Theor Appl Genet (1986) 73:20–26



# Nuclear DNA changes within *Helianthus annuus* L.: cytophotometric, karyological and biochemical analyses

**A. Cavallini [1], C. Zolfino [1], G. Cionini [2], R. Cremonini [3], L. Natali [1], O. Sassoli [1] and P. G. Cionini [3]**

[1] Istituto di Genetica della Università, Via G. Matteotti 1/A, [2] Istituto di Mutagenesi e Differenziamento del C.N.R., Via Svezia 10, [3] Dipartimento di Scienze Botaniche della Università, Via L. Ghini 5; I-56100 Pisa, Italy

Received February 11, 1986; Accepted April 22, 1986
Communicated by F. Mechelke

**Summary.** Cytophotometric measurement of the root meristems of seedlings after Feulgen-staining reveals that large differences (up to 58.16%) in nuclear DNA content may occur in the thirty-one cultivated varieties or lines of *Helianthus annuus* tested. Significant variations (not exceeding 25%) in the amount of DNA, which does not differ between the root and the shoot meristems of a single seedling, are also found to exist within cultivars or lines; even seedlings obtained from seeds collected from different portions of single heads of plants belonging to a selfed line may vary one from the other in this respect. Variations in the number of chromosomes or alterations in the chromosome structure do not account for the differences observed in nuclear DNA content. Karyometric analyses demonstrate that the surface area of squashed interphase nuclei and metaphase chromosomes and the total length of the latter increase with the increase in Feulgen/DNA absorption. DNA thermal denaturation and reassociation kinetics indicate that a frequency variation in repeated DNA sequences goes hand in hand with changes in the size of the genome. These results, supporting the concept that a plant genome is highly flexible, are discussed in relation to other data to be found in the literature on the intraspecific variation in the nuclear DNA content and in relation to the way in which it is produced in *H. annuus*.

**Key words:** *Helianthus annuus* – Intraspecific DNA changes – Cytophotometry – Karyology – Chemicophysical DNA characterization

## Introduction

Extraordinary plasticity, at both the morphological and physiological levels, is a common feature of plants and is due to their inability to escape a changing environment as well as to the mode of their reproduction and development. The suggestion that genome flexibility might be an aspect of this changing ability has raised objections in the past, on the basis of the widely accepted concept of the absolute primacy of DNA over all biological phenomena, thus postulating its constancy (Lima-De-Faria 1983).

Today, there is a growing consensus of opinion that proliferation or deletion of nuclear DNA sequences does not occur only in the case of divergence and evolution of species (Price 1976; Hutchinson et al. 1980). Both developmental and physiological stimuli and the response to stressing environmental conditions may also give rise to rapid genomic changes resulting in variations in the nuclear DNA content (Walbot and Cullis 1985). These variations may originate in different ways: polyploidy commonly occurs in plant populations and chromosome endoreduplication is a well-known factor in tissue differentiation in pteridophytes and the vast majority of angiosperms. Changes in the amount of certain genome fractions alone may originate from the occurrence or the variation in number of supernumerary chromosomes, aneuploidy, deletion or duplication of chromosome segments produced by unequal crossing-over or by other chromosomal aberrations; they may also originate from such processes as extra DNA synthesis (DNA amplification), non-replication of certain sequences during a DNA synthetic phase (DNA underreplication) or elimination of certain DNA sequences (DNA loss). Genomic variations may become stabilized and/or be inherited with relative ease in plants, due to *de novo* organ formation throughout life, absence of a discrete germ line, and the ability to regenerate *in vitro*.

In this paper, we present evidence indicating that, in the sunflower, variations in genome size and organization continuously occur during reproduction and diversify cultivated varieties or lines.

## Materials and methods

### *Plant material*

Seeds (achenes) of different cultivated varieties and selfed lines of *Helianthus annuus* were germinated in damp ver-

miculite under sterile conditions at 20 °C and the seedlings were used as experimental material.

