Frequency-dependent fertilisation involving rye B chromosomes

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The transmission of B chromosomes in rye was studied using both an isozyme locus (*Got-3*) and B chromosomes as markers of the gametes involved in fertilisation. Four experimental populations were established in which male parents carried 0 or 2Bs at different frequencies and were homozygous or heterozygous for *Got-3*. Female parents carried 0B or 2B and were all homozygous. The origin of the pollen types involved in fertilisation was recognized in the descendants by determining their B number and their *Got-3* phenotype. The data showed firstly, that pollen produced by 0B or 2B males did not fertilise at random when these males were present at different frequencies. Secondly, that pollen from 2B plants was preferentially involved in fertilisation when the proportion of plants carrying 2B was low and, conversely, pollen from 0B plants was preferentially involved in fertilisation when the proportion of 2B plants was high. The selfish nature of B chromosomes is discussed.

INTRODUCTION

The non-Mendelian mode of transmission of rye B chromosomes is well known at the cytological level, with Bs undergoing directed non-disjunction to the nucleus giving rise to the gametes (Müntzing, 1946; Håkanson, 1948). The processes result in the accumulation of Bs in a proportion of the gametes, and this accumulation is compensated for by the low fitness of plants carrying a high number of Bs (Müntzing, 1963; Puertas *et al.*, 1985).

The mode of transmission of Bs at the population level, however is still not completely understood. In a recent paper (Puertas *et al.*, 1986) we proposed that B chromosome behaviour is selfish-like, since the advantage of gametes carrying Bs seemed to be of benefit only to the B chromosomes themselves. The advantage or disadvantage of gametes carrying Bs depended on the frequency of B chromosomes in the population. Pollen carrying B chromosomes became relatively more advantageous when its frequency declined. Therefore, we proposed that frequency-dependent selection occurs to maintain the B chromosome polymorphism in rye.

Rye plants with Bs produce male and female gametes with and without Bs at different frequencies. Thus, it is usually impossible to determine

whether a 0B plant is descendant from a 0B × 0B, 0B × B or B × B cross. In the present work we have used an isozyme locus (*Got-3*) as a marker of the gametes involved in fertilisation, in order to determine the differential fertilisation of gametes with or without B chromosomes, depending on the frequency of Bs in the population.

MATERIAL AND METHODS

Secale cereale of the variety JNK was used. This variety naturally carries B chromosomes.

The seeds were first germinated on moist filter paper and the chromosome number of each plant was then established in the root tips. Roots were fixed in 1:3 acetic alcohol and then stained by the Feulgen method.

When these plants reached a convenient stage of development a sample of their leaves was analyzed by protein electrophoresis to determine their phenotypes for glutamate oxaloacetate transaminase (GOT). In particular, we have studied the GOT-3 isozyme. This is a dimeric enzyme controlled by a single locus (*Got-3*) with two alleles (Pérez de la Vega and Allard, 1984), so that, three isozyme phenotypes can be detected: *11*, *12* and *22*. In the following, numbers in italics will refer to alleles or phenotypes for *Got-3*.

Electrophoresis was carried out using crude leaf extracts absorbed in paper wicks (Whatman No. 3, 0.2×0.5 cm) and inserted in 8 per cent horizontal polyacrylamide gels. Tris-citric acid, pH 7.75 was the gel buffer, and NaOH-boric acid was the electrode buffer. The staining procedure was that described by Decker and Rau (1963), with minor modifications.

After determining the B chromosome number and the *Got-3* phenotype of each individual plant, the appropriate plants were transplanted in an experimental field and arranged in four plots, as given in table 1.

Rye has hermaphroditic flowers and is mainly cross-pollinating. To be certain that no self-pollination occurred within plots, however, the spikes of plants that were used as females were emasculated. Nothing was done to plants used as males, and in the following, we shall name emasculated plants "females" and non-emasculated plants "males".

In all plots (i) the males were sown in the centre of the plot with the females surrounding them, (ii) plants with different numbers of B chromosomes and GOT phenotypes were sown at random within each plot, (iii) at the time of anthesis each plot was covered with a thin white cotton fabric to allow free pollination within each plot, and to prevent pollination among plots, so that pollen was potentially able to fertilise at random all the females within a given plot, (iv) offspring were collected individually from females only, (v) the

number of flowers per plant was counted in the males (table 1).

