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D. Carputo · L. Monti · J. E. Werner · L. Frusciante

Uses and usefulness of endosperm balance number

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Abstract The Endosperm Balance Number (EBN) hypothesis was developed in the early '80s to explain the basis for normal seed development after intra- and inter-specific crosses, first in the potato and then in several other crop species. According to this hypothesis, each species has a genome-specific effective ploidy, the EBN, which must be in a 2 : 1 maternal to paternal ratio in the hybrid endosperm for normal development of the endosperm itself. This paper reviews how the EBN may act as a powerful isolating mechanism in sexual reproduction, maintaining the genome integrity of the species and playing an important role in the speciation of polyploids from diploids. We also provide further evidence that EBN is more important than chromosome ploidy in determining the success or failure of interspecific crosses. In fact, results from interploidy and inter-EBN crosses to infuse 1EBN *Solanum commersonii* into 4EBN *S. tuberosum* demonstrated that the knowledge and manipulation of EBN is a useful tool in designing breeding schemes and in predicting the offspring ploidy and EBN. In this paper we also discuss the exceptions to the 2 : 1 EBN ratio, and report the evidence for endomitosis in the polar nuclei to explain exceptions to the EBN model in the potato.

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D. Carputo (✉) · L. Frusciante
Department of Agronomy and Plant Genetics,
via Universita' 100, 80055 Portici, Italy
Fax: +39-81-7753579
E-mail: carputo@unina.it

L. Monti
CNR-IMOF, Research Institute for Vegetable and Ornamental
Plant Breeding, via Universita' 133, 80055 Portici, Italy

J. E. Werner
Department of Botany and Plant Sciences,
University of California Riverside, Riverside, CA 92521, USA

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Introduction

It is well known that in Angiosperms double fertilization occurs; one sperm nucleus fuses with the egg to generate a zygote, the other fuses with the polar nuclei of the central cell of the female gametophyte to form the primary endosperm nucleus. The endosperm is a major food and feed source, and a unique and fundamental tissue in Angiosperms. It provides food materials for the developing embryo and, in many cases, for the young seedlings. Furthermore, the endosperm is extremely important in relation to seed formation or failure in interploid and interspecific crosses, in that endosperm breakdown is usually the main cause of seed failure after double fertilization (Brink and Cooper 1947).

Over the years many studies have been carried out to explain the basis for normal seed development. Early hypotheses suggested a need for a particular balance of chromosome sets between the fundamental parts of the developing seed (i.e. maternal tissue, embryo and endosperm) for normal seed growth (Boyes and Thompson 1937; Valentine 1956). However, during studies on haploid extraction in potatoes through $4x \times 2x$ crosses, von Wangenheim et al. (1960) found that normal endosperm development occurred with various balances in the chromosome sets of the above mentioned tissues. This suggested that endosperm development could depend upon its own constitution, and not on that of the embryo or the maternal tissue. Strong support for this idea was provided by cytological studies in corn by Lin (1975). This author tested a number of hypotheses for the cause of endosperm failure using the indeterminate gametophyte (*ig*), which allows the functioning of various numbers of polar nuclei during secondary fertilization, and employing a reciprocal translocation as a marker for the male genome. Endosperms with ploidy levels ranging from diploid to nonaploid were generated following crosses between the diploid mutant (*igig*) as female and either diploid (*IgIg*) or tetraploid (*IgIgIgIg*) normal males. It was found that only two types of endosperm were normal at maturity, the standard 3*x* (2*x* female and 1*x* male contribution) from *igig* × *IgIg* crosses, and the 6*x* (4*x* female and 2*x* male contribution) from *igig* × *IgIgIgIg* crosses. This provided convincing evidence that neither the maternal tissue, which was 2*x* in all cases, nor the embryo, which was 2*x* and 3*x* following the *igig* × *IgIg* and the *igig* × *IgIgIgIg* crosses respectively, had any effect on endosperm development. It was also demonstrated that a 2 : 1 maternal to paternal genome dosage in the endosperm was fundamental for the normal growth of the endosperm in maize.

