

## Genome size variation in *Vicia faba*

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Findings obtained using different approaches indicated the occurrence of genomic size variations within *V. faba*. Significant differences in the basic amount of nuclear DNA (up to 34.6 per cent) between 39 local populations collected from the Mediterranean Basin were observed by means of Feulgen/DNA cytophotometry. By contrast, no difference in genome size was found when five commercial varieties were compared. Dot blot hybridization of *FokI* *V. faba* repeats to genomic DNAs showed up to fourfold differences in the redundancy of these sequences in the nuclear DNA of different accessions. In agreement with the cytophotometric findings, a significant, positive correlation was determined between the DNA contents of populations and the copy numbers of DNA sequences related to *FokI* repeats. Significant differences between accessions were found in the length of the chromosome complement at metaphase, and these differences were particularly apparent in certain chromosome pairs. A positive correlation was found between the length of the complement and the genome size of the populations. These results are discussed in relation to other data in the literature on intraspecific nuclear DNA changes.

**Keywords:** DNA cytophotometry, *FokI* DNA sequences, intraspecific DNA changes, karyometry, repetitive DNA, *Vicia faba*.

### Introduction

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. Indeed, many results obtained in different materials have shown that fluid domains, which are capable of rapid quantitative changes, may exist in the genome. These DNA domains, which are generally made up of repeated sequences, possibly occur more commonly, and express their ability to vary more frequently, in plant genomes than in animal genomes (reviewed for plants by Walbot & Cullis, 1985; Cionini, 1989; Bassi, 1990; Cullis, 1990; Nagl, 1990).

Redundancy variation of DNA sequences may accompany certain developmental processes. It has been suggested that they play a regulatory role in development (Frediani *et al.*, 1994 and references therein). Intraspecific alterations of the basic amount of nuclear DNA may also correlate with differing environmental factors; thus, these alterations have been explained as adaptive (Ceccarelli *et al.*, 1992 and references therein). This view seems to be supported

by results indicating that quantitative changes in nuclear DNA can affect various phenotypic characters at the cellular and organismal level (Ceccarelli *et al.*, 1993 and references therein).

Because they may be of great biological importance and since many of their aspects are poorly understood, the above-mentioned genomic changes deserve thorough investigation. These changes have been shown to occur in many plant species (see Cavallini & Natali, 1991), but we do not know to what extent they are of common occurrence. The DNA sequences involved in the variations do not seem to be the same in different instances. To quote but two examples, certain medium repeated sequences mainly show redundancy variations in *Helianthus annuus* (Natali *et al.*, 1993), while quantitative differences in the nuclear DNA between populations of hexaploid *Festuca arundinacea* are mainly the result of changes in a fraction of highly repeated sequences (Ceccarelli *et al.*, 1992). The chromosomal organization of varying DNA sequences, as well as the mechanism by which intraspecific alterations in genome size and organization are produced and controlled, are almost entirely unknown.

We investigated the nuclear DNA changes in *Vicia faba* by studying a number of local populations

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collected from the Mediterranean Basin. Results are reported of DNA cytophotometry, karyometry and molecular hybridization experiments using tandemly repeated *FokI* sequences, already shown to be frequent in the *V. faba* genome (Kato *et al.*, 1984).

## Materials and methods

### *Plant material*

Seeds of 39 accessions of *Vicia faba* were kindly provided by the Istituto del Germoplasma, CNR, Bari, Italy. The seeds were washed in tap water and germinated in damp vermiculite under sterile conditions at room temperature in the dark. The main root and shoot apices of the seedlings were fixed in ethanol-acetic acid 3:1 (v/v) or in 10 per cent neutral formalin to be used for DNA cytophotometry. Lateral roots to be squashed for karyological analyses were treated with a 0.05 per cent aqueous solution of colchicine (Sigma) for 4 h at room temperature and fixed in ethanol-acetic acid. The roots of plantlets grown for 15 days in water changed twice a day under the same conditions as above were collected and used for DNA extraction.

