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A CYTOLOGICAL ANALYSIS OF A NATURALLY OCCURRING *HELIANTHUS* HYBRID

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One of the most interesting examples of natural hybridization in the genus *Helianthus* (family Compositae) is that involving *H. salicifolius* A. Dietr. and *H. grosseserratus* Martens. The first species is a perennial sunflower with tall, smooth stems bearing many drooping, alternate lance-linear leaves; the disk corollas may be either purple- or yellow-colored. It is found in dry prairies from Missouri to Texas but is sometimes cultivated in northeastern United States for its unusual foliage and showy flowers. The second species is a common weed that grows along roads and fencerows throughout much of the Midwest. The plant produces tall, generally smooth stems with ascending, alternate, lanceolate or lance-ovate leaves; the disk corollas are yellow. The hybrid of these species is *H.* × *Kellermani* Britton pro sp. named for Dr. W. A. Kellerman who first collected the plant near Columbus, Ohio, in 1897 (Kellerman, 1902). Since that time only a few collections have been made in widely separated places from Ohio to Wisconsin.

In the course of a previous investigation, an artificial F_1 hybrid was produced and compared morphologically and cytologically to a natural hybrid from Columbus, Ohio (Long, 1955). A preliminary study of microsporogenesis showed that irregularities were present in both hybrids. This report deals with the results of a detailed examination of microsporogenesis in a new collection (23) of putative *H.* × *Kellermani* from near Leonardsburg, Ohio, made in 1954. The investigation was undertaken primarily to obtain data with which to evaluate the taxonomic relationship of *H. salicifolius* and *H. grosseserratus.*

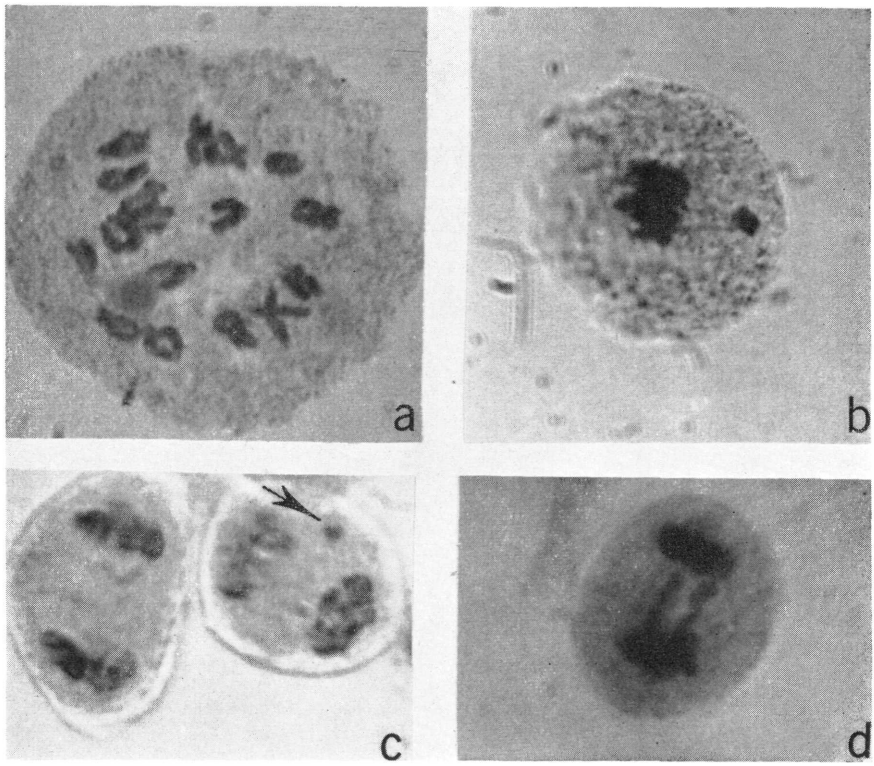
METHODS AND MATERIALS

Transplants of *H. salicifolius*, *H. grosseserratus*, and the new collection of *H.* × *Kellermani* were grown in an experimental garden during 1954. The culture of *H. salicifolius* (115) was grown from seed originally obtained from the Pearce Seed Company. Two cultures of *H. grosseserratus* (10 and 78) were kindly supplied by Dr. C. B. Heiser of Indiana University; they were collected in New York and Illinois, respectively. Specimens of these plants were prepared and placed in the Herbarium of Ohio Wesleyan University.

