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ORIGINAL ARTICLE

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Variability of chromosomal DNA contents in maize (*Zea mays* L.) inbred and hybrid lines

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Abstract The flow karyotypes of different maize (*Zea mays* L.) inbred and hybrid lines were analyzed. The accumulation and isolation of large quantities of high-quality metaphase chromosomes from root tips was achieved from many kinds of maize lines. The chromosome suspensions were prepared by a simple slicing method from synchronized maize root tips and analyzed by flow cytometry. Variations of experimental flow karyotypes were detected among inbred and hybrid lines in terms of the positions and/or the numbers of chromosome peaks. The 2C DNA amount among eight inbred lines ranged from 5.09 to 5.52 pg. The selection of appropriate maize lines is critical for sorting specific single chromosome types. At least five different chromosome types can be discriminated and sorted from five maize lines. The variability of DNA content in maize chromosome 1 was 9.1%, ranging from 0.685 to 0.747 pg. Differences were detected in the DNA content of homologous chromosome 1 of hybrid lines.

Keywords Chromosomal DNA content · Chromosome isolation · Flow cytometry · Flow karyotype · *Zea* (chromosomal DNA)

Abbreviations CV coefficient of variation · PI propidium iodide

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Introduction

Maize has 10 chromosomes ($n = 10$) with relatively large differences in size. The ten chromosomes are all morphologically distinguishable by their structural characteristics, such as relative length, centromere position, satellite, and numbers and positions of chromosomal knobs. Maize inbred lines have different sets of knobs with a total of 23 locations on 15 out of 20 arms (McClintock et al. 1981). The size and numbers of knobs vary in different maize inbred lines (Longley 1941). These morphological characters permit unquestionable identification of individual maize chromosomes. There are also many cytological stocks with a large number of chromosomal translocations useful for sorting of individual chromosome by flow cytometry.

Flow-cytometric analyses of isolated nuclei or chromosomes have been proven to be a rapid and reliable research tool in molecular cytogenetics. For example, flow cytometry has been used to determine plant ploidy levels and to detect aneuploidy (reviewed in Dolezel 1991; Bashir et al. 1993; Pfosser et al. 1995; Lee et al. 1998). Flow cytometry can be used for the classification of chromosomes based on DNA content and the identification of chromosomal variation within species, or the detection of abnormal chromosomes (Gary et al. 1988; Dolezel and Lucretti 1995; Lee et al. 1996, 2000; Gill et al. 1999; Lysak et al. 1999; Vrana et al. 2000). In addition, flow-sorted chromosomes have been used for the generation of chromosome-specific DNA libraries, for physical mapping of a specific chromosome, and for cloning of defined regions of a complex genome (Van Dilla and Deaven 1990; Wang et al. 1992; Macas et al. 1993; McCormick et al. 1993; Arumuganathan et al. 1994).

For the construction of chromosome-specific libraries, it is necessary that chromosome peaks containing only single chromosome types be separated from the other chromosome peaks. Among different maize lines, it is known that there is variation in genome size

and the positions and/or contents of chromosomal knobs (Longley 1941; McClintock et al. 1981). A change in chromosomal knob constitution might account for the variation in flow karyotype among lines. Therefore, it is necessary to find lines with as many single chromosome types in the flow karyotype as possible. However, the qualitative and quantitative isolation of plant chromosomes for flow cytometric analysis and chromosome sorting is difficult. Several reports of successful flow karyotyping in different plants have been published (De Laat and Blaas 1984; Conia et al. 1987; Arumuganathan et al. 1991; Lucretti et al. 1993; Gualberti et al. 1996; Lee et al. 1996, 1997; Lee and Arumuganathan 1999; Vrana et al. 2000; Svirshchevskaya and Dolezel 2001).

The purpose of this research was twofold: (i) to compare the flow karyotypes of maize lines, and the DNA amount of individual chromosomes among these lines; and (ii) to determine the number of single chromosome types that can be discriminated from other chromosomes for specific chromosome sorting, and to predict the experimental flow karyotypes of hybrid lines.