*DNA cytophotometry*

For DNA cytophotometry, root or shoot apices, fixed in ethanol-acetic acid 3 : 1 (v/v), were squashed in a drop of 45% acetic acid after treatment with a 5% solution of pectinase (Sigma) for 1 h at 37 °C. The cover-slips were removed by the dry-ice method and the preparations were Feulgen-stained after hydrolysis in N HCl at 60 °C for 8 min. After staining, the slides were subjected to three 10-min washes in $SO_2$ water prior to dehydration and mounting in DPX (BDH). Since simultaneous processing was not possible due to the large number of preparations to be analyzed, squashes made with the root tips of a single plantlet of *Vicia faba* (4C = 53.31 pg: Bennett and Smith 1976) were concurrently stained for each group of slides and used as standards in order to make all results comparable and to convert relative Feulgen units into picograms of DNA. Feulgen/DNA absorptions in individual cell nuclei were measured at the wavelength of 550 nm using a Barr and Stroud GN5-type integrating microdensitometer.

*Karyology*

Roots were treated with a 0.05% colchicine (Sigma) solution for 4 h at room temperature and preparations of apices were made as described above. The surface area of the 2C ($G_1$) and 4C ($G_2$) interphase nuclei and of the chromosomes in euploid C-metaphases was measured using a Leitz MPV3 microscope photometer equipped with a mirror scanner and a HP 85 computer. Metaphase chromosome length was measured in microphotographs.

*DNA extraction*

DNA was extracted from lyophilized tissues according to the method developed by Bendich et al. (1980), modified by Durante et al. (1985). Roots were ground in a mortar and lysed with a solution containing 0.05 M Tris-HCl pH 8.0, 1 mM EDTA, 0.15 M NaCl and 1% sodium dodecylsarcosinate. The lysate was heated at 60 °C for 10 min, then preincubated pronase (Sigma) was added to a final concentration of 250 µg/ml. After incubation at 37 °C for 3 h, the mixture was centrifuged at 20,000 × g for 15 min. Solid CsCl and ethidium bromide from a stock solution (10 mg/ml) were added to the supernatant up to a final concentration of 0.8 g/ml and 300 µg/ml, respectively. The solution was centrifuged at 44,000 rpm in a Beckman L5-65 ultracentrifuge using the 50 Ti rotor and the DNA band, visualized under long-wave UV illumination, was collected and recentrifuged. Ethidium bromide was then removed by gentle inversion of the solution with n-butanol.

*Thermal denaturation*

Thermal denaturation was performed in 0.1 × SSC using a Perkin-Elmer 576 ST spectrophotometer equipped with a temperature programme controller, and the increase in hyperchromicity at 260 nm was continuously followed by a X-Y recorder.

*Reassociation kinetics*

DNA was sheared by sonication in a MSE sonicator at medium energy output for 5 × 5 s with 10-s intervals at 4 °C. Sedimentation in neutral sucrose gradients according to Clewell and Helinski (1969) revealed that the fragments of different DNA samples were constantly of a relatively homogeneous length of about 400 base pairs. Sheared DNA, dissolved in 0.12 phosphate buffer at a concentration of 50 µg/ml, was denatured for 10 min at 103 °C and allowed to renature according to Britten et al. (1974). The reassociation process was monitored in a closed thermostatically-controlled cuvette using the same equipment employed for the analysis of the thermal denaturation kinetics. *E. coli* DNA (Sigma) was used as a standard after shearing in the same conditions as above: its $C_0t\ ½$ value was found to be 3.95 moles × $l^{-1}$ × s in our renaturation system. Repetitive DNA frequencies were calculated by means of Scatchard-type analysis (Marsh and MacCarthy 1974).

## Results

*Cytological determinations*

The mean Feulgen-absorptions of early prophases (=4C) in the root meristems of seedlings and the calculated DNA content per haploid nucleus in each of thirty-one cultivated varieties or lines of sunflower we studied are reported in Table 1. Amounts of DNA differ widely from one cultivar or line to another: for example, taking the most variant values, a 58.16% variation exists between the A selfed line (5.33 pg of DNA per haploid nucleus) and the 'Romsun HS 52' (3.37 pg) cultivar. It is worth noting that the nuclear DNA content of the 'Stromboli' hybrid (4.03 pg) is half way between those of the two parentals: HA89 (4.60 pg) and Restorer RHA271 (3.41 pg) lines. In Table 1, seed provenance and certain distinctive features of each cultivar or line are also listed: no relationship seems to exist between the genome size and the reported characteristics.

