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C-value paradox in natural and artificial polyploids of the *Christella (Cyclosorus) parasitica* complex

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Abstract

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A C-value paradox was detected within tetraploid members of the *Christella parasitica (Cyclosporus parasiticus)* polyploid complex. However, synthetic autotetraploids generated from the diploid member of this group (*Ch. hispidula*) as well as the triploid hybrid *Ch. parasitica* × *Ch. hispidula* did not comply to this trend. Contrary to expectation, increased nuclear DNA values could be detected. The mechanism of this phenomenon is discussed.

Introduction

Polyploidy is one of the most important processes in speciation (Stebbins 1950, 1966, Ohno 1970, Vida 1973, Jackson 1976, Sparrow and Nauman 1976, Lovis 1977). About 30–35 percent of flowering plants have multiple basic chromosome number of their taxonomic groups (Stebbins 1971). Both auto- and alloploidy as well as aneuploidy and cryptopolyploidy are characteristic of evolution (Vida 1976, Jones 1970).

The so called C-value paradox (non-linear relationship between ploidy level and DNA/cell value) associated with polyploidy is well known to evolutionary biologists. Many data concerning this phenomenon accumulated recently but actual causes and mechanisms have not been proposed (Thomas 1971, Sparrow et al. 1972, Price et al. 1975, Hinegardner 1976, Nagl 1976, Schaeffer and Miksche 1977). Moreover, the C-value paradox is not observable in every case (Vida and Mohay 1980).

Polyploidy seems to be particularly widespread among pteridophyte taxa. We tried to clarify the relationship between ploidy level and nuclear DNA content, in the *Ch. parasitica (Cyclosorus parasiticus)* polyploid complex. (For nomenclature see Holttum 1976.) Genomic relationships between members of this group were presented by Panigrahi and Manton (1958) as well as by Ghatak et al. (1971).

The diploid member of this complex, *Ch. hispidula* (Decne) Holttum, is widely distributed in tropical and subtropical areas. Ferns possess the capability for apospory:

Dedicated to Prof. T. Reichstein on the occasion of his 90th birthday

young leaves of sporophytes can generate prothallia under proper circumstances. The chromosome number of these gametophytes corresponds to that of the sporophytes. This character presents an opportunity for raising synthetic autotetraploids.

Investigations were directed towards analysis of DNA content of plants which reached the diploid and tetraploid level during evolution. A further aim was to compare the DNA content at these levels and of synthetic hybrids and tetraploids without any changes in genetic material during evolution.

Materials and methods

Plant material

The following four taxa of the genus *Christella* (formerly known as *Cyclosorus;* see synonyms in Holttum 1976) were studied:
Christella hispidula (Decne) Holttum – diploid, n = x = 36; collector Prof. R. E. Holttum 1958, Sarawak, Borneo.
Christella parasitica (L.) Lév. – tetraploid, n = 2 x = 72; collector Prof. I. Manton and W. A. Sledge 1950, Ceylon, designated P 285.
Christella malabariensis (Fée) Holttum – tetraploid, n = 2 x = 72; collector Prof. I. Manton and W. A. Sledge 1950, Ceylon, designated P 199.
Christella dentata (Forsk.) Brownsey & Jermy – tetraploid, n = 2 x = 72; collector Prof. I. Manton and W. A. Sledge 1949, Madeira.

Spores were kindly presented in 1969 by Prof. I. Manton. Progenies were raised and have since been cultivated in the Budapest Greenhouse of the Institute of Botany of the Hungarian Academy of Sciences.

Cultivation, crossing, induction of apospory

Spores were sown either on the surface of sterilized Knop's medium, solidified with 1% agar, or on sterilized vermiculite moistened with Knop's solution, or directly on peat.

Hybridizations were carried out by immersing mature prothallia in a drop of distilled water for 2–4 h. Aposporously produced prothallia were carefully separated and treated alone in order to obtain self-fertilized homozygous sporophytes.

Induction of apospory: Primary or secondary fronds of very young sporophytes were excised and placed on the surface of sterilized agar medium. Surface sterilization of the leaves was made by dipping them into 5% sodiumhypochlorite for 5 min. The next step was rinsing with 1% para-chlor-benzoate solution for 10 min. After washing in sterilized distilled water the fronds were placed on the media. The application of natural peat for aposporous regeneration made frond-sterilization unnecessary.

Cytology

Mitosis: Root-tips of mature sporophytes were treated with 0.1% colchicine solution for 4 h at room temperature, or treated with 0.004 M 8-hydroxyquinoline solution in a glass tube kept cool under running tapwater. Thereafter the root-tips were fixed for 12 h according to the method of Sharma et al. (1977). Composition of the fixative in the proportions of 5 : 1 : 2 : 1 : 1 abs. ethanol : glacial acetic acid : 2N HCl : formaldehyde : chloroform, plus 0.25 g ammonium oxalate per 100 ml of the mixture. If softening of the tissue seemed to be necessary, roots were digested with 6% Helicase for 1 h at 37 °C. Chromosomes were stained with aceto-carmine. Meiosis: For genome analysis sporangia were stained and squashed according to the aceto-carmine method.