Materials and methods

Plant materials

Eight maize (*Zea mays* L.) inbred lines (A188, A619, B73, B79, KYS, N28, Oh43, and W23), and eight hybrid lines (A188/B73, B73/W23, B73/A619, A619/B73, A619/A188, A619/Oh43, A188/Oh43, and Oh43/A188) were used in this study. The pedigree and origin of the eight maize inbred lines are shown in Table 1. Seeds of the eight inbred lines and eight hybrid lines studied were obtained from the Agronomy farm of the University of Nebraska-Lincoln (from Dr. Kaeppler).

Estimation of 2C DNA content and nucleotide composition

Maize nuclei were isolated from four 7-day-old seedlings in nuclear isolation buffer (50 mM KCl, 10 mM $MgSO_4$, 5 mM Hepes, 3 mM dithiothreitol, 0.25% Triton X-100) by the chopping method as described by Arumuganathan and Earle (1991). After the isolated nuclear suspension was filtered through a 30-μm nylon mesh, the suspension was stained with one of the following three fluorochromes: for calculating total nuclear DNA, propidium iodide (PI) was used at a final concentration of 100 μg/ml (incubation 30 min); for measuring the percentage GC composition, mithramycin was used at a final concentration of 50 μg/ml (incubation 30 min); and for determining the percentage AT composition, bisbenzimide Hoechst 33258 was used at a final concentration of 5 μg/ml (incubation 10 min). The nuclei isolated from chicken red blood cells (CRBC) were used as internal standards (2C = 2.33 pg, 42.7% GC composition; Galbraith et al. 1983) for the determination of 2C nuclear DNA content and nucleotide composition. The base composition was estimated using Eqs. 1 and 2 developed by Godelle et al. (1993):

$$\%AT_{sample} = \%AT_{standard}(R_{HO}/R_{Eb})^{1/5} \quad (1)$$

$$\%GC_{sample} = \%GC_{standard}(R_{Mi}/R_{Eb})^{1/3} \quad (2)$$

where R_{Eb} = $intensity_{sample}/intensity_{standard}$ with an intercalating dye: R_{HO} with bisbenzimide Hoechst 33258, and R_{Mi} with mithramycin.

Each line was analyzed four times, and each nuclear-suspension sample was run twice (i.e. a total of eight replicates per line).

Cell cycle synchronization and metaphase chromosome isolation

Cell cycles were synchronized and metaphase chromosomes isolated as described previously (Lee et al. 1996) with minor modifications. Briefly, seedlings with radicles about 0.5 cm long were incubated in 10 ml Hoagland's solution (Sigma) containing 5 mM hydroxyurea for 18 h at 28 °C in the dark, washed three times with distilled water, incubated for 1 h in 10 ml Hoagland's solution, and treated 1 μM trifluralin (a gift from DowElanco, Indianapolis, Ind., USA) for 4 h. Metaphase chromosomes were prepared in chromosome isolation buffer (50 mM KCl, 10 mM $MgSO_4$, 5 mM K_2HPO_4, 5 mM Hepes, 2 mM dithiothreitol, 0.2% Triton X-100, 25 μg/ml PI) by chopping. Sixteen root tips were used to prepare one chromosome suspension sample.

Flow cytometry and flow analysis

The isolated nuclei or chromosomes stained with PI were analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry system, San Jose, Calif., USA), and the isolated nuclear suspensions stained with mithramycin or Hoechst were analyzed using a FACS vantage flow cytometer and sorter (Becton Dickinson Immunocytometry system) equipped with an Argon-ion laser. The laser was tuned to UV light (351–365 nm) at 100 mW for the analysis of Hoechst-stained samples, and the fluorescence was collected through a 424-nm long-pass filter. For analysis of mithramycin-stained samples, the laser was tuned to 457.9 nm at

Table 1. DNA content and base composition (%GC) of 2C nuclear DNA from eight different maize (*Zea mays*) inbred lines. The DNA content of chicken red blood cells (CRBC) was used as an internal standard (2C = 2.33 pg, 42.7% GC base composition). Propidium iodide (PI) was used for 2C DNA content, and bisbenzimide Hoechst 33342 and mithramycin for base composition. Four independent nuclear isolations were performed for each line. Analyses were repeated twice for four nuclear suspensions per line. Data are means ± SD

