Further Studies on Sex Linked Chromosome Abnormalities

By

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The author has expressed the opinion that so-called sex chromosomes are meiotic abnormalities induced by previous hybridization of the species, and that these abnormalities have been encouraged to persist over a number of generations through the advent of the dioecious condition in an otherwise monoecious or perfect flowering genus. That infers that the so-called sex chromosomes are the product of a disturbance in the meiotic process, hybridization, and the peculiar circumstances attendant upon the development of unisexuality. Investigations of plants exhibiting a wide variety of cytological and genetical conditions is necessary to thoroughly establish such a contention. In fact, criticism of the conventional theory of sex chromosomes has repeatedly pointed to errors whose stimulation has been the restricted source of evidence upon which the theory was based. To avoid similar embarrassment as well as to continue the construction of the ideas as set forth in a recent paper on the origin of so-called sex chromosomes in the Angiosperms, Jensen 1939, the author submits a study of microsporogenesis in three species, namely; *Xanthorrhiza apiifolia* L'Her, *Chamaelirium luteum* L., and *Smilax herbacea* L. Since each was studied for a particular reason, theoretical considerations will be included with the descriptive material under the heading of each species.

Material for these investigations was collected in the Swannanoa valley of western North Carolina, was preserved in modifications of Carnoy's fluid, was treated and imbedded according to the Jeffrey technique (Jeffrey 1928) and stained with Heidenhain's iron-alum haematoxylin, staining and decoloring slowly to obtain maximum differentiation. Zeiss apparatus was used in the microscopy.

Xanthorrhiza apiifolia L'Her. As the author has previously indicated, Jensen 1939, the cytological condition of monotypic, unisexual species is important to the argument in that recent hybridization of the species seems impossible and, if the author's opinion be correct, no evidence of so-called sex chromosomes should be found. *Xanthorrhiza apiifolia* is monotypic and polygamo-dioecious. Some plants were found which apparently produced only flowers of one sex, although the mixed condition was more frequent. Because the

sexual condition of this species is evidently in a state of flux, presumably evolving into the strictly unisexual type, the behavior of its reproductive chromosomes should be of interest. Would there be some indication of sex chromosome evolution—, that is the question.

The haploid number of chromosome for this species appears to be fifteen, fig. 3, as seen from the polar view of the heterotypic metaphase. Some of the chromosomes appear to retain their old spireme connections up to this stage. Generally, however, they are distinct after the dissolution of the nuclear membrane. Fig. 4 shows this as seen in a side view of the same stage. Usually the metaphase configuration was flat, although a few chromosomes were occasionally a bit out of line. The early separation of some of the smaller chromosomes leads to an uneven figure during the late metaphase and early anaphase of the first division. With but one exception, shown in fig. 1, the chromosomes were found to move simultaneously toward the pole of the spindle. No evidence of heterochromosomes was discovered. As seen in fig. 4, the chromosomes appear to consist of identical units. No extruded chromatin was observed in the cytoplasm, nor were extra nuclei formed. The second meiotic division was quite regular and the pollen appeared to be structurally perfect.

Thus, *Xanthorrhiza apiifolia*, a monotypic species approximating the dioecious state, reveals no meiotic abnormality which could suggest the development of sex chromosomes. As was expected, no diagnostic hybrid characteristics were found in the meiotic process, and therefore no evidence of recent hybridization of the species. This compares with the conditions found in *Pyrularia pubera*, Jensen 1939, and in so far as it is an additional case, contributes to the argument that so-called sex chromosomes will only be found in species capable of recent hybridization. Though monotypic species may well be of hybrid origin, the absence of parental or related forms in the genus would certainly suggest that such an origin lay far in the past—, far enough so that whatever meiotic irregularities might once have existed have long since disappeared.