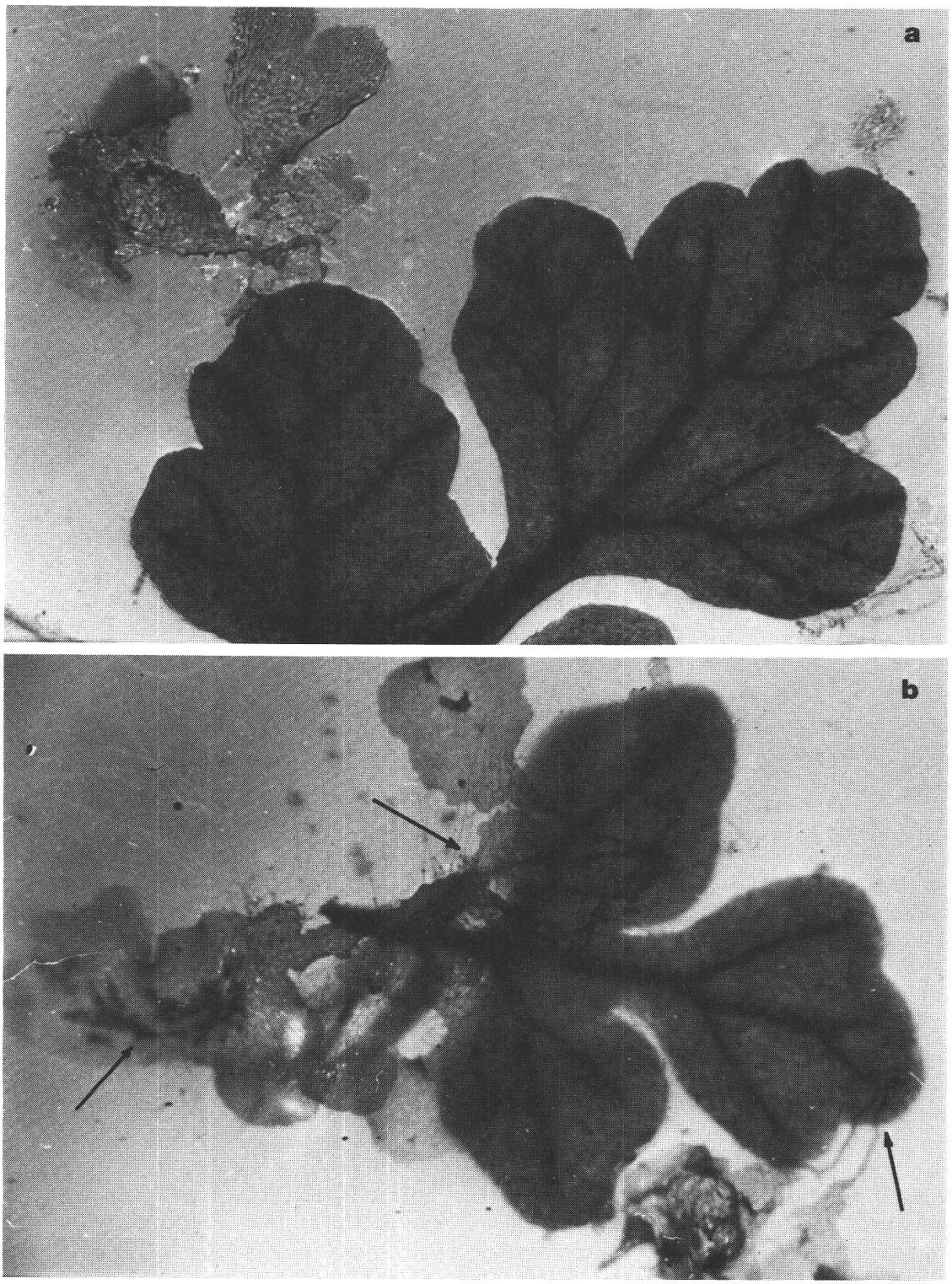


Fig. 1. Excised secondary leaf of *Christella hispidula* with aposporous prothallia: a) Young plate-shaped prothallia with filamentous connective elements (×10). b) Arrows show filamentous connective elements and archegonia on the lower surface of the gametophytes (×6).

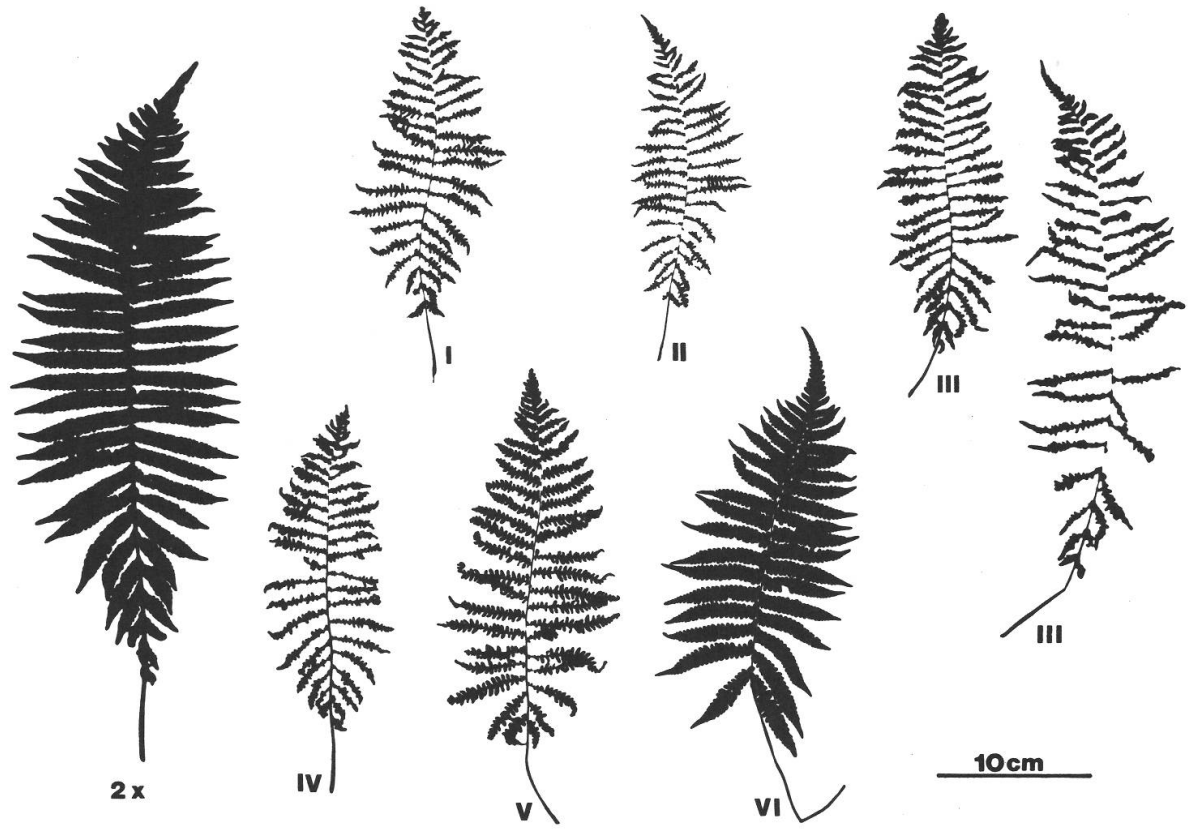


Fig. 2. Silhouettes of the fronds of diploid *Ch. hispidula* (2x) and autotetraploids originating from apospory (I–VI).

