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Cytological Studies on Some Members of the Family Pontederiaceae

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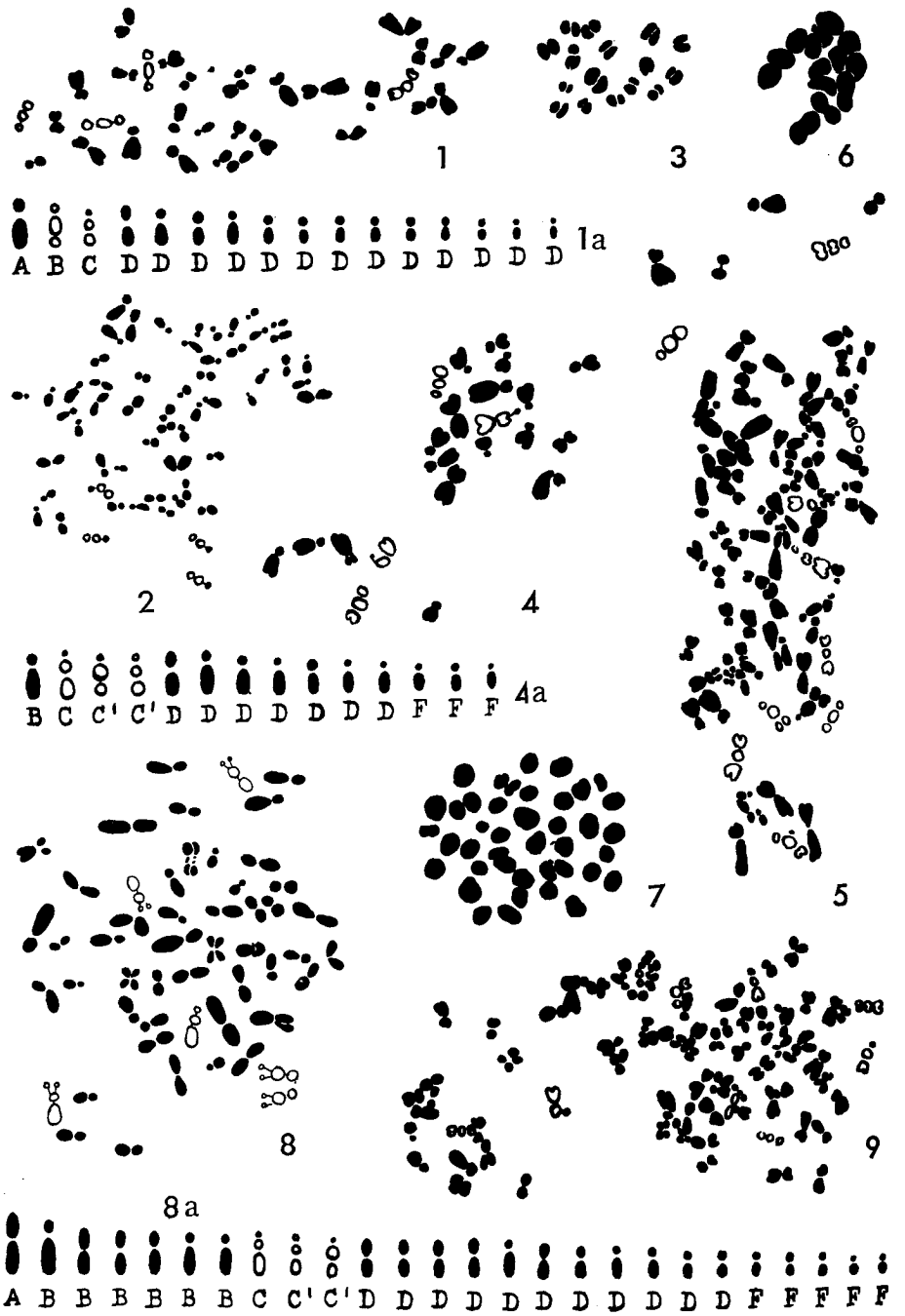
Introduction

The family Pontederiaceae in India is represented principally by two genera, *Eichhornia* and *Monochoria*. Only one species of *Eichhornia* i.e. *E. crassipes* occurs extensively in lakes and fresh water streams. Their extensive vegetative reproduction and capacity for retention of water help them to invade wide territories. Often an entire lake may be covered by one individual species. Of *Monochoria* only two species namely, *M. hastaefolia* and *M. vaginalis* are found, of which the former is mainly restricted to tropical zones, whereas the latter extends up to the temperate zones as well. In both of them vegetative reproduction is profuse, though not so effective as in *Eichhornia crassipes*.

