Chromosome Studies in Natural Populations of *Isoetes pantii*, with Heterosporous Sporangia

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Variable chromosome numbers are known in a large number of plants and animals including man (Müntzing 1967, White 1954, Levan 1969). Such incon- stancy among individuals of the same species may either be due to variations in number of A or B or both types of chromosomes. Nevertheless, supernumerary chromosomes often exert detectable changes in individuals (Müntzing 1967) and it becomes imperative to investigate such variables and assign their probable cause to specific chromosomes.

Isoetes L. is already a peculiar pteridophytic genus by virtue of being a "rem- nant" of giant lycopods of Carboniferous age. There are hence, accumulated numerous morphological features of tremendous significance (Eames 1936, Bier- horst 1971). A recent discovery of *Isoetes pantii* (Goswami and Arya 1968, 1970) showing an unusual feature of occurrence of megaspores inside the microsporangia has further been of great academic interest. Keeping this in mind a systematic study of chromosome analysis was initiated and the possibility of variable chromo- some numbers was first observed in August 1967. Present paper, is a compilation of the studies on chromosome variations and year to year changes in the frequency of supernumerary chromosomes, since then.

Materials and methods

Isoetes pantii Gosw-Ary. plants have been repeatedly collected from two ori- ginal localities at Narsingharh, M. P. Fresh young roots were immediately put in sat. aq. paradichlorobenzene at the spot of collection. Numerous uprooted plants were maintained for a week or more in boquets and the water was changed on alternate days. After 3-4 hrs' pre-treatment either with paradichlorobenzene or 0.25% colchicine, roots were transferred to NewComer's fixative which also acted as preservative (Cohn 1964).

Roots were hydrolysed in N HCl for 8-10 min at 58-60°C and stained with conventional aceto-orcein (1.5%) method. Few preparations were also made permanent.

When varying frequency of chromosomes was ascertained, mature mega- sporangia were also kept with the roots of each plant and a separate tube was taken for another plant. This helped in accurate record of chromosomal counts and spore characters of the plant for species diagnosis. Few roots were directly fixed, without pre-treatment, in order to study the normal mitosis in bisporangiate plants.

Specific hours of fixing and changes in pretreatments are shown in the legend of figures with magnifications.

Results

1. Normal mitosis

In majority of cells, root tip mitosis was normal except a few occasional observations showing lagging chromosomes (Fig. 1). It is however important that these laggards were always either 2 small triangular (telocentric), 2 metacentric chromosomes and or a large acrocentric chromosome.

Figs. 1-4. 1, a clumped metaphase (nontreated) showing two metacentric, two acrocentric and one large chromosomes behaving as laggards. $\times 2150$ strain B. 2, root tip squash showing $44+1$ chromosomes in strain B. $\times 2150$; Colchicine, 3 hrs. 3, root tip squash from the same plant showing $44+3$ chromosomes. Pointer indicates a large faintly stained chromosome (X-chromosome) and arrow indicates two acrocentric chromosomes as in Fig. 1. $\times 2150$; Colchicine, 3 hrs. 4, diploid count of $36+3$ chromosomes from the roots fixed at 10 p.m. All chromosomes except the two exhibited typical coils and "fuzziness." $\times 2150$; strain A; Colchicine, 4 hrs.

2. Pretreated mitosis

Not only the roots of plants of similar megaspore morphology show different chromosome counts but also the roots of the same plant show such variables (Figs. 2 and 3). A pointer indicates in Fig. 3, a faintly stained long "fuzzy" chromosome; below the pointer on the left side are two chromosomes giving a false appearance of being one. Fig. 4 typically demonstrates the spirally coiled chromonemata

seen in certain chromosomes of pteridophytic genera such as *Equisetum* (Manton 1950). While all chromosomes have loosened their chromonemata, there are 3 chromosomes (two are small) which have exhibited no change in their normal morphology. Another bisporangiate plant exhibited 1 or 2 long acrocentric chromosomes but majority of cells have showed 44 chromosomes (Figs. 5 and 6). Similarly, seven plants have exhibited constant diploid number of 36 along with 1 or 2 supernumeraries (Figs. 7 and 8).

Figs. 5-8. 5, root tip squash showing 44+1 in strain A; note the acrocentric chromosome (arrowed) $\times 1000$; Paradichlorobenzene, 4 hrs. 6, root tip squash showing a pair of long acrocentric (T) chromosomes along with one dumbbell (I) shaped small metacentric and 44 other chromosomes. $\times 2150$; strain C; Paradichlorobenzene, 3 hrs. 7, $2n=36$ chromosomes; note the presence of long (T) X-chromosome. $\times 2000$; Colchicine, 4 hrs. 8, $2n=36$ from the same root but without the X-chromosome. $\times 1850$; strain A.