Analysis of spore size

In order to obtain a true picture of the types of spores produced by different plants, we collected carefully isolated mature spores. By means of a Laborscale Analyser PS-L and PS-A, freshly moistened and dispersed spores were examined in 0.9% NaCl solution.

Cytophotometry

Root-tips were fixed for 24 h according to the method of Östergren and Heneen (1962). Fixative: 60 ml methanol, 30 ml chloroform, 20 ml distilled water, 1.0 g picric acid, 1.0 g 2,4-dinitrophenol, 1.0 g $HgCl_2$. After fixation roots were carefully washed with 70% methanol and stored below 0 °C until used. Before hydrolysis roots were hydrated in a 50–30–10% methanol series and in distilled water each for 3 min, then hydrolized in 1 N HCl for 12 min at 60 °C. Hydrolysis was stopped in ice-chilled distilled water for 3 min. Treatment in Feulgen reagent for 3 h gave the best staining. The DNA content was determined with a Vickers M 85 scanning microdensitometer in arbitrary units. At least 10 metaphases were measured in each root-tip and a minimum of 10 roots were stained from each plant. Usually the number of examined cells was between 100 and 300.

Results

Apospory

The first prothalloid regeneration appeared in the fourth to eight month, usually on the margins of the excised fronds. Only the primary and secondary fronds possessed the

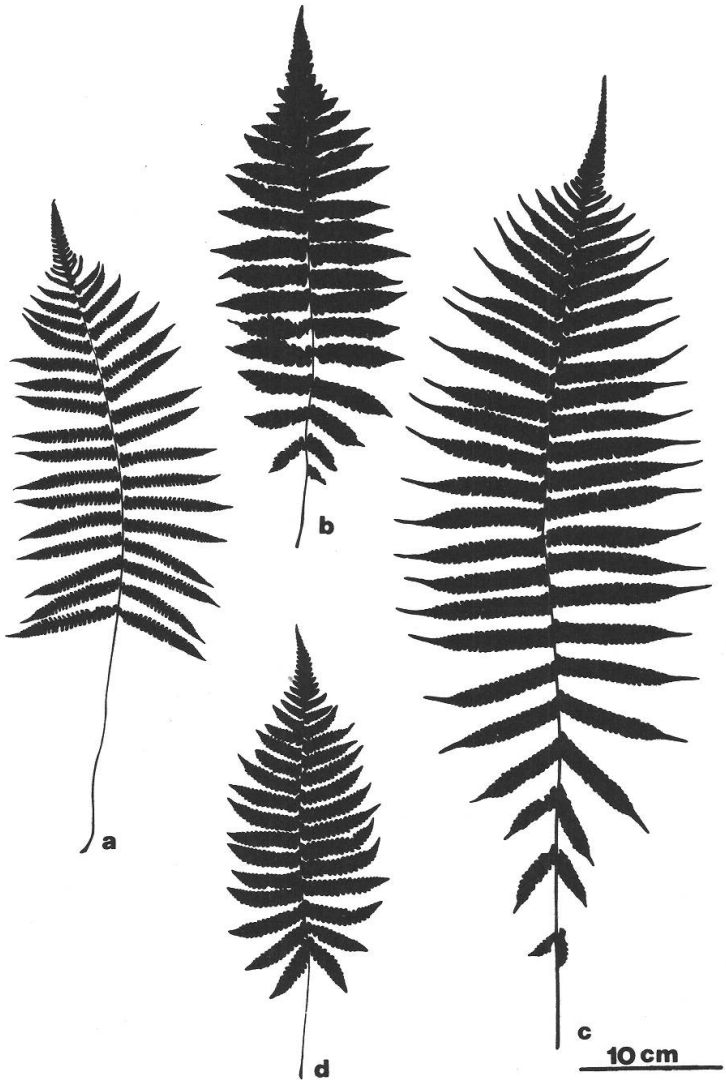


Fig. 3. Silhouette of a frond of the triploid hybrid between *Ch. parasitica* and *Ch. hispidula* showing frond characters of the tetraploid parent. a) *Ch. parasitica;* b) *Ch. dentata;* c) *Ch. malabariensis;* d) *Ch. parasitica* × *Ch. hispidula.*

power for aposporous regeneration. The medium should be without any carbohydrates (cf. Hirsch 1975). According to our observations, first one-celled protuberations emerged at the terminations of veins. After this stage they developed into filamentous and some weeks later into normal, plate-shaped gametophytes. At later stages they did not show any difference as compared to normal prothallia. Their growth rate decreased, however. On normal gametophytes (n) antheridia and archegonia appeared within 2–3 months. On diploid (2n) prothallia the length of this period doubled. Gametophytes often remained in contact with the excised fronds by a thin filament. Mehra and Sulklyan (1969) reported aposporous gametophytes of *Ampelopteris prolifera* which became laminated after having been separated from the original leaf. In our experiments aposporous prothallia became laminated normally when connected with the leaves (Fig. 1). A similar development was observed in another experiment with *Pteridium aquilinum* similar to Sheffield's observations (1984).

