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(with Plate XXIII)

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AN INVESTIGATION ON THE CYTOLOGY OF *MONOCHORIA HASTAEFOLIA* PRESL.

by

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(with Plate XXIII)

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The family Pontederiaceae in India is mainly represented by three species belonging to two different genera *Monochoria* and *Eichhornia*. The two species of *Monochoria*, viz., *Monochoria hastaeifolia* and *M. vaginalis* are the elements of Eastern Asia but the other genus is native of America. They are abundantly found as growing profusely in ponds and lakes propagating both through reproductive and vegetative means. *M. vaginalis*, however has got a restricted distribution in India and is mainly found in the lakes of dry regions of Himalayas.

A thorough search into the literature reveals the scanty data available as to the cytological behaviour of these genera. So far as the genus *Monochoria* is concerned, different basic numbers have been reported in the species. BANERJI and HALDER (1942) report the chromosome number of *M. hastaeifolia* as twenty-eight and MORINAGA's (1931) work reveals the presence of fifty-two chromosomes in *M. vaginalis*. In case of water Hyacinth the 2n number is thirty-two (TAYLOR, 1925).

The two different basic numbers, seven and thirteen amongst the allied species seem quite interesting, there being a possibility also of one as a derivative of the other.

In view of the scanty data of the cytogenetics of these species and because of the fact that the plants are easily available, a detailed cytological investigation seemed highly desirable for a solution of ancestry of these species. Fortunately as the text would reveal, results have been obtained in *M. hastaeifolia*, suggesting a basic number far less than the original reports which may also be responsible for the evolution of other species. A detailed work of the family is already in view and the present report deals with the cytological behaviour of *M. hastaeifolia*.

MATERIALS AND METHODS.

For the study of the somatic chromosomes, root-tips were fixed in Lewitsky's fluid and different modifications of the same viz., 1: 1, 1: 2 and 1: 3 with increased proportion of Formalin and the most satisfactory results were obtained in materials fixed in Chromic-Formalin mixture in the proportion of 1:3. Temporary smear preparations of the root-tips were obtained following Aesculin treatment (SHARMA and BAL, 1953) for thirty minutes in cold, followed by hydrolysis in Orcein (2 % solution) - Hydrochloric acid (normal) mixture (9:1) and subsequent squashing in Orcein (1 % solution). Excellent results were obtained following this technique, the slides revealing well spread metaphase plates with constrictions brought out with clarity.

For the study of meiotic chromosomes, anthers of suitable size were smeared and fixed in Navaschin and stained following Crystal Violet Iodine technique.

Drawings were made using an Olympus compensating eyepiece $\times 20$, and 1.3 apochromatic objective and an aplanatic condensor with 1.4 N. A., at a table magnification of $\times 3000$ approximately.

OBSERVATIONS.

The somatic chromosome number of the species has been found to be $2n = 28$ (Figs. 1 and 2). On the basis of the size difference existing between the chromosomes, they can be classified into five pairs of long chromosomes, three pairs of medium-sized chromosomes and six pairs of short chromosomes. It is to be noted, however, that the size differences existing between the chromosomes are not very marked and one gradually merges into the other. The lengths of the chromosomes vary between 1 micron to 2.7 micron. Taking into account the size and position of the primary and secondary constrictions, the chromosomes can be classified into the following types (Fig. 3):

Type A. One pair of long chromosomes with median primary constriction and a prominent satellite at the end of one arm, connected with the body by a conspicuous thread.

Type B. One pair of long chromosomes with sub-median primary constriction and a prominent secondary constriction at the sub-median portion of the longer arm.

Type C. One pair of long chromosomes with sub-median primary constriction and a small satellite attached to a thread at the end of the long arm.

Types D & E. Two pairs of long chromosomes with subterminal and submedian primary constrictions, of which one pair (D) is comparatively longer than the other.

Type F. Three pairs of medium-sized chromosomes with sub-median primary constrictions.

Type G. Four pairs of small chromosomes with sub-median primary constrictions.

Type H. Two pairs of very short chromosomes with median primary constrictions.

