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Chromosome breakage-fusion-bridge-cycle and phenotypic instability in isochromosome lines of tomato

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Summary. Cultivated tomato lines with 7–9 copies of extra chromosomes, which were fully heterochromatic, were found to have normal growth and reproduction. Cytological evidence suggested that all extra chromosomes in the 24 plants investigated were derived from a single isochromosome of the short arm of chromosome 2. In spite of their common origin, the individual extra chromosomes within and between plants varied considerably with respect to size and morphology. The morphological variation of these extra chromosomes was found to result from chromosome- or chromatid breakage-fusion-bridge-cycle (BFBC). In order to verify whether BFBC could induce "genomic shock" and instability, the phenotypes of more than 10,000 seedlings from some of the progeny were examined. There was a 3- to 4-fold increase in the occurrence of the number of chlorophyll variegated sectors in the progeny of plants with BFBC when compared to the control variety.

Key words: Tomato – Isochromosomes – Breakage-fusion-bridge-cycle – Phenotypic instability

Introduction

The cultivated tomato (*Lycopersicon esculentum*, $2n=2x=24$) is one of the few crop plants in which a critical mass of classical genetic information is available. More than one thousand genes have been recorded so far, and nearly a quarter of these have already been assigned to locus sites on linkage maps. Among many other favourable aspects, the feasibility of maintaining plants in vitro and in vivo (seed and vegetative propogation, cell and tissue culture etc.), the availability of a large number of good marker genes – which include several isozyme loci, and the possibility of identifying pachytene chromosomes, make tomato especially favourable for both genetic studies and manipulation.

A unique feature of the chromosomes of tomato is that, with one exception, each chromosome arm is clearly differentiated into eu- and heterochromatin. The one exception is the short arm of chromosome 2 (2S), which is entirely heterochromtic (Moens and Butler 1963). Heterochromatin in tomato, as in other organisms, appears to be devoid of Mendelian genes (Khush and Rick 1968), nevertheless ribosomal RNA genes (rDNA) are present in the nucleolus organising region (Nor) of the 2S.

The discovery of an isochromosome of 2S, derived spontaneously from a trisomic of chromosome 2 (Moens 1965), opened the way for manipulating the dosages of heterochromatin and rDNA in tomato. Through appropriate crossing and selection, Quiros (1976a) obtained tomato plants with as many as 8 copies of isochromosomes in their cells. Such plants are valuable for an elucidation of cytogenetic phenomena such as chromosome pairing, dosage effect of heterochromatin on genetic stability and recombination, and the behaviour of structurally altered forms of chromosomes during mitosis and meiosis.

While investigating the behaviour of isochromosomes in the lines selected by Quiros (1976a), we observed the phenomenon of breakage-fusion-bridge-cycle (BFBC) involving the extra chromosomes in these plants. Some of the observations on BFBC and on the relevance of this phenomenon for phenotypic instability in these plants form the subject of this article.

Materials and methods

Seeds of the isochromosome lines, Δ4475L355 and Δ4481L476 were obtained from Prof. C. M. Rick of the University of California. These lines were indicated as 2S.2S stocks derived from parents that had several extra chromosomes. Some of the details on the origin of these lines are described by Quiros (1976a).

Fifteen plants from each of the families were grown during the summer of 1984, partly in the greenhouse and partly in the field.

Chromosome numbers were determined in all plants in root tips of young seedlings and pollen mother cells of mature plants. Somatic chromosomes were counted by the following method: the root tips were treated with an aqueous solution of 0.002 M 8-hydroxyquinoline for 2½–3 h, fixed in 3:1 ethanol acetic acid solution for 48 h, hydrolysed in 1 N HCl for 8 min at 60 °C, stained with Feulgen reagent for 2 h and squashed in 1% acetocarmine.

For meiotic chromosomes, young anthers were fixed in 3:1 ethanol propionic acid for 48 h or more and squashed in 2% acetocarmine.

Giemsa c-banding of both mitotic and meiotic chromosomes was carried out as described by Ramachandran and Ramanna (1985).

Plants were selfed and intercrossed in an insect-free greenhouse.

