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M. Baranyi · J. Greilhuber

Flow cytometric and Feulgen densitometric analysis of genome size variation in *Pisum*

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Abstract A DAPI and ethidium bromide flow cytometric and Feulgen densitometric analysis of genome size variation in *Pisum* was conducted. The material included 38 accessions of *P. sativum* of widely different geographic origin and altogether 14 samples of *P. elatius*, *P. abyssinicum*, *P. humile* and *P. fulvum*. The relative genome size values obtained with the three staining methods were strongly correlated. No evidence for genome size variation was found among *P. sativum* cultivars. In particular, certain Italian cultivars, for which strongly deviating C-values have been reported, proved to be invariant. The only occasion when ambiguous evidence for marginal genome size variation was found was when all 38 accessions taxonomically affiliated with *P. sativum* were considered. *Pisum abyssinicum* and *P. fulvum* differed from *P. sativum* by about 1.066- and 1.070-fold, respectively; 1 accession of *P. humile* differed by 1.089-fold, and 2 of *P. elatius* by 1.122- and 1.195-fold, respectively (ethidiumbromide comparison), while the other accessions of these taxa were not different from *P. sativum*. This variation may indicate taxonomic inhomogeneity and demands further investigation. Cultivated *P. sativum* has long been suspected of not being constant with respect to genome size. As shown here, these findings were not based on genuine differences, but rather were technical in origin.

Key words *Pisum sativum* · Wild peas · Genome size variation · Flow cytometry · Feulgen densitometry · 4′, 6-Diamidino-2-phenylindole (DAPI)

Introduction

The genus *Pisum* (Fabaceae) is traditionally classified into four or five annual species, *P. sativum*, *P. elatius* (= *P. sativum* subsp. *elatius*), *P. abyssinicum*, *P. humile* and *P. fulvum* (Lehmann and Blixt 1984). In contrast to the huge amount of genetic information available on *P. sativum*, relatively little is known about the chromosomal variation in the genus itself, especially in the wild peas, where only limited information on translocations and inversions is available (Hâkansson 1936; Rosen 1944; Lamprecht 1964; Saccardo 1971; Ben-Ze'ev and Zohary 1973; Conicella and Errico 1985, 1990; Errico et al. 1991).

The purpose of the investigation presented here was to analyze genome size variation in the genus *Pisum*, with special reference to the problem of variability within *P. sativum*. *Pisum sativum* is a variable taxon that has been classified into three or more subspecies. It is widely distributed in different climates; it is known to occur in varying states of cultivation, from wild to high-bred; it has been under human selection for hundreds of years; and it is an inbreeder. These conditions seem to be in keeping with a variability of DNA values, as seen from the lists of Bennett and Smith (1976, 1991), and with recent reports on considerable genome size variation within this species obtained with Feulgen densitometry (Guerra 1983; Mukherjee and Sharma 1986; Cavallini and Natali 1990; Cavallini et al. 1993) and flow cytometry (Arumuganathan and Earle 1991). On the other hand, Greilhuber and Ebert (1994) and Baranyi and Greilhuber (1995) found no densitometric and flow cytometric evidence for genome size variation in very different accessions of *P. sativum*. These discrepancies prompted us to continue these studies with a set of peas that included wild accessions, landraces, and cultivars, in particular those between which up to 1.29-fold differences have been reported (Cavallini and Natali 1990; Cavallini et al. 1993). For comparison, samples of *P. elatius*, *P. abyssinicum*, *P. humile* and *P. fulvum* were tested. Using the efficient technology of flow cytometry with ethidium bromide and high-resolution DAPI staining, and Feulgen densitometry as a control, we were able to demonstrate that there is no occurrence of genome size variation within *P. sativum* except to

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M. Baranyi · J. Greilhuber (✉)
Institute of Botany, University of Vienna, Rennweg 14, A 1030 Vienna, Austria

marginal degrees, if any. On the other hand, *P. abyssinicum*, some *P. humile* accessions, *P. fulvum* and some accessions of *P. elatius* were clearly seen to have larger genomes than *P. sativum*, thereby providing new evidence for a taxonomic differentiation within the genus. However, our main finding is that *P. sativum* has a largely stable genome and that all previous results indicating variable genome size in the pea have apparently resulted from inappropriate methods.

Materials and methods

The samples tested and the seed bank and other sources are listed in Tables 1, 2 and 3. In particular, 5 Italian cultivars which also appear in the study of Cavallini and Natali (1990) and Cavallini et al. (1993) were purchased in an Italian seed shop. Five partly other cultivars appearing in these studies were received upon request from the Istituto del Germoplasma (I.D.G.), Bari, Italy. These samples were genetically inhomogeneous (seeds mixed mottled and non-mottled, or round and wrinkled) and were treated by us with due precaution regarding purity. The correct name of the cultivar termed 'Nano Progress' by Cavallini and co-workers is obviously 'Progress 9', because no 'Nano Progress' exists according to the 'Gemeinsamer Sortenkatalog für landwirtschaftliche Pflanzenarten' (Anonymous 1994), and the commercial packing we obtained is labelled 'Pisello nano Progress 9'. In the tables, synonymy is updated when necessary, but the synonyms as given in the seed bank lists are quoted as a footnote.

As a methodological principle, for comparative measurements in flow cytometry every nuclear isolation was jointly done for 1 individual of the test material and 1 of an internal standard, thereby ensuring identical conditions for both during isolation and further processing.

Table 1 List of *Pisum* accessions as obtained from the Institut für Pflanzengenetik und Kulturpflanzenforschung (I.P.K. Gatersleben), Sachsen-Anhalt, Germany; the Nordic Gene Bank (N.G.B.), Alnarp, Sweden; the Botanical Garden (H.B.), Coimbra; and the John Innes Institute (J.I.I.), Norwich, England

