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On the toleration of duplications and deletions by the *Vicia faba* genome

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Summary. From eight pairs of crosses between differently reconstructed diploid karyotypes of *Vicia faba,* the progeny after selfing of plants heterozygous for both parental chromosome reconstructions were inspected for occurrence and transmission of duplications and deletions of defined chromosome segments, comprising together about one third of the metaphase genome length. The duplications and deletions studied involved either one or more chromosome segments of the respective karyotype (0.8%–9.1% of the metaphase length). They arose during meiosis in double heterozygotes by crossing over between partially homologous chromosomes or by mis-segregation from multivalents. While most duplications, provided they were not accompanied by deletions and in dependence on the segment involved, were viable and transmissible, even in homozygous state, deletions had lethal effects on gametes of both sexes.

Key words: *Vicia faba* – Duplication – Deletion – Interchange heterozygotes – Pseudoaneuploidy

Introduction

Plasticity of a diploid genome as inferred from its capability to tolerate duplications or deletions is of theoretical interest and importance for stable inheritance of gene dosage effects. In *Vicia faba* ($2n = 12$; 2C DNA content = 29 pg), toleration and rate of transmission of chromosome structural rearrangements is quite high. Aneuploidy is tolerated and transmitted poorly, and polyploidy is survived only as autotetraploidy; other ploidy variants, inclusive allopolyploidy (until now no interspecific cross resulted in viable progeny, Ramsey and Pickersgill 1986), seem to be lethal (for review see Schubert et al. 1982, 1986).

In this paper, we describe the capability of the *Vicia faba* genome to tolerate duplications and corresponding deletions in hetero- or homozygous condition. The duplicated or deleted regions comprise 0.8%–9.1% of the metaphase genome length and consist of 1–3 independent chromosome segments (Table 3). A total of about 38% of the genome were duplicated or deleted segments and tested with respect to their effects on the viability of the plants affected. Individuals homozygous for two different reciprocal translocations with one chromosome involved in both were crossed. The self-progeny of double heterozygotes was inspected for the presence of duplications or deletions. These originated via crossing over between partially homologous chromosomes or via mis-segregation from multivalents (Schubert and Rieger 1985). While most of the duplications (provided they were not accompanied by deletions) proved to be viable and could even be maintained in a homozygous state, the deletions occured neither in the hetero- nor in the homozygous state.

Materials and methods

The reconstructed karyotypes of *V. faba* used for crossing are described in Table 1 with respect to the chromosomes involved in reconstructions and location of original breakpoints. For description of karyotypes, the chromosome nomenclature of Michaelis and Rieger (1959) and, for localization of breakpoints, an arbitrary subdivision of the standard karyotype into 28 segments (Döbel et al. 1973; Fig. 1) were used. Letters denote single primary reconstructions. Lines above or below the letters symbolize different versions of secondary karyotype reconstructions, which occur via crossing over between chromosomes involved in the corresponding primary karyotype recon-

Table 1. Description of reconstructed karyotypes of *V. faba*, which were used for crossing with the aim to produce duplications and deletions, respectively

Karyotype	Type of reconstruction	Chromosomes involved	Segmental position of original breakpoints
A	translocation	satellite arm chromosome I	4
		long arm chromosome III	14
C	translocation	long arm chromosome I	8
		long arm chromosome VI	27
D	translocation	satellite arm chromosome I	4
		short arm chromosome III	13
E	translocation	long arm chromosome IV	20
		long arm chromosome V	23
F	translocation	short arm chromosome II	9
		long arm chromosome III	14
G	translocation	satellite arm chromosome I	3
		long arm chromosome II	11
J	translocation	satellite arm chromosome I	4
		short arm chromosome V	21
K	translocation	satellite arm chromosome I	4
		long arm chromosome VI	26
T140	translocation	satellite arm chromosome I	3
		long arm chromosome IV	18
$\overline{FD}$	secondary reconstruction via crossing over between	chromosome F III	13–14
		chromosome D III	13–14
DK14	compensating tertiary trisomy obtained by crossing of karyotypes D and K, see Table 3, case 3		

structions (Fig. 6). Karyotype analyses were performed using permanent slides obtained from main roots of seedlings after Feulgen staining or Giemsa banding according to Schwarzacher et al. (1980). Localization of breakpoints that resulted, via consecutive rearrangements, in primary reconstructions was based on Giemsa banding and determination of the relative arm length of metaphase chromosomes. The estimation of size of duplicated or deleted regions (Table 2) rests on former measurements of Feulgen-stained metaphase chromosomes (Michaelis and Rieger 1971).

