OCCURRENCE OF SUPERNUMERARY CHROMOSOMES IN *AEGILOPS SPELTOIDES* TAUSCH

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Summary : By studying meiotic behaviour in six strains of Aegilops speltoides TAUSCH., occurrence of extra chromosomal material has been revealed. A single plant of a particular strain (G - 1045) showed the presence of a pair of supernumerary homologous chromosomes (B bivalent) constantly in all PMCs examined. The extra chromosomes were of a typical, constant morphology, smaller than the normal members of the complement, with subterminal centromere, usually forming a rod - bivalent. Further observations concerning the offspring of this particular plant led to the conclusion that there exist supernumerary chromosomes in Aegilops speltoides, transmissible through the female parent, yet without appearing in root - tips due to somatic instability.

1. Introduction

Aegilops speltoides Tausch., a species belonging to the section Sitopsis of the genus *Aegilops*, is remarkably distinctive within it, having some common characteristics with wheat and participaring in the first polyploid step taken within *Triticum*. (McKey, 1968). Its genome (S genome), one among the most stabile and isolated of the genus, has not contributed to the synthesis of any tetraploid or hexaploid *Aegilops* species (Kihara 1954).

Aegilops speltoides consists of two taxa or subspecies, namely *ligustica* and *aucheri* which seem exophenotypically different (Eig, 1929a). According to Sears, (1941a) they just represent two botanical varieties, having mutually differentiated by «awn character» through genic speciation, whereas Zohary and Imber (1963) have supported the view that *ligustica* and *aucheri* plants are interconnected by a common mating system forming only one dimorphic, widely symmictic

population. Besides, various strains of *Ae. speltoides* seem to be fairly heterogeneous, due to its breeding system, with respect to floral biology (mostly outbreeders), a condition resebling that of *Seeale cereale*, in which genetical stability has been obtained through the balanced mechanism of heterozygosity (Rees, 1955).

Chennaveeraiah, (1960), in an attempt to elucidate interspecific, evolutionary relationships, studied the meiosis of many *Aegilops* species, but failed to include *Ae. speltoides*. Therefore, we found it interesting to initiate a meiotic investigation of this, by studying various strains of different geographic origin, at the interpopulational level.

The present paper deals only with one side of the problem, i.e. qualitative variation of meiosis, due to the presence of extra chromosomal material, ranging from minute fragments up to supernumerary chromosomes of B category. This chromosomal polymorphism has been frequently observed in allogamous groups inside Triticeae, such as *Seeale cereale* (Müntzing 1958) and *Dactylis glomerata* (Shah 1963), in contrast to the constancy of karyotypes within *Aegilops* (most representatives selfers), where B chromosomes have only been reported in two cases, in *Aegilops mutica* by Mochizuki (1957) and by Chennaveeraiah and Löve in *Ae. eylindrica* and *Ae. columnaris* (1959). So far, there has been no mention of B chromosomes having occurred in *Ae. speltoides* *.

Materials and methods

The initial meterial, kindly provided by Dr. B. L. Johnson, of the Riverside University, included seed from six botanical strains, different in origin. Three of them, (G - 1080, G - 1039 and G - 1045) originated in Turkey, one derived from North Iraq (G - 724), another (G - 617) from Israel and a sixth one (G - 943) is supposed to be of unknown source, having been received from Japan.

Morphologically, almost all strains correspond to the subspecies *aucheri* and they breed true, with the exception of G - 943, which consists of a dimorphic, halanced segregating population (*aucheri* and *ligustica*).

The plants were grown under experimental conditions, in a grouth

* This paper was approved for publication in December 1971. In the meanwhile several important pieces of work on the same subject have been published by English and Israeli scientists. (Cromosoma 33, 63-69, 38, 77-94, 38, 367-386).

chamber adjusted to cycling system of temperature and artificial illumination.

Temp.	16^h 22^o,	8^h 16^o
Illum. initial	12^h light,	12^h dark
subsequent	16^h light,	8^h dark
Light intensity:	2600 lumen	

Spikes were collected firstly seventy days after planting and a subsequent sampling took place over a period of two months. When collection of spikes was accomplished, the plants were transplanted in the green house where they remained till maturity.

Ten plants were preliminarily checked from each strain and three of them used for final cytological analysis. (Number of cells scored per group 220 - 420). Observations of permanent slides (FEULGEN technique) were mostly done at MI, with additional information from AI, MII and tetrad stage.

A second experiment, under the same conditions and techniques, was carried out, by using the progenies of some initial plants, in particular one individual plant, which performed unusual cytological behaviour, evidenced by the results of the first experiment.