The 2 : 1 genome-dosage hypothesis was consistent with the results from interploid intraspecific crosses. However, it did not account for the results obtained with some interploid interspecific crosses in various genera. For example, Ramanna and Abdalla (1970) reported that tetraploid *Solanum polytrichon* was easily crossable to diploid *Solanum tuberosum* Group Phureja (*phu*), giving rise to triploid hybrids associated with a pentaploid endosperm where a 4 : 1 maternal to paternal genomic ratio was generated. On the other hand, crosses between *S. polytrichon* and tetraploid *S. tuberosum* Group Tuberosum (*tbr*) failed, in spite of the fact that a 2 : 1 genomic ratio was guaranteed. Similarly, Gill and Waines (1978) reported that 4*x* × 2*x* crosses between *Triticum boeticum* and *Aegilops squarrosa* did produce seeds with a 4 : 1 maternal to paternal chromosome ratio in the endosperm. To explain failures in both intraspecific and interspecific crosses in *Solanum*, Johnston et al. (1980) developed a model which extended the 2 : 1 genomic ratio hypothesized by Lin (1975) to include interspecific crosses. This model relies on a balance of qualitative genetic factors (Endosperm Balance Numbers, EBNs), and not chromosome sets, for normal endosperm development. In the present paper we review how the EBN may play an important role in the speciation of polyploids from diploids, and we present different hypotheses to explain deviations from the EBN model. In addition, we provide further evidence that the knowledge and manipulation of EBN is useful in designing breeding schemes aimed at the exploitation of sexually isolated species.

The endosperm balance number hypothesis

According to the EBN hypothesis, each species has a genome-specific effective ploidy level, the EBN, which is not necessarily equivalent to the chromosome ploidy. It is the EBN which must be in a 2 : 1 maternal to paternal ratio in the hybrid endosperm for normal development of endosperm and, consequently, of the hybrid embryo. EBNs have been experimentally assigned to a number of *Solanum* species after crosses with standard species whose EBN was arbitrarily established, and assuming the 2 : 1 ratio as a prerequisite for normal endosperm development. *Solanum chacoense* ($2n = 24$) was first chosen as a standard species by Johnston and Hanneman (1980) and given an EBN of 2. All species which resulted in successful crosses with *S. chacoense* were assigned an EBN of 2. In fact, when species with the same EBN are crossed, the maternal to paternal EBN ratio in the developing endosperm will always be 2 : 1, regardless of the parental ploidies and the direction of the cross. Following these test crosses, a number of species including diploids (e.g. *Solanum verrucosum* and *Solanum boliviense*), and tetraploids (*Solanum acaule* and *Solanum stoloniferum*) have been assigned an EBN of 2. In the same study, Johnston and Hanneman (1980) assigned an EBN of 4 to *tbr* and the hexaploids *Solanum demissum* and *Solanum oplocense* after they successfully crossed them with a colchicine-induced tetraploid 4EBN *S. chacoense* tester.

Some diploid species (*Solanum brevidens*, *Solanum cardiophyllum*, *Solanum commersonii*) strongly isolated from other diploids were also used by Johnston and Hanneman (1982) to test the applicability of the EBN. It was found that *S. cardiophyllum* could be crossed with 2EBN diploid standards only in the artificially produced 4*x* form or through the function of 2n gametes. Thus, it was assigned an EBN of 1, as were *S. brevidens* and *S. commersonii* (*cmm*). Recent studies by Hawkes and Jackson (1992), Ochoa (1992) and Hanneman (1994) have contributed to assigning an EBN to a larger number of *Solanum* species.

Although the potato has been used as the model crop in studying the EBN hypothesis, evidence for EBN-like systems has also been reported in other species. Based on the results of interspecific crosses reported in the literature, Parrott and Smith (1986) provided evidence that EBN is operating in *Trifolium*, and assigned EBN values from 2 to 8 in clover species. Ehlenfeldt and Hanneman (1992) evaluated the crossability relationships within *Lycopersicon* species, and found them consistent with the EBN system. The species belonging to the *L. esculentum* crossability group were classified as 2EBN, whereas higher EBN values were found in the *L. peruvianum* complex. Recently, Katsiosis et al. (1995) proposed that the hypothesis of polar nuclei activation, formulated in *Avena* (Nishyama and Yabuno 1978), and EBN in the potato can be considered as the same biological concept, and they thus grouped oat species into four EBN groups, 1 to 4.

