PLOIDY BARRIER TO ENDOSPERM DEVELOPMENT IN MAIZE

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ABSTRACT

Maize kernels inheriting the indeterminate gametophyte mutant (*ig*) on the female side had endosperms that ranged in ploidy level from diploid (2x) to nonaploid (9x). In crosses with diploid males, only kernels of the triploid endosperm class developed normally. Kernels of the tetraploid endosperm class were half-sized but with well-developed embryos that regularly germinated. Kernels of endosperm composition other than triploid or tetraploid were abortive.——Endosperm ploidy level resulting from mating *ig/ig* × tetraploid *Ig* similarly was variable. Most endosperms started to degenerate soon after pollination and remained in an arrested state. Hexaploid endosperm was exceptional; it developed normally during the sequence of stages studied and accounted for plump kernels on mature ears. Since such kernels have diploid maternal tissues (pericarp) but triploid embryos, the present finding favors the view that endosperm failure or success in such circumstances is governed by conditions within the endosperm itself.——Whereas tetraploid endosperm consisting of three maternal genomes and one paternal genome is slightly reduced in size but supports viable seed development, that endosperm having two maternal and two paternal chromosome sets was highly defective and conditioned abortion. Thus, development of maize endosperm evidently is affected by the parental source of its sets of chromosomes.

IN crosses between plants of different ploidy levels, endosperm impairment frequently occurs, often preventing successful hybridization. Therefore, many cases in which diploid-diploid and tetraploid-tetraploid crosses are successful, diploid-tetraploid intercrosses fail from endosperm abortion.

Since this "ploidy barrier" also intervenes in crosses between a diploid and its derived autotetraploid, in which no qualitative genetic difference can be suspected, attempts have been made to explain it in terms of genome balance. Inasmuch as a seed consists of three distinct components, of maternal, endosperm and embryo tissues, endosperm failure and the ploidy barrier have been variously attributed to imbalance of genome ratios, as: (1) deviation from a 2:3:2 ratio in ploidies of maternal tissue, endosperm and embryo (MÜNTZING 1930); (2) deviation from a 3:2 ratio of endosperm and embryo (WATKIN 1932); (3) deviation from a 2:3 ratio of maternal tissue and endosperm (VALENTINE 1954).

More recent discussions have considered the possibility that endosperm failure may somehow be a function of the genetic constitution of endosperm itself. WANGENHEIM (1957) hypothesized an upset in balance between the plasmon (including all non-nuclear genes) and the nuclear genome within the endosperm. Other interpretations consider the nuclear genotype to be of prime importance. SARKAR and COE (1971) regarded triploidy *per se* as an essential condition for normal endosperm development. NISHIYAMA and INOMATA (1966) inferred that, in addition to endosperm ploidy, the parental source of endosperm chromosomes is also critical and proposed, without supporting data, a 2:1 ratio of maternal and paternal chromosomes as being essential.

Two additional problems may be raised. One is the assumption that ploidy barriers have a common controlling mechanism in a great diversity of plants, whereas these might be various in some genera. An example of the latter appears to be Musa, in which an astonishing range of ploidy combinations can exist within seeds formed by diploids and triploids. An extreme case is of triploid maternal tissue sustaining viable heptaploid embryos and endosperm presumably containing 13 sets of chromosomes. In most instances, not only are the diverse seeds potentially viable but they are equally well filled and indistinguishable in appearance within the same inflorescence or even the same fruit (K. SHEPHERD, personal communication). Similar cases apparently occur in the genus Beta (MOCHIZUKI 1953).

The other problem is that of criteria for assessment of differential endosperm development. There is, in fact, no commonly recognized definitions for what is normal or abnormal. For instance, MÜNTZING (1933) reported diploid-tetraploid crosses as normal in Galeopsis on the basis of embryo viability, whereas HOWARD (1939) regarded them as defective in Brassica on the basis of seed size. These findings may not be mutually exclusive. Recently, ESEN and SOOST (1973) have used both criteria on diploid-tetraploid crosses in Citrus but appear to have used them separately to support divergent conclusions. Both embryo viability and seed size are considered in the present study.

A number of hypotheses for the causes of endosperm failure are tested in this study by using the indeterminate gametophyte mutant (*ig*) in maize. With appropriate chromosomal and genetic markers, it is possible to identify a series of ploidy levels of endosperm and confirm the parental contributions, all with diploid maternal tissue and with diploid or triploid embryos. Moreover, by relating results from diploid-diploid and diploid-tetraploid crosses, tetraploid endosperms that have two different levels of paternal genome contribution can be compared.

