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Condensed Chromatin in Diploid and Allopolyploid *Microseris* Species with Different Genome Size: a Quantitative Electron Microscopic Study*

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Summary. The species-specific proportion of chromatin in the condensed state was estimated by quantitative electron microscopic morphometry of nuclear sections in 9 diploid and 5 allopolyploid species of *Microseris* (Asteraceae). A positive correlation between the genome size (haploid DNA content, or C value) and the percentage of chromatin in the condensed state (as visible in ultrathin sections) was found in diploids (r = 0.89). Nuclei of allopolyploid (tetraploid) species exhibit condensed chromatin in a percentage which corresponds to the average of the values found in the parents. This suggests that each parental genome controls chromatin condensation at interphase independently within the nucleus, and that the degree of condensation is not directly determined by the nuclear DNA content per se. Genome size differences among *Microseris* species may depend preferentially, but not entirely, on DNA fractions located in, and perhaps being the cause of, condensed chromatin.

Key words: *Microseris* – DNA content – Condensed chromatin – Polyploidy

Introduction

The proportion of chromatin in the condensed state (eu- and heterochromatin) has been shown to be species-specific in plants and depends primarily on the nuclear DNA content (2C value; Nagl 1979; Nagl and Fusenig 1979). Variation of the species-specific chromatin texture occurs during the cell cycle, but not in relationship to tissue differentiation and transcription activity as in higher animals, except in cells of the gametophyte or other highly specialized cells. So far, the positive correlation between DNA content and chromatin condensation has been studied in several unrelated plants and in some species of Anthemideae (Fuhrmann and Nagl 1979). In this study we have tested the situation in the genus *Microseris*, among which deploid and tetraploid species with various 2C values are known (Price and Bachmann 1975). It was found that the correlation between genome size and proportion of condensed chromatin holds also for diploid *Microseris* species, but that all polyploids exhibit less condensed chromatin than expected on the basis of their total nuclear DNA content.

Material and Methods

Root tips of the species listed in Table 1 were fixed with glutaraldehyde (6.25%), post-fixed with osmium tetroxide (1%) in PIPES buffer, pH 7.2 (PIPES = piperazine-N,N' -bis-2-ethanesulfonic acid), and embedded according to Spurr (1969). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 10 electron microscope. Micrographs were magnified to a final magnification of 10.000. The proportion of condensed chromatin was determined using the semi-automatic equipment MOP-Digiplan (Kontron) according to the formula:

$$\% \text{ Cond. Chrom.} = \frac{\text{Area}_{\text{Cond. Chrom.}} \times 100}{\text{Area}_{\text{Nucleus}} - \text{Area}_{\text{Nucleolus}}}$$

Only non-cycling nuclei from root tips were used (about 2 mm above the tip). Statistic analysis was made with a PDP-8 computer (Digital Equipment).

The reliability of this quantitative electron microscopic morphometric method has been tested in a number of comparative light and electron microscopic studies (Nagl 1979, in press, and unpublished results). Non-cycling nuclei of angiosperms show only minimal structural variation, except in the gametophyte and certain cells of the ovule (W. Nagl unpublished). The probability of evaluating an atypical nuclear section is reduced by (a) eliminating nuclei of very small area ('cup-sections'), and (b) eliminating the nucleolar area (if present), which generally cannot be occupied by condensed chromatin. This leads to similar values for condensed chromatin in nuclei sectioned in the region of the nucleolus and nuclei sectioned in a different region.

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In general, the probability of obtaining a real figure of the proportion of condensed chromatin is higher the more evenly the chromatin is distributed in the nuclear cavity. Therefore, the number of measurements have been higher the less condensed chromatin present, and the more scattered it is over the nucleus in a given species. As a good criterion for the necessary number of measurements, a standard deviation of about 10% can be seen, which is similar to that obtained in scanning cytophotometric DNA measurements.

Results

Table 1 lists the results of our determinations. The figure given there for the percentage of chromatin in condensed form includes both the constitutive heterochromatin and the species-specifically condensed euchromatin, neither of which can be distinguished apart in electron micrographs. As the elaborate methods for obtaining percentage values for the fraction of condensed chromatin in a nucleus limit the number of determinations, it is not clear how much of the variability is due to sampling error and how much to real biological differences. Even if the total variability is real, though, there are clear and obvious differences in the amounts of chromatin in condensed form in the various species of the genus *Microseris*. Figure 1 gives examples for nuclei of species with little and much condensed chromatin, respectively. The standard deviation of the means are on the order of ten percent, indicating that the values can be taken as reliable according to our criterion.