To determine the degree of phenotypic instability, the seeds were sown in greenhouses with relatively uniform growing conditions. Seedlings were carefully evaluated for the presence or absence of chlorophyll variegated sectors or any other morphological deviations. Deviating seedlings were recorded from their the cotyledon stage (10–15 days old) up to 4th leaf stage (4–5 weeks old). Plants with marked phenotypic variability were grown to maturity for further observations as well as propogation. The cultivar 'Moneymaker' was used as a control.

Results and discussion

Characteristics of extra chromosomes: in 24 randomly selected plants from two lines previously reported to contain isochromosomes the number of isochromosomes were estimated in both root tips and anthers (Table 1). Between plants, the number of extra chromosomes varied from 2–9 in somatic cells. Generally, the numbers were constant in different cells of a plant but variation was observed in some cases. Therefore, only the maximum number counted in each case was taken into account (Table 1).

With a few exceptions, the number of extra chromosomes in pollen mother cells was lower than in the root tips of the same plant (Table 1) and the numbers varied from 1–7. As in somatic cells, the number of extra chromosomes in pollen mother cells varied slightly, only the maximum numbers were taken into account. During late pro- and metaphase I stages of meiosis, the extra chromosomes remain mostly unpaired and therefore they could be easily distinguished from the bivalents.

When metaphase chromosomes of somatic and meiotic cells were stained with Feulgen stain and aceto-

Table 1. Chromosome numbers, frequencies of bivalent-like structures and dicentrics in isochromosome plants

Code[a] 2S	Chromosome nos.		No. cells studied	Frequencies of bivalent-like structures and dicentrics			
	Mitosis 24+	Meiosis 24+		Bivalent-like structures			Dicentrics
				Total (per cell)	(%) Symmetric	(%) Asymmetric	(%)
1	5	4	132	38 (0.29)	33 (86.8)	5 (13.2)	–
2	9	7	166	81 (0.49)	71 (87.7)	10 (22.3)	–
3	5	3	132	15 (0.11)	13 (86.7)	2 (13.3)	–
4	5	4	125	24 (0.19)	22 (91.7)	2 (8.3)	–
5	4	3	132	29 (0.22)	–	–	–
6	9	7	145	68 (0.47)	43 (63.2)	25 (36.8)	11 (7.6)
7	5	3	135	20 (0.15)	19 (95.0)	1 (5.0)	–
8	3	1	130	0 (0)	0 (0)	0 (0)	–
9	4	4	145	21 (0.14)	18 (85.7)	3 (14.3)	–
10	7	6	132	35 (0.27)	30 (85.7)	5 (14.3)	–
11	5	5	138	21 (0.15)	20 (95.2)	1 (4.8)	–
12	5	4	142	27 (0.19)	14 (51.8)	13 (48.1)	9 (6.3)
13	5	3	136	18 (0.13)	12 (66.7)	6 (33.3)	7 (5.15)
14	7	6	152	30 (0.20)	30 (100)	0 (0)	–
15	6	6	160	32 (0.20)	16 (50.0)	16 (50.0)	11 (6.9)
16	3	3	132	15 (0.11)	15 (100)	0 (0)	–
17	3	2	132	8 (0.06)	8 (100)	0 (0)	–
18	4	4	132	18 (0.14)	17 (94.4)	1 (5.6)	–
19	6	4	165	30 (0.18)	22 (73.3)	8 (26.7)	5 (3.0)
20	8	6	132	26 (0.20)	21 (80.8)	5 (19.2)	–
21	4	2	134	13 (0.10)	11 (84.6)	2 (15.4)	–
22	3	2	130	8 (0.06)	8 (100)	0 (0)	–
23	6	6	165	26 (0.16)	21 (80.8)	5 (19.2)	9 (5.4)
24	9	3	154	18 (0.12)	8 (44.4)	10 (55.6)	5 (3.3)

