# SHORT PAPER

# On the nonautonomy of the small-kernel phenotype produced by B–A translocations in maize

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### SUMMARY

Several of the B–A translocations of maize produce a small-kernel phenotype which is associated with hypoploid endosperms from fertilizations by sperm which result from nondisjunctional events of the B centromere at the second microspore division. Lin (1975) demonstrated that the small-kernel phenotype was not a consequence of the deficient nature of said endosperms, but involved a differential effect of male and female chromosomal regions.

The TB-A effect has been examined for evidence of autonomy in mosaics for the most extreme such case known, i.e. the small-kernel effect produced by the compound B–A translocation, 1La-5S8041. Although reduced-sized kernels are formed when the paternal contribution of the 1L-5S element is totally lacking from the endosperm, sectorial loss has no detrimental effect on dry weight. This phenomenon is therefore considered to be nonautonomous.

## 1. INTRODUCTION

The translocations between the supernumerary, B, chromosomes and the normal A set in maize allow the production of deficient or duplicate endosperms due to the property of the B centromere to undergo nondisjunction at the second microspore division, giving rise to nonconcordant sperm. Upon the induction of the first such B–A translocations, it was immediately obvious (Roman, 1947) that hypoploid endosperms lacking certain regions of the genome were considerably smaller than the euploid and hyperploid siblings. This effect was thought to be due to a chromosomal deficiency until the elegant experiments of Lin (1975; see also Carlson, 1978), revealed that the small-kernel phenotype was conditioned by the absence of a paternal contribution to the endosperm rather than by their deficient nature.

Using TB-10L's of varying lengths, Lin (1975) showed that a region on chromosome ten produced small kernels in 4 ♀:0 ♂ and 2 ♀:0 ♂ dosages, but normal ones in 2 ♀:1 ♂ and 2 ♀:2 ♂ constitutions. Since endosperms of identical dosages were clearly different, an explanation of the phenomenon must take into account the parental origin of the chromosomes or perhaps the lack of a paternal contribution.

Several other B–A translocations exhibit the small-kernel phenotype (see Beckett, 1978) with the most extreme case being TB-1La-5S8041. This compound B–A translocation was synthesized from TB-1La and A–A translocation 1L-5S8041

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(Robertson, 1975) and therefore contains the proximal portion of 1L (1L 0·20–0·80) and the distal region (5S 0·10 to tip) of 5S. The extreme small-kernel phenotype results from the cumulative effects of factors on 1L and 5S (Robertson, 1975).

Various investigators have noted the high frequency of mosaic kernels in material involving B–A translocations (Roman, 1947; Bianchi *et al.* 1961; Carlson, 1970), the mosaicism arising from nondisjunction or loss of the B–A chromosomal element. Such kernels were present in our cultures of TB-1La-5S8041 but exhibited no mosaicism for the small-kernel phenotype. This nonautonomous behaviour is in contrast to numerous other characters studied in mosaics (see McClintock, 1951; Rhoades & Dempsey, 1973; Coe & Neuffer, 1977) and as such, is a unique property of this class of genes.

## 2. MATERIALS AND METHODS

### (i) *Stocks*

The *A a2 C C2 R* tester was obtained from the Maize Genetics Stock Center, Urbana, Illinois. The TB-1La-5S8041 stock was kindly provided by Dr D. S. Robertson. TB-1La-3L5242 and the *a A2 C C2 R-scm-2* tester were synthesized as previously described (Birchler, 1980). The *A, A2, C, C2,* and *R* loci are in chromosomes 3L, 5S, 9S, 4L and 10L, respectively, the total complement of these genes being required for anthocyanin expression.

### (ii) *Crosses*

The *a2* tester was used as a female for hyperploid heterozygotes (1, $1^B$, $B^{1L·5S}$, $B^{1L·5S}$, $5^1$) of TB-1La-5S8041 but homozygous for *A2*. The *a* tester line was used as females for hyperploid heterozygotes (1, $1^B$, $B^{1·3L}$, $B^{1·3L}$, $3^1$) of TB-1La-3L5242.
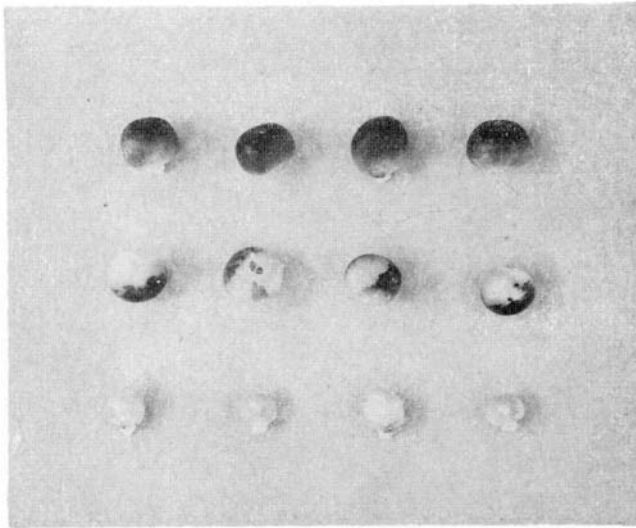
### (iii) *Determination of mean mass*

Kernels were removed from five ears and classified into *A2, a2,* and *A2/a2* mosaic classes. Each kernel was individually weighed to the nearest milligram and means with their standard errors calculated. Mosaic kernels were examined under a binocular microscope for observations on cell size in the different sectors.

