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The Significance of Genic Balance to Endosperm Development in Interspecific Crosses

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Summary. The endosperm has played a significant role in the evolution of angiosperms because of its physiological and genetic relationships to the embryo. One manifestation of this evolutionary role is its abnormal development in interploidy crosses. It is now established that the endosperm develops abnormally in interploidy-intraspecific crosses when the maternal: paternal genome ratio deviates from 2:1 in the endosperm itself. We propose an Endosperm Balance Number (EBN) hypothesis to explain endosperm development in both interploidy-intraspecific and interspecific crosses. Each species is assigned an EBN on the basis of its crossing behavior to a standard species. It is the EBN which determines the effective ploidy in the endosperm of each species, and it is the EBNs which must be in a 2:1, maternal:paternal ratio. The EBN of a species may be determined by a few genes rather than the whole genome. This hypothesis brings most intraspecific-interploidy and interspecific crossing data under a single concept with respect to endosperm function. The implications of this hypothesis to isolating mechanisms, 2n gametes, the evolution of disomic polyploids, and reciprocal differences in seed development are discussed.

Key words: Endosperm – Evolution – Interspecific crosses – Solanums – 2n gametes

Introduction

The endosperm is one of the products of double fertilization in angiosperms. It is a tissue peculiar to angiosperms and unique in both the plant and animal kingdoms in that it is the only product of fertilization which does not form germ cells. In some as yet unexplained manner the evolution of the endosperm in the Cretacous (Beck 1976) is probably closely linked to the rapid diversification and expansion of the angiosperms (Darlington 1976).

The evolutionary significance of the endosperm is certainly due to its role in the development of the seed (Stebbins 1976). Following fertilization the endosperm grows rapidly and differentiates into the nutritive tissue for the embryo. In some species the endosperm is quite ephemeral; in others it persists through germination. The success of the embryo depends on the normal development of the endosperm in almost all species (Brink and Cooper 1947).

The endosperm is genetically related to the embryo, making the evolutionary significance of its physiological connection to the embryo even more important. In most angiosperms the central cell of the female gametophyte, which upon fertilization becomes the endosperm, has two exact duplicates of the chromosomes of the egg. Thus, since the male nuclei delivered to the central cell and the egg are the same, the nuclear constitution of the endosperm differs from the embryo only in having one extra set of the maternal chromosomes. The development of the endosperm, then, is dependent on the same genes as its accompanying embryo, though in different doses (Fig. 1).

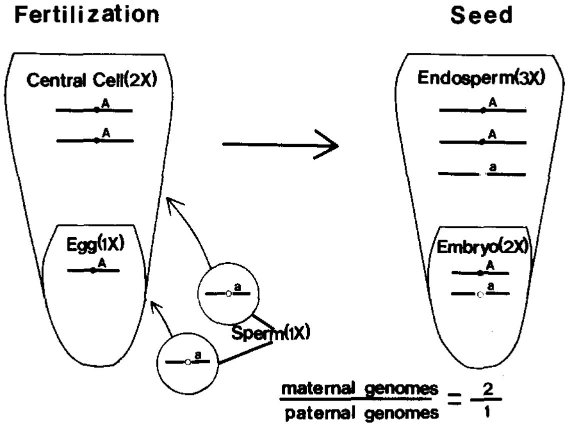

Fig. 1. Normal fertilization. Maternal chromosomes have solid circles depicting centromeres (——●——), paternal have open circles (——○——)

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The importance of the genetic relationship between the endosperm and the embryo to the evolution of higher plants has been almost completely ignored (e.g. Grant 1971; Stebbins 1974; Beck 1976).

Interploidy Crosses

One of the most interesting and enigmatic aspects of the endosperm is its failure to develop normally in interploidy crosses. For example, in many intra- and interspecific 2x × 4x crosses the seed aborts early in development due to the collapse of the endosperm (Brink and Cooper 1947; von Wangenheim 1957; Woodell and Valentine 1961). The problem is clearly not ploidy per se – the endosperm in this cross is tetraploid (two genomes from the female and two from the male) and tetraploid plants are often vigorous and fertile. Apparently, in this cross tetraploidy is acceptable in all tissues except the endosperm. Nor can the failure of the endosperm in most crosses be attributed to species differences. Clearly, genome incompatibility is not the problem in crosses between a diploid and its induced autotetraploid. Even in interspecific, interploidy crosses the problem is not necessarily due to incompatibility between the genomes of the species, since it is often overcome by the functioning of 2n gametes or the induced chromosome doubling of one of the species (Hanneman and Peloquin 1968; Johnston and Hanneman in press).

