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Genome size variations within *Dasypyrum villosum*: correlations with chromosomal traits, environmental factors and plant phenotypic characteristics and behaviour in reproduction

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Abstract Feulgen/DNA cytophotometric determinations carried out in the root meristem of seedlings showed that substantial quantitative alterations in the nuclear genome are present between and within 15 natural populations of *Dasypyrum villosum* in Italy. When the most variant values are considered, there is a 17.6% difference between the mean genome size of the populations, and a 66.2% difference between the genome size of individual plants within a population. A highly significant, positive correlation was found to exist between the genome size of *D. villosum* plants and the altitude of their stations, and differences in DNA contents between individual plants were greater in populations from mountain sites. Karyological analyses showed all chromosome pairs to differ largely in size between plants with differing DNA contents. A highly significant, positive correlation was found to exist between genome size and both the length of the chromosome complement at metaphase and the length and arm ratio of pair VII. Significant correlations were also found between DNA content and certain phenotypic characteristics of the plants. The mean genome size of the populations was negatively correlated with the mean leaf length and width. In contrast, the genome size of individual plants was positively correlated with the weight of the seed from which they originated and their flowering interval. A large range of genome sizes was found in the half-sib progeny of a plant having a relatively large genome. In contrast, in the half-sib progeny of a plant having a small genome, the genome sizes of the individual plants were less divergent and similar to that of the mother plant. All siblings from crosses between plants with differing genome sizes had similar DNA contents, which were intermediate between those of the parental plants, even if closer to the DNA content of the parent plant having the smaller genome size. Size polymorphism within pairs was never observed in plants obtained from these crosses or in half-sibs whose genome size differed from that of the mother plant. The intraspecific alterations observed in the nuclear genome and their effects on plant development and phenotype are briefly discussed as evolutionary factors which allow *D. villosum* populations to withstand different environmental conditions as well as the variability of conditions in a given environment.

Key words *Dasypyrum villosum* · Intraspecific genomic changes · Environmental adaptation · Developmental regulation · Reproduction

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Introduction

A number of reports, which have been accumulating in the literature since the mid-1980s, indicate that variations in the basic amount of nuclear DNA can occur within species (for plants, Bennett and Leitch 1995). Accordingly, there is a growing consensus that, in addition to more stable portions, fluid domains also exist in the nuclear DNA, which is intrinsically plastic due to its content of independent replicative units. Changes in genome size and organization may then differentiate populations within species and/or individuals within populations (Cavallini and Natali 1991; Ceccarelli et al. 1992, 1995; Cavallini et al. 1996).

Intraspecific changes that occur in the fluid domains of nuclear DNA may be of special importance to plants

because of the necessity of adaptive responses, which is particularly stringent in plants due to their immobility and their way of development and reproduction. In spite of this, our understanding of this phenomenon is only in its initial stages, and many aspects are still subject to debate. As a rule, alterations in the redundancy of repeated DNA sequences are involved in these genomic changes (Cavallini et al. 1996). The ability to produce them could be grounds for the presence of repetitive DNA in eukaryotic genomes and for its particular abundance in those of plants, independent of whether the quantitative alterability of nuclear DNA is simply a consequence, rather than the cause, of the presence in it of repeated sequences (Charlesworth et al. 1994). However, to what extent intraspecific changes in the size and organization of the nuclear genome commonly occur, the mechanism(s) by which they are produced and controlled, the structure and chromosomal organization of the DNA sequences involved and their correlation with adaptive stimuli and phenotypic characteristics are still poorly understood.

The high variability of chromosomal traits observed within *Dasypyrum villosum* (Blanco et al. 1996, and references therein) is indirect evidence of the existence of fluid domains in the genome, and it has been shown that its size may actually differ between populations as well as, within populations, between plants from caryopses differing in size and colour (yellow or brown caryopses; Cremonini et al. 1994; Frediani et al. 1994). Moreover, results obtained using different investigation methods showed rapid alterations in the basic amount of nuclear DNA, which occur to different extents during seed germination in seedlings from yellow or brown caryopses. Redundancy modulations of subtelomeric and other repeated DNA sequences were proven to be involved in these genomic changes (Frediani et al. 1994).

Given the importance of intraspecific alterations in the size and organization of plant genomes, we thought that a further study of such alterations within *D. villosum* would be of interest. Data on the relationships of these genomic changes with chromosomal traits, environmental factors and plant phenotypic characteristics and on their behaviour in reproduction are reported and discussed in this paper.

Materials and methods

Plant material

Seeds (caryopses) were collected from natural populations of *Dasypyrum villosum* (L.) Candargy in Italy at stations having different geographical and environmental parameters (Table 1). Other seeds were obtained by open pollination of plants grown in the experimental field. Crosses between plants were made by emasculating and then bagging the seed parent, followed by controlled fertilization. Seeds were germinated in petri dishes on wet paper under sterile conditions at room temperature in the dark, and the seedlings were then used as experimental materials. Other seedlings were grown first in Jiffy pots and then set out in the experimental field after the tip of the seminal root was removed and collected when the latter was 30 mm in length. Collected materials were fixed in ethanol-acetic acid 3:1 (v/v). For karyological analyses, root tips were treated with a saturated aqueous solution of alpha-bromonaphthalene for 4 h at room temperature before being fixed as above. Since, in *D. villosum*, the genome size may differ between plants obtained from seeds with different coloured caryopses (see Introduction), only plants from seeds in yellow caryopses were used in this investigation.