Chamaelirium luteum L. This is one of two species which comprise the genus *Chamaelirium*. In view of the author's findings in several native species of *Smilax*, Jensen 1937, which is a large genus of some 300 species (Engler-Diels 1936), it was thought advisable to investigate a small dioecious genus of the same family. The distinguishing features of the two species are so slight that there is every indication that they are closely related. Consequently, one might expect to find meiotic irregularities or evidence of heterochro-

mosomes. However, in microsporogenesis the first reproductive division proceeds with regularity. The haploid number of chromosomes appears to be twelve and composed of two duplicate sets of six each. Two large chromosomes stand out in contrast with the others, fig. 7 and fig. 8. The metaphase finds all the chromosomes gathered at the plate, fig. 10, and a bit irregularly dispersed in the early anaphase, fig. 9. At first there seemed to be several instances of heterochromosome formation. Closer study however, and comparison with other pollen mother cells revealed that all the chromosomes were composed of similar units. Occasionally a few chro-

Figs. 1-4. *Xanthorrhiza apiifolia*. 1. Only instance of laggards seen. 2. Heterotypic anaphase. 3. Polar view of heterotypic metaphase. 4. Early anaphase showing equal sets of chromosomes. Figs. 5-10. *Chamaelirium luteum*. 5. Heterotypic anaphase. 6. Middle anaphase showing odd laggards. 7 and 8. Polar view of anaphase complements in the first division. 9. Early heterotypic anaphase. 10. Heterotypic metaphase.

mosomes would lag on the spindle, fig. 6, but at no time were the laggards extruded into the cytoplasm and stranded there. All the chromosomes were included in the daughter nuclei, as checked with counts, and no extra nuclei were seen. The second division is quite normal and the pollen appears to be structurally perfect. Seed development depends upon fertilization because female plants which had been protected from pollen produced no seed. If the occurrence of an odd laggard during the heterotypic division can be credited with much significance, it is the sole evidence of previous hybridization of the species. However, the chromosome complement of the somatic cells seems to be tetraploid and possibly this may be one of those frequent cases of doubling the number and thereby eliminat-

ing the disturbance in the meiotic process. However, this remains largely problematical. The fact remains that no evidence was found which might suggest that heterochromosomes or sex chromosomes were present in this species.

Smilax herbacea L. The author has already reported his study of microsporogenesis in *Smilax glauca* and *S. rotundifolia*, Jensen 1937. Although it belonged to another section of the genus, *S. herbacea* was just another species which ought to be looked at. Even though it does not compare with material of *Lilium*, *Trillium*, *Allium*, or *Tradescantia*; the carrion flower is a good subject for a study of the origin of meiotic chromosomes. Exact knowledge of what takes place before the reduction division is vital to a discussion of the theory of sex chromosomes. This theory depends upon the successful maintenance of chromosome individuality, which in turn theoretically depends upon the successful operation of synapsis or active pairing. Because observations on numerous species have given rise to the suspicion that the active pairing of chromosomes prior to the reduction division is far from a universal fact, the convenience of *S. herbacea* was utilized. A description of spireme and chromosome formation follows.

Two observations stood out over others when the synizetic stage of the spireme was studied. The first was the general occurrence of a second and smaller nucleolus attached to the original and principal nucleolus. The smaller nucleolus always faced the weft of contracted spireme, as shown in fig. 11. In fig. 12 the spireme had expanded once more and the strands were considerably thicker. However, there was a striking absence of free ends of chromatids. As was later revealed, the haploid chromosome count for this species seems to be thirteen. That would call for twenty six chromatids, and since each chromatid must have a minimum of two ends, fifty two ends should present themselves. Without question, the spireme in this stage is practically continuous. In whole nuclei only two or three free ends could be discovered and in most pollen mother cells none could be found. The doubleness of the spireme was partly discernible as early as the synizetic stage at which time there was also a dearth of free ends to the supposed chromatids. Prior to diakinesis, fig. 13, the chromosomes become distinctly segmented in the spireme into individual chromosomes or blocks of chromosomes. At this stage the spireme is clearly tetrapartite and separated into chains of chromosomes (that is, no longer continuous). The chromosome next to the nucleolus and the one at the top of the nucleus reveal the four parted nature of the twisted spireme. Fig. 14 is the conventional diakinetik figure and similar conditions

in other pollen mother cells led the author to believe that the haploid chromosome count for the species was nine. However, when the nuclear membrane disappeared, more than nine chromosomes were visible in the cytoplasm. Presumably eight chromosomes are associated in four groups of two each. Fig. 15 is a polar view of a heterotypic metaphase showing thirteen chromosomes, while fig. 16 is the same of a slightly earlier stage. The four double associations may be seen in the latter, and are marked with a D.