During meiosis, regular bivalent formation can be noticed during all the stages of first division till metaphase and various configurations of bivalents as a result of different degrees and patterns of terminalization of chiasmata can be noticed. Twenty eight chromosomes form clear fourteen bivalents during diakinesis (Fig. 4). In some of the bivalents even chiasmata at three points are also not of infrequent occurrence (Fig. 6). In addition to normal fourteen bivalents in diakinesis, cases are also of occurrence with thirteen bivalents and two univalents (Fig. 5). Howfar these univalents are to be regarded as univalents in the strict sense or formed as a result of early separation of chromosomes need further investigation. However, as such cases are quite rare, it seems likely that they represent the early separation of some of the bivalents.

Long thread-like connections connecting different bivalents have also been noticed (fig. 6), in some of the P.M. Cs. Such interbivalent connections are also on record in different members of the family Commelinaceae (SHARMA, unpublished) and also in the genus *Cicer* (THOMAS and REVELL, 1946). These have been considered as due to heterochromatic fusion during early stages which are also responsible for secondary associations in the later stages of meiosis. (THOMAS and REVELL, *l. c.*). A tendency of one of the bivalents to associate in groups is evident during diakinesis. This tendency of association and interbivalent connections are also quite marked in prometaphase stage of meiosis (fig. 7).

During metaphase I, clear fourteen bivalents can be seen oriented at the equatorial region of the spindle (figs. 8 & 9). Prominent secondary association between some of the bivalents is the characteristic feature of the metaphase of this species. Random readings from different P.M.Cs show the maximum association or least grouping as $1(5) + 1(4) + 1(3) + 1(2)$ of bivalents (fig. 8). The association of bivalents that occurs in maximum frequency (fig. 9) is $2(3) + 2(2) + 4(1)$ — Vide Table I.

TABLE I.

Different Types of Secondary Association		No. of times observed out of 100 clear plates	Max. Association	Units of groupings
(1)	1 (5) + 4 (2) + 1 (1)	3		6
(2)	1 (4) + 3 (2) + 4 (1)	9		8
(3)	3 (3) + 1 (2) + 3 (1)	6		7
(4)	1 (4) + 1 (3) + 2 (2) + 3 (1)	9		7
(5)	1 (5) + 1 (3) + 2 (2) + 2 (1)	3		6
(6)	5 (2) + 1 (3) + 1 (1)	3		7
(7)	1 (4) + 4 (2) + 2 (1)	6	1 (5) + 1 (4) + 1 (3) + 1 (2)	7
(8)	1 (4) + 2 (3) + 1 (2) + 2 (1)	6		6
(9)	2 (3) + 2 (2) + 4 (1)	15		8
(10)	1 (5) + 1 (3) + 1 (2) + 4 (1)	3		7
(11)	5 (2) + 4 (1)	3		9
(12)	4 (2) + 6 (1)	6		10
(13)	1 (5) + 1 (4) + 1 (3) + 1 (2)	3		4
(14)	1 (3) + 3 (2) + 5 (1)	6		9
(15)	1 (4) + 1 (2) + 8 (1)	3		10
(16)	2 (4) + 2 (2) + 2 (1)	3		6
(17)	1 (4) + 1 (3) + 1 (2) + 5 (1)	3		8
(18)	1 (3) + 4 (2) + 3 (1)	3		8
(19)	3 (2) + 8 (1)	1		11
(20)	1 (5) + 1 (4) + 1 (3) + 2 (1)	3		5
(21)	1 (4) + 2 (3) + 2 (2)	3		5

Anaphasic segregation has been found to be normal and clear fourteen chromosomes in each of the two nuclei are found to be present during second division metaphase (fig. 10). The tendency of association of chromosomes to associate among themselves are also marked at this stage. The rest of the division is found to be quite normal.

DISCUSSION

A study of the Karyotype of the species investigated shows that the size ranges between the chromosomes are not so marked and are quite divisible into the types as mentioned in the text. The absence of marked size difference amongst the members of the complement is also quite conspicuous during meiotic stages.

Study of meiosis reveals one of the characteristic feature of the species, that is the manifestation of secondary association between bivalents. If the theory of secondary association — as a means of finding out the basic number of chromosomes be considered as correct then the number of chromosomes present in the original basic set of the ancestral type should be considered as four.

