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Numeral Stability and Morphological Instability of Chromosomes in Spore Calli of *Osmunda japonica*, a Fern

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Karyotype analysis was performed on spore calli of *Osmunda japonica* on MS media containing 2, 4-D and kinetin in order to clear up the cytogenetical behavior of callus cells from the haploid cells of a fern.

The spore calli showed a fair stability in haploid chromosome number ($n=22$). However, these calli showed a remarkable variation in karyotype as follows. The normal karyotype corresponding to that of the sopre donor was observed only in a few callus cells. The other karyotypes in many spore calli were multiple, and not systematically consolidated. These karyomorphological aspects were concluded that gross rearrangements of chromosome structures with maintaining a fair stability in haploid chromosome number are associated with spore cultures of *O. japonica* as a nuclear response to callus induction and to established culture condition.

It has been reviewed that chromosome variabilities in number and/or in structure are frequently observed in anther or ovary cultures in many angiospermous species.[1-3] For example, some callus cell lines in ploidy, namely, haploid maintaining a stability in chromosome number for a long term, diploid by doubling chromosomes and mixoploid of haploid and diploid have been derived from isolated pollen cultures of poenies.[4] In the culture of *Brachycome dichromosomatica*, gross structural rearrangements without changing chromosome number from the original number in explants were observed in the C-banded chromosome complements of callus cells.[5] Sacristan[6] has shown that haploid cultures of *Crepis capillaris* produce a higher frequency of chromosome rearrangements than a diploid culture.

In ferns, karyological investigations in haploid tissue cultures have been performed only on callus cells from gametophytes of *Osmunda cinnamomea*.[7] This investigation showed that the chromosome number, $n=22$, of a callus cell from the gametophyte is equal to a half the chromosome number in a sporophyte used as a spore donor. However, it has not been determined whether cultured cells from a fern gametophyte are stable or not in karyotypic feature.

The present author has been carrying out to induce callus cells from a fern gametophyte into haploid sporophytes. In this paper, the karyotype analysis was performed on callus cells from spores of *Osmunda japonica* in order to clear up the cytogenetical behavior of the haploid tissue cultures.

Materials and Methods

The karyotype analysis was made on spore donors and spore calli over six months old induced by the sporangium culture of *Osmunda japonica*. The procedure for the present culture was followed by the steps as shown in the anther culture of *Nicotiana tabacum*.[8]

Some of sterilized sporangia were planted onto the half-strength Knop media with 8 g/*l* agar for determining whether the spores were damaged or not by the sterilizing treatment, and it was made sure

that these spores had the normally germinating ability into the native gametophytes as shown in **Fig. 1** A. The other sporangia were planted onto MS media containing 1 mg/*l* 2, 4-D, 0. 5 mg/*l* kinetin, 30 g/*l* sucrose and 8 g/*l* agar for the spore callus induction (**Fig. 1** B). These cultures were continuously illuminated by a fluorescent lamp in an incubator at 25℃.

Preparations for karyotype analysis were followed by the aceto-orcein staining and squashing method. The spore calli were fixed with a mixture (alcohol 6 : acetic acid 3 : chloroform 1) at room temperature, but they were not treated with 8-hydroxyquinoline and 1 N HCl. On the other hand, root

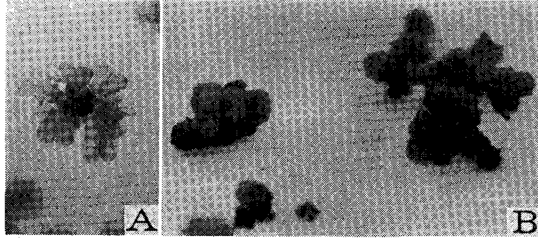

Fig. 1. Photomicrographs of gametophytes and haploid calli from spores of *Osmunda japonica*. A, normal gametophytes growing on half-strength Knop medium. B, spore calli propagating on MS medium with 1 mg/*l* 2, 4-D, 0. 5 mg/*l* kinetin and 30 g/*l* sucrose.