In spite of the interest provided by the wide distribution of this family in India, its strict ecological preference and effective vegetative means of propagation, no cytological work has yet been done extensively on species of this family. Species propagating by vegetative means provide numerous cytological peculiarities in the somatic tissue which aid in the origin of the new genotypes (Sharma 1956, Sharma and Sharma 1959). A large number of chromosomal biotypes are therefore expected in individuals. Moreover the adaptibility of such biotypes to the ecological conditions has been shown to be a promising field of investigation. In view of the scanty cytological data on the species of this family, the interest provided by the distribution, ecological preference and propagation of the constituent members, the present cytological work was undertaken. Species and varieties on *Monochoria* and *Eichhornia* were collected from different regions of India during periodic tours in the Himalayas and plains and cytological investigation has been carried out on them. Such an investigation throws considerable light on the issue mentioned above.

Materials and methods

Four species under the family Pontederiaceae have been investigated. Two of them were obtained from the gardens attached to the University of Calcutta, one from Shillong and one from Nepal. For somatic study temporary aceto-orcein squash preparations were made, using 0.002 M solution of Hydroxyquinoline at 12°–14°C as the pretreatment chemical for all the materials. For meiotic study both temporary aceto-carmine smear preparation and permanent crystal violet stain-



Figs. 1-9. 1 and 1a, *Eichhornia crassipes*. Somatic metaphase with 2n=32 chromosomes and idiogram respectively. ×2000. 2 *E. crassipes*. Somatic variation nuclei with 2n=58 chromosomes. ×2000. 3, *E. crassipes*. Meiosis showing n=16 bivalents at metaphase I. ×2000. 4 and 4a, *Monochoria hastaefolia*. Somatic metaphase with 2n=28 chromosomes and idiogram

ed paraffin block sections were observed. In the figures the chromosomes with secondary constrictions were drawn in outline.

Observations

1. *Eichhornia crassipes*, Solms., 2n=32; $A_2+B_2+C_2+D_{26}$ (Fig. 1)

Taking into account the size and position of the primary and secondary constrictions the chromosomes can be classified into the following types:

Type A—One pair of medium-sized chromosomes with nearly submedian constriction (3.5μ). Type B—One pair of medium-sized chromosomes each with primary and secondary constrictions, both of which are located at the submedian positions; the middle segment being the longest one (3μ). Type C—One pair of medium chromosomes with two constrictions, primary and secondary, one nearly median and the other nearly subterminal in position (2.5μ). Type D—Thirteen pairs of medium to short chromosomes with primary constrictions, which vary from median to nearly submedian in position (2.5μ–1.5μ).

Besides normal number, 2n=30 and 58 were also obtained as variant numbers (Fig. 2). In meiosis n=16 bivalents are found in metaphase I (Fig. 3).

2. *Monochoria* spp.

Somatic chromosome number of the three species under the genus *Monochoria* varies from 2n=26 to 2n=80. On critical analysis of the karyotypes, the following types of chromosomes can be distinguished: Type A—Medium-sized chromosome longest in the set with nearly median primary constriction (4.25μ). Type B—Medium-sized chromosome with median to submedian primary constriction (3.75μ-2.75μ). Type C—Medium-sized chromosome with two constrictions, primary and secondary, one is median to submedian and the other one nearly subterminal at the distal end of the shorter arm (3.25μ–2.75μ). Type C′—Medium-sized chromosome with nearly median primary constriction and a satellite at the distal end of the short arm (3μ–2.25μ). Type D—Medium to short chromosome with median to submedian primary constriction (3μ–2μ). Type E—Short chromosome with two constrictions, primary and secondary, both submedian in position. The middle arm is the longest one (2μ). Type E′—Resembles type E, the only difference is that the middle segment is not the longest one (2.25μ–2μ). Type F—Short chromosomes with median to nearly median primary constrictions (2μ–1μ).