The results shown in Table 2 demonstrate that the variations in the amount of nuclear DNA are not confined only to roots. Indeed, no significant difference exists between early prophase Feulgen-absorptions in the root and the shoot meristems of a single seedling. On the other hand, it may be seen from Table 2 that significant differences in the DNA content exist between seedlings within a selfed line; these variations, never exceeding 25%, occur as a rule within every cultivar or line analyzed.

Table 3 contains the results of cytophotometric determinations carried out on the root meristems of seedlings obtained from seeds (achenes) collected head by head in five plants of the E selfed line: the data given indicates that genome size may differ within the progeny of a single homozygous plant. Table 3 also shows that: i) a relationship exists between the amount of DNA and the position in the inflorescence in which the seed has developed, since, in all heads, the nuclear DNA content of the seedlings obtained from seeds developed at the periphery of the head is significantly greater than that of the seedlings obtained from seeds developed in the remaining part of the head; ii) there

**Table 1.** Characteristics, Feulgen-absorption of early prophases (=4C) and calculated DNA content per haploid nucleus of thirty-one cultivars, hybrids or lines of *Helianthus annuus*. Each Feulgen-absorption value is the mean of one hundred determinations carried out in root meristems: twenty prophases for each of five seedlings

| Name | Cultivar, hybrid or line | Seed provenances | Distinctive features | Feulgen absorption (a.u.; mean ±SE) | Mean DNA content per haploid nucleus (pg) |
|---|---|---|---|---|---|
| 'Ala' | cultivar | Italy | – | 30.4±0.67 | 4.26 |
| 'Argentario' | cultivar | Italy | – | 34.4±0.47 | 4.83 |
| 'Cernianka' | cultivar | Italy | short plants | 31.1±0.61 | 4.36 |
| 'Gianni' | cultivar | Italy | – | 31.7±0.60 | 4.45 |
| 'IH 155' | cultivar | Hungary | – | 26.2±1.52 | 3.68 |
| 'RO 150' | cultivar | Rumania | – | 35.0±0.45 | 4.91 |
| 'RO 155' | cultivar | Rumania | – | 27.9±0.39 | 3.91 |
| 'Romsun HS 52' | cultivar | Rumania | – | 24.0±0.46 | 3.37 |
| 'Romsun HS 53' | cultivar | Rumania | – | 28.9±0.47 | 4.05 |
| 'Romsun HS 301' | cultivar | Rumania | – | 24.4±0.37 | 3.42 |
| 'Sannace' | cultivar | Italy | – | 30.8±0.61 | 4.32 |
| 'Stromboli' | hybrid (Restorer RHA271×HA89) | France | tall plants | 28.7±0.51 | 4.03 |
| HA 89 | inbred line (HA89×Maintainer A) | Italy | male-sterile | 32.8±0.49 | 4.60 |
| Restorer RHA 271 | selfed line | Italy | restorer | 24.3±0.65 | 3.41 |
| Maintainer A | selfed line | Italy | maintainer | 32.7±0.65 | 4.59 |
| 4-22 | selfed line | Italy | oil-enriched seeds; tall plants | 32.0±0.48 | 4.49 |
| 4-31 | selfed line | Italy | oil-enriched seeds; tall plants | 32.6±0.46 | 4.57 |
| 4-34 | selfed line | Italy | oil-enriched seeds | 28.1±0.65 | 3.94 |
| 4-36 | selfed line | Italy | oil-enriched seeds; tall plants | 33.1±0.51 | 4.64 |
| 4-32 | selfed line | Italy | protein-enriched seeds; tall plants | 29.1±0.47 | 4.08 |
| 4-33 | selfed line | Italy | protein-enriched seeds; tall plants | 33.8±0.65 | 4.74 |
| 4-37 | selfed line | Italy | protein-enriched seeds; tall plants | 25.2±0.62 | 3.53 |
| 4-38 | selfed line | Italy | protein-enriched seeds; tall plants | 25.1±0.60 | 3.52 |
| A | selfed line | Italy | – | 38.0±0.70 | 5.33 |
| GIO | selfed line | Italy | short plants | 37.7±0.47 | 5.29 |
| E | selfed line | Italy | – | 36.8±0.61 | 5.16 |
| M | selfed line | Italy | tall plants | 36.7±0.61 | 5.15 |
| H | selfed line | Italy | tall plants | 35.4±0.52 | 4.96 |
| G | selfed line | Italy | short plants | 33.7±0.59 | 4.73 |
| L | selfed line | Italy | tall plants | 33.3±0.63 | 4.67 |
| F | selfed line | Italy | tall plants | 30.9±0.73 | 4.34 |