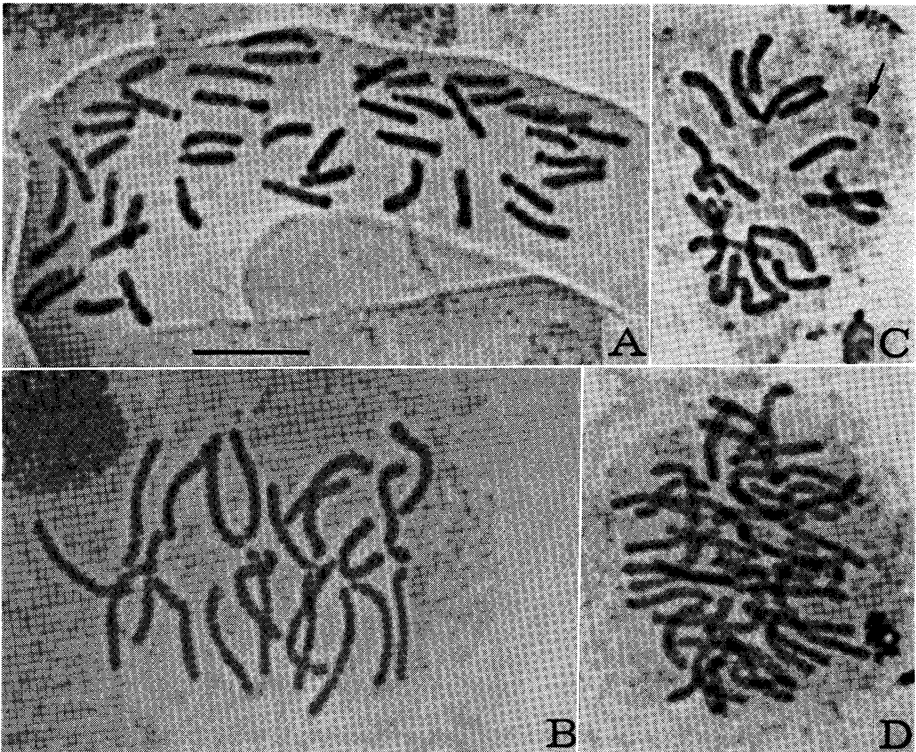

Fig. 2. Photomicrographs of somatic chromosomes at metaphase in a root tip cell of a spore donor and in spore callus cells of *Osmunda japonica* ($2n=44$). A, a root tip cell. B, a normal haploid callus cell with 22 chromosomes. C, a hypo-haploid callus cell with 19 chromosomes, showing a fragment chromosome (arrow). D, a polyploid callus cell with over 44 chromosomes. Bar : 10 μm.

Table 1. Number of various ploid cells in spore donors and in spore calli of *Osmunda japonica*.

Ploidy	No. (%) of cells observed in			
	Root tips from spore donors	Spore calli		
		6.5 month old	8 month old	total
Diploid ($2n=44$)	15 (100)			
Haploid ($n=19$–24)				
normal haploid		10	14	24 (77.4)
hypo-haploid		1		1 (3.2)
hyper-haploid		2		2 (6.5)
polyploid or allopolyploid		1	3	4 (12.9)