DNA cytophotometry

Fixed root apices were treated with a 5% aqueous solution of pectinase (Sigma) for 1 h at 37°C and squashed under a coverslip in a drop of 45% acetic acid. The coverslips were removed by the solid CO_2 method, and the preparations were Feulgen-stained after hydrolysis in 1 *N* HCl at 60°C for 8 min. After staining, the slides were

Table 1 Geographical and environmental parameters of the stations of the *Dasypyrum villosum* populations studied

Populations	Location			Mean temperature during the year (°C)	Rainfall (mm per year)
	Latitude north	Longitude east	Altitude (m a.s.l.)		
Apulia 1	41°52′	15°21′	5	15.5	569
Basilicata 1	40°15′	16°04′	500	12.8	1100
Basilicata 2	40°12′	16°04′	960	8.8	1206
Basilicata 3	40°08′	15°58′	1205	–	–
Basilicata 4	40°09′	16°00	1350	–	–
Calabria 1	39°17′	16°14′	250	16.5	1041
Calabria 2	39°23′	16°17′	330	16.5	1041
Latium 1	42°32′	12°45′	350	15.9	842
Latium 2	42°31′	12°47′	505	14.1	939
Latium 3	42°33′	12°57′	900	9.0	1354
Latium 4	42°33′	12°54′	1010	7.8	1486
Molise 1	41°34′	14°38′	685	14.4	842
Sardinia 1	40°30′	8°28′	565	14.1	698
Sicily 1	36°42′	15°05′	160	18.3	671
Tuscany 1	43°42′	10°23′	5	14.8	845

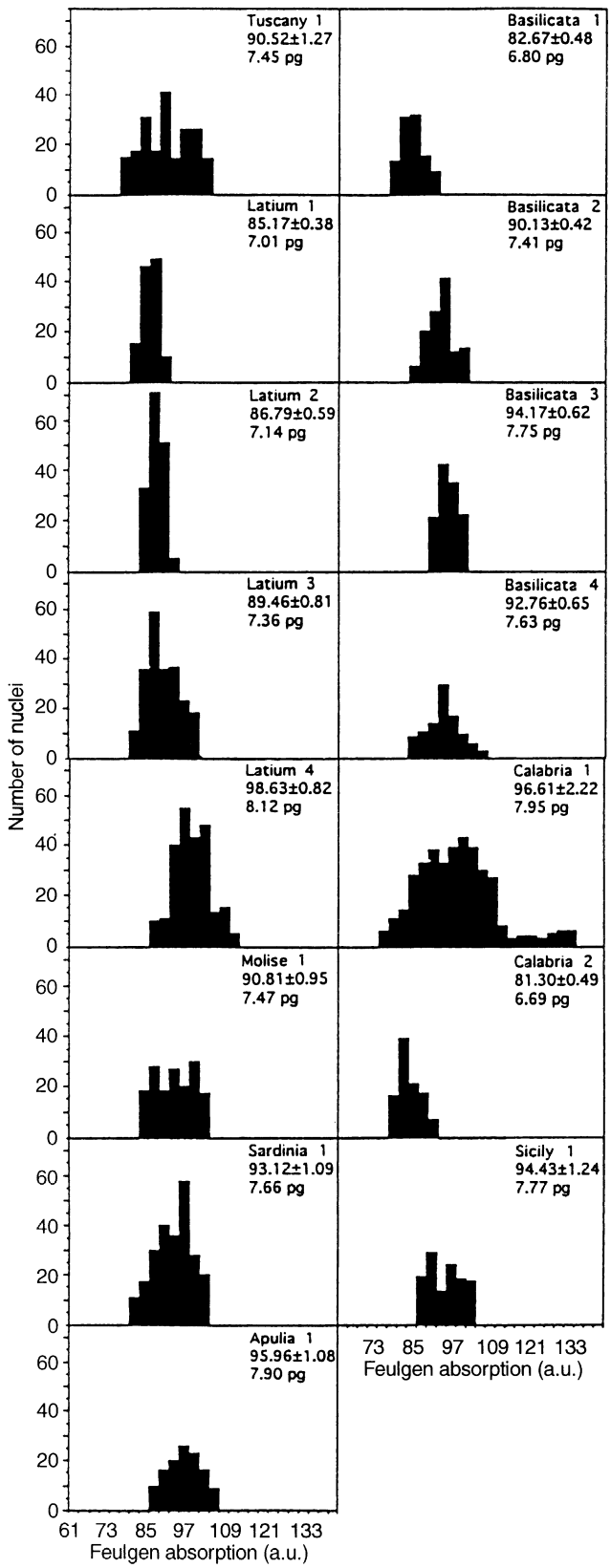

Fig. 1 Feulgen absorptions of early prophases (= 4C) in the root meristem of seedlings obtained by germinating seeds collected from natural populations of *D. villosum*. At least 5 seedlings per population were studied, and 20 prophases per seedling were analysed. Mean Feulgen absorptions (± SE) and DNA contents calculated per 1C are shown