In the last 10 years interest has arisen in studying the genetic control of EBN. In potato, Ehlenfeldt and Hanneman (1988 a) developed a threshold-type genetic model based on the assumption that three unlinked loci with additive effects, and with an equal EBN gene value within a species, control the EBN. Their results also indicated that the three genes involved are homozygous in each species. Under this additive model, genetic recombination in the hybrids may produce gametes with various EBN gene values. Gametes with equal EBN gene values from both parents will give the required 2:1 EBN ratio in the endosperm, and thus the offspring will be normal. Camadro and Masuelli (1995) developed a similar model to explain the results from crosses between 2EBN *S. acaule* (2n = 48) and either 1EBN *cmm* (2n = 2*x*) or 4EBN *S. gourlayi* (2n = 48). However, they hypothesized that the EBN of these species is under the control of two, and not three, independent loci, with alleles in homozygosity. Recently, Johnston and Hanneman (1996) confirmed that more than one gene and more than one chromosome are responsible for the genetic control of EBN in *Solanum* and *Datura*.

Exceptions to the 2:1 EBN requirement have been reported in the literature. For example, Tarn and Hawkes (1986) produced triploid hybrids from crosses between tetraploid *cmm* (2EBN) and diploid *cmm* (1EBN). Even though according to the EBN hypothesis only tetraploid offspring are expected, Chavez et al. (1988) obtained tetraploid and hexaploid hybrids by crossing *S. acaule* (2EBN) with a tetraploid *S. etuberosum-S. pinnatisectum* hybrid (2EBN). Also deviating from the EBN model is the production of inter-EBN hybrids between *cmm* (1EBN) and *S. chacoense* (2EBN) (Ehlenfeldt and Hanneman 1988 a), *S. circaeifoilium* (1EBN) and the *tbr* haploid (2EBN) (Louwes et al. 1992), *S. acaule* (2EBN) and *S. gourlayi* (4EBN) (Camadro and Masuelli 1995); and *S. stoloniferum* (2EBN) and *tbr* (4EBN) (Janssen et al. 1997). Recently, an exceptional tetraploid fertile somatic hybrid *cmm* (+) *tbr* haploid obtained by Cardi et al. (1993 a) has been used in reciprocal inter-EBN crosses with 4EBN tetraploid varieties (Carputo et al., in press). Viable seeds could be obtained from both types of crosses, although the viable to aborted seed ratio was 1:1.1 after 4EBN × 3EBN crosses (female EBN excess) and 1:11.6 after 3EBN × 4EBN crosses (male EBN excess). Ehlenfeldt and Hanneman (1988 a) also reported that a slight excess in maternal EBN dosage was tolerated better than an excess in male EBN dosage, and small but viable seeds could be obtained following 2EBN × 1.5EBN crosses.

Different hypotheses can explain the success or failure of inter-EBN crosses. The most obvious is that 2n gametes occurred, and equalized the parental EBNs. Following various 2*x* × 2*x* inter-EBN crosses, triploid hybrids were produced through the function of 2n gametes from the lower EBN parent (Ehlenfeldt and Hanneman 1988 b; Masuelli et al. 1992). When 2n gametes do not account for exceptions to the EBN ratio requirement, other hypotheses have been postulated. For example, Hermsen (1987) suggested that the different degree of compatibility found between species and genotypes can be explained by assuming that EBN is a quantitative, rather than a qualitative, factor. Camadro and Masuelli (1995) and Masuelli and Camadro (1997) explained triploid hybrid production from inter-EBN crosses between *S. acaule* (2n = 48, 2EBN) and *cmm* (2n = 24, 1EBN) in terms of segregation of the EBN loci. They also hypothesized that EBN could be part of a more complex system of interspecific barriers acting at pre- and post-zygotic levels. Johnston and Hanneman (1995) hypothesized an incomplete penetrance of the EBN ratio requirement, so that random environmental events may allow an occasional endosperm to develop normally. They also maintained that the 2:1 EBN ratio requirement may not be as leaky as it appears, and that the exceptions found may be manifestations of unusual fertilization and mitotic events. For example, due to unusual fertilization, two pollen tubes may fertilize the same ovule, delivering two generative nuclei to the central cell and one to the egg. In 4*x* (4EBN) × 2*x* (2EBN) crosses this would give a 3*x* embryo and a 6*x* endosperm with a balanced EBN ratio. Alternatively, it is possible that mitotic abnormalities occur after inter-EBN crosses, and that loss or gain of chromosomes during the proliferation of the endosperm cells restores a 2:1 EBN balance.