[a] 1–6 was from the line Δ4475L355 and the rest from Δ4481L476

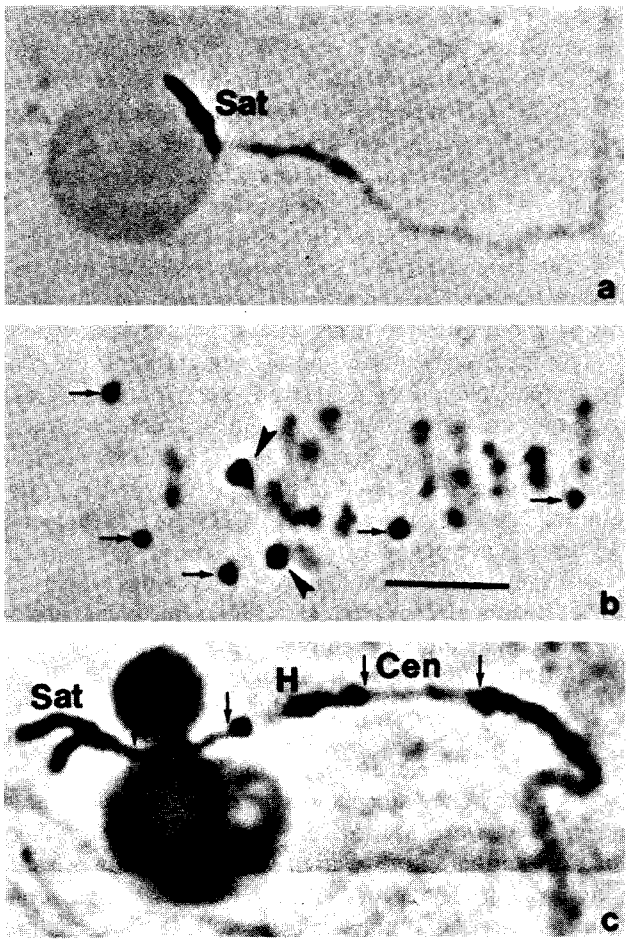


Fig. 1a–c. Cytological evidence for the origin of extra chromosomes from 2S. **a** A pachytene bivalent showing Giemsa c-banding pattern. Note: only the satellite region is darkly stained (c-banded); **b** Metaphase I of meiosis in which only the nucleolar bivalent (*arrow heads*) and five extra chromosomes (*arrows*) are c-banded (bar = 10 μm); **c** A pachytene bivalent stained with acetocarmine in which typically the centromere (Cen); heterochromatic segment (H); nucleolus organising region (Nor) and satellite (Sat) are visible

carmine respectively, it was not possible to determine whether all the extra chromosomes in a plant were morphologically similar to or different from each other. Nor was it possible to determine whether they were similar to the normal chromosomes of the complement. Through Giemsa c-banding of metaphase chromosomes, however, the following two facts could be established: (i) all the extra chromosomes were similar in that they had a block (or blocks) of c-banded heterochromatin (c-heterochromatin) both at mitosis and meiosis; (ii) a large block of c-heterochromatin was present only in the satellite part of the nucleolar chromosome but not in any other chromosome of the normal complement (Fig. 1 a and b). Thus, only the extra chromosomes and the satellite arm of chromosome 2 share the property of having large blocks of c-heterochromatin. This observation was consistant with the fact that all the extra chromosomes in the plants investigated were derived from the isochromosomes of 2S.

Despite their common origin, there were significant morphological and size differences between the extra chromosomes. Such differences were associated with drastic structural differentiation which could be established by a detailed analysis at the pachytene stage of meiosis.

A notable feature of the pachytene chromosomes of tomato is that a detailed morphological characterization of all the chromosomes, including the extra chromosomes, is possible: when they are stained with common nuclear stains such as Feulgen stain or acetocarmine, different parts of bivalents, like centromeres, telomeres, proximal heterochromatin, euchromatin and chromomeres are clearly differentiated. These structures provide convenient landmarks for a clear identification of all the 12 bivalents as well as extra chromosomes. Using the routine acetocarmine staining method, the proximal heterochromatin is visible in all 24 arms of the 12 bivalents. Based mainly on staining and a few other criteria (including lack of chiasmata, absence of Mendelian genes; Khush and Rick 1968) the short arm of chromosome 2 has been regarded as "entirely heterochromatic". Nevertheless, this arm (Fig. 1 c) is not a homogeneous mass of one type of heterochromatin. Through Giemsa c-banding, the 2S can be clearly differentiated into the following three parts: (i) a heterochromatic segment or H-segment, which extends from the centromere up to Nor; (ii) the Nor, which is usually the point of attachment to the nucleolus, or appears as a lightly stained gap, and (iii) a c-banded heterochromatic segment or CH-segment. The order and identity of these three regions are of importance for the detection of structural variation in the extra chromosomes.

