Original Article

Cytogenet Cell Genet 93:175–181 (2001)

Cytogenetics and Cell Genetics

Alteration of chromosome numbers by generation of minichromosomes – Is there a lower limit of chromosome size for stable segregation?

I. Schubert

Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben (Germany)

Abstract. Practical applications of minichromosomes, generated by de novo composition or by truncation of natural chromosomes, rely on stable transmission of these chromosomes. Functional centromeres, telomeres and replication origins are recognized as prerequisites for minichromosome stability. However, it is not yet clear whether, and if yes, to what degree the chromatin content has a qualitative or quantitative impact on stable chromosome transmission. A small translocation chromosome, which arose after X-irradiation of a reconstructed field bean karyotype, comprised ~ 5% of the haploid metaphase complement and was found to consist of three pieces of duplicated chromatin and a wild-type centromere. This chromosome was stably transmitted through all meristematic and pollen grain mitoses but was frequently lost during meiosis (66% loss in hemizygous and 33% in homozygous condition). This minichromosome was only a little smaller than stably segregating translocation chromosomes (comprising ~ 6% of the genome) of a euploid field bean karyotype. The duplications specific for this minichromosome did not influence meiotic segregation when associated with non-duplicated chromatin of other chromosomes. In comparison with minichromosomes of other species, the possibility of a lower size limit for a stable chromosome transmission must therefore be considered which might be based, for instance, on insufficient lateral support of centromeres or on insufficient bivalent stability due to the incapability of chiasma formation.

Artificial chromosomes of eukaryotes are exciting tools for many scientific and biotechnological purposes. Such extrachromosomal linear structures are available for yeasts, are being established for mammals but have not yet been constructed for plants. Also for plants, artificial chromosomes (PLACs) would provide useful vehicles for transfer of genetic information into target organisms which, once established, could be manipulated by conventional crossing without (or only rare) recombination with the host genome. Compared to other methods of gene transfer, the advantage of artificial chromosomes is their high carrying capacity. They enable the transfer of complex traits and allow to study stability, function and modification of large chromatin domains as separately segregating entities in homologous and heterologous systems. Theoretically, it is even possible to design artificial chromosomes in a way suitable for controlled self-destruction (e.g., by inducible restriction endonucleases).

There are two main strategies for construction of artificial chromosomes: (i) systematic fragmentation of natural chromosomes and (ii) de novo composition including centromeres and telomeres (Choo, 1997). Disadvantages of de novo composition of PLACs are the limited knowledge about functional DNA sequences of plant centromeres and the limited amount of DNA which can be cloned in bacteria or yeasts. Therefore, it is essential to address the question as to the minimal size for stable mitotic/meiotic transfer of (artificial) chromosomes.

Received 2 April 2001; manuscript accepted 20 April 2001.

Request reprints from Ingo Schubert, Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstrasse 3, D–06466 Gatersleben (Germany); telephone: +49 (0) 39482 5239; fax: +49 (0) 39482 5137; e-mail: schubert@ipk-gatersleben.de

KARGER

Fax + 41 61 306 12 34
E-mail karger@karger.ch
www.karger.com

0301–0171/01/0934–0175$17.50/0

Accessible online at:
www.karger.com/journals/ccg

Table 1. Possible size limits for stable transmission of artificial chromosomes may depend on genomic DNA content

Species	Minichromosome size	Mitosis	Meiosis	DNA content (1C)	References
Yeast	≥150 kb	100%	100%	10 Mb	Murray et al., 1986
	<55 kb[a]	<100%	<100%		Murray and Szostack, 1983
Drosophila	1.3 Mb	100 %	~50 %	150 Mb	Williams et al., 1998
	0.2 Mb[b]	~ 87 %	0.3-14%		
Mouse	~60 Mb[c]	~99 %		3.300 Mb	Telenius et al., 1999
	~30 Mb[d]		<1-33%		Tomizuka et al., 1997
	4.5 Mb[e]	>99% in vitro	<20%		Shen et al., 1997, 2000
		30-80% in vivo			
Human	~ 50 Mb	100 %[f]		3.500 Mb	Mills et al., 1999
	~ 8 Mb	95 %[f]			
	~ 2.4 Mb	88-92 %[g]			
	~ 5 Mb	~99 %[h]			Ikeno et al., 1998
					Harrington et al., 1997
					Heller et al., 1996
	>10 Mb	99.8 %[i]			Kuroiwa et al., 2000
	5-20 Mb	>90%	32-38%[j]		Shinohara et al., 2000
					Tomizuka et al., 2000
	5-10 Mb	>99%	31-35%[k]		Voet et al., 2001
Field bean	≤ 700 Mb	100 %	17.0%[l]	13.500 Mb	this paper
			66.7%[m]		

[a] Linear centric plasmid.
[b] Neocentric fragment.
[c] sat-DNA-based artificial chromosome.
[d] Human Y-derived artificial chromosome.
[e] Human artificial chromosome with mouse sat-DNA.
[f] In hamster cells.
[g] In chicken DT 40 cells.
[h] In human HT 1080 or CHO cells.
[i] In chicken DT40, mouse ES cells and chimeric mouse.
[j] Up to F3 in mice.
[k] F1 in mice.
[l] Hemizygous.
[m] Homozygous.