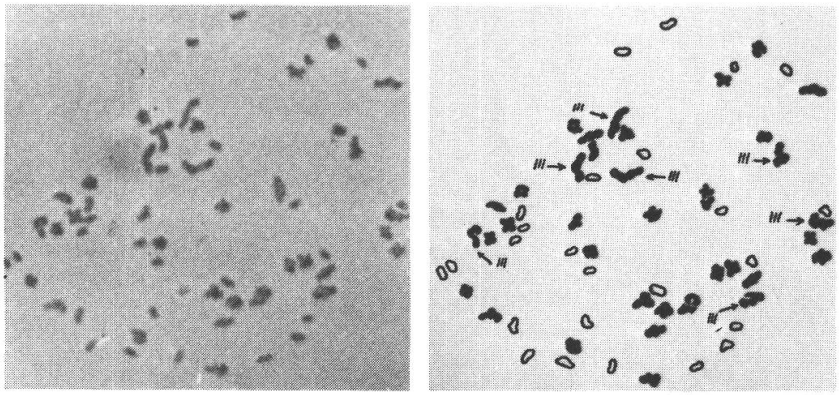


Fig. 4. Cytology of the triploid hybrid *Ch. parasitica* × *Ch. hispidula:* meiotic plate (×500).

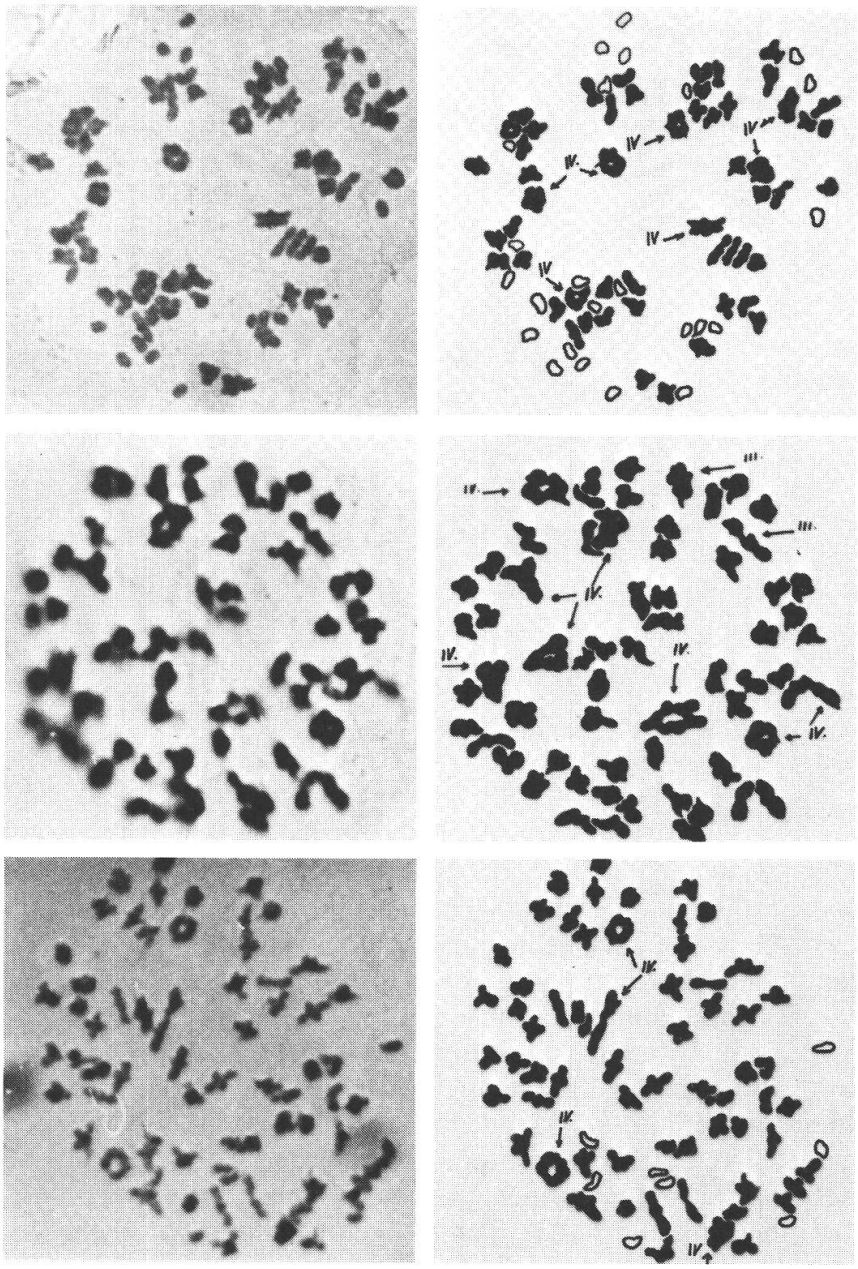


Fig. 5. Analysis of meiosis of the autotetraploid plants designated as I, II, III (×800, ×1000, ×800).