**Table 2.** Mean Feulgen-absorption (arbitrary units) of early prophases (=4C) in the shoot and root apices of three seedlings of the A selfed line. Twenty prophases were measured in each apex

| Seedling | Shoot apex | Root apex |
|---|---|---|
| 1 | 40.85 | 41.54 |
| 2 | 37.05 | 38.31 |
| 3 | 35.63 | 33.85 |

| Source of variation | Degrees of freedom | Sum of squares | Mean squares | F |
|---|---|---|---|---|
| Plants | 2 | 58.94 | 29.47 | 29.77* |
| Apices within a plant | 5 | 4.97 | 0.99 | 0.52 |
| Nuclei within an apex | 114 | 217.12 | 1.90 | 0.81 |
| Total | 119 | 281.03 | 2.36 | – |

* Significant at $P \leqq 0.01$

**Table 3.** Feulgen-absorption (arbitrary units; mean ±SE) of early prophases (=4C) in the root meristems of seedlings obtained from seeds developed in different portions of the heads of five plants of the E selfed line. Each Feulgen-absorption value is the mean of sixty determinations; twenty prophases for each of three seedlings

| Head no. | Head portion | | |
|---|---|---|---|
| | Peripheral | Intermediate | Central |
| 1 | 39.01±0.46 | 34.13±0.34 | 34.50±0.40 |
| 2 | 40.21±0.53 | 35.15±0.22 | 37.34±0.41 |
| 3 | 38.86±0.52 | 36.24±0.53 | 35.99±0.43 |
| 4 | 39.08±0.31 | 35.08±0.28 | 33.59±0.25 |
| 5 | 38.79±0.15 | 37.34±0.29 | 36.61±0.23 |

**Table 4.** Karyological analyses in the root meristems of five seedlings of the 'Stromboli' hybrid and its HA89 and Restorer RHA271 parental lines

| Hybrid or line | Mean no. of chromosomes per nucleus | Mean surface area ($\mu m^2 \pm SE$) of squashed nuclei and chromosomes | | |
|---|---|---|---|---|
| | | 2C ($G_1$) nuclei | 4C ($G_2$) nuclei | Euploid C-metaphases |
| Restorer RHA 271 | 30.2 | 155.3±3.3 | 301.4±4.2 | 271.6±5.0 |
| 'Stromboli' | 30.2 | 175.2±2.1 | 320.2±4.4 | 299.4±4.8 |
| HA 89 | 30.8 | 189.5±3.8 | 341.3±5.6 | 322.1±4.4 |

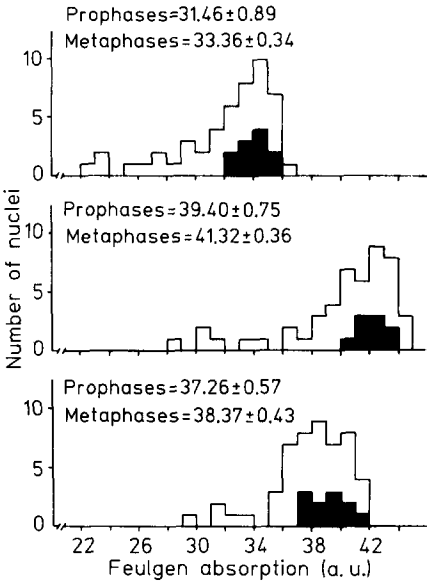



**Fig. 1.** Feulgen-absorptions of early prophases (*open bars*) and euploid C-metaphases (*solid bars*) in the root meristems of three seedlings of the A line

are no significant differences either in the highest or in the lowest values obtained in each head: it follows that the variations found in one single head cover the whole range of variation occurring within the line.