These unusual fertilization events cannot explain the production of triploid progeny following 2*x*(2EBN) × 4*x*(4EBN) crosses, as reported by Hanneman and Peloquin (1968), because the 2:1 EBN ratio in the endosperm would not be re-established. To further clarify the occurrence of triploids from 2*x* × 4*x* crosses, we have studied megagametogenesis in some *tbr* haploid × wild species hybrids (2EBN) used in 2*x* × 4*x* crosses. It was observed that they had unusual female gametophytes with larger polar nuclei and larger nucleoli in the polar nuclei, in comparison to that of the nuclei and nucleoli of the egg and synergids (Table 1). The diameter of the enlarged polar nuclei was 13–15 units (1 unit = 0.57 μm), as compared with 10.0–11.0 units for the egg nuclei. Similarly, the diameter of the nucleoli of the polar nuclei was 3.5–4.0 units, as compared with 2.0–3.0 units for the egg nucleolus. Normally, the size of the nuclei in the egg, synergids and polar nuclei is similar. In the unusual female gametophyte, the size of the egg and synergids remained unchanged (representing 1n gametophytes), while the enlarged size of polar nuclei and the size and/or number of their nucleoli indicated a 2n female gametophyte. These results can be explained in terms of endomitosis in the polar nuclei, which caused a duplication of chromosomes but without cell or nuclear division. Following endomitosis, each of the polar nuclei would be 2EBN, and since the sperm from the 4*x*(4EBN) male parent would also be 2EBN, 2*x*(2EBN) × 4*x*(4EBN) crosses would give rise to normal endosperm development (with a 2:1 EBN ratio) associated with a 3*x* embryo. It is interesting to note that the frequency of triploids from 2*x* × 4*x* crosses for the clone L199 (7.5%)

Table 1 Frequency of female gametophytes with enlarged polar nuclei (FGEPN) in *S. tuberosum* Group Tuberosum haploid × wild species hybrids. Floral buds were collected and processed according to the Mayer's hemalum-methyl salicyclate method (Stelly et al. 1984)

Hybrid	Pedigree[a]	No. of female gametophytes analyzed	FGEPN	
			No.	%
P129.4	*tbr* × *ber*	57	2	3.5
L199	*tbr* × *chc*	81	7	8.6
L335	*tbr* × *chc*	48	2	4.2
L355	*tbr* × *chc-ifd*	58	1	1.7
T145	*tbr* × *chc-ifd*	70	1	1.4
T710	*tbr* × *chc*	90	1	1.1

[a] *tbr* = *S. tuberosum* Group Tuberosum haploid; *ber* = *S. berthaultii*; *chc* = *S. chacoense*; *ifd* = *S. infundibuliforme*

corresponded to the frequency of the enlarged polar nuclei observed in the same clone (8.6%). The larger polar nuclei could also be the result of the fusion of supernumerary nuclei. However, extra nuclei were not observed, indicating that endomitosis of the polar nuclei is the most probable explanation.

The EBN in potato speciation

Solanum species have poorly differentiated genomes (Peloquin et al. 1989) and chromosomes pair normally in most cases, thus allowing the production of potentially fertile interspecific hybrids. This means that genomic differentiation can hardly account for maintaining the integrity of so many *Solanum* species. Thus, there must be another mechanism which operates to maintain diversity even between species with same ploidy. The development of the EBN model has helped to clarify this point. In fact, it is the EBN that acts as a powerful isolating mechanism in the sexual reproduction of *Solanum* species. Differences in EBN create effective incompatibility barriers between sympatric species, leaving their genotypic integrity intact. Johnston et al. (1980) hypothesized that a new species could arise after a mutation leading to a new EBN. If fertilization involves gametes with matching EBNs, a new genetically isolated, but sympatric, population would arise, and it could eventually form a new species, without changes in ploidy, environmental isolation or the size of the original population. Ehlenfeldt and Hanneman (1988 a) suggested that speciation via EBN may have involved a duplication of the genes controlling the EBN without a concomitant doubling of genomes, or else a change in the level of regulation of these genes.