MATERIALS AND METHODS

Indeterminate gametophyte (ig): Against a genetic background of inbred W23, this allele not only conditions male sterility in the homozygous state but is responsible for several abnormalities in embryo sacs derived from megaspores that contain it. The most important and relevant of these is the function of varying numbers of polar nuclei in secondary fertilization (KERMICLE 1971; LIN 1978). Defective kernels amount to 45% in *ig/ig* plants, half as many in *Ig/ig*.

Translocated chromosomes: *T6-10a* translocation breaks are at L0.75 in chromosome *6* and at L0.15 in chromosome *10* (LONGLEY 1961). Based on RHOADES' (1950) measurement of meiotic

TABLE 1

Chromosome numbers of endosperms produced following ig/ig *(homozygous standard)* × Ig/Ig *(homozygous* T6-10a) *matings*

Endosperm ploidy	*T6-10a* dosage	No. of endosperms	(%)
2x	1	3	(1.5)
3x	1	114	(56.2)
4x	1	54	(26.6)
5x	1	19	(9.4)
6x	1	6	(2.9)
7x	1	5	(2.4)
8x		2	(1.0)
9x	1		
Total		203	(100.0)

chromosomes, the translocated chromosome having the centromere of chromosome *6* (6^{10}) is about the length of chromosome *2* and has an arm ratio of 1:4.15. The complementary chromosome with the centromere of chromosome *10* (10^{6}) is about two-thirds the length of chromosome *10*, and its arm ratio is 1:1.3. In somatic metaphase, it appears to be metacentric and is readily distinguished from the subtelocentric, shorter chromosomes of the standard complement.

Genetic markers for endosperm pigmentation: The two *R* alleles (*r-g*: colorless embryo and aleurone; *R-st*: colorless embryo and stippled aleurone) used in this study were described by BRINK (1964). *M-st* is an intensifier of *R-st* and located 5.7 crossover units distal to it (ASHMAN 1960); the effect of *R-st* + *M-st* on intensity of stippling is proportional to dosage (KERMICLE 1971) and, therefore, can reveal parental genome contributions.

Cytological determination of endosperm ploidy: Developing ears were harvested 5–12 days following hand pollination. Ovules were collected and pretreated with 8-hydroxyquinoline, sucrose and aeration, and glusulase was used to spread the cells (LIN 1977a).

RESULTS

Endosperm development in 2x × 2x: Cytological analyses were done on samples 5 days after pollination, when differences in endosperm size were not yet detectable. Table 1 gives results of the cytological analysis of endosperms following the cross *ig/ig* (homozygous standard) × *Ig/Ig* (homozygous *T6-10a*). Unlike the *Ig* endosperm whose basic ploidy was uniformly 3x (LIN 1977b), the ploidy of endosperm of *ig* maternity was variable, ranging from 2x to 9x. Of 203 *ig* endosperms studied, approximately half (56.2%) were triploid. The tetraploid and pentaploid endosperms stood, respectively, second and third in rank, at 26.6 and 9.4%. Endosperms of other ploidy levels constituted only minor portions of the total. The single 9x endosperm listed was observed in a separate sample and, therefore, is excluded from the frequency tabulation.

The presence of diploid endosperms was of particular interest and was confirmed by a study of older samples, at 10 days after pollination when size differences were evident. Five of 300 endosperms were small and similar in structure and relative position in the nucellus, and each was about one-fourth the size of the nucellar tissue. Three proved to be diploid; the others were pentaploid and hexaploid.

