Interspecific Hybridization and Karyotype Analysis in Two Species in the Genus *Collinsia*

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The genus *Collinsia* (Scrophulariaceae) includes 22 species, 19 of which are found in the western half of the United States. Most of these species are found in the foothills and low mountain meadows of the western coastal states. Three species *C. violacea*, *C. parviflora*, and *C. verna* are found east of the Mississippi River (Rickett 1966). Several species in the genus have been studied cytogenetically with the first papers appearing over 50 years ago (Hiorth 1930, 1931, 1933a, 1933b). Garber, 1956, grouped species based on chiasmata frequencies and morphological characters into two main categories: I—sessile flowers, variable populations and relatively low chiasmata frequencies; II—pedicelled flowers, uniform populations and high chiasmata frequencies.

Root tip chromosomes are thin and hairlike and therefore not suitable for karyotypic studies. As a result, comparative karyotype studies have been neglected or based on studies of pollen mother cells at metaphase I. Based on these meiotic observations it has been concluded that the various species possess 7 pairs of more or less metacentric chromosomes, one of which is slightly smaller than the remaining six pairs (Garber 1975). Material for hybrid meiotic cytological investigations was obtained from several interspecific hybridization experiments. Past studies have shown considerable chromosomal repatterning and chromosomal formulae have been developed to explain this repatterning based on reciprocal translocations and paracentric inversions (Garber 1975).

Collinsia verna ("blue-eyed Mary") is a spring flowering annual with a scattered distribution from New York on the east coast of the United States, through the midwest and south to northern Arkansas (Rickett 1966). It is an understory herb, often found on floodplains, where it grows in small, discontinuous populations which are widely separated from each other, often by several miles. *C. verna* has not been extensively studied, crossed with other species or genetically investigated. With the exception of its inclusion in group II (pedicelled flowers, presumed uniform populations, "high" chiasmata frequencies) by Garber (1975), no mention is made of it in the extensive *Collinsia* literature.

Collinsia heterophylla, a west coast species, is included in group I by Garber (1956). It grows in rocky mountain meadows of the foothills of the west coast mountain ranges. *C. heterophylla* has been successfully crossed with 9 different

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Collinsia species, failed to cross with 5 others and had not been crossed with *C. verna* (Garber 1975).

The purpose of this investigation was to compare the karyotypes of the two species as well as to hybridize them, to analyze their genetic relationships and to cytologically examine the interspecific hybrids.

Materials and methods

Plants of *C. verna* were field collected when they were two to five centimeters high. They were brought to the greenhouse at the University of Notre Dame where each plant was individually potted in a 10 cm. clay pot with a 1:1 mixture of potting soil and sand. Twelve populations were collected from various locations in Indiana, Illinois and Kentucky. Of these, two, Saint Patrick's (from northern Indiana) and Kentucky (from northern Kentucky), were used in this study. *Collinsia heterophylla* seeds were kindly supplied by Dr. R.W. Allard of the University of California at Davis. These were collected from elevations of approximately 1,500 feet in the area surrounding Davis, California.

"Growlux" fluorescent bulbs were employed at a uniform height of three feet above the bench top to simulate a day length of fourteen hours. Plant populations were assigned bench space using a random numbers table to assure unbiased placement. The greenhouse temperatures were set at 16 ± 5 degrees C during the day and 8 ± 5 degrees C during the night. Day length parameters and temperatures were selected to approximate the natural environment of the species. Individual plants were tied to stakes as necessary to prevent sprawling and all populations were watered every other day. Procedures for germinating seeds, raising plants in the greenhouse and making crosses were the same as those reported in detail by Gorsic (1957) and Greenlee and Rai (1984). Anthers from young flower buds were dissected in 45% acetic acid and squashed in 1% aceto-orcein stain.

Karyotypes were constructed from photographs of the few pre-meiotic mitoses observed in both species. The photographs showing the least chromosomal overlap were traced, cut out, and assembled into karyotypes. Measurements were taken and relative arm lengths were calculated for a comparative analysis.

Interspecific crosses were made between *C. heterophylla* and populations of *C. verna* from northern Indiana and northern Kentucky. Field collected, mature plants of *C. verna* from northern Indiana were used as pollen donors and six plants of *C. heterophylla* were emasculated and hand cross pollinated. *Collinsia heterophylla* and *C. verna* from northern Kentucky were also used in reciprocal crosses involving two plants of each species. Seeds were collected from the cross involving *C. heterophylla* female and *C. verna* male parents. The reciprocal cross was completely sterile and did not yield any seeds. The F_1 seeds were germinated and cytology of two F_1 hybrid plants which lived to flowering stage was done. These hybrids were backcrossed to *C. heterophylla* male plants. Seeds were collected and germination was attempted.