In plot 1 all males were 0Bs, and 50 per cent of them were 11 for *Got-3* and 50 per cent 12. Females were all 11, but 50 per cent were 0B and 50 per cent 2B. Therefore, this plot serves as a control of the equal viability of male gametes carrying alleles 1 or 2 on 0B or 2B females.

In plots 2 to 4 all females were homozygous 11, but 50 per cent carried 0B and 50 per cent 2B. Males were homozygous or heterozygous for *Got-3*, and had 0B or 2B in variable proportions in order to detect possible frequency-dependent deviations from random expectations.

The seeds of the offspring were analyzed to establish the GOT phenotype, the number of Bs, or both, by the techniques described above.

RESULTS

Table 2 shows the comparison between observed and expected *Got-3* phenotypes in the offspring of both the 0B and 2B females.

It should first be noted that the proportion of males given in this table is different from that given in table 1. This is because rye plants form a variable number of spikes per plant, and a variable number of flowers per spike. The total mass of pollen involved in fertilisation will be dependent on the number of flowers, rather than the number of plants. We therefore counted the number of flowers in males and the corresponding proportions of males were estimated.

Expected distribution of descendants were calculated as follows.

Plot 1: 3/4 of male gametes carried allele 1, while 1/4 carried allele 2. Since all females were 11, 3/4 of descendants will be 11 and 1/4 12, on both the 0B and the 2B females. Observed offspring coincide almost exactly with expectations.

Plot 2: the male flower proportion was ten 12 to one 11; therefore, we expect 12/22 of descendants to be 11 and 10/22 to be 12, on both the 0B and the 2B females. The difference between the observed and the expected offspring of 0B females was significant. Following the same reasoning in plot 3, in which the male proportion was five 12 to one 11, we expect 7/12 of descendants to be 11 and 5/12 to be 12. In plot 4 the expectation is 3.5/5 descendants 11 and 1.5/5 12.

In plots 3 and 4 the observed results did not differ significantly from the expected ones. It should also be noted that observed and expected distributions coincide almost exactly in the descendants of 2B females.

Table 1 Composition of the plots

Plot		No. plants	No. flowers	No. Bs	<i>Got-3</i> phenotype
1	Females	5		0	11
		5		2	11
	Males	7	978	0	11
		7	985	0	12
2	Females	7		0	11
		7		2	11
	Males	12	3121	0	12
		3	316	2	11
3	Females	7		0	11
		7		2	11
	Males	3	620	0	11
		12	2948	2	12
4	Females	7		0	11
		7		2	11
	Males	7	766	0	11
		7	1136	2	12

Table 2 Comparison between observed and expected offspring for *Got-3* phenotypes assuming random fertilisation

Plot	Males Propor.	Bs	GOT		GOT phen. in offspring of 0B females			GOT phen. in offspring of 2B females		
					11	12	χ^2	11	12	χ^2
1	1	0	11	obs.	93	30	ns	94	32	ns
	1	0	12	exp.	92.25	30.75		94.5	31.5	
2	10	0	12	obs.	120	74	*	115	79	ns
	1	2	11	exp.	105.8	88.2		106.2	87.8	
3	1	0	11	obs.	270	174	ns	70	52	ns
	5	2	12	exp.	259	185		71.2	50.8	
4	1	0	11	obs.	175	65	ns	88	37	ns
	1.5	2	12	exp.	168	72		87.5	37.5	

ns Non-significant.

* Significant at 0.05 level.

In addition to *Got-3* phenotype, the number of B chromosomes was determined in a portion of the progeny of 0B females in plots 2, 3 and 4. The observed and expected data are shown in table 3.

For the calculation of the expected distributions two approaches can be used. The first one is based on the fact that 2B plants produce 4 per cent of pollen grains without Bs. This frequency has been estimated by direct observation of first and second pollen grain mitosis (Kishikawa, 1965; Puertas *et al.*, 1979). Let us take plot 2 as an example. In this plot there was a ratio of 10 males with 0B 12 to 1 male with 2B 11. 0B males produce gametes 0B 1 and 0B 2 in a proportion of 10/22 each. Therefore, the probability of obtaining 0B 11 progeny is $10/22 + 0.04$ (2/22), the probability of obtaining 0B 12 progeny is 10/22, and the probability of 11 progeny with Bs is 0.96 (2/22). The expectations for plots 3 and 4 were calculated in a similar manner.

