The transmission of B chromosomes in *Secale cereale* and *Secale vavilovii* populations. II. Dynamics of populations

M. J. Puertas,
A. Ramirez and
F. Baeza

Departamento de Genética, Facultad de Biologia, Universidad Complutense 28040 Madrid, Spain.

Experimental populations were established with individuals with and without B chromosomes at different frequencies. Two species were used: the cross-pollinating *Secale cereale*, naturally carrying B chromosomes, and the self-pollinating *S. vavilovii* in which Bs were introduced artificially. The plants were allowed to fertilise by open pollination and the offspring produced were studied for the distribution of B chromosomes over several successive generations. In *S. cereale* the equilibrium polymorphism was reached in only one generation. The mean number of Bs per plant and their distribution was different depending on the initial frequency of Bs in the established populations. In *S. vavilovii*, plants with many Bs were formed because of its mating system, which did not allow an equilibrium to be reached in one generation. The existence of equilibrium polymorphism for Bs in populations with a percentage of self-pollination is discussed.

INTRODUCTION

B chromosomes are found at different frequencies in natural populations. This can be due to several causes which are not mutually exclusive: (a) Genetic differences among populations, which could modify the values of parameters affecting the transmission of B chromosomes, *i.e.*, accumulation mechanisms, fitness of carriers, etc. (b) Environmental differences, which could also affect these values. (c) Differences in the frequencies at which B chromosomes were initially introduced into the population.

In rye, it has been demonstrated that differences in the variables related to B chromosome transmission (B univalent loss, rate of non disjunction and preferential distribution, etc.) affect the frequency of B chromosomes at equilibrium (Matthews and Jones, 1982, 1983).

Also in rye, it has been reported that environmental factors, such as the age of anthers, influence the rate of non disjunction (Puertas *et al.* 1979).

The present work studies the third hypothesis. For this, experimental populations were established, with plants carrying B chromosomes at different frequencies. The distribution of Bs in the offspring was studied in several successive generations. Two species of *Secale* were studied: *S.*

cereale which naturally carries B chromosomes, and *S. vavilovii* into which Bs were artificially introduced.

MATERIALS AND METHODS

The cross-pollinating species *Secale cereale* var. JNK (2n = 14) carrying B chromosomes, and the self-pollinating species *S. vavilovii* (2n = 14) were used. The latter naturally lacks B chromosomes.

Bs were introduced into *S. vavilovii* from *S. cereale* by hybridising both species and backcrossing to *S. vavilovii* for six successive generations. this line was named "Charito" (Puertas *et al.*, 1985)

The following experimental populations were established, each with 50 plants:

JNK with 20 per cent 0B and 80 per cent 2B plants

Charito with 20 per cent 0B and 80 per cent 2B plants

JNK with 20 per cent 2B and 80 per cent 0B plants

Charito with 80 per cent 0B and 20 per cent 2B plants

In the following we will name 2C those populations derived from *S. cereale* and established with 20 per cent 2B plants, 8C to those established with

80 per cent 2B plants, and correspondingly 2V and 8V to those drived from *S. vavilovii* with 20 per cent 2B and 80 per cent 2B plants, respectively.

Two replicates of each population were made, which we will name *a* and *b* (2Ca, 2Cb, etc.). Each replicate was sown in 1 m^2 plot. Individuals with and without B chromosomes were distributed at random in each plot. The 8 plots were adjacent and simultaneously sown. At the moment of anthesis each plot was covered with a thin white cotton fabric to prevent cross-pollination among plots, allowing open pollination within each one.

A sample of 400 seeds was taken from each 8C and 2C plot, and 200 from 8V and 2V. About 100 seeds were used for counting the number of B chromosomes, and the remainders were sown to obtain a new generation. Two successive generations were obtained in JNK and three in Charito.