Vicia faba shows an average of about 40% of self-pollination. Growing of genetically differing plants in the same field will therefore allow "free cross-pollination" by insects.

One of the karyotypes (DK14) with duplications arose from 'cross-pollination' experiments, continued for 2–3 years. In seven cases, intentional crosses were performed and the self-progeny of the double heterozygotes was examined as to the presence of karyotypes with duplications and deletions, respectively.

One duplication occurred spontaneously in a heterozygous state. It represents a tandem duplication of the secondary constriction and the satellite (segments 1p and 2) in chromosome A*III* of the otherwise homozygous, multiple reconstructed karyotype ACB (as described in Döbel et al. 1973).

The karyotypes involved in crosses are characterized by reciprocal translocations (A, C, D, E, F, G, J, T140). In one case, plants with a secondarily reconstructed karyotype (FD) were crossed with pseudoaneuploid individuals (karyotype DK14) characterized by seven pairs of acrocentric chromosomes.

Two routes of origination of duplications or deletions are possible: (1) Crossing over between partially homologous chromosomes forming meiotic multivalents in doubly heterozygous individuals, for example, in KC het karyotype (Fig. 6); and (2) Mis-segregation from multivalents resulting in hyper- ($n=7$ acrocentrics) and hypoploid ($n=2$ metacentrics + 3 acrocentrics) gametes, respectively (Schubert and Rieger 1985; Fig. 2). The latter route is possible for heterokaryotypes JK het, DK het, AJ het, GJ het, JT140 het and $\overline{FD}$DK14 het; only in karyotype JE het, mis-segregation led to euploid gametes (Fig. 5).

Results

Table 3 shows the chromosome segments for which occurrence of duplications or deletions was tested in the self-progeny from eight crosses between plants with differently reconstructed karyotypes.

Case 1

A self-progeny (694 seeds) obtained from doubly heterozygous JK het plants was analyzed karyotypically. 248 individuals showed duplication of segments 25 and 21q in heterozygous and 24 in homozygous (karyotype JK14) condition. Such duplications result from mis-segregation of seven acrocentric chromosomes from the multivalent formed at meiosis of the JK heterokaryotype (Fig. 2). The corresponding deletions, which occur in complemen-