There have been many hypotheses suggested to explain the failure of the endosperm in interploidy crosses over the last fifty years. Because there are three tissues in close contact in the seed – maternal, endosperm, and embryo – which vary in genetic composition and ploidy, many were attracted to the idea that the seed failed when the normal ploidy ratios of these tissues varied. Müntzing (1930) suggested that a ploidy ratio of 2:3:2 of the maternal tissue: endosperm:embryo was necessary for normal seed development. Watkins (1932) proposed that only the -:3:2 ratio of endosperm:embryo mattered. Valentine (1954) hypothesized that it was the 2:3:- ratio of maternal tissue:endosperm which was important. Some interspecific crosses, however, did not meet any of the above criteria for success or failure. To solve this Stephens (1942) suggested that cotton species be assigned different values or 'strengths' in order to make their crossing behavior conform to the -:3:2 endosperm:embryo ratio. Valentine (1956) used the same reasoning to make the results of crosses in *Primula* meet the 2:3:- maternal tissue:endosperm ratio criterion.

More recently there has been support for an idea originally proposed by Nishiyama and Inomata (1966). They suggested that the success of the endosperm depended on a 2:1 ratio of the maternal:paternal genomes in the endosperm itself, regardless of the ploidy level of the maternal tissue or embryo. The earlier embryological work in potato by von Wangenheim et al. (1960) supports this hypothesis, though they did not give this interpretation to their work. They found normal endosperm development in 4x × 2x crosses with maternal:endosperm:embryo ploidy ratios of 2:3:2, 2:3:1, and 2:3:0 (i.e. normal endosperm development with no embryo present). Since the endosperm is normal when 2n eggs function in 2x × 4x crosses, a 1:3:2 ratio is also acceptable. Therefore, endosperm development does not depend exclusively on either a 2:3:2, 2:3:-, or a -:3:2 ratio.

To date the seminal work on the role of ploidy in endosperm development has been that done in maize by Lin (1975). Using a gene (*ig*) which conditions mitotic abnormalities in the female gametophyte, he was able to produce 1x to 8x central cells on a 2x female. From the results of crosses of *igig* diploids with 2x and 4x males, combined with elegant cytology, he concluded that: 1) Neither the ploidy of the maternal parent nor the embryo influences the development of the endosperm, and 2) Not only must the endosperm have a ploidy that is a multiple of 3x for normal development, but the ploidy must consist of a two maternal: one paternal genomes ratio. A 6x endosperm with a maternal:paternal genome ratio of 4:2 was normal; however, a 6x endosperm with a ratio of 5:1 was abortive.

A survey of the literature on interploidy, *intra*specific crosses (Wellensiek 1955; Avery et al. 1959; von Wangenheim et al. 1960; Tsuchiya 1960) indicates almost all the results of crossing can be explained on the basis of the 2:1 hypothesis. The proposal of Nishiyama and Inomata (1966), proved in maize by Lin (1975), has done much to clarify the basis of endosperm failure in interploidy crosses.

Discussion

The Endosperm Balance Number (EBN) Hypothesis

In potato all intraspecific, interploidy crosses conform to the 2:1 hypothesis (von Wangenheim et al. 1960; Marks 1966). However, it is not directly applicable to all interspecific crosses. For example, *Solanum acaule* is a tetraploid (2n = 4x = 48) which does not form hybrids with other S. American tetraploids, often due to endosperm breakdown (von Wangenheim 1955). Yet, *S. acaule* readily produces plump seeds with 3x embryos and normal endosperms when crossed with diploid species (2n = 2x = 24) or even the haploids (2n = 2x = 24) extracted from *S. tuberosum* tetraploids (2n = 4x = 48) (Irikura 1968). This type of crossing behavior is not unique to *S. acaule*. Several other tetraploids and at least one hexaploid (*S. demissum*) also seem to violate the 2:1 hypothesis

(Johnston and Hanneman, in press). This anomalous behavior is not peculiar to potato; similar results have been reported in wheat (Gill and Waines 1978), oats (Nishiyama and Yabuno 1978), alfalfa (Lesins 1961), *Datura* (Avery et al. 1959), cotton (Stephens 1942), and tomato (Cooper and Brink 1945).

We propose an Endosperm Balance Number (EBN) hypothesis as an expansion of an Endosperm Balance Factor hypothesis developed by Johnston and amplified by den Nijs and Peloquin (Johnston and Hanneman 1978; den Nijs and Peloquin 1977c). The EBN hypothesis extends the 2:1 proposal to many interspecific crosses. Under this hypothesis the genome of each species is assigned a specific value in the endosperm. The value may be different for species of the same ploidy. It is the EBN which determines the effective ploidy in the endosperm and which must be in a 2 maternal:1 paternal ratio (Fig. 2).