In the following, the number 0, 1, 2 or 3 will indicate the generation studied, being the generation 0 that corresponding the year of the first sowing, 1 the first offspring, and so on. For example, the population named 2C1a correspond to the replicate *a* of the population derived from *S. cereale* initiated with 20 per cent 2B plants, obtained the first year. Table 1 shows a scheme of all populations studied.

For chromosome counts, root tips were fixed in acetic alcohol 1:3, and stained by the Feulgen method.

RESULTS

The variety JNK of *Secale cereale* was received in our laboratory in 1974. Then, we studied the distribution of B chromosomes present in that sample. This material was maintained in our laboratory by open pollination till 1981, when a new control of B number was made. A χ^2 contingency test between both distributions resulted non significant. Weighted frequencies were calculated, and they will be considered the equilibrium frequencies for this variety (table 2).

The descendants obtained in two successive generations of 8C and 2C experimental populations are shown in table 3. Similarly, the descendants obtained in three successive generations of 8V and 2V populations are shown in table 4.

In order to compare the distribution of B chromosomes in the populations we have used χ^2 contingency tests. Since 1B and 3B plants are usually present in a low number, we have followed the criterion of summing 1B plants to 2B ones and/or 3B plants to 4B ones when less than 5 plants were present in a class. Plants with more than 4Bs were very rare and they were not considered.

Two by two replicates of the same generation, successive generations, and different replicates of different generations were compared in all possible combinations. In the case of populations derived from *S. cereale* they were also compared with the equilbrium values of the original variety JNK.

In both 8C and 2C populations the distribution of B chromosomes and the mean number of Bs per plant was maintained in two successive generations (table 3). In only one case (2C1a and 2C2b) significant differences were found, but it has to be noted that the latter had the lowest number of plants analysed. Nevertheless, in both populations these values were significantly different to the original JNK variety. The mean values of the number of Bs per plant cannot be compared by "t" tests since the distribution of Bs in the populations is not normal.

In *S. vavilovii* (table 4) it can be firstly observed that there were no significant differences between replicates. Differences between the first and second generation were highly significant in all cases; however, differences between the second and third generation were non significant with only one exception (8V2b and 8V3b, table 4). Obviously, it was not necessary to calculate the χ^2 values between the first and third generation.

Since *S. vavilovii* lacks B chromosomes in nature, it was not possible to compare these populations with a known equilibrium values.

Table 1   Scheme of the experimental populations studied

Year of sowing	Populations derived from *Secale cereale*				Populations derived from *Secale vavilovii*			
	JNK 20% 0B+80% 2B (8C)		JNK 20% 2B+80% 0B (2C)		Charito 20% 0B+80% 2B (8V)		Charito 20% 2B+80% 0B (2V)	
1981–82	8C0a	8C0b	2C0a	2C0b	8V0a	8V0b	2V0a	2V0b
1982–83	8C1a	8C1b	2C1a	2C1b	8V1a	8V1b	2V1a	2V1b
1983–84	8C2a	8C2b	2C2a	2C2b	8V2a	8V2b	2V2a	2V2b
1984–85					8V3a	8V3b	2V3a	2V3b

Table 2 Frequencies of individuals carrying B chromosomes in *Secale cereale* var. JNK

| | B chromosome number of individuals | | | | | |
	0	1	2	3	4	Total
1974 control	47	10	38	7	17	119
	0·395	0·084	0·319	0·059	0·143	
1981 control	152	80	125	52	89	498
	0·305	0·161	0·251	0·104	0·179	
Total weighted	199	90	163	59	106	617
frequencies	0·322	0·146	0·264	0·096	0·172	

Table 3 Number of individuals carrying B chromosomes in succesive generations of populations derived from *Secale cereale*

| Population | B chromosome number of individuals | | | | | Total | Mean No. of Bs per plant |
	0	1	2	3	4		
8C1a	28	4	34	2	40	108	2·20
8C1b	34	–	41	1	43	119	2·16
8C2a	31	5	23	6	27	92	1·92
8C2b	21	8	36	3	28	96	2·09
2C1a	44	3	41	–	7	95	1·19
2C1b	47	2	34	5	13	101	1·36
2C2a	46	5	33	3	7	94	1·15
2C2b	41	3	20	1	12	77	1·22