It is to be mentioned, however, that the validity of this theory has recently been questioned by THOMAS and REVELL (*l. c.*). It has been pointed out by them that between some of chromosomes, *random fusion* of heterochromatic segments takes place during the early stage, the result of which is the manifestation of interbivalent connection during later nuclear cycle. Finally, it has been claimed that secondary association is the end result of the process. As the fusion has been claimed to be occurring at random, such associations are stated to have no bearing on the basic number of the species.

In members of Commelinaceae (SHARMA, unpublished) as well as during the present investigation, interbivalent connections as well as secondary associations have been noted. Contrary to the observations of THOMAS and REVELL, it has been emphasized (SHARMA, *l. c.*) that the fusion of heterochromatic segments which are also responsible for interbivalent connections and secondary groupings do not occur at random but specific chromosomes are involved in the process. This implies in all probability the fusion of heterochromatin between distantly related chromosomes. The support of this theory has been obtained in the presence of connections only between members of groups constituting metaphase with maximum association. It has been suggested that the fusion of heterochromatin might be attributed to their least change during evolution being to some extent genetically inert. This sug-

gestion seems to be applicable in the present case too and as such the basic number of the species is to be considered as four.

It is apparent from the observation during meiosis that the groupings as noted during maximum association are $1(5) + 1(4) + 1(3) + 1(2)$. The least groupings or maximum association, and not the one occurring in maximum frequency, has been taken into account in deriving the basic number. It is needless to mention, that the chances of all these distantly related chromosomes to associate specifically are remote in view of several factors operating within the nucleus which may stand in their way of association and as such, the maximum association whenever encountered, should be given consideration.

A glance at the karyotype too, reveals the presence of five pairs of long chromosomes, three pairs of medium-sized chromosomes, four pairs of short chromosomes and two pairs of very short chromosomes. These four different types of chromosomes may be visualized to behave in a way as noted during the maximum association at metaphase. It is apparent that five pairs belonging to «long» category include three pairs of nucleolar ones. This is quite explainable in view of considerable structural changes of chromosomes taking place during evolution resulting in a change of the karyotype and even loss of satellites in some. If one is to consider four as the basic number of chromosomes in *Monochoria hastaeifolia* then not only pure and simple amphidiploidy but rather considerable duplication and subsequent changes of the original set are to be visualized.

It is significant that the chromosome numbers of all the members of Pontederiaceae so far worked out are the multiples of four, as for example in *M. vaginalis* the $2n$ number is 52 and in *Eichhornia crassipes* the number is 32. In the light of the present investigation, it seems quite likely that the original suggestion of the presence of two different basic numbers within the genus *Monochoria* is debatable. A thorough search into the structure and behaviour of chromosomes in different members of the family may reveal the presence of a single line of evolution leading to the differentiation of different genera and species, and starting with a common original set of four chromosomes. An investigation in this direction is highly desirable.

SUMMARY

A detailed mitotic and meiotic study of *M. hastaeifolia* has been carried out. Refined techniques devised in this laboratory have been adopted for the clarification of the karyotype.

A karyotype analysis of twenty eight chromosomes has been made and

the presence of six chromosomes with secondary constrictions have been brought out.

Regularity in the meiotic behaviour has been noticed though occasional univalents are not of infrequent occurrence.

Distinct secondary associations and interbivalent connections of bivalents have been observed and the basic number as revealed is four. The origin of interbivalent connections and its bearing on secondary association have been discussed. Confirmation of four as the basic number of the species has also been obtained from an analysis of karyotype.

A review of the cytological knowledge of the other species and genus of the family has been made and a thorough critical search into the cytology of these types has been suggested in view of the data obtained during the present investigation.

