

The structure, meiotic behaviour and effects of B chromosomes in *Briza humilis* Bieb. (Gramineae)

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Abstract

A single population of *Briza humilis* contained two types of B chromosome, one a large (B^L) and the other a small (B^S) acrocentric. DNA measurements show that the B^L chromosome contains approximately twice as much DNA per unit length as the members of the regular complement. The meiotic pairing behaviour of the Bs is variable and B^L and B^S are seen to pair in some cells. The presence of B^L depresses the chiasma frequency of the regular complement but the chiasma frequency of A and B chromosomes does not appear to be related. The transmission rate of the B chromosomes is variable and the B^L shows a non-disjunction mechanism during microsporogenesis that is absent during megasporogenesis. For the B^S chromosome the transmission rate is very low and there is no evidence of a non-disjunction mechanism. In general seeds containing B^L chromosomes germinate more slowly than those without B chromosomes.

Introduction

In a previous paper (Murray, 1979) a supernumerary or B chromosome of *Briza humilis* Bieb. (syn. *Briza spicata* Sibth. & Sm.) was found to pair at low frequency with a pair of autosomes in a plant with abnormal meiotic behaviour. The A-B associations appear to be chiasmata and occurred in about 10% of pollen mother cells. Although some preliminary evidence was presented to confirm that the supernumerary was a B and not a trisomic, it was desirable that more data on these supernumeraries should be accumulated. Therefore, this paper describes the structure, meiotic behaviour, pattern of inheritance and the effect of the B^L on seed germination time. A shorter B (B^S) occurred in the same population, and its behaviour is also described.

Material and methods

The original plants that contained the supernumeraries were collected from a large population growing near Ionannina, Epirus, Greece. Seeds were also obtained from other populations in Greece, Bulgaria and Turkey. Mitosis and meiosis were studied using standard aceto-orcein staining techniques. Nuclear DNA content was measured by Feulgen microdensitometry of isolated nuclei following the schedule of Paroda and Rees (1971). Different plants with known B chromosome constitution were used for each replicate. All the slides in each replicate were hydrolysed and stained in the same container to ensure that hydrolysis and staining time were the same for all B chromosome classes and a preparation of *Allium cepa* root tip nuclei was included in each replicate as a standard. Photometric measurements were made on a Vickers M85 integrating microdensitometer. Measurements were taken on 25 small presumed 2C nuclei and 5 large presumed 4C nuclei.

Since *B. humilis* is self-incompatible crosses were made without emasculation by bagging together inflorescences from two different plants until seed had set.

To investigate the effect of Bs on seed germination time, seeds were sown on moist filter paper in petri dishes and kept at either room temperature or in an incubator at constant temperature. Germinating seedlings were collected at daily intervals and their chromosome number determined.

Results

Distribution and morphology of the B chromosomes

The population that contained the B chromosomes was growing in a habitat typical for the species, gravelly soil at the side of the road. The population contained approximately 200–300 plants. Twenty three plants were sampled and 10 of these contained B chromosomes. Six had a large B chromosome (B^L), three had $2B^L$ and one had $1B^L$ and one smaller B (B^S). Both types of B chromosome appear to be euchromatic. Plants with higher numbers of Bs of both types and in various combinations were produced by controlled crosses. Plants with up to $4B^L$ flowered readily but plants with more than this number showed no sign of flowering at any time in their life span.

The B^L chromosome is approx. $5.5 \mu\text{m}$ long at metaphase (Fig. 1b) and is only $1.0 \mu\text{m}$ shorter than the smallest A chromosome. The small B (B^S) is $3.0 \mu\text{m}$ long (Fig. 1a) and the long arm of B^S is more or less the same length as the short arm of B^L . Both types of B are in general mitotically stable with the same number of Bs being present in root tip and pollen mother cells of any particular individual. However, two plants out of a total of 154 studied were found in which the B^L was present in only 25% of pmcs but a constant number of Bs was found in root tip cells.