Monochoria hastaefolia, Presl., 2n=28; $B_2+C_2+C_4'+D_{14}+F_6$ (Fig. 4)

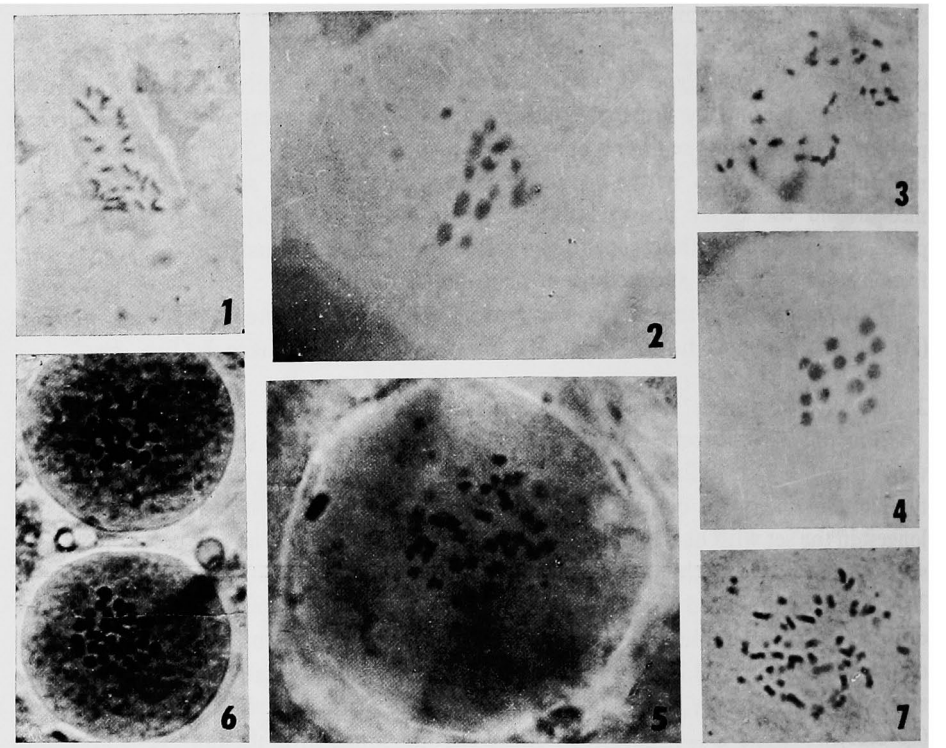
Besides normal number, cells with 2n=34, 40, 70, 76, 80, 82 chromosomes are also observed in the somatic tissue (Fig.5). In meiosis in normal metaphase I n=14 bivalents (Fig. 6) were obtained and abnormal cells were also recorded with n=40,

respectively. ×2000. 5, *Monochoria hastaefolia*. Somatic variation nuclei with 2n=82 chromosomes. ×2000. 6, *Monochoria hastaefolia*. Meiosis shwoing n=14 bivalents at metaphase I. ×2000. 7, *Monochoria hastaefolia*. Meiotic variation with n=41 bivalents at metaphase I. ×2000. 8 and 8a, *Monochoria vaginalis* I. Somatic metaphase with 2n=52 chromosomes and idiogram respectively. ×2000. 9, *Monochoria vaginalis* I. Somatic variation nuclei with 2n=80 chromosomes. ×2000.

41, 42 bivalents (Fig. 7).

M. vaginalis, Presl.

Three types of this species are obtained from two different collections. Type I and II were collected from Shillong in September, Type III collected from the same place in May.



Figs. 1–7. 1, *Eichhornia crassipes*. Somatic metaphase plate showing 2n=32 chromosomes. 2, *E. crassipes*. Meiotic plate showing n=16 bivalents. 3, *Monochoria hastaefolia*. Somatic metaphase plate showing 2n=28 chromosomes. 4, *M. hastaefolia*. Meiotic plate showing n=14 bivalents. 5, *M. hastaefolia*. Meiotic variation plate. 6, *M. vaginalis* III. Meiotic plate showing n=40 bivalents at metaphase I. 7, *M. vaginalis* var. *plantaginea*. Somatic metaphase plate showing 2n=52 chromosomes.