From a morphological point of view, an isochromosome is expected to consist of two identical arms flanking the centromere. Accordingly, an isochromosome of 2S may be depicted as shown in Fig. 2c, in which each arm has a H-segment, Nor and a CH-segment. The presence of such a complete isochromosome in the original parent was convincingly demonstrated by Moens (1965). However, none of the 24 plants used in the present study contained a complete isochrome 2S.2S; instead, only the modified forms were present. Based on their morphology and pairing behaviour, the origin of two major types of extra chromosomes, referred to as type I and type II, could be envisioned as illustrated in Figs. 2 and 3.

Type I probably originated by an "internal deletion" of the H-segment, Nor and a part of the CH-segment in one of the arms of an isochromosome with a transposition of a part of CH-segment adjacent to the centromere (Figs. 2 d and 3 a); the other 2S being intact. Because of the presence of the Nor in the intact 2S, the type I was usually associated with the nucleolus along with the normal nucleolar bivalent during prophase I

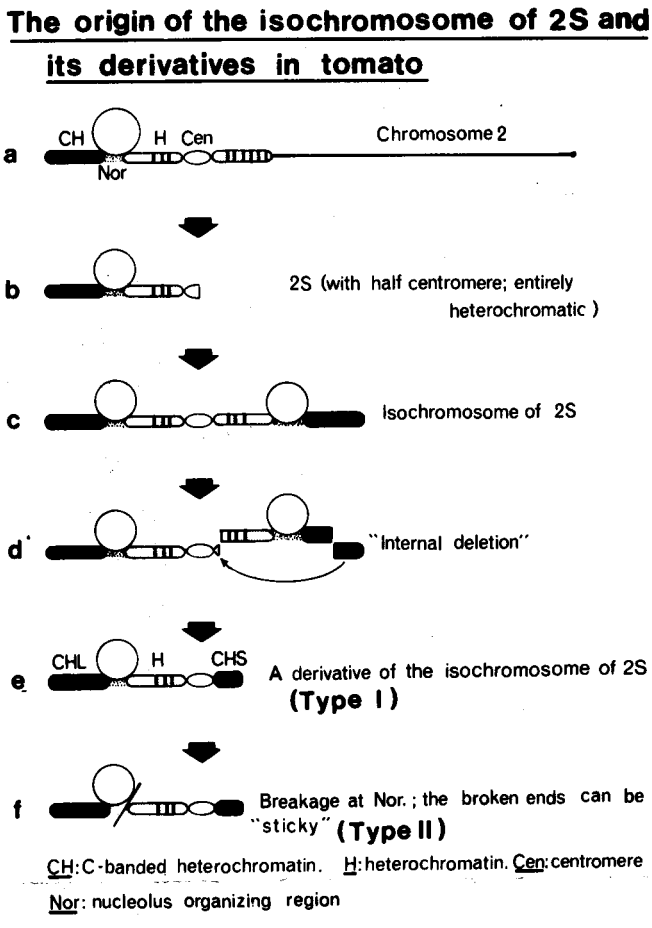


Fig. 2. A schematic representation of the origin of the two major types of extra chromosomes that are found in the plants studied

Fig. 3a–c. The origin of type II extra chromosome from type I (bar = 5 μm). **a** A type I chromosome with CHS (c-banded heterochromatic short segment); Cen (centromere); H (H-segment); Nor (nucleolus organizing region) and CHL (c-banded heterochromatin long segment). **b** An acentric fragment with Nor and CHL. **c** A type II extra chromosome with a CHS, Cen and H-segment – the end of which can be "sticky"

stages. Only in some cases (8–10%) does this extr chromosome organise a small nucleolus independentl (Fig. 3a), or none at all. It should be noted that i type I the CH-segment of the intact 2S is larger tha the one that solely constitutes the other arm (Figs. 2 and 3a). For the sake of convenience, the large an small CH-segments of type I extra chromosomes ar abbreviated as CHL and CHS, respectively.