These supernumerary chromosomes are of three types; small metacentric and telocentric and large acrocentric. During five-year survey, attention was paid to record the variation in number of these chromosomes but not to their respective types in each cell (Table 1). Such analysis, however, has been started now with a view to reveal the detailed karyotypic analysis of the species. At present it can be

Table 1. Frequencies of supernumerary chromosomes in spread metaphases of *I. pantii*

Year	No. of metaphases	None		One		Two		Three		Diploid count.
		N	f.	N.	f.	N.	f.	N.	f.	
1967	15	3	0.20	2	0.13	3	0.20	7	0.46	24, 33+1, 33+3
1968	38	3	0.07	8	0.21	18	0.47	9	0.23	24+1, 33
1970	26	7	0.26	7	0.26	4	0.15	8	0.30	44, 44+1, 33+2, 44+3
1971	35	5	0.14	9	0.25	6	0.17	15	0.42	24, 44, 44+1, 33+3
1972	67	9	0.13	16	0.23	15	0.22	27	0.40	22+2, 33+3, 44+1
Total	181	27	0.14	42	0.23	46	0.25	66	0.36	

N: number of supernumerary chromosomes.

f.: frequency.

visualised that *I. pantii* shows, unlike any species of the genus, an unstable karyotype which is under the rigid enforcement of natural selection. No concluding remark can be made but it would not be out of place to mention regarding a recent discovery of a strain of *Isoetes pantii* (strain C) possessing three types of sporangia; *megasporangia*, containing normal megaspores; *microsporangia*, containing only normal microspores and *mixed sporangia* containing both megaspores and microspores (Goswami 1974). Figs. 5 and 6 are the root cells of one such plant.

3. Meiosis

As typical with Indian *Isoetes* (Pant and Srivastava 1965) *I. pantii* also shows only a few bivalents during meiosis. Whole process is highly irregular. During August 1971 the species was differentiated to comprise of three strains. Tables 2 and 3 are compiled on the total meiotic data; averages of chromosome configura-

Table 2. Mean chromosome configuration in different strains of *Isoetes pantii* over five years

Year	Strain(s)	No. of spore mother cells observed	Mean chromosome configuration		
			Univalent	Bivalent	Trivalent
1968	—	12	25.5	3.5	1.5
1970	—	8	22.0	7.0	—
1971	A	4	20.0	4.0	4.0
	B	5	24.0	6.0	—
	C	7	23.0	9.0	1.0
1972	A	4	25.0	4.0	3.0
	B	12	20.5	8.5	1.5
	C	9	18.0	10.0	2.0
1973	A	3	17.5	10.5	2.0
	C	4	25.0	8.0	1.0
Total		68	210.5	70.5	16.0
Average :			21.05	7.05	1.6
Expected :			(22.0)	(8.0)	(2.0)
$\chi^2 =$			0.04	0.11	0.08

I. pantii: Chromosome number $2n$ (ca)=44

tions are calculated as per separate records on every spore mother cell. While Table 2 estimates the probable averages of uni, bi and trivalents and computes the diploid number as 44 chromosomes, Table 3 indicates, from the same data, that strain C reveals "restoration" in pairing by showing 9 bivalents (instead of 5.8 or 7.5). The trivalents observed are never true trivalents, instead, just irregular association of three chromosomes. Normally strain C shows one such configuration, 8 bivalents and 26 univalents (Figs. 9 and 10). A spore mother cell also exhibited a heterobivalent (Fig. 11); its univalents were unusually "stretched".

Figs. 9-12. 9, a megaspore mother cell in diakinesis showing 4 SAT-chromosomes attached to the nucleolus. 'T' indicates large acrocentric univalent suspected as X chromosome. $\times 1000$, strain C. 10, a close up of the same figure showing one terminally attached trivalent, 8 bivalents and 26 univalents. 'T' indicates enlarged X chromosome exhibiting deeply staining (euchromatic) and faintly staining (heterochromatic) regions. $\times 2000$. 11, a portion of a megaspore mother cell in diplotene showing 12 univalents and 2 bivalents. Note the pairing of unequal chromosomes. $\times 3000$. 12, the young sporangial tissue exhibiting lobed nuclei, only one spore mother cell was detected in this sporangium. $\times 2000$; strain C. Note pedunculated lobes and their variables.

Table 3. Diplotene-diakinesis analysis of chromosomal configuration in *Isoetes pantii* complex

Strain	Mean chromosome configurations		
	Univalents	Bivalents	Trivalents
A Total	110.0	29.0	10.5
Average	22.0	5.8	2.1
B Total	42.5	14.5	1.5
Average	21.25	7.25	0.75
C Total	66.0	27.0	4.0
Average	22.0	9.0	1.3

Other bivalents in this spore mother cell were dark and tightly paired.