Maize line	Pedigree/origin	2C DNA content (pg)	Base composition (% GC)
A188	4-29 (Silver King)/46 (N.W.Dent)4^4	5.09 ± 0.10	45.16 ± 0.24
A619	(A171/Oh43)Oh43	5.25 ± 0.11	45.22 ± 0.21
B73	Iowa stiff stalk synthetic C5	5.16 ± 0.13	45.49 ± 0.18
B79	BS10 (Iowa 2-ear synthetic)	5.36 ± 0.15	45.74 ± 0.19
KYS	Yellow selection, pride of Saline	5.52 ± 0.13	45.78 ± 0.17
N28	Stiff stalk synthetic	5.20 ± 0.11	45.58 ± 0.15
Oh43	Oh40B/W8	5.29 ± 0.13	45.32 ± 0.16
W23	Golden Glow	5.23 ± 0.18	45.72 ± 0.19

100 mW and the fluorescence was collected through a 475-nm longpass filter. Approximately 2,000 G1 nuclei per line were analyzed. Analyses were repeated twice for each nuclear suspension. Fluorescence pulse area was measured in all cases. At least 2,000 particles were analyzed to generate each flow karyotype.

Numbers of expected chromosome types in each chromosome peak were determined as the proportion of total events of each chromosome peak in linear flow karyotypes. The DNA content of individual chromosome peaks was calculated based on the relative PI fluorescence intensity of individual chromosome peaks and G1 nuclei. We measured the 2C-DNA in G1 phase and 4C-DNA in metaphase chromosomes. Since even 2C DNA usually denotes the nuclear DNA content of the diploid nucleus in the G1 phase of the cell cycle, we used 2C DNA for comparison of the DNA content in interphase with the DNA content in metaphase chromosomes in Table 2. Each peak revealed in the flow karyotypes was sorted and the content of the peak was analyzed under the fluorescence microscope for identification of clumps, chromosomes, chromatids, or cell debris as described previously (Lee et al. 1996). The instrument settings were adjusted to place the peak of the largest chromosome (chromosome 1) of the B73 inbred line at channel 600. The same instrument settings were used for analyses of chromosomes isolated from other inbred lines.

Results and discussion

The 2C nuclear DNA contents and percentage GC base-pair composition of maize inbred lines are shown in Table 1. Four nuclear isolations were performed for each line. Analyses were repeated twice for four nuclear suspensions per line.

The variation in genome size was observed to be 8.5%, ranging from 5.09 to 5.52 pg/2C nucleus. Other researchers also found significant variation of genome size in maize (Laurie and Bennett 1985; Rayburn and Auger 1990; Bashir et al. 1994). These previous studies revealed about 26% variation in genome size, ranging from 4.7 to 5.9 pg. The authors suggested that the variation was caused by the number and size of heterochromatic knobs (McClintock et al. 1981; Laurie and Bennett 1985). Races of maize vary in knob numbers and positions, and so far a total of 23 knob locations have been identified on the 10 maize chromosomes. Rayburn et al. (1985) found a positive correlation between 2C nuclear DNA content and C-banded heterochromatic knob number. KYS, W23 and Oh43 inbred lines have five large heterochromatic knobs (references in Rayburn et al. 1989). These lines, which were used in our study, have a relatively large genome size compared with other inbred lines. The GC base-pair composition of the eight inbred lines studied did not show appreciable variability. The variation was 1.3%, ranging from 45.16 to 45.74% GC composition. The estimation of the AT base-pair content of the eight inbred lines studied showed a similar percentage of 100 minus % GC base-pair composition (100 minus % GC).

The 10 pairs of maize chromosomes ($n = 10$) can be classified into 5 groups based on the relative chromosome size data from Bennett and Laurie (1995), as shown in the theoretical flow karyotype of the maize line Seneca 60 constructed with a coefficient of variation (CV) of 3% (Fig. 1a). A maize theoretical flow karyotype was modeled as described in Lee et al. (1996). The theoretical model showed two peaks with single chromosome types (chromosomes 1 and 10) and three

Table 2. DNA content (mean ± SD) of chromosome 1 and individual chromosome peaks based on relative PI fluorescence intensity from different maize lines. It should be noted that total 2C DNA content based on relative PI fluorescence intensity in metaphase chromosomes may be less than that of interphase nuclei due to chromatin coiling and fluorochrome binding difference