Previously we have shown the occurrence of and the reasons for an upper size limit for stable chromosome transmission (Schubert and Oud, 1997; Schubert et al., 1998).

There are (also) indications for a (possibly species-specific) lower size limit for stable chromosome transfer, particularly during meiosis (Table 1).

In yeast (1 C ≅ 10 Mb), minichromosomes of 150 kb show normal stability, while centric linear plasmids below 55 kb are clearly less stable. In Drosophila (1 C ≅ 150 Mb), minichromosomes of 1.3 Mb segregate normally, while neocentric fragments of 0.2 Mb are partially lost during mitosis and are poorly transmitted meiotically. Mammalian mini- or artificial chromosomes of 4 to 60 Mb are fairly transmitted during mitosis in homologous and heterologous systems. Transmission via germ cells has so far been reported in a few cases: a human minichromosome (~ 30 Mb) was transmitted through female mice (33%), while transmission via males was below 1% (Tomizuka et al., 1997); a human minichromosome (~ 4.5 Mb) with mouse satellite sequences through three generations with a transmission rate between 0 and 20% (Shen et al., 2000, 2001) and other human minichromosomes of 5–20 Mb with a transmission rate between 31% and 38% up to F3 in mice (Shinohara et al., 2000; Tomizuka et al., 2000; Voet et al., 2001) (Table 1).

Based on these data it is tempting to speculate that for stable mitotic transmission artificial chromosomes should contain ≥1% of the host's genome size and clearly more for perfect meiotic stability. In order to study the possible effect of chromosome size reduction on transmission frequency in a higher plant with a large genome, we used field bean lines with reconstructed karyotypes.

Using an established procedure for experimental alteration of diploid chromosome numbers in the field bean *Vicia faba* (2n = 12), karyotypes with 2n = 14 chromosomes were generated (Schubert and Rieger, 1985). This procedure is based upon individuals heterozygous for two translocations both involving the same metacentric but different acrocentric chromosomes with breakpoints close to the centromeres. In such individuals, hexavalents are formed during meiosis. Usually, the parental karyotypes segregate into the gametes but in some cases missegregation occurs (the two translocated metacentrics of the hexavalent migrate to one and the four corresponding acrocentrics to the other pole). The hyperploid gametes (n + 1) contain small duplications comprising centromeric/short arm regions while the hypoploid ones (n – 1) suffer from deletions of the same regions (and proved to be inviable in the cases tested so far for *V. faba*). Fusion of hyperploid gametes yields progenies with a homozygously increased diploid chromosome number (2n + 2 = 14).

