept in refrigerator at 10°-15° C for seven days. Germinated seeds were planted directly into 3.5 inch clay pots filled with sandy loam soil (one to two seeds per pot). Eleven hours of light per day for the first six weeks was sufficient. A subsequent raise to 14 hours of light per day promoted flowering if accompanied by an increase in temperature to 25°- 30° C during the light period. Under these conditions, staked plants developed flower buds within 60-70 days.

Removal of anthers (using forceps) from plants, chosen to be used as the maternal parents in crosses, was performed when the upper lip of flowers began to rise. Five days after emasculation the flowers were fully open, and the stigma was dusted with pollen, which has been collected on the brush-like tip of a rolled piece of torn paper toweling. Forty-eight hours after a successful pollination, the corolla wilted and was usually shed. Ripe seeds, collected from spontaneously opened capsules, may be put to germination without delay to start a new crop. Seeds remain viable for 6 to 7 years.

RESULTS

Five gene loci - one of them having multiple alleles - have been identified. The names of characters and the analytical data of hybridization experiments are given in Table 1.

Dotted (F^p) and Spotted (F^s)

In *C. concolor* the *F* locus, controlling formation of the white markings on the upper surface of cotyledons and leaves, showed the same mode of inheritance as the *F* locus in *C. heterophylla*, a closely related species (Gorsic 1957, Hiorth 1930, 1931).

Plants homozygous for dotted (F^p/F^p) produced few small, transient white dots on the cotyledons and leaves (Fig. 1A), whereas plants homozygous for spotted (F^s/F^s) had numerous, large white spots that were recognizable as long as cotyledons and leaves remained green.

Crosses between plants having no white markings (*f/f*) and dotted (F^p/F^p) or spotted (F^s/F^s) plants produced offspring having few small white dots on the cotyledons and leaves. Both hybrids, F^p/f and F^s/f, produced in the F_2 dotted or spotted and pure green plants in 3 : 1 ratios (Table 1).

The distinctness of the F^p and F^s alleles was further supported by the segregational data of the cultures in which the *R* (red-veined, see below) gene was coupled with the F^p allele, and the *Ld* (dark-dotted, see below) gene was coupled with the F^s allele. A cross between an F^pRld/F^pRld and an F^srLd/F^srLd plant produced trihybrid plants (F^pRld/F^srLd) having on the cotyledons and leaves small white dots and maroon dots, and a red vein on their leaves. Plants of the F_2 progenies of these trihybrids having no red vein on the leaves (F^srLd/F^srLd) exhibited large white spots and pronounced maroon dots, whereas plants exhibiting the red vein, F^pRld/F^srLd and F^pRld/F^pRld, had few small white dots (the latter could be distinguished from the former only by the absence of maroon dots).

Red-tinged (F^{pR}, F^{sR})

The white dots on cotyledons and leaves of *C. concolor* were pure white (F^{p}, F^{s}) or red-tinged (F^{pR}, F^{sR}) with the red pigment concentrated especially around the edges forming red-rimmed whitish dots. A cross between a plant with large red-tinged spots (F^{sR}/F^{sR}) and a pure green (*f*/*f*) plant produced F_1 plants having small red-tinged spots. The F_2 progeny of this hybrid (F^{sR}/f) segregated in red-tinged spotted and pure green plants in a ratio of 3 : 1 (Table 1). The F_2 progeny of the F^{pR}/F^{s} hybrid with small red-tinged dots segregated for plants having small red-tinged dots and plants with large pure white spots in a 3 : 1 ratio (Table 1).

The genetic uniformity of the F_1s and the F_2 segregation ratios of four hybrids, involving the *F* locus (listed in Table 1), clearly suggest that the *f*, F^{p}, F^{s}, F^{pR} and F^{sR} are members of the same multiple allelic series.

The complete and incomplete white veins on the cotyledons and leaves of *C. concolor* were less clearly expressed as the white veins (F^{v}, F^{i}, F^{z}) of the cotyledons and/or leaves of *C. heterophylla* (Gorsic 1957). The mode of inheritance of the white veins in *C. concolor* has not been pursued.

Dark-dotted (*Ld*) and Red-veined (*R*)

Plants with the dark-dotted (*Ld*) phenotype exhibited transient maroon dots on the cotyledons and leaves. In plants with the red-veined (*R*) phenotype the basal part of the midrib of leaves was reddish-maroon rather than green. The F_2 progenies of both hybrids, *Ld*/*ld* and *R*/*r*, segregated in typical monohybrid ratios (Table 1). The *Ld* and *R* genes were completely linked with the *F* locus (Table 2). The *Ld* was found only in the coupling phase with the F^{s} and F^{sR}, but the *R* was observed in both the coupling and repulsion phase with the F^{p}.