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 analysed, squashes made with the root tips of a single plantlet of *Vicia faba* were concurrently stained for each group of slides and used as standards in order to make the results comparable. All the notable differences in Feulgen/DNA absorption between preparations observed using the above method of comparison were further checked by analysing preparations that were made again and processed all together. Feulgen/DNA absorptions in individual cell nuclei were measured at a wavelength of 550 nm using a Leitz MPV 3 microscope photometer equipped with a mirror scanner. Relative Feulgen/DNA units were converted into picograms of DNA by assuming a 4C DNA content of 53.3 pg (Bennett and Smith 1976) in the *V. faba* plant used as standard. By this method, the C-value (the DNA content of an unreplicated haploid nucleus) of individual plants was calculated.

Karyology

Alpha-bromonaphthalene-treated root apices to be used for karyological analysis were Feulgen-stained and squashed in a drop of 1% acetic orcein. The preparations were then processed as described above without washing in SO_2 water. Metaphase chromosomes were measured under the light microscope with the aid of a nonium-equipped ocular or on microphotographs.

Biometry and data analysis

The growth rate of plants was determined by measuring the height of the main culm every week. The following characteristics were studied in plants at anthesis (a plant was considered at anthesis when 50% of its flowers on the main culm had visible anthers): (1) length and maximum width of the flag leaf; (2) height (from the soil to the base of the spike) of the highest culm; (3) number and weight of the seeds; (4) flowering time (days from seed germination to anthesis); and (5) flowering interval (days needed for anther dehiscence in all flowers).

Results

Intraspecific genome size variation and its correlation with environmental factors

The Feulgen absorptions of early prophases (= 4C) in the root meristem of seedlings obtained from seeds collected from different populations (Table 1) and the calculated DNA C-values are given in Fig. 1. Significant differences are clearly present between populations. When the most variant values are considered there is a 17.6% difference between the mean genome size of the Latium 4 population (DNA C-value = 8.12 pg) and that of the Calabria 2 population (6.69 pg), taking the former value as the standard. A notable scattering of values can occur within populations, due

to differences in the genome size between individual plants. This is particularly apparent in the Calabria 1 population, where these differences may be greater than those between the mean genome sizes of the populations. Indeed, in one group of plants studied the smallest genome (DNA C-value = 6.39 pg) reaches only 66.2% of the largest one (10.62 pg) (Fig. 2).

The populations from Basilicata and Latium were found at stations located at different altitudes in restricted areas (Table 1). As is shown in Fig. 3a, b, a highly significant ($P \leq 0.0001$) correlation exists between the genome size of *D. villosum* plants and the altitude of their stations; the higher the altitude, the larger the genome size. From the same figure it can also be seen that the genome size of individual plants tends to vary more in populations which are found at higher altitudes. Indeed, in the 2 population groups of Fig. 3a and b, the divergence of DNA content between plants having extreme values never exceeds 6% in populations from stations at elevations less than 500 m a.s.l., whereas it is larger in populations from stations at higher elevations, reaching 11.5% in the Latium 4 population (1010 m).