In the summer of 1955 flower buds were collected from plants in the experimental garden, fixed in Carnoy's solution (3:1) for 12 hours, and then transferred to 70 percent ethyl alcohol. Pollen mother cells were examined using the aceto-carmine smear technique. Chromosome behavior was studied during diakinesis (late prophase I), metaphase I, anaphase I, and telophase II. Photomicrographs were made using a Leitz Makam camera. Estimates of pollen fertility are based on differential staining of microspores with anilin blue in lactophenol. Fertile pollen stains dark blue; non-fertile pollen remains unstained. Approximately 200 grains were examined from each plant.

RESULTS

The data obtained from an examination of meiosis in the putative hybrid and



EXPLANATION OF FIGURES IN PLATE I

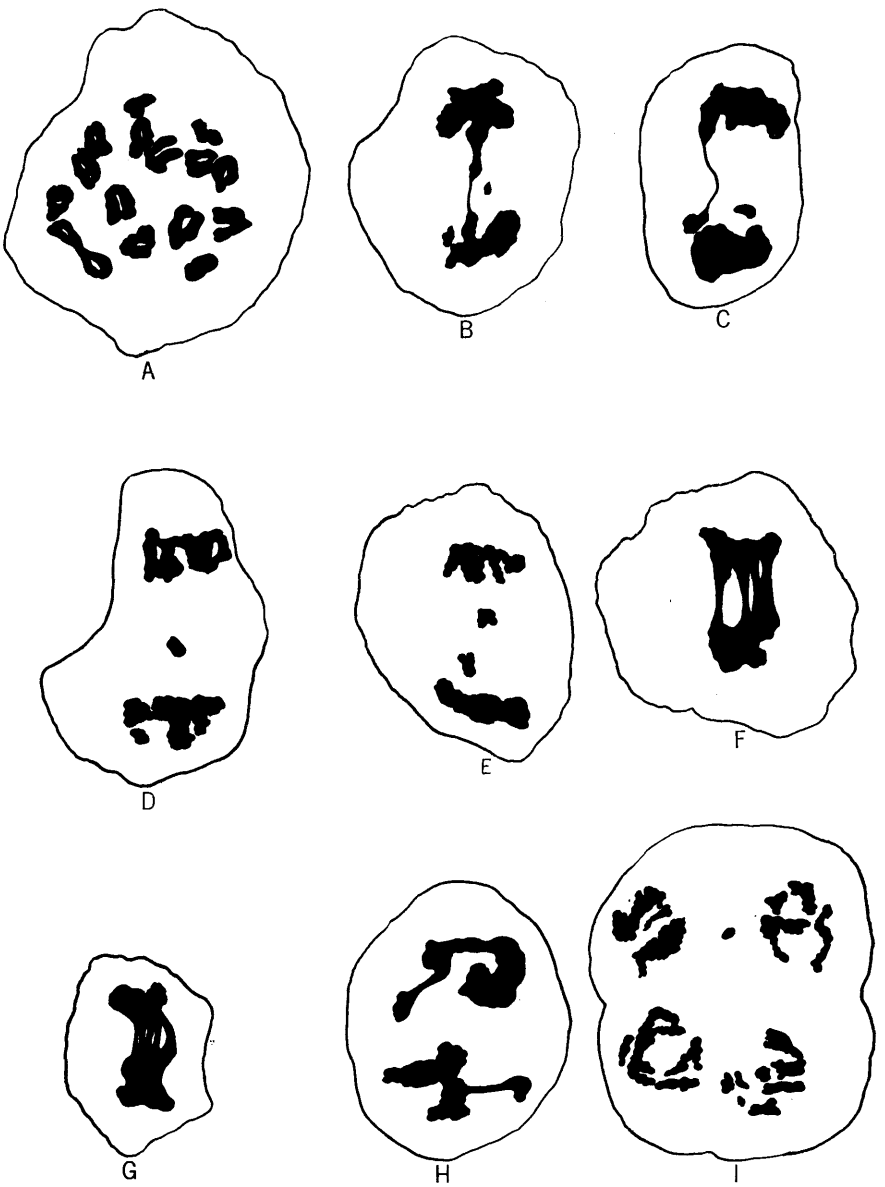
Photomicrographs of representative cells of *H.* × *Kellermani* during microsporogenesis (approximately × 1180).

a. 17 bivalents, normal diakinesis.
b. lagging bivalent, metaphase I.
c. lagging chromosome (arrow), early telophase I.
d. two chromatid bridges (fused chromatids), early telophase I.