The type II extra chromosome is similar to type except that the Nor and the CHL-segment from the intac 2S have been deleted (Figs. 2f and 3c). Thus, in type I only a CHS-segment and an H-segment flank the cen tromere. Deletion of CHL and Nor from type I ca occur as a result of breakage at the Nor and subsequen loss of an acentric fragment (Fig. 3b). Such acentri fragments were observed in plants with type I chromo somes. The similarities between CHS- and H-segmen in these two types of extra chromosomes were evider when they paired homologously. Unlike the type I, th type II was not associated with a nucleolus nor organised one independently.

Although the distinction between CH- and H-seg ments is based on the c-banding pattern of the extr chromosomes, these segments could be unmistakabl identified in acetocarmine-stained preparations an their pachytene morphologies could be compare (Fig. 4). A remarkable feature was that the morpholog of extra chromosomes varied within and between di ferent plants. In type I, the variation was evident fro either partial or complete absence of the H-segment c the Nor from the intact 2S. This suggests that the interna deletion in type I was not uncommon. In type II th variation was mostly confined to H-segments whic varied in length.

For a comparison of differences in morphology an size between extra chromosomes the bivalent-lik structures that were observed at pachytene stage wer most useful (Fig. 5). Such structures arise from random association of the centromeres in both orienta tions (i.e., the arms may be homologously arranged c in an opposing orientation). In both cases the bivalen like structures could be classified either as symmetr (both H-segments similar) or as asymmetric (the H-seg ments dissimilar). The proportion of symmetric an asymmetric and bivalent-like structures may be con sidered as a rough estimate of the variation of th type II extra chromosomes in a plant. Their frequencie in different plants are given in Table 1. In some of th plants (6, 12, 15 and 24, Table 1) a relatively hig proportion of bivalent-like structures were asymmetri One notable feature was that the plants with a high proportion of asymmetric bivalent-like structures ha also dicentric extra chromosomes. In view of this, it wa interesting to examine whether dicentric chromosom formation was a cause of variation of morpholog

Fig. 4. A pollen mother cell in pachytene stage in which five type II extra chromosomes (*arrow heads*) can be identified (bar = 10 μm)