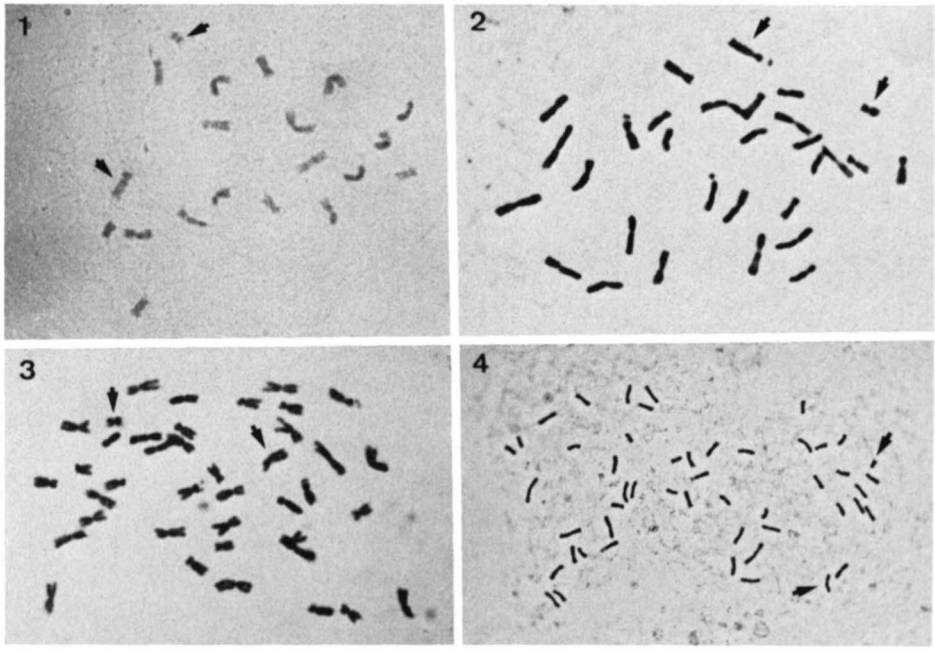

FIGURE 1.—Chromosomes of a diploid (2x) endosperm cell derived from the cross *ig/ig* (homozygous standard) × *Ig/Ig* (homozygous *T6-10a*). The two translocation chromosomes are marked with an arrow.

FIGURE 2.—Chromosomes of a triploid (3x) endosperm cell derived from the same ear as that in Figure 1. The two translocation chromosomes are marked with an arrow.

FIGURE 3.—Chromosomes of a tetraploid (4x) endosperm cell derived from the same ear as that in Figure 1. The two translocation chromosomes are marked with an arrow.

FIGURE 4.—Chromosomes of a pentaploid (5x) endosperm cell derived from the same ear as that in Figure 1. The two translocation chromosomes are marked with an arrow.

The paternal contribution to the *ig* endosperms was uniformly one genome throughout the sample collected. Only one dose of the short metacentric chromosome 10^6 was present, irrespective of the ploidy level. Similarly, only one dose of the long translocated chromosome 6^{10} was found in cells in which this chromosome was distinguishable from the normal chromosome *6* (Figures 1–8). Accordingly, it was confirmed that the maternal contribution was responsible for the ploidy variation found.

Three classes of mature kernels were produced following the mating *ig/ig* × *Ig/Ig*: normal, miniature and abortive (Figure 9). The "normal" class does not differ appreciably from the typical seeds obtained in *Ig/Ig* × *Ig/Ig* crossing involving strains of the same genetic background. Kernels of the miniature class are well filled and plump but are only half the size of normals. Kernels of the abortive class are variable in size but always smaller than miniature kernels; the pericarp is wrinkled, and the endosperm is empty or only partly filled. Within the inbred W23 background, viability of normal and miniature kernels was high (Table 4), but viability of abortives was low (>5% germination).