### *Cytophotometry*

Fixed root or shoot apices of seedlings were treated with a 5 per cent aqueous solution of pectinase (Sigma) for 1 h at 37°C and squashed under a coverslip in a drop of 45 per cent acetic acid. The coverslips were removed by the solid CO<sub>2</sub> method and the preparations were Feulgen-stained after different hydrolysis durations in N HCl at 60°C: 8 min for those made with material fixed in ethanol-acetic acid and 20 min for those made with formalin-fixed material. After staining, the slides were subjected to three 10 min washes in SO<sub>2</sub> water prior to dehydration and mounting in DPX (BDH). As simultaneous processing was not possible because of the large number of preparations to be analysed, squashes made with the root tips of a single plantlet of *Vicia faba* cv. Superaguadulce were concurrently stained for each group of slides and used as standards in order to make the results comparable. All 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 the wavelength of 550 nm, using a Leitz MPV 3 microscope photometer equipped with a mirror scanner and an HP 85

computer. Relative Feulgen/DNA units were converted into picograms of DNA by assuming a 4C DNA content of 53.31 pg (Bennett & Smith, 1976) in the *V. faba* plant used as a standard.

### *DNA extraction and probe preparation*

For DNA preparation, about 0.5 g of fresh root tissue was pulverized with a pestle and mortar in the presence of liquid nitrogen and homogenized in 5 mL of a pH 8.0 buffer containing 0.1 M Tris, 0.005 M EDTA, 0.5 M NaCl and 0.01 M mercaptoethanol. DNA extraction and purification were performed as described by Maggini *et al.* (1978). The isolated DNA was then suspended in TE buffer (0.01 M Tris-HCl pH 8.0 plus 0.001 M EDTA pH 8.0) and stored at +4°C.

Tandem arrays of *FokI* repeats about 300 bp in length were isolated from *V. faba* genomic DNA by digestion with *Sau3AI* and *RsaI* restriction endonucleases, and subcloned in pBS- vectors, as described by Maggini *et al.* (1994).

### *Dot blot hybridization and calculation of sequence copy numbers*

Replicated samples of 50, 25 or 12.5 ng of genomic DNA were suspended in 10 µL of TE buffer and applied to a Zeta Probe (Bio-Rad) filter using the Mini-fold I apparatus (Schleicher and Schuell). On the same filter,  $5 \times 10^8$ ,  $5 \times 10^9$ , or  $5 \times 10^{10}$  copies of *FokI* sequences were also delivered (*FokI* dilution spots). The filter was heated at 80°C in a vacuum oven for 2 h, prehybridized for 5 h using 0.2 mL cm<sup>-2</sup> of hybridization buffer (5 × SSC, 0.1 per cent *N*-lauroylsarcosine, 0.02 per cent SDS, 1 per cent blocking reagent) and then hybridized for 10 h using 0.025 mL cm<sup>-2</sup> of hybridization buffer containing 200 ng of freshly denatured *FokI* repeats which were labelled with digoxigenin-11-dUTP (Boehringer) using a random primed DNA labelling kit (Promega). After hybridization, filters were washed twice for 5 min at room temperature with 2 × SSC containing 0.1 per cent SDS and twice for 15 min at 65°C with 0.1 × SSC containing 0.1 per cent SDS. Immunological detection of digoxigenin haptens in DNA-DNA hybrids was performed using a DigDNA detection kit (Boehringer). Densitometric scanning of the coloured spots was performed using an LKB 2202 Ultra Scan laser densitometer. The amounts of absorbance of the incident 590 nm laser beam were then measured with an LKB 2210-062 potentiometric recorder and used to evaluate the relative colour development and therefore the relative amounts of hybridization of the spotted DNA to the labelled probe. The linear regression equation relating the

natural logarithm of the copy number of *FokI* repeats in the dilution spots and the natural logarithm of the corresponding densitometric readings was used to calibrate the relationship between the copy number of the sequence probed in the samples of *V. faba* genomic DNA and the amount of absorbance as detected densitometrically.

### Karyometry

For the karyological analyses, the meristems of colchicine-treated and fixed roots were Feulgen-stained and then squashed and mounted as described above. The lengths of metaphase chromosomes were measured on microphotographs. The data obtained were converted into microns by comparison with microphotographs of a micrometric slide.