### (iv) *Rationale*

The *A2* locus resides in the region of chromosome arm 5S which is present in the distal portion of TB-1La-5S8041. In the crosses of the *a2* tester by male TB-1La-5S8041, the resulting genotypes of the triploid endosperms will be *a2/a2/−, a2/a2/A2* or *a2/a2/A2/A2*. These classes arise due to the property of the B centromere to undergo nondisjunction at the second microspore division, giving rise to the two maize sperm. The *a2/a2/−* endosperms are the small kernels and lack a paternal contribution to the genotype because the nondisjoined chromosomes were present in the sperm that fertilized the egg nucleus. The complementary class *a2/a2/A2/A2* results when the nondisjoined chromosomes are present in the sperm that fertilizes the polar nuclei. The class of interest here is *a2/a2/A2* which results from those cases of disjunction of the B centromere thereby transmitting a single dose of 1L-5S to both the egg and polar nuclei.

If, during endosperm development, this $B^{1L·5S}$ element nondisjoins or fails to be included in a daughter nucleus, a mosaic kernel will be produced and can be recognized by the sectorial loss of the dominant *A2* marker – giving rise to a pigmentless area on an otherwise pigmented background. Such kernels can thus be analysed for autonomy or lack thereof with respect to the small-kernel effect.

Top, large kernels with *A2* phenotype; middle, mosaic kernels for the
*A2/a2* marker; bottom, small-kernel class of *a2/a2/−* genotype

## 3. RESULTS

Twenty-three kernels mosaic for *A2/a2* were examined for evidence of defective or reduced growth in the *a2* patches. In no case was there evidence of an effect of sectorial paternal loss. A substantial fraction of these mosaics resulted from events occurring at the first or second division of the primary endosperm nucleus. These kernels showed loss of the *A2* marker for $\frac{1}{2}$–$\frac{1}{4}$ of the endosperm, but both the *A2* and *a2* sectors appeared normal and no demarcation line was evident at or near the anthocyanin border (see Plate 1).

The results of kernel mass determinations confirm these observations. Although there is a highly significant difference between the means of the *A2* and *a2* classes, the mosaics fall clearly into the large kernel class with no detrimental effect on total mass due to their mosaic nature. The mean masses are given in Table 1.

Table 1. *Dry weight comparisons of* A2, a2 *and* A2/a2 *mosaic kernels*

| Phenotype | No. kernels | Mean ± s.e. (mg) | % A2 |
|-----------|-------------|------------------|------|
| A2 | 208 | $220 \pm 23$ | 100 |
| a2 | 55 | $84 \pm 3$ | 38 |
| Mosaic | 23 | $218 \pm 6$ | 99 |

Each kernel of the respective classes was individually weighed to the nearest milligram. The difference between the means of the *A2* and *a2* classes is significant at the 99 % confidence level.
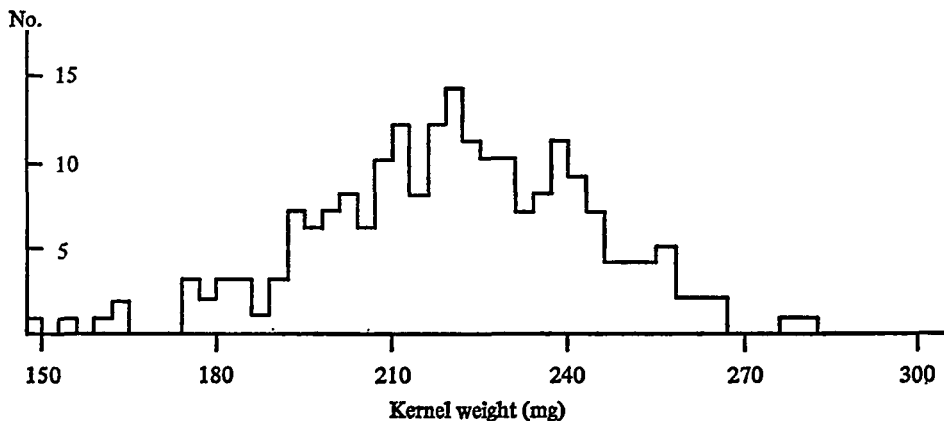


Fig. 1. Histogram of the dry weight of individuals from the *A2* large-kernel class

Endosperms of *a2/a2/A2* and *a2/a2/A2/A2* genotypes are not genetically distinguishable in our crosses. We wished to determine whether the additional dose of 1L-5S in the latter case made the endosperm larger than the normal triploid *a2/a2/A2*. The individual weight of each kernel was plotted in a histogram to examine the data for evidence of bimodality within this phenotypic class. This histogram is presented in Fig. 1 and shows no obvious clustering of kernel weights. We conclude therefore that the extra dose of 1L-5S beyond the triploid level has no additive effect upon kernel mass in this genetic background – a situation typical of regions which condition the small-kernel effect.