A discussion of these differences can be based on the comparison of the total DNA amount contained in the haploid genome (1C DNA value) of each species with the amount of chromatin in the condensed form. The relationship is illustrated in Figure 2 for diploid species of *Microseris*. An inspection of this figure shows a general positive correlation between the two parameters, although there is some spread among species with similar DNA content. The coefficient of correlation is r = 0.89, the slope of the regression line 12.66% condensed chromatin per pg DNA.

Table 1. The proportion of condensed chromatin in *Microseris* species as determined by electron microscopic morphometry of ultrathin sections of nuclei

Species	Strain	Ploidy	N_m[a]	% c.c.[b]
M. laciniata	A60	2x	20	35.5 ± 3.5
M. nutans	B73	2x	16	31.0 ± 2.9
M. howellii	B74	2x	27	50.5 ± 4.9
M. lindleyi	A22	2x	22	20.0 ± 1.5
M. douglasii	B14	2x	20	20.5 ± 0.8
M. bigelovii	A25	2x	18	11.5 ± 1.2
M. elegans	B36	2x	22	13.2 ± 3.0
M. pygmaea	B95	2x	25	10.7 ± 2.0
M. acuminata	B41	4x	11	13.4 ± 1.8
M. campestris[c]	B55	4x	19	16.9 ± 1.5
M. decipiens[d]	B20	4x	30	15.6 ± 1.2
M. heterocarpa[c]	B10	4x	26	20.5 ± 1.8
M. scapigera	B94	4x	14	38.9 ± 2.3

[a] Number of electron micrographs measured
[b] Percentage of chromatin in the condensed form (means + standard deviations)
[c] Parents: *M. elegans* and *M. douglasii*
[d] Parents: *M. lindleyi* and *M. bigelovii*
[e] Parents: *M. lindleyi* and *M. douglasii*.

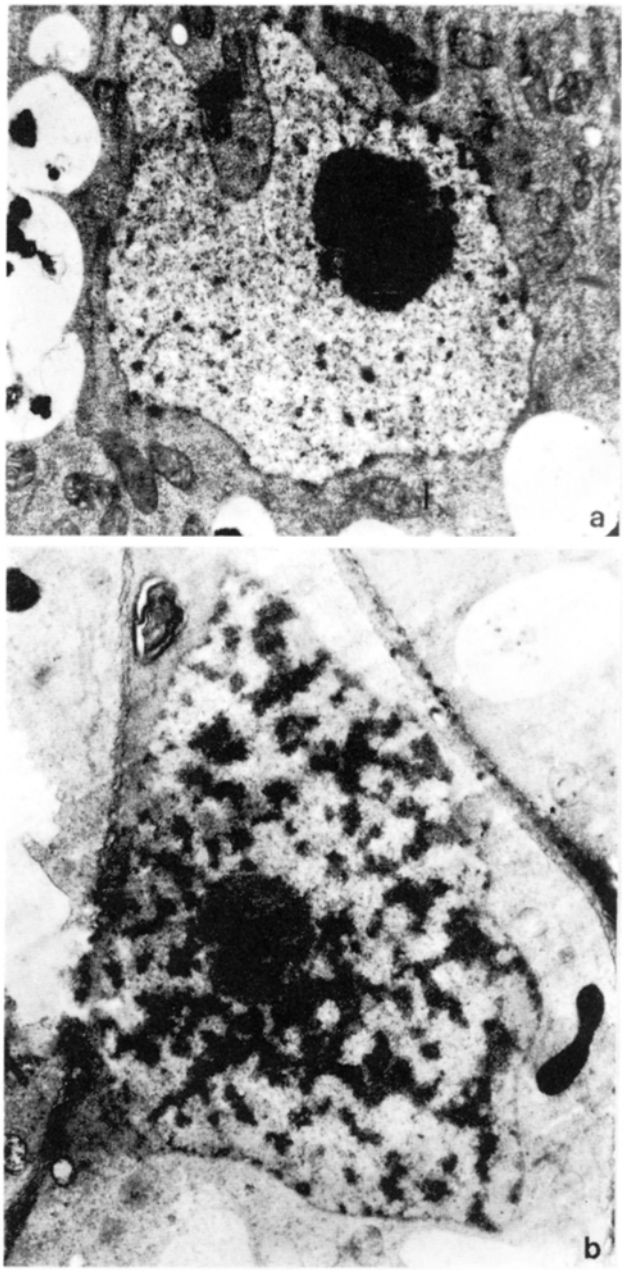

Fig. 1a and b. Electron micrographs of root tip nuclei in *Microseris pygmaea* (a) and *M. howellii* (b). Note the enormous difference in the amount of condensed chromatin (× 10.000)