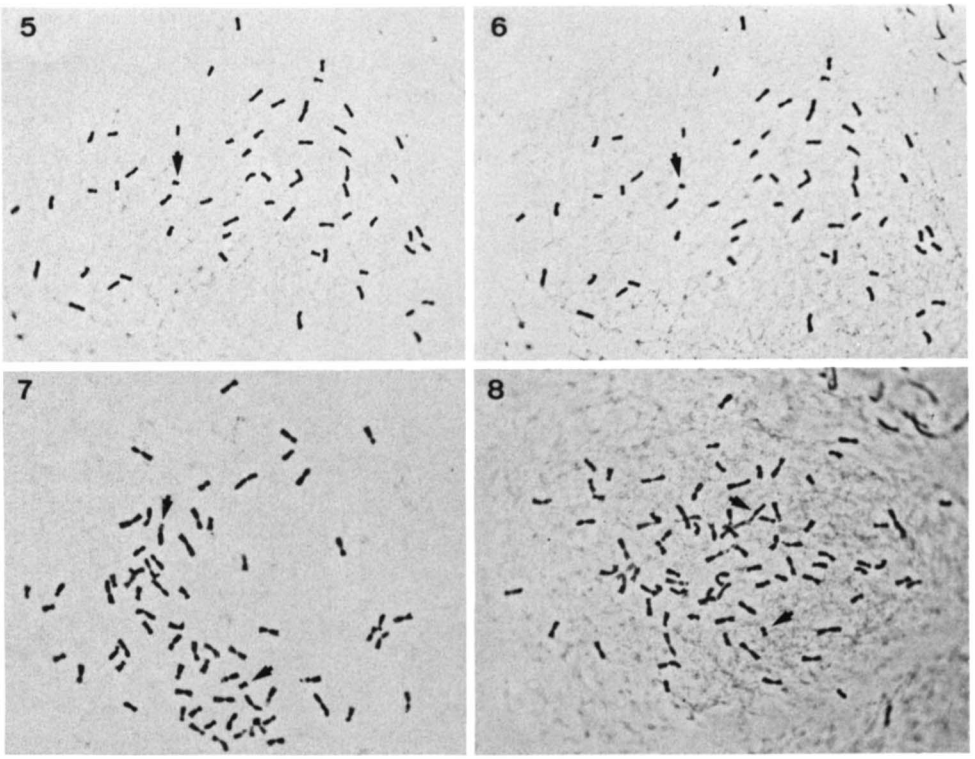

FIGURE 5.—Chromosomes of a hexaploid (6x) endosperm cell derived from the same ear as that in Figure 1. Only the short translocation chromosomes is marked with an arrow.

FIGURE 6.—Chromosomes of the same cell as Figure 5 at different focus.

FIGURE 7.—Chromosomes of a heptaploid (7x) endosperm cell derived from the same ear as that in Figure 1. The two translocation chromosomes are marked with an arrow.

FIGURE 8.—Chromosomes of a nonaploid (9x) endosperm cell derived from an ear different than that in Figure 1. The two translocation chromosomes are marked an arrow.

Normal kernels constituted the majority class, 58.9%, which is comparable to the frequency of juvenile triploid (3x) endosperms (56.2%, Table 1). The frequency of miniature kernels (25.6%) agrees with that of tetraploid (4x) endosperms (26.6%). The frequency of the abortive kernels (15.5%) is comparable to the sum of ploidy levels other than 3x and 4x (17.2%). These results indicate that endosperm of normal kernels is triploid and that of the miniature kernels tetraploid. The abortive kernels contained endosperm with a ploidy other than 3x and 4x. This conclusion is of interest, since it indicates that tetraploid endosperms can support the development of viable embryos.

Table 2 presents results for aleurone stippling of mature kernels, graded on an intensity scale from 1–10. The first three entries are standard crosses illustrating the range of grades in triploid *Ig* endosperms with one, two and three *R-st* + *M-st* alleles present. In the cross *ig/ig*; *r-g/r-g*; *m-st/m-st* × *Ig/Ig*; *R-st/R-st*; *M-st/M-st* (entry 4, Figure 10), gradings for plump normal kernels corresponded to the range and frequencies for a single dose in the standard triploid endosperm.

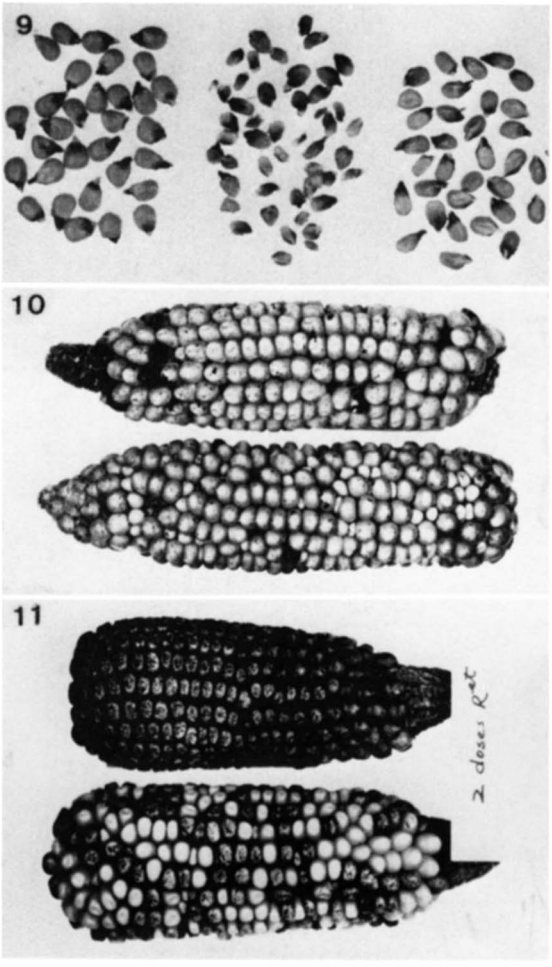

FIGURE 9.—Three classes of kernels obtained from the cross *ig/ig* × *Ig/Ig*. Left to right, respectively: normal, abortive and miniature.