Dissected leaf (*D*)

C. concolor has an opposite (decussate) leaf arrangement. The leaf blades of the experimental plants were either entire or lobed (dissected). The lobed leaves exhibited a barely recognizable, a shallow, or a deep cut on one or both sides of the blade, or a pair of basal primary lobes without any secondary lobing (Fig. 1A). The lobing was expressed in leaves of the lowermost one to three (rarely four to five) nodes.

Crosses between plants having leaves with a pair of primary lobes (*D*/*D*) and plants having entire leaves (*d*/*d*) produced offspring with variably lobed leaves. The F_2 progenies segregated in 3 : 1 ratios for plants having a pair of primary lobes or variably lobed leaves and plants with entire leaves (Table 1). The dissected leaf (*D*) behaved as a semidominant trait with variable expressivity.

Floral characters

Flowers of *C. concolor* are borne single in the axil of bracts arranged in whorls of two to five on the peduncle. Corolla is 1-1.4 cm long, pentamerous and bilabiate, having a two-lobed upper lip and a three-lobed lower lip. The middle lobe of the lower lip is folded into a keel, which harbors four nonspurred, slightly didynamous stamens and a two-carpellate pistil. The ovary contains 20-25 ovules yielding 15-20 seeds when ripe.

By comparison with the flowers of *C. heterophylla*, the flowers of the experimental plants of *C. concolor* were relatively uniform in morphology and coloration. The upper lip was white to pinkish and, in some plants, violet tipped. Between the cleft of the upper lip and the mouth of corolla tube the following maroon features were observed (Fig. 1B-C): a ring (*Ur*), a group of dots (*Ud*), and a crossline of disjoined dots (*Uc*). The mode of inheritance of these features has not been determined, although it should be pointed out that similar traits are inherited as monogenic dominant characters in *C. heterophylla* (Gorsic 1957).

The lateral lobes of the lower lip were violet with or without a distinctive midvein (veinless). The inheritance of the midveinless in *C. concolor* has not been investigated, but in *C. heterophylla* the veinless (*ll*) was established as a monogenic recessive trait (Hiorth 1930).

Three additional floral variants were observed but not fully investigated: (1) variegated flower, whose lateral lobes of the lower lip were violet-carnea sectored, (2) folded upper lip, whose upper lip lobes were vertically folded backward (Fig. 1B), and (3) hairy keel, whose middle lobe of the lower lip was hairy at the external distal end.

Hairy calyx (*Ch*)

The hairiness of the outer surface of calyx was established as a monogenic dominant trait (Table 1). A similar dominant gene *Ch* is known to control the formation of hairs on the calyx in *C. heterophylla* (Gorsic 1957).

Plants of *C. concolor*, grown for this investigation, exhibited two patterns of glandular hair distribution: (1) glands present on the keel of flowers, the inner and outer surfaces of calyx, and the pedicel, and (2) glands present on the keel, calyx, pedicel, and the peduncle. The mode of inheritance of these two gland distribution patterns in *C. concolor* has not been investigated, but the same two patterns in *C. heterophylla* are known to be controlled by two alleles: *gl*, producing glands on floral parts (as in type 1 above), and gl^2 producing glands on floral parts and peduncle (Gorsic 1957). A dominant gland inhibitor (like *Gi* gene in *C. heterophylla*, Gorsic 1957) is suspect to operate in *C. concolor* as well, but it remains to be proven.

DISCUSSION

The progenies of 60 self-pollinated plants of *C. concolor*, grown from seed of the wild collection, yielded a total of 18 variants; whereas the progenies of 70 self-pollinated *C. heterophylla* plants, grown from seed of the wild collection, yielded more than 50 genetic variants. In the wild both species practice cross-pollination by insects. However, in the greenhouse culture, *C. concolor* plants were noticeably more apt to produce seeds by self-pollination than the plants of *C. heterophylla*. The ability to self-fertilize more readily may be a factor restricting, by some degree of inbreeding, the genetic diversity of *C. concolor*.

One of the more complex loci in the genome of *Collinsia* is the *F* locus. Genes of the *F* locus do not interfere with chlorophyll formation; the mesophyll tissue below the white

markings is green. The white features are brought about by the total reflection of light from structurally changed epidermal layer above them (Hiorth 1931).