Since extensive aneusomaty has been shown to occur in *H. annuus* (Cavallini and Cremonini 1985; Cremonini and Cavallini 1986), the possibility could not be ignored that this phenomenon might account for the variations in nuclear DNA content that had been observed. That this is not the case is clearly demonstrated by the data given in Fig. 1 and Table 4. Figure 1 gives the Feulgen-absorptions of individual prophases and euploid C-metaphases in the root meristems of three plants belonging to the A selfed line. The occurrence of aneusomaty and its different frequency in the three meristems is evident when the Feulgen-absorptions of prophases are considered, since large ranges of values are observed. However, the Feulgen/DNA contents of euploid metaphases are different in the three seedlings; moreover, in each seedling, their mean value does not differ significantly from that obtained from the measurements of the prophases. Chromosome counts in the C-metaphases of the root meristems of seedlings belonging to the 'Stromboli' hybrid and its HA89 and Restorer RHA 271 parental lines, all three differing in genome size (Table 1), demonstrate that the mean number of chromosomes per nucleus is practically the same (Table 4); as is already known, they are rather uniform in size in *H. annuus* (see Kodama 1974). Chromosome numbers exceeding the diploid number (=34) or alterations in the chromosome structure were never observed.

Table 4 also gives the mean surface areas of squashed 2C ($G_1$) and 4C ($G_2$) interphase nuclei and euploid C-metaphases: significant differences occur between the meristems studied; these differences parallel the variation in DNA content as was cytophotometrically assessed. Chromosome length also increases with the increase in the amount of DNA: the mean length of the entire chromosome complement is 108.9± 1.25 μm in the GIO line (5.29 pg of DNA per haploid nucleus: Table 1) and 100.4± 1.87 μm in the F line (4.34 pg).

*Biochemical analyses*

Figure 2 contains the melting profiles and the first derivative curves of the DNA extracted from the roots of seedlings of the GIO, G and F selfed lines. No significant difference is seen between the three DNAs as far as the Tm values, and hence the guanine-citosine contents, are concerned. However, clear-cut differences are observed both on the light and the heavy sides of the derivative curves, indicating that particular DNA families are differently represented in the three genomes.

The three DNAs, and particularly their repetitive fractions, which are most likely to be involved in the variations in the genome size, were also studied by means of reassociation kinetics. Analysis of the reassociation curves (Fig. 3), which is detailed in Table 5, reveals the existence of rather complex differences in the organization of the three genomes. It must be stressed that there is a difference in the frequency of the repeated DNA sequences, which is particularly evident in the case of the highly repeated sequences: as is also inferable from comparison of the melting profile

**Table 5.** Analysis of the reassociation curves shown in Fig. 3

| DNA sequences | Lines | Frequency | $C_0t$ ½ observed (moles×l$^{-1}$×s) | $C_0t$ ½ pure (moles×l$^{-1}$×s) | Kinetic complexity (nucleotide pairs) | Redundancy |
|---|---|---|---|---|---|---|
| Highly repeated | GIO | 0.119 | $5.9\times10^{-3}$ | $7.02\times10^{-4}$ | $7.93\times10^{2}$ | $7.26\times10^{5}$ |
| | G | 0.102 | $1.0\times10^{-2}$ | $1.02\times10^{-3}$ | $1.15\times10^{3}$ | $3.84\times10^{5}$ |
| | F | 0.083 | $1.2\times10^{-2}$ | $9.96\times10^{-4}$ | $1.12\times10^{3}$ | $2.94\times10^{5}$ |
| Medium repeated | GIO | 0.142 | $1.2\times10^{0}$ | $1.70\times10^{-1}$ | $1.92\times10^{5}$ | $3.58\times10^{3}$ |
| | G | 0.137 | $9.3\times10^{-1}$ | $1.27\times10^{-1}$ | $1.44\times10^{5}$ | $4.12\times10^{3}$ |
| | F | 0.129 | $8.5\times10^{-1}$ | $1.10\times10^{-1}$ | $1.24\times10^{5}$ | $4.13\times10^{3}$ |