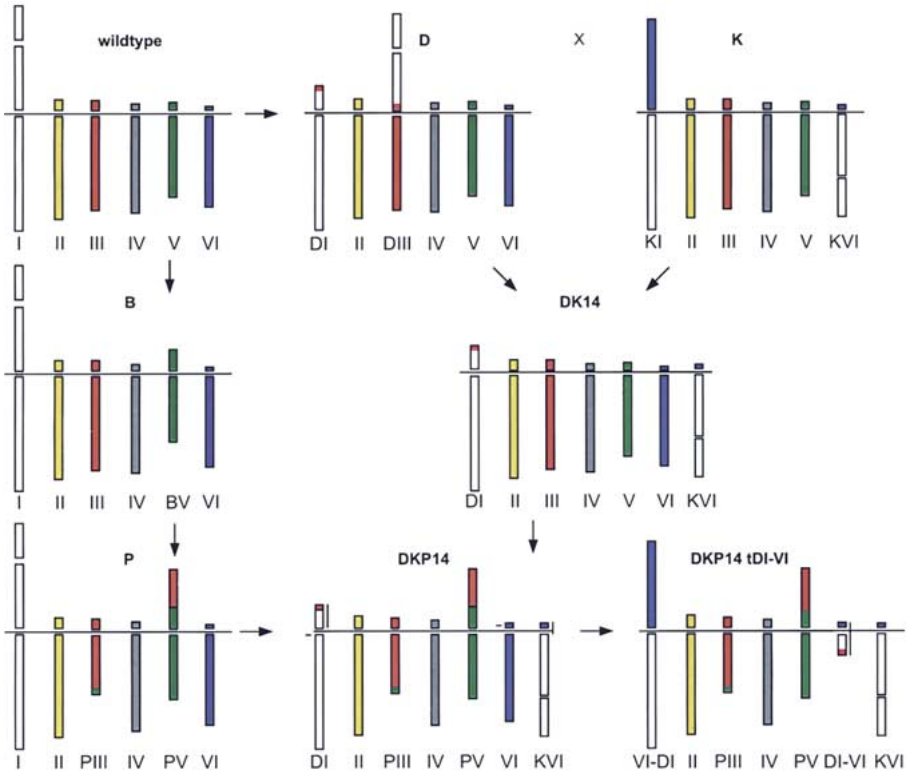


Fig. 1. Scheme of the wild-type chromosome complement of *Vicia faba* and its derivatives resulting via combination of rearrangements in the karyotype DKP tDI-VI. The duplicated regions of the karyotypes with seven chromosomes are marked by vertical bars and the breakpoints of translocation DI-VI by horizontal bars within the karyogram of DKP14; each chromosome of the wild-type is distinguishable by different color.

Presoaked seeds of one of these karyotypes (DKP14, see Figs. 1 and 3) with 14 chromosomes and therefore with small duplicated regions have been X-irradiated. Among the progeny of the irradiated individuals, plants were found with a translocation resulting in a large metacentric and in a small chromosome (~ 5% of the metaphase genome, Figs. 1 and 3). The small translocation chromosome was perfectly stable during mitosis but was frequently absent in progenies reducing their chromosome number from 14 to 13 or 12. To study the possible reasons for that loss, the chromatin composition of the small translocation chromosome as well as meiosis and male gametophytic mitoses of the new translocation karyotype have been studied.

Materials and methods

For description of the reconstructed field bean karyotypes (EF, DKP 14 and its derivatives) used in this study see Results section. Letters specify the chromosome reconstructions (reciprocal translocations or inversions), Roman numbers (I–VI) the wild-type chromosomes and S or L the short and long arms; t stands for translocation and Arabic numbers refer to the chromosome number of the respective karyotype. Translocation chromosomes are designated by the letter for the corresponding translocation and the Roman numbers for the chromosomes involved with that providing the centromere in first position.

Chromosome preparations from root tip meristems, pollen mother cells and pollen grains were made by gentle squashing after HCl-hydrolysis and Feulgen-staining or after acetocarmine-staining (meiosis) according to routine procedures. Giemsa banding followed the schedule of Schwarzacher et al. (1980). FISH was done according to Fuchs and Schubert (1995); for probe

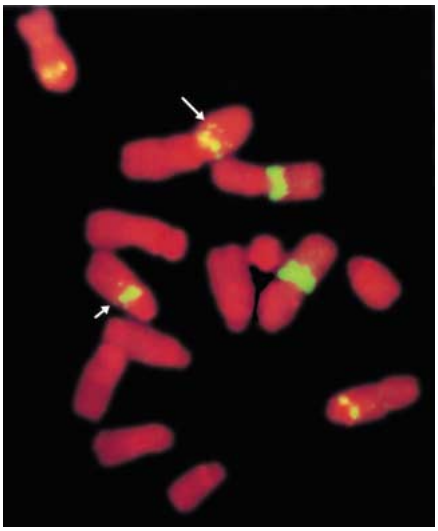


Fig. 2. Complete metaphase cell of the heterozygous karyotype DKP/DKP tDI-VI with all 14 chromosomes individually recognizable after FISH with a digoxigenin-labeled probe of the 321-bp tandem repeat pVf7 (Fuchs et al., 1998); a single FITC-signal occurred on chromosome pairs PV and KVI and a double signal on the acrocentric chromosome VI and on the metacentric translocation chromosome VI-DI. The weaker signal on chromosome VI (short arrow) and on the shorter arm of chromosome VI-DI (long arrow) confirm the translocation between chromosomes DI and VI (compare Fig. 1). Magnification: 3,000×.