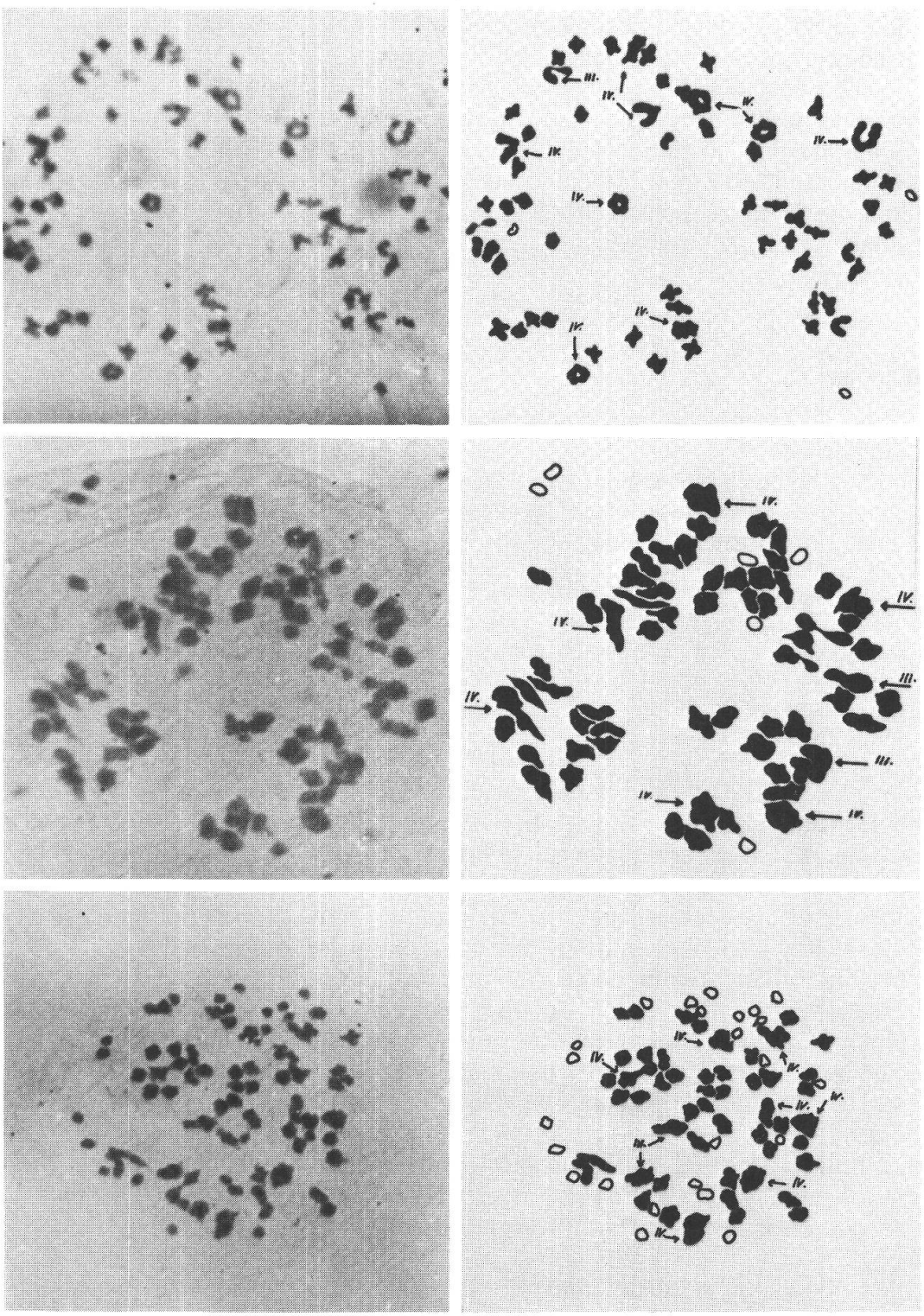


Fig. 6. Chromosome associations during meiosis of autotetraploids numbered IV, V, VI (×800, ×1000, ×800).

Selfing of 50 aposporous gametophytes resulted in 12 aposporous autotetraploids only, in spite of the fact that the presence of spermatozoids and archegonia was observed in each case. Sporophytes developed very slowly, and six out of the 12 plants lived for some weeks only. The surviving individuals showed a retarded growth rate. The first fertile fronds appeared only on plants 2 years old. The non-aposporous control plants matured within a year. The autotetraploids exhibited significant differences both in size and shape: fronds were about half of that of the normals and dissection was very characteristic (Fig. 2). This anomalous appearance could be detected already in the very first stage.

Hybridization.

In order to obtain triploid plants, we attempted hybridization in the following combinations:
Ch. dentata (4×) × *Ch. hispidula* (2×) (20 prothalli);
Ch. malabariensis (4×) × *Ch. hispidula* (14 prothalli);
Ch. parasitica (4×) × *Ch. hispidula* (35 prothalli).

One plant from the last hybridization developed successfully. Morphologically this individual was near to the tetraploid parent. The lobing of the leaf segments was shallower, it had a creeping rhizome and anthocyanin-coloured petioles (cf. Manton and Ghatak 1958, Fig. 3).

Cytology

The diploid and the natural tetraploid species show normal, regular mitosis and meiosis. Root-tip mitosis exhibited $2n = 72$ and $2n = 144$ chromosomes, respectively. Observed meiotic configurations were 36 bivalents and 72 bivalents, respectively. Panigrahi and Manton (1958) as well as Ghatak et al. (1971) elucidated the relationships within this group by genome analysis. The diploid *Ch. hispidula* and the tetraploid *Ch. parasitica* seem to share a common genome.

In our triploid hybrid the somatic chromosome number was $2n = 108$. This plant produced sporangia abundantly, but the sporogenic tissue degenerated early in the development. One of the spore mother cells was analysable. Irregular meiosis resulted in 25 univalents, 31 bivalents and 7 trivalents. We could not detect even in a single case such regular chromosome pairing ($36_I + 36_{II}$) as mentioned by Panigrahi and Manton (1958, Fig. 4).

The mitotic plates of aposporous autotetraploids showed in all cases $2n = 144$ chromosomes. Analysis of their meioses, as expected, resulted in an irregular picture: the chromosome associations varied from univalents up to quadrivalents (Fig. 5 and Fig. 6).

Table 1 shows the results of meiotic analysis of these aposporous tetraploids. The amount of spores produced varied notably. The largest amount was produced by plant III.

Spore size

Distribution of spore sizes of different species is shown in Fig. 7. The diploid *Ch. hispidula* had the smallest spores and the least deviations. The spores of tetraploid

Tab. 1. Analysis of the meiotic chromosome pairing in aposporous autotetraploids

Plant number	Chromosome associations			
	univalents	bivalents	trivalents	quadrivalents
I	24	46	–	7
	14	53	4	3
	13	60	1	2
II	–	53	2	8
III	8	60	–	4
	8	60	–	4
	4	54	–	8
IV	8	60	–	4
	10	51	–	8
	3	51	1	9
V	6	54	2	6
VI	26	37	–	11
	24	42	–	9

Each line represents a different spore mother cell

species proved to be approximately twice as large (in diameter) as those of the diploid specimen. Plant III of the autotetraploids produced spores in a quantity suitable for analysis. The maximum value corresponds to that of the tetraploids, but the shape of distribution curve is flatter. Our triploid hybrid, mainly with aborted spores and debris, showed the typical abnormal distribution, with the widest range of deviation.