Genebank, accession no.	Taxon, cultivar	Origin, source
I.P.K., Gatersleben		
PIS 1106/84	*P. sativum* subsp. *sativum* convar *axiphium* var *dinocarpum*	China 1956: Agr. Exp. Stn. Hulon
PIS 180/89	*P. sativum* subsp. *sativum* convar *axiphium* var *giganteum*	Unknown
PIS 127/92	*P. sativum* subsp. *sativum* convar *axiphium* var *kappertii* Wachserbse von Prof. Kappert	R. Schreiber & Söhne, Quedlinburg
PIS 2116/88	*P. sativum* subsp. *sativum* convar *axiphium* var *minus*	USSR (Georgian SSR)
PIS 611/84	*P. sativum* subsp. *sativum* convar *medullare* var *pervicax*	R. Maly, Italy 1950
PIS 1697/83	*P. sativum* subsp. *sativum* convar *medullare* var *schneebergeri*	Landrace, Poland 1976
PIS 365/74	*P. sativum* subsp. *sativum* convar *medullosaccharatum* var *majakense* Weibulls Apollo	R. Schreiber & Söhne, Quedlinburg
PIS 968/79	*P. sativum* subsp. *sativum* convar *medullosaccharatum* var *prasinum* Zegalowa	Agriculture Exhibition Moscow 1954
PIS 2016/83	*P. sativum* subsp. *sativum* convar *sativum* var *fonticulorum*	South Italy 1980
PIS 1165/74	*P. sativum* subsp. *sativum* convar *sativum* var *mesomelan*	Mongolia 1964
PIS 496/73	*P. sativum* subsp. *sativum* convar *speciosum* var *ankoberense*	Hindukush 1935
PIS 389/77	*P. sativum* subsp. *sativum* convar *speciosum* var *capucinorum*	Sortiment H. Heyn, Rastatt, Bad.
PIS 132/84	*P. sativum* subsp. *transcaucasicum*	R. Schreiber & Söhne, Quedlinburg; Gruzija, USSR
PIS 1143/92	*P. elatius*	Unknown
PIS 1150/80	*P. elatius*	Unknown
PIS 1223/73	*P. abyssinicum* var *vavilovianum*	VIR Leningrad USSR, Kat. VIR 2759; Ethiopia
PIS 154/79	*P. abyssinicum* var *viridulogriseum*	Ethiopia
PIS 1318/89	*P. humile*[c]	Unknown
PIS 1319/91	*P. fulvum*	Unknown
N.G.B., Alnarp		
WL 0226	*P. elatius*	Unknown
NGB 100808 (WW 808)	*P. abyssinicum*	Unknown; donor: Rosen, G. von
NGB 102033	*P. humile*, Keeran pea	Katmandu, Nepal; donor: IPG Poznan
NGB 102148 (WW 2148)	*P. fulvum*	Israel; collector and donor: Lie and Ladizinski
H. B. Coimbra		
	P. sativum subsp. *sativum*[d]	Coimbra periphery, cult.
J.I.I., Norwich		
JI 4[a]	*P. sativum* Roi des Gourmands	Donor: Lamm, R.
JI 261	*P. elatius*	Donor: Dodds, K. S.
JI 225	*P. abyssinicum*	Unknown
JI 241	*P. humile*	Donor: Zohary, D.
JI 224[b]	*P. fulvum*	Donor: Zohary, D.

[a] Lamprecht's standard line N.G.B. 110 (M. Ambrose, personal communication) as used by Errico et al. (1991)
[b] Compare Errico et al. (1991)
[c] Specified as *P. syriacum* in I.P.K.
[d] Misidentified as *P. sativum* subsp. *elatius* in the seed catalogue of H.B. Coimbra

Table 2 DAPI and ethidium bromide flow cytometric data of *P. sativum* cultivars, either of commercial origin (comm) or from an anonymous donor (a.d.), as obtained from various sources in Austria, England, Italy and the Senegal market at Dakar. DAPI C.V. refers to the mean of a number of test seedlings (up to 4), with the best run of each single test seedling taken. If not stated otherwise, each run included *P. sativum* 'Kleine Rheinländerin' as the internal standard. All peaks were unimodal and symmetric, and thus provided evidence against distinct differences in terms of DAPI DNA fluorescence. EB data are presented as the ratio (%) of *P. sativum* versus *H. vulgare* 'Ditta'. For each accession the average ratio, its standard deviation (SD, with reference to N_r), number of seedling pairs tested (N_s) and number of total runs (N_r) is given

Source	Sample	DAPI, mean C.V. (G1)	EB, ratio *P. sativum*/*H. vulgare*			
			Mean	SD	N_s	N_r
Cambridge, P.B.I.	Minerva Maple	1.25	86.45	1.05	2	6
Senegal (comm)	*P. sativum* cultivar[a]	1.45	87.21	0.77	2	5
Austria (comm)	Kleine Rheinländerin		87.31	1.34	6	29
Austria (comm)	Frühe Heinrich[b]	1.99	91.05	1.88	1	2
Austria (comm)	Frühe Maiperle[b]	1.81	89.46	1.26	1	3
Austria (comm)	Frühe Volltragende[b]	1.68	88.82	2.65	1	3
Austria (comm)	Lancet[c]	1.81	86.09 (86.83)[d]	1.74	3	5
Austria (comm)	Linga[b]	1.94	89.52	1.97	1	2
Austria (comm)	Maierbse[c]	1.73	87.69 (87.54)[d]	0.94	2	6
Austria (comm)	Perfektion[c]	1.95	88.21 (87.83)[d]	1.13	3	12
Austria (comm)	Wunder von Kelvedon[c]	1.94	88.30 (88.10)[d]	0.96	4	14
Italy (a. d.)	Davina	1.60	86.69	1.11	2	8
Italy (a. d.)	Frila	1.59	87.18	0.52	2	5
Italy (a. d.)	Karina	1.16	87.95	1.03	2	8
Italy (a. d.)	Revolution	1.44	87.84	1.17	2	7
Italy (a. d.)	Traxy	1.63	87.42	0.80	1	4
Italy (comm)	Espresso Generoso	1.46	87.13	0.94	6	26
Italy (comm)	Progress 9	1.44	86.58	1.01	6	14
Italy (comm)	Rondo	1.80	85.73	0.92	3	9
Italy (comm)	Senatore	1.47	87.38	0.00	1	3
Italy (comm)	Telefono	1.33	87.70	1.35	2	6

[a] An 'afila' variant, with leaflets transformed into tendrils
[b] Values from hydrated seeds only
[c] Values from hydrated seeds and seedlings
[d] Values from seedlings

The standards belonged to one of the following taxa: *Pisum sativum* 'Kleine Rheinländerin', *Hordeum vulgare* 'Ditta', *Triticum monococcum* and, rarely, the F_1 hybrid corn line *Zea mays* 'Sundance', *Allium cepa* 'Stuttgarter Riesen' and *A. cepa* 'Frühstamm'. The standards were cross-compared (Table 4) and found to be genomically stable according to flow cytometric criteria.

Seeds were germinated on plates. In *Pisum* the seeds were sometimes also used directly after hydration for 3 h. A nuclear suspension was prepared basically following Galbraith et al. (1983) and De Laat et al. (1987), and DAPI staining was as described in Baranyi and Greilhuber (1995).

For ethidium bromide (EB) staining, 0.95 ml of the nuclear suspension, prepared as above, was filtered through a 50-µm nylon gauze, mixed with RNase (Sigma) to a final concentration of 0.15 mg/ml and digested at 37 °C for 30 min. One part of the nuclear suspension was then mixed with five parts of the EB staining solution (60 µg/ml EB in distilled water containing 0.4 *M* Na_2HPO_4, 10 m*M* sodium citrate, and 25 m*M* sodium sulfate) and measured after at least 20 min. The instrument used for the measurements was a Partec CA II flow cytometer equipped with a 100 W high pressure mercury lamp and different photomultipliers for DAPI and EB (optic arrangement for DAPI: KG 1, BG 38, UG 1 filters, TK 420 dichroic mirror, 40 × 0.80 quartz objective, GG 435 barrier filter; for EB: KG 1, BG 38, BG 12 filters, TK 500 dichroic mirror, 40 × 1.25 glycerin immersion objective, RG 570 barrier filter).

Feulgen densitometry (Greilhuber and Ebert 1994) was used to corroborate the results obtained with flow cytometry.

A single classification analysis of variance, Scheffé test, and correlation analysis were done with the SPSS for Windows 6.0 package (SPSS, Chicago, Ill.) and a nested analysis of variance with the NESTAN routine of the BIOM-pc Vers.2.1. package (Rohlf 1992).