EXPLANATION OF FIGURES IN PLATE II

Camera lucida drawings of representative cells during microsporogenesis of *H.* × *Kellermani* (approximately × 1300).

A. 15 bivalents and circle of four chromosomes ("figure eight"), diakinesis.
B. Chromatid bridge and fragment, early telophase I.
C. Chromatid bridge (fused chromatids), early telophase I.
D. Lagging chromosome, early telophase I.
E. Lagging chromosomes, early telophase I.
F. Multiple chromatid bridges, early telophase I.
G. Multiple chromatid bridges, early telophase I.
H. Chromatid bridges (fused chromatids), anaphase II
I. Lagging chromosome, telophase II.



species cultures are presented in table 1. The haploid chromosome number of 17 reported for these sunflowers (Geisler, 1931; Heiser, 1950) is confirmed.

The most common meiotic abnormality in the hybrid was the formation of rings and chains of from 3 to 6 chromosomes during diakinesis (22 percent of cells examined). Usually this was a chain of four chromosomes. Chains were also found in species' cultures, especially in *H. salicifolius* (14.1 percent).

The second most commonly observed abnormality was lagging univalents and bivalents during metaphase (21.5 percent). Laggers occurred in all the other garden plants (Plates I and II).

During anaphase and early telophase I, chromatid bridges with fragments, bridges without fragments ("fused chromatids"), and lagging chromosomes were observed. Several cells contained multiple bridges. All of these aberrations were rare in the other cultures.

Telophase II apparently proceeds normally in the hybrid as well as in the other plants. Regular tetrads are formed almost without exception. No significant number of abnormalities was observed although a few cells contained laggers and micronuclei.

TABLE 1

Meiosis in garden cultures and putative wild hybrid

Plant	Diakinesis				Metaphase I			Anaphase I				Telophase II		
	no.	17 II	rings, chains	I's	no.	normal	with laggers	no.	normal	bridges	with laggers	no.	normal	abnormal
grosseserratus														
#78	56	98.2	1.8	0.0	216	97.2	2.8	305	100.0	0.0	0.0	209	98.6	1.4
#10	44	100.0	0.0	0.0	204	92.0	8.0	308	98.4	0.8	0.8	206	99.3	0.7
salicifolius														
#115	52	84.6	14.1	1.3	209	97.4	2.6	313	98.2	0.9	0.9	202	100.0	0.0
× *Kellermani*														
#23	41	75.5	22.0	2.5	212	78.5	21.5	307	90.5	2.4	7.1	208	99.4	0.6

Approximately 12 cells were studied during diakinesis in each plant to obtain chiasma frequencies. It was not possible to study meiosis at earlier stages in this material. The chiasma frequency per chromosome pair in *H. grosseserratus* (78) was $1.41 \pm .01$; in *H. salicifolius* $1.48 \pm .01$; in the hybrid $1.62 \pm .06$. Most of the chiasmata were terminal although X-shaped bivalents were numerous.

The hybrid was vigorous and normal in every external respect during observations that extended over two growing seasons. The morphological intermediacy of the new collection (23) was readily apparent when it was placed along side the putative parental species. Pollen fertility was high in both species cultures and hybrid (90 to 100 percent stainable pollen) although seed-set was poor in open-pollinated heads.

DISCUSSION

In most hybrids between closely related species the chromosomes are either partially or completely paired during prophase and metaphase. Any subsequent lagging, bridge-fragment configurations or other phenomena are associated with lack of chromosome homology. Generally, the chiasma-frequency is somewhat lower than that found in the parental species (Stebbins, 1945). Preliminary counts gave a higher chiasma-frequency in the hybrid than in the putative species. However, the difference for the hybrid is not statistically significant. The putative hybrid exhibited regular chromosome pairing in approximately 75 percent of the cells studied. Lagging was observed during metaphase and early telophase. Bridge-fragments and "fused chromatids" were also formed.