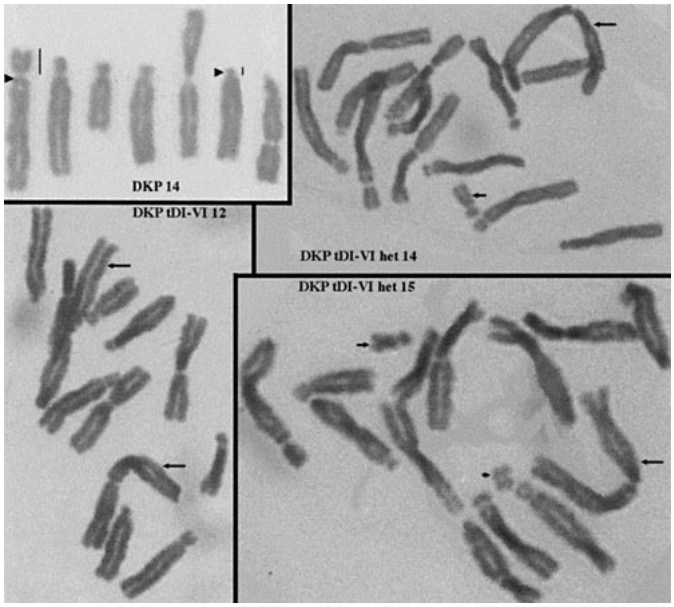
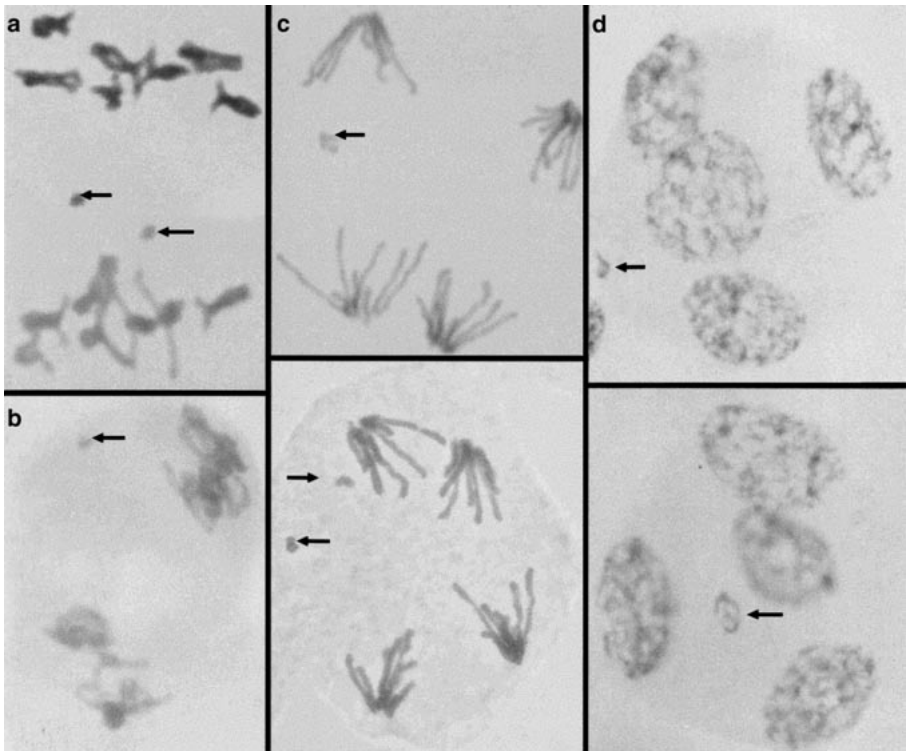
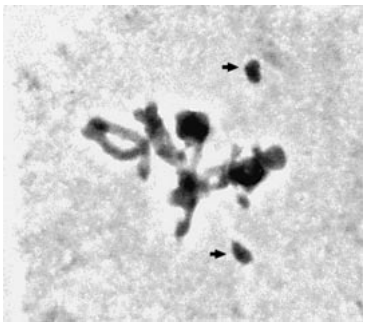


Fig. 3. Ideogram of karyotype DKP 14 and complete metaphases of its derivatives DKP/DKP t DI-VI 14 (heterozygous), DKP/DKP t DI-VI 15 (heterozygous) containing an additional small translocation chromosome DI-VI with a partially deleted arm, and DKP t DI-VI 12 (homozygous) with both small translocation chromosomes lost (Feulgen-staining); duplicated regions (bars) and translocation breakpoints (arrow heads) are marked in DKP14; chromosomes VI-DI, DI-VI and its deleted variant are marked by long, short and very short arrows, respectively. Magnification: 3,000×.



4



5

Fig. 4. Acetocarmine-stained meiotic stages of the karyotype DKP t DI-VI with 13 chromosomes (due to the loss of one of the small translocation chromosomes DI-VI); (**a**) two metaphase I cells, (**b**) prometaphase II, (**c**) two anaphase II cells, (**d**) two telophase II cells. Arrows mark univalents and the micronuclei resulting from exclusion of the small translocation chromosome DI-VI. Magnification: 2,000×.