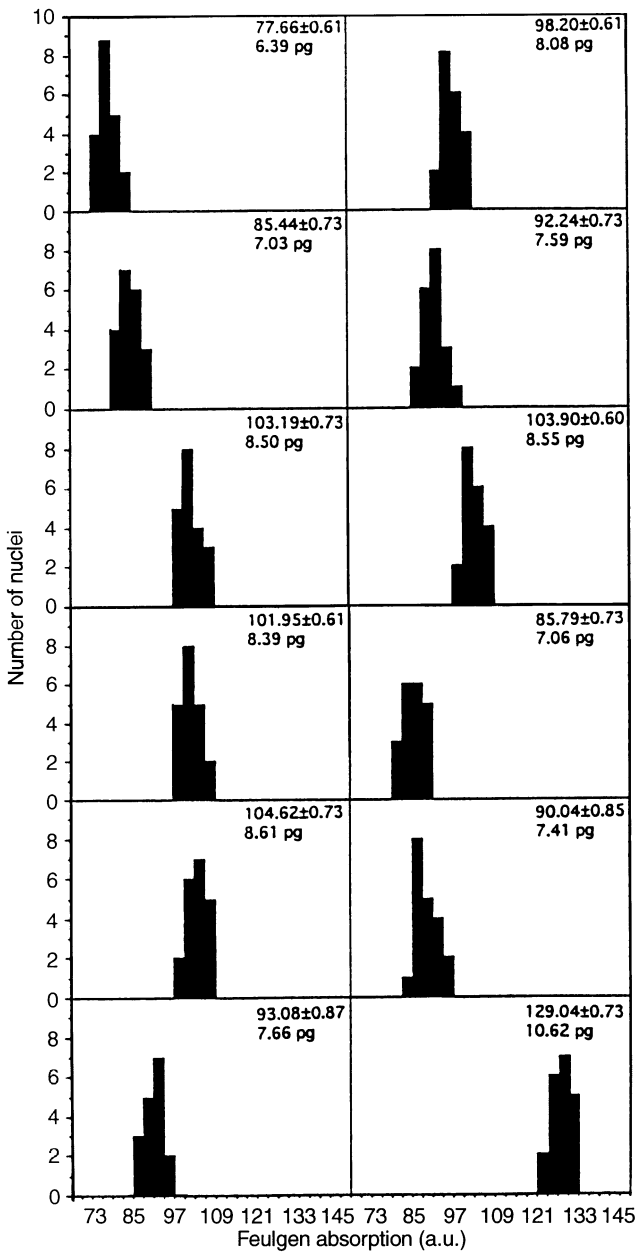

Fig. 2 Feulgen absorptions of early prophases (= 4C) in the root meristem of individual plants belonging to the Calabria 1 population. Mean Feulgen absorptions (± SE) and DNA contents calculated per 1C are shown

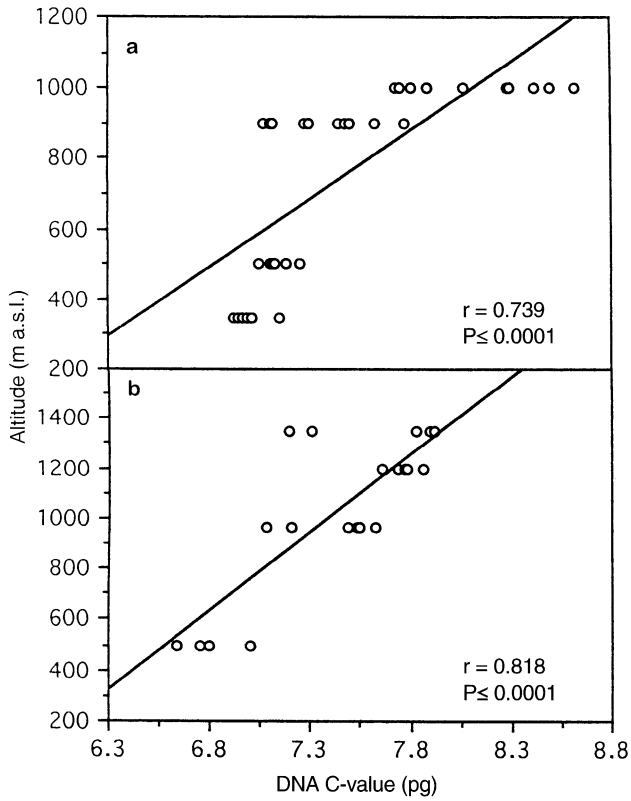

Fig. 3a, b Correlation between the genome size of individual plants and altitude. Plants belonging to populations of Latium (**a**) and Basilicata (**b**) from stations located at different altitudes were studied

Phenotypic characteristics at the cellular and organismal level, and correlations with genome size

Metaphase plates from 2 plants belonging to the Calabria 1 population whose genome sizes differ greatly are shown in Fig. 4a, b. The chromosomes of the two complements differ remarkably in size and are larger and longer in the plant with the greater amount of nuclear DNA. Results of a detailed analysis of the

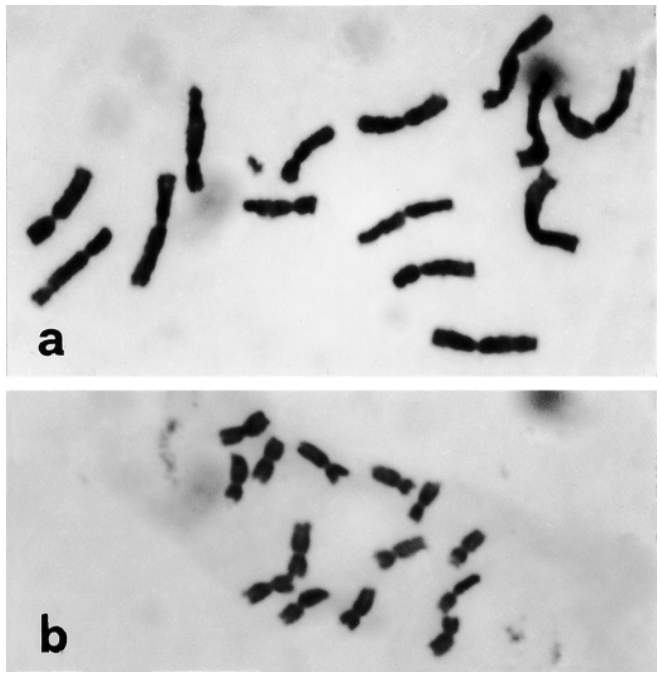

Fig. 4a, b Metaphase plates in the seminal root meristems of 2 plants belonging to the Calabria 1 population with genome sizes that differ greatly. **a** DNA C-value = 8.39 pg, **b** DNA C-value = 6.39 pg. Feulgen counterstained with acetic orcein. × 2050

metaphase chromosomes of these 2 plants are given in Table 2. The two complements differ significantly in the length of each arm of each chromosome pair except for the shorter arm of pair VII. That length variations are not evenly distributed in the chromosome arms also appears when calculating the arm ratios. Indeed, these differ significantly between the two complements in three pairs (V, VI and VII).