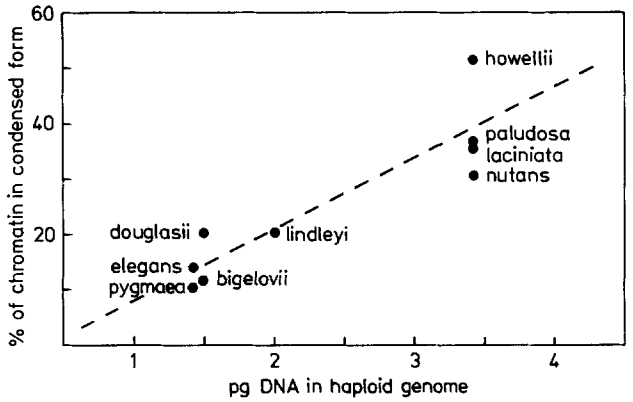

Fig. 2. Diagram to illustrate the relationship between haploid DNA content (C value) and percentage of chromatin in the condensed form in diploid species of *Microseris*

One could suggest that chromatin condensation is controlled by the amount of repetitive DNA in the genome (for discussion see Nagl 1979). The spread of values for condensed chromatin among species with similar DNA content reinforces, however, earlier observations by Bachmann and Price (1977) who found that genome size differences in diploid *Microseris* species are not due to various amounts of repetitive DNA being added in the different species to a constant common basic genome. Just as genome size differences do not result from a simple addition of non-coding repetitive DNA to a basic genome in this genus (but evidently in *Lathyrus, Vicia, Dermestes* and other genera – reviewed by Nagl 1978), so we do not find various amounts of condensed chromatin added to a constant amount of diffuse chromatin. There is, however, a basic trend towards a higher percentage of condensed chromatin in species with more nuclear DNA (species whose haploid DNA content amounts to about 1.5 pg DNA cluster round 15% condensed chromatin, the species with 2.0 pg show 20%, and most species with about 3.5 pg are grouped at 35%). In addition, the total variation of decondensed chromatin (in absolute terms) is only 1 : 2.35, while that of condensed chromatin exceeds up to 1 : 4.81 among the diploid species. As the DNA concentration in condensed chromatin is much higher than in decondensed chromatin, there is evidence that much of the variation in genome size (C value) must be attributed to variation of the condensed chromatin.

While the maximum percentage of condensed chromatin in the annual species is 20% (*M. douglasii*), the lowest percentage for a perennial is 31% (*M. nutans*). The total range of values extends from 10.7% (*M. pygmaea*) to 50.5% (*M. howellii*). This variation among very closely related species is striking (for the systematic relationships in *Microseris* see Chambers 1955; Bachmann and Chambers 1978). These values look somewhat like the saltatory change in various repetitive DNA fractions which must be postulated to explain the genome structure of the various *Microseris* species (Bachmann and Price 1977). The possibility that one specific kind of DNA, characterized, for example, by its degree of sequence repetition, occurs in condensed chromatin and another kind of DNA occurs in less condensed chromatin, cannot yet be ruled out, but the pattern of genome structure and the pattern of chromatin texture cannot be correlated at present.

That the quality rather than the gross amount of DNA is related to the fraction of chromatin in condensed form can be deduced from the data obtained in allopolyploid species of which the parental species can be identified (Chambers 1955). Concerning the genome sizes of any of these species we know that they are near the value obtained by adding the parental values. The amounts of condensed chromatin represent, however, not the sums of the values measured in the parental species, but they are rather near the mean of them (Figs. 3, 4). This is particularly striking in the two derivatives of *M. lindleyi*, which reflect the striking differences in chromatin structure between the respective other parental species. The parents of *M. scapigera* are not yet known but it can be predicted from the proportion of condensed chromatin that the one should have more than 38.9% (which is expected to be the average of the parents as in the other polyploids), probably *M. howellii*.

Discussion

The finding that one cannot extrapolate nucleotypic effects from diploid to polyploid species simply by con-

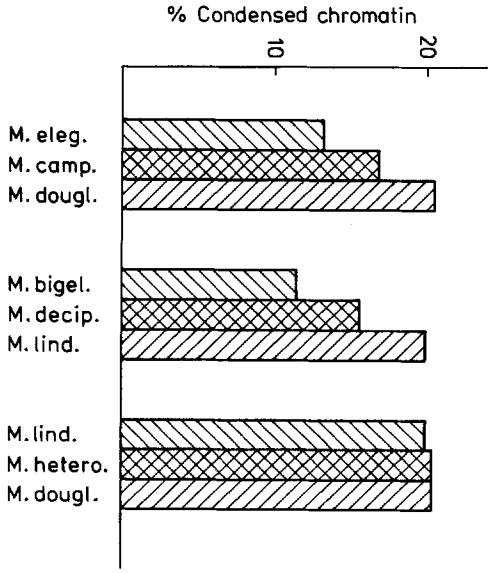

Fig. 3. Diagram to illustrate the proportion of condensed chromatin in allopolyploid species of *Microseris* and their parents. The middle columns represent the polyploid hybrids