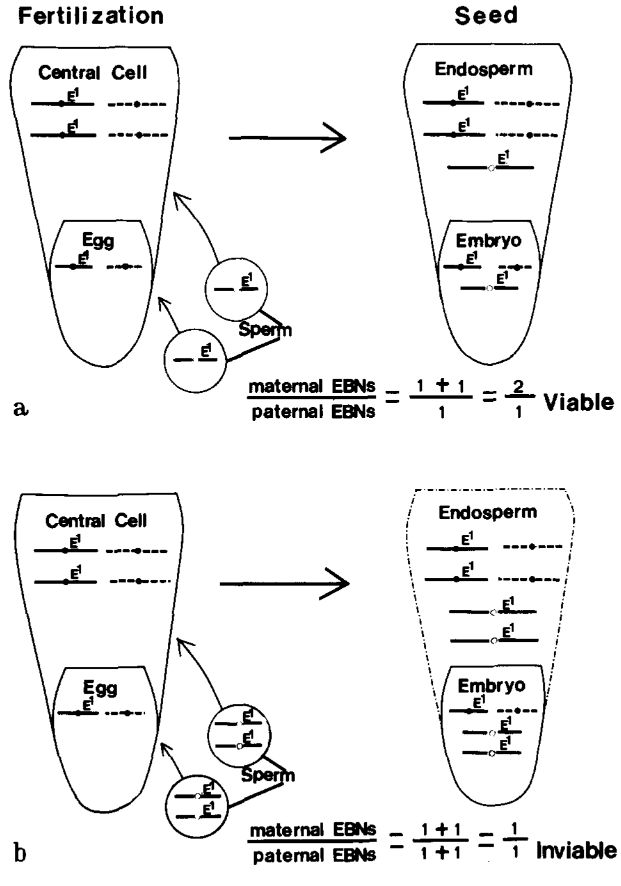

Fig. 2a and b. a 4x(2EBN) *S. acaule* X 2x(2EBN) *S. chacoense.* Successful interspecific, interploidy, intra-EBN cross in *Solanum* b 4x(2EBN) *S. acaule* X 4x(4EBN) *S. chacoense.* Unsuccessful interspecific, intraploidy, inter-EBN cross. Maternal chromosomes have solid circles depicting centromeres (—•—). Paternal chromosomes have open circles (—○—).
E^1 represents hypothetical factor(s) determining the Endosperm Balance Number (EBN). In this example it is assumed *S. acaule* is a 4x(2EBN) species with inactivation of the EBN factors in one genome

For example, let us arbitrarily assign an EBN of two to 2x *S. chacoense.* This species crosses with 4x *S. acaule* yielding 3x offspring from plump seeds. Therefore, *S. acaule* would be assigned an EBN of two also. Colchicine-induced 4x(4EBN) *S. chacoense* forms plump seeds when crossed with 4x *S. tuberosum*, but not when crossed with *S. acaule* or 2x *S. chacoense.* Therefore, 4x *S. tuberosum* would be assigned an EBN of four. It follows from the EBN hypothesis that the hybrids would have normal endosperm when *S. acaule* is crossed with 2x(2EBN) *S. tuberosum* haploids, but, abortive when crossed with 4x(4EBN) *S. tuberosum.* However, colchicine-induced 8x(4EBN) *S. acaule* should cross with 4x(4EBN) *S. tuberosum.* All these predictions of the EBN hypothesis are met (Johnston and Hanneman in press; Swaminathan 1954; and Johnston unpublished).

Further tests of the EBN hypothesis have been conducted in potato (Johnston and Hanneman in press). They indicate that the 2:1 EBN ratio is a necessary condition for successful endosperm development in interspecific crosses. Apparently, the genome of each species has an effective number which may not be a direct reflection of its ploidy (e.g. we have found 4x(2EBN), 4x(4EBN), 3x(3EBN), 3x(2EBN) and 6x(4EBN) species). The EBN of each species seems constant in different interspecific crosses indicating that the effective endosperm ploidy is determined in the same way in different species.

The EBN hypothesis also appears to clarify the results of interspecific crosses in several other genera including cotton, (Stephens 1942), tomato (Cooper and Brink 1945), and *Datura* (Avery et al. 1959). Nishiyama and Yabuno (1978) have proposed a similar idea to explain interspecific crossability in oats. The results of their extensive interspecific crosses were best explained by assigning an arbitrary value to one species and then determining the values for other species assuming that the maternal:paternal values had to be in a 2:1 ratio in the endosperm for successful seed development.