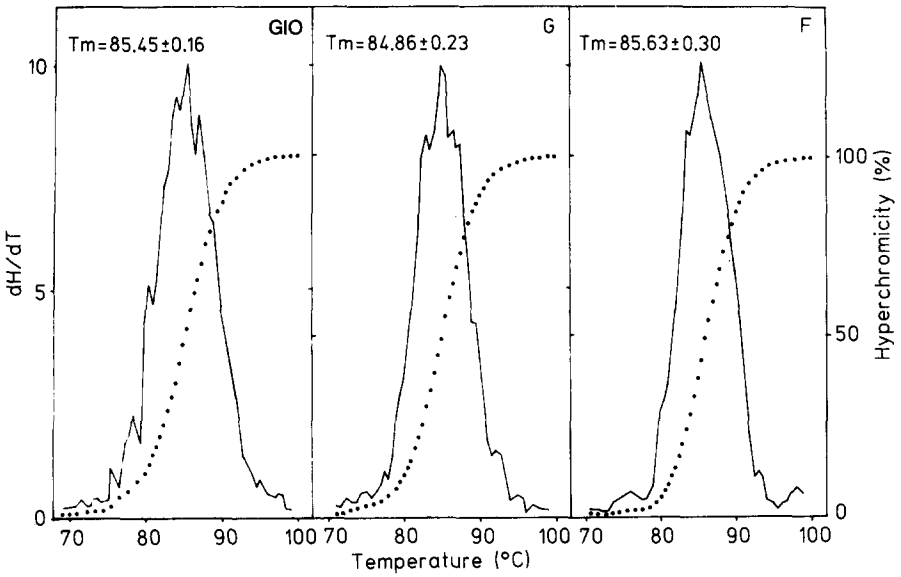
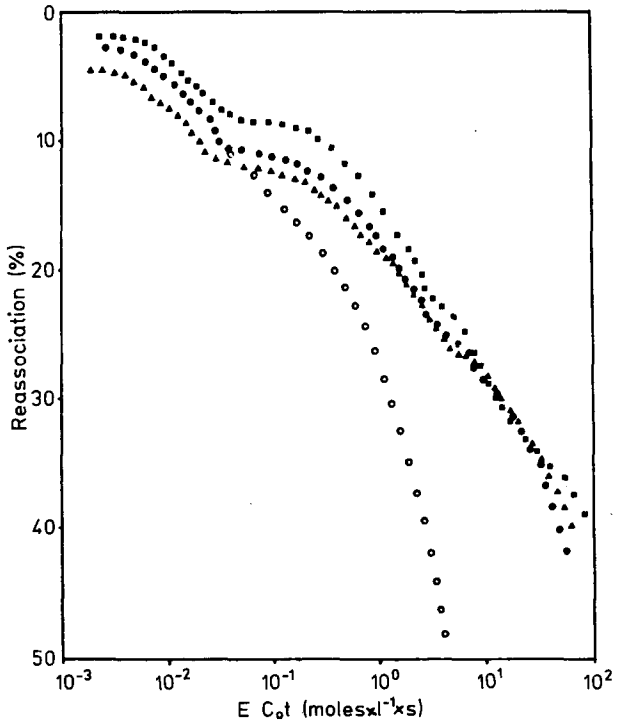



**Fig. 2.** Melting profiles and first derivative curves of the DNAs extracted from the roots of seedlings of the GIO, G and F lines. Graphs are obtained from the averaged values of four repetitions: two for each of two different DNA extractions




derivative curves (Fig. 2), repeated sequences are progressively less frequently represented in the DNA of the GIO, G and F lines (5.29, 4.73 and 4.34 pg of DNA per haploid nucleus, respectively: Table 1).

## Discussion

Mutually supporting one another, our cytological and biochemical results suggest that; i) in sunflower, genome changes are continuously occurring during reproduction in spite of selfing and homozygosity (Table 3); ii) mean DNA content (Table 1) and genome organization (Figs. 2 and 3 and Table 5) may consistently differ from one cultivated variety or line to another, even if variability within them must make us cautious as to the actual extent of the divergencies

**Fig. 3.** Reassociation kinetics of the DNAs extracted from the roots of seedlings of the GIO (▲), G (●) and F (■) lines. Each point is the mean of the values obtained in four repetitions: two for each of two different DNA extractions. *E. coli* DNA (○) was used as a marker

found. The cytophotometric estimates find support in the results of the karyometric determinations showing that the surface area of squashed nuclei and chromosomes (Table 4) and the length of the latter increase with the increase in the Feulgen-absorptions. Both nuclear and chromosome volume are indeed well-established correlates with DNA content in angiosperms (see Price 1976). The cytophotometric (Fig. 1) and karyological (Table 4) analyses also make it clear that variations in chromosome number or gross structure do not account for the changes in nuclear DNA content, which, on the contrary, are paralleled by the frequency variation of repeated DNA sequences, as shown by the biochemical results (Fig. 2 and Table 5).