Cytophotometry

Cytophotometric data are presented in Table II. Each value represents metaphasic cells, i.e., the 4C and 8C levels in arbitrary units: mean ± S.D. of 100–300 nuclei. These data show that the nuclear DNA content of all three natural tetraploids in this complex is significantly lower than expected (twice that of the diploid).

In the case of the triploid hybrid, the mean value also differs significantly, being higher than expected (45.02 versus 41.29). This trend appeared to a greater extent in the aposporous plants. The highest densities were measured in plants designated as V and VI (Fig. 8). Endoploidy was not observed. The cell size of diploids was inferior to that of tetraploids in all tissue types. Values of the DNA content of the various ploidy levels varied within broad limits (Fig. 9). The values of diploid metaphases show little variation. The range is much wider in the case of tetraploids. Values for aposporous tetraploids showed also an extremely wide scattering. Moreover, interphasic nuclei of the root-tip meristems showed a strongly fluctuating capacity for staining. Measured densities did not correspond to the expected 2C–4C or 4C–8C proportions, respectively. Surprisingly, this type of anomaly was observed in the case of prophases and highly condensed telophases, too. Usually they showed decreased levels.

Tab. 2. Cytophotometric estimates of nuclear DNA content in arbitrary units

Plant number	Species/cross	Ploidy level	Integrated optical density ($\bar{x} \pm$ S.D.)
1	*Ch. hispidula*	2x	28.27±1.68
2	*Ch. hispidula*	2x	28.86±3.36
3	*Ch. hispidula*	2x	30.26±4.46
4	*Ch. hispidula*	2x	30.31±2.64
5	*Ch. hispidula*	2x	30.34±2.27
6	*Ch. hispidula*	2x	31.52±4.77
7	*Ch. parasitica* × *Ch. hispidula*	3x	45.02±1.53
8	*Ch. parasitica*	4x	49.86±2.35
9	*Ch. parasitica*	4x	50.09±2.51
10	*Ch. parasitica*	4x	51.72±7.80
11	*Ch. parasitica*	4x	52.63±0.43
12	*Ch. parasitica*	4x	52.67±1.96
13	*Ch. parasitica*	4x	52.99±1.47
14	*Ch. parasitica*	4x	53.91±5.37
15	*Ch. parasitica*	4x	54.72±3.97
16	*Ch. parasitica*	4x	55.23±3.28
17	*Ch. dentata*	4x	56.34±11.19
18	*Ch. dentata*	4x	59.13±5.41
19	*Ch. malabariensis*	4x	52.91±6.19
20	*Ch. malabariensis*	4x	54.02±6.37
I	Aposporous *Ch. hispidula*	4x	67.51±5.59
II	Aposporous *Ch. hispidula*	4x	66.41±4.67
III	Aposporous *Ch. hispidula*	4x	64.10±8.38
IV	Aposporous *Ch. hispidula*	4x	72.39±5.39
V	Aposporous *Ch. hispidula*	4x	83.25±8.65
VI	Aposporous *Ch. hispidula*	4x	80.88±7.28

Discussion

Reduction of the nuclear DNA amount of natural polyploids and an increased DNA content of triploid hybrids and synthetic autotetraploids requires some explanation. Whether the decrease of DNA in natural tetraploids is attributable to elimination of some genetic material during evolution, or whether it is an artifact caused by heterochromatinization processes is still doubtful (Verma and Rees 1974, Grant 1969, Taper and Grant 1973, De Maggio et al. 1971, Nirula et al. 1961, I.-Jung Cheng and Grant 1973). Lower values measured in our experiments and variations within data referring to telophases probably could be explained by high densities of compact nuclei and problems mentioned by Miksche et al. (1979). Prophasic low values are also questionable. We succeded in achieving reproducible results with metaphases and with the cells of the calyptra. The latter seemed to be in G_0 according to their integrated densities.

Reports showing increased DNA values also can be found in the literature (Cullis 1979, Nagl 1979). Dhillon et al. (1983) detected an increase of DNA content and