Nuclear DNA content of the B^L chromosome

The DNA content of plants with 0, 2 and 4 B^L chromosomes was estimated by Feulgen microdensitometry and the results are given in Table 1. They show that the DNA content of the A chromosomes

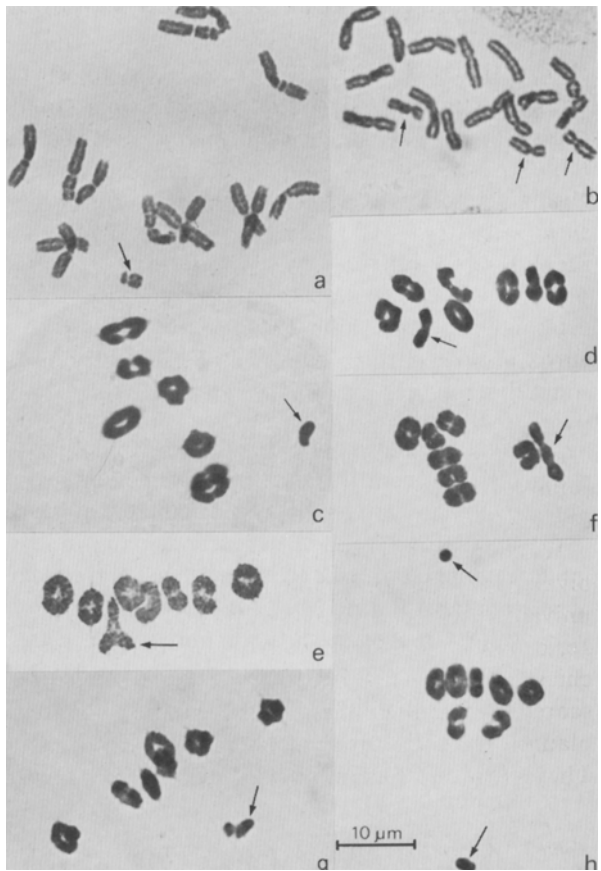


Fig. 1. Mitotic and meiotic metaphase in plants of *B. humilis* with different types and numbers of B chromosomes (indicated by arrows): (a) mitotic metaphase with $1B^S$; - (b) Mitotic metaphase with 3 B^L ; - (c) Meiotic metaphase I showing a B^L univalent; - (d) Meiotic metaphase I with B^L bivalent; - (e and f) Meiotic metaphase I showing B^L trivalents; - (g) Meiotic metaphase I showing a B^L/B^S heteromorphic bivalent; - (h) Meiotic metaphase I with B^L and B^S unpaired as univalents. Scale = $10 \mu\text{m}$.

Table 1. Nuclear DNA amounts for plants of *B. humilis* with 0, 2 and 4 B^L chromosomes, 2C values converted to pg DNA using *Allium cepa* (2C value = 33.55 pg) as standard.

No. of Bs	Replicate			Mean	pg DNA
	1	2	3		
0B	21.32	22.96	22.93	22.40	10.75
2B	26.43	-	26.55	26.50	12.72
4B	31.55	29.23	31.13	30.63	14.70

is 10.75 pg DNA/2C nucleus and that each additional B^L chromosome contributes approximately one pg of DNA to the 2C nucleus. Total chromo-

some length and chromatid width was measured in 10 cells at mitotic metaphase which contained B^L chromosomes. Chromatid width was found to be uniform within cells and the B^L chromosome comprises 5% of the total chromosome length. If the DNA were uniformly distributed among A and B chromosomes the addition of one B^L chromosome should increase the DNA content by 5%. However, the difference in DNA content between 0B and 2B plants was approximately 20% and therefore indicates that the DNA content per unit length of the B^L chromosomes must be twice that of the A chromosomes.

Meiotic behaviour and effects on the A chromosomes

When more than one B^L chromosome is present bivalent and multivalent formation is common and univalents are formed only at low frequency (Fig. 1c, d, e and f). Chromosome configurations and chiasma frequencies for the B^L chromosome were scored at metaphase I in 25 plants with 2B^L and 19 plants with 3B^L and the results are given in Table 2. They show that there is considerable variation be-

Table 2. Mean meiotic configurations with ranges in brackets and chiasma frequencies of the B^L chromosomes of *B. humilis*. 20 pmcs were scored for each plant. I = univalent, cII = chain bivalent, oII = ring bivalent and III = trivalent.

Chromosome configuration					Mean chiasmata per cell
2B plants (25 individuals)					
2I	cII	oII			
2.6	15.64	1.76			1.04
(0-7)	(9-19)	(0-8)			(0.90-1.35)
3B plants (19 individuals)					
3I	cII, 1	oII, 1	III		
5.95	1.79	10.00	2.26		1.27
(2-11)	(0-5)	(5-16)	(0-7)		(1.00-1.60)
4B plant (1 individual)					
cII, 2I	cII, cII	oII, cII	III, 1		
5	2	1	12		1.95

tween plants in the frequency of the chromosome configurations and in the number of chiasmata that are formed. Only one plant with 4B^L was analysed and it is interesting to note that quadrivalents were not observed in this individual and a trivalent and univalent was the most common configuration.