Karyometry was carried out in several plants from different populations whose genome sizes had been shown to differ on the basis of Feulgen/DNA cytophotometry. Particular attention was given to chromosome pair VII which, according to the data presented in Table 2, does not undergo variations in its shorter arm and can be easily recognized due to the satellite. Neither aneuploidy nor recurrent aneusomaty were ever observed. The lengths of the chromosome complements, as well as the length and arm ratio of pair VII, were found to vary greatly between the plants studied. Figure 5 shows that a highly significant ($P \leqslant 0.0001$), positive correlation exists between the size of the genome and the length of the chromosome complement at metaphase. As an indication that DNA content variations follow definite patterns in the chromosomes, the arm ratio in pair VII is significantly correlated with both its length ($P = 0.0042$; Fig. 6a) and the genome size of the plant ($P = 0.0102$; Fig. 6b).

Highly significant correlations were also found to occur between the genome size and certain phenotypic characteristics of the plants (Fig. 7a–c). The mean genome size of the populations is negatively correlated with the mean leaf length ($P = 0.0044$; Fig. 7a) and width ($P = 0.043$; not shown). In contrast, the genome size of individual plants is positively correlated with the weight of the seed from which they originated ($P \leq 0.0001$; Fig. 7b) and their flowering interval ($P \leq 0.0001$; Fig 7c). Significant correlations were not found between genome size and the other plant phenotypic characteristics considered (see Materials and methods).

Genome size variations in reproduction

Seeds were obtained by open pollination (half-sib progenies) or by crossing those plants from the Calabria 1 population having different genome sizes. The Feulgen absorptions of early prophases in the root meristem of seedlings obtained from these seeds are reported in Table 3. A large range of genome sizes in the half-sib progeny of a plant having a relatively large genome (DNA C-value = 8.39 pg) can be observed, with only the highest values being similar to that of the mother plant. In contrast, in the half-sib progeny of a plant having a small genome (C-value = 6.39 pg), the genome sizes of individual plants are more similar to that of the mother plant, and their mean (6.65 pg) does not differ significantly.

Reciprocal crosses were made between 2 plants having DNA C-values of 6.41 pg and 7.82 pg, respectively. Crossing was much more successful when the plant having the lower genome size was the mother instead of the pollen donor. In this case, 41 plump seeds were obtained from 80 flowers which had been emasculated and pollinated, while only 5 plump seeds were obtained from an equal number of reciprocal crosses. Apart from this, significant differences were not found between the DNA contents of plants from the two crosses. The data are pooled in Table 3. It can be seen that all siblings have similar genome sizes, which are intermediate between those of the parental plants. However, the values are closer to the DNA C-value of the parent plant having the smaller genome size. Indeed, the mean 1C DNA content of the siblings (6.95 pg) differs by 0.54 pg from the C-value of the smaller genome parent and by 0.87 pg from the DNA content of the parent plant having the larger genome. Taken together, the data given in Table 3 indicate that the genome sizes of both parents affect that of the offspring, but that the smaller genome has the greater effect.

Size polymorphism within chromosome pairs was never observed in plants obtained by crossing parents having differing genome sizes or in half-sibs whose genome size differed from that of the mother plant. No apparent structural differences exist between the two

Table 2 Means of metaphase chromosomes length, length of the arms (μm ± SE) and arm ratio (± SE) in the seminal root meristems of 2 plants with very different genome sizes. Ten metaphase plates per plant were studied, and the results of *t*-test are given. The pairs were arranged according to Blanco et al. (1996)

Chromosome pair	DNA C-value = 8.39 pg				DNA C-value = 6.39 pg			
	Entire length	Longer arm	Shorter arm	Arm ratio	Entire length	Longer arm	Shorter arm	Arm ratio
I	5.61 ± 0.09	2.88 ± 0.06	2.73 ± 0.04	1.05 ± 0.01	3.64 ± 0.04	1.87 ± 0.02	1.77 ± 0.02	1.06 ± 0.01
II	7.43 ± 0.04	4.21 ± 0.03	3.22 ± 0.03	1.31 ± 0.02	4.51 ± 0.03	2.58 ± 0.03	1.93 ± 0.02	1.34 ± 0.02
III	6.57 ± 0.03	3.80 ± 0.02	2.77 ± 0.03	1.37 ± 0.02	4.22 ± 0.04	2.49 ± 0.02	1.73 ± 0.02	1.44 ± 0.02
IV	5.99 ± 0.06	3.57 ± 0.04	2.42 ± 0.04	1.48 ± 0.02	4.00 ± 0.04	2.36 ± 0.01	1.64 ± 0.03	1.44 ± 0.02
V	6.33 ± 0.03	4.26 ± 0.02	2.07 ± 0.03	2.06 ± 0.03	3.98 ± 0.02	2.56 ± 0.02	1.42 ± 0.01	1.80 ± 0.02
VI	4.74 ± 0.07	3.15 ± 0.03	1.59 ± 0.04	1.98 ± 0.06	3.69 ± 0.03	2.33 ± 0.05	1.36 ± 0.03	1.71 ± 0.07
VII	4.46 ± 0.10	3.07 ± 0.08	1.39 ± 0.04	2.21 ± 0.08	3.46 ± 0.05	2.14 ± 0.03	1.32 ± 0.02	1.62 ± 0.02
Entire complement	82.26 ± 0.30				55.00 ± 0.18			