## Results

### Cytophotometry

All the cytophotometric data given below were obtained from material fixed in ethanol-acetic acid. Comparable results were obtained when formalin-fixed material was used. The mean Feulgen absorptions of early prophase ( $=4C$ ) in the root meristems of seedlings obtained from seeds of 39 local populations of *V. faba* and the C-values (the DNA contents of unreplicated haploid chromosome complements) calculated are given in Table 1. Significant ( $P \leq 0.01$ ) variations in the basic amount of DNA occur between accessions; considering the most variant values, there is a 34.6 per cent difference between accession no. 111927 from Italy (C-value = 10.94 pg) and accession no. 112082 from Morocco (C-value = 14.73 pg), with the former as standard. On the contrary, the genome sizes of individual plants do not differ significantly within a single accession. Neither do significant differences exist between the DNA contents in the root and shoot meristems of one and the same plant, as proven by comparisons between the respective Feulgen/DNA absorptions made in a number of seedlings belonging to different accessions (data not shown). Significant correlations were not found between the basic DNA contents of the populations and the latitudes, longitudes or altitudes of their places of origin. However, the mean genome size of accessions from Europe ( $1C = 12.81 \pm 0.10$  pg) differs significantly ( $P \leq 0.05$ ) from that of accessions from Africa ( $1C = 13.39 \pm 0.11$  pg).

In contrast to the cytophotometric results obtained

by studying local populations, Feulgen absorptions of early prophase in the root meristems of seedlings from seeds of five commercial varieties were practically the same, even if the places of origin of the seeds were different (Table 2).

### Molecular hybridizations

Variations in the genome size between populations are also suggested by the results of molecular hybridization experiments. The densitometric absorptions of dot blot filters loaded with genomic DNAs from seedlings obtained by germinating seeds of seven accessions differing in genome size, and hybridized with digoxigenin-labelled *FokI* *V. faba* repeats, are given in Fig. 1. The sequences probed hybridize to these DNAs to extents that differ significantly. The copy number of *FokI* sequences in the genome and the percentage of the nuclear DNA that they represent were calculated and are given in Table 3. It can be seen that *FokI* repeats represent a considerable portion of the genome of *V. faba* (3.09–9.47 per cent in the accessions studied). Their redundancy varies largely between populations; it is four times higher in the accession with the largest genome than in that with the smallest genome ( $21.51 \times 10^6$  copies vs.  $5.39 \times 10^6$  copies, respectively). These data indicate that repetitive DNA is involved in the variations of genome size between populations, and are in agreement with the cytophotometric findings. Indeed, the positive, significant ( $P = 0.0127$ ) correlation shown in Fig. 2 proves that the redundancy in the nuclear genome of DNA sequences related to the *FokI* repeats is higher the larger is the basic DNA content of the accessions as determined cytophotometrically (Table 1).

### Karyometry

All local populations studied were found to be diploid ( $2n = 2x = 12$ ). The occurrence of supernumerary chromosomes, never reported in *V. faba*, or recurrent aneusomy in the accessions were not observed.

Table 4 contains data obtained by measuring on microphotographs of metaphase plates the length of each chromosome pair of the complement, which consists of a long, satellited, submetacentric (M) pair and five subtelo-centric (S) pairs. The total length of the complement differs significantly between the seven populations differing in genome size. Differences in length, all of which go in the same direction within each accession, occur in all the chromosome pairs. However, by observing the coefficients of variation given in Table 4, it appears that the extents of length

**Table 1** Feulgen absorption of early prophases (= 4C) and DNA content calculated per 1C in 39 local populations of *Vicia faba*