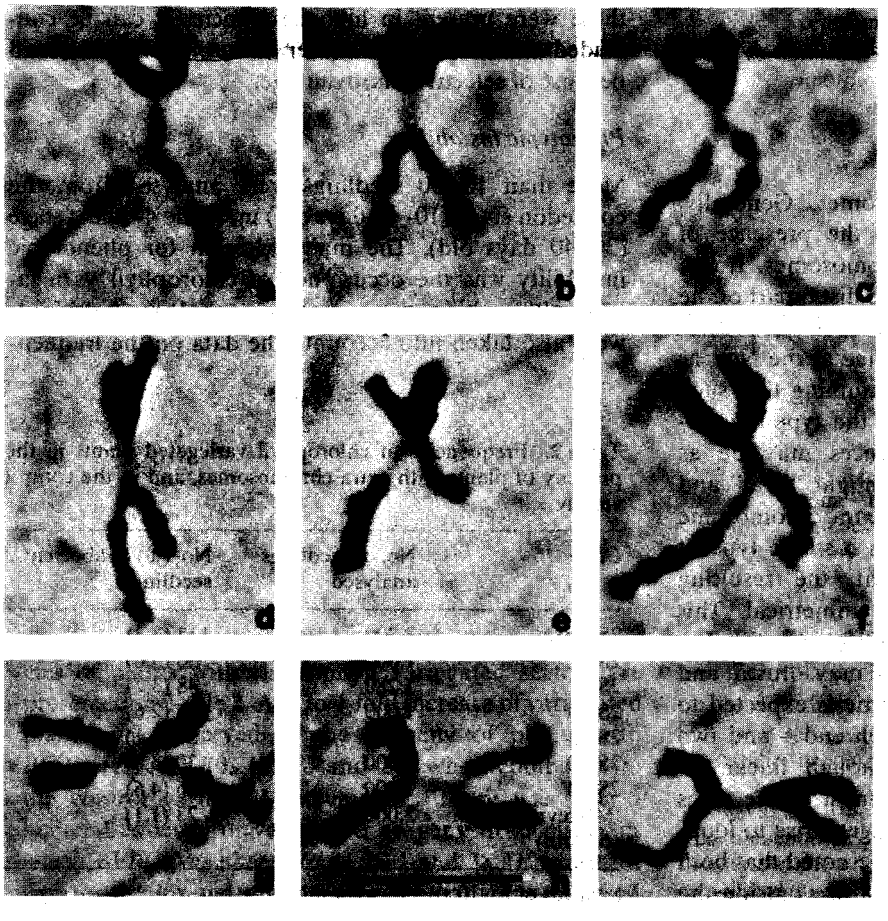


Fig. 5a–i. Bivalent-like structures resulting from the association of type II extra chromosomes at centromeres during pachytene stage. **a–c** Symmetric bivalent-like structures with homologous orientation; **d–f** Asymmetric bivalent-like structures with homologous orientation; **g–h** Symmetric structures with opposite orientation and **i** Asymmetric structure with opposite orientation (bar in h = 5 μm)

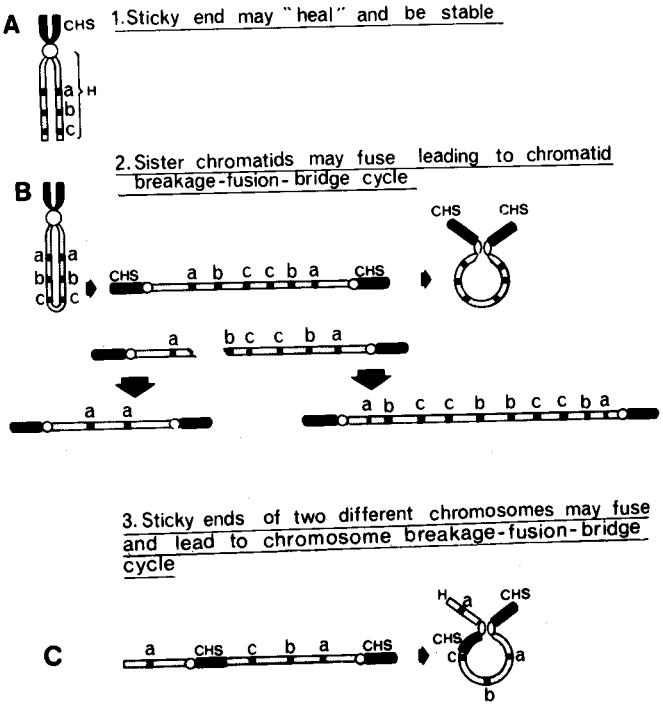


Fig. 6. A schematic representation of the consequences of the presence of sticky ends in type II extra chromosomes