Flow chr Peak[a]	Maize line													
	A188		A619		B73		B79		KYS		N28		Oh43		W23	
	DNA[b] (pg)	Chr No.[c]	DNA (pg)	Chr No.	DNA (pg)	Chr No.	DNA (pg)	Chr No.	DNA (pg)	Chr No.	DNA (pg)	Chr No.	DNA (pg)	Chr No.	DNA (pg)	Chr No.
1	0.701 ± 0.016	1	0.685 ± 0.018	1	0.698 ± 0.013	1	0.687 ± 0.019	1	0.747 ± 0.012	1	0.688 ± 0.013	1	0.687 ± 0.012	1	0.696 ± 0.015	1
2	0.552 ± 0.021	3	0.594 ± 0.011	1	0.543 ± 0.015	3	0.550 ± 0.017	4	0.607 ± 0.019	3	0.616 ± 0.015	2	0.626 ± 0.012	2	0.556 ± 0.016	3
3	0.475 ± 0.010	2	0.519 ± 0.017	3	0.470 ± 0.012	2	0.463 ± 0.011	1	0.521 ± 0.013	1	0.480 ± 0.025	3	0.542 ± 0.016	2	0.488 ± 0.011	2
4	0.388 ± 0.010	2	0.440 ± 0.011	2	0.388 ± 0.007	2	0.392 ± 0.014	3	0.443 ± 0.013	2	0.388 ± 0.011	2	0.467 ± 0.011	2	0.397 ± 0.010	2
5	0.340 ± 0.011	2	0.354 ± 0.008	3	0.346 ± 0.007	2	0.340 ± 0.008	1	0.377 ± 0.010	2	0.337 ± 0.009	2	0.400 ± 0.012	2	0.346 ± 0.008	2
6									0.324 ± 0.010	1			0.338 ± 0.007	1		
Total	4.763		4.778		4.735		4.866		5.053		4.810		5.095		4.826	

[a]Chromosome peaks of flow karyotypes are described by numerical designation. Chromosome peak 1 for all flow karyotypes is expected to be a chromosome 1

[b]2C DNA content of each chromosome peak. The mean value for DNA content of each chromosome peak was calculated as the average DNA content from the total number of chromosome particles in each chromosome peak. Approximately 2,000 particles were analyzed to generate each flow karyotype

[c]Numbers of expected chromosomes were calculated as the proportion of total events of each chromosome peak in linear flow karyotypes

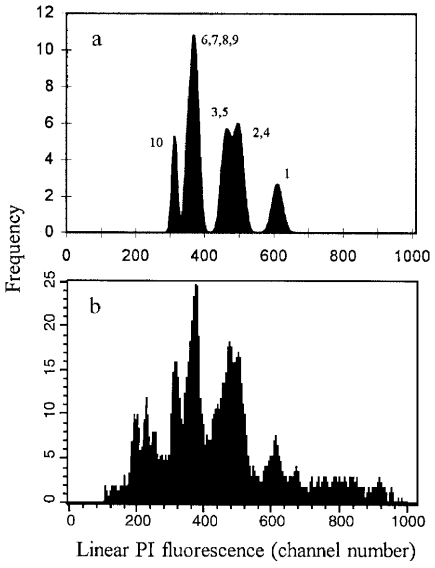

Fig. 1. Theoretical flow karyotype (**a**) and experimental flow karyotype (**b**) of the maize (*Zea mays*) line Seneca 60. The theoretical flow karyotype was calculated on the basis of relative chromosome size and CV = 3% (Bennett and Laurie 1995). Peaks corresponding to chromosomes 1 and 10, and three composite peaks corresponding to chromosomes 2, 4, chromosomes 3, 5, and chromosomes 6, 7, 8 and 9 are shown. The experimental flow karyotype (**b**) closely matches the theoretical flow karyotype (**a**)