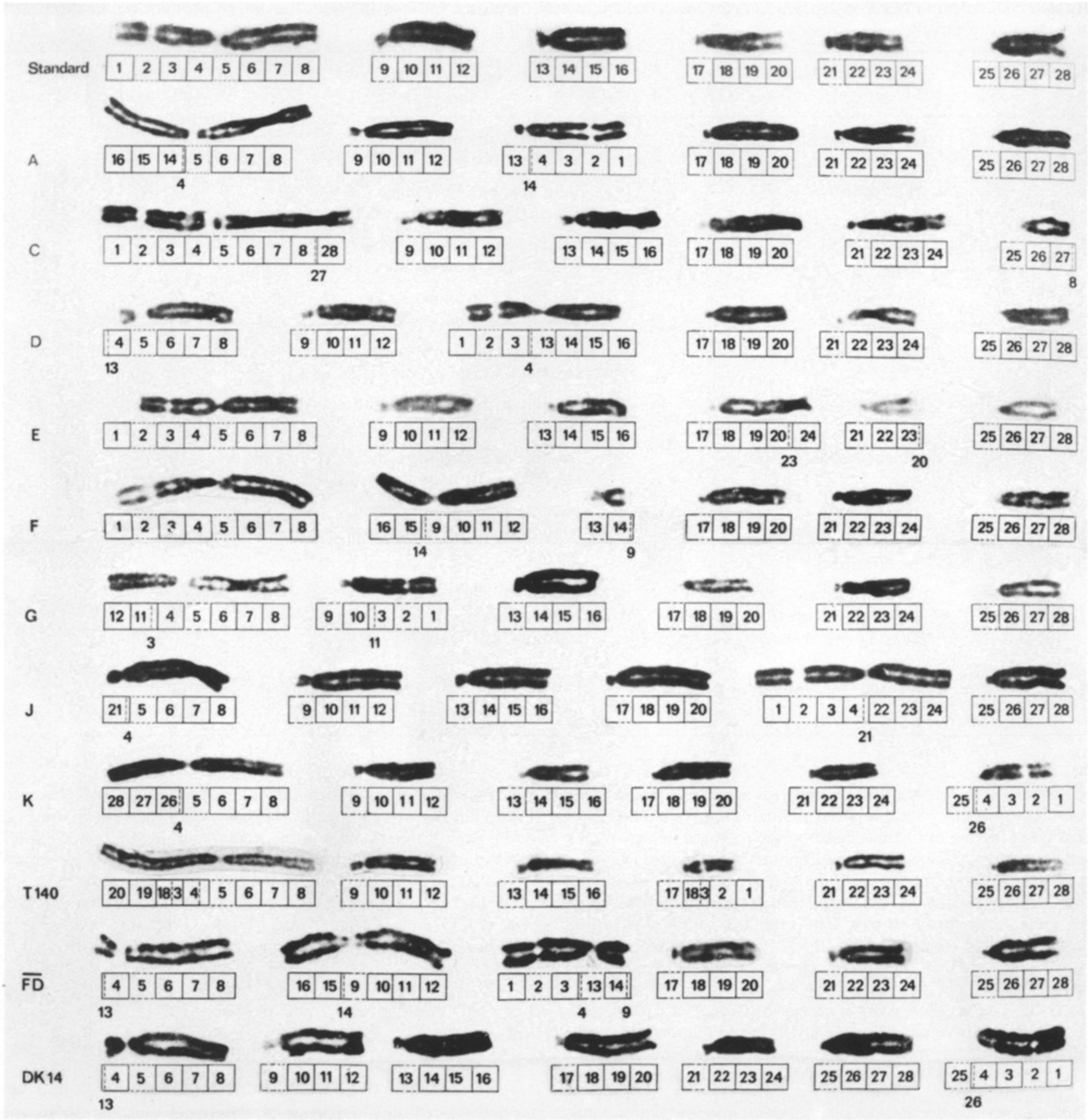

Fig. 1. Haploid chromosome complements of the standard karyotype and those reconstructed karyotypes of *Vicia faba* used for production of duplications and deletions and their subdivision into 28 segments. The sequence of segments denotes the original breakpoints that led to reconstruction events

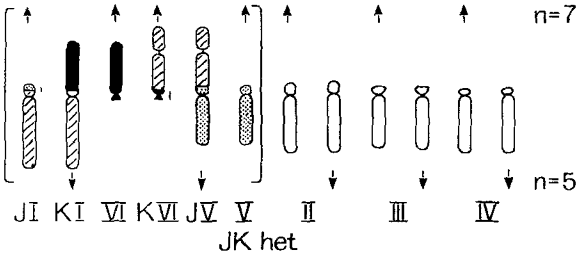

Fig. 2. Heterokaryotype JK het schematically. *Empty*: standard chromosomes *II, III, IV*; *hatched*: parts of the original chromosome *I*; *black*: parts of the original chromosome *VI*; *dotted*: parts of the original chromosome *V*; *in brackets*: multivalent consisting of chromosomes J*I*, J*V*, K*I*, K*VI*, *V*, *VI*. *Arrows* indicate mis-segregation into hyperploid ($n=7$) and hypoploid ($n=5$) gametes, respectively. Consequently, segments 25 and 21q, labelled by bars, are duplicated in hyper- and deleted in hypoploid gametes

tary hypoploid gametes containing two metacentric and three acrocentric chromosomes, were not observed neither in hetero- nor in homozygous condition. Apparently hypoploid gametes did not contribute to offspring production.