FIGURE 10.—Top, Ear produced following the cross *Ig/Ig*; *r-g/r-g*; *m-st/m-st* × *Ig/Ig*; *R-st/R-st*; *M-st/M-st*, showing the stippled expression of kernels containing one dose of *R-st* + *M-st*. Bottom, Ear produced following the cross *ig/ig*; *r-g/r-g*; *m-st/m-st* × *Ig/Ig*; *R-st/R-st*; *M-st/M-st*. The stippled expression of kernels on this ear is comparable to that of control kernels carrying one dose of *R-st* + *M-st*.

FIGURE 11.—Top, Ear produced from the cross *Ig/Ig*; *R-st/R-st*; *M-st/M-st* × *Ig/Ig*; *r-g/r-g*; *m-st/m-st*, showing stippled expression of kernels carrying two doses of *R-st* + *M-st*. Bottom, Ear derived from the cross *Ig/ig*; *R-st/r-g*; *M-st/m-st* × *Ig/Ig*; *r-g/r-g*; *m-st/m-st*. The stippled expression of this ear is similar to that of kernels carrying two doses of *R-st* + *M-st*.

The cross *Ig/ig*; *R-st/r-g*; *M-st/m-st* × *Ig/Ig*; *r-g/r-g*; *m-st/m-st* segregated colorless kernels (Figure 11), and entry 5 in Table 2 represents only the normal colored kernels, 36% of the normal class. Apart from the few expected crossovers between *R-st* and *M-st* loci, the gradings agreed well with a double maternal dose of stippling. The inference is reinforced that the normal class of

TABLE 2

Aleurone stippling intensity of kernels produced following intercrosses of R-st-*carrying plants associated with* Ig *or* ig

Entry	Crosses[a]	No. of kernels according to grade of stippling										*R-st* dosage
		1	2	3	4	5	6	7	8	9	10	
1	a	1	86	61	2							1
2	b				4	51	83	12				2
3	c							8	52	75	14	3
4	d	0	82	67	1							1
5	e	3	2		5	57	76	8				2

[a] a: *Ig/Ig; r-g/r-g; m-st/m-st* × *Ig/Ig; R-st/R-st; M-st/M-st.*
b: *Ig/Ig; R-st/R-st; M-st/M-st* × *Ig/Ig; r-g/r-g; m-st/m-st.*
c: *Ig/Ig; R-st/R-st; M-st/M-st* selfed.
d: *ig/ig; r-g/r-g; m-st/m-st* × *Ig/Ig; R-st/R-st; M-st/M-st.*
e: *Ig/ig; R-st/r-g; M-st/m-st* × *Ig/Ig; r-g/r-g; m-st/m-st.*

TABLE 3

Chromosome numbers of endosperms produced following ig/ig × Ig/Ig/Ig/Ig *crosses*

Endosperm size	No. of endosperm (%)					
	5 Days	10 Days		12 Days		
	Uniform	Large	Small	Large	Medium	Small
3x						
4x	51 (57.3)	2 (2.9)	46 (95.8)		7 (25.9)	46 (93.9)
5x	25 (28.1)	48 (69.5)	1 (2.1)	7 (36.8)	16 (59.3)	3 (6.1)
6x	9 (10.1)	16 (23.2)		9 (47.4)	1 (3.7)	
7x	4 (4.5)	2 (2.9)	1 (2.1)	2 (10.5)	2 (7.4)	
8x		1 (1.5)		1 (5.3)	1 (3.7)	
9x						
Total	89 (100)	69 (100)	48 (100)	19 (100)	27 (100)	49 (100)

kernels has triploid endosperm of the basic 2x maternal: 1x paternal constitution, whether it is derived from *Ig* or *ig* embryo sacs.

Endosperm development following ig/ig × Ig/Ig/Ig/Ig *matings*: Table 3 presents a ploidy analysis of *ig* endosperms collected 5, 10 and 12 days after pollination by tetraploid plants. At 5 days they were of uniform size, and it was possible to take an unbiased sample. By 10 days they had started to differentiate in size and shape and had become markedly different by 12 days after pollination.

The ploidy levels of the earliest set ranged from 4x to 7x. The frequency distribution agreed well with that from the diploid-diploid cross of Table 1, when allowance was made for one additional paternal genome ($\chi^2 = 4.90$, 6 d.f., $P > 0.5$). The diploid nature of the pollen was confirmed by its use on an *Ig* diploid plant; an ear with uniformly abortive kernels was produced (see also RANDOLPH 1935).