M. vaginalis Type I, 2n=52; $A_2+B_{12}+C_2+C'_4+D_{22}+F_{10}$ (Fig. 8)

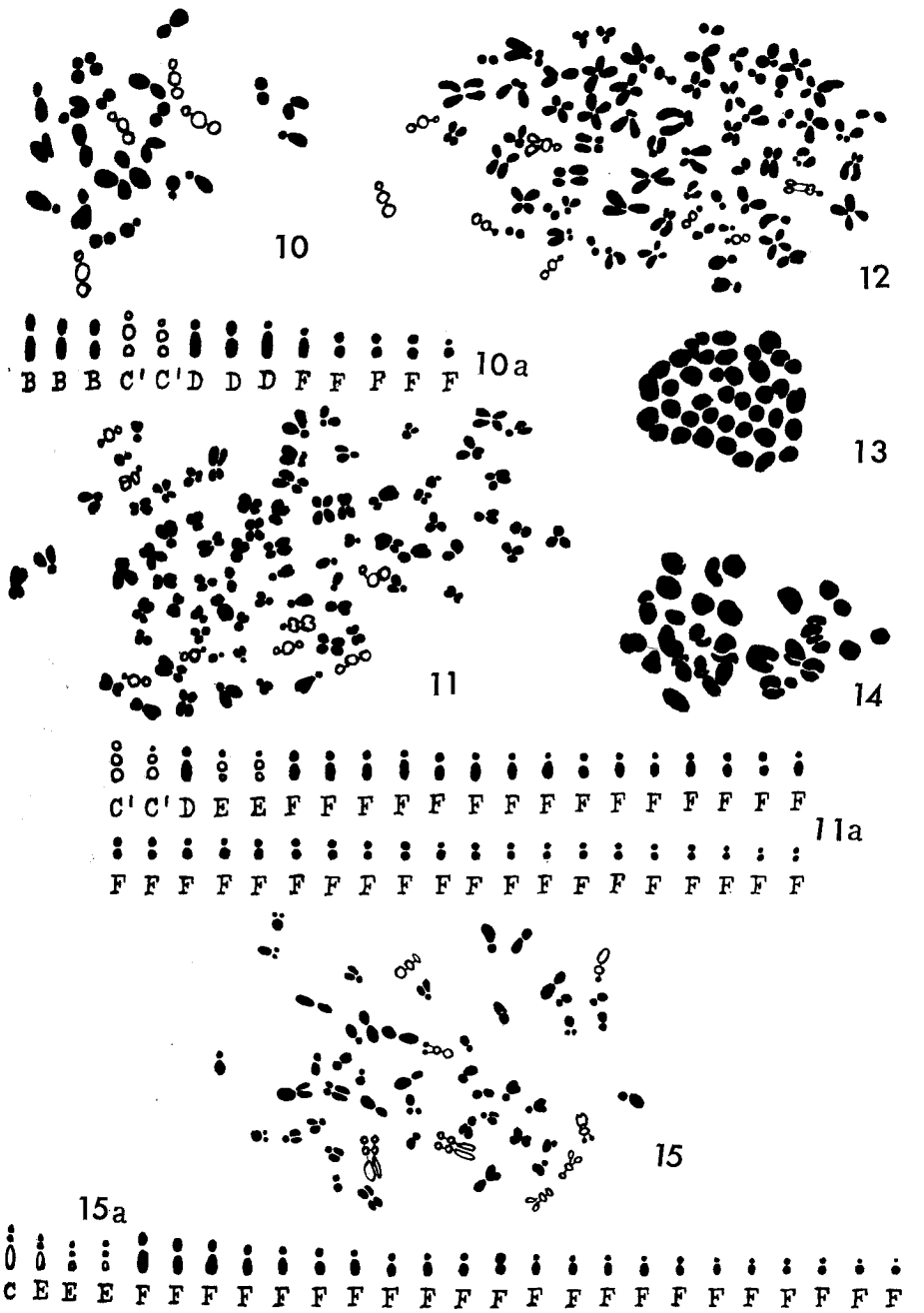
Besides normal number, 2n=28, 58, 80, 87 are obtained in different somatic cells within the same plant (Fig. 9).

M. vaginalis Type II, 2n=26; $B_6+C'_4+D_6+F_{10}$ (Fig. 10)

M. vaginalis Type III, 2n=80; $C'_4+D_2+E_4+F_{70}$ (Fig. 11)

Besides the normal number, 2n=72, 74 are also obtained in different somatic cells (Fig. 12).