Accession	Country	Latitude north	Longitude	Altitude (m a.s.l.)	Feulgen absorption (a.u.; mean ± SE)	Mean DNA C-value (pg)
106858	Greece	38°56'	23°10'E	100	25.40 ± 1.52	11.28
106961	Greece	39°45'	20°45'E	500	28.14 ± 0.87	12.50
110340	Italy	41°50'	15°48'E	150	31.01 ± 1.55	13.77
110343	Italy	41°43'	15°55'E	450	30.92 ± 1.01	13.73
110440	Italy	41°19'	14°44'E	550	29.47 ± 0.22	13.09
110442	Italy	41°16'	15°02'E	500	25.40 ± 1.22	11.28
110443	Italy	41°16'	15°02'E	500	29.38 ± 0.15	13.05
110444	Italy	41°16'	15°02'E	500	29.66 ± 0.73	13.17
110951	Italy	40°49'	15°28'E	1000	27.03 ± 0.42	12.01
111837	Italy	40°32'	15°34'E	720	29.90 ± 0.35	13.28
111924	Italy	39°30'	16°07'E	550	27.41 ± 0.61	12.07
111927	Italy	39°19'	16°21'E	650	24.62 ± 0.58	10.94
113063	Italy	37°09'	14°45'E	460	29.37 ± 0.18	13.05
113064	Italy	37°09'	14°45'E	450	29.91 ± 0.33	13.28
113071	Italy	37°40'	13°23'E	600	29.29 ± 0.32	13.01
113072	Italy	37°42'	13°21'E	660	31.47 ± 0.35	13.98
113074	Italy	37°57'	12°42'E	200	31.36 ± 0.60	13.93
113079	Italy	37°57'	12°42'E	200	30.57 ± 0.18	13.58
113747	Italy	38°20'	16°19'E	340	25.24 ± 0.67	11.21
115029	Italy	40°49'	15°28'E	1000	28.62 ± 0.50	12.71
107139	Spain	37°32'	4°59'W	100	30.89 ± 0.49	13.72
107141	Spain	37°59'	4°10'W	350	30.94 ± 0.46	13.74
107151	Spain	37°45'	2°59'W	850	27.21 ± 0.30	12.08
107214	Spain	41°29'	0°59'W	440	29.05 ± 1.64	12.90
110726	Egypt	24°33'	33°03'E	0	30.74 ± 0.48	13.66
110729	Egypt	24°46'	32°58'E	0	29.23 ± 0.51	12.98
110730	Egypt	25°00'	32°59'E	0	29.94 ± 0.26	13.30
111891	Libya	32°09'	12°58'E	450	26.87 ± 0.40	11.94
111892	Libya	32°05'	12°37'E	300	28.77 ± 0.31	12.78
111893	Libya	32°45'	13°51'E	60	29.94 ± 0.55	13.30
111895	Libya	25°40'	15°07'E	310	31.87 ± 0.62	14.16
106725	Tunisia	34°35'	9°10'E	874	30.21 ± 1.39	13.42
106744	Tunisia	33°53'	10°06'E	20	29.50 ± 0.48	13.10
106773	Tunisia	36°20'	10°22'E	100	31.96 ± 0.60	14.20
106361	Algeria	30°50'	1°59'E	530	30.51 ± 0.29	13.56
106363	Algeria	31°00'	2°40'E	580	28.11 ± 0.58	12.49
106367	Algeria	33°31'	6°03'E	100	32.07 ± 0.53	14.29
112082	Morocco	32°00'	8°55'W	180	33.16 ± 1.15	14.73
112083	Morocco	31°09'	8°39'W	730	29.26 ± 0.70	12.99

Source of variation	d.f.	MS	F	P
Accessions	38	4.08	1.78	≤0.01
Plants within an accession	156	6.17	0.37	—
Nuclei within a plant	3705	6.17	0.98	—

The latitudes and longitudes of the places of origin of the seeds are indicated. Twenty prophases were analysed in the root meristem of each of five seedlings per accession.

differences are not the same in all the chromosomes or chromosome portions. As shown in Fig. 3, a significant ( $P=0.013$ ) positive correlation exists between the length of the chromosome complement and the basic DNA content of the accessions.