Table 2. Chromosome segments found to be involved or not involved in duplications and deletions and their relative metaphase length

Segments involved	%	Segments not involved	%	Chromosome
		1q	0.5	I
1p	5.0	3q	0.7	
2		5	16.6	
3p	3.9	6		
4	4.6	7		
(4p	2.5)	8		
9	2.0			II
(9q	1.0)			
10	4.4	11q	2.1	
11p	2.3	12	4.4	
13	1.4	14q	9.3	III
(13p	0.7)	15		
14p	3.6	16		
17	0.7	18q	11.8	IV
18p	1.5	19		
		20		
21q	0.4	21p	7.9	V
24	4.0	22		
		23		
25	0.4	26	8.7	VI
28	3.8	27		
	38.0		62.0	

Case 2

The no. of karyotyped individuals obtained by self-pollination of plants with the doubly heterozygous karyotype AJ het was 858. Duplication of segments 13 and 21q was shown by 294 plants in heterozygous and by 33 in homozygous (karyotype AJ14) condition. The corresponding deletions obviously could be transmitted via hypoploid gametes.

Case 3

Among the progeny from "free cross-pollination" experiments involving plants with translocation karyotypes D and K, plants with karyotype DK14 ($n=7$ acrocentrics and duplication of segments 4p, 13q, and 25) were observed in either hetero- or homozygous state. Again, no corresponding deletions were observed.

Case 4

After selfing of plants heterozygous for translocations G and J, 1604 offspring individuals were karyotyped. No pseudoaneuploid karyotypes with 10, 11, 13, or 14 chromosomes were found. This means that neither hypoploid gametes with $n=5$ (deletion of segments 9, 10, 11p, 21q and duplication of segment 4) nor hyperploid gametes with $n=7$ (duplication of segments 9, 10, 11p, 21q and deletion of segment 4; Fig. 3) participated in progeny production from GJ het.

Case 5

Comparable to case 4, among 776 seeds from self-pollinated plants of heterokaryotype JT140 none were pseudoaneuploid. Therefore, neither duplication of segments 17, 18p, 21q and deletion of segments 3p, 4 (origi-

Table 3. Occurrence of duplications or deletions in certain genome positions among the progeny after selfing of different doubly heterozygous karyotypes

Chromosomes involved in reconstructions	Original hetero-karyotype	Progeny scored	Deletion	Duplication	Segments concerned	% of total metaphase genome length
I, V, VI	JK	694	–	+	25, 21q	0.8
I, III, V	AJ	858	–	+	13, 21q	1.8
I, III, VI	DK		–	+	4p, 13q, 25	3.6
I, II, V	GJ	1,604	–		4	4.6
			–		9, 10, 11p, 21q	9.1
I, IV, V	JT140	776	–		3p, 4	8.5
			–		17, 18p, 21q	2.6
I, II, III, VI	F̅D̅DK14	114		+	9q, 13, 14	5.0
			–		9q, 13p, 14p	5.3
I, IV, V	JE	1,223	–	+[a]	24	4.0
I, VI	KC	1,502	–	+[a]	28	3.8
AIII[b]				+[a]	1p, 2	5.0

[a] In heterozygous state only; for detailed description see text

[b] Observed as spontaneous heterozygous tandem duplication in an individual with karyotype ACB