Table 2 Continued

Chromosome pair	Entire length		Longer arm		Shorter arm		Arm ratio	
	t-test	*P*	*t*-test	*P*	*t*-test	*P*	*t*-test	*P*
I	17.09	0.0001	16.72	0.0001	16.48	0.0001	0.56	0.58
II	60.63	0.0001	27.66	0.0001	32.01	0.0001	1.04	0.32
III	60.90	0.0001	54.97	0.0001	21.14	0.0001	1.68	0.13
IV	22.12	0.0001	24.95	0.0001	15.13	0.0001	0.87	0.41
V	51.42	0.0001	41.11	0.0001	20.77	0.0001	6.08	0.0002
VI	14.09	0.0001	13.74	0.0001	4.07	0.0030	2.86	0.019
VII	10.72	0.0001	11.27	0.0001	1.29	0.23	7.28	0.0001
Entire complement	57.54	0.0001						

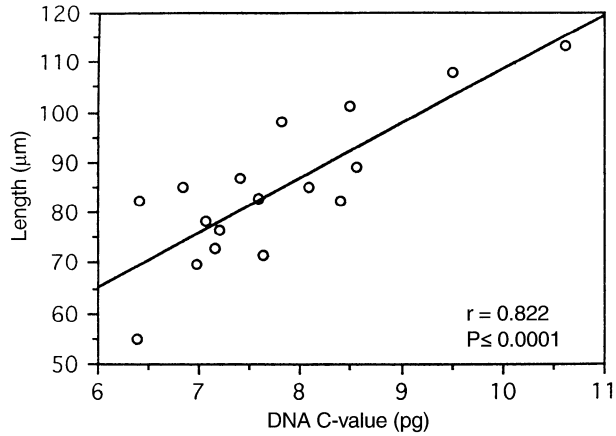

Fig. 5 Correlation between the genome size of individual plants belonging to different populations and the length of their chromosome complement at metaphase

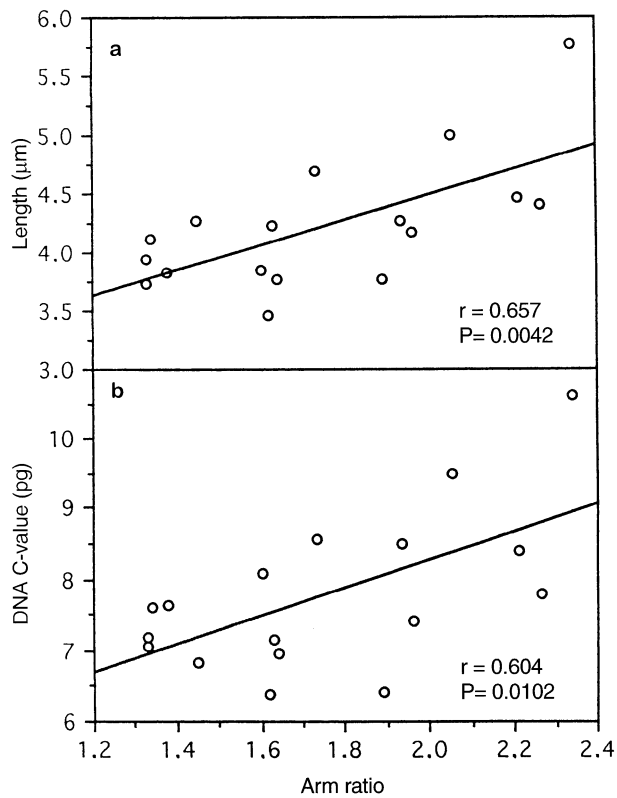

Fig. 6 a, b Correlation, in the same plants as in Fig. 5, between the longer arm/shorter arm ratio in chromosome pair VII and its length (**a**) or the genome size of the plant (**b**)

members of each pair of metaphase chromosomes in a plant having a DNA C-value of 6.85 pg which was obtained by open pollination of a plant having a DNA C-value of 8.39 pg (Fig. 8).