Results and discussion

Methods and standards

In flow cytometric estimations of plant nuclear DNA content DAPI has been established as a DNA-specific fluorochrome (Sgorbati et al. 1986; Ulrich and Ulrich 1986, 1991; De Laat et al. 1987; Ulrich et al. 1988; Doležel et al. 1989, 1992; Doležel 1991; Ulrich 1992). For certain purposes it is preferable over EB or propidium iodide because of its highly stoichiometric binding and therefore sharp resolution (coefficient of variation, C.V., on average 1.8% in our hands) that easily enable differences as low as 4% in single tests to be detected. Therefore, during a part of the present study, *P. sativum* 'Kleine Rheinländerin' was used as internal standard, and peak unimodality at low C.V. was taken as evidence for genome size homogeneity (Tables 2, 3, 5; Fig. 1). Ethidium bromide clearly has the advantage over DAPI of not being base-composition-dependent (Le Pecq and Paoletti 1967), which results in unbiased DNA quantity measurements. The somewhat larger C.V. (2.7% on average in our hands), which is typical for EB, necessitates the inclusion of an internal standard

Table 3 DAPI flow cytometric results in *P. sativum* as obtained from Istituto del Germoplasma (I.D.G.), Bari, and *Pisum* accessions as listed in Table 1. If not stated otherwise each run included *P. sativum* 'Kleine Rheinländerin' as the internal standard (peak 1, mode set to 100). Solitary *Pisum* peaks were unimodal and symmetric, indicating the absence of distinct differences to *P. sativum* 'Kleine Rheinländerin' in terms of DAPI DNA fluorescence. Deviating *Pisum* accessions produced a second peak, whose mean mode position and standard deviation is given. C.V.s are given for all *Pisum* peaks and refer to the mean of a number of test seedlings (up to 12), with the best run of each single test seedling taken. For some of the accessions *H. vulgare* was included as another standard, whose mean peak position and corresponding standard deviations is given

Genebank no.	*Pisum* (G1)					*Hordeum* (G1)	
	Peak 1	Peak 2		C. V.		Mean	SD
		Mean	SD	Peak 1	Peak 2		
I.D.G., Bari							
Subsp. *arvense* (100001)	100			1.48		84.44	1.10
	100[e]			1.65		84.31	0.16
Rondo (100212)	100			1.48		83.01	1.03
	100[e]			1.47		83.38	0.34
Dolce Provenze (100241)	100			1.34		83.88	0.50
	100[e]			1.50		83.78	0.39
Senatore (100329)	100			1.48		82.95	0.38
	100[e]			1.46		82.26	1.08
Telefono (101111)	100			1.48		83.77	0.52
	100[e]			1.66		84.15	0.75
I.P.K., Gatersleben							
PIS 1106/84	100			1.47		–	–
PIS 180/89	100			1.92		83.61	0.81
	100[e]			1.63		83.78	0.80
PIS 127/92	100			1.96		–	–
PIS 2116/88	100			1.81		82.02	0.57
	100[e]			1.33		82.74	1.38
PIS 611/84	100			1.43		–	–
PIS 1697/83	100			1.44		–	–
PIS 365/74	100			1.46		–	–
PIS 968/79	100			1.47		–	–
PIS 2016/83	100			1.43		–	–
PIS 1165/74	100			1.49		–	–
PIS 496/73	100			1.34		–	–
PIS 389/77	100			1.47		–	–
PIS 132/84	100			1.46		–	–
PIS 1143/92[a]	100	114.99	1.52	1.65	1.70	83.06	1.09
PIS 1150/80[a]	100			1.64		–	–
PIS 1223/73[b]	100	107.68	0.88	1.78	1.85	–	–
PIS 154/79[b]	100	108.01	1.08	1.56	1.90	82.67	1.01
PIS 1318/89[c]	100	109.07	1.55	1.52	1.66	82.20	0.98
PIS 1319/91[d]	100	107.82	0.94	2.18	2.02	83.06	0.41
N.G.B., Alnarp							
WL 0226[a]	100			1.60		83.10	0.89
	100[e]			1.49		82.54	0.37
NGB 100808[b]	100	108.45	1.15	1.59	1.39	82.91	1.27
NGB 102033[c]	100			1.40		82.93	0.86
	100[e]			1.30		82.71	0.60
NGB 102148[d]	100	109.18	–	1.52	1.40	83.67	–
H. B. Coimbra							
P. sativum	100			1.65		83.07	0.96
	100[e]			1.96		83.55	1.19
J.I.I., Norwich							
JI 4	100			1.81		88.93	0.66
JI 261[a]	100	117.39	2.18	1.63	1.71	82.51	1.41
JI 225[b]	100	106.96	0.96	1.85	2.13	82.95	0.77
JI 241[e]	100			2.29		83.20	1.22
JI 224[d]	100	106.97	0.78	2.23	2.21	82.84	1.20

[a] *P. elatius*
[b] *P. abyssinicum*
[c] *P. humile*
[d] *P. fulvum*
[e] *Pisum* test material not combined with *P. sativum* 'Kleine Rheinländerin'

which does not overlap but which is also not too different from pea (to avoid linearity problems) and is readily available. The inclusion of an internal standard was a methodological principle of the present work, because we mistrust the practice of calibrating the instrument once and doing subsequent runs over many hours without any further internal reference.

Feulgen densitometry is an established methodology for DNA measurements that has produced surprisingly divergent genome size estimates in *P. sativum*. However, Greilhuber and Ebert (1994) have shown in numerous *P. sativum* accessions that variation is apparently absent if the technique is properly applied. In the present analysis, we used Feulgen densitometry as a control for flow cytometry (Tables 6,7). We want to emphasize that all three techniques in *Pisum* gave comparable results (Table 8), with correlation coefficients of 0.9506 (Feulgen-EB), 0.9559 (Feulgen-DAPI) and 0.9815 (EB-DAPI).

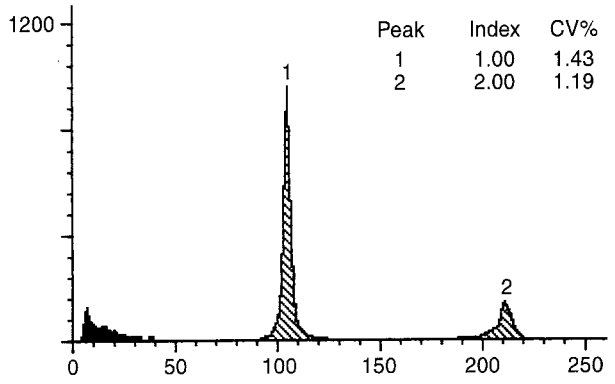

Fig. 1 Simultaneous DAPI flow cytogram of the Italian *P. sativum* cultivars 'Espresso Generoso' and 'Progress 9', demonstrating genome size constancy in these pea strains