In meiosis normal cells show n=40 bivalents (Fig. 13) in metaphase I. Varying



Figs. 10–15. 10 and 10a, *Monochoria vaginalis* II. Somatic metaphase with 2n=26 chromosomes and idiogram respectively. ×2000. 11 and 11a, *Monochoria vaginalis* III. Somatic metaphase with 2n=80 chromosomes and idiogram respectively. ×2000. 12. *Monochoria vaginalis* III. Somatic variation nuclei with 2n=72 chromosomes. ×2000. 13, *Monochroia vaginalis* III. Meiosis with n=40 bivalents at metaphase I. ×2000 14, *Monochoria vaginalis* III. Meiotic variation with n=30 bivalents at metaphase I. ×2000 15 and 15a. *Monochoria vaginalis* var. *plantaginea*. Somatic metaphase with 2n=52 chromosome and idiogram respectively. ×2000.

number like n=30 are also obtained (Fig. 14).

M. vaginalis, Presl. var. *plantaginea*, Solms.—Laubch., 2n=52; $C_2+E_6'+F_{44}$ (Fig. 15).

Discussion

Of the different species and varieties of the different populations of *Eichhornia crassipes* studied during the present investigation, all have shown 2n=32 chromosomes. Sixteen bivalents have been observed clearly in meiotic cells. Such a regular constancy in chromosome number in a species with extensive vegetative propagation is quite remarkable. However, the explanation of such a stability possibly lies in the fact that in addition to vegetative propagation, seed setting is profuse and sexual reproduction is normal. In absence of any other peculiarity in chromosome behaviour the 16 chromosomes may for the present be considered as not only haploid but even the basic set for the genus *Eichhornia*.

In *Monochoria hastaefolia* the chromosome number is 2n=28 which also confirms the previous report (Majumdar 1953). But during meiosis clear 14 free bivalents have been noted in contrast to Majumdar's (Majumdar 1953) obserbation where bivalents were supposed to form secondary association, possibly indicating a lower basic number. Such discrepancies in observation between the two populations may be due to the structural changes continually occurring in evolution resulting in the complete loss of homology with the ancestral chromosomes so that even the capacity for secondary grouping has been lost. In the present observation, in any case, fourteen chromosomes seem to form, possibly, the basic set. In *M. hastaefolia*, though abnormalities in chromosome number have been observed in the somatic tissues, there appears to be a selection barrier again in their participation in the growing of the daughter shoots, as all populations have been found to show a remarkably constant number of 2n=28 chromosomes.

An interesting feature that has been observed in *M. hastaefolia* is the increase in variant nuclei of the somatic tissue. Plants collected from the plains of Bengal have been grown in the University College gardens. After a year the frequency of the varying nuclei increased, though the normal number occurred in the highest frequency. Such variations not only range to a very high number, but may even participate in the formation of the meiotic cells where such irregularities have been recorded. But as mentioned already, possibly there is a selection barrier against this type of pollen forming male gametes. As such the number of 2n=28 chromosomes becomes constant in the species.

In *M. vaginalis* four populations have been studied, three belonging to *M. vaginalis* and one to *M. vaginalis* var. *plantaginea*.

In *M. vaginalis* the different chromosome numbers have been noted in different populations, namely types I and III, containing 2n=52 and 80 chromosomes respectively. Meiosis on the other hand does not show any multivalents, even in types with 2n=80 chromosomes. All the three populations show clear bivalents, suggesting that multivalents formation is possibly controlled by certain inhibiting gene or genes. Here every population shows variation in chromosome number

in somatic tissue and as such the origin of such types through vegetative propagation appears likely. An interesting record is that in all the populations, growing in different areas of Shillong, a distinct chromosome number is fixed for a particular population (Morinaga and Fukushima 1931). Such correlation between a particular chromosome number and a distinct population brings out clearly a cyto-ecological correlation within this species.

The type II with 2n=26 chromosomes deserves special mention. This type originated in the experimental plots at the University College compound from the parental stock with 2n=52 chromosomes collected from Shillong. The parental stock had been growing in water-logged soil for two years, which forms the natural environment for the species. Later a portion of the shoot started growing on ordinary soil being pushed off by the parental stock from the water-logged area. This portion of the shoot on examination revealed 2n=26 instead of 2n=52 chromosomes of the parental stock. This shoot was then detached from the mother plant and grown separately in a separate water logged area. Since then a population has originated out of this new stock, members of which are characterised by 2n=26 chromosomes. They are bieng maintained for the last three years. This is a remarkable example of the way through which plants with different genotypes originate through vegetative reproduction. There is the possibility that cells with such reduced chromosome number occur spontaneously in the somatic tissue. Such cells were selected as they became adapted to the new conditions to which they were exposed on being pushed off by the parental stock. This possibility supported by large scale variations occurring in somatic tissue and the variant nuclei having being found to contain even 28 chromosomes. Whatever may be the inducing factor such a significant decrease in chromosome number can only originate through somatic reduction (Huskins and Choulnard 1950, Sharma and Sharma 1959). After originating possibly through somatic reduction there is evidence to show that the chromosomes of this new type (Type II) have further undergone structural changes during evolution, borne out by a comparison of the karyotypes of types I and II in which the latter does not exactly represent the haploid complement of the former.