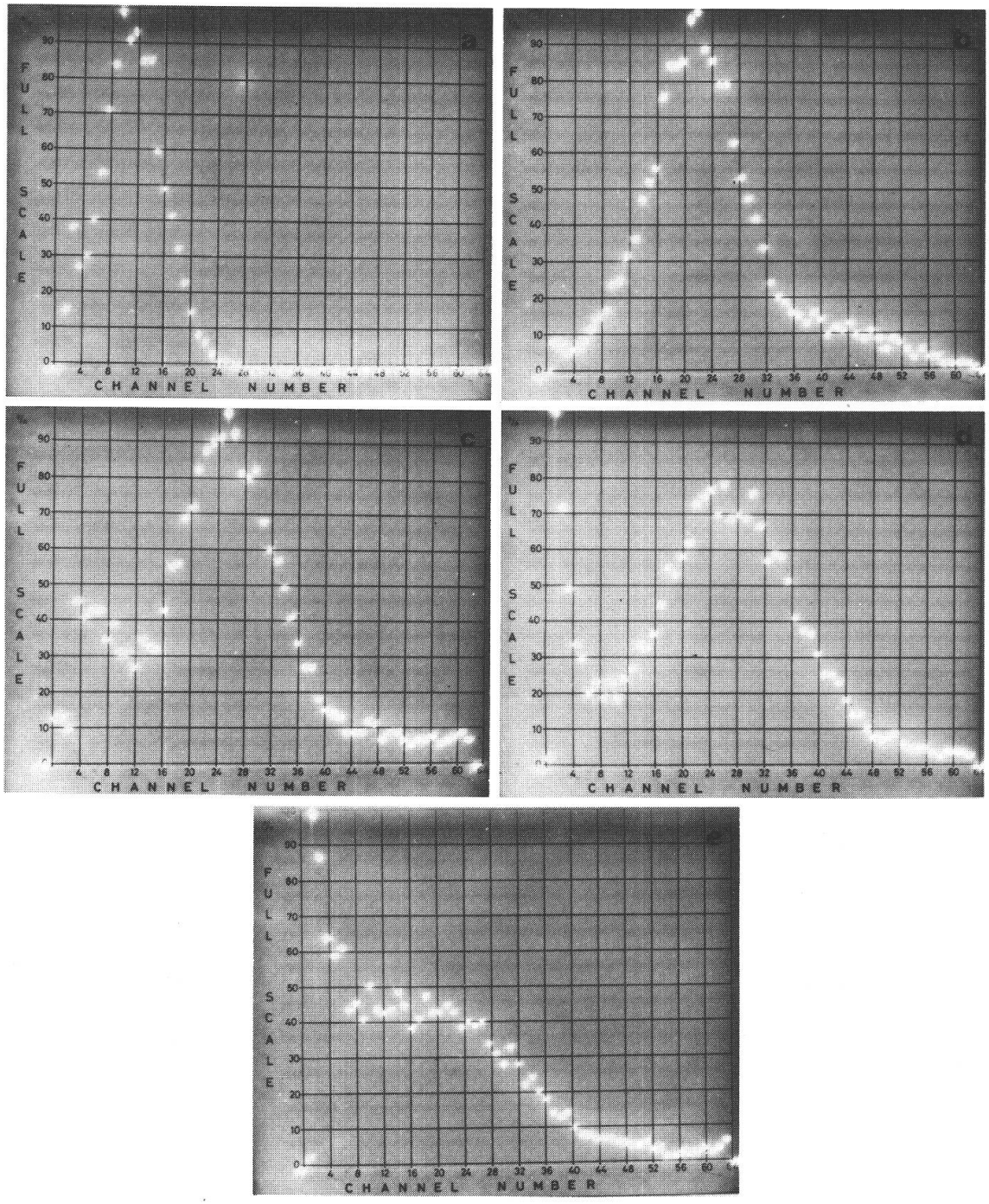
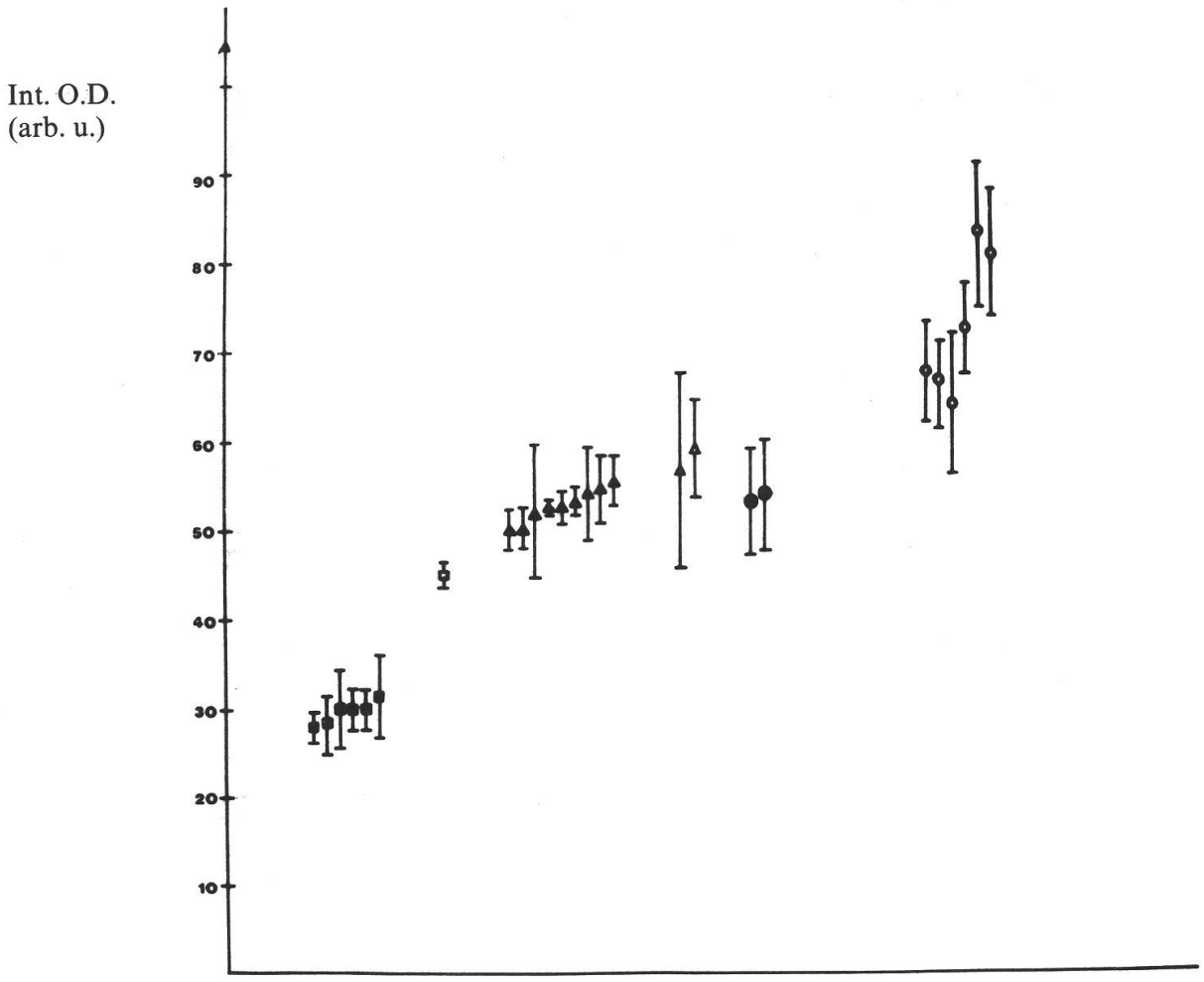


Fig. 7. Distribution of spore size: a) *Ch. hispidula,* b) *Ch. parasitica,* c) *Ch. malabariensis,* d) aposporous tetraploid III, e) *Ch. parasitica* × *Ch. hispidula,* 3x hybrid

heterochromatinic fractions in dihaploid tobacco without any changes in chromosome number. They reported a 12% increase of heterochromatic elements, suggesting amplification of certain specific sequences. They found these alterations to be stabilized in the progeny. This process was coupled with lower leaf yield and growth than that of the parental lines. A relatively great variation in nuclear DNA amount and disturbed mei-