The requirement of an internal standard for DNA measurements is well-known (Bennett and Smith 1976; Greilhuber 1988). The following species and cultivars were considered for this purpose: (1) *Pisum sativum* 'Kleine Rheinländerin', because it is readily available and is genetically closely related to the material tested in the present study; (2) *Hordeum vulgare* 'Ditta', because it does not differ too much in genome size from the vegetable pea; (3) *Triticum monococcum*, because it does not overlap with peas of elevated genome size; (4) *Zea mays* 'Sundance', which is a F_1 hybrid corn and is supposed to be homogeneous in knob endowment, and therefore genome size; (5) *Allium cepa* 'Stuttgarter Riesen', which has been a preferred reference cultivar in the present laboratory, and *A. cepa* 'Frühstamm' have only rarely been used, because of unsuitable genome size (data not shown). With DAPI, repeated runs of combined nuclear suspensions of these taxa demonstrated practically constant peak distances. *Zea mays* 'Sundance' was $49.3 \pm 0.9\%$ of *Hordeum vulgare* 'Ditta' (10 independent tests, on average three repeats per nuclear isolate). *Hordeum vulgare* 'Ditta' was $82.9 \pm 0.5\%$ of *P. sativum* 'Kleine Rheinländerin' (10 tests, two repeats each). *Allium cepa* 'Stuttgarter Riesen' was 505.4% of *P. sativum*. Onion was not generally used because of its being too different from pea. However, barley was suitable (Tables 2, 3), and corn was also used in certain cases (Table 6). Owing to the sharp resolution of DAPI peaks,

Table 4 Comparison of species used as internal standards using ethidium bromide flow cytometry. The ratio (%) of test versus reference species, its standard deviation (SD, with reference to N_r), the number of seedling-pairs (N_s) and the total number of runs (N_r) are given

Test species	Reference species	Ratio		N_s	N_r
		Mean	SD		
P. sativum Kleine Rheinländerin	*A. cepa* Frühstamm	25.35	0.37	6	10
P. sativum Kleine Rheinländerin	*A. cepa* Stuttgarter Riesen	25.32	0.47	7	15
P. sativum Kleine Rheinländerin	*T. monococcum*	74.24	1.03	10	33
P. sativum Kleine Rheinländerin	*H. vulgare* Ditta	87.31	1.34	6	29
T. monococcum	*H. vulgare* Ditta	117.71	1.35	8	24
Z. mays Sundance	*H. vulgare* Ditta	50.05	1.00	5	10
Z. mays Sundance	*P. sativum* Kleine Rheinländerin	59.31	1.26	1	2

Table 5 DAPI flow cytometric data either of single *P. sativum* cultivars or of 2 or more *P. sativum* cultivars run simultaneously. Mean C.V. refers to the mean of a number of test seedlings or test seedling sets (up to 4), with the best run of each single test set taken, All *Pisum* peaks including those containing several cultivars were unimodal and symmetric, generally with low C.V., indicating the absence of distinct variation between cultivars in terms of DAPI DNA fluorescence

Cultivars	Mean C.V. (G1)
Minerva Maple	1.97
Kleine Rheinländerin	1.78
Fruhe Maiperle	2.56
Frühe Voltragende	2.08
Perfektion	2.36
Wunder von Kelvedon	1.28
Frühe Maiperle + Perfektion	1.22
Frühe Maiperle + Frühe Voltragende	1.25
Frühe Voltragende + Wunder von Kelvedon	2.36
Kleine Rheinländerin + Frühe Maiperle + Frühe Volltragende + Wunder von Kelvedon	1.46
Kleine Rheinländerin + Frühe Maiperle + Frühe Voltragende + Perfektion + Wunder von Kelvedon	2.42
Minerva Maple + Kleine Rheinländerin + Frühe Maiperle + Frühe Volltragende + Perfektion + Wunder von Kelvedon	1.67
Progress 9 + Espresso Generoso	1.45
Rondo + Espresso Generoso	1.23
Telefono + Espresso Generoso	1.47

P. sativum 'Kleine Rheinländerin' was even preferred as the internal reference for testing the homogeneity or inhomogeneity of combined nuclear isolates in pea (Tables 2, 3, 5; Figs. 1, 2).

Peak distances with EB clearly differed from those with DAPI (Table 4; Figs. 2, 3). *Triticum monococcum* was only used with EB. Our observations are in good agreement with the data of Doležel et al. (1992) in *P. sativum*, *Z. mays* and *A. cepa*.

Pisum cultivars with alleged genome size variation

Pisum sativum 'Minerva Maple', a recommended standard for DNA measurements, has been reported to deviate 0.82-fold from vegetable pea (Guerra 1983). This cultivar was compared with *P. sativum* 'Kleine Rheinländerin' and a number of other vegetable peas using DAPI (Tables 2, 5), and its genome size was determined using EB and *H. vulgare* and *T. monococcum* as standards (Tables 2, 9). 'Minerva Maple' was clearly not different from vegetable peas, which is in accordance with the previous Feulgen results of Greilhuber and Ebert (1994).

We were especially interested in a set of Italian cultivars for which up to 1.29-fold variation has been reported (Cavallini and Natali 1990; Cavallini et al. 1993). Five cultivars were of commercial origin (Table 2), but a partly congruent sample was also obtained from a germ plasm source (Table 3). Altogether 7 of the 10 lines of Cavallini and Natali (1990) were tested, covering the whole range of DNA content variation reported. All tests with DAPI, EB and Feulgen proved genome size homogeneity with other *P. sativum* lines (Tables 2, 3, 5, 6, 9).

Separate tests were made with 'Progress 9' and 'Espresso Generoso', those lines showing the largest differences in genome size and associated nucleotypic parameters (Cavallini et al. 1993). A comparison was made with DAPI of each of these and 'Kleine Rheinländerin' using *Z. mays* as the standard (Table 6). 'Progress 9', 'Espresso Generoso' and 'Kleine Rheinländerin' were 256.8%, 254.4%, and 256.9% of corn, respectively; this variation being non-significant. In other DAPI tests, low-ranking cultivars of Cavallini and Natali (1990), 'Rondo', 'Telefono' and 'Progress 9', were measured simultaneously with the high-ranking 'Espresso Generoso' and determined not to be different (Table 5; Fig. 1). With EB, a separate analysis involving cvs 'Progress 9', 'Espresso Generoso' and 'Kleine Rheinländerin' showed again that these are not significantly different; the 2 Italian cultivars only differed by 0.6% (Table 2; Fig. 3). A comparison of expanded leaflets of plants 13 days old showed only a 0.4% non-significant difference (data not shown). The Feulgen measurements corroborated these results (Table 6).