ACKNOWLEDGEMENTS

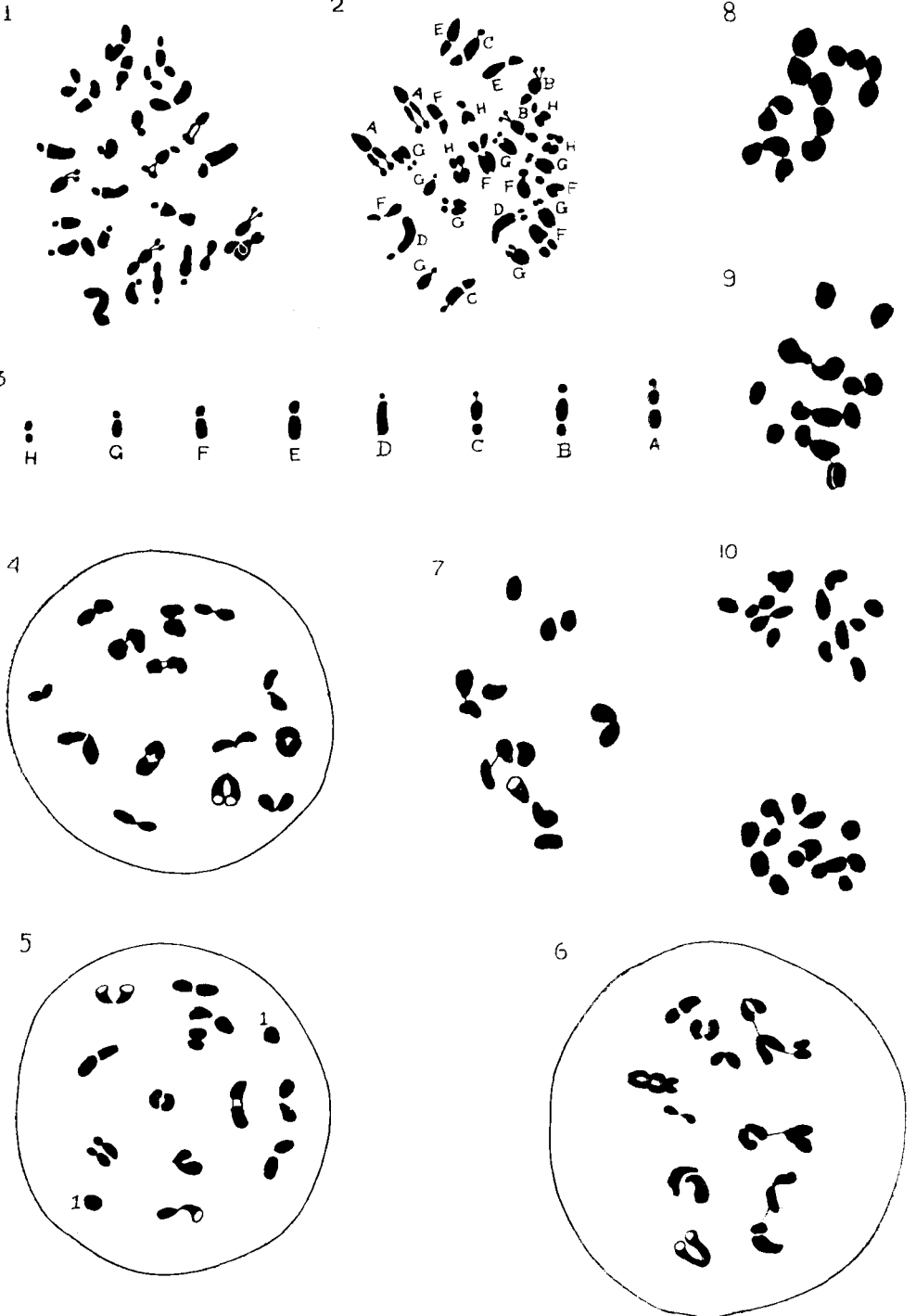
In conclusion, I wish to express my sincere gratitude to Mr. A. K. SHARMA, for his kind guidance and profound interest during the investigation and to Prof. P. C. SARBADHIKARI for the facilities provided.

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Explanation of Plate XXIII.

- Figs. 1-10. - Mitotic and meiotic behaviour in *Monochoria hastaeifolia* Presl.
- Figs. 1-3. - Somatic metaphase plates and idiogram of the same respectively. Anaphasic splitting of some of the chromosomes is evident in fig. 2.
- Figs. 4-6. - Diakinesis stages showing 14 clear bivalents; 13 bivalents and 2 univalents and interbivalent connections respectively.
- Fig. 7. - Prometaphase showing secondary association and interbivalent connection.
- Figs. 8 & 9. - Metaphase I, polar view showing a maximum association of 1(5) + 1(4) + 1(3) + 1(2) and most frequent association of 2(3) + 2(2) + 4(1) respectively.
- Fig. 10. - Metaphase II, polar view showing 14 chromosomes in each nucleus. Association to some extent is noticeable here.



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