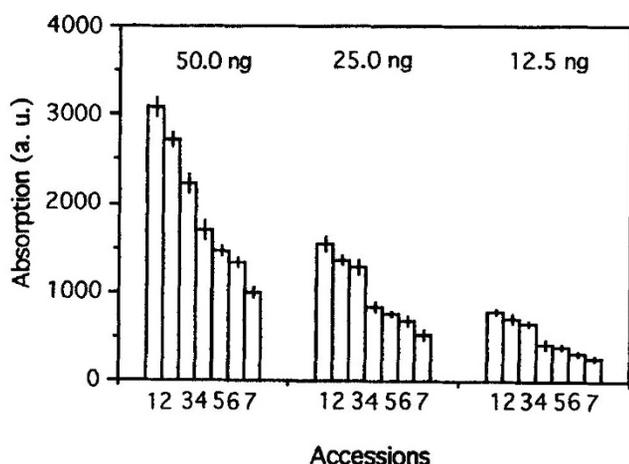
**Discussion**

Results obtained using different approaches indicate that changes in the nuclear DNA affecting the C-value differentiate local populations of *V. faba*. Indeed, the

**Table 2** Feulgen absorption of early prophase in five cultivars of *V. faba*

Cultivar	Seed provenance	Feulgen absorption (a.u.; mean $\pm$ SE)	Mean DNA C-value (pg)
Aprilia	Italy	29.79 $\pm$ 0.75	13.23
Loreta	Holland	29.46 $\pm$ 0.47	13.09
Superaguadulce	Morocco	29.70 $\pm$ 0.33	13.20
Super Set	Morocco	29.62 $\pm$ 0.63	13.16
Super Simonia	Morocco	29.41 $\pm$ 0.81	13.07

Twenty prophase were analysed in the root meristem of each of five seedlings per cultivar.



**Fig. 1** Densitometric absorptions of dot blot filters loaded with 50.0, 25.0 or 12.5 ng of genomic DNA from the roots of seedlings of seven local populations differing in genome size. 1, accession no. 112082 from Morocco; 2, accession no. 110343 from Italy; 3, accession no. 106363 from Algeria; 4, accession no. 106961 from Greece; 5, accession no. 110726 from Egypt; 6, accession no. 111927 from Italy; 7, accession no. 106858 from Greece. The filters were probed with digoxigenin-labelled *FokI* *V. faba* DNA repeats. Each value is the mean of six repetitions, three for each of two DNA extractions. Confidence intervals are for 99 per cent.

results of Feulgen/DNA cytophotometry, indicating significant differences in the basic DNA content between accessions (Table 1), find confirmation in those of molecular hybridization experiments, showing that tandemly-repeated DNA sequences may be differently represented in the genomes (Fig. 1 and Table 3). The existence of intraspecific quantitative variations in the nuclear DNA is further suggested by the results of karyometry, indicating that significant differences between populations may occur in the lengths of their chromosome complements (Table 4). The linear correlations existing between these data (Figs 2 and 3)

may be regarded as further proof of their reliability. Thus, *V. faba* can be added to the number of species within which variations in genome size and organization have been found (see Cavallini & Natali, 1991). Our findings are in line with evidence reported for other species which shows that domains that are capable of intraspecific fluidity do exist in the genome of plants (see Introduction). Redundancy variations of sequences in these DNA domains may differentiate even plants within a population, as in *Dasypyrum villosum* (Frediani *et al.*, 1994) and individuals within a single progeny of homozygous plants, as in *Helianthus annuus* (Natali *et al.*, 1993).