Fig. 5. Acetocarmine-stained metaphase I of the homozygous karyotype DKP tDI-VI with 14 chromosomes; showing both chromosomes DI-VI as univalents (arrows) apart from the equatorial plane. Magnification: 1,500×.

description see legend to Fig. 2. Measurements of propidium iodide-stained root tip nuclei of karyotypes DKP tDI-VI14, DKP tDI-VI12 and wild type using a FACStar^{Plus} flow cytometer (Becton Dickinson) yielded DNA contents of 26.972, 25.793 and 25.978 pg/2C, respectively. This showed that DKP tDI-VI14 nuclei contain ~ 4.6% more DNA than DKP tDI-VI12 or wild type nuclei ($P < 0.05$), confirming the size estimation of ~ 5% for the small translocation chromosome DI-VI which was based on length measurement of the metaphase chromosome complements.

Results

The karyotype DKP14 and the translocation tDIL-VIS

The karyotype DKP14 arose by several consecutive events. At first, a fusion of two n+1 gametes, resulting from meiotic mis-segregation within an individual heterozygous for the translocations D (t IS-IIIS) and K (t IS-VIL), resulted in karyo-

type DK14. Into this karyotype the translocation P (between the short arm of the pericentrically inverted chromosome BV and the long arm of chromosome III) was introduced (Fig. 1). Due to the combination of chromosomes DI and VI of karyotype D and KVI and III of karyotype K, the karyotypes DK14 and DKP14 contain duplications comprising the short arm of chromosome DI (composed of the distal half of the short arm of wild-type chromosome III and the proximal region of the satellite arm of wild-type chromosome I) and the centromere and the short arm of wild-type chromosome VI. The new X-ray-induced translocation (t DIL-VIS) resulted in a large metacentric (VI-DI) and a small chromosome (DI-VI). The latter contains all regions duplicated within karyotype DKP14 (Fig. 1). This became evident from chromosome morphology, Giemsa banding (not shown) and FISH with the repetitive element pVf7 (Fig. 2). The size and banding pattern of the shorter arm of the large metacentric translocation chromosome precisely correspond to that of the long arm of wild-type chromosome VI which is characterized by four Giemsa marker bands (Fuchs et al., 1998). It also contains the two loci of the tandem repeat pVf7 typical only for the long arm of chromosome VI (Fuchs et al., 1998). The longer arm of the small translocation chromosome represents the short arm of chromosome DI while its short arm is somewhat longer than that of wild-type chromosome VI. Therefore, one breakpoint must have occurred within the long arm of chromosome DI between the centromere and the proximally adjacent heterochromatin band and the other within the tiny short arm of wild-type chromosome VI. The individual heterozygous for the karyotypes DKP14 and DKP14 tDI-VI (DKP14 tDI-VI het) should produce two types of gametes with the corresponding karyotypes, respectively, which should equally contribute to the progeny. In fact, among the 52 gametes contributing to a progeny of 26 individuals, 15 (28.8%) are of karyotype DKP14, and 8 (15.4%) of karyotype DKP14 tDI-VI. The remaining 29 gametes were derived from DKP14 tDI-VI by loss of the small translocation chromosome DI-VI. Only one individual was homozygous for karyotype DKP14 tDI-VI. Altogether, nine gametes (17.3%) transferred the small translocation chromosome DI-VI (in one case as additional chromosome to karyotype DKP14). Individuals heterozygous for karyotypes DKP14 and DKP14 tDI-VI without the small translocation chromosome have 13 chromosomes per cell. The progeny of such individuals also showed a deviation from Mendelian expectation with a bias in favour of the karyotype with six chromosomes, i.e., without the small chromosome which carries the segments duplicated in DKP14 (gamete ratio: 25:17 instead of 21:21).

From these data it is inferred that gametes containing small duplications (~ 5% of the genome) are at a disadvantage as compared to euploid ones. The ideograms of karyotype DKP14, the heterozygous translocation karyotype DKP tDI-VI14 and its derivatives DKP tDI-VI12 and DKP tDI-VI15 are shown in Fig. 3. The chromatin content of karyotype DKP tDI-VI with only 12 chromosomes corresponds to that of the wild type. The centromere of chromosome VI is present twice, due to the substitution of centromere I within the new large metacentric translocation chromosome.