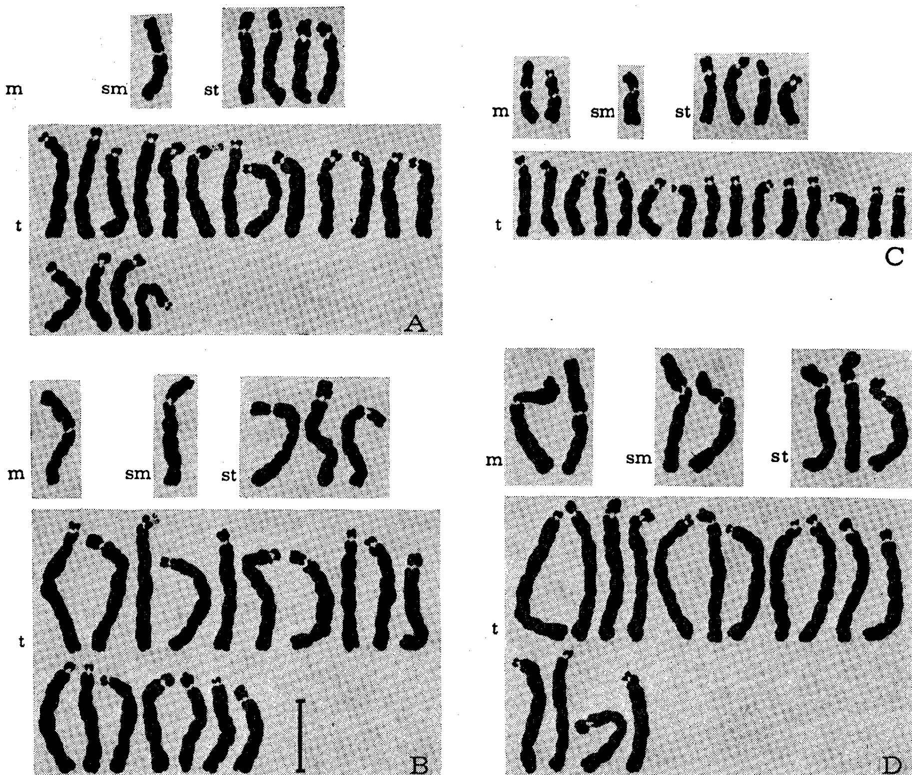

Fig. 3. Somatic metaphase chromosomes of callus cells from spores of *Osmunda japonica* classified by arm-ratio. m, median chromosomes. sm, submedian chromosomes. st, subterminal chromosomes. t, terminal chromosomes. A, chromosome complement of a haploid showing an original karyotype; $n=22=0(0)$ m$+1(4.5)$ sm$+4(18.2)$ st$+17(77.3)$ t. B–D, chromosome complements of haploids showing the derivative karyotypes. B, $n=22=1(4.5)$ m $+1(4.5)$ sm$+3(13.6)$ st$+17(77.3)$ t. C, $n=22=2(9.1)$ m$+1(4.5)$ sm$+4(18.2)$ st $+15(68.2)$ t. D, $n=22=2(9.1)+2(9.1)$ sm$+3(13.6)$ st$+15(68.2)$ t. Scale: 5 μm.

tips from spore donors were pretreated with 0.002 M 8-hydroxyquinoline aqueous solution, at room temperature for 4 h, and then they were fixed by the same mixture as used for the calli. The fixed tips were macerated with 1 N HCl at 60°C for 1 min.

For a description of karyotypic features, the metaphase chromosomes of these materials were classified by criterion on arm-ratio according to Levan *et al.*[9] into four types, namely median, submedian, subterminal and terminal.

Results and Discussion

Karyomorphological features, for example, number and arm-ratio of chromosomes, of the present spore donors (**Fig. 2** A) coincided with those in the previous papers.[10-12] This shows that these features are cytogenetically common in sporophytes of *O. japonica*.

In spore calli slowly propagating, cells showed the numeral and morphological features of chromosomes as follows.

The spore callus cells of this species showed a little variation in ploidy (**Table 1**, **Fig. 2** B-D) in contrast with the observation in *O. cinnamomea*.[7] These cells were classified into normal haploid with $n=22$ chromosomes (**Fig. 2** B), allohaploid (hyper- or hypo-haploids) shown in **Fig. 2** C, and polyploid or allopolyploid over diploid chromosome number (**Fig. 2** D). In the callus cells observed, the proportion of the normal haploids was about 80%, the allohaploids about 10% and the polyploids about 10% (**Table 1**). This observation on the chromosome number concluded that the spore calli of *O. japonica* fairly maintains the numeral stability of chromosomes without any apparent influence of the culture conditions.