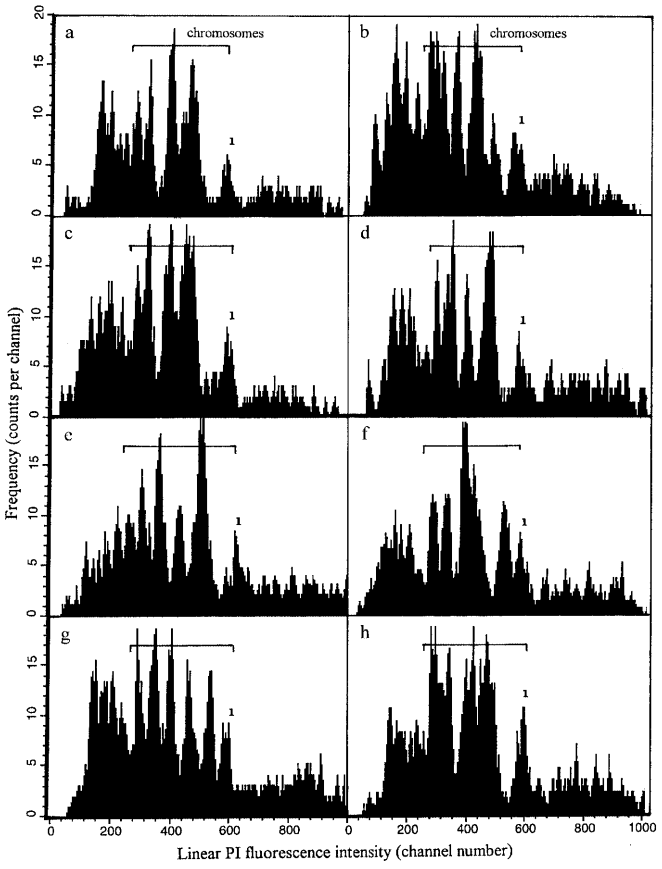

Fig. 2a–h. Variability of flow karyotypes in maize inbred lines. **a** A188, **b** A619, **c** B73, **d** B79, **e** KYS, **f** N28, **g** Oh43, **h** W23. The instrument settings were adjusted to channel 600 for the largest chromosome (chromosome 1) of the B73 inbred line. The same instrument settings were used for analyses of chromosomes isolated from other inbred lines. Chromosome 1 is indicated (*1*) for all flow karyotypes

composite chromosome peaks (chromosomes 2 and 4, chromosomes 3 and 5, and chromosomes 6, 7, 8 and 9). The experimental flow karyotype of Seneca 60 (Fig. 1b) closely matched the theoretical flow karyotype (Fig. 1a). However, the experimental flow karyotype might be different among maize lines since genome size and/or number of heterochromatic knobs vary among lines (McClintock et al. 1981; Laurie and Bennett 1985; Rayburn et al. 1985).

The flow karyotypes presented in Fig. 2 provide information on eight maize inbred lines regarding the chromosomal DNA content of each peak, the number of chromosome peaks, and different chromosomal types. The CVs of the individual chromosome peaks analyzed ranged from 1.7 to 3.8 %. Each peak revealed in the flow karyotypes was sorted and the content of the peak was analyzed under the fluorescence microscope for identification of clumps, chromosomes, chromatids, and cell debris. Five to six chromosome peaks resolved from each inbred line ranged from channel 279 for the smallest chromosome to channel 642 for the largest chromosome. The peak of the largest chromosome (chromosome 1) was located between channel 589 and channel 642.

The DNA content of individual chromosome peaks and the number of chromosome types in the chromosome peak are shown in Table 2. The DNA content of each chromosome ranged from 0.324 to 0.747 pg. In each inbred line, the difference in DNA content between the largest and the smallest chromosome peak was nearly 2-fold. The variation of DNA content in chromosome 1 was 9.1% among the eight inbred lines. The highest DNA content of chromosome 1 was 0.747 pg in KYS, while the lowest content was 0.685 pg in A619. At least five single chromosome types could be discriminated on flow karyotypes from four inbred lines. The total genomic DNA content added up from each chromosome peak ranged from 4.735 to 5.095 pg. These values were on average 7.5% lower than those of the 2C DNA content calculated from interphase nuclei. This discrepancy may result from differences in fluorochrome binding and/or chromatin coiling between interphase and metaphase chromosomes. The proportion of total events of each chromosome peak in linear flow karyotypes was calculated and this number was taken to be the expected chromosome number. Based on the 2C DNA content of each chromosome peak and numbers of expected chromosomes, the expected flow karyotypes can be generated.