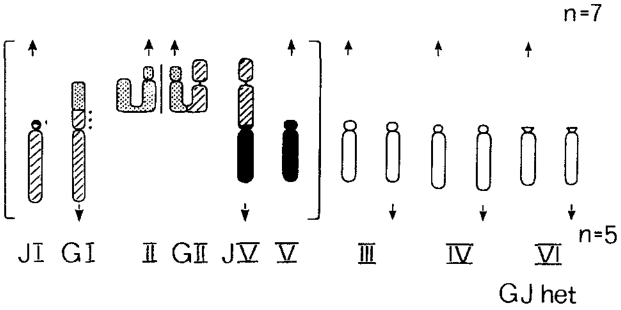

Fig. 3. Heterokaryotype GJ het schematically. *Empty*: standard chromosomes *III, IV, VI*; *hatched*: parts of original chromosome *I*, *black*: parts of original chromosome *V*; *dotted*: parts of original chromosome *II*; *in brackets*: multivalent consisting of chromosomes J *I*, J *V*, G *I*, G *II*, *II*, *VI*. *Arrows* indicate mis-segregation into hyperploid ($n=7$) and hypoploid ($n=5$) gametes, respectively. Segments 21q, 9, 10, 11p, marked by *bars*, are duplicated in hyper- and deleted in hypoploid gametes and segment 4, marked by a *dotted line*, is duplicated in hypo- and deleted in hyperploid gametes

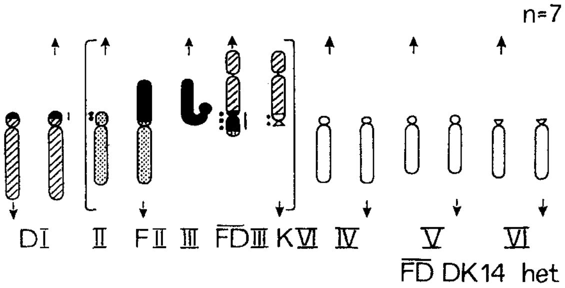

Fig. 4. Heterokaryotype $\overline{\mathrm{FD}}$DK14 het schematically. *Empty*: standard chromosomes *IV, V, VI*; *hatched*: parts of original chromosome *I*; *black*: parts of original chromosome *III*; *dotted*: parts of original chromosome *II*; *in brackets*: multivalent consisting of chromosomes *II*, *III*, K *VI*, F *II* and $\overline{\mathrm{FD}}$ *III*, the latter representing the result of secondary karyotype reconstruction by crossing over between chromosomes F *III* and D *III* during meiosis of karyotype FD het. *Arrows* indicate mis-segregation into a hyperploid ($n=7$) gamete with duplication of segments 9q, 13q, 13p, 14p (*bars*) and the reciprocal segregation product with 12 chromosomes, duplication of segment 25, and deletion of segments 9q, 13p, 14p (labelled by *dotted lines*)

nating from hyperploid gametes) nor the opposite situation (originating from hypoploid gametes) occurred.

Case 6

From plants heterozygous for the secondarily reconstructed karyotype $\overline{\mathrm{FD}}$ and the pseudoaneuploid karyotype DK14 (contributing seven acrocentric chromosomes, see case 3), 114 individuals of the self-progeny were checked for the presence of duplications or deletions. Two seedlings showed karyotype $\overline{\mathrm{FD}}$14 with homozygous duplication of segments 19q, 13, 14p, but no karyotype with 12 chromosomes (2 × {D*I*, F*II*, *IV*, *V*, *VI*, K *VI*}), containing duplication of segment 25 and deletion of segments 9q, 13p, 14p (see Fig. 4) was found.

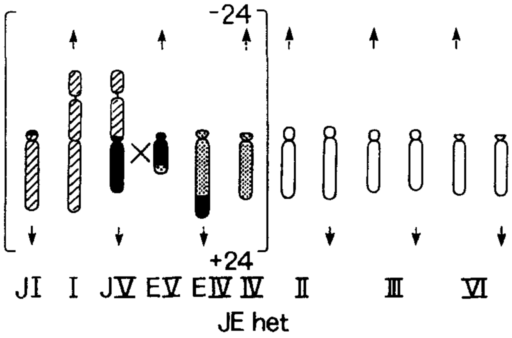

Fig. 5. Heterokaryotype JE het schematically. *Empty*: standard chromosomes *II, III, VI*; *hatched*: parts of original chromosome *I*; *black*: parts of original chromosome *V*; *dotted*: parts of original chromosome *IV*; *in brackets*: multivalent consisting of chromosomes J *I*, *I*, J *V*, E *V*, E *IV*, *IV*. *Broken arrows* indicate mis-segregation of chromosomes E *IV* and *IV* leading to duplication (*below*) or deletion (*above*) of segment 24. *X* marks the region of potential crossing over between chromosomes J *V* and E *V*, which in addition may result in the secondary reconstructed chromosome $\overline{\mathrm{JE}}$ *V* (not shown)

