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The transmission of B chromosomes in populations of *Secale cereale* and *Secale vavilovii* 1. Offspring obtained from 0B and 2B plants

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The transmission of rye B chromosomes in experimental populations of *Secale cereale* and *S. vavilovii* was studied. B chromosomes were introduced from *S. cereale* into *S. vavilovii* by hybridisation and backcrossing. Populations were established with plants carrying 0B or 2B chromosomes at different frequencies. Each population was isolated and allowed to fertilise by open pollination. Seeds were collected individually from each 0B or 2B plant. The number of B chromosomes in the offspring was analysed. The results showed that there was not a direct relationship between the frequencies at which each type of pollen was formed and the offspring obtained. On the contrary, the effectiveness in fertilisation by pollen with or without B chromosomes was mainly dependent on the frequencies of plants with and without B chromosomes in the population; that is, pollen carrying B chromosomes became relatively more advantageous when its frequency declined. We discuss the proposal that frequency-dependent selection occurs for rye B chromosomes.

INTRODUCTION

The nature of the genetic mechanisms controlling the non-Mendelian mode of transmission of B chromosomes is still incompletely understood.

B chromosomes are transmitted following species-specific rules. In rye, (*Secale cereale L.*), their meiotic segregation is mainly normal although some univalents can be formed (Kishikawa, 1965). At first mitotic division of both male and female gametophytes B chromosomes undergo non disjunction and preferential distribution to the nucleus giving rise to the gametes (Müntzing, 1946; Håkanson, 1948). This mode of transmission tends to accumulate B chromosomes in populations. The accummulation is compensated by the low fitness of plants carrying a high number of B chromosomes (Müntzing, 1963; Puertas *et al.*, 1985). These mechanisms also explain the variation of B chromosome number among the individuals within populations.

This work describes investigations into the transmission of rye B chromosomes in experimental populations in two *Secale* species: *S. cereale* which naturally carries B chromosomes, and *S. vavilovii* which does not. In the latter, B chromosomes were introduced from *S. cereale.*

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 does not naturally carry B chromosomes.

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

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

Population	Abbreviation
S. cereale with 20 per cent 0B +80 per cent 2B plants	8C
S. vavilovii with 20 per cent 0B +80 per cent 2B plants	8V
S. cereale with 20 per cent 2B +80 per cent 0B plants	2C
S. vavilovii with 20 per cent 2B +80 per cent 0B plants	2V

The chromosome numbers of the parent plants were determined by root tip screening before

planting. The roots were fixed in acetic alcohol 1:3 and stained by the Feulgen method.

Two replicates of each population were sown in $1m^2$ plots. Individuals with and without B chromosomes were distributed at random in each plot. The 8 plots were adjacent and all were planted at the same time.

At the time of anthesis each plot was covered with a thin white cotton fabric to prevent cross-pollination among plots, but allowing open-pollination within each one.

Seed was collected individually from each 0B or 2B plant, in order to know the female parent chromosome number of the offspring. From each plot two samples were taken: about 100 seeds from 0B plants and 100 seeds from 2B plants.

The chromosome number of the offspring was determined in the root tips.

RESULTS

The between-replicate heterogeneity χ^2 was non significant in all cases; and the replicate data were therefore pooled for further analysis.

Cross-pollination was expected to occurr among the plants of populations derived from *S. cereale*, while self-pollination was expected in the plants of populations derived from *S. vavilovii* due to the natural mating system of these species.

To establish the expected frequencies of the descendants of each experimental population under cross- or self-pollination, it is necessary to know the types and frequencies of gametes formed by plants carrying 2B chromosomes.

The formation of male gametes mainly depend on the loss of B univalents during meiosis and on the frequency of directed non disjunction during pollen mitosis. Both parameters can be estimated directly from microscopic observations. The values given in table 1 are the frequences obtained by Kishikawa (1965) and Puertas *et al.* (1979)

Table 1 Types and frequencies of gametes produced in *S. cereale* (JNK) and *S. vavilovii* (Charito) plants with 2B chromosomes. Data of pollen were obtained from direct microscopic observations, data of egg-cells were obtained from 2B×0B crosses

		B chromosome number of the gametes				
		0B	1B	2B	3B	4B
JNK	Pollen	0·0366	0·2383	0·6329	0·0135	0·0183
	Egg-cell	0·2512	0·0151	0·7114	0·0	0·036
Charito	Pollen	0·057	0·2211	0·7087	0·0119	0·0013
	Egg-cell	0·12	0·0	0·88	0·0	0·0

The types and frequencies of female gametes have to be indirectly estimated from 2B×0B crosses. The values shown in table 1 correspond to a number of crosses made during several years in our laboratory which are not previously published.

It has to be noted that in all tables presented in this work gametes or individuals with more than 4B chromosomes were not considered since their frequency was always very low. These classes were added to the 4B one.