Results

The arm ratios measured in *C. heterophylla* and *C. verna* gave long arm/short arm ratios listed in Fig. 1. The chromosomes were designated 1 to 7 based on their decreasing length. The overall length decreased by 50% from chromosome 1 to 7 in both species. The longest chromosome measured approximately 11 microns and the shortest 5 microns. The centromere in chromosomes 1 and 2 showed large differences in position between the 2 species. The differences seen in the other chromosomes were much smaller. *C. verna* showed chromosomes 1, 2, 4, 5 and 6 approximately metacentric and 7 submetacentric and knobbed. *C. heterophylla* showed chromosomes 2, 3, 4, 5 and 6 approximately metacentric with chromosomes 1 and 7 submetacentric, chromosome 7 possessed a knob also.

Seed set in *C. verna* averaged 2.45 per capsule with a range of 1 to 6 and *C. heterophylla* seed set average 6.9 with a range of 1 to 14. Seed set was extremely low in all interspecific hybrid crosses. Zero to thirteen seeds per plant were collected from crosses involving *C. heterophylla* female and *C. verna* male. Eight F₁ seeds from two plants did germinate, but six died before reaching 5 cm. in height. Two plants lived to flowering and cytology revealed many meiotic abnormalities such as desynapsis shown in Figure 2. Also, at diakinesis two heteromorphic pairs of chromosomes were seen (Fig. 3). Phenotypically, the hybrid plants were small in size, and flower characters were the same as those of *C. heterophylla*.

Seed set in the two backcross plants was somewhat higher than those of the F₁'s. One plant had 8 seeds in one capsule and the other plant had 11, 3, 9, 9, 8, 7, 6, 5, 1 seeds per capsule for a total of 65 seeds. Ten backcross seeds germinated. The one plant that lived to the budding stage was cytologically normal in the few dividing cells observed. No meiotic material was obtained from other backcross plants due to death before reaching the budding stage.

Fig. 1. Karyotypes of *C. verna* and *C. heterophylla* constructed from photographs. A 50% decrease in size from the largest to the smallest chromosome is seen in each species as well as change in centromere position in chromosomes one and two between the species.

Figs. 2-3. 2, desynapsis of chromosome bivalents at metaphase I in pollen mother cells in the hybrid of *C. heterophylla* female $\times$ *C. verna* male. 3, arrows indicate large differences in chromosome size in two bivalents in pollen mother cells from the hybrid of *C. heterophylla* female $\times$ *C. verna* male.

Discussion

The arm ratios of both chromosomes 1 and 2 showed a change in centromere position between the two species. Chromosome 1 is metacentric in *C. verna* and submetacentric in *C. heterophylla*. Chromosome 2 is metacentric in *C. heterophylla* and submetacentric in *C. verna*. Both of these changes in centromere position are probably due to chromosome breakage and an inversion of the segment containing the centromere (Swanson *et al.* 1981). However, the small number of dividing cells observed at prophase I showed no evidence of looping out of one homologue or other anaphase I configurations diagnostic of inversions. The arm ratios were similar but not exactly the same in the remaining chromosomes. These small differences may partially be due to cell to cell variation in degree of chromosome condensation.

The interspecific hybrids between *C. verna* and *C. heterophylla* were neither vigorous nor meiotically normal plants. The predominant abnormality was premature desynapsis at metaphase I. Chromosomes showed effective pairing and chiasmata formation at diplotene (Fig. 3). However, by metaphase I (Fig. 2) most were present as univalents. The differential sizes of the homologues were quite pronounced in 2 bivalents (Fig. 3). Differences in length of the chromosomes could be from DNA duplication and/or deletions (Price 1976, Hinegardner 1976, Chooi 1971) or from loss of chromosomal material following breakage and rearrangement such as inversions (Burnham 1977, Swanson *et al.* 1981). The heteromorphic bivalents (Fig. 3) are visual evidence for changes in chromosome length, and possible changes in the amount of DNA. The amount of DNA in *C. verna* Saint Patrick's and *C. heterophylla* has been previously determined by Feulgen cytophotometry (Greenlee and Rai 1984) to be 28.6 pg. and 10.6 pg. respectively. This difference is larger than can be explained by the observed hybrid chromosomal heteromorphism and could be a manifestation of differences in packing or condensation of the chromatin (Swanson *et al.* 1981).