The presence of rings and chains of chromosomes during prophase suggests that chromosomal interchanges have taken place and the two species differ by at least one translocation. It would appear that adjacent chromosomes usually pass to opposite poles since pollen fertility is high.

Lagging bivalents were frequent in the hybrid and were also observed in all parental cultures. Most hybrids between closely related species have normal spindle behavior. Lagging bivalents in these sunflowers may be caused by certain internal factors that upset the spindle mechanism and terminalizations' timing relations (Stebbins, 1945). Certain environmental factors, such as temperature, might also tend to interfere with normal spindle behavior. This lagging did not seem to affect the later course of meiosis, however, at least to any extent.

During anaphase and early telophase chromosome behavior was normal except for an occasional cell with bridge-fragment configurations, "fused chromatids," and laggers. Cross-overs in regions of inversions were assumed to be the cause of bridge-fragments, and probably some of the fused chromatids were formed in the same manner. In addition, fused chromatids may be caused by non-disjunction of one or more pairs of homologues and the persistent association of distal ends of homologous chromatids (Sax, 1932). Restitution nuclei were not seen at telophase I nor were characteristic "dumbbell" shaped nuclei of persistent chromatid bridges observed. The low frequency of bridges and absence of any detectable effect on later stages of meiosis argue against considering them of much importance contributing to genetic isolation. It would appear that inversions, along with translocations, have occurred in *H. salicifolius* and *H. grosseserratus* but that these chromosomal alterations have not been directly important in the evolution of races of the species.

The degree of chromosome homology, vigor, and pollen-fertility in *H.* × *Kellermani* demonstrate that *H. salicifolius* is closely related, cytogenetically, to *H. grosseserratus*. Morphological differences between the two species are quite striking, chiefly because of the unusually narrow leaves of *H. salicifolius*. Both species, however, possess alternate leaves and loose, attenuate phyllaries, and these are probably more important from the taxonomic standpoint. It seems advisable, from the evidence at hand, to include *H. salicifolius* in the "Giganteus" section of the genus (Long, 1954). This will necessitate expansion of the morphological description of the section to include species with disk-corollas red to purplish-brown, as well as those with disk-corollas yellow.

SUMMARY

A cytological analysis of a new collection of *Helianthus* × *Kellermani* has been undertaken to obtain data for evaluating the taxonomic relationship of *H. salicifolius* and *H. grosseserratus*. Approximately 75 percent of the pollen mother cells examined from the hybrid showed regular chromosome-paring. Structural differences were evidenced by the formation of rings and chains of chromosomes and chromatid bridges. Disturbances at metaphase I and anaphase I were also noted, but none of these phenomena interfere with the formation of large amounts and proportions of fertile pollen.

It was concluded that *H. salicifolius* and *H. grosseserratus* are closely related. *Helianthus salicifolius* should be included in the "Giganteus" section of the genus.

LITERATURE CITED

Geisler, F. 1931. Chromosome numbers in certain species of *Helianthus*. Butler Univ. Bot. Studies 11: 53–62.

Heiser, C. B., Jr. 1950. Report of progress. Yearbook Amer. Philos. Soc. 1949: 181–183.

Kellerman, W. A. 1902. Minor plant notes, No. 4. Ohio Nat. 2: 179–181.

Long, R. W., Jr. 1954. A biosystematic investigation of *Helianthus giganteus* L. and related species. Ph. D. thesis. University Microfilms A54–1659. Ann Arbor, Michigan.

———. 1955. Hybridization between the perennial sunflowers *Helianthus salicifolius* and *H. grosseserratus*. Amer. Midl. Nat. 54: 61–64.

Sax, K. 1932. Meiosis and chiasma formation in *Paeonia suffruticosa*. Jour. Arnold Arboretum 13: 375–384.

Stebbins, G. L. 1945. The cytological analysis of species hybrids. Bot. Rev. 11: 463–486.