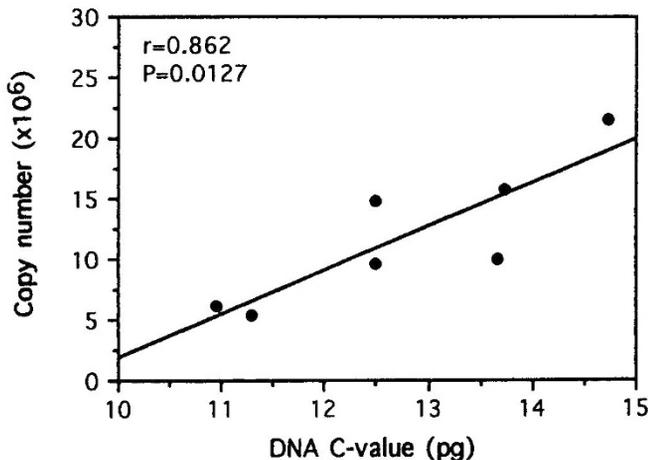
As well as in *D. villosum* (Frediani *et al.*, 1994), *H. annuus* (Cavallini *et al.*, 1986; Natali *et al.*, 1993), *Festuca arundinacea* (Ceccarelli *et al.*, 1992), *Pisum sativum* (Cavallini *et al.*, 1993) and several other plant species (see Cavallini & Natali, 1991), repetitive DNA is involved in these genomic changes. Our results point out a particular family of repeated sequences which represent a considerable portion of the nuclear genome of *V. faba* (Table 3). On this point, our findings are in agreement with those of Kato *et al.* (1984), who calculated that there are  $5 \times 10^6$  to  $5 \times 10^7$  copies of *FokI* elements per diploid genome. However, when comparing the range of variation of these repeats (Table 3) with the differences in DNA content between accessions as assessed cytophotometrically (Table 1), it appears that modulations within other families of DNA sequences must be involved in the creation of the genome size variations within *V. faba*.

The positive correlation between the length of metaphase chromosomes and the basic DNA contents of the accessions (Fig. 3) strongly suggests that quantitative modulations of DNA sequences affect the size of the chromosome complement. Also in other species, changes in the redundancy of repeated DNA sequences were found to correlate with chromosome length variations. It has been suggested that these variations can be due to alterations in the sequence repetitiveness *per se*, as well as to different rearrange-

**Table 3** Copy number of *FokI* repeats in the genome and percentage of the genome that they represent in seven local populations of *V. faba*, as calculated from the results of dot DNA blot hybridizations

Accession	DNA C-value (pg)	No. of <i>FokI</i> repeats per 1C DNA ( $\times 10^6$ )	Percentage of the genome that is <i>FokI</i> repeats
112082	14.73	21.51	9.47
110343	13.73	15.73	7.41
106363	12.49	14.73	7.63
106961	12.50	9.66	5.00
110726	13.66	9.98	4.71
111927	10.94	6.16	3.64
106858	11.28	5.39	3.09

DNA C-values are taken from Table 1.



**Fig. 2** Correlation between the copy number of sequences related to the *FokI* DNA repeats in the haploid genome (1C) of the seven local populations in Fig. 1 and their basic DNA content as determined by cytophotometry (Table 1).

ments of the chromatin superstructure that these alterations may induce (see Vogt, 1992 and references therein). Length differences between accessions occur to different extents in different chromosome pairs (Table 4). This suggests that redundancy variations of the same DNA sequences do not take place to the same extent in all the chromosomes and/or that variations concern DNA sequences which are not present in all the chromosomes, as in the case of *FokI* repeats, which are located only in S pairs (Yakura *et al.*, 1987).

Also in the light of the finding that the genome size of European accessions differs significantly from that of African accessions, it seems proposable that quanti-

tative changes in the nuclear genome of *V. faba* play some role in environmental adaptation. Also in other species, this biological role is strongly suggested by the existence of correlations with such environmental factors as latitude or altitude (see Cavallini & Natali, 1991), temperature (as in *F. arundinacea*; Ceccarelli *et al.*, 1992) or substratum composition (as in flax; see Cullis & Cleary, 1986).

In agreement with Bennett & Smith (1976), our findings show that genome size variations do not occur between commercial varieties of *V. faba* (Table 2), and this result represents a good internal control of the reliability of our cytophotometric data. When environmental adaptation is assumed as the cause of intraspecific variation in the nuclear genome, the lack of this variation between commercial varieties may be explained by the reduction of environmental selective pressure due to a standardized cultivation. Moreover, commercial varieties are genetically stabilized in order to maintain agronomically favourable phenotypical characters.

Quantitative variations in the nuclear genome may play a role in environmental adaptation by affecting developmental dynamics at the cellular and organismal level through nucleotypic effects (Bennett, 1985). Work is in progress to test this possibility in *V. faba* by comparing plant development in different local populations.