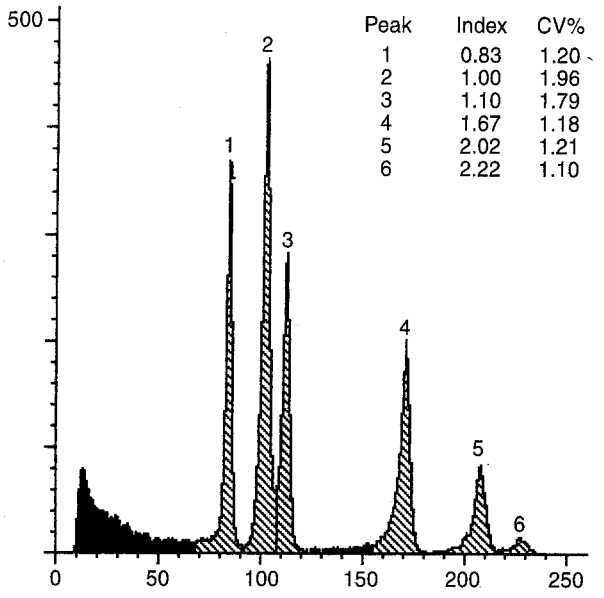

Fig. 2 Simultaneous DAPI flow cytogram of *H. vulgare* 'Ditta' as standard (*peaks 1, 4*), *P. sativum* 'Kleine Rheinländerin' (*peaks 2, 5*) and *P. humile*, PIS 1318/89 (see Table 2; *peaks 3, 6*). For comments on *H. vulgare* peak position, see text

Table 6 DAPI flow cytometric and Feulgen densitometric comparison of 3 *P. sativum* cultivars, the first two purportedly differing 1.29-fold in genome size (see text). For DAPI comparison, *Z. mays* 'Sundance' was used as the standard. Cultivars were non-significantly different, but individuals of the same cultivar were significantly different ($P < 0.001$). In Feulgen densitometric comparisons, telophase nuclei, 10 per primary root tip and slide, were measured. Integrated absorbance is given in arbitrary units (A.U.). Hierarchical analysis of variance indicated significant differences between slides ($P < 0.001$, $F_{10,117} = 5.0490$), but no significant differences between the cultivars ($F_{2,10} = 1.0805$) (N_s number of seedling pairs, N_r total number of runs)

P. sativum cultivar	DAPI-flow cytometry				Feulgen densitometry		
	Ratio *Pisum/Zea* (G1)		N_s	N_r	DNA content (A.U.)		n
	Mean	SD			Mean	SD	
Progress 9	256.8	3.6	9	17	29.43	0.95	50
Espresso Generoso	254.4	1.9	6	21	29.96	1.00	50
Kleine Rheinländerin	256.9	2.8	8	19	29.56	0.86	30
Grand mean	256.0	3.0	23	57	29.66	0.97	130

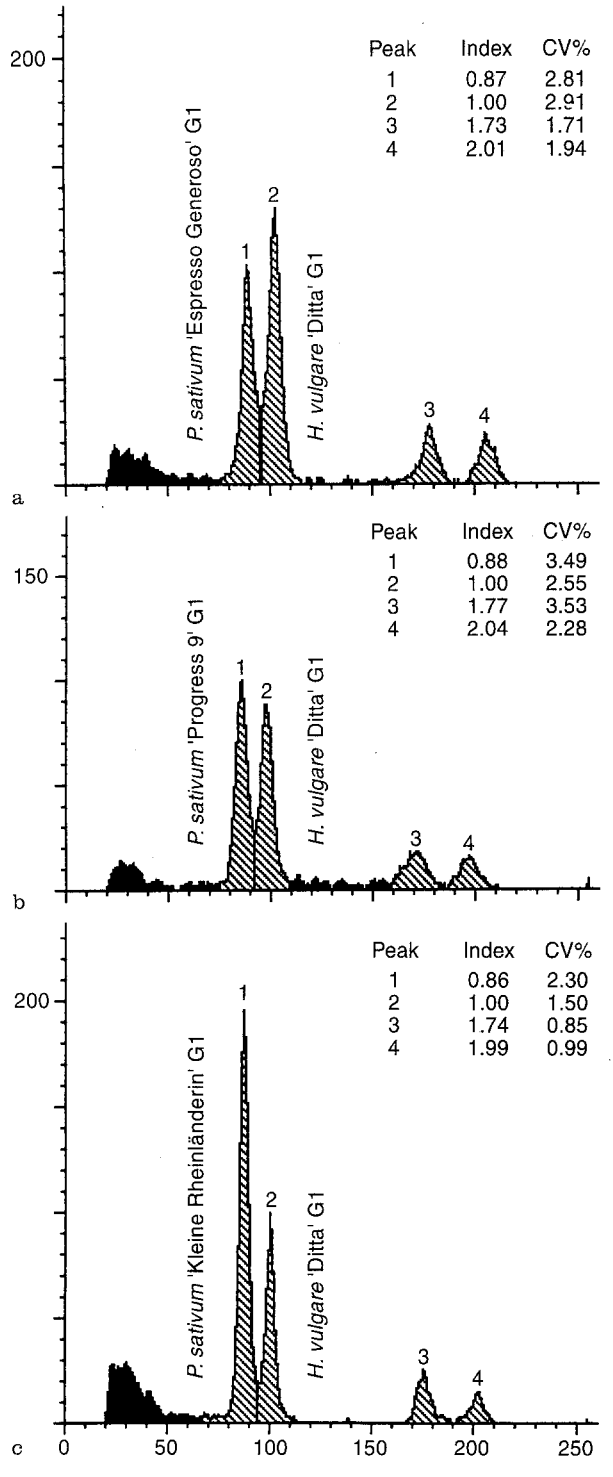

Fig. 3a–c Simultaneous ethidium bromide flow cytograms of *P. sativum* cultivars and *H. vulgare* 'Ditta' (as standard), demonstrating genome size constancy in the pea strains. **a** *P. sativum* 'Espresso Generoso', **b** *P. sativum* 'Progress 9', **c** *P. sativum* 'Kleine Rheinländerin'

Other *P. sativum* accession

An additional 16 cultivars, including 'Roy des Gourmands' (Lamprecht's standard line N.G.B. 110), 9 *P. sativum* subsp. *sativum* varieties without cultivar status and 1 *P. sativum* subsp. *transcaucasicum* were analyzed. With DAPI, pairwise and multiple simultaneous comparisons of pea cultivars provided no evidence of genome size variation (Tables 2, 3, 5). Even a simultaneous run of 6 cultivars did not result in a larger coefficient of variation than usually occurs with a single seedling. EB analyses with *H. vulgare* as the standard confirmed these observations (Table 2). Variation between all 25 cultivars (data from hydrated seeds excluded, because of supposedly less regular staining, see Table 2) was non-significant upon ANOVA (between cultivars $F = 1.3361$, n.s.; between individuals, within cultivars $F = 2.1558$, $P < 0.001$).

The whole span of EB-DNA variation including cultivars and non-cultivars was only 1.036-fold, which is significant upon hierarchical ANOVA ($P < 0.05$), but non-significant with the Scheffé test ($P > 0.05$).

Feulgen measurements in 4 lines, among these 'Roy des Gourmands', confirmed its invariance relative to 'Kleine Rheinländerin' (Table 7).