Results

Meiotic variation showed a spectrum including:

1) Extra fragments, centric or acentric, occasionally ring shaped and apparently small, in some cases minutes. Their frequency was found to range between 2.18% (G - 1080) and 7.81% (G - 943). Strain G - 724 was the only one in which no fragments were noticed. The commonest form was that of acentric or telocentric fragment (Fig. I) possibly resulting from neocentric activity (BÖSEMARK 1956b), whereas the observed rings seemed to represent isochromosomes (symmetric) or inversion isochromosomes (asymmetric). The latter were, in particular, met with in G - 1080 accompanied by lagging bridges at AI.

Strain G - 943 (dimorphic population) showed as expected, the highest degree of variation, including as well one plant completely normal. Most fragments were ring - like, as if had been formed through internal pairing (JOHN and LEWIS, 1965). A single PMC (among 320 analysed) contained two extra rings.

In groups G - 617 and G - 1039 all plants examined showed frag-

ments in their sporocytes. (Corresponding frequencies 3.66% and 4.54%). One PMC from G - 617 (0.33%) included two fragments not showing any evidence of homology.

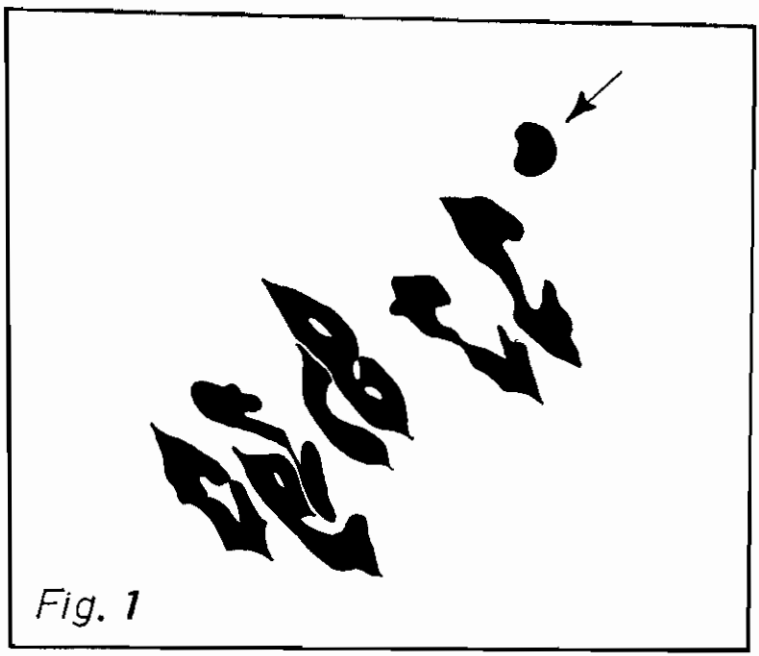

Fig. 1. G - 1080. MI cell with 7 bivalents and an acentric fragment.

2) Another deviation from the typical meiotic figure was the occurrence of differential phases (asynchrony of bivalents) inside the same PMC. In G - 1080, cells were noticed with 3 pairs at MI, 3 pairs at AI and one pair intermediate. The figure was observed as frequently as 3.65%, associated with micronuclei in tetrads.

Univalents were mostly found in cells of G - 724 (18.12%) and G - 943 (8.12%). The former showed a constant behaviour expressed hy all plants investigated, whereas in the latter a striking variation between plants was seemed.

3) B chromosomes.

Complete subterminal chromosomes or centric fragments consisting of at least one chromosome arm (telocentrics) were occasionally observed in G - 943, G - 1039 and G - 617. Moreover, extra bivalents, that is one pair of homologues, in addition to the seven pairs of the

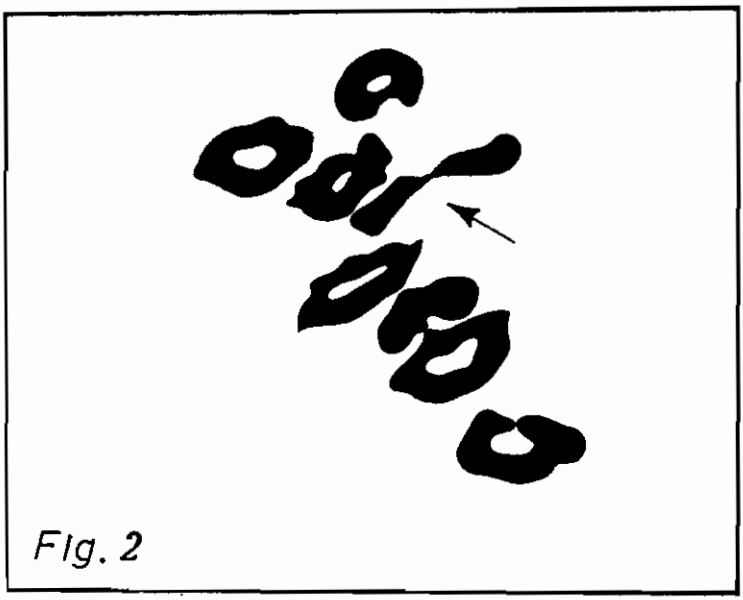

Fig. 2. G - 1045 - 2. PMC with 8 pairs of chromosomes. B pair indicated by arrow.