Once the female and male gamete frequencies were known in both species, the expected frequencies for the different types of descendants were calculated for each experimental population. These frequencies are shown in table 2. Obviously, the values calculated for populations derived from *S. cereale* were different from the 8C and 2C populations, since they were estimated assuming cross-pollination. However, the values estimated for populations derived from *S. vavilovii* were the same for both 8V and 2V populations, since they were estimated assuming self-pollination. In this case, the seeds produced by a plant do not depend on the other plants of the population.

Table 2 Frequencies of descendants expected in the experimental populations. In *S. cereale* (JNK) these frequencies were calculated for cross-pollination, in *S. vavilovii* (Charito) they were calculated for self-pollination

Population	Female parent	Offspring 0B	1B	2B	3B	4B
JNK 8C	0B	0·2293	0·2389	0·5063	0·0108	0·0146
	2B	0·0576	0·0635	0·2939	0·1775	0·4075
JNK 2C	0B	0·8073	0·0597	0·1266	0·0027	0·0037
	2B	0·2028	0·0272	0·6070	0·0451	0·1179
Charito	0B	1·00				
	2B	0·0068	0·0265	0·1353	0·1960	0·6354

Table 3 shows the descendants observed in 8C and 2C populations obtained on 0B and 2B plants, and the values expected according to the frequencies calculated in table 2. It can be seen that the differences between observed and expected values are very large. χ^2 values were highly significant in all cases.

Table 4 shows the same for populations derived from *S. vavilovii*: 8V and 2V. Also in this case the differences between observed and expected values were remarkable.

The significant differences between observed and expected values in the descendants indicate that the gametes involved in fertilisation were not

Table 3 Offspring obtained from populations derived from *S. cereale* and values expected from cross-pollination. All χ^2 tests were significant

Population	Female parent		Offspring 0B	1B	2B	3B	4B	Total
JNK 8C	0B	obs.	107	7	58	6	33	210
		exp.	48·15	50·17	106·32	2·27	3·06	
	2B	obs.	28	5	71	13	103	220
		exp.	12·67	13·97	64·66	39·05	89·65	
JNK 2C	0B	obs.	144	10	54	4	8	220
		exp.	177·61	13·13	27·85	0·60	0·81	
	2B	obs.	31	2	81	11	56	181
		exp.	36·71	4·92	109·87	8·16	21·33	

Table 4 Offspring obtained from populations derived from *S. vavilovii* and values expected from self-pollination. All χ^2 tests were significant

Population	Female parent		Offspring 0B	1B	2B	3B	4B	Total
Charito 8V	0B	obs.	83	0	11	4	50	148
		exp.	148					
	2B	obs.	3	1	15	13	148	180
		exp.	1·22	4·77	24·35	35·28	114·37	
Charito 2V	0B	obs.	201	0	10	1	26	238
		exp.	238					
	2B	obs.	2	1	34	5	92	134
		exp.	0·91	3·55	18·13	26·26	85·14	

in the same frequencies as those expected according to direct pollen observations and egg-cell estimations. In view of this discrepancy it is therefore necessary to estimate the types and frequencies of gametes that actually took part in fertilisation in each population.

The descendants obtained on 0B plants indicate the types and frequencies of pollen involved in fertilisation, since 0B plants produce only gametes without Bs, and the distribution of Bs among their descendants is a direct reflection of the types of pollen that actually fertilised them.

Assuming that the mass of pollen nuclei that fertilised 0B plants was the same that fertilised 2B plants, then the types and frequencies of female gametes actually fertilised can also be estimated. Let p, q, r, s, t be the frequencies of 0, 1, 2, 3 and 4B carrying female gametes to be estimated; let p', q', r', s', t' be the the frequencies of the corresponding male gametes (which are known from descendants of 0B plants), and let P, Q, R, S, T be the frequencies of descendents with 0, 1, 2, 3 and 4B obtained in the population. The frequencies of female gametes can thus be easily estimated, since $pp' = P$; $pq' + p'q = Q$; $pr' + qq' + rp' = R$, etc.

The values estimated for female and male gametes formed (based on table 1) and actually fertilised in populations derived from *S. cereale* are shown in table 5.

Table 6 shows these values calculated for populations derived from *S. vavilovii*. In this case female fertilised gametes cannot be estimated due to self-pollination. The assumption that the mass of pollen that fertilised 0B plants was the same that fertilised 2B plants cannot be made.

The values estimated in tables 5 and 6 were used to obtain the ratios between the gametes with Bs and without Bs formed and fertilised in each population. The ratios have been represented in a Dewitt graph (figure 1)

DISCUSSION

Populations derived from S. cereale

The deviation between observed values of descendants obtained in 8C and 2C populations and those expected assuming cross-pollination (table 3) was very large. For example, in the 8C population the descendants without Bs collected on 0B plants were more than double the number expected. A percentage of self-pollination could be supposed to account for this deviation. However, this seems not to be the case due to two reasons: (a) in this

Table 5 Frequencies of gametes formed and taking part in fertilisation in populations derived from *S. cereale*

	8C population				2C population			
Number of Bs	total pollen formed	total pollen fertil.	egg-cells formed	egg-cells fertil.	total pollen formed	total pollen fertil.	egg-cells formed	egg-cells fertil.
0B	0·2293	0·5095	0·2512	0·2498	0·8074	0·6545	0·2512	0·2617
1B	0·2389	0·0333	0·0151	0·0282	0·0597	0·0454	0·0151	0·0
2B	0·5063	0·2762	0·7114	0·4962	0·1266	0·2455	0·7114	0·5856
3B	0·0108	0·0286	0·0	0·0544	0·0027	0·0182	0·0	0·0449
4B	0·0146	0·1571	0·0360	0·1714	0·0036	0·0364	0·0360	0·1078

Table 6 Frequencies of pollen formed and taking part in fertilisation in populations derived from *S. vavilovii*

	8V population		2V population	
Number of Bs	total pollen formed	total pollen fertilised	total pollen formed	total pollen fertilised
0B	0·2456	0·5608	0·8114	0·8445
1B	0·1769	0·0	0·0442	0
2B	0·5670	0·0743	0·1417	0·0420
3B	0·0095	0·0270	0·0024	0·0042
4B	0·0010	0·3378	0·0003	0·1092

population 4B descendants were obtained with a much higher frequency than expected and, (b) in the 2C population many less 0B descendants were obtained from 0B plants than expected. If self-pollination occurred many more would have been found. Therefore, even accepting that a percentage of self-pollination can occurr in these populations, this was evidently not the main cause of the difference between observed and expected offspring.

In order to find an explantion for the results, we estimated the frequencies of the male and female gametes actually involved in fertilisation, following the method previously described (table 5).

The estimates for fertilised female gametes with 0B coincide in the 8C and 2C population with the frequencies of 0B gametes formed estimated from 0B × 2B crosses (table 5). This is a consistent result, since no variation was expected in the types of female gametes depending on different populations. That is, the ratio between female gametes with Bs/without Bs was constant in all types of estimates and populations. However a difference was observed in 2B and 4B classes between the frequency of female gametes formed and fertilised.

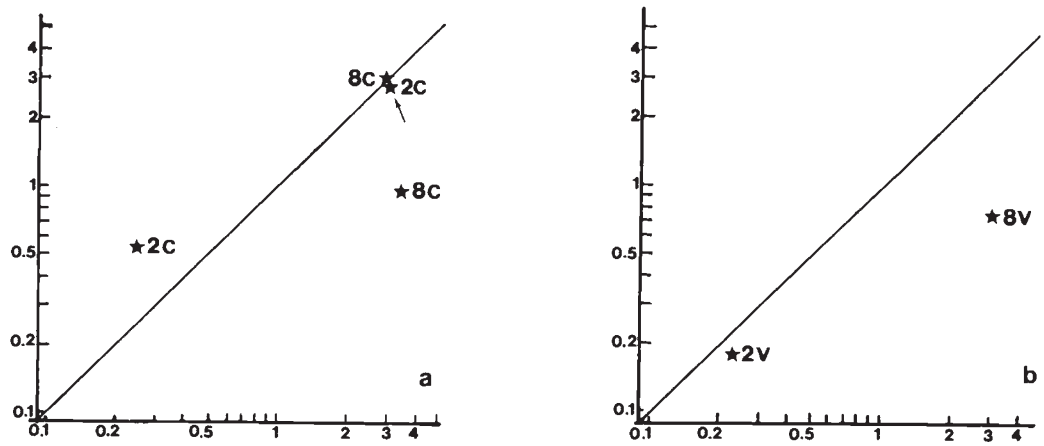

Figure 1 Graphs representing the deviation from random fertilisation in the four studied populations. Abscissae are the ratios between gametes with Bs/without Bs formed in the parental population. Ordinates are the ratios between gametes with Bs/without Bs really fertilised. The scale is logarithmic. *a.* Populations derived from *S. cereale.* Female gametes (arrow) fertilised at the same frequency that they were formed. Male gametes carrying Bs were more effective than expected in population 2C and less effective in 8C. *b.* The same in populations derived from *S. vavilovii.*

This is because the estimation has to be considered as only approximate, since the estimation of q (1B) depend on p (0B), the estimation of r (2B) depend on p and q, and so on; so that the errors are cummulative. However, it seems that for 0B female gametes the estimated frequencies can be accepted.

In the case of the male gametes large differences were observed between the frequencies of pollen B-types formed and the frequencies actually taking part in fertilisation, but they are of different direction in the 8C and 2C populations.

In the 8C population in which there were 80 per cent plants with Bs, much pollen with Bs was formed, but the frequency of 0B pollen taking part in fertilisation was double the amount expected. In the case of 2B pollen the amount involved in fertilisation was only half of the amount actually produced. Therefore, when there are many B chromosomes in the population pollen without Bs becomes advantageous, and pollen with Bs disadvantageous.

On the contrary, in the 2C population in which there were 20 per cent plants with Bs, pollen without Bs was involved in fertilisation at about $\frac{3}{4}$ of the frequency at which it was formed, while 2B pollen took part in fertilisation at double the frequency of its formation. Therefore, when there are few Bs in the population, pollen with Bs becomes advantageous and pollen without Bs disadvantageous.

The deviation from randomness can be represented in a Dewitt graph (fig. 1a), the abscissae are the ratios between pollen with Bs/without Bs formed in the parental populations; the ordinates are the ratios between pollen with Bs/without Bs taking part in fertilisation. In the case of female gametes these ratios coincide. However, a competition between the different types of pollen is clearly manifested: pollen with Bs becomes advantageous when its frequency is low and vice versa.

Populations derived from S. vavilovii

Table 4 shows that the offspring obtained differed significantly from expectations assuming total self-pollination.

The mating system of this species can be considered as totally self-pollinating, since a complete lack of variation for many isoenzyme loci was demonstrated (Perez de la Vega and Allard, 1984). However, our results are a proof that selfing was not complete in this experiment since 0B plants gave rise to descendants with Bs, and it seems evident that the cross-pollination detected is due to B chromosomes.

The frequencies of the different pollen B-types involved in fertilisation were also estimated for the 8V and 2V populations (table 6). Female gametes actually fertilised cannot be estimated in this case due to selfing. In *S. cereale* we supposed that the mass of pollen involved in fertilising 0B plants was the same that which fertilised the 2B plants, but this assumption cannot be made for *S. vavilovii.*

In population 8V, that is with many Bs, we can observe that 0B pollen was involved in fertilisation at double the frequency at which it was produced, while pollen with Bs took part in fertilisation at a frequency which was much lower than that at which it was formed. Therefore both in *S. cereale* and *S. vavilovii* when there are many Bs in the population pollen without Bs becomes advantageous.

In the 2V population there is almost no difference between the types of pollen formed and the types involved in fertilisation, *i.e.*, in this case the advantage of pollen carrying Bs is not as evident as in *S. cereale.* However it has to be noted that gametes carrying Bs have broken the mating system of the species. That is, pollen carrying Bs was sufficiently competitive to take part in fertilisation in about 15 per cent of the cases, when it was expected to fertilise none due to self-pollination. These data are represented in figure 1 b.

Two main conclusions can be drawn from the data presented in this paper. First: the effectiveness of pollen carrying B chromosomes is mainly dependent on the frequency of plants with and without Bs in the population; pollen carrying Bs becomes advantageous when its frequency declines. Second: this behaviour is dependent on B chromosomes themselves, since the competence among the different types of pollen is comparable in both species *S. cereale* and *S. vavilovii.*

This behaviour could be explained assuming a pollen-style interaction, in such a way that the mating ability of each type of pollen would depend on this interaction. That is, if the style belongs to a 2B plants, a 0B pollen would have a higher probability being involved in fertilisation, and conversely, if the style belongs to a 0B plant a pollen carrying Bs would have the higher opportunity. Therefore, in a population with a high frequency of 0B plants, pollen with Bs would be advantageous, and vice versa. Then, the equilibrium for B chromosomes polymorphism could be established by a frequency-dependent selection.

Finally, it has to be noted that pollen carrying 1B always took part in fertilisation with a lower frequency than expected, while pollen with 3 or 4 Bs was involved with a frequency higher than

expected in all populations (tables 5 and 6). In other words, pollen with 3 or 4 Bs was advantageous irrespective of the frequency of B chromosomes in the population.

This can be explained as follows: in the present work the frequencies of pollen types involved in fertilisation were calculated from descendants collected on 0B plants. If we suppose than on 0B style, pollen with Bs is advantageous due to pollen-style interactions, the higher the number of Bs in pollen, the higher the advantage of such pollen. Then, pollen with many Bs lying on 0B styles will be very effective, despite the fact that the zygotes will carry many B chromosomes, and therefore, they will have a low fitness.

This behaviour, which can be qualified as parasitic, together with the fact that the advantage of B carrying gametes seems to be only of benefit to the B chromosomes themselves, makes clear that B chromosomes can be considered as selfish DNA. We believe that this idea first proposed by Jones (see Jones, 1985 for a review) can be definitively accepted.

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