Case 7

A total of 1223 seeds from self-pollinated plants heterozygous for translocations J and E were karyotyped. Due to mis-segregation from the hexavalent formed by meiotic pairing of chromosomes J*I*, *I*, J*V*, E*V*, E*IV*, *IV*, ten plants contained chromosomes E*IV*, instead of *IV*, together with chromosomes *V* or J*V*, i.e., were heterozygous for duplication of segment 24 (Fig. 5). This situation was observed in karyotypes J hom (5 ×), J het (2 ×), $\overline{\mathrm{JE}}$ het, E het and standard (1 × each). The opposite variant (segregation of standard chromosome *IV* together with E*V* or $\overline{\mathrm{JE}}$*V*) resulting in deletion of segment 24 was not observed. Two of the ten plants with an additional segment 24 were fertile and produced 64 seeds after self-pollination. Remarkably, the duplication of segment 24 was not transmitted at all.

Case 8

A total of 1502 seeds from self-pollinated plants heterozygous for translocations K and C were analysed cytologically. Due to crossing over in the quadrivalent formed by meiotic pairing of chromosomes K*I*, C*I*, K*VI*, C*VI* between either the short arm of K*I* and the long arm of C*VI* or the long arm of K*I* and the long arm of C*I*, either deletion ($\overline{\mathrm{KC}}$*I*) or duplication ($\underline{\mathrm{KC}}$*I*) of segment 28 was produced by secondary reconstruction of former chromosome K*I* (Fig. 6). In six heterozygous individuals, chromosome $\overline{\mathrm{KC}}$*I* with deletion of segment 28 was found. For all these plants, however, aneuploidy com-

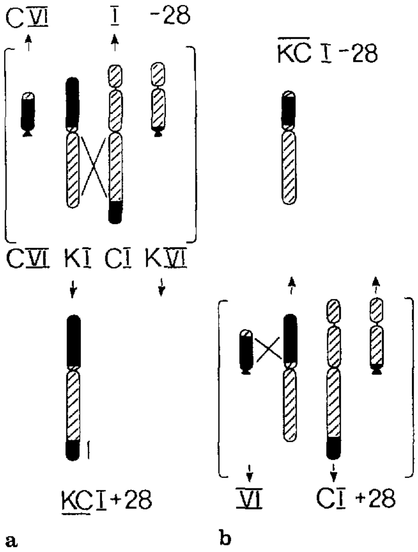

Fig. 6a and b. Quadrivalent formed by reconstructed chromosomes C *VI*, K *I*, C *I* and K *VI* of heterokaryotype KC het schematically (**a** above; **b** below). *Hatched*: parts of original chromosome *I*; *black*: parts of original chromosome *VI*: *X* marks regions of potential crossing overs that may give rise to secondary reconstructions. **a** *below*: secondarily reconstructed chromosome KC *I* with duplication of segment 28 (*bar*). Since this chromosome always cosegregates not only with chromosome K *VI* but also with chromosome C *VI*, the reciprocal segregation product (*arrows upward*) with chromosomes *I* and C *VI*, i.e., deletion of segment 28, could not be found. **b** *above*: secondarily reconstructed chromosome KC *I* with deletion of segment 28. In addition to chromosome K *VI*, the reciprocal crossing over product, chromosome *VI*, always (illegitimately) cosegregated with chromosome KC *I* resulting de facto in trisomy for chromosome C *VI* and compensation of the loss of segment 28. Therefore, the reciprocal regregation product with chromosomes *VI* and C *I*, i.e., duplication of segment 28, was not observable

pensating for the segment lost was established. The complementary situation (chromosome KC*I* with duplication of segment 28) was detected in heterozygous state in 56 plants.

Astonishingly, however, in all cases an additional chromosome C*VI* was present rendering these karyotypes de facto trisomic for a complete chromosome *VI*.