At 10 days postpollination endosperms consisted of two size classes: large and small. The large endosperms filled approximately two-thirds of the nucellar cavity and, like the 3x endosperms of *Ig* material collected at the same stage, had a round, dome-shaped crown. The small endosperms filled less than one-fourth of the nucellus and had a shrunken top resembling the 4x endosperms produced following ordinary diploid × tetraploid crosses.

The two classes had quite different ploidy distributions. The large endosperms were mainly 5x or 6x (69.5 and 23.2%, respectively) and, at this stage, they appeared to be growing equally well. The small ones were almost all 4x and were already retarded and abnormal.

Variation in endosperm size was even more evident at 12 days. The large ones completely filled the nucellar cavity and were most often 5x or 6x again, never 4x. A class of medium-sized endosperms filled two-thirds of the nucellus like the large class previously; they were mostly 5x, sometimes 4x and only once 6x. The smallest group were again solidly 4x, with occasional 5x.

When all three young stages were considered, there was an interesting divergence of behavior between the common ploidy levels. All developed normally up to at least 5 days after pollination, but, by 10 days, the majority of the tetraploid tissues were already retarded, and all of them were retarded by 12 days. On the other hand, 5x endosperms underwent a regular development through the 10-day stage, but most of them were abnormal 2 days later. At the final sampling time, only one of the hexaploid endosperms had faltered in growth, and this one was found to have 58 chromosomes instead of 60. LIN (1975) concluded, on the basis of his study of miniature seeds on tetraploid plants, that a deficient chromosome constitution, as such, is the basis of endosperm failure.

This endosperm development pattern is consistent with the frequency distribution of mature seeds. On an average, the frequency of normal plump kernels on an *ig/ig* ear pollinated by pollen of a tetraploid plant is 12%, which is comparable to the frequency (10%) of endosperm with a chromosome constitution of 6x of the 5-day sample (Table 3). These results, therefore, indicate that only 6x endosperms developed normally up to maturity. Endosperms of other ploidy levels started to degenerate at early stages of development and became abortive by maturity.

Maturation and germination of kernels possessing tetraploid endosperms: Two distinctive sources of tetraploid endosperms were compared for their effects on mature kernel form and viability. One was from the series of crosses of *ig/ig* as female with three different *Ig/Ig* strains as male, which produced tetraploid endosperms containing three maternal genomes and one paternal (3x:1x), as demonstrated previously. The crosses behaved similarly; evident 4x (3x:1x) kernels were comparable but smaller than the 3x (2x:1x) ones on the same ears. In tetraploid kernels, after the thin and transparent pericarp was removed, the exposed endosperm was plump, well filled and colored by carotenoids. A shell of dense (horny) starch enveloped a central pocket of soft starch. Embryos were normal in shape but slightly smaller than those associated with 3x (2x:1x).

TABLE 4

Weights and germination of sib seed classes containing 3x and 4x endosperm

Entry	Parentage		Seed weights (mg)			Seed germination (%)		
	Female	Male	Large seed[a]	Small seed[b]	Small/ large	Large seed	Small seed	Small/ large
1	*ig/ig* (W23)	*Ig/Ig* (W22)	246.4[c]	128.8	0.52	100.0[d]	100.0	1.00
			249.2	115.7	0.46	97.7	94.4	0.96
			181.1	90.7	0.50	100.0	100.0	1.00
			218.1	114.7	0.53	98.8	90.9	0.92
2	*ig/ig* (W23)	*Ig/Ig* (W187)	220.0	110.0	0.50	100.0	96.9	0.97
			199.7	97.3	0.48	100.0	91.7	0.92
			235.4	132.2	0.56	96.3	94.9	0.98
			238.4	136.7	0.57	98.2	97.1	0.99
3	*ig/ig* (W23)	*Ig/Ig* (B190)	224.7	129.2	0.57	96.1	97.5	1.01
			196.1	107.6	0.54	95.9	96.3	1.00
			210.8	108.2	0.51	97.5	97.1	0.99
			242.0	123.0	0.51	97.5	100.0	1.02
4	Ig/Ig[e] (W23)	*Ig/Ig* (BN751)	247.0	27.9	0.11	98.6	1.4	0.01
		Ig/Ig/Ig/Ig (BN751)	253.0	25.0	0.10	100.0	1.4	0.01

[a] Seeds contain 3x endosperm.
[b] Seeds contain 4x endosperm.
[c] Average weight of 70 seeds.
[d] Percentage of 70 seeds germinated.
[e] Each individual ear was pollinated by monoploid and diploid pollen grains. See the text for details.