Fig. 3. G - 1045 - 2. Eight pairs of chromosomes. Supernumerary bivalent as typical, short rod - bivalent.

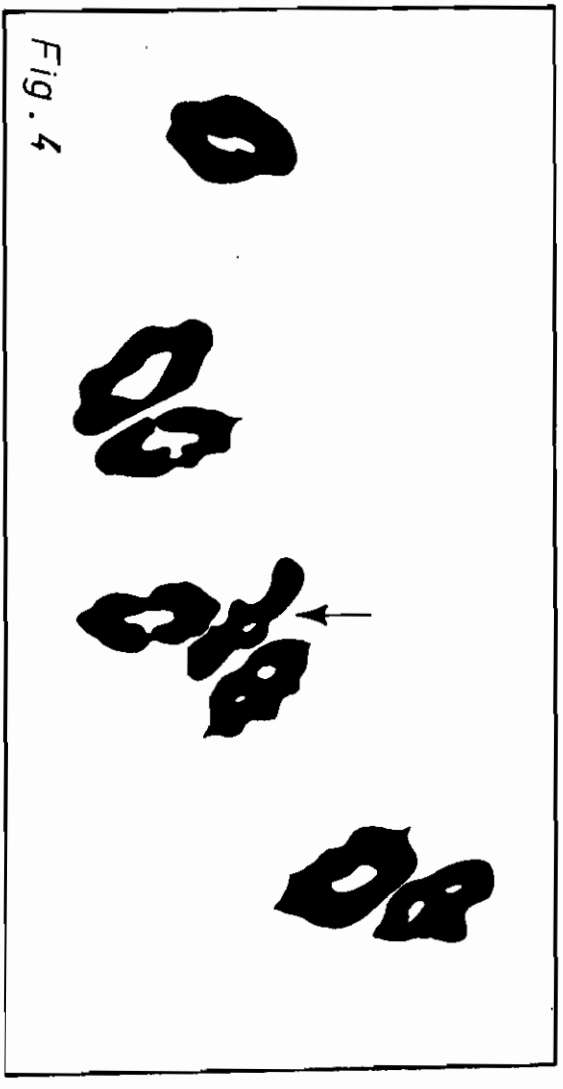

Fig. 4. G - 1045 - 2. Eight pairs of chromosomes. B - bivalent almost as dictiocentric.

normal complement, were sporadically found in G - 724 (0.37%) and G - 1039 (1.81%).

The results can be summarised in the following table. (T. I).

As it is evident from the above data there exists a striking difference between all other groups and group G - 1045. The result has actually been derived from one single plant of the strain, which contained a supernumerary bivalent constantly present in all sporocytes. (240 cells from 3 tillers scored, and 7 more tillers checked). Therefore its karyotypical set was $2n = 16 (14 + 2B)$. The extra bivalent appeared constant in morphology as well. (Fig. 2, 3, 4). It consists of two subterminal members, resembling telocentrics, usually paired by one chiasma,

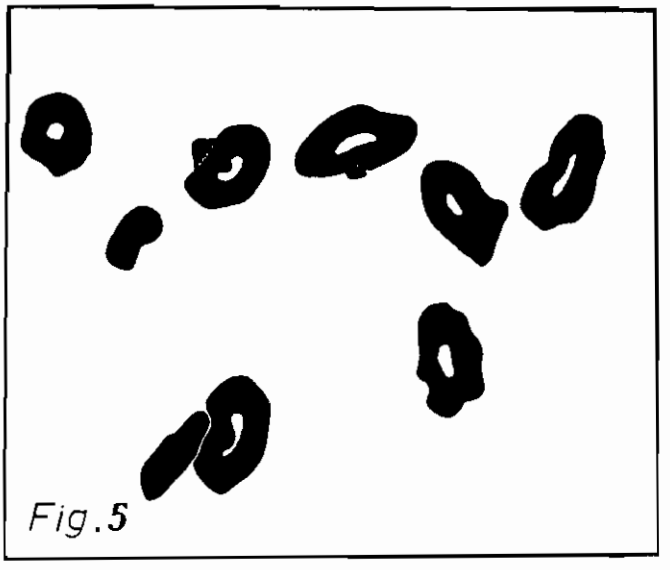

Fig. 5. G - 1039. PMC with 8 bivalents. B - chromosomes appear as univalents.