Figure 3 shows flow karyotypes of eight maize hybrid lines. The homologous chromosome 1 of hybrid lines B73/A619 (Fig. 3c, d), A619/A188 (Fig. 3e), and Oh43/A188 (Fig. 3g, h) revealed different chromosome peaks. In Table 2, the DNA content of chromosome 1 of B73,

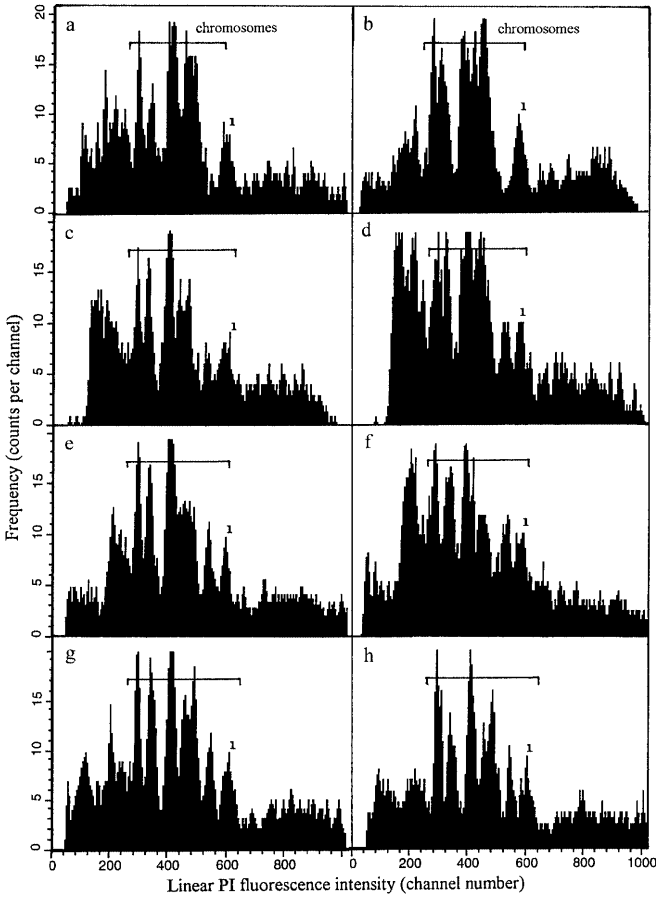

Fig. 3a–h. Flow karyotypes of maize hybrid lines. **a** A188/B73, **b** B73/W23, **c** B73/A619, **d** A619/B73, **e** A619/A188, **f** A619/Oh43, **g** A188/Oh43, **h** Oh43/A188. The instrument settings were the same as for Fig. 2. The same instrument settings were used for analyses of chromosomes isolated from other hybrid lines. Chromosome 1 is indicated (*1*) for all flow karyotypes. The flow karyotypes generated from reciprocal crosses were closely similar to each other: **c** vs. **d**, and **g** vs. **h**. Homologous chromosome 1 of hybrid lines (B73/A619, A619/A188, A619/Oh43, A188/Oh43) revealed different chromosome peaks

A619, A188, and Oh43 is 0.689, 0.685, 0.701, and 0.687, respectively. The differences in DNA content in chromosome 1 of those lines were 1.9%, 2.3%, and 2.0% respectively. The high variation of chromosome size/DNA content between homologous chromosomes might decrease the genetic variability among their progenies due to prevention of homologous pairing during meiosis. If so, the flow karyotypes can be utilized for selection of breeding parents. Hybrid lines derived from reciprocal crosses produced very similar flow karyotypes [Fig. 3c (B73/A619) vs. Fig. 3d (A619/B73); Fig. 3g (A188/Oh43) vs. Fig. 3h (Oh43/A188)].

Using a flow karyotype of the F_1 hybrid line, the chromosomal variations between two parental lines can be identified. Figure 4 shows theoretical flow karyotypes of the Oh43 (Fig. 4a), A188 (Fig. 4b) and Oh43/A188 (Fig. 4c) hybrid lines based on DNA content of individual chromosome peaks in Table 2. These theoretical flow karyotypes closely matched their experimental flow