Observed and expected values differed significantly in plots 2 and 3, while in plot 4 the differences were nonsignificant. For the calculations of χ^2 values the class 0B 12 in plots 3 and 4 was not considered since the expected number is very low.

The second approach was based on the estimation of 0B pollen grains formed in 2B plants that were involved in fertilisation. The frequencies of zygotes with or without Bs formed in 0B females can be estimated from the data shown in table 3. For example, in plot 3 six 12 plants with 0B were obtained; therefore, $6/48 = 0.125$ is the frequency of 0B gametes involved in fertilisation, and so on. These estimations are shown in table 4. They were used for calculating the expected progeny of each plot in a similar manner to that of the first approach, changing the value 0.04 by 0.15, 0.125, or 0.214, respectively. The deviations from random expectations were similar using both approaches.

Table 3 Isozyme phenotype and B chromosome number in descendants from 0B females

Plot	Observed						Expected 1			Expected 2		
	<i>Got-3</i>	0B	1B	2B	3B	4B	0B	with Bs	χ^2	0B	with Bs	χ^2
2	11	27	5	11	0	1	31.14	5.91	***	31.84	5.26	***
	12	24					30.94	—		30.90	—	
3	11	39	14	21	0	3	22.92	50.00	***	27.33	45.57	*
	12	6	17	22	1	2	2.08	50.00		6.51	45.57	
4	11	52	8	30	2	3	56.44	39.45	ns	63.59	32.30	ns
	12	9	7	25	0	1	1.64	39.45		8.79	32.30	

*** Significant at 0.001 level.

* Significant at 0.05 level.

ns Non-significant.

Table 4 Estimated frequencies of zygotes formed in 0B females which were fertilised by 2B males

No. of Bs in pollen	Plot			Pollen formed*
	2	3	4	
0	0.15	0.125	0.214	0.04
1	0.25	0.354	0.167	0.27
2	0.55	0.458	0.595	0.64
3	0.00	0.021	0.000	0.02
4	0.05	0.042	0.024	0.03

* Data from Kishikawa (1965) and Puertas *et al.* (1979).

DISCUSSION

In previous papers (Puertas and Lacadena, 1974; Puertas *et al.*, 1986) we have demonstrated that rye B chromosomes are not transmitted at random. The different types of B-carrying pollen do not fertilise at the frequencies at which they are produced, but the involvement of pollen in fertilisation is also dependent on the frequency of plants with or without Bs in the population. The results reported in this work are in agreement with our previous data. We have used an isozyme marker locus (*Got-3*) for the unambiguous identification of the pollen involved in fertilisation when various males (with or without Bs) were in competition.

The design of this experiment could be improved using only homozygous 11 and 22 plants, instead of 11 and 12. However, the frequency of 22 plants is low in this population; furthermore, the frequency of 22 plants with a given number of Bs is so low that the experiment would be unattainable.

Firstly, we have analyzed the progeny obtained with respect to *Got-3* phenotypes (table 2). Plot 1 serves as a control for the Mendelian transmission of *Got-3* alleles, since males did not carry Bs. Observed and expected offspring coincide both on 0B and 2B females. Therefore, it can be concluded that *Got-3* is a good marker for our purpose and the deviations from random found in other plots can be attributed to the effects of the B chromosomes.

In plot 2 there were more male parents without Bs. In the offspring of 0B females there are more 11 descendants observed than expected. This must be due to an excess of fertilisation by gametes carrying the allele 1. Since male parents with Bs were 11, this suggests that pollen formed in plants with Bs were preferentially involved in fertilisation.

In 2B females the difference between observed and expected offspring is not statistically sig-

nificant, although it can be seen that there are also more 11 descendants than are expected.

In plot 3 the proportion of plants with and without Bs was contrary to plot 2: there were more male parents with Bs. The difference between the observed and expected offspring of the 0B females is not significant, but it can be seen that there are more 11 plants. Since in this case males with Bs were 12, it seems that pollen from plants with Bs took part in fertilisation less frequently than expected. This proposal will be considered below using the data in table 3.