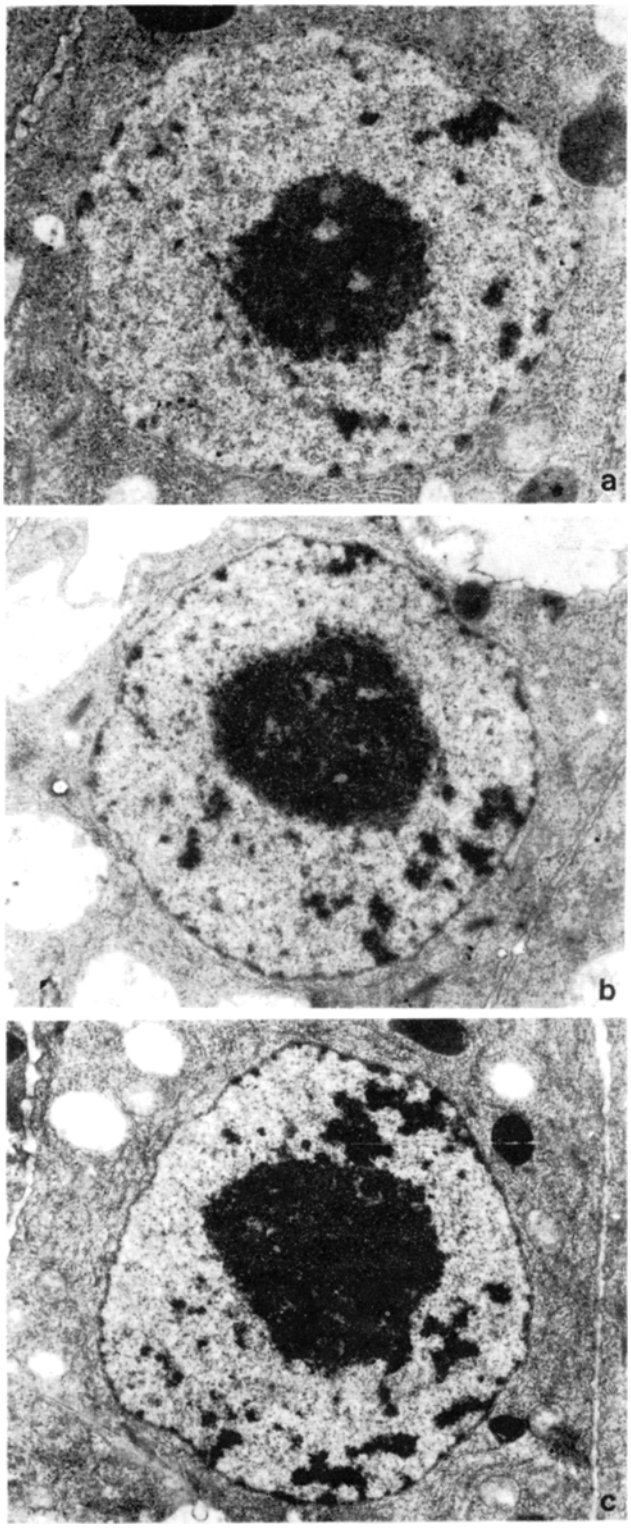

Fig. 4a-c. Electron micrographs of representative nuclei of the allopolyploid species *M. decipiens* (b) and its diploid parents, *M. bigelovii* (a) and *M. lindley* (c) (× 10.000)

sidering total nuclear DNA amount also applies to other quantitative characters such as cell cycle duration (reviewed by Nagl 1978). These and other results reveal the necessity to elucidate how nucleotypic effects originate. They evidently cannot be due to a direct influence of the total DNA amount per se, at least with reference to chromatin condensation. The quantitative effects of genome size must be mediated by a qualitative difference between small and large genomes (e.g. different amount and/or distribution of a histone H1-binding repetitive DNA sequence). Evidence for a relation between genome organization and chromatin texture comes, in addition, from the fact that there is a gross correlation between the 1C value and the percentage of condensed chromatin, but the regression is different in different genera and families with respect to the coefficient of correlation, the slope of the regression line, and its cross with the y axis (Nagl, in press).

It seems that condensed chromatin in plants represents silenced portions of the genome, which have been added to the functional genome during evolution eventually by nucleotypic reasons (influences on cell and plant size, cell cycle and minimal generation time etc.). ^{3}H-Uridine autoradiography in nuclei from species with little and much condensed chromatin showed that their absolute transcription activity is nearly identical (Sokol and Nagl, in preparation). Experiments are now planned to elucidate the molecular determinants of chromatin condensation in plants as well as its physiological significance.

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