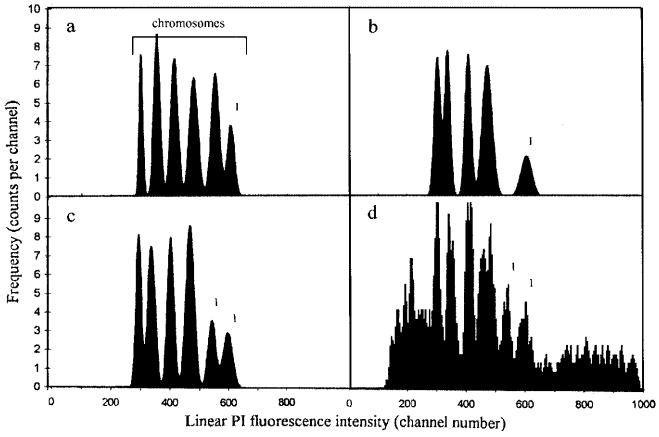

Fig. 4. Theoretical flow karyotypes of maize lines Oh43 (**a**), A188 (**b**) and Oh43/A188 (**c**), and experimental flow karyotype of Oh43/A188 (**d**). Theoretical flow karyotypes were generated based on the DNA content of individual chromosome peaks in Table 2. These theoretical flow karyotypes closely matched their experimental flow karyotypes in Fig. 2a, g, and Fig. 3h or Fig. 4d. This correspondence validates the numbers of expected chromosomes calculated as the proportion of total events of each chromosome peak in linear flow karyotypes in Table 2

karyotypes in Fig. 2a, g, and Fig. 3h or Fig. 4d. This correspondence validates the numbers of expected chromosomes calculated as the proportion of total events of each chromosome peak in linear flow karyotypes in Table 2. This result also validates the homologous chromosome 1 peaks of hybrid lines B73/A619 (Fig. 3c, d), A619/A188 (Fig. 3e) and Oh43/A188 (Fig. 3g, h), which were separated in flow karyotypes.

In conclusion, high-quality metaphase chromosomes from maize root tips can be accumulated in large quantities and isolated from many kinds of maize lines. Knowledge of the experimental flow karyotype of maize lines is useful for sorting specific chromosomes. For example, all maize lines are suitable sources for sorting chromosome 1; B79 and KYS inbred lines are good for sorting at least three single chromosome types; the Oh43 inbred line is a better choice for sorting chromosomes 1 and 10. The variability of DNA content between homologous chromosomes can be useful information for the study of maize genome analysis, genetic variation, or hybrid vigor among their offspring. Variations in flow karyotyping suggested that the fingerprint patterns of the flow karyotype might be used for pedigree analysis, or chromosomal analysis.

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References

Arumuganathan K, Earle ED (1991) Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol Biol Rep 9:229–233

Arumuganathan K, Slattery JP, Tanksley SD, Earle ED (1991) Preparation and flow cytometric analysis of metaphase chromosomes of tomato. Theor Appl Genet 82:101–111

Arumuganathan K, Martin GB, Telenius H, Tanksley SD, Earle ED (1994) Chromosome 2-specific DNA clones from flow-sorted chromosomes of tomato. Mol Gen Genet 242:551–558

Bashir A, Auger JA, Rayburn AL (1993) Flow cytometric DNA analysis of wheat-rye addition lines. Cytometry 14:843–847

Bashir A, Bullock DG, Rayburn AL (1994) Nuclear DNA amount, growth, and yield parameters in maize. Theor Appl Genet 88:557–560

Bennett MD, Laurie DA (1995) Chromosome size in maize and sorghum using EM serial section reconstructed nuclei. Maydica 40:199–204

Conia J, Bergounioux C, Perennes C, Muller P, Brown S, Gadal P (1987) Flow cytometric analysis and sorting of plant chromosomes from *Petunia hybrida* protoplasts. Cytometry 8:500–508

De Laat AMM, Blaas J (1984) Flow-cytometric characterization and sorting of plant chromosomes. Theor Appl Genet 67:463–467

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

Dolezel J, Lucretti S (1995) High-resolution flow karyotyping and chromosome sorting in *Vicia faba* lines with standard and reconstructed karyotypes. Theor Appl Genet 90:797–802

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

Godelle B, Cartier D, Marie D, Brown SC, Siljak-Yakovlev S (1993) Heterochromatin study demonstrating the non-linearity of fluorometry useful for calculation genomic base-composition. Cytometry 14:618–626

Gary JW, Trask B, Van den Engh G, Silva A, Lozes C, Grell S, Schonberg S, Yu L, Golbus MS (1988) Application of flow karyotyping in prenatal detection of chromosome aberrations. Am J Hum Genet 42:49–59