Nuclear DNA content variation within *H. annuus* had already been reported (Nagl and Capesius 1976) and recently confirmed (Olszewska and Osiecka 1983; Michaelson and Price 1985; Cavallini and Cionini 1986). Moreover, results to be found in the literature suggest intraspecific genome size variation occurs at a level detectable by either Feulgen cytophotometry or chemical methods in other animal and plant species. In plants, even though this finding has not always been confirmed by subsequent studies, this occurrence has been described in several conifers and cereal crops (see Bennett and Smith 1976) and, more recently, in the genus *Microseris* (Price et al. 1980, 1981) and in *Collinsia verna* (Greenlee et al. 1984).

In certain instances, intraspecific genome size variation is clearly linked to changes in growth environment and influences the phenotype, as in the case of flax (see Cullis 1983); in other instances, as is the case with certain *Pinaceae,* in which DNA variations are correlated with changes in latitude, it has been suggested that environmental variants such as climate are factors in the variation (Miksche 1968, 1971; El-Lakany and Sziklai 1971). In several other instances, such as *Pinus resinosa* (Dhir and Miksche 1974), nuclear DNA changes have no correlation with determinable environmental factors, nor do they influence the phenotype. With *H. annuus,* no correlation is discernible between DNA content and seed provenance or the phenotypic characters given in Table 1. With reference to the factors able to induce the DNA changes, since seedlings with different genome sizes are obtained from seeds not randomly located on the head (Table 3), one hypothesis might be that genome changes arise in response to microenvironmental variations possibly due to differences in the availability of water and nutrients in different head portions and/or to differences in the times at which reproduction processes and embryo development occur there.

Our data do not allow us to advance any hypothesis as to the developmental stage(s) at which DNA changes are produced. In this connection, however, it seems worthwhile to consider the behaviour of aneusomaty, since it also produces nuclear changes in *H. annuus,* which is thought to be a species of polyploid origin (Jackson and Murray 1983). A recent study by Cremonini and Cavallini (1986) clearly shows that aneusomaty arises and becomes progressively stabilized during embryogenesis, since the frequency of aneusomatic cells in the root and the shoot apices continuously increases during embryo development and remains unchanged in seedlings.

A further question concerns the way, of all those mentioned in the Introduction, in which the DNA changes that we have observed originate. On the basis of our results, the most likely mechanism of variation would seem to be serial lengthwise repetitions or deletions of encoded DNA base sequences due to extra synthesis or underreplication during DNA synthetic phases. It was suggested that this explains intraspecific variation in genome size in other plant species (Miksche 1971; Miksche and Hotta 1973; Price 1976). Frequency variation of repeated DNA sequences due to such molecular processes has also been shown to occur concomitantly with plant cell functional activity (Cremonini and Cionini 1977) and with such developmental events as cell differentiation (Bassi et al. 1984) and dedifferentiation (Natali et al. 1986) or phase change (Schaffner and Nagl 1979). In this connection, the suggestion put forward by Cullis (1983) that the plant genome might be organized into constant and fluid domains is intriguing. Our data may be added to other evidence progressively accumulating in the literature to support this view.

Work is in progress to answer further questions raised by our results, concerning the developmental stage(s) at which the unstable DNA sequences vary, the molecular process involved and the mechanism controlling the range of variation, the structure and organization at the chromosomal level of unstable DNA.

*Acknowledgements.* We wish to thank Prof. S. Baroncelli and Prof. G. P. Vannozzi (Institutes of Genetics and of Agronomy, University of Pisa) for their generous gift of *H. annuus* seeds. Research work supported by C.N.R., Italy, Special grant I.P.R.A., Subproject 1, Paper No. 916.