Recently Hawkes and Jackson (1992) discussed the taxonomic implications of the EBN hypothesis and reported that the postulated evolution of potatoes previously described by Hawkes (1990) was consistent with the EBN model. They hypothesized that the 1EBN condition represented the original state of wild diploid species in Mexico, and that the 2EBN condition arose in South America as an isolating mechanism in the evolution of 2EBN species of the series Yungasense from 1EBN species of the series Commersoniana. 1EBN species present in South America (e.g. *cmm*) are considered a remnant of the migration from Mexico to South America.

EBN incompatibility may have also played an important role in the speciation of polyploids from diploids, complementing the role of 2n gametes in the polyploid evolution of potato species. It is well known that 2n gametes provide the opportunity for sexual polyploidization in several plant species (Bretagnolle and Thompson 1995). The genetic determination, the consequences of sexual polyploidization, and the widespread occurrence of 2n gametes strongly suggest that 2n gametes also played a key role in the evolution of the tuber-bearing *Solanum* species (den Nijs and Peloquin 1977; Iwanaga and Peloquin 1982). den Nijs and Peloquin (1977) proposed an evolutionary scheme where n and 2n gametes link together all ploidy levels, thus overcoming the ploidy barriers and providing the opportunity for gene flow throughout sympatric species with different chromosome numbers. The evolutionary scheme they proposed is not only consistent with the EBN hypothesis, but clearly shows that EBN can be considered a powerful screen for 2n gametes. Indeed, sexual polyploidization doubles both ploidies and EBN values, so that in crosses between a 2EBN diploid and a 4EBN tetraploid the 2:1 EBN requirement favors 2n gametes of the parent with a lower EBN. Bamberg and Hanneman (1990) did not find any recombination and segregation for EBN genes in gametes of hybrids between *cmm* and Mexican species, and concluded that EBN genes have been preserved in the species studied. This also provided indirect evidence that only 2n gametes can allow inter-EBN crosses, and thus gene flow, between species. It should be pointed out that this complementary role of EBN and 2n gametes has an important meaning not only because it facilitates gene introgression from diploids to tetraploids, but also because it maintains the ploidy integrity of the two parental species.

Ortiz and Ehlenfeldt (1992) hypothesized that the tetraploid *S. acaule* could have originated through bilateral sexual polyploidization after the fusion of 2n gametes from two different diploid 1EBN species. It is unlikely that it originated from diploid species with different EBN values, since this would cause endosperm disfunction due to an unbalanced EBN ratio. Under the EBN hypothesis, *tbr* may have arisen from the fusion of 2n gametes of 2EBN diploid species. Hawkes (1990) reported that it is likely that the two diploid species involved were *Solanum stenotomum* and *Solanum sparsipilium*, both 2EBN.

The EBN also sheds new light on the origin of the so called odd ploidy in polyploid potato evolution. den Nijs and Peloquin (1977) have already recognized the potential of triploids in sexual polyploidization, and hypothesized that they may have represented a link between diploid and tetraploid species in the process of polyploid evolution. Recently, Ehlenfeldt and Ortiz (1995) reported that the production of triploid hybrids between sympatric species with different EBNs may be considered as both a genetic bridge and a genetic buffer in speciation. Triploids can be generated through 2*x*(1EBN) × 2*x*(2EBN) crosses when the parent with a lower EBN produces 2n gametes. Alternatively, triploids can be produced following 4*x*(2EBN) × 2*x* (2EBN), or following 2*x*(2EBN) × 4*x*(4EBN), crosses if endomitosis occurs in the polar nuclei of the 2*x* parent. All these possibilities support the validity of the EBN hypothesis.