or, occasionally, asynaptic. Since there was no multivalent found in any of the sporocytes analysed, it is concluded that the extra chromosomes belong to the category of B's, i.e. they represent a specific type of polysomy, in which extra chromosomes do not give any indication of homology to normal members of the complement (LEWIS and JOHN, 1963). Their persistence implicates that the cause of irregularity cannot be attributed to premeiotic errors, but, more probably, to transmission through earlier ontogenetic stages, as primordia of inflorescences. We

TABLE I

Observed meiotic configuration in different stroins of Aegilops speltoides (Absolute volues and percentages)

Strains	Total number of cells	Univa-lents	Fragments or B-chrom.	Laggards -bridges	Supernu-merary bivalents (B)	Total of aberrant cells	Normal
G - 1080	275	21 (7.63)	6 (2.18)	9 (3.27)	0	36 (13.08)	239 (86.92)
G - 724	265	48 (18.12)	0	8 (3.01)	1 (0.37)	57 (21.50)	208 (78.50)
G - 943	320	26 (8.12)	25 (7.81)	4 (1.25)	0	55 (17.18)	265 (82.82)
G - 617	300	24 (8.00)	11 (3.66)	18 (6.00)	0	53 (17.66)	247 (82.34)
G - 1039	220	3 (1.36)	10 (4.54)	9 (4.09)	4 (1.81)	26 (11.80)	194 (88.20)
G - 1045	420	18 (4.28)	6 (1.42)	27 (6.42)	210 (50.00)	261 (62.12)	159 (37.88)

prefer this explanation, since there had been no sign of any extra chromosome in the root-tips of the initial seedling.

By examination of pairing ability concerning extra bivalents, we found an apparent variation between tillers. In one case a relatively high frequency of univalents was scored (20% of PMCs) yet the majority of tillers showed either extremely big pairing (over 95%) or complete absence of univalents. (Table II). Since B bivalents mostly appear as rod - bivalents, the short arms of participating homologues can hardly be distinguished, thus giving the impression of ditelocentrics. (Fig. 2, 4). However, at a percentage as high as 25%, supernumerary bivalents appeared ring - shaped, forming one chiasma per arm. Even in this situation, B's can be clearly seen, smaller than normal bivalents and more intensely stained.

Cells observed at AI showed extra chromosomes as typically subterminal with an arm ratio approximating 4:1. Disjunction between homologues seemed to be fairly normal, as reported for other Gramineae too, that is in *Zea mays* L. by BLACKWOOD (1956) and in *Secale cereale* by MÜNTZING (1954).

Discussion - Conclusions

Although *Aegilops speltoides* has been widely used in cytological experiments (RILEY, 1960), no reference dealing with supernumerary chromosomes in it appeared so far. However, it should be taken into account that in experimental work done towards investigating wheat diploidisation (5B system) a particular strain of the species has always been used, probably representing a balanced cytological condition. Instead, the material used in this study, heterogeneous by nature, manifested, in general, cytological instability.

The phenomenon was more strikingly shown by strain G - 1045, a single plant of which (G - 1045-2) contained constantly a supernumenary bivalent in its PMC's. This indication of instability, in association with the lack of similar observations for the species, has encouraged us to search for further evidence towards explaining the mode by which the above B's are transmitted, maintained or eliminated.

Therefore, seeds were received from 5 ears of this particular plant and put to germinate for root - tipping, one hundred somatic cells having, totally, been analysed. All of them were found to contain the normal somatic complement. ($2n = 14$). We could, of course, at first conclude that extra chromosomes cannot be transmitted through the female parent, due to selective elimination, being given that the plant

TABLE II

Pairing degree of B - bivalents in plant G-1045-2

Tiller-samples*	Number of cells	Pairing of B-chromosomes			Number of chiasmata per B-bival.			% ring bival.
		2 unival.	1 bival.	% bival.	0	1	2	
1	160	32	128	80	32	98	30	18.75
2	140	0	140	100	0	103	37	26.42
3	120	6	114	95	6	84	30	25.00
	420	38	382	91.6				

* Each sample: two slides.

was taken as outbreeder, while an effort towards artificial selfing did not succeed. However, we could, as well admit that B's are mitotically unstable, not seen in all cells of an individual. In order to clarify this point, the seedlings from which root - tips had been removed, were ubsequently put to grow and anthers were squashed for meiotic examination (second experiment).