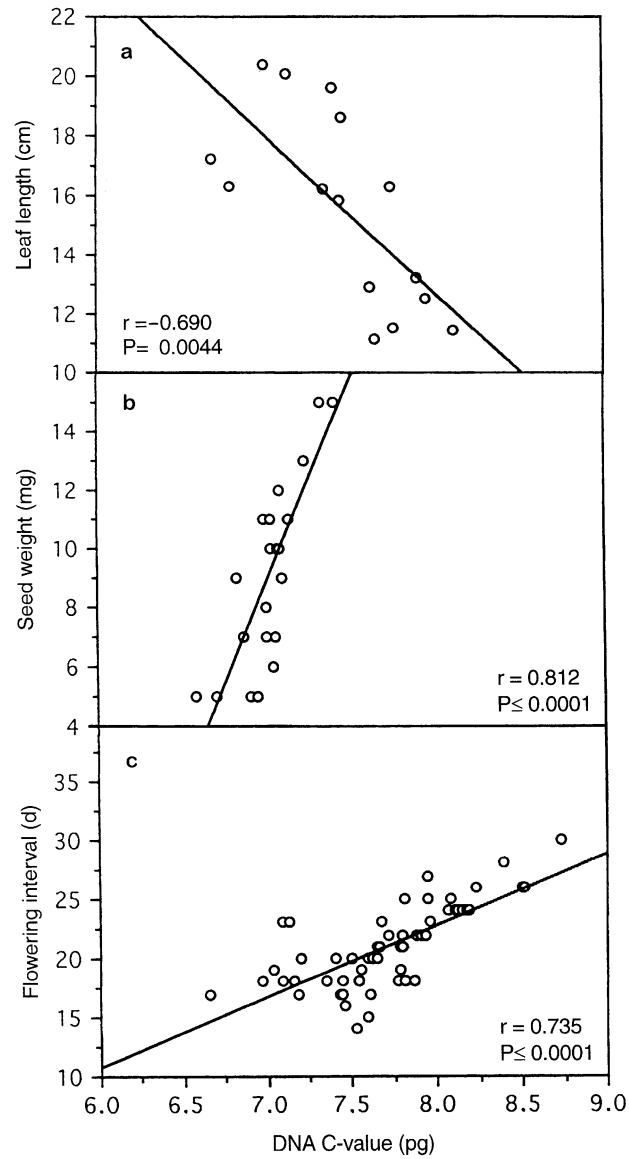

Fig. 7a–c Correlations between genome size and phenotypic characteristics of plants belonging to various populations. **a** Mean genome size and mean leaf length, **b** genome size of individual seedlings and weight of the seeds from which they originated, **c** genome size of individual plants and their flowering interval

Discussion

Variations in the basic amount of nuclear DNA between *D. villosum* populations and, within them, between plants from seeds in different kinds of caryopsis have already been shown to occur (Cremonini et al. 1994; Frediani et al. 1994). Our present findings confirm the occurrence of significant differences in the mean genome size between populations (Fig. 1) and show that even larger differences may exist, within certain populations, between individual plants, independent of the colour of the caryopsis from which they

Table 3 Feulgen absorptions of early prophases (= 4C; a.u.) and, in parentheses, DNA contents calculated per 1C (pg) in parent plants and plants belonging to their progenies obtained by open pollination or cross. Each Feulgen absorption value is the mean (± SE) of 20 determinations carried out in the root meristem

Parental plants	Open pollination		Cross
Individuals in the progeny	101.94 ± 0.76 (8.39)	77.63 ± 0.71(6.39)	77.88 ± 0.73(6.41) × 95.01 ± 0.73(7.82)
1	95.01 ± 0.73 (7.82)	77.88 ± 0.73 (6.41)	83.71 ± 0.49 (6.89)
2	93.19 ± 0.97 (7.67)	88.45 ± 1.21 (7.28)	79.95 ± 0.12 (6.58)
3	88.94 ± 0.73 (7.32)	84.93 ± 0.97 (6.99)	85.05 ± 0.12 (7.00)
4	103.27 ± 0.48 (8.50)	82.38 ± 0.85 (6.78)	86.26 ± 0.36 (7.10)
5	83.23 ± 0.61 (6.85)	80.68 ± 0.73 (6.64)	85.41 ± 0.48 (7.03)
6	78.73 ± 0.73 (6.48)	81.04 ± 0.49 (6.67)	83.96 ± 0.48 (6.91)
7	87.84 ± 0.36 (7.23)	74.48 ± 0.61 (6.13)	84.44 ± 0.24 (6.95)
8		82.25 ± 0.61 (6.77)	85.78 ± 0.73 (7.06)
9		74.96 ± 0.73 (6.17)	85.54 ± 0.36 (7.04)
Means of the progeny	90.03 ± 0.66 (7.41)	80.78 ± 0.74 (6.65)	84.46 ± 0.38 (6.95)

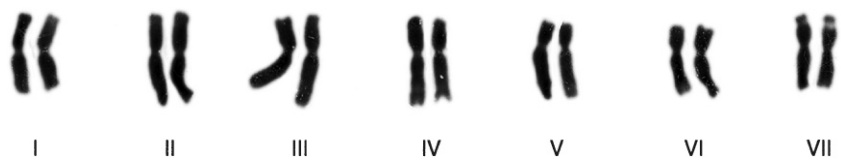

Fig. 8 Chromosome complement at metaphase from a plant belonging to the Calabria 1 population whose genome size differs greatly from that of the mother plant (DNA C-values = 6.85 pg and 8.39 pg, respectively). Size polymorphism cannot be observed within pairs. Feulgen counterstained with acetic orcein. × 2050

originated (Fig. 2). Thus, a large and complex field of genomic variability occurs and is maintained by reproduction within the species (Table 3). The changes in the basic amount of nuclear DNA can affect phenotypic characteristics at the cellular and organismal levels (Figs. 4–7) and may be correlated with an environmental factor, altitude (Fig. 3).