The offspring collected on 2B females coincide almost exactly with expected, indicating that pollen from 0B or 2B male parents fertilised 2B females at random.

In Plot 4 the same number of 0B and 2B male plants was sown, in order to study the effect of absence of competition. However, the variation in flower number resulted in a slightly greater proportion of male flowers with Bs. The results of this plot are similar to those of plot 3.

In addition to the *Got-3* phenotype, the number of Bs was counted in a portion of the descendants of 0B females in plots 2, 3 and 4. Table 3 shows the phenotype of plants with respect to both the number of Bs and the *Got-3* locus. This double mark serves to determine more precisely the origin of pollen that participates in fertilisation. With the analysis of only the *Got-3* locus we cannot know how many 11 plants were the progeny of 0B or 2B males, because allele 1 was present in both. However, by counting the number of Bs we are sure that all 11 plants with Bs must be formed by pollen from 2B males carrying allele 1.

The observed and expected offspring shown in table 3 were significantly different in plots 2 and 3. These data demonstrate: firstly, the nonrandom fertilisation by pollen produced on 0B or 2B males. Secondly that, pollen from 2B plants preferentially took part in fertilisation when there were few 2B males (plot 2) and, conversely, that pollen from 0B plants preferentially took part in fertilisation when there were few 0B males (plot 3). For example: 5.91 plants with Bs were expected in plot 2, but 17 were obtained; 100 plants with Bs were expected in plot 3, but 80 were obtained.

In plot 4 in which the 0B and the 2B males were approximately equal in proportion, the observed progeny did not differ significantly from the expected, indicating that pollen from 0B and 2B males participates in fertilisation at random when there is no competition.

A surprising result of this work is the difference between the frequencies of the different types of

pollen grains formed in a 2B plant, as estimated by direct observation of pollen mitoses, and the frequencies of pollen grains involved in fertilisation, as estimated from the observed progeny (table 4). The proportion of 0B pollen formed is only 4 per cent, while it seems that it fertilises in a much higher proportion (12.5 to 21 per cent). This can occur if 0B pollen is relatively more successful in fertilisation than pollen with Bs, or 0B zygotes are more viable, or both. However, this is not in contradiction with our previous conclusion, since pollen formed in 2B plants can be advantageous or disadvantageous depending on the frequency of plants with or without Bs in the population, while 0B pollen grains or 0B zygotes can be always more viable. Therefore, it seems that the competitive ability of pollen from 0B or 2B plants is sporophytically determined, irrespective of whether or not that pollen, or the zygotes, with or without Bs have different viabilities.

Our results strongly suggest that the competitive ability of pollen formed by 0B or 2B plants on 0B females varies according to the frequency of plants with or without Bs in the population. It seems that when pollen grains land on a 0B stigma the competitive ability of pollen of different parents is dependent on, and inversely related to their own proportion. This mechanism is favourable for B chromosomes because it tends to maintain them at a moderate frequency in a population (plants with many Bs have a very low fitness).

On the contrary, it also seems that on 2B females there is no competition between pollen from 0B or 2B plants, since the observed offspring coincides very well with expectations. If the main strategy of Bs is to maintain themselves, this absence of competition is also favorable for Bs since 2B females transmit B chromosomes to the next generation irrespective of the male. However, this conclusion is not firmly established since we only have data of *Got-3* and not of the number of Bs in descendants of 2B females, because it is not possible to distinguish if their Bs come from male, female or both parents.

The main conclusion of this work is that pollen formed by 0B and 2B plants has a differential fertilising ability on 0B females which is dependent

on the frequency of 0B or 2B plants. By this mechanism the polymorphism for B chromosomes can be maintained in equilibrium by frequency-dependent selection.

These properties make rye B chromosomes one of the best examples of selfish DNA: they do not have any known gene function, their effects are neutral or deleterious for carriers (see Jones and Rees, 1982 for a review), their transmission mechanisms tend to increase their number in spite of their deleterious effects, and finally their maintenance is frequency-dependent which is very appropriate for a parasitic type of behaviour.

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