In the haploid cells, karyotypes remarkably varied in contrast with the numeral constancy of chromosomes (**Fig. 3**). The regular members corresponding to the half set of the chromosome complement of a cell of the spore donor were found only in a few cells. These members are shown in the next karyotype, $n=22=0$ (0) m+1 (4.5) sm+4 (18.2) st+17 (77.3) t (**Fig. 3** A). On the other hand, karyotypes of almost all of the haploid cells were multiple as shown in **Fig. 3** B, C and D, and they were found not to be systematically consolidated. The median chromosomes with or without satellite in these karyotypes were new members derived from the modification of the original chromosome complements. In addition to this observation, a chromosome fragment was found in a hypohaploid callus cell with $n=19$ chromosomes (**Fig. 2** C). The occurrence of these chromosomes shows that the chromosome complements in the spore callus cells are remarkably rearranged by the following structural change of chromosome; fragmentation, translocation, inversion and the other. Therefore, it is concluded that the present culture conditions for inducing and establishing the spore calli make the chromosomes structurally rearrange with maintaining the numeral stability of chromosome as shown in the previous report of *B. dichromosomatica*.[5]

References

1) Kasha, K. J., L. Kott, G. Seguin-Swartz, 1982. In "Plant Tissue Culture" (ed. by Fujiwara, A.), p. 405-408, Maruzen, Tokyo.
2) Hu, H., Z. Xi, J. Jing, X. Wang, 1982. In "Plant Tissue Culture" (ed. by Fujiwara, A.), p. 421-424, Maruzen, Tokyo.
3) Phillips, R. L., A. S. Wang, 1984. In "Cell Culture and Somatic Cell Genetics of Plant" (ed. by Vasil, I. K.), Vol. 1, p. 712-727, Academic Press, London.
4) Ono, K., S. Harashima, 1982. In "Plant Tissue Culture" (ed. by Fujiwara, A.), p. 437-438, Maruzen, Tokyo.
5) Gould, A. R., 1982. In "Plant Tissue Culture" (ed. by Fujiwara, A.), p. 431-432, Maruzen, Tokyo.
6) Sacristan, M. D., 1971. Chromosoma, **33**: 273-283.
7) Morel, G., R. H. Wetmore, 1951. Am. J. Bot., **38**: 141-143.
8) Sunderland, N., 1984. In "Cell Culture and Somatic Cell Genetics of Plant" (ed. by Vasil, I. K.), p. 283-292, Academic Press, London.

9) Levan, A., K. Fredga, A. A. Sandberg, 1964. Hereditas, **51** : 201-220.
10) Okuno, S., 1936. Bot. Mag. Tokyo, **50** : 332-337.
11) Kurita, S., 1963. J. Coll. Arts Sci., Chiba Univ., Nat. Sci. Ser., **4** : 43-52.
12) Tatuno, S., H. Yoshida, 1966. Bot. Mag. Tokyo, **79** : 244-252.

≪和文要約≫

ゼンマイ胞子起源カルスにおける染色体の数的安定性と形態的不安定性

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シダ植物の半数性組織起源培養細胞の細胞遺伝学的特性を明らかにする目的で，ゼンマイの胞子嚢培養によって作出された胞子由来カルスの核形態学的観察を行った．

培養開始後6.5〜8カ月を経た胞子起源カルス細胞の約80%が正半数体（n=22）で，異数性半数体（n=19，24等）や倍数体（n=36，44等）は極少数であった．この染色体数の安定性とは対照的に，大多数の半数体の核型は，多種多様で，不統一的であって，既報の胞子嚢供与体の核型と同じ核形態学的特性を示す基本核型が極少数の半数体でのみ見出されたにすぎなかった．低半数体では，染色体断片がみられることもあった．

したがって，ゼンマイ胞子由来カルスの誘導期や増殖期には，半数性染色体数が維持されるが，不特定多数の染色体に複雑な構造変化が高頻度で誘起されると考えられる．