Of special mention are two univalents labelled in Figs. 9 and 10. The large acrocentric and differentially stained chromosome labelled T, is in close vicinity of a telocentric triangular chromosome. Both are of common occurrence in certain number of root tip cells (See also Figs. 5 and 6). At the first anaphase spore mother cells often revealed unequal, some time tripolar segregation. This further confirms the irregularity in meiosis. The fate of such spores is quite predictable. Certain cells in a megasporangium also exhibited "polyhedral" nuclei (Fig. 12), a feature of quite unusual significance. As per common belief, nodulated or lobed nuclei are not common in plants.

Discussion

Chromosomal variations in natural population

Except *I. hystrix* ($n=10$) which probably is an aneuploid (Manton 1950) majority of species have demonstrated $2n=22$ and or their multiple variables (Ninan 1958, Pant and Srivastava 1965). While Indian species of the genus typically exhibit diploid, triploid and hexaploid forms there have been reports of deviant chromosome numbers elsewhere. For example, Dunlop (1949) reported $2n=24-26$ chromosomes in *I. muricata*; Manton (1950) mentioned about $2n=ca. 100$ chromosomes in *I. lacustris* and *I. echinospora* and recently, Hall (1971) has counted 29 bivalents ($2n=ca. 58$) in *I. tenuifolia*. Haploid numbers like $n=11, 22, 35, ca. 55$ and also $n=10$ (aneuploid) indicate that there is a polyploid series in this genus based on the number $n=11$ as held by others. Furthermore, among Indian forms, *I. coromandelina* shows intraspecific polyploidy with the basic chromosome set $n=11$ (22, 33 and 44; See Pant and Srivastava 1965). All forms studied cytologically, except *I. sampathkumarinii* ($2=66$) show the occurrence of an extrachromosome (? B chromosome). The possibility of an extrachromosome however, can not be ruled out in all other species reported from elsewhere because of above mentioned conflicting reports (Dunlop 1949, Manton 1950).

Five year record on chromosome counts in *I. pantii* has made it clear that the genome of *Isoetes* has an inherent tendency to increase its chromosome set; *I. pantii* is a step ahead for having demonstrated the polymorphism. An interesting feature about polyploids in India is the formation of only few bivalents with no

normal multivalents (see Ninan 1958, Pant and Srivastava 1965). Apparently, the absence of multivalents precludes the possibility of species having been originated by autopolyploidy (although, not always so, see Müntzing 1967). Size of the chromosomes is also not small enough to check the chiasma formation; should it then be suspected that Indian species of the genus have originated by interspecific hybridization in the past? Unfortunately, there is no specific clue to this assumption. We however know that due to antiquity of species, internal changes in chromosomes may accumulate and may prohibit bivalent formation. Conversely, as Manton (1950) mentioned in *Pteris cretica* and held by Indian authors on *Isoetes*, apogamy might have arisen by internal changes in the chromosomes.

Both on morphological as well as cytological grounds *I. pantii* is understood

Figs. 13-14. 13, a portion of a section of sporangium showing mixed occurrence of microspores and megaspores. $\times 200$. 14, a germinated megaspore from mixed sporangium bearing young sporophyte. $\times 20$. RT=Root; SP=Spore; FL=First leaf.

to have comprised of three strains tentatively referred as A, B and C. Strain A seems to contain $2n=22+2$ and $33+1$ or 2 chromosomes; strain B shows $33+3$ in many root tip cells while strain C mainly possesses 44 (+1 or 2) chromosomes. Over all population is characterised by fluctuating number and presence of small telocentric and metacentric chromosomes and a large acrocentric chromosome, probably the X-chromosome.

A probable role of X-chromosome

It has to be ascertained whether this chromosome is responsible for the development of megaspores in the microsporangium (Fig. 13). Nevertheless, it is imperative to suspect its role for following reasons:

- i) This chromosome has always been found in a root of a plant possessing heterosporous sporangia;
- ii) The chromosome behaves as a laggard or it arrives late at metaphase plate in mitosis; since not all cells of the same tissue possess, it is quite possible that it does not always divide into two chromatids for equal separation at anaphase;
- iii) During anaphase I the long chromosome can be easily identified and like other "aberrant" univalents this moved to the poles with unbent arms (the centromere does not precede the chromosome arms in movement).
- iv) Despite basic similarities its all three strains have differed both on cytological and morphological grounds. The genome of *I. panti* has "exercised all possible trials" and chromosome sets with variable numbers of three types of supernumeraries (Table 1) have been encountered. And finally, strain C now possesses certain plants which bear true mixed sporangia and their roots have exhibited $2n=44$ chromosomes including the large acrocentric X-chromosome (Goswami 1974).

Furthermore, the megaspores contained in the true mixed sporangium are capable of producing a young sporophyte (Fig. 14). The spore germinated (? apogamously) in a Petridish containing garden soil.

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