When the large and small B chromosomes are present in the same plant pairing between them at

Table 3. Mean cell chiasma frequencies of A and B chromosomes in plants of *B. humilis* with and without B chromosomes. Values are the means of 20 cells per plant. 20 plants of the 0 to 3 B^L category were studied, one of the 4B^L and 4 of the 1B^S.

0B ^L	1B ^L	2B ^L		3B ^L		4B ^L		1B ^S
		A	B	A	B	A	B	
14.10	13.35	10.65	1.15	12.50	1.20	12.80	1.95	14.15
13.95	12.35	12.95	0.95	13.70	1.45			12.85
14.05	13.35	13.00	1.10	13.70	1.50			13.70
13.65	12.40	13.75	1.15	13.35	1.00			13.55
13.80	13.75	13.10	0.80	13.45	1.60			
14.05	13.50	13.20	1.05	13.05	1.60			
13.95	14.10	12.90	1.05	11.90	1.30			
13.75	14.10	12.80	1.10	11.75	1.15			
13.75	13.40	10.95	0.90	12.40	1.50			
14.05	13.85	12.50	1.30	10.90	0.80			
14.05	13.45	12.35	1.10	12.60	1.40			
14.00	10.40	10.80	0.90	12.70	1.15			
13.70	11.60	13.60	0.75	13.35	1.00			
14.00	13.40	13.80	0.75	13.00	1.15			
13.75	14.00	11.60	0.90	13.00	1.30			
13.80	13.30	12.85	0.95	12.35	1.10			
13.95	13.80	12.30	1.35	11.80	1.25			
13.75	13.40	12.00	1.20	13.00	1.10			
13.90	13.40	12.50	1.05	12.10	1.05			
13.90	13.50	13.50	1.35	13.50	1.55			
13.90	13.22	12.56	1.04	12.71	1.26	12.80	1.95	13.56

meiosis was common (Fig. 1g) although the frequency of bivalents and univalents (Fig. 1h) were not recorded. Where bivalents were formed they appeared as rod bivalents but it is not possible to say whether chiasmata can occur in both arms of the B^S when it forms bivalents with the B^L.

In 92.5% of anaphase I cells (53 cells), when a single B^L chromosome is present it migrates with the A chromosomes in a regular manner to one of the spindle poles. In the remaining 7.5% of cells the univalent B divided into its two component chromatids but they migrated to the same pole.

The presence of B^L chromosomes appears to depress the chiasma frequency of the A chromosomes (Table 3). An analysis of variance indicates that there are significant differences between the B chromosome classes in mean chiasma frequency ($F = 15.144, P < 0.001$). From Table 3 it appears that the effects of the Bs are additive, but only up to 2Bs since the means of the 3B and 4B classes are very similar to that of the 2B class. B^L chromosomes increase both the within plant and between plant variance in chiasma frequency. For the 0B plants the within plant variance is 0.29 whereas for the 3B plants it is 2.09 with corresponding values of 0.02 and 0.53 for the between plant variances. The B^S chromosome appears to have no effect on

A-chromosome chiasma frequency (Table 3) but insufficient plants were analysed to make any meaningful comparison of the effects B^L and B^S on A-chromosome chiasma frequency. The relationship between A- and B-chromosome chiasma frequency was also investigated but no clear correlation between A- and B-chromosome chiasma frequency/cell was observed ($r = 0.2365, P = NS$).

Inheritance

Crosses were made between plants with and without B^L chromosomes and the results are presented in Table 4. They show that the transmission rate and pattern of inheritance varies between different crosses. In the majority of crosses the results show that there is a non-disjunction mechanism that operates in the male but not the female germ line. In seven out of eight 0B × 1B crosses the 2B class is the largest class of the B-containing progeny, and in only one cross (h) this is not the case. In cross h the 1B class is the largest followed by the 3B class; only one progeny plant had 2Bs. However, this inheritance pattern did not persist in the progeny of these plants as the male parent (the 1-B-containing plant) in cross g was an F₁ plant from cross h. Crosses between 1B females × 0B males produce

Table 4. The inheritance of B^L in *B. humilis*.