and size in type II extra chromosomes. Generally, dicentric chromosomes arise due to the presence of "sticky" ends of newly ruptured chromosomes. It can thus be expected, for example, that the distal part of the H-segment of type II extra chromosomes might possess such sticky ends in view of the breakage at the Nor, as pointed out before (Figs. 2 and 3). Assuming that such sticky ends were present in some of the type II chromosomes, the cytological consequences may be as follows (Fig. 6): (i) the broken ends might "heal" and give rise to a stable form of type II extra chromosome (Fig. 6A). When many copies of such a stable type of chromosome accumulate in a plant, the resulting bivalent-like structures are mostly symmetrical. This was indeed found in some plants (Table 1). (ii) The sticky ends of two sister chromatids may "fuse" and give rise to a dicentric. Such a dicentric is expected to have two CHS-segments – one at each end – and two centromeres (Figs. 6B and 7a). Although linear dicentrics were observed, as in 7a, in most of the cases the two centromeres were paired and gave rise to loop-like structures (Fig. 7b–d). It should be noted that both CHS-segments in such a configuration are outside the loop. As a consequence of the presence of two centromeres, the dicentric is expected to rupture in the succeeding anaphase and to produce type II chromosomes of different sizes. Thus, the presence of asymmetric bivalents in some of the plants might be the result of chromatid breakage-fusion-bridge-cycle during pre-meiotic mitosis. (iii) The sticky end of a H-segment may fuse with a sticky end of a CHS-segment and thus give rise to a dicentric (Figs. 6C and 7e). In this case only one of the CHS-segments is terminal, the other being interstitial. When the two centromeres were associated, they formed a loop-like structure which was quite different from those observed in the previous case (see Figs. 6B and 7b–d). Only one of the CHS-segments is outside the loop and the other inside. This configuration might rupture in the subsequent anaphase and the behaviour may be comparable to chromosome breakage-fusion-bridge-cycle (McClintock 1951).

In addition to these main types, there were also cases in which a CHS-segment was fused with a similar segment of another type II extra chromosome. Such configurations were rare and are therefore not illustrated.

As a result of the breakage-fusion-bridge-cycle various other morphological types of extra chromosomes may be expected to occur. Some of the types which varied in size and morphology are shown in Fig. 8. Since none of these were present in high frequencies it can be concluded that they were either unstable or eliminated because of selective disadvantage.

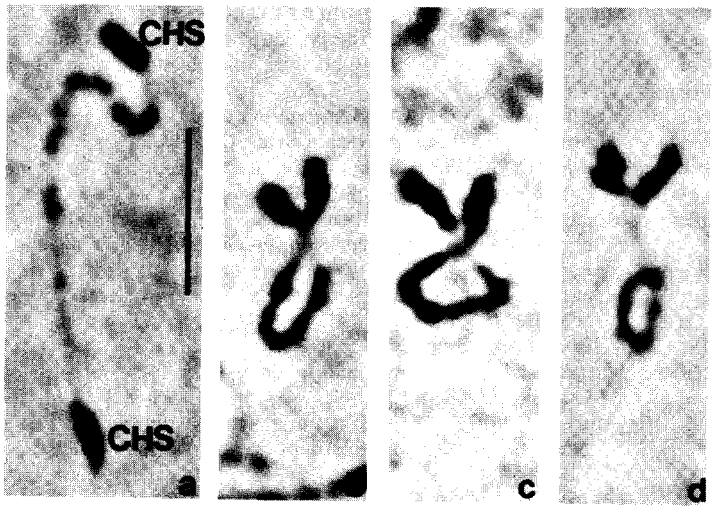
Phenotypic instability

More than 10,000 seedlings were analysed from the cotyledon stage (10–15 days old) up to the 4–5 leaf stage (30–40 days old). The main criterion for phenotypic instability was the occurrence of chlorophyll variegation, although in some cases morphological deviants were also taken into account. The data on the frequen-

Table 2. Frequencies of chlorophyll variegated plants in the progeny of plants with extra chromosomes, and in the control variety

Code	No. of seedlings analysed	No. (%) of aberrent seedlings
2S-mix[a]	3,300	113 (3.3)
2S-1	1,000	30 (3.0)
2S-3	1,280	48 (3.7)
2S-10	1,000	26 (2.6)
2S-15	1,120	34 (3.1)
2S-20	1,000	30 (3.0)
2S-24	1,000	46 (4.6)
'Moneymaker' (control)	2,000	23 (1.1)