Behavior of small chromosomes during meiosis and pollen grain mitosis: translocation chromosome DI-VI

Meiosis and pollen grain mitoses were studied within an individual with 13 chromosomes, homozygous for the translocation karyotype DKP tDI-VI but lacking one of the homologues of the small translocation chromosome (DI-VI). Anthers at various developmental stages were acetocarmine-stained. Pollen mother cells in metaphase I revealed six bivalents properly arranged in the equatorial plane and a dot-like univalent which in 82.5% of 103 cells was clearly apart (Fig. 4a). During prometaphase II the univalent often remains in separate position (Fig. 4b). In anaphase II the small chromosome was found to be left in the equatorial plane or separated from the spindle area in 36% of 111 cells (Fig. 4c). In 18.2% of 44 early telophase II cells the decondensing chromatids of the small chromosome were located outside the four daughter nuclei and 13.5% of 104 tetrads showed one or two micronuclei (Fig. 4d). In ana- and telophases of the first pollen grain mitosis, lagging of small chromosomes was no longer observable. The microspores had either already lost chromosome DI-VI or, if still present, it showed normal segregation. These data show that the small translocation chromosome in monosomic condition becomes only randomly incorporated into the nuclei of microspores and is frequently lost during meiosis. Among the progeny of plants with the karyotype constitution DKP tDI-VI 13 which had only 13 chromosomes due to the loss of one of the small translocation chromosomes DI-VI, three individuals were homozygous for karyotype DKP tDI-VI 14. From these plants six seeds were harvested. Four of these were hemizygous for the small translocation chromosome, i.e., the transmission rate from homozygous parents is only ~ 66%. Investigation of meiotic cells of an individual with a homozygous karyotype DKP tDI-VI 14 revealed that in 40% of metaphase I cells chromosomes DI-VI were excluded from the equatorial plane and/or formed no bivalent (Fig. 5). These chromosomes segregate randomly into the daughter nuclei or get lost.

Translocation chromosome FIII

For comparison, meiotic stages were investigated in individuals homozygous for karyotype EF (Schubert and Rieger, 1991) with two translocations (E: tIVL-VL and F: tIIS-IIIL) which contain a pair of small translocation chromosomes (FIII) only slightly larger (~ 6% of metaphase genome length) than translocation chromosome DI-VI. In the case of karyotype EF, the six bivalents including the one formed by the small translocation chromosome FIII showed normal meiotic behavior and proper chromosome segregation during meiosis and pollen grain mitosis in all inspected pollen mother cells and microspores. Also individuals of karyotype DKP14 revealed normal formation of (seven) bivalents and chromosome segregation.

Seed setting in plants of karyotypes DKP14, DKP12, and EF was compared to that of the wild type. For EF, DKP14, DKP12 and wild-type plants (21, 20, 21 and 26 individuals, respectively) the average number of pods/seeds per plant was: 19.6/56.8 (wild type), 12.5/30.3 (EF), 14.3/18.5 (DKP14) and 5.4/8.7 (DKP tDI-VI12).

Discussion

The data obtained from translocation karyotype DKP14 tDI-VI show that (i) reduction of chromosome number may occur by loss of chromosomes consisting exclusively of duplicated/dispensable chromatin and (ii) in the described case chromosome loss is due to improper meiotic segregation of the small chromosome even under homozygous condition and although it contains a normal centromere (that of wild-type chromosome I).

Theoretically, it cannot be excluded that the presence of duplicated chromatin is "recognized" and becomes actively eliminated. Such elimination is not possible when duplicated chromatin is associated with non-duplicated chromosome regions as in chromosomes of DKP 14 and other field bean karyotypes with 2n = 14 chromosomes. The reduced transmission of these karyotypes from individuals heterozygous with a euploid chromosome complement is explainable by a slower migration of generative pollen nuclei containing the duplications as compared to strictly euploid ones. However, interpreting the low transmission rate of chromosome DI-VI as a consequence of active elimination bears the difficulty to explain how chromatin duplication can cause meiotic malfunction of an apparently normal centromere and why larger univalents of polysomic chromosomes are often transferred with a higher frequency, for instance in cereals (Tsuchiya, 1960, 1967).

Except for the cases listed in Table 1, there are reports on meiotic loss of small chromosomes. A very small translocation chromosome 7^{15} of the mouse (<1% of the haploid metaphase complement, Schriever-Schwemmer and Adler, 1993) and a short arm telocentric of a rye B chromosome (with a longitudinal extension not surpassing the width of a chromatid, J. Sybenga, personal communication) were not transferred to the next generation, while their centromeres functioned properly during mitotic divisions. Although it cannot be excluded that the centromere of the rye B telocentric had lost by fission components essential for proper functioning during meiosis, it might be possible that its size simply became too small for meiotic transmission.