The EBN in potato breeding and genetics

It is well known that wild tuber and non-tuber bearing *Solanum* species represent an extremely useful source of genes for potato breeding. Genetic variability has been found for many traits, including resistance to biotic and abiotic stresses, tuber quality, and earliness (Hanneman and Bamberg 1986). In addition, wild species contribute allelic diversity to optimize heterotic combinations which are important for polygenic traits, such as tuber yield (Peloquin and Ortiz 1992). The establishment of EBNs makes it possible to logically predict the success or failure of crosses between *Solanum* species of interest. In addition, it allows the prediction of the ploidy and EBN of the offspring, even when previous crossability data are not available. The predictive value of EBN is also useful for designing breeding schemes and for the exploitation of *Solanum* species which have developed reproductive isolating mechanisms.

For example, *cmm* (2n = 24, 1EBN) has several valuable traits, including resistance to low temperature stress and cold acclimation capacity, resistance to pathogens and pests, and a high dry matter content of tubers (Hanneman and Bamberg 1986). Knowledge of its EBN and the occurence of 2n gametes have been used to manipulate whole sets of chromosomes for direct germplasm transfer from *S. commersonii* to 4EBN *tbr* (Carputo et al. 1995, 1997). The EBN and ploidy of *cmm* was doubled through a tissue-culture cycle (Cardi et al. 1993 b), and 2EBN tetraploid *cmm* was crossed to 2EBN *phu-tbr* diploid clones to produce 2EBN triploid hybrids (2n = 36). Through the functioning of 2n eggs, 4EBN pentaploid hybrids were then generated following triploid (2EBN) × *tbr* (4EBN) crosses (Table 2). It should be pointed out that in this breeding scheme, 2n eggs are important not only as balanced functional gametes of an odd-ploidy parent, but also because they allow a compatible 2:1 EBN ratio. The following breeding steps are quite straight forward in that pentaploids have an effective ploidy (EBN) of four, and can be easily backcrossed with 4EBN *tbr* varieties to obtain tetraploid/near-tetraploid breeding lines (Table 2). This direct EBN manipulation, coupled with the use of 2n gametes, represents the simplest, most efficient and reproducible method for the exploitation of 1EBN species. The same breeding scheme was used by Ehlenfeldt and Hanneman (1984, 1988 b) to infuse the 1EBN species *S. brevidens* and *cmm* into the *tbr* form. Knowledge and manipulation of EBN served also for germplasm introgression to potato from 2EBN tetraploid species of the series Acaulia (Watanabe et al. 1992) and the series Longipedicellata (Adiwilaga and Brown 1991).

Table 2 Summary of results from 3*x*(2EBN) × 4*x*(4EBN), and 5*x*(4EBN)-4*x*(4EBN) crosses performed to introgress *S. commersonii* (2n = 2*x* = 24, 1EBN) into the tetraploid (2n = 4*x* = 48, 4EBN) gene pool of the cultivated potato *S. tuberosum* Group Tuberosum (from Carputo et al. 1997)

Cross[a]	Berry set (%)	Average no. of seeds/berry
3*x*(2EBN) × 4*x*(4EBN)	36.7	1.5[b]
5*x*(4EBN) × 4*x*(4EBN)	47.7	27.2
4*x*(4EBN) × 5*x*(4EBN)	28.5	9.5

[a] 3*x* (2EBN) hybrids come from 4*x* *S. commersonii* × Phureja-Tuberosum diploid hybrids; 5*x*(4EBN) hybrids come from 3*x* × 4*x* crosses; 4*x*(4EBN) genotypes include various Tuberosum varieties

[b] Only fruits with seeds were considered

EBN also represents a basic concept for the synthesis of highly heterozygous tetraploid 4EBN hybrids through sexual polyploidization crossing schemes. The value of sexual polyploidization as a tool for potato breeding has been extensively reviewed by Peloquin and Ortiz (1992). It is based on the knowledge of EBN, on the use of 2EBN *tbr* haploid-wild species hybrids, and of 2n gametes to re-establish the tetraploid 4EBN condition of the cultivated potato. Various crossing schemes can be performed between diploid 2EBN parents which produce 2n gametes (2*x* × 2*x* crosses), or between a diploid 2EBN parent which produces 2n gametes and tetraploid 4EBN *tbr* (4*x*-2*x* crosses). Sexual polyploidization relies on the fact that, according to the EBN model, 2n gametes are favored and progenies of intermediate ploidy (3*x*) are not expected, due to the collapse of the endosperms with unbalanced EBN ratios.