■ = *Ch. hispidula* 2x 29.93; □ = *Ch. parasitica* × *Ch. hispidula* 3x 45.02; ▲ = *Ch. parasitica* 4x 52.65; △ = *Ch. dentata* 4x 57.74; ● = *Ch. malabariensis* 4x 53.47; ○ = Aposporous 4x 72.42

Fig. 8. Microspectrophotometric determination of nuclear DNA content in root-tip meristems of *Christella* species, synthetized triploid hybrid, and aposporously raised autotetraploids. Each point represents mean ± SD of metaphases for individuals in arbitrary units.

osis of autotetraploid and hybrid *Christella* also indicate reorganization processes in the genome during polyploidization and hybridization. The spectacular irregularity of the frond morphology might also be a consequence of genomic instability.

Manton (1950) reported similar meiotic behaviour of chromosomes in aposporous *Osmunda.* She detected similar deviations in the development of the polyploid series, too.

The unexpectedly high cytophotometric values may be a consequence not only of amplification but also of staining anomalies caused by rearrangements. Differences in heterochromatinization and overestimation of diffused chromatin might also lead to the same results. Great differences in staining of individual interphasic nuclei can be caused by disparity of differentiation. Fröhlich and Nagl (1979) reported similar stain-

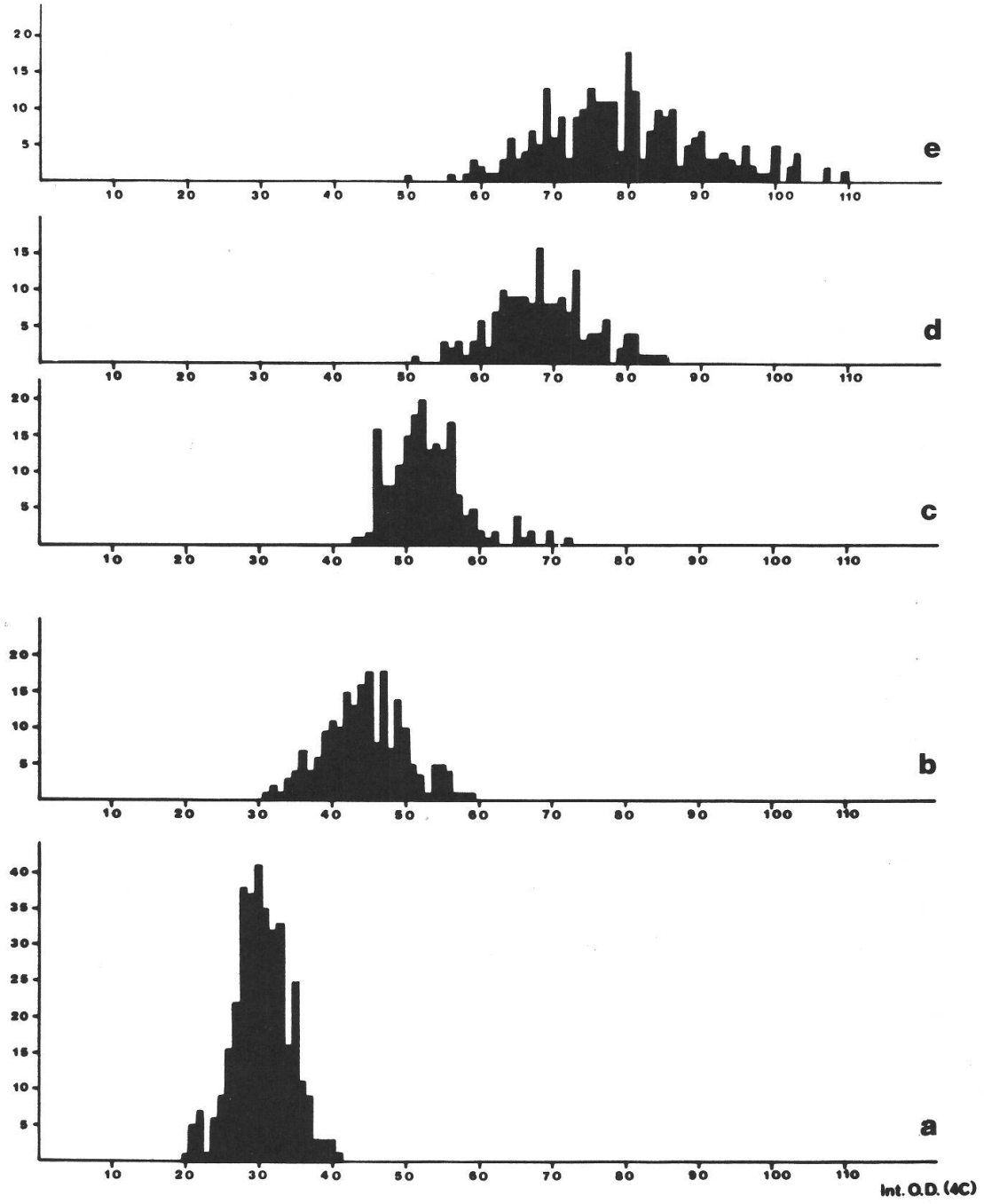


Fig. 9. Cytophotometric analysis of Feulgen-stained metaphases of different ploidy levels. The values for nuclei of diploid species are distributed over the narrowest region. The values for higher ploidy levels are characterized by wider ranges. a) *Ch. hispidula:* 30.26±4.46; b) *Ch. parasitica*×*Ch. hispidula:* 45.02±1.53; c) *Ch. malabariensis:* 52.91±6.19; d) aposporous autotetraploid I: 67.51±5.59; e) aposporous autotetraploid VI: 80.88±7.28.

ing irregularities in *Rhoeo discolor* during floral differentiation. Integrated optical density values distributed over diversely broad intervals at different ploidy levels were reported by De Maggio et al. (1971).

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