## References

Bassi P, Cionini PG, Cremonini R, Seghizzi P (1984) Underrepresentation of nuclear DNA sequences in differentiating root cells of *Vicia faba.* Protoplasma 123:70–77

Bendich AJ, Anderson RS, Ward BL (1980) Plant DNA: long, pure and simple. In: Leaver CJ (ed) Genome organization and expression in plants. Plenum, New York London, pp 31–33

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc Lond, Ser B 274:227–274

Britten RJ, Graham DE, Neufeld BR (1974) Analysis of repeating DNA sequences by reassociation. Meth Enzymol 29:363–405

Cavallini A, Cionini PG (1986) Nuclear DNA content in differentiated tissues of sunflower (*Helianthus annuus* L.). Protoplasma 130:91–97

Cavallini A, Cremonini R (1985) Aneusomaty in sunflower (*Helianthus annuus* L.). Z Pflanzenzücht 95:118–124

Clewell DB, Helinski DR (1969) Supercoiled circular DNA-protein complex in *Escherichia coli:* purification and induced conversion to an open circular DNA form. Proc Natl Acad Sci USA 62:1159–1166

Cremonini R, Cavallini A (1986) Origin and fate of aneusomaty in sunflower (*Helianthus annuus* L.). Z Pflanzenzücht 97:89–92

Cremonini R, Cionini PG (1977) Extra DNA synthesis in embryo suspensor cells of *Phaseolus coccineus*. Protoplasma 91:303–313

Cullis CA (1983) Environmentally induced changes in plants. CRC Crit Rev Plant Sci 1:117–131

Dhir NK, Miksche JP (1974) Intraspecific variation of nuclear DNA content in *Pinus resinosa* Ait. Can J Genet Cytol 16:77–83

Durante M, Tagliasacchi AM, Avanzi S (1985) Fast reannealing sequences of DNA in *Allium cepa:* characterization and chromosomal localization. Cytobios 44:263–271

El-Lakany MH, Sziklai O (1971) Intraspecific variation in nuclear characteristics of Douglas fir. Adv Front Plant Sci 28:363–378

Greenlee JK, Rai KS, Floyd AD (1984) Intraspecific variation in nuclear DNA content in *Collinsia verna* Nutt. (*Scrophulariaceae*). Heredity 52:235–242

Hutchinson J, Narayan RKJ, Rees H (1980) Constraints upon the composition of supplementary DNA. Chromosoma 78: 137–145

Jackson RC, Murray BG (1983) Colchicine induced quadrivalent formation in *Helianthus:* evidence for ancient polyploidy. Theor Appl Genet 64:219–222

Lima-De-Faria A (1983) Molecular evolution and organization of the chromosome. Elsevier, Amsterdam New York London

Kodama A (1974) Karyotype analyses of crown gall cells of the sunflower. Bull Hiroshima Agric Coll 5:15–21

Marsh JL, McCarthy BJ (1974) Effect of reaction conditions on the reassociation of divergent deoxyribonucleic acid sequences. Biochemistry 13:3382–3388

Michaelson M, Price HJ (1985) Nuclear DNA content variation within *Helianthus annuus* L. Am J Bot 72:877

Miksche JP (1968) Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus banksiana*. Can J Genet Cytol 10:590–600

Miksche JP (1971) Intraspecific variation of DNA per cell between *Picea sitchensis* (Bong) Carr provenances. Chromosoma 32:343–352

Miksche JP, Hotta Y (1973) DNA base composition and repetitious DNA in several conifers. Chromosoma 41: 29–36

Nagl W, Capesius I (1976) Molecular and cytological characteristics of nuclear DNA and chromatin for angiosperm systematics: basic data for *Helianthus annuus* (*Asteraceae*). Plant Syst Evol 126:221–237

Natali L, Cavallini A, Cremonini R, Bassi P, Cionini PG (1986) Amplification of nuclear DNA sequences during induced plant cell dedifferentiation. Cell Differ 18:157–161

Olszewska MJ, Osiecka R (1983) The relationship between 2C DNA content, life cycle type, systematic position, and the dynamics of DNA endoreduplication in parenchyma nuclei during growth and differentiation of roots in some dicotyledonous herbaceous species. Biochem Physiol Pflanz 178:581–599

Price HJ (1976) Evolution of DNA content in higher plants. Bot Rev 42:27–52

Price HJ, Bachmann K, Chambers KL, Riggs J (1980) Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. Bot Gaz 141:195–198

Price HJ, Chambers KL, Bachmann K (1981) Genome size variation in diploid *Microseris bigelovii* (*Asteraceae*). Bot Gaz 142:156–159

Schaffner KH, Nagl W (1979) Differential DNA replication involved in transition from juvenile to adult phase in *Hedera helix*. Plant Syst Evol (Suppl) 2:105–110

Walbot V, Cullis CA (1985) Rapid genome changes in higher plants. Annu Rev Plant Physiol 36:367–396