Gill KS, Arumuganathan K, Lee J-H (1999) Isolating individual wheat (*Triticum aestivum*) chromosome arms by flow cytometric analysis of ditelosomic lines. Theor Appl Genet 98:1248–1252

Gualberti G, Dolezel J, Macas J, Lucretti S (1996) Preparation of pea (*Pisum sativum* L.) chromosome and nucleus suspensions from single root tips. Theor Appl Genet 92:744–751

Laurie DA, Bennett MD (1985) Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation. Heredity 55:307–313

Lee J-H, Arumuganathan K (1999) Metaphase chromosome accumulation and flow karyotypes in rice (*Oryza sativa* L.) root tip meristem cells. Mol Cells 9:436–439

Lee J-H, Arumuganathan K, Kaeppler SM, Kaeppler HF, Papa CM (1996) Cell synchronization and isolation of metaphase chromosomes from maize (*Zea mays* L.) root tips for flow-cytometric analysis and sorting. Genome 39:697–703

Lee J-H, Yen Y, Kaeppler SM, Baenziger PS, Arumuganathan K (1997) Synchronization of cell cycle in root-tips and flow karyotype of metaphase chromosomes of common wheat (*Triticum aestivum* L.). Genome 40:633–638

Lee J-H, Yen Y, Arumuganathan K, Baenziger PS (1998) The absolute DNA content of wheat chromosomes at interphase measured by laser-based flow cytometry. Theor Appl Genet 95:1300–1304

Lee J-H, Arumuganathan K, Chung Y-S, Kim K-Y, Chung W-B, Bea K-S, Kim D-H, Chung D-S, Kwon D-C (2000) Flow-cytometric analysis and chromosome sorting of barley (*Hordeum vulgare* L.). Mol Cells 10:619–625

Longley AE (1941) Chromosome morphology in maize and its relatives. Bot Rev 7:262–289

Lucretti S, Dolezel J, Schubert I, Fuchs J (1993) Flow karyotyping and sorting of *Vicia faba* chromosomes. Theor Appl Genet 85:665–672

Lysak MA, Cihalikova J, Kubalakova M, Simkova H, Kunzel G, Dolezel J (1999) Flow karyotyping and sorting of mitotic chromosomes of barley (*Hordeum vulgare* L.) Chromosome Res 7:431–444

Macas J, Dolezel J, Lucretti S, Pich U, Meister A, Fuchs J, Schubert I (1993) Localization of seed storage protein genes on flow-sorted field bean chromosomes. Chromosome Res 1:107–115

McClintock B, Kato-Y TA, Blumenschein A (1981) Chromosome constitution of races of maize. Colegio de postgraduados, Chapingo, Mexico

McCormick M, Campbell E, Deaven L, Moyzis R (1993) Low frequency chimeric yeast artificial chromosome libraries from flow-sorted chromosomes 16 and 21. Proc Natl Acad Sci USA 90:1063–1067

Pfosser M, Amon A, Lelley T, Heberle-Bors E (1995) Evaluation of sensitivity of flow cytometry in detecting aneuploidy in wheat using disomic and ditelosomic wheat-rye addition lines. Cytometry 21:387–393

Rayburn AL, Auger JA (1990) Genome size variation in *Zea mays* ssp. *mays* adapted to different altitudes. Theor Appl Genet 79:470–474

Rayburn AL, Price HJ, Smith JD, Gold JR (1985) C-band heterochromatin and DNA content in *Zea mays*. Am J Bot 72:1610–1617

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 40:1179–1184

Svirshchevskaya AM, Dolezel J (2001) Karyological characterization of sugar beet gynogenetic lines cultured in vitro. J Appl Genet 42: 21–32

Van Dilla MA, Deaven LL (1990) Construction of gene libraries for each chromosome. Cytometry 11:208–218

Vrana J, Kubalakova M, Simkova H, Cihalikova J, Lysak MA, Dolezel J (2000) Flow-sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). Genetics 156:2003–2041

Wang ML, Leitch AR, Schwarzacher T, Heslop-Harrison JS, Moore G (1992) Construction of a chromosome-enriched HpaII library from flow-sorted wheat chromosomes. Nucleic Acids Res 20:1897–1901