Figs. 11-16. *Smilax herbacea*. 11. Synizesis. 12. Continuous and double spireme. 13. Partially segmented spireme showing tetrapartite nature. 14. Diakinesis. 15. Polar view of heterotypic metaphase. 16. Early heterotypic metaphase. All stages taken from microsporogenesis.

The remainder of microsporogenesis proceeds with utmost regularity. Cytokinesis occurs after both divisions. Four microspores are formed and though completely mature pollen was not observed, grains which had obtained most of their growth gave no indication of abortion.

The author is aware of the following determinations of chromosome number in the genus *Smilax*.

<i>S. herbacea</i>	12	Humphrey	1914
"	12-13	Elkins	1914
"	13	Lindsay	1930
"	13	Jensen	1939
<i>S. hederacea</i>	15	Nakajima	1937
<i>S. Oldhami</i>	16	"	1937
<i>S. China</i>	15	"	1937
<i>S. glauca</i>	14	Jensen	1937
<i>S. rotundifolia</i>	16	"	1937

Apparently there is no consistent basic chromosome number yet discernible among the determinations already made. However, the fact that there is considerable variation in the numbers would suggest that the genus is a good subject for investigation, particularly since there are some three hundred species.

Taken by itself, the significant point to be made from the study of *S. herbacea* is the absence of free chromatids during the prophase of meiosis. It is difficult to conceive how the spireme could overcome the engineering difficulties and pursue the act of pairing in a successful manner. The condition of the spireme in *S. herbacea* is basically similar to the conditions found in *S. glauca* and *Dioscorea quarternata*, Jensen 1937, *Rumex acetosa*, *R. acetosella* and *Rheum rhaponticum*, Jensen 1936, and *Ilex opaca* Jensen 1939. Though considerable work remains to be done on this moot point, enough evidence is forthcoming to warrant skepticism over the more conventional assumptions about the meiotic process. Careful manipulation of the staining technique and patient microscopy will often reveal the continuous nature of the spireme when more superficial methods of study would lead one to accept the presence of individual chromatids in the earlier premeiotic nuclear stages. If the spireme from which the meiotic chromosomes arise is fundamentally continuous, a more reasonable explanation would be to consider the doubleness to have arisen by means of a longitudinal splitting of the spireme. Of course, such a conclusion rather embarrasses the hypothesis of strict chromosome individuality; and therefore reflects upon the theory of sex chromosomes. This substantiates the position taken by Jeffrey and Haertl 1938 in regard to *Trillium grandiflorum* in which they cast definite doubt upon the entire synaptic arrangement. As a consequence, even if one were to find heterochromosomes in *S. herbacea*, and subsequently find that they fitted into a sex chromosome pattern, their function as determinants of sex would be out of the question in that the individuality of the chromosomes is probably lost somewhere before the initiation of the reduction division. The author to some degree appreciates how much nicer it would be to roll along with the structural mechanisms so vitally needed by the Weismann school of cyto-genetics. Nevertheless, impartial observations on the development of the meiotic chromosomes in this species makes another conclusion more in keeping with the facts.

Summary

Meiosis in male plants of *Xanthorrhiza apiifolia* L'Her is strictly regular and gives no indication of any abnormality or

inequality among the chromosomes which might be interpreted as a sex chromosome complex. No diagnostic hybrid characteristics were found. This bears out the author's prediction and adds another case which is cited in support of the opinion that so-called sex chromosomes will not be found in monotypic species because recent hybridization has not been possible in such species. The chromosome number appears to be $n-15$.

Meiosis in *Chamaelirium luteum* L. is also quite normal, without any indication that a sex chromosome complex is present. Little evidence was found for recent hybridization of the species. The chromosome number was determined as $n-12$.

The origin of the meiotic chromosomes was studied in *Smilax herbacea* L., as a result of which the mechanism of active pairing or synapsis is questioned. The meiotic chromosomes seem to arise from a fundamentally continuous spireme which splits longitudinally and later becomes segmented into the haploid number of chromosomes. Eight of the meiotic chromosomes are associated during diakinesis in groups of two each. The chromosome number has been determined as $n-13$. Meiosis is normal with no trace of diagnostic hybrid characteristics.

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