Up to now, lacking or impaired meiotic transmission concerned minichromosomes in monosomic condition. Thus, it might be possible that the reduced transfer is a consequence of their incapability to form bivalents during meiosis I. The higher transmission frequency of some polysomic chromosomes of cereals in univalent condition could possibly depend on the chromosome involved and/or the genetic background.

Frequent loss (~ 33%) of the small chromosome DI-VI even occurs in homozygous condition. There is no obvious reason why its centromere should function improperly during meiosis. Either the chromatin on either side of the centromere is specifically incapable of forming chiasmata for bivalent stabilization or there is indeed a general requirement of a certain (species-specific) amount of chromatin to form stable bivalents that can properly be separated during meiosis I. Shen et al. (2001) describe variable mitotic stability of minichromosomes (1.7 to 6.7 Mb in size) in different host species (and tissues) depending on correct centromere formation rather than on minichromosome size. Nevertheless, the reported mitotic transmission rate decreased during culture without selection, even in cells where transmission frequency is high. Transmission of these minichromosomes through meiosis was generally below that of normal chromosomes (Shen et al., 2000; Tomizuka et al., 1997; Shinohara et al., 2000; Voet et al., 2001). So far, we are not aware of perfect meiotic transfer of chromosomes with a size of ~ 1% of the host genome. Birds having minichromosomes as regular components of their genomes might represent an exception with a particular adaptation for transfer of naturally occurring minichromosomes.

Therefore, it might be possible that impaired meiotic transmission of chromosome DI-VI, even in homozygous condition, is caused by its "too small" size, and that the translocation chromosome FIII, which is only little larger (~ 6% of metaphase genome length), is demarcating the lower size limit for stable transmission. To exclude the possibility that the failure of chiasma formation is a specific feature of the chromosome arms of chromosome DI-VI and the poor meiotic transfer is not only due to the small chromosome size, the generation of other minichromosomes of comparable size within the field bean karyotype is required.

Another reason (except impaired chiasma formation) for a lower size limit below which stable chromosome transmission, particularly during meiosis, is no longer possible could be the requirement of a "minimum lateral support" for proper centromere function, which has to be provided by a certain (species-specific) amount of chromatin flanking a centromere. Phosphorylation of histone H3 at serine position 10 occurs at active centromeres and the surrounding pericentromeric regions during mitosis and meiosis II and along the entire chromosomes, including univalents, during meiosis I in plants (Houben et al., 1999; Manzanero et al., 2001), while single chromatids resulting from equationally divided univalents show no phosphorylation during meiosis II and form laggards (Manzanero et al., 2001). Therefore it might be possible that a certain amount of chromatin around centromeres available for H3 phosphorylation is required as a lateral support for correct segregation.

Investigations of more minichromosomes in diverse species are needed to decide whether indeed a species-specific lower tolerance limit of chromosome size or the specific chromatin content is responsible for impaired minichromosome transmission.

Acknowledgements

The excellent technical assistance of Rita Schubert and Martina Kühne is gratefully acknowledged. I thank Martin Barow for flow cytometric DNA measurement, Merten Menke for help with artwork, Jaap Sybenga and Fritz Matzk for helpful discussion and Rigomar Rieger, Gottfried Künzel and Holger Puchta for critical reading of the manuscript.

References

Choo KHA: The Centromere (Oxford University Press, Oxford 1997).

Fuchs J, Schubert I: Localization of seed protein genes on metaphase chromosomes of *Vicia faba* via fluorescence *in situ* hybridization. Chrom Res 3:94–100 (1995).

Fuchs J, Strehl S, Brandes A, Schweizer D, Schubert I: Molecular-cytogenetic characterization of the *Vicia faba* genome – heterochromatin differentiation, replication patterns and sequence localization. Chrom Res 6:219–230 (1998).

Harrington JJ, Van Bokkelen G, Mays RW, Gustashaw K, Willard HF: Formation of *de novo* centromeres and construction of first-generation human artificial microchromosomes. Nature Genet 15:345–355 (1997).

Heller R, Brown KE, Burgtorf C, Brown WRA: Mini-chromosomes derived from the human Y chromosome by telomere-directed chromosome breakage. Proc natl Acad Sci, USA 93:7125–7130 (1996).

Houben A, Wako T, Furushima-Shimogawara R, Presting G, Künzel G, Schubert I, Fukui K: The cell cycle dependent phosphorylation of histone H3 is correlated with the condensation of plant mitotic chromosomes. Plant J 18:675–679 (1999).

Ikeno M, Grimes B, Okazaki T, Nakano M, Saitoh K, Hoshino H, McGill NI, Cooke H, Masumoto H: Construction of YAC-based mammalian artificial chromosomes. Nature Biotechnol 16:431–439 (1998).