In addition to these cases, a spontaneous heterozygous tandem duplication of the satellite and the nucleolus organizing secondary constriction (segments 1p and 2, comprising ca. 5% of the metaphase genome length) was found for chromosome A*III* of a completely sterile individual homozygous for the multiply reconstructed karyotype ACB.

Discussion

The data presented in this paper show the degree of tolerance for duplications and deletions of defined chromosome segments (comprising about 38% of the genome) of *V. faba* in a quasi diploid karyotype background. Most of them were obtained after intentional crossing of suitable, reconstructed karyotypes (Fig. 7).

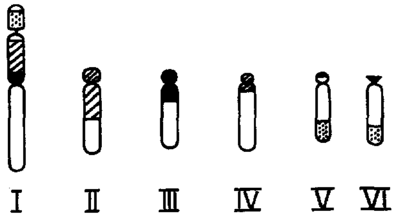

Fig. 7. Haploid chromosome set of *V. faba* standard karyotype schematically. *Black regions* mark segments found to be viable and transmissible in duplicated form. *Dotted regions* occurred as duplications in heterozygous state only. *Hatched regions* symbolize segments that not only were lethal when deleted, but did not occur as duplications too, probably because the corresponding gametes always contained additional deletions

With respect to duplications, it may be concluded that most of them (individually comprising maximally 5% and totally 8.3% of the genome) are tolerated, even in homozygous condition, and sexually transmissable provided they are not accompanied by deletions of other chromosome regions in the respective gametes. Some of them, e.g., segments 1p and 2 or 24, occur only in heterozygous condition and are seldomly transmitted to progeny; others, such as those for segment 28, occur only in quasi trisomic karyotypes, even in heterozygous state, and are almost never transmissable by gametes.

The rate of sexual transmission of duplications is apparently not only dependent on the size of the duplicated segment in question but also on other parameters, such as the genetic information of the duplicated segment or its nucleotypic function. This may be inferred from the finding that 2 out of 114 seeds obtained from heterokaryotype FDDK14 (with 3 alternative segregation possibilities) were found to be homozygous for duplication of 5% of the total metaphase genome length. Duplication of segment 24 (ca. 4% of total metaphase genome length), however, was never transmitted to 64 progeny seeds obtained after selfing of plants heterozygous for this duplication. The high frequency of hyperploid chromosome sets in the offspring of heterokaryotpyes AJ and JK het indicates the frequent occurrence of pseudoaneuploid gametes (30%–50%). Their contribution to the progeny depends, however, on the degree to which viability and function of gametes is affected by the respective duplications or deletions. This gains further support from pollen competition experiments without castration of mother plants. In reciprocal crosses between a pure diploid karyotype EFD and a pseudoaneuploid one (DK14), which is self-fertile inspite of small homozygous duplications, there is a considerable bias in production of progeny heterozygous for duplications. Pollination of pseudoaneuploid mothers with euploid pollen resulted in 61.3% heterozygotes in a progeny of 986 individuals, while the reciprocal cross yielded, under comparable conditions, 0.3% heterozygotes in a progeny of 965 seedlings (Schubert, unpublished).

Contrary to most of the duplications, no deletions were tolerated, even in heterozygous state. This shows that, in spite of the large genome size and high redundancy of DNA in *V. faba*, the deletions (comprising from 0.8%–9.1% of metaphase genome length, in toto 33% of the genome) are lethal in both sexes during haplophase. Possible reasons for this may be "genic" or "nucleotypic" but are presently unknown, since almost no gene mapping has been done up to now in this species. It remains an open question too, whether all or only some (e.g., 4p,13p,21q) of the deleted segments, which in combination are lethal, would also be lethal when occurring as singular deletions.

Basically similar results an in *Vicia faba* have been reported after crossing of interchange lines of *Drosophila* (Muller and Prokofyeva 1935, Muller 1956) and barley (Hagberg 1962, Hagberg and Hagberg 1978). In both cases homozygous duplications were obtained. In *Zea mays* even small deletions were found to be viable (Gopinath and Burnham 1956, Kasha 1979).

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