Table 4 Number of individuals carrying B chromosomes in succesive generations of populations derived from *Secale vavilovii*

| Population | B chromosome number of individuals | | | | | Total | Mean No. of Bs per plant |
	0	1	2	3	4		
8V1a	13	1	10	7	36	67	2·78
8V1b	8	1	5	1	30	45	2·98
8V2a	47	–	18	–	16	81	1·23
8V2b	40	–	23	–	18	81	1·46
8V3a	48	–	16	–	32	96	1·67
8V3b	56	1	10	–	20	87	1·16
2V1a	67	1	6	5	17	96	1·00
2V1b	29	1	–	1	21	52	1·69
2V2a	78	–	3	–	3	84	0·21
2V2b	67	–	4	–	3	74	0·27
2V3a	89	–	3	–	2	94	0·15
2V3b	80	–	–	–	5	85	0·23

DISCUSSION

The frequencies of individuals carrying B chromosomes in the rye variety JNK was constant in our laboratory over 7 years (table 2). This indicates that the polymorphism for B chromosomes can be maintained in equilibrium.

The causes of the maintenance of polymorphisms for Bs have been discussed by a number of authors (Jones and Rees, 1982 for a review). Two main causes have been proposed: (a) B chromosomes in some way provide an adaptive advantage to carriers, (b) Bs are maintained due to the balance between their tendency to increase in number and the low fitness of the carriers of a high number of Bs. This behaviour can be considered parasitic or selfish (Jones, 1985), since Bs would not provide any adaptive advantage to the carrier, and their accummulation mechanisms would be sufficient to maintain themselves.

In rye, the second hypothesis seems to be true. It has been found that plants with Bs show a low fitness (Müntzing, 1963) and evidences for the selfish nature of Bs have been reported *i.e.*: the accumulation mechanisms and the effects on carriers are due to B chromosomes themselves, irrespective of the genetic background (Puertas *et al.*, 1979, 1985). In addition, it seems that B polymorphism is maintained by a frequency-dependent selection (Puertas *et al.*, 1986) which is expected in the case of parasitic-like behaviour.

Therefore, if the maintenance of B chromosomes is mainly due to their own genetic system it would be expected that polymorphism would be maintained at a wide range of frequencies. However, this range must be limited since (a) a proportion of individuals without Bs must exist to ensure 0B × B crosses, giving rise to descendants with a low number of Bs, and (b) the proportion of individuals with many Bs must be low, otherwise the load imposed on the population would be too high.

1. Secale cereale

It can be observed that the differences between successive generations are not significant, both in 8C and 2C populations. It seems that the mean number of Bs per plant was also conserved. Therefore, it can be concluded that equilibrium polymorphism was reached in only one generation in both cases, although the equilibrium frequencies were different in 8C, 2C and JNK.

However, the main difference is due to 0B and 4B classes, the frequency of 2B plants being similar in all cases.

This can be interpreted as follows: when the population was initiated with a high number of Bs, the number of plants with 4B in the next generation was high, due to non disjunction of Bs. Nevertheless, 4B plants would give few offspring due to their low fitness. On the other hand, the

crosses $0B \times 2B$ would be able to maintain B chromosomes in the population. The mean number of Bs in the population is very high, due to 4B plants.

By contrast, when the population was initiated with a low number of plants with Bs (as in 2C) the proportion of 4B plants remained low, but $0B \times 2B$ crosses would produce plants carrying Bs in the next generation. The mean number of Bs in the population is low.

It can be concluded that 2B plants are most important in maintaining the polymorphism, and the distribution of Bs depend on their frequency at the moment of the establishment of the population.

In natural populations of rye, different distributions and mean number of Bs are found. The initial frequency at which B chromosomes are introduced in the population would be a determinant of both variables.