To test whether these results were perhaps peculiar to the *A2* marker, crosses of the *a A2 C C2 R-scm-2* tester by TB-1La-3L5242 were screened for partial losses of pigment. This stock allows anthocyanin expression in scutellar as well as endosperm tissue and

will give a genetic confirmation of the type of kernels which are mosaic. Seven $A/a$ sectored endosperms were found. In all cases the scutellum was fully pigmented confirming the expectation that most recognizable losses of the B–A element occur in endosperms with but a single dose of this chromosome.

This compound B–A translocation also exhibits the small-kernel phenotype (Birchler, 1980), but to a much lesser degree than TB-1La-5S8041. All of the $A/a$ sectored kernels showed nonautonomy with respect to kernel size. This result indicates that the phenomenon is not peculiar to a particular chromosome arm nor due to a locus specific mottled expression of the marker genes.

## 4. DISCUSSION

The maternal and paternal forms of most genes with known allelic alternatives are both expressed in the maize endosperm (see Coe & Neuffer, 1977), However, a major exception involves the genes controlling kernel size and shape (see discussion by Schwartz, 1965). He points out that a longstanding observation in maize genetics is that the kernel size is strictly dependent upon the maternal genotype and, in the absence of specific mutant genes, totally independent of the genetic constitution of the endosperm itself. Indeed reciprocal crosses between a line with exceptionally large kernels (Gourdseed) and one with diminutive kernels (Super Gold Popcorn) give $F_1$ ears with caryopses, not of intermediate size, but similar to the respective maternal line (Birchler, unpublished). This observation has been interpreted as an example of gene imprinting in which the maternal form is expressed but not the paternal as a result of events occurring in the respective gametophytic generations (Schwartz, 1965; Kermicle, 1978). However, the possibility that the maternal genome (female gametophyte) synthesizes the necessary products which are then present in the cytoplasm even before fertilization occurs has not been ruled out.

The observation that kernel size is normally maternally determined, by whatever means, makes the study of Lin (1975) quite intriguing. As stated above, he found that endosperms with identical dosages of a region on 10L were of different sizes depending upon the parental origin of the segments. That is, $4\,♀:0\,♂$ endosperms were small, whereas $2\,♀:2\,♂$ dosages were normal. In fact the $4\,♀:0\,♂$ class was similar in size to the $2\,♀:0\,♂$ group. These results have been interpreted as an example of gene imprinting such that the paternal allele of the seed size genes on 10L are active but the maternal alleles are not, or at least much less so. Birchler (1979) has found that for yet another B–A translocation, 1La, a lack of a 1L paternal contribution to the endosperm results in the small-kernel phenotype regardless of 1L dosage in a similar but not identical fashion as occurs with 10L. It appears then that this effect discovered by Lin (1975) has general applicability to the numerous small-kernel effect regions in the maize genome. Given Lin's interpretation, there appears to be a dichotomy between the normal maternal determination of kernel size and the presumptive greater expression of paternally derived alleles of seed size genes. The two phenomena, however, could be reflections of independent processes.

Phenomenologically the absence of a paternal contribution of selected regions to the endosperm results in a less than normal sized kernel within the realm of experiments involving B–A translocations. It is not our intention to imply that the presence of at least one paternal allele will universally result in normal development, for this is certainly not the case. Many of the primary trisomics (especially of chromosome one) of maize produce small kernels in crosses as females by normal males. These kernels have four maternal and one paternal dose of the particular chromosome in question. Thus the data, presently known, indicate that small-kernel phenotypes result when the female to male dosage relationship for specific regions is greater than the normal $2\,♀:1\,♂$. The lack of a

paternal contribution to the endosperm in TB-A crosses would fall into this characterization.

An additional unusual property of this system is reported here, i.e. despite the fact that small kernels result when no paternal contribution is present in the endosperm as a whole, mosaic losses of the paternal chromosome do not cause defective sectors. One possibility to explain this nonautonomy might be that a substance produced in the *A2* cells diffuses into the deficient regions to alleviate the small endosperm effect. Although this must be considered as a possible explanation, Coe & Neuffer (1977) list 40 loci which exhibit virtual cell autonomy in maize. In no case is there evidence that cross feeding in the endosperm can traverse more than one or a few cell layers. Such an explanation in this case would require an unusual property of the diffusible substance.

A second possibility is that the size of the endosperm is a genetically determined process which is defined in the primary endosperm nucleus. The maternal effect described above is consistent with an early determination. Perhaps the paternal allele of the responsible genes plays a role in this process during the short period between fertilization and the first mitotic division of the endosperm nucleus.

Yet a third explanation might be that kernel size is a function of the quantity and quality of long-lived molecules produced from these genes before cell wall formation occurs and sufficient amounts of these gene products for normal development are partitioned into individual cells during the numerous subsequent divisions. The data reported here do not discriminate among these or other explanations, but perhaps the recognition of the nonautonomy of the small-kernel effect is a step in the understanding of this complex developmental problem.

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