B constitution of parents		No of Bs in progeny					No. of progeny	Total Bs	Mean B no of progeny	
		0	1	2	3	4			Observed	Expected
0 × 1	a	11	0	1	0	0	12	2	0.17	0.5
	b	21	0	3	0	0	24	6	0.25	0.5
	c	20	0	4	0	0	24	8	0.33	0.5
	d	12	0	5	0	0	17	10	0.59	0.5
	e	20	0	10	0	0	30	20	0.66	0.5
	f	23	6	11	0	0	40	28	0.70	0.5
	g	43	3	27	1	0	74	60	0.81	0.5
	h	1	22	1	14	0	48	66	1.38	0.5
1 × 0	a	31	4	0	0	0	35	4	0.11	0.5
	b	42	15	0	0	0	57	15	0.26	0.5
	c	9	5	0	0	0	14	5	0.36	0.5
0 × 2	a	3	1	27	0	0	31	55	1.77	1.0
	b	1	0	28	0	0	29	56	1.93	1.0
2 × 0	a	0	7	0	0	0	7	7	1.00	1.0
	b	0	8	0	0	0	8	8	1.00	1.0
	c	0	12	1	0	0	13	14	1.07	1.0
0 × 3		0	0	11	0	1	12	26	2.17	1.5
3 × 0		0	6	5	0	0	11	16	1.45	1.5
2 × 2		0	3	3	8	2	17	47	2.76	2.0

(16B)

Table 5. The inheritance of B^S in *B. humilis*.

B constitution of parents	No. of Bs in progeny			No. of progeny	Total Bs	Mean no. Bs/plant
	0	1	2			
0 × 1	3	2	1	6	4	0.66
	11	0	0	11	0	0
	8	0	0	8	0	0
1 × 0	6	5	0	11	5	0.45
	10	0	0	10	0	0
	13	0	0	13	0	0
	12	0	0	12	0	0

Table 6. Seed germination time in plants of *B. humilis* with and without B^L chromosomes. Numbers in brackets are the number of plants scored.

	Temp.	Mean germination time ± SEM in days			
		0B	1B	2B	3B
Exp. 1	Room	6.32 ± 0.17 (139)	6.84 ± 0.31 (49)	6.97 ± 0.37 (32)	7.12 ± 0.41 (26)
Exp. 2	20°	6.89 ± 0.14 (142)	7.20 ± 0.16 (74)	7.88 ± 0.16 (126)	8.75 ± 0.12 (136)
Exp. 3	10°	9.71 ± 0.09 (234)	9.12 ± 0.15 (58)	9.37 ± 0.17 (41)	9.94 ± 0.15 (31)

offspring that have either 0B or 1B thus confirming the absence of a non-disjunction mechanism in the female germ line. In general these inheritance patterns persist when plants with more than 1B^L are used in crosses.

In the eight 0B × 1B combinations the transmission rate, expressed as the mean number of Bs per progeny plant ranges from 0.17 to 1.38. Some of these crosses show a substantial accumulation of B chromosomes in the progeny (d-h) over expectation on the basis of random B transmission. In other crosses (a-c) there is a net loss of B chromosomes. The reciprocal cross combinations (1B^L × 0B) resulted in a net loss of B chromosomes in all cases. Crosses involving plants with higher numbers of Bs also showed some variation in transmission rate although in many cases the observed B frequency in the progeny is very similar to the expected frequency.

Fewer crosses were made with plants with the B^S chromosome. In two crosses the transmission rate was close to the expected value based on random transmission but in the other five crosses the B^S was not transmitted to the progeny. There was no indication of a non-disjunction mechanism (Table 5).

To check whether any selfing was occurring, 28 inflorescences from eight plants were isolated in

crossing bags and shaken each day to ensure good pollen dispersal. Only two plants set any selfed seed and in both cases the level of seed set was less than 5% of that produced by crossing. It therefore seems reasonable to conclude that the self-incompatibility system was operational in these plants.

Effects of B^L chromosomes on seed germination time

Seeds were germinated either at room temperature (20 ± 5 °C) or at constant temperatures (10° and 20 °C) in incubators. The results of these experiments are given in Table 6. They show that in all cases the seeds with Bs seem to take longer to germinate than those without Bs and also that at low temperature seed germination takes longer. However, the relationship between B chromosomes and seed germination time may not be causal since germination is affected by a variety of factors and the differences between the B classes are small.

Discussion

Several features of the B chromosomes of *B. humilis* are of interest. The DNA content per unit

length of the B^L chromosome indicates a difference in the pattern or degree of chromosome coiling when compared to the A chromosomes. Similar results were obtained by Jones and Rees (1968) and Ayonoadu and Rees (1971) for the B chromosomes of rye and maize. In rye and maize the Bs are heterochromatic or partly heterochromatic but in *B. humilis* they appear to be euchromatic so the difference in DNA content per unit length is not simply a consequence of heterochromatinization.