The other source was of 2x:2x endosperm arising from the diploid-tetraploid cross *Ig/Ig* × *Ig/Ig/Ig/Ig*. Again, it was matched with standard 2x:1x endosperms on the same ear produced by divided pollination. This was accomplished by dividing the core of silks into two halves and pollinating one-half with pollen from the tetraploid parent and the other half with pollen from an *Ig/Ig* diploid. The control kernels were large, plump and solid, whereas those with 2x:2x were small, shriveled and hollow. The pericarp of the defective kernels was thick and gray colored. Beneath the pericarp were small remnants of the endosperm associated with a reduced, wrinkled and irregularly shaped embryo.

Kernel weights (Table 4) emphasize the difference between the 3x:1x and 2x:2x endosperms. Seeds with the former were about half the weight of the 2x:1x controls on the same ear, and agreement between the crosses was good ($F = 0.83$, 2 and 9 d.f., $P > 0.1$). The 2x:2x kernels, however, were only about one-tenth of the weight of the corresponding controls, and of this the pericarp and embryo formed the major part.

Germination tests (Table 4) showed that small kernels from three *ig/ig* × *Ig/Ig* crosses germinated at the same frequency as the large kernels, and no

TABLE 5

Genomic constitution and development of maize endosperm at maturity

Genomic constitution of endosperm (♀:♂)	Endosperm development			Source
	Normal	Subnormal	Abortive	
1x:1x			+	*ig/ig* × *Ig/Ig*
2x:1x	+			Same
3x:1x		+		Same
4x:1x			+	Same
5x:1x			+	Same
6x:1x			+	Same
7x:1x			+	Same
2x:2x			+	*ig/ig* × *Ig/Ig/Ig/Ig*
3x:2x			+	Same
4x:2x	+			Same
5x:2x			+	Same
6x:2x			+	Same
2x:1x	+			*Ig/Ig* × *Ig/Ig*
2x:2x			+	*Ig/Ig* × *Ig/Ig/Ig/Ig*

statistical difference was detected between them ($F = 2.00$, with 2 and 9 d.f., $P > 0.1$). The evidence suggests that, beyond a certain point, viability of embryos does not reflect the developmental condition of the endosperms. In the case of *Ig/Ig* × *Ig/Ig/Ig/Ig* crosses, the small kernels germinated poorly at 1.4%. The germination was improved to 10 to 15% when the pericarps were opened artificially during germination.

In summary, kernels with 3x:1x endosperms were subnormal in size, normal in morphology and normal in viability. Those with 2x:2x were severely defective and had a low viability. Since the two endosperm types both contained a simple additional genome, of maternal origin in the former (3x:1x) and of paternal origin in the latter (2x:2x), these results suggest that the difference in the parental source of a single genome has significant developmental consequences for the endosperm.

DISCUSSION

The ploidy structure and developmental fate of *ig* endosperms, and two regular ones, are summarized in Table 5. Of the 12 types borne on *ig/ig*, only two were evidently normal at maturity, namely, the standard 3x (2x female:1x male) associated with 2x embros, or 1x in few cases (see also KERMICLE 1969), and 6x (4x:2x) associated with 3x embryos. One 4x tissue (3x:1x) was subnormal but supported fully viable 2x embryos. It is very probable that all other combinations of maternal and paternal genomes aborted.

Hypotheses involving genome balance between maternal tissue, endosperm and embryo can be readily dismissed. The maternal tissue was 2x in all cases, yet one class of 6x endosperm succeeded as well as the standard 3x ones. The

endosperm to embryo ratio differs substantially between the two classes of normal kernels, 6:3 and 3:2 or 3:1. It appears that neither the maternal tissue nor the embryo had any critical effect on endosperm development in this material.

The data are also instructive with respect to theories based on the genetic constitution of the endosperm itself. The notion of SARKAR and COE (1971), that triploidy or multiples of it are basic to normal endosperm development, fails on the unequal performance of two classes of 6x tissue. The 5x:1x class (Figures 5 and 6) should grow as well as the 4x:2x class if total ploidy were the only critical factor. But, as mentioned previously, of the five most defective endosperms selected from 300 in *ig/ig* × *Ig/Ig* at age 10 days, one was hexaploid; frequencies of seed abortion on mature ears likewise indicate that no seeds of the 5x:1x class survive. Triploidy (or multiples of it) may be necessary but are not alone sufficient for normal endosperm development.