This predictability capacity of the EBN is important also to understand the results from inter-EBN and inter-ploidy crosses in genetic studies. As already mentioned, we have recently developed triploid and pentaploid sexual hybrids between *cmm* and *tbr* (Carputo et al. 1995, 1997). These odd-ploidy hybrids have been used in intra- and inter-EBN crosses with various testers

Table 3 Results from intra- and inter-EBN crosses involving 2EBN triploid ($2n = 3x = 36$) and 4EBN pentaploid ($2n = 5x = 60$) hybrids of *S. commersonii-S. tuberosum* and 1EBN or 2EBN diploid ($2n = 2x = 24$) and 4EBN tetraploid ($2n = 4x = 48$) testers

Cross combination[a]	Seeds	Ploidy of the offspring
$3x$(2EBN) × $2x$(1EBN)	No	–
$3x$(2EBN) × $2x$(2EBN)	Yes	$2n = 29$–36
$3x$(2EBN) × $4x$(4EBN)	Yes	$2n = 60$
$5x$(4EBN) × $2x$(1EBN)	No	–
$5x$(4EBN) × $2x$(2EBN)[b]	Yes	$2n = 48$–60
$5x$(4EBN) × $4x$(4EBN)	Yes	$2n = 48$–60
$4x$(4EBN) × 5x(4EBN)	Yes	$2n = 48$–60

[a] $2x$(1EBN) testers = *S. cardiophyllum*, *S. brachistotrichum*, *S. brevidens*; $2x$(2EBN) testers = *S. tuberosum* Group Phureja, *S. tuberosum* Group Tuberosum haploid, *S. tuberosum-S. berthaultii* hybrid; $4x$(4EBN) testers = cultivars Blondie, Carmine and Tollocan, and advanced selection Wis 482

[b] $2x$(2EBN) tester = *S. tuberosum* Group Phureja

(Table 3), and the results obtained can be logically explained in terms of EBN. Triploids have two genomes of *cmm* (0.5EBN + 0.5EBN) and one of *phu-tbr* (1EBN), so that their EBN = 2. As expected from the EBN model, they successfully crossed as female parents only with 2EBN testers. Interestingly, mitotic analysis of $3x \times 2x$ progeny revealed that the number of chromosomes in hybrids ranged from 29 to 36, while usually the number of chromosomes in $3x \times 2x$ progeny approaches that of the diploid parent. Since triploids can theoretically produce gametes with chromosome numbers ranging from 12 to 24, this indicated that only gametes with 17–24 chromosomes were functional. Knowledge of EBN can shed light on this intriguing result. If gametes from triploids can have from 12 to 24 chromosomes, this means that their gametic EBN values may range from 0.5, equivalent to a haploid genome of *cmm*, to 1.5, equivalent to a haploid genome of *cmm* (0.5EBN) plus a haploid genome of *phu-tbr* (1EBN). Under the additive genetic model proposed by Ehlenfeldt and Hanneman (1988 a), gametes with a higher chromosome number have a higher probability of having EBNs matching those of the gametes from the 2EBN parent. In the $3x \times 2x$ crosses performed, EBN acted as a screen for those gametes, promoting haplontic selection. Triploid hybrids also crossed successfully with tetraploid 4EBN varieties. Based on the EBN model, it can be predicted that any plant obtained from $3x$(2EBN) × $4x$(4EBN) crosses would be pentaploid, arising from the fertilization of a 2n(2EBN) egg of the triploid by n(2EBN) pollen of the tetraploid. Mitotic analysis confirmed that the hybrids obtained were pentaploid or near-pentaploid (Carputo et al. 1997).

Results from crosses involving pentaploid hybrids are also consistent with the EBN model. Pentaploid hybrids have two genomes of *cmm*, each of which has an EBN value of 0.5, and three of *tbr*, each of which has an EBN of 1. As a result, the EBN of the pentaploid is four. $5x