Implications

The EBN hypothesis also has evolutionary and genetic implications which are important.

Isolating Mechanisms: Crosses between species of different EBN yield almost no hybrids of intermediate ploidy even when many fertilizations are effected. Since the EBN is not directly related to ploidy, two species of the same ploidy but different EBN can be strongly incompatible as are 4x(2EBN) *S. acaule* and 4x(4EBN) *S. tuberosum.* EBN incompatibility may also serve as a barrier between diploids in both oats (Nishiyama and Yabuno 1978) and potato (Johnston unpublished). There is even evidence that there may be an EBN barrier between some diploid species in the genus *Datura*, which does not have a ploidy series (Avery et al. 1959).

Through the EBN isolating mechanism, a new species could arise rapidly if there are only a few genes which determine the EBN. This is because mutations leading to a new EBN would either be lethal due to endosperm failure (if fertilization involved gametes of normal EBN) or would form a new true-breeding, genetically isolated but sympatric population (if fertilization involved gametes having the new EBN). Such an isolating mechanism may in part explain the endosperm's role in the rapid diversification of the angiosperms.

2n Gametes: Occasionally, plump seeds are formed in inter-EBN crosses. In potato most of these are due to the functioning of 2n gametes (Hanneman and Peloquin 1967; den Nijs and Peloquin 1977a,b; Johnston and Hanneman in press). For example, 2x(2EBN) *S. chacoense* × 4x(4EBN) *S. tuberosum* will yield an occasional plump seed that gives rise to a 4x(4EBN) plant. Apparently, a 2n egg in *S. chacoense* is fertilized and the resulting endosperm has a 2:1 EBN ratio. Thus, the 2:1 EBN requirement can serve as a strong selective screen for the functioning of the 2n gametes of the lower EBN species in inter-EBN crosses. This would allow for recurrent, direct introgression from the lower EBN species to the higher, while still maintaining the ploidy integrity of each. In this way the EBN hypothesis compliments the growing realization of the importance of 2n gametes in evolution (den Nijs and Peloquin 1977a,b).

Disomic Inheritance: Invariably, in the species surveyed, 4x(2EBN) species (i.e. tetraploids that behave as some diploids in the genus with respect to endosperm development in interspecific crosses) have bivalent pairing. Those that have been analyzed apparently have disomic inheritance. This correlation may be accidental or it may reflect the nature of evolution of these species. For example, a 4x(2EBN) may have arisen from two 2x(1EBN) species via bilateral sexual polyploidization (Mendiburu and Peloquin 1976). The newly arisen 4x(2EBN) species would be reproductively isolated from the progenitor diploids due to EBN differences. It would be isolated from 2x(2EBN) species as the hybrids would be highly sterile triploids. However, disomic inheritance would not be a prerequisite for this type of evolution. One would expect to find both disomic and tetrasomic 4x(2EBN) species. Only disomic 4x(2EBN) species have been found.

On the other hand, a 4x(2EBN) species may be the result of the diploidization itself after bilateral sexual polyploidization involving 2x(2EBN) species. If the genes controlling EBN were inactivated in one genome a stable EBN could only be maintained by the pairing only between chromosomes of the same species. Clausen and Cameron (1950) have shown that this type of process has taken place with chlorophyll genes in tobacco, and Garcia-Olmedo et al. (1978) have evidence suggesting that only portions of the A, B, and D genomes are active in wheat.

Reciprocal Differences in Interspecific Crosses: Inherent in the EBN hypothesis is the potential for explaining reciprocal differences in endosperm development in inter-EBN crosses. The embryos in reciprocal crosses would be genetically the same. The endosperms, however, would have different EBN ratios. For example, a 4EBN × 2EBN cross would have a maternal:paternal EBN ratio of 4:1 in the endosperm. The reciprocal cross would have ratio of 1:1. Several workers have noted differences in endosperm development in reciprocal crosses (Cooper 1951; Shevtsov 1972; Nishiyama and Inomata 1966; Lin 1975) and Nishiyama and Yabuno (1978) have proposed rules to explain the difference based on endosperm ratios.

Concluding Comments

The EBN hypothesis is probably a simplification of the situation. Certainly other interactions are also important in determining endosperm development. For example, Gill and Waines (1978) have described a situation in wheat where imprinting is important in determining endosperm development in some interspecific crosses.

The value of the EBN hypothesis, however, is that it: 1) Helps predict the success of and resultant ploidy from interspecific crosses; 2) Adds coherence to the interpretation of the data on endosperm development in interspecific crosses; 3) Points to the fundamental role of the endosperm in evolution; 4) Focuses future work on the genetic and physiological factors which underlie the EBNs.

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