The normal condition of 4x:2x endosperms from *ig/ig* × *Ig/Ig/Ig/Ig* crosses also renders very unlikely the supposition of interaction between cytoplasm and nucleus (WANGENHEIM 1957). The endosperms contain a hexaploid nucleus surrounded by cytoplasm that would be "balanced" only by a triploid nucleus, since the maternal plant is diploid. In other words, there is a shortage of cytoplasm which, according to WANGENHEIM (1957), would result in an endosperm failure. To bring the present finding into conformity with WANGENHEIM'S hypothesis, it is necessary to suppose that these endosperms resulted from *ig* embryo sacs that underwent an additional mitotic division before cellularization in conjunction with a change in cytoplasmic "ploidy" level. Embryological study (LIN 1981) suggests that an extra mitosis indeed occurs during *ig* megagametogenesis, but its frequency (about 5%) can only account for the formation of endosperms with ploidy higher than 6x (6.4%, the sum of endosperms with 6x or higher ploidy in Table 1). Thus, it is unlikely that *ig* embryo sacs that gave rise to the 4x:2x endosperm had experienced an extra mitotic division. Moreover, the results indicating a differential performance of the two 4x endosperms in the same cytoplasm background also disagree with the hypothesis.

A difference in action of maternal and paternal genomes, as speculated by NISHIYAMA and INOMATO (1966), appears to be consistent with results of *ig* matings. Of the 12 endosperm classes borne on *ig/ig* plants, only two, 2x:1x and 4x:2x, conform to this ratio, and these are the only two that undergo normal growth. Endosperms representing parental ploidy ratios other than 2:1 are either subnormal or abortive.

The effect of parental source was termed "imprinting" by CROUSE (1960) to describe the elimination of paternal chromosomes in Sciara. This term was later adopted by KERMICLE (1975), BROWN and CHANDRA (1977) and LIN (1982). The imprinting hypothesis can further be applied to the growth of tetraploid endosperms. Of the two tetraploid endosperms compared, one is of the 3x:1x constitution and the other 2x:2x. The former is substandard in size but of normal viability, whereas the latter is abortive. The evidence suggests that the presence of an extra genome in the endosperm causes an adverse

developmental effect. The magnitude of the effect depends upon the parental source of this genome. When the extra genome is received maternally, the resulting endosperm is defective to a lesser degree. This is in accord with the imprinting hypothesis, since the magnitude of chromosome excess in terms of the standard maternal contribution is 1/2 in this situation as opposed to 1/1 when the extra genome is paternally contributed.

Also consistent with the imprinting view is the developmental condition of 1x:1x endosperms. These endosperms are produced in *ig/ig* × *Ig/Ig* crosses. They are the result of fusion of a single polar nucleus and a sperm (Figure 1). Such endosperms would be expected to be as severely defective as the 2x:2x endosperms, since both possess the same ratio of maternal to paternal chromosomes, *i.e.*, a 1:1 ratio. This indeed is the case. As shown previously, of five most defective endosperms selected from 300 10-day-old endosperms, three were diploid. It appears that these endosperms diverged from a normal developmental path at a very early stage. This degeneration, according to the parental imprinting hypothesis, is due to deviation from a 2:1 parental ratio.

The imprinting activity of genes affecting endosperm growth was also shown by LIN (1982). Using a *10-B* translocation, termed *TB-10 (19)*, he synthesized, in a single cross, two endosperms that had the same chromosome makeups but differed in the parentage of the long arm of chromosome *10* (*10L*). One endosperm included only four maternal *10L*s, *i.e.*, a 4 *10L*:0 *10L* constitution, and the other endosperm contained the same *10L* dosage but is of the 2 *10L*:2 *10L* combination. At maturity, the former was subnormal and similar to the miniature kernels produced on *ig* plants, whereas the latter was normal and appeared the same as the control. Furthermore, the same ear also yielded a third endosperm that possessed only two maternal *10L*s; *i.e.*, a 2 *10L*:0 *10L* constitution and which was subnormal. Since the three types of endosperms grew on an individual ear, a maternal influence was unlikely to be the foundation of different endosperm growth. LIN also ruled out the possible effects of marker genes or of the translocation used in the study. He concluded that the parental source of *10L* was responsible for the different endosperm growth. Whenever a paternal representative of *10L* is absent as in 2 *10L*:0 *10L* and 4 *10L*:0 *10L* cases, the endosperm is debilitated no matter how many maternal *10L* are present. It is evident that *10L* carries genes, termed endosperm factors, which function only when they are paternally inherited.

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