From preliminary observation (detailed data will be given elsewhere) of PMCs from 10 plants, we found most of them to contain B chromosomes. However, the progenies showed considerable variation in number of supernumeraries. Eight plants showed to contain 3 supernumeraries at a proportion higher than 50% of PMCs observed. Those extra chromosomes must be homologous as evidenced by the frequent formation of trivalents. Two plants were identical to the maternal one, showing the presence of a B bivalent. Consequently, supernumerary chromosomes must have been transmitted though a certain route not including adventitious roots, but probably following the central part of the plant to be refound in inflorescenses and germ cells. This particular distribution has been described by MÜNTZING (1948c) for *Poa alpina*.

According to PUTEYEVSKY and ZOHARY (1970), who studied supernumerary chromosomes in *Dactylis glomerata*, non - disjunction and selective transmission tend to increase the number of B's in progenies, chiefly on the male side, by contrast to the female. However, the above authors had also to admit that transmission of supernumerary chromosomes is much more complicated than it superficially seems. With regard to our observation it seems that only somatic elimination of B's takes place through EMC, whereas a directed post - meiotic non - disjunction leading to accumulation of B's in the offspring is the probable mechanism of transmission. The meiotic spectrum of the progenies is a reflection of this instability, showing that B chromosomes are subject to considerable numerical variation, both within and between generations (JOHN and LEWIS, 1968).

Conclusively, we can summarise as following:

Supernumerary chromosomes can exist in certain population of *Aegilops speltoides*, maintainable by somatic instability. A single plant was found as meiotically uniform, but, even in this case, uniformity seemed to be confined into this very stage. Instead, instability should have been the rule during earlier steps of development. Thus, B chromosomes are transmissible through the female parent, reappearing in

the progenies and resulting rather to increase than elimination of their numher, without occurring in root meristems.

The problem, certainly, needs, further elucidation. Consequently we are attempting artificial selfing of F1 progeny (1045 - 2 family) and crossing of the strain with a standart one, not containing B chromosomes.

ΑΝΕΥΡΕΣΙΣ ΥΠΕΡΑΡΙΘΜΩΝ ΧΡΩΜΑΤΟΣΩΜΩΝ ΕΙΣ ΤΟΝ AEGILOPS SPELTOIDES TAUSCH.

Ὑπὸ

ΕΛΛΗΣ ΚΟΥΚΟΛΗ καὶ ΧΡ. ΠΑΠΟΓΛΟΥ

ΠΕΡΙΛΗΨΙΣ

Διὰ τῆς μελέτης τῆς μειώσεως εἰς ἓξ (6) ὁμάδας (πληθυσμοὺς) τοῦ εἴδους Aegilops speltoides Tausch. διεπιστώθη ἡ παρουσία ὑπεραρίθμου χρωματοσωμικοῦ ὑλικοῦ, ἤτοι χρωματοσωμικῶν θραυσμάτων ἤ, σπανιώτερον, ὁλοκλήρων ὑπεραρίθμων χρωματοσώμων τῆς κατηγορίας τῶν Β.

Εἰς ἓν φυτὸν ἐκ τῆς ὁμάδος G-1045, ἀνευρέθη ἓν ζεῦγος ὁμολόγων ὑπεραρίθμων σταθερῶς εἰς ἅπαντα τὰ ἀναλυθέντα σποριοκύτταρα. Τὰ χρωματόσωμα ἦσαν σταθερᾶς μορφολογίας, ὑπακροκεντρικά, μικρότερα τῶν κανονικῶν μελῶν τοῦ καρυοτύπου, σχηματίζοντα κατὰ τὸ πλεῖστον ἓν ραβδόμορφον δισθενές.

Περαιτέρω προκαταρκτικαὶ παρατηρήσεις ἐπὶ τῶν ἀπογόνων τοῦ ἀνωτέρω φυτοῦ ἀποδεικνύουν ὅτι, εἰς τὸ ὑπὸ μελέτην εἶδος ὑφίστανται Β χρωματόσωμα, ἐπιλεκτικῶς μεταβιβάσιμα διὰ τοῦ θήλεως γονέως, ἀλλὰ σωματικῶς ἀσταθῆ, δεδομένου ὅτι δὲν ἀνευρέθησαν εἰς κύτταρα ἀκρορριζίων.

Διὰ τῆς παρούσης ἐπισημαίνονται τὸ πρῶτον Β χρωματόσωμα εἰς τὸν Aegilops speltoides.

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