2. *Secale vavilovii*

The study of the dynamics of B chromosomes in this species offers special interest since it is self-pollinating. None of the hundreds of plant species in which B chromosomes have been reported to date is self-pollinating. The only case in which Bs have been intorduced into a self-pollinating species is the "Lindstrom" line obtained by Müntzing (1973).

It is supposed that selfing is incompatible with the existence of Bs since non-disjunction tends to increase the number of Bs each generation and plants carrying a high number of Bs have a low fitness. Therefore, Bs can only be maintained in cross-pollinating populations in which crosses between plants with and without Bs are frequent.

Let us make an estimation of the amount of selfing compatible with the existence of B chromosomes. Let us consider a population with a proportion S of self-pollination and $(1-S)$ of cross-pollination. Let us name B the proportion of plants with Bs, and $(1-B)$ the proportion of plants without Bs.

A proportion SB of plants will give rise to plants with many Bs in the next generation, a proportion $S(1-B)$ will give rise to plants with 0Bs, a proportion $(1-S)B^2$ will give rise to plants with many Bs, a proportion $(1-S)2B(1-B)$ will give rise to plants with a low number of Bs, and finally, a proportion $(1-S) (1-B)^2$ will give rise to plants without Bs. Therefore, for the maintenance of B chromosomes in the population it is necessary that

$$SB + (1-S)B^2 < (1-S)2B(1-B)$$

That is, the proportion of plants giving rise to an offspring with many Bs (which have a low fitness) must be lower than the proportion of plants giving a low number of Bs. Then,

$$SB + B^2 - SB^2 < 2B - 2B^2 - 2BS + 2B^2S,$$

$$S(3B - 3B^2) < 2B - 3B^2,$$

$S < (2\text{-}3B)/(3\text{-}3B)$, in this quotient, the denominator is always positive since B is always less than 1; then, when $2 > 3B$ the quotient will be positive; therefore, giving values to $B < 2/3$, the limit for S can be calculated. For example: for $B = 0.05$, $S < 0.65$; for $B = 0.10$, $S < 0.63$; for $B = 0.50$, $S < 0.33$, for $B = 0.66$, $S < 0.02$ etc.

This estimation shows that to maintain B chromosomes in populations a proportion of Bs lower than 2/3 is required, and when the frequency of Bs is increasing selfing must decrease.

The results of this work show that the distribution of B chromosomes in the first generation of *S. vavilovii* was different from that of the second generation, while the difference between the second and third generations was non significant. Is this a demonstration that the equilibrium for B chromosomes was reached also in *S. vavilovii*?

It can be observed that the population initiated with 80 per cent 2B plants (8V) formed a high proportion of 4B plants in the next generation (8V1) due to selfing of 2B plants, and a low proportion of 2B plants probably due to $0B \times 2B$ crosses. This situation is impossible to maintain due to the low fitness of 4B plants.

At the next generation (8V2) the number of 4B plants decreased, as expected, since the proportion of 2B plants was low in the preceding generation, but 2B plants increased in number, which is only possible if $0B \times 2B$ cross-pollination occurred. The values of the next generation (8V3) were not significantly different of 8V2; however, it is doubtful whether this absence of statistical differences reflect that equilibrium was really reached rather the loss of B chromosomes is now slow and undetectable in only one generation.

A similar situation was found in 2V populations. In the first generation (2V1) the proportion of 4B plants was higher than 2B ones, which is impossible to maintain. The 4B plants tended to decrease in the following generations, and a low proportion of 2B plants is maintained without statistical differences between 2V2 and 2V3 generations. Again, it is difficult to predict the final fate of Bs in this population. Our hypothesis is that if pollen carrying B chromosomes is able to pollinate 0B plants in a sufficient proportion, the poly-

morphism for B chromosomes will be maintained, even at a very low frequency of Bs; while if $0B \times 2B$ crosses occur less frequently, B chromosomes will be lost, the number of generations depending on the ability of pollen carrying Bs to pollinate 0B plants.

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