In the phenotypes associated with the decrease in chromosome number, no significant qualitative change could yet be recorded. No blooming has been observed within the last three years, which might have been the effect of the change of the genotypic condition. In any case the population size has been continually increasing and the number is still stable in water logged environment. Such synthesis of new chromosomal biotypes bring forth clearly the role of variation in karyotype in somatic tissue in the origin of new genotypes through vegetative reproduction (Sharma 1956, Sharma and Bhattacharyya 1962).

M. vaginalis var. *plantaginea* was collected from near Bagmati river of the Kathmandu valey (Nepal Himalayas–altitude about 4000 ft.). In this population the chromosome number 2n=52 is no doubt being maintained, but the karyotype shows certain differences from the karyotype collected from Shillong (Khasia Hills–altitude 3000–4000 ft.). The differences principally involve the number and the morphological details of chromosomes with secondary constriction. In the pheno-

type too, the plants show an overall reduction in size. As mentioned before, in all cases variation in the karyotype, both numerical and structural, have been noted in somatic tissue in all the populations studied. The normal karyotype occurs in highest frequency in cells whereas the variant nuclei do not generally exceed ten percent of the dividing nuclei. The population from Kathmandu therefore is an example where such a structurally changed karyotype has been adapted in selection.

A fact which needs special mention is the size of the chromosomes in *M. vaginalis* type III and *M. vaginalis* var. *plantaginea*. A study would reveal that the total amount of haploid chromatin length in type III with 2n=80 chromosomes is nearly identical with type I with 2n=52 chromosomes. *M. vaginalis* var. *plantaginea* with 2n=52 chromosomes also has a very reduced total chromatin length as compared to that of type I. This is clear index of the fact that evolution at least at an intraspecific level in this taxon has been associated with diminution in chromosome size as well. Such diminution in chromosome size is not infrequent in nature (Stebbins 1951). Such reduction in size may either be due to more compact spiralization or elimination of heterochromatic matter which is yet to be ascertained.

The evidence so far obtained therefore indicate that polyploidy, aneuploidy and structural changes of chromosomes have played effective roles in the evolution of species of *Monochoria*. The correlation of the chromosomally altered individuals with their distribution is also quite distinct in this taxon. Furthermore the study of the two genera shows that even though n=16 is deep seated for *Eichhornia* and n=13 or 14 for *Monochoria*, the two genera certainly represent a natural assemblage under Pontederiaceae as suggested by the taxonomists. This is principally shown by their karyotypes which have a remarkable similarities in the gross morphology with each other. In all of them the chromosomes are mostly medium to short represented in the graded karyotype. The constrictions vary mostly from median to submedian positions. All these factors taken together certainly confirm the homogeneity of Pontederiaceae as far as these two genera are concerned.

Summary

Four species under two genera of the family Pontederiaceae have been investigated, namely, *Eichhornia crassipes*, Solms. (2n=32, n=16), *Monochoria hastaefolia*, Presl. (2n=28, n=14), *M. vaginalis*, Presl. (2n=I 52, II 26, III 80, n=III 40), *M. vaginalis*, Presl. var. *plantaginea*, Solms.-Laubch. (2n=52). In *Eichhornia crassipes* 2n=32 chromosomes and n=16 bivalents have been observed in different populations of this species. Such a regular constancy in chromosome number in a species with extensive vegetative propagation is quite remarkable. The evidences so far obtained indicate that polyploidy, aneuploidy and structural changes of chromosomes have played effective roles in the evolution of species of *Monochoria*. The study of the two genera shows that even though n=16 is deep seated for *Eichhornia* and n=13 or 14 for *Monochoria* the two genera certainly represent a natural assemblage under Pontederiaceae as suggested by the taxonomists. This is principally shown by their karyotypes which have remarkable similarities in the gross morphology with each other.

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