Interspecific hybridization has been a large area of study in *Collinsia* with 90 of 153 possible combinations previously studied. Meiotic analysis of the species' hybrids has revealed the role of chromosomal repatterning in speciation, including reciprocal translocations and paracentric inversions (Garber 1960). Garber derived chromosomal formulae based on the exchange of terminal chromosomal segments during reciprocal translocations and their patterns at metaphase I. Some of the hybrids previously tested between Group II species were fertile, but the degree of fertility ranged from high seed set and high viability to low seed set and small percentage germination (Bell and Garber 1961). Also, previous studies reported bidirectional fertility in the hybrids (Garber 1960).

Previous *Collinsia* studies with meiotic chromosomes revealed seven pairs of metacentric chromosomes with six pairs approximately equal in size and one pair shorter (Garber 1975). The present study revealed distinct differences in chromosome size and centromere positions. Some differences may be due to differential condensation among meiotic versus mitotic chromosomes. During metaphase I of meiosis, the stage some researchers have studied, the chromosomes are highly condensed and dumbbell shaped, often leading to difficulties in karyotype characterization. The hybrid from the cross *C. heterophylla* female $\times$ *C. verna* male differs in several ways from previous *Collinsia* hybrids. It is the first successful hybrid between Group I and Group II species, and it shows unidirectional crossing compatibility, with the *C. verna* female $\times$ *C. heterophylla* male cross being completely sterile. Also, this hybrid shows evidence of pericentric inversions which are previously unreported in *Collinsia*.

Abstract

Karyotype analysis of *Collinsia verna* and *C. heterophylla* revealed seven pairs of chromosomes with a decrease in length of chromosomes across each genome from approximately 11 microns to 5 microns. The smallest pair is knobbed in both

species. A change in centromere position in the two longest chromosomes between the two species is indicative of the presence of chromosomal rearrangements such as pericentric inversions. Interspecific hybrids produced meiotic cells with many abnormalities including desynapsis and heteromorphic bivalents. This analysis revealed new cytological information and involved a hybrid cross between two groups of *Collinsia* species which had previously produced no fertile hybrids.

References

- Bell, S. L. and Garber, E. D. 1961. The genus *Collinsia* XII. Cytogenetic studies of interspecific hybrids involving species with sessile flowers. *Bot. Gaz.* **122**: 210–218.
- Burnham, C. R. 1977. Discussions in Cytogenetics. St. Paul, Minn.
- Chooi, W. Y. 1971. Variation in nuclear DNA content in the genus *Vicia*. *Genetics* **68**: 195–211.
- Garber, E. D. 1956. The genus *Collinsia* I. Chromosome number and chiasma frequency of species in the two sections. *Bot. Gaz.* **118**: 71–72.
- 1960. The genus *Collinsia* IX. Speciation and chromosome repatterning. *Cytologia* **25**: 233–243.
- 1975. *Collinsia*. In: R. C. King (ed.) Handbook of Genetics, Vol. 2. Plenum Press, New York.
- Gorsic, J. 1957. The genus *Collinsia* V. Genetic studies in *C. heterophylla*. *Bot. Gaz.* **118**: 208–223.
- Greenlee, J. K., Rai, K. S. and Floyd, A. D. 1984. Intraspecific variation in nuclear DNA content in *Collinsia verna* Nutt. (Scrophulariaceae). *Heredity* **52**(2): 235–242.
- Hinegardner, R. 1976. Evolution of genome size. In: F. J. Ayala (ed.) Molecular Evolution. Sinauer Assoc. Inc. Sunderland.
- Hiorth, G. 1930. Genetische Versuche mit *Collinsia bicolor* I. *Z. indukt. Abstammungs-Vererbungsl.* **55**: 117–144.
- 1931. Genetische Versuche mit *Collinsia* II. Die Blatt- und Kotyledonenzeichnungen von *Collinsia bicolor*. *Z. indukt. Abstammungs-Vererbungsl.* **59**: 236–269.
- 1933a. Genetische Versuche mit *Collinsia* III. Koppelungsuntersuchungen bei *Collinsia bicolor*. *Z. indukt. Abstammungs-Vererbungsl.* **65**: 253–277.
- 1933b. Genetische Versuche mit *Collinsia* IV. Die Analyse einer nahezu steilen Artbastardes. Die Diploiden Bastarde zwischen *Collinsia bicolor* und *C. bartsiaefolia*. 1. und 2. Teile. *Z. indukt. Abstammungs-Vererbungsl.* **66**: 106–157, 245–274.
- Price, H. J. 1976. Evolution of DNA content in higher plants. *The Botanical Review* **42** (1): 27–52.
- Rickett, W. H. (ed.) 1966. Wildflowers of the United States. Vol. 1. Part 2. McGraw Hill Book Co. New York. 401–402.
- Swanson, C. P., Merz, T. and Young, W. J. 1981. Cytogenetics. Prentice-Hall, Inc., New Jersey.
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