Large genetic variability within *D. villosum* has also been proven by the results of another investigation on 7 Italian populations (De Pace and Qualset 1995). In particular, 10% of this variability was observed between populations and 90% within populations for two enzyme systems and rDNA RFLP phenotypes. A similar partitioning of the variability between and within populations occurred for phenotypic characteristics, such as days to heading, while for other characteristics such as plant height and number of culms per plant, the difference between populations accounted for 20–26% of the total variability. These findings agree with our own, which show that the changes in genome size are larger within populations than between populations. This concordance of data and especially the highly significant correlations found between genome size and different parameters such as an environmental factor (Fig. 3), chromosomal traits (Figs. 4–6) and plant phenotypic characteristics (Fig. 7a–c) render our cytophotometric findings quite reliable. As a matter of fact, the determination of DNA content by scanning cytophotometry of Feulgen-stained nuclei may be subject to certain reservations. Indeed, factors such as the chemical nature and spatial configuration of the chromatin due to its proteinaceous component and functional state, the presence of particular substances in the cells, especially in differentiated tissues, or the size of the nuclei measured, may interfere with DNA stainability and/or the measurement of the amount of the stain (Frölich and Nagl 1979; Mello and Vidal 1980; Greilhuber 1986). However, none of these conditions differed in the cells we analysed, since Feulgen/DNA absorptions were measured on early prophases and each time root meristems of plants at the same developmental stages were compared. It has already been shown that fixation in alcohol-acetic acid does not cause subsequent distorsions in Feulgen/DNA absorptions in our material, nor are alterations in genome size within *D. villosum* confined to roots, since the same amounts of DNA are found, as a rule, in the root and shoot apices of the same plant (Frediani et al. 1994).

The highly significant correlation between the genome size of plants and the altitude of their stations strongly suggests that the alterations in the basic amount of nuclear DNA observed within *D. villosum* play a role in environmental adaptation. It should be noted that differences in genome size between plants within populations may be greater in those from mountain sites (Fig. 3a, b), where the environment is expected to be particularly limiting and/or variable. Therefore, the ability to alter genome size can be thought of as an evolutionary factor which allows *D. villosum* populations to withstand different environmental conditions as well as the variability of conditions in a given environment.

Intraspecific variations in the basic amount of the nuclear DNA which may be related to environmental adaptation have been shown to occur in several plant species (Cavallini and Natali 1991; Ceccarelli et al. 1992, 1995). For example, complex genomic changes very similar to those observed in *D. villosum* have been found to occur in *Helianthus annuus*. Also in this species, the mean DNA content may differ within cultivated varieties or lines and, within these, genomic changes are continuously produced during reproduction even with selfing and homozygosity (Cavallini et al. 1986, 1989).

Our data show that intraspecific alterations in the basic amount of nuclear DNA can affect given phenotypic characteristics of the plants (Fig. 7a-c). This result may be obtained by either producing specific changes in the expression of the genome or by simply altering the nucleotype. Indeed, it is known that certain characteristics of the nucleus, such as its mass and volume, can affect certain traits of the cell such as the timing of the mitotic cycle or enlargement with differentiation. In turn, these cellular characteristics can affect developmental dynamics and the phenotype at the organismal level (Bennett 1995, 1987). At the intraspecific level, correlations between changes in genome size and phenotypic characteristics at the cellular and organismal level have already been observed in species such as *Festuca arundinacea* (Ceccarelli et al. 1993), *Pisum sativum* (Cavallini et al. 1993), *Helianthus annuus* (Natali et al. 1993) or *Vicia faba* (Minelli et al. 1996), even if different characteristics may be involved in each instance. Therefore, adaptation may be affected by optimizing development and phenotype in relation to environmental stimuli rather than by buffering the effects of environmental conditions so as to avoid or limit developmental and phenotypic changes. In light of this consideration, intraspecific alterations of genome size may help to explain the extraordinary plasticity within plant species at both the morphological and physiological levels.

Our findings show certain features of the remarkable plasticity that characterizes the nuclear genome of *D. villosum* and also raise other, as yet unanswered, questions. For example, if the strict correlation between DNA content and chromosome size (Figs. 5, 6) is taken into account, polymorphism within pairs might be expected in plants whose genome size differs largely from that of the mother plant. Conversely, no polymorphism is observed within the chromosome pairs of such plants (Fig. 8). This finding suggests that imbalances of DNA sequence redundancy between the members of a chromosome pair are recognized and overcome rapidly after fertilization. However, it is currently impossible to speculate on the way in which this is achieved, as well as why small genome sizes are somehow 'dominant' in determining DNA contents during reproduction. Research is currently in progress in an attempt to shed more light on this and other aspects of the genome plasticity within *D. villosum*.

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