Pisum elatius

According to Lehmann (1954), *P. elatius* (syn. *P. sativum* subsp. *elatius*) is a wild climbing pea with a distribution that extends from the Mediterranean and Asia Minor to Tibet and India. It is the only wild species having gritty seeds, although not always, and the pods are explosively dehiscing when mature (Lehmann and Blixt 1984). The samples, which we received under the above-mentioned name (Table 1) were heterogeneous in genome size according to DAPI, EB and Feulgen staining (Tables 3, 7–9). Two samples, PIS 1150/80 and WL 0226, were not different from *P. sativum* 'Kleine Rheinländerin', but 2 others strongly deviated from the latter, being on average 113.7% (PIS 1143/92, with indistinctly gritty testa) and 118.3% (JI 261, with distinctly gritty testa), respectively, of the standard cultivar. Among those samples not deviating there was 1 (PIS 1150/80) with unambiguously gritty seeds, the key character of *P. elatius*. It is not surprising that genome size in a taxon often classified as a subspecies of *P. sativum* does not deviate from the typical genome size of this species, but the existence of remarkably larger genomes calls for a taxonomic reappraisal of the corresponding accessions. *Pisum elatius* JI 261 has already been measured (as *P. sativum*, weed from Turkey) by Schweizer and Davies (1972), who found only 105.1% of vegetable pea, which is significantly less than the 118.3% we found. It would be important to know whether the genome size differences coincide with a certain translocation, the same one that also occurs in a southern population of *P. humile* (Ben-Ze'ev and Zohary 1973).

Pisum abyssinicum

This taxon occurs in southern Arabia and Ethiopia and is sometimes cultivated there (Lehmann 1954; Lam-

Table 7 Feulgen absorbance measurements in *Pisum* accessions (see Table 1) relative to that of *P. sativum* 'Kleine Rheinländerin'. Root tips of test material and *P. sativum* 'Kleine Rheinländerin' as the standard were jointly Feulgen-stained. Each test sample was calibrated against an equal number of standard nuclei. Ten telophase nuclei per primary root tip meristem and slide were measured. The mean relative DNA content is given in percentage of the standard. The standard deviation (SD) refers to the number of nuclei (N) of the test sample and represents the relative weighted standard deviation combining variation of test material and standard (for formula see Greilhuber and Ebert 1994). Accessions jointly tested are labelled with the same number

Taxon and source (no.)	Test material			Test no.
	N	Mean (relative to standard)	SD	
I.P.K., Gatersleben				
P. sativum subsp. *sativum* convar *axiphium var giganteum* (PIS 180/89)	40	97.93	4.42	2
P. sativum subsp. *sativum* convar *axiphium* var *minus* (PIS 2116/88)	50	98.51	5.16	3
P. elatius (PIS 1143/92)	40	113.80	5.68	2
P. elatius (PIS 1150/80)	50	99.56	5.02	1
P. abyssinicum var *vavilovianum* (PIS 1223/73)	10	109.55	3.51	4
P. abyssinicum var *viridulogriseum* (PIS 154/79)	40	106.98	3.98	3
P. humile[a] (PIS 1318/89)	40	107.82	4.99	2
P. fulvum (PIS 1319/91)	30	105.00	4.52	1
N.G.B., Alnarp				
P. elatius (WL 0226)	30	98.26	4.76	5
P. abyssinicum (NGB 100808)	30	105.63	4.85	5
P. humile (NGB 102033)	50	98.24	4.55	4, 5
P. fulvum (NGB 102148)	10	107.63	3.11	4
H. B. Coimbra				
P. sativum	20	100.17	3.98	2
J.I.I., Norwich				
P. sativum Roi des Gourmands (JI 4)	100	101.49	5.57	6, 8
P. elatius (JI 261)	90	118.15	5.81	7
P. abyssinicum (JI 225)	40	110.91	4.67	6
P. humile (JI 241)	100	100.69	4.81	6, 8
P. fulvum (JI 224)	70	106.90	4.42	7

[a] Listed as *P. syriacum* in I.P.K. Gatersleben

Table 8 Comparison of measurements with Feulgen densitometry and flow cytometry with DAPI and ethidium bromide in *Pisum* accessions other than *P. sativum* (see Table 1). The values given are means expressed in percentage of *P. sativum* 'Kleine Rheinländerin'. Unity values refer to homogeneity with *P. sativum* 'Kleine Rheinländerin' in combined runs. EB values were calibrated with *T. monococcum* and converted taking the EB value for *P. sativum* 'Kleine Rheinländerin' as 100.00%. The correlation coefficients ($P < 0.001$ in all cases) in *Pisum* accessions were 0.9815 (21 comparisons) between DAPI and EB, 0.9559 (19 comparisons) between DAPI and Feulgen and 0.9420 (15 comparisons) between EB and Feulgen

Taxon and source (no.)	Feulgen	DAPI	relative EB
I.P.K., Gatersleben			
P. elatius (PIS 1143/92)	113.80	114.99	112.20
P. elatius (PIS 1150/80)	99.56	100	100.34
P. abyssinicum var *vavilovianum* (PIS 1223/73)	109.55	107.68	106.45
P. abyssinicum var *viridulogriseum* (PIS 154/79)	106.98	108.01	106.68
P. humile (PIS 1318/89)	107.82	109.07	108.93
P. fulvum (PIS 1319/91)	105.00	107.82	107.09
N.G.B., Alnarp			
P. elatius (WL 0226)	98.26	100	100.38
P. abyssinicum (NGB 100808)	105.63	108.45	106.61
P. humile (NGB 102033)	98.24	100	99.69
P. fulvum (NGB 102148)	107.63	109.18	–
H. B. Coimbra			
P. sativum	100.17	100	–
J.I.I., Norwich			
P. sativum Roi des Gourmands (JI 4)	101.49	100	98.88
P. elatius (JI 261)	118.15	117.39	119.50
P. abyssinicum (JI 225)	110.91	106.96	106.65
P. humile (JI 241)	100.69	100	98.61
P. fulvum (JI 224)	106.90	106.97	106.92

precht 1974). Lamprecht (1974) maintains that typical *P. abyssinicum* is endemic to the extremely hot parts of Ethiopia with two short summers and two rain periods. It is characterized by deeply dentated or serrated leaflets, which strongly resemble those of typical *P. humile*. In the present study 4 samples designated as *P. abyssinicum* were investigated (Table 1), all of them having serrated leaflets. All 4 samples consistently showed 106.6% of *P. sativum* 'Kleine Rheinländerin' (Tables 5, 7–9). This agrees with the results of Baranyi and Greil-

Table 9 Ethidium bromide flow cytometric comparison of *Pisum* accessions using *T. monococcum* as the standard. The ratio (%) of test versus reference species, its standard deviation, (SD, with reference to N_r), the number of seedling-pairs (N_s), and total number of runs (N_r) are given. Homogeneous groups according to the Scheffé test are given the same letter

Reference Table	Sample	Scheffé groups	Mean	SD	N_s	N_r
2	Minerva Maple	a	73.55	0.56	8	24
2	*P. sativum* (Senegal)	a	73.33	0.80	7	19
2	Kleine Rheinländerin	a	74.24	1.03	10	33
2	Senatore	a	74.05	1.12	1	4
2	Telefono	a	75.14	0.24	1	3
5	Telefono (I.D.G. Bari)	a	73.92	1.19	1	2
1	PIS 127/92	a	73.38	1.63	1	2
1	PIS 1143/92[a]	d	83.30	0.83	2	10
1	PIS 1150/80[a]	a	74.49	1.06	1	4
1	PIS 1223/73[b]	b	79.03	0.79	9	23
1	PIS 154/79[b]	b	79.20	0.73	7	15
1	PIS 1318/89[c]	c	80.87	0.74	5	12
1	PIS 1319/91[d]	b	79.50	1.08	6	11
1	WL 0226[a]	a	74.52	0.41	1	3
1	NGB 100808[b]	b	79.15	0.76	6	18
1	NGB 102033[c]	a	74.01	0.90	6	16
1	JI 4	a	73.41	0.68	2	7
1	JI 261[a]	e	88.72	0.96	11	16
1	JI 225[b]	b	79.18	0.89	6	22
1	JI 241[c]	a	73.21	0.83	5	21
1	JI 224[d]	b	79.38	0.92	6	16

[a] *P. elatius*
[b] *P. abyssinicum*
[c] *P. humile*
[d] *P. fulvum*

huber (1995) in another accession of this taxon. *Pisum abyssinicum*, JI 225, has already been measured by Schweizer and Davies (1972), who found 105.7% compared with vegetable pea, which is compatible with our findings.