[a] Seeds of 2S-10 and 15 were mixed



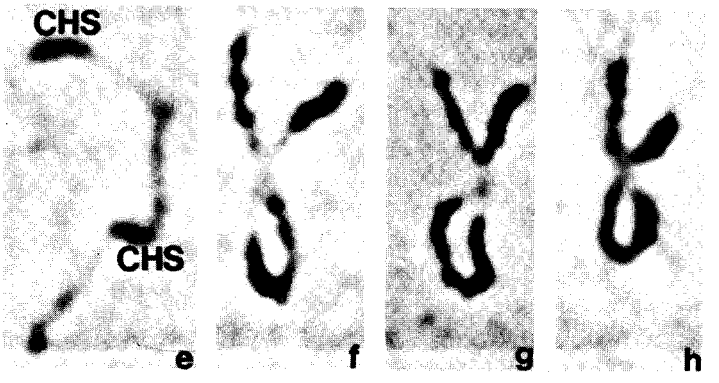


Fig. 7a–h. Two major types of dicentric extra chromosomes identified at pachytene stages. **a–d** Dicentrics resulting from the fusion of sister chromatids: **a** a linear dicentric in which the two centromeres are present immediately adjacent to the two CHS-segments; **b–d** dicentrics in which the two centromeres are associated giving rise to loop-like structures. **e** A linear dicentric resulting from the fusion of a H-segment of one extra chromosome with a CHS-segment of another. **f–h** Loop-like configurations resulting from the association of the two centromeres of a dicentric as in Fig. 7e. Note: One of the CHS-segment is inside the loop (bar in a = 5μm)

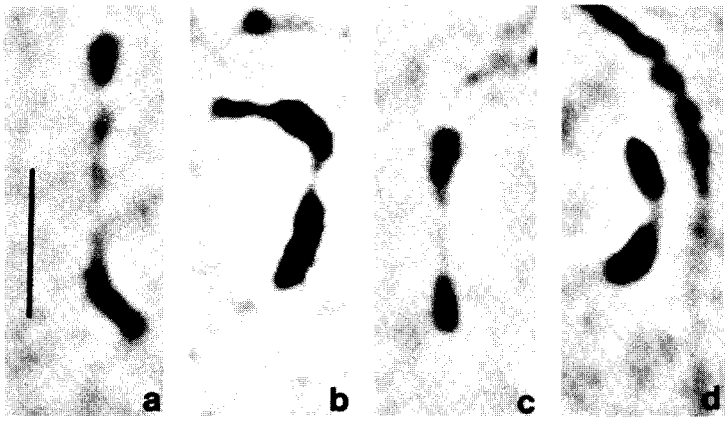


Fig. 8a–d. Some modified forms of extra chromosomes that differ markedly from the types I and II. **a** Lacks Nor; **b–d** Two CH-segments flank the centromeres (bar in a = 5 μm)

cies of plants with chlorophyll variegated sectors are presented in Table 2. A 3- to 4-fold increase of variegated plants was observed in the progeny of plants with BFBC (e.g., 2S-10, 15, 20 and 24) when compared to the control variety 'Moneymaker'. There was also a higher frequency of variegated progeny from plants in which BFBC was absent (e.g., 2S-1 and 3). However, in view of the limited number of cells that were analysed in these plants, it is by no means certain that BFBC was absent in these cases.

Any reasonable explanation for the observed phenotypic instability in some of the progeny must be deferred until more data become available. However, among several possible explanations, the effect of extra heterochromatin and the phenomenon of BFBC may be considered. Quiros (1976b) investigated the effect of extra heterochromatin, caused by the increased number of isochromosomes, in tomato. Out of the

five loci he investigated, two (*marm* and *fd*) were clearly affected. Based on these observations, he concluded that "... constitutive heterochromatin, although perhaps devoid of structural genes, possesses active regulator genes capable of interfering with transcriptional process of certain genes". Although clear proof for the existence of such genes are yet to emerge, it must be noted that classical geneticists have recognized for a long time the existence of different classes of genes in heterochromatin (Mather 1954; Mather and Jinks 1982).

Apart from the dosage effect of heterochromatin, the phenomenon of BFBC deserves a consideration. At least in maize it is well established that BFBC can induce "genomic shock" and cause genetic instability (McClintock 1950, 1984; Peterson 1953; Bianchi et al. 1969; Döerschung 1973). It would be of interest to investigate whether the instability observed in tomato is analogous to BFBC-induced instability in maize.

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