The B^S would appear to be a deletion product of B^L as the long arm of B^S is the same size as the short arm of B^L . As these chromosomes readily pair and form chiasmata this provides further support for the origin of one type of B from the other. B chromosomes in many species are often polymorphic undergoing various types of structural change, for example in *Allium schoenoprasum* Bougourd and Parker (1979) have described nine different types of B chromosomes from populations growing along the river Wye. The B^S in *B. humilis*, if it is derived from the B^L , must be of more recent origin than B^L and it may not be a permanent feature of populations of this species since its transmission rate is so low.

The behaviour of B chromosomes at meiosis is variable. In some species such as *Plantago serraria* (Frost, 1959), the Bs remain unpaired, regardless of the number in the cell, at metaphase I. Other species such as *Haplopappus gracilis* (Pritchard, 1968) and *Aegilops speltoides* (Mendelson & Zohary, 1972) show bivalent formation in the presence of two Bs, but the bivalents are seen only in a proportion of cells. In *Secale cereale* several studies have been made on the pairing behaviour of Bs (Sarvella, 1959; Kishikawa, 1965, 1966; Sybenga & De Vries, 1972). The plants used in these studies showed a very wide range in the frequency of pairing between Bs; some plants had mostly univalent configurations whereas in others pairing was fairly regular. Thus the pattern of B chromosome pairing at meiosis need not be considered to be constant for a species. In *B. humilis* there is some variation between plants in the frequency of B^L chromosome pairing but the degree of variation is much less than in rye. At this stage it is not clear whether this variation has a genetic basis or whether it is largely environmentally induced and it should be noted that no consistent relationship was observed between A and B chromosome chiasma frequency.

Despite the lack of a correlation between A and B^L chromosome chiasma frequency the B chromosomes do have an effect on the chiasma frequency of the A chromosomes. This is to reduce the frequency of chiasmata in the A chromosomes. However, it appears that the presence of more than $2B^L$ has no further effect on A chromosome chiasma frequency. The means for the $2B^L$ and $3B^L$ classes are very similar. Plants with more than $2B^L$ were not found in the original population and in general the plants in natural populations of grasses seldom contain high numbers of Bs. In *B. humilis* it would appear that there is a threshold, which corresponds to the maximum number of Bs in the population, beyond which additional B^L chromosomes have no further depressing effect on chiasma frequency. These results provide a contrast to observations on *B. media* and *B. elatior* where the B chromosomes appear to have no effect on A-chromosome chiasma frequency (Murray, 1976). However, the B chromosome effects on chiasma frequency are similar to those observed by Cameron and Rees (1967) in *Lolium perenne* and Zarchi *et al.* (1972) in *Aegilops speltoides*, but differ from those in many other species where Bs increase chiasma frequency (see Jones, 1975 for references).

The inheritance of the large B in general conforms to the 'grass' pattern. Presumably there is directed non-disjunction at the first pollen grain mitosis resulting in 0B and 2B progeny in crosses between 0B females and 1B males but this is not proven since B chromosome behaviour was not observed during pollen grain mitosis. However, this non-disjunction process is not completely efficient and in some crosses anomalous results were obtained. Variation was observed between crosses in the efficiency of transmission of the B^L ; in some crosses it was transmitted at a very low rate resulting in a net loss whereas in others the transmission rate was much higher. Nevertheless in the majority of crosses there was a deficiency of B chromosomes over expectation amongst the progeny. In view of the fact that the B^L showed highly regular meiotic behaviour, even when present as a univalent this would suggest that the B containing pollen is of reduced viability, or that B containing embryos/seedlings are less viable than those without Bs. The inheritance of the B^S was erratic for they showed no evidence of an accumulation mechanism and in the majority of crosses were not transmitted from par-

ent to progeny. It would appear that the gene or chromosome segment controlling non-disjunction is on the long arm of B^L and these have been lost in the deletion product B^S. With its very low transmission rate it is difficult to see how B^S is maintained in the population and its presence may be only transitory. The difference between crosses in the transmission rate of the B chromosomes suggests that either the A-chromosome genotype has an important effect or that this is a highly plastic character that is influenced by the environment. Even more extensive variation than this has been described by Parker *et al.* (1982) in *Hypochoeris maculata*. This species shows different transmission rates through pollen and egg both within and between plants and variation in the pollen transmission rate was also found between different years. Since *B. humilis* is an annual, comparisons between years is not possible but it is clear that further work needs to be done before we can have a complete understanding of the transmission of B chromosomes.

The supernumerary chromosome that is the main subject of this paper is large and is in fact only marginally smaller than the smallest A chromosome. However, the results of this study clearly show that this supernumerary exhibits many of the required characteristics of B chromosomes (Jones, 1975), and therefore is not a primary trisomic.

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