Pisum humile

Pisum humile occurs in the Near East (Zohary and Hopf 1973). It is non-climbing, semiprostrate (Lamprecht 1974) and adapted to dry, low grassland well away from the coastal regions (Smartt 1984). According to Lehmann and Blixt (1984), whose classification is declaredly an artificial one, *P. humile* (syn. *P. syriacum*) shares with *P. abyssinicum* dentation of the leaflets, but differs in flower characters, has an anthocyanin-spot in the axils of the stipules and has pods explosively dehiscing at maturity. In the present study 3 samples were investigated (Table 1) and found to be heterogeneous in genome size. All 3 samples were devoid of deeply incised leaflets. Only 1 accession (PIS 1318/89, Table 1) deviated with EB, and to very similar degrees with DAPI and Feulgen; it was 108.9% of *P. sativum* 'Kleine Rheinländerin' (Tables 3, 7–9). This accession did not conform to the description given above in that it has narrow stipules and leaflets, the latter being undentated. One non-deviating sample, designated as 'Keeran Pea' (NGB 100808), originates from Nepal, which does not fit into the accepted geographical distribution of *P. humile*. Another non-deviating sample (JI 241) had moderately dentated leaflets, a residual anthocyanin ring in the axils of the stipules (which fits better to *P. sativum* sensu lato) and anthocyanin-shaded leaflet margins. The taxonomic affiliation of these accessions should be reconsidered. It would be interesting to test whether the genome size differences we observed are correlated with the presence or absence of a translocation that is present in a southern population of *P. humile* (Ben-Ze'ev and Zohary 1973).

Pisum fulvum

This species is distributed throughout the dryer and stony areas of the Near East. Of all wild peas, *P. fulvum* is unique with respect to its flower color: the standard and wings, salmon-ochreous; the keel, cream colored (Lamprecht 1974). It is this taxon whose species status has been the least debated due to the strong, although not complete, crossing barriers that correlate positively with karyotype reconstructions (see Smartt 1984). A recent opinion is that *P. fulvum* is the only species in the genus in addition to *P. sativum*, the latter covering the other taxa as subspecies and varieties (Davis 1970; Smartt 1984).

Three accessions of *P. fulvum* (Table 1) were measured, all showing with all three techniques a very similar genome size of about 107.0% of *P. sativum* 'Kleine Rheinländerin' (Tables 3, 7–9; Fig. 4). A third nucleolar constriction was seen in Feulgen slides of all these accessions, which is in agreement with the observations of Errico et al. (1991), whose line JI 224 was included in the present study. The seeds had the typical velvety testa. Two lines had deeply incised leaflets, but in JI 224 these were only weakly dentated. The latter accession has already been measured by Schweizer and Davies (1972), who found only 96.3% of vegetable pea, which is a noteworthy deviation from our findings.

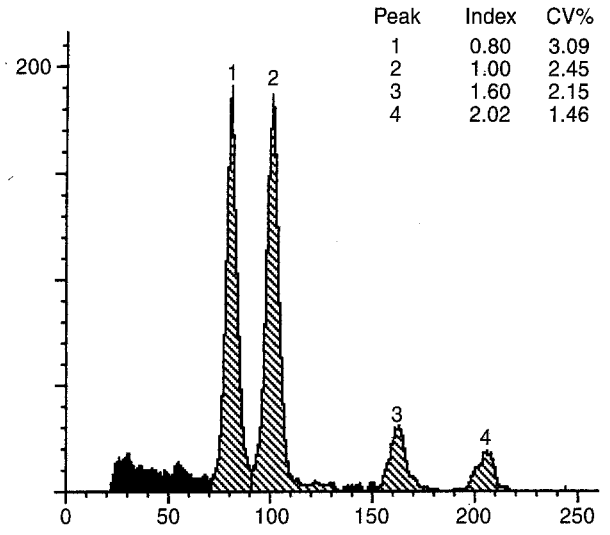

Fig. 4 Simultaneous ethidium bromide flow cytogram of *P. fulvum*, JI 224 (see Table 5; *peaks 1, 3*), and *T. monococcum* as standard (*peaks 2, 4*)

Absolute C-values in *Pisum*

Based on the EB fluorescence of *P. sativum* 'Kleine Rheinländerin', we estimated the following 1C values for groups of accessions, which appear to be fairly homogeneous in genome size: all *P. sativum* accessions, *P. elatius* (WL 0226, PIS 1150/80) and *P. humile* (NGB 102033, JI 241) have 4.42 pg, the value obtained by Greilhuber and Ebert (1994) with Feulgen densitometry using *A. cepa* with 16.75 pg as reference value; *P. elatius* PIS 1143/92 has 5.03 pg; *P. elatius* JI 261 has 5.23 pg; *P. humile* PIS 1318/89 has 4.81 pg; all *P. abyssinicum* have 4.71 pg; and all *P. fulvum* have 4.73 pg. Our *P. sativum-H. vulgare* EB ratio is 0.873, which fits well to the Feulgen ratio given by Bennett and Smith (1976) and Bennett et al. (1982) of 0.875 and our own Feulgen ratio (unpublished) of 0.850. However, the 1C value for *P. sativum* of 4.87 pg given by Bennett and Smith (1976) is, according to our results, too high, obviously because of a discrepancy with the *A. cepa* standard. Doležel et al. (1992) using propidium iodide found a pea/onion ratio of 0.2609. Taking the 1C value of onion by convention as being 16.75 pg, a genome size for *P. sativum* of 4.37 pg results, which is very close to our Feulgen value of 4.42 pg (Greilhuber and Ebert 1994). In comparison, the data of Michaelson et al. (1991), which indicates that the *P. sativum* genome size is 76.9% of *H. vulgare*, appear somewhat distorted.

Genome size variation and systematics in *Pisum*

One result of our study is our observation that all accessions affiliated with *P. sativum* are practically constant in genome size. We do not claim that some marginal variation would never occur, but the strong variation reported in the literature is obviously wrong. Therefore, we consider that a discussion of the ecological significance of infraspecific genome size variation in terms of the nucleotype theory (Bennett 1985; Rayburn et al. 1989; Graham et al. 1994) is superfluous in the case of *P. sativum*. In contrast to genome size constancy in *P. sativum*, the accessions affiliated with *P. elatius* and *P. humile* were variable: in part they were congruent in genome size with *P. sativum*, and in part they were higher. The deviation was particularly strong in some *P. elatius*. A number of the *P. humile* and the *P. abyssinicum* and *P. fulvum* accessions of the present study were higher than *P. sativum* to a very similar degree. We think that genome size, which has not previously been considered as a trait in *Pisum* taxonomy, is an important new character that may be successfully used to identify biologically related groups. *Pisum abyssinicum*, some *P. humile*, and *P. fulvum* thus may be biologically more closely related with each other than they are with *P. sativum*, some *P. elatius* and part of *P. humile*. It remains to be shown whether these relationships correlate with translocations, and the role of hybridization and introgression needs to be investigated. As a working hypothesis, we consider the possibility that the constant genome size of typical *P. sativum* is an indicator of a past common gene pool and therefore indicates the conspecificity, as *P. sativum* sensu lato, of all the accessions so characterized. Vice versa, those accessions deviating in genome size appear to have had gene pools isolated from *P. sativum* sensu lato and may represent separate taxa.