In addition to the recessive *f* (null) allele, there are four groups of the dominant *F* alleles (Gorsic 1957, Hiorth 1930, 1931): (1) alleles that control the formation of white dottings: F^f, F^p, F^s; (2) alleles controlling the development of white veins: F^a, F^i, F^v, F^z; (3) alleles regulating the formation of both the white dottings and white veins: F^{pv}, F^{sa}, F^{sv}; and (4) alleles that produce red-tinged and red-rimmed white dottings: F^{pR}, F^{sR}. The F^s allele of *C. concolor*, reported in this article, and F^s allele of *C. heterophylla* (Hiorth 1931) both produce white spots on cotyledons and leaves, but the spots in *C. concolor* appear larger. The F^{pR} and F^{sR} alleles, first reported here, were observed in *C. heterophylla* and *C. tinctoria* as well.

Whether the *F* locus comprises a true multiple allelic series or a group of very tightly linked genes (for dots, veins, pigmentation), cannot be answered at this time; no decisive evidence of recombination between the subunits (genes) of any double superscript *F* alleles (F^{pv}, F^{sa}, F^{pR}, etc.) is available.

The *F* locus is a member of the closely linked *F-R-Ld* gene cluster, whose gene order is not yet fully established. In *C. heterophylla* the *F* and *R* loci are 0.1 crossing-over unit apart (Hiorth 1930), and the recombination between *R* and *Ld* is ca 1.8 percent (Gorsic 1957).

Both F^p or F^s and *Ld* or *R* may be expressed in the same plant; therefore, the dots and spots are not white because of the lack of anthocyanin in cotyledons and leaves (*Ld* and *R* being genetic markers for anthocyanin). The F^p and F^s alleles may be looked upon (1) as the products of recombination by crossing-over between completely linked genes (pseudoalleles) F^{pR} and f^{pr} resulting in F^{pr} (=F^p) and f^{pR}, and recombination between F^{sR} and f^{sr} resulting in F^{sr} (=F^s) and f^{sR} respectively (f^{pr} and f^{sr} being null activity elements, f^{pR} and f^{sR} being ancient dark-dotted genes), or (2) the F^p and F^s may be considered the degenerate F^{pR} and F^{sR} genes, whose pigment regulating function has been completely eliminated by a mutation or by an insertion or removal of controlling elements affecting the *R*-segment of the F^{pR} and F^{sR} genes.

On the other hand, today's F^R (F^{pR}, F^{sR}) alleles may have been derived by the reverse process from revertant F^{pr} and F^{sr} genes in which the pigment producing function of the *r*-segment has been partly or completely restored by similar chromosomal changes described for the anthocyanin marker *R* in corn (Kermicle 1980). Another possible origin of F^R genes could be the f^{pR} and f^{sR} alleles in which the mutation or transposition of controlling elements affected the spotting *p*- and *s*-segments of the f^R genes.

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Table 1. The F_2 phenotypic segregation[a] of characters in *Collinsia concolor*.

Character and Symbol	Genotye	No.of domin.	No. of reces.	Total	Chi-Sq.	P
		Cotyledon and leaf characters				
Dotted, F^p F^p/f	32	12	44	0.031	.8	
Spotted, F^s	F^s/f	87	37	124	1.548	.2
Red-tinged, F^R	F^sR/f	52	13	65	0.867	.4
	F^pR/F^s	26	9	35	0.000	–
Dark-dotted, *Ld*	*Ld/ld*	139	49	188	0.063	.8
		Leaf characters				
Red-veined, *R*	*R/r*	74	20	94	0.695	.3
Dissected, *D*	*D/d*	166	70	236	2.734	.1
		Floral characters				
Hairy calyx, *Ch*	*Ch/ch*	30	9	39	0.008	.9

[a] - Expected ratio 3:1

Table 2. Pairs of genes deviating significantly from independent segregation ratio of 9:3:3:1.

Gene pairs	Segregation	AB	Ab	aB	ab	Total	
F^s/f *R/r*	F_2	29	–	–	9	38	
F^s/f *Ld/ld*	F_2	81	–	–	23	104	
Ld/ld R/r	F_2	29	–	–	9	38	

Figure 1. Hereditary variants of *Collinsia concolor*.
A. Dotted cotyledons and leaves (F^p); Dissected leaves (*D*).
B. Maroon markings on the upper lip: Ring (*Ur*), Dotts (*Ud*), Crossline (*Uc*); Folded upper lip.
C. Upper lip without Ring.

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