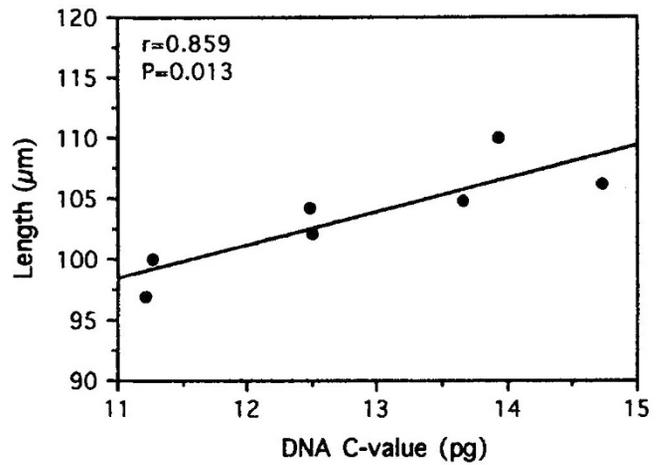
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**Table 4** Length of the entire chromosome complement and of each chromosome or chromosome portion in seven local populations of *V. faba* differing in genome size

Accession	Country	1C DNA content (pg)	Entire chromosome complement ( $\mu\text{m}$ ; mean $\pm$ SE)		Chromosome pairs							Coefficient of variation			
			Long arm	Short arm minus satellite	Satellite	S1	S2	S3	S4	S5					
112082	Morocco	14.73	8.19 $\pm$ 0.17	4.75 $\pm$ 0.12	2.94 $\pm$ 0.07	8.15 $\pm$ 0.19	7.68 $\pm$ 0.16	7.53 $\pm$ 0.16	6.82 $\pm$ 0.13	6.91 $\pm$ 0.15					
113074	Italy	13.93	8.65 $\pm$ 0.19	5.03 $\pm$ 0.11	3.05 $\pm$ 0.06	8.53 $\pm$ 0.18	7.99 $\pm$ 0.17	7.82 $\pm$ 0.17	7.18 $\pm$ 0.17	6.80 $\pm$ 0.13					
110726	Egypt	13.66	8.15 $\pm$ 0.20	4.80 $\pm$ 0.13	2.91 $\pm$ 0.08	8.18 $\pm$ 0.17	7.70 $\pm$ 0.14	7.46 $\pm$ 0.17	6.58 $\pm$ 0.13	6.83 $\pm$ 0.15					
106961	Greece	12.50	8.09 $\pm$ 0.16	4.57 $\pm$ 0.10	2.85 $\pm$ 0.05	7.97 $\pm$ 0.15	7.42 $\pm$ 0.14	7.20 $\pm$ 0.14	6.52 $\pm$ 0.12	6.55 $\pm$ 0.12					
106363	Algeria	12.49	8.24 $\pm$ 0.14	4.85 $\pm$ 0.11	2.88 $\pm$ 0.04	8.08 $\pm$ 0.17	7.47 $\pm$ 0.15	7.27 $\pm$ 0.12	6.63 $\pm$ 0.12	6.73 $\pm$ 0.13					
110442	Italy	11.28	7.88 $\pm$ 0.14	4.65 $\pm$ 0.09	2.82 $\pm$ 0.06	7.77 $\pm$ 0.13	7.21 $\pm$ 0.13	7.11 $\pm$ 0.15	6.39 $\pm$ 0.12	6.24 $\pm$ 0.10					
113747	Italy	11.21	7.60 $\pm$ 0.15	4.35 $\pm$ 0.07	2.74 $\pm$ 0.04	7.43 $\pm$ 0.12	7.06 $\pm$ 0.09	6.95 $\pm$ 0.12	6.11 $\pm$ 0.09	6.19 $\pm$ 0.08					
		—	4.15	4.63	3.32	4.33	4.20	3.98	5.05	4.52					

10 C-metaphase plates in the root meristem of five plantlets per accession were analysed. The coefficients of variation of the means are reported as an estimation of the extents of variation between the values obtained in different accessions.



**Fig. 3** Correlation between the length of the chromosome complement of the seven local populations in Table 4 and their genome size.

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