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References

Anonymous (1994) Gemeinsamer Sortenkatalog für landwirtschaftliche Pflanzenarten. *Pisum sativum* L. Amtsbl Eur Gemeinschaft 18:323–346

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Baranyi M, Greilhuber J (1995) Flow cytometric analysis of genome size variation in cultivated and wild *Pisum sativum* L. (Fabaceae). Plant Syst Evol 194:231–239

Bennett MD (1985) Intraspecific variation in DNA amount and the nucleotypic dimension in plant genetics. In: Freeling M (ed) Plant Genetics (UCLA symposia on molecular and cellular biology, new series, vol 35). Alan R Liss, New York, pp 283–302

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

Bennett MD, Smith JB (1991) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London Ser B 334:309–345

Bennett MD, Smith JB, Heslop-Harrison JS (1982) Nuclear DNA amounts in angiosperms. Proc R Soc London Ser B 216:179–199

Ben-Ze'ev N, Zohary D (1973) Species relationship in the genus *Pisum* L. Isr J Bot 22:73–91

Cavallini A, Natali L (1990) Nuclear DNA variability within *Pisum sativum* (Leguminosae): cytophotometric analyses. Plant Syst Evol 173:179–185

Cavallini A, Cionini G, Gennai D (1993) Nuclear DNA variability within *Pisum sativum* (Leguminosae): nucleotypic effects on plant growth. Heredity 70:561–565

Conicella C, Errico A (1985) Identification of the chromosomes involved in translocations of *P. abyssinicum* and *P. fulvum*. In: Eucarpia Meet Pea Breed. Plant Breeding Center C.N.R., Portici, Italy, pp 86–101

Conicella C, Errico A (1990) Karyotype variations in *Pisum sativum* ect. *abyssinicum*. Caryologia 43:87–97

Davis PH (1970) *Pisum* L. In: Davis PH (ed) Flora of Turkey, vol 3. University Press, Edinburgh, pp 370–372

De Laat AMM, Göhde W, Vogelzang MJDC (1987) Determination of ploidy of single plants and plant populations by flow cytometry. Plant Breed 99:303–307

Doležel J (1991) Flow cytometric analysis of nuclear DNA content in higher plants. Phytochem Anal 2:143–154

Doležel J, Binarova P, Lucretti S (1989) Analysis of nuclear DNA content in plant cells by flow cytometry. Biol Plant 31:113–120

Doležel J, Sgorbati S, Lucretti S (1992) Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiol Plant 85:625–631

Errico A, Conicella C, Venora G (1991) Karyotype studies on *Pisum fulvum* and *Pisum sativum*, using a chromosome image analysis system. Genome 34:105–108

Galbraith DW, Harkins KR, Maddox JM, Ayres NA, Sharma DP, Firoozabady E (1983) A rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220:1049–1051

Graham MJ, Nickell CD, Rayburn AL (1994) Relationship between genome size and maturity group in soybean. Theor Appl Genet 88:429–432

Greilhuber J (1988) "Self-tanning" – a new and important source of stoichiometric error in cytophotometric determination of nuclear DNA content in plants. Plant Syst Evol 158:87–96

Greilhuber J, Ebert I (1994) Genome size variation in *Pisum sativum*. Genome 37:646–655

Guerra M dos S (1983) Variaçao no conteúdo de DNA nuclear de *Pisum sativum* L. Cienc Cult 35:1661–1663

Håkansson A (1936) Die Reduktionsteilung in einigen Artbastarden von *Pisum*. Hereditas 21:215–222

Lamprecht H (1964) Partielle Sterilität und Chromosomenstruktur bei *Pisum*. Agric Hortic Genet 22:56–148

Lamprecht H (1974) Monographie der Gattung *Pisum*. Steiermärkische Landesdruckerei, Graz.

Lehmann CO (1954) Das morphologische System der Saaterbsen (*Pisum sativum* L. sens. lat. GOV. ssp. *sativum*). Zuchter 24:316–337

Lehmann CO, Blixt S (1984) Artificial infraspecific classification in relation to phenotypic manifestation of certain genes in *Pisum*. Agric Hortic Genet 42:49–74

Le Pecq J-B, Paoletti C (1967) A fluorescent complex between ethidium bromide and nucleic acids. J Mol Biol 27:87–106

Michaelson MJ, Price HJ, Ellison JR, Johnston S (1991) Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. Am J Bot 78:183–188

Mukherjee S, Sharma AK (1986) Estimation of in situ DNA content in organs of different strains of *Pisum sativum* L. Nucleus 28:236–239

Rayburn AL, Auger JA, Benzinger EA, Hepburn AG (1989) Detection of intraspecific DNA content variation in *Zea mays* L. by flow cytometry. J Exp Bot 40:1179–1183

Rohlf FJ (1992) BIOM. A package of statistical programs to accompany the text "Biometry". Applied Biostatistics, New York

Rosen G von (1944) Artkreuzungen in der Gattung *Pisum*, insbesondere zwischen *P. sativum* L. und *P. abyssinicum* Braun. Hereditas 30:261–400

Saccardo F (1971) Crosses among *Pisum* species. Pisum Newsl 3:38

Schweizer D, Davies D (1972) Nuclear DNA contents of *Pisum* genotypes grown in vivo and in vitro. Planta 106:23–29

Sgorbati S, Levi M, Sparvoli E, Trezzi F, Lucchini G (1986) Cytometry and flow cytometry of 4',6-diamidino-2-phenylindole (DAPI)-stained suspensions of nuclei released from fresh and fixed tissues of plants. Physiol Plant 68:471–476

Smartt J (1984) Evolution of grain legumes. I. Mediterranean pulses. Exp Agric 20:275–296

Ulrich I, Ulrich W (1986) Flow cytometric DNA-analysis of plant protoplasts stained with DAPI. Z Naturforsch 41c:1052–1056

Ulrich I, Ulrich W (1991) High-resolution flow cytometry of nuclear DNA in higher plants. Protoplasma 165:212–215

Ulrich I, Fritz B, Ulrich W (1988) Application of DNA fluorochromes for flow cytometric DNA analysis of plant protoplasts. Plant Sci 55:151–158

Ulrich W (1992)) Simultaneous measurement of DAPI-sulforhodamine 101-stained nuclear DNA and protein in higher plants by flow cytometry. Biotechnic Histochem 67:73–78

Zohary D, Hopf M (1973) Domestication of pulses in the Old World. Science 182:887–894