Kuroiwa Y, Tomizuka K, Shinohara T, Kazuki Y, Yoshida H, Ohguma A, Yamamoto T, Tanaka S, Oshimura M, Ishida I: Manipulation of human minichromosomes to carry greater than megabase-sized chromosome inserts. Nature Biotechnol 18:1086–1090 (2000).

Manzanero S, Arana P, Puertas MJ, Houben A: The chromosomal distribution of phosphorylated histone H3 differs between plants and animals at meiosis. Chromosoma 109:308–317 (2000).

Mills W, Critcher R, Lee C, Farr CJ: Generation of a 2.4 Mb human X centromere-based minichromosome by targeted telomere-associated chromosome fragmentation in DT40. Human Mol Genet 8:751–761 (1999).

Murray AW, Szostak JW: Construction of artificial chromosomes in yeast. Nature 305:189–193 (1983).

Murray AW, Schultes NP, Szostak JW: Chromosome length controls mitotic chromosome segregation in yeast. Cell 45:529–536 (1986).

Schriever-Schwemmer G, Adler I-D: A mouse stock with 38 chromosomes derived from the reciprocal translocation t(7;15)33Ad. Cytogenet Cell Genet 64:122–127 (1993).

Schubert I, Oud JL: There is an upper limit of chromosome size for normal development of an organism. Cell 88:515–520 (1997).

Schubert I, Oud OJL, Pich U: Unscheduled apoptosis in meristematic plant cells is triggered via terminal deletions in artificially elongated chromosome arms. Theor Appl Genet 96:1022–1026 (1998).

Schubert I, Rieger R: A new mechanism for altering chromosome number during karyotype evolution. Theor Appl Genet 70:213–221 (1985).

Schubert I, Rieger R: Catalogue of chromosomal and morphological mutants of faba bean in the Gatersleben Collection. Faba Bean Inform Serv Newslet 28/29:14–22 (1991)

Schwarzacher T, Ambros P, Schweizer D: Application of Giemsa banding to orchid karyotype analysis. Plant Syst Evol 134:293–297 (1980).

Shen MH, Mee PJ, Nichols J, Yang J, Brook F, Gardner RL, Smith AG, Brown WRA: A structurally defined mini-chromosome vector for the mouse germ line. Curr Biol 10:31–34 (2000).

Shen MH, Yang J, Loupart M-L, Smith A, Brown W: Human mini-chromosomes in mouse embryonal stem cells. Human molec Genet 6:1375–1382 (1997).

Shen MH, Yang JW, Yang J, Pendon C, Brown WRA: The accuracy of segregation of human mini-chromosomes varies in different vertebrate cell lines, correlates with the extent of centromere formation and provides evidence for a *trans*-acting centromere maintenance activity. Chromosoma 109: 524–535 (2001).

Shinohara T, Tomizuka K, Takehara S, Yamauchi K, Katoh M, Ohguma A, Ishida I, Oshimura M: Stability of transferred human chromosome fragments in cultured cells and in mice. Chrom Res 8:713–725 (2000).

Telenius H, Szeles A, Keresö J, Csonka E, Praznovskzy T, Imreh S, Maxwell A, Perez CF, Drayer JI, Hadlaczky G: Stability of a functional murine satellite DNA-based artificial chromosome across mammalian species. Chrom Res 7:3–7 (1999).

Tomizuka K, Shinohara T, Yoshida H, Uejima H, Ohguma A, Tanaka S, Sato K, Oshimura M, Ishida I: Double trans-chromosomic mice: maintenance of two individual human chromosome fragments containing Ig heavy and [kappa] loci and expression of fully human antibodies. Proc natl Acad Sci, USA 97:722–727 (2000).

Tomizuka K, Yoshida H, Uejima H, Kugoh H, Sato K, Ohguma A, Hayasaka M, Hanaoka K, Oshimura M, Ishida I: Functional expression and germline transmission of a human chromosome fragment in chimaeric mice. Nature Genet 16:133–143 (1997).

Tsuchiya T: Cytogenetic studies in trisomics in barley. Jpn J Bot 17:177–213 (1960).

Tsuchiya T: The establishment of a trisomic series in a two-rowed cultivated variety of barley. Can J Genet Cytol 9:667–682 (1967).

Voet T, Vermeesch J, Carens A, Dürr J, Labaere C, Duhamel H, David G, Marynen P: Efficient male and female germline transmission of a human chromosomal vector in mice. Genome Res 11: 124–136 (2001).

Williams BC, Murphy TD, Goldberg ML, Karpen GH: Neocentromere activity of structurally acentric mini-chromosomes in *Drosophila*. Nature Genet 18:30–37 (1998).