



Variation for maize B chromosome preferential fertilization: a component of the B chromosome drive mechanism

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Abstract

The B chromosome in maize is a supernumerary chromosome that due to its dispensability is present in only some lines of maize. Over its evolution, the B chromosome has developed a two-part drive mechanism that ensures its continued presence in maize populations. Its drive mechanism involves nondisjunction at the second pollen mitosis in which two sperm cells are produced and preferential fertilization by the sperm with the two B chromosomes more often joining with the egg as opposed to the central cell in the process of double fertilization. Previous work had suggested some lines of maize exhibit a different response and that this was controlled by the female parent. We sought to examine the variation for this trait by testing a wide spectrum of characterized maize lines. Most inbred lines exhibit the canonical preference for the egg cell, some appear to have random fertilization, and one inbred line (B73) shows a preference for the B containing sperm to fertilize the central cell.

Keywords B chromosome · Genetic drive · Fertilization

Introduction

B chromosomes are nonessential, supernumerary chromosomes found in select organisms [9]. These chromosomes are present in varying copy numbers between individuals and show mechanisms of inheritance inconsistent with Mendelian transmission [9]. B chromosomes across various organisms have been identified as having unique drive mechanisms that perpetuate the continuation of the B chromosome into the next generation, ensuring these chromosomes continue to exist in the population despite their dispensability [9].

A B chromosome is found in certain lines of maize in addition to the normal 20 chromosome number typical in maize. Its presence is nondetrimental to the maize plant except at high copy numbers [2]. B chromosome emergence is suggested to have occurred millions of years ago, with

evidence of continuous evolutionary introduction of transposed genes from the A chromosomes [3].

The addition of genes to the B chromosome has allowed it to evolve various mechanisms that alter cellular functions in ways essential to its perpetuation [2]. Studies have indicated that the B chromosome can modulate gene expression of the normal A chromosomes [14, 15, 28]. Additionally, as an unpaired univalent, the B chromosome has developed a mechanism that, through premature poleward migration of the chromosome, the univalent form is stabilized, which prevents its loss during meiosis [7, 11, 12]. The presence of B chromosomes also increases the frequency of chiasmata formation in A chromosomes during meiosis on all chromosomes, particularly in centromeric heterochromatic regions [1, 5, 8, 13, 18, 20, 23, 29]. This increase is greater during male meiosis, where increased crossing over on the B chromosome itself ensures orderly segregation into spores preceding the aspects of the drive mechanism [5, 23].

Critical to the continuation of the B chromosome's survival in the genetic lineage of maize is the drive mechanism of the B chromosome. The drive mechanism consists of two parts, namely, nondisjunction of the chromosome at the second pollen mitosis [24] (Fig. 1), which is followed by preferential fertilization of the egg during double fertilization [25] (Fig. 2).

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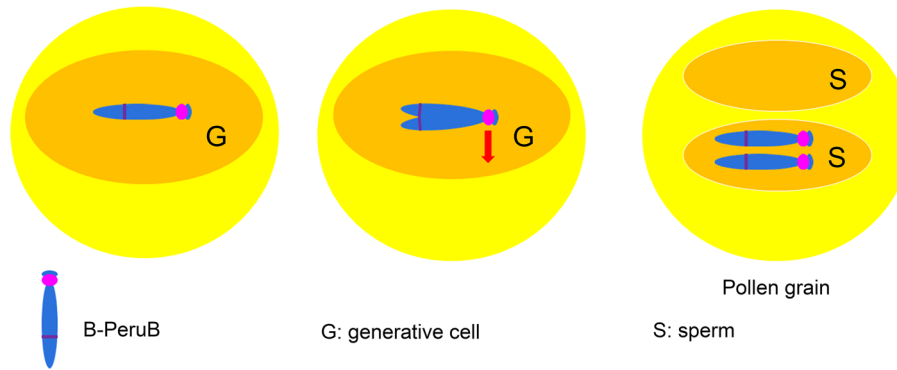


Fig. 1 The first component of the B drive mechanism. Diagram of nondisjunction of the B chromosome in maize. The B chromosome (blue with a magenta centromere) is shown in the generative nucleus (G) after the first pollen mitosis. The B chromosome has an insertion of a construct containing the *B-Peru* gene, which is a transcription factor for the anthocyanin pathway. Thereafter, we used B-PeruB to

refer to the B chromosome with the *B-Peru* insertion. After replication, the two chromatids proceed to the same pole (red arrow) at the second pollen mitosis in the vast majority of divisions. Thus, most mature pollen grains contain two sperm cells (S) with only one containing the B chromosomes (color figure online)

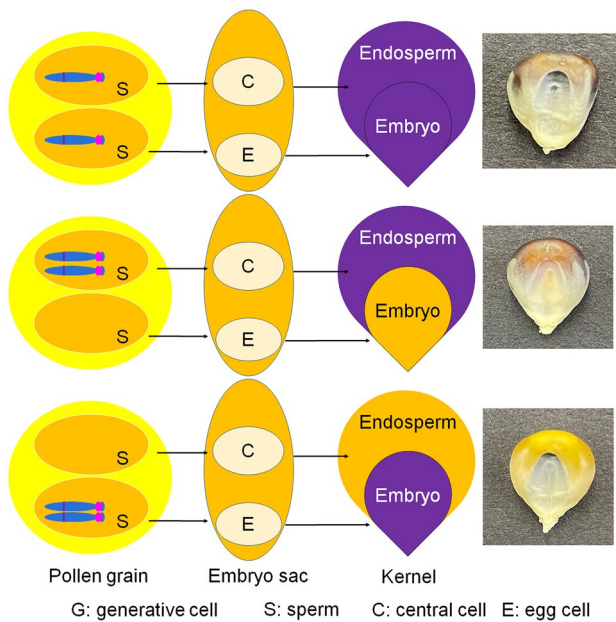


Fig. 2 Depiction of preferential fertilization, the second component of the B drive mechanism. For most lines of maize, the sperm with the two B chromosomes will preferentially fertilize the egg (E), as compared with the central cell (C) in the process of double fertilization. The fertilized egg develops into the next generation embryo and the fertilized central cell develops into the endosperm. The B-PeruB chromosome allowed us to distinguish preferential fertilization by observing the phenotype of the embryo and endosperm. If the sperm with two B chromosomes fuses with the central cell, the endosperm is purple (anthocyanin present or “colored”) and the embryo is yellow (no anthocyanin or “colorless”). The kernel would show a colored embryo and colorless endosperm if the sperm with the B chromosomes fertilized the egg cell. If there is no B chromosome nondisjunction, both embryo and endosperm would be colored (color figure online)

Nondisjunction at the second pollen mitosis occurs as B chromosome sister chromatids fail to separate at the centromeric region, producing one sperm with a pair of B chromosomes and the other sperm without a copy. In preferential fertilization, the sperm containing the two B chromosomes preferentially fertilizes the egg cell at a greater frequency than the central cell [25], resulting in an enhanced frequency of B chromosome transmission into the embryo and thus the individuals of the next generation [25]. The preference of the B chromosome for the egg cell shows variation across maize populations, with differences in preference dependent on the female parent [4, 6]. Previous research with Argentinian maize lines indicated that just one gene may be responsible for variation in preference [10, 12, 26]. Characterization of the variation in preferential fertilization across maize lines could provide a route to identify and evaluate the genetic factor(s) important for preferential fertilization and to evaluate the population dynamics of the B chromosome drive mechanism.

Nested Associated Mapping (NAM) is a trait dissection strategy that allows researchers to analyze trait differences across a substantial portion of maize variation [30]. With the selection and sequencing of twenty-six inbred lines as NAM founders, these lines are well characterized [16]. Because the *trans* factor responsible for determination of preferential fertilization of the B chromosome is from the female parent, evaluation of preferential fertilization using female NAM founder lines will thus provide a representative characterization of the variation in preferential fertilization across maize varieties.

Methods and materials

Crosses were made between female lines and males that carried two copies of the phenotypically marked B chromosome [17] that had been converged to an *r1-r* W22 line as evidenced by colored embryos in kernels with a colorless (i.e. no anthocyanin) endosperm. The frequency of nondisjunction in this line is 99% as evidenced by the frequency of nonconcordant kernels with regard to purple pigment in the embryo and endosperm. From each cross to the various female lines, individual kernels were classified as having anthocyanin pigment in the embryo or the endosperm or both. Pigment in the embryo alone is an indication that the nondisjoined B chromosome fertilized the egg (Fig. 2). Pigment in the endosperm alone is an indication of fertilization of the central cell by the B containing sperm (Fig. 2). Pigment in both tissues would be an indication of the lack of nondisjunction of the B chromosome in the microspore that produced the two sperm that fertilized the embryo sac giving rise to those kernels (Fig. 2) or potentially cases of heterofertilization in which two pollen tubes enter the ovule and fertilization of both the egg and the central cell occurs with a B chromosome containing sperm.

Results

Characterization of diverse maize lines for fertilization preference of the B chromosome

Preferential fertilization in maize NAM founders was assessed through phenotypic analysis of the progeny of each line. Because variance in fertilization preference is determined by the female parent, progeny of crosses between female NAM founder inbred lines and DRDsB (Direct Repeat Ds on B) males [17] were used to determine rates of preferential fertilization. DRDsB males are from a line that contains the transgene *B1-Peru* allele of the maize *bl* regulatory gene [19, 27] on the B chromosome, which confers a purple color to tissues that contain the B chromosome (Fig. 3). The purple phenotype produced by *B-peru* B allows fertilization preference to be assessed by the naked eye, with preferential fertilization of the egg cell producing a kernel with a purple embryo and a colorless endosperm, while preferential fertilization of the central cell produces a kernel with a purple endosperm and a colorless embryo.

Crosses were made over two growing seasons during the summers of 2021 and 2022. Maize ears produced by female parents, pollinated by B-PeruB pollen, were

removed from the plants, labeled, and dried for five days. Each individual ear was then scored, with removal and classification of all kernels based on the presence or absence of purple pigment in the embryo and endosperm.

The percentage of preferential fertilization of the egg cell was determined for each ear with greater than fifty kernels (a cutoff to ensure sufficient numbers to determine fertilization trends) by dividing the number of kernels that showed the purple embryo phenotype by the total number of kernels. A percent greater than 50% indicates preference for the egg, while a percent less than 50% suggests preference for the central cell. A chi squared test was performed on the two classes of fertilization of each individual to test the statistical significance. Random fertilization would result in equal fertilization of the egg cell and central cell and serves as the null hypothesis. This analysis was performed on every individual ear that met the fifty-kernel limit, as well as with group data for each analyzed line. The rate of preferential fertilization of the egg cell was calculated by summing all the purple embryo kernels for a given line and dividing it by the total number of kernels for that line.

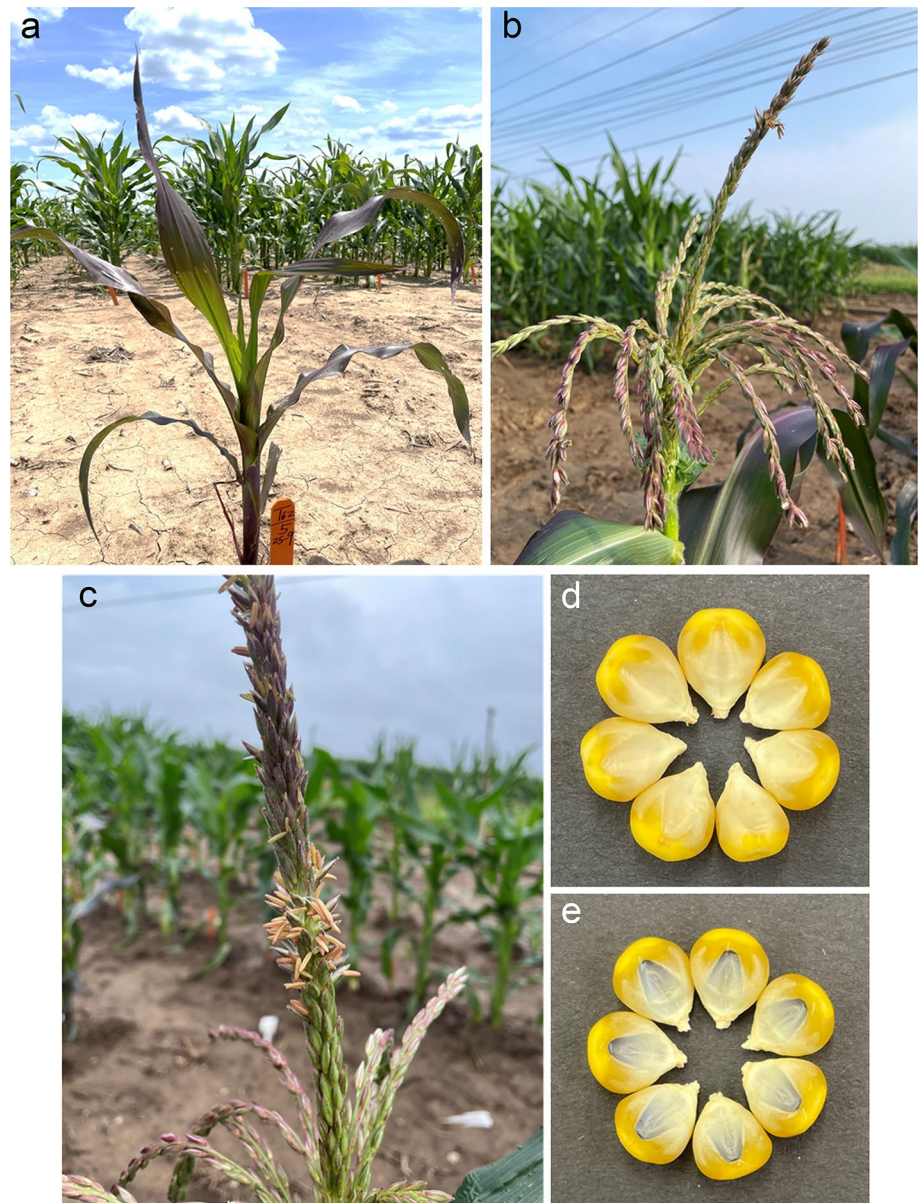
Of the twenty-six maize NAM founder inbred lines, fifteen lines were able to be characterized. No materials of CML228, CML52, CML69, Hp301, M162W, and Mo18W inbred lines were recovered due to considerable differences in flowering time with the male parent. CML322 contains a color inhibitor that prevents the phenotypic expression of *B1-Peru*. Lines Oh7b, Tx303, NC358, B97, Mo17, P39, Tzi8, CML103, M37W, NC350, CML247, and Ms71 demonstrated statistically significant preferential fertilization of the egg cell (Table 1). Phenotypic analysis of Ky21, CML277, and Oh43 as a bulked group resulted in no statistically significant preference in fertilization (Table 1). B73 displayed statistically significant preferential fertilization of the central cell (Table 1).

Discussion

The full spectrum of the types of fertilization by the B containing sperm was found in this study. The most prevalent is the preference for the egg as is generally known to be the case. However, several lines did not show a significant difference between the predicted random fertilization of either the egg or the central cell. In one line, B73, there is a switched preference for the central cell for fertilization by the B containing sperm—a behavior that has been previously described in an uncharacterized line [6].

In some backgrounds, the B chromosome can have very detrimental effects, namely causing chromosomal breakage at heterochromatic knob sites and the production of triploids and trisomies of the A chromosomes [21, 22]. It is possible that some populations could have evolved

Fig. 3 The B-PeruB phenotype in different tissues. **a** Plant, note that the anthocyanin level in the tip and the base of the leaf is different. This is because the B-Peru in plants requires another transcription factor *pl1* that is induced by sun exposure to produce anthocyanin. This is also the case in tassel (**b**) and anther (**c**). **d** Kernels without the B-PeruB. **e** kernels with the B-PeruB (anthocyanin present). The anthocyanin accumulation in the kernel requires *B-Peru* and *CI*, which provides a similar function as *Pl1*



to eliminate the female trait of allowing the preferential fertilization of the egg or even to have evolved to have the B containing sperm fertilize the central cell to eliminate the B chromosome from the lineage given the detrimental effects of the B chromosomes. This property could have been separated from a background in which B chromosomes are detrimental during the development of inbred lines. The documentation of the types of variation for this property reported here open the opportunity in the future to identify the genic differences between lines with different

preferences, which might shed light on the basis of preferential fertilization.

In this study, fifteen well characterized and sequenced maize lines were tested for fertilization preference for the B chromosome containing sperm. Twelve lines showed a statistically significant preference for the egg cell, three lines showed random fertilization between the egg and central cell. One line, B73, shows a preference for the central cell. These data provide the information for a future molecular understanding of fertilization preference.

Table 1 Tests for fertilization preference of diverse inbred lines of maize

Line	Fertilization preference			Percentage E.F. ^a	Expected E.F. ^b	Delta ^c	X ²	Null ^d
	Egg cell	Central cell	Total					
B73	270	381	651	41.5	325.5	55.5	9.46	Reject
Oh7b	352	278	630	55.9	315.0	37.0	4.35	Reject
Tx303	1216	847	2063	58.9	1031.5	184.5	33.00	Reject
NC358	459	245	704	65.2	352.0	107.0	32.53	Reject
B97	257	137	394	65.2	197.0	60.0	18.27	Reject
Mo17	272	157	429	63.4	214.5	57.5	15.41	Reject
P39	138	82	220	62.7	110.0	28.0	7.13	Reject
CML247	149	104	253	58.9	126.5	22.5	4.00	Reject
CML103	128	68	196	65.3	98.0	30.0	9.18	Reject
M37W	367	274	641	57.3	320.5	46.5	6.75	Reject
Tzi8	41	18	59	69.5	29.5	11.5	4.48	Reject
NC350	667	413	1080	61.8	540.0	127.0	29.87	Reject
Ms71	259	184	443	58.5	221.5	37.5	6.35	Reject
Ky21	231	200	431	53.6	215.5	15.5	1.11	Accept
CML277	64	49	113	56.6	56.5	7.5	1.00	Accept
Oh43	1146	1216	2362	48.5	1181	35	1.04	Accept

^a Abbreviation for Egg Fertilization

^b Number expected if no preferential fertilization

^c Observed-Expected

^d Null hypothesis: There is no preferential fertilization

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Author contributions JB designed the experiments; VB and HY conducted the experiments and analyzed the data; VB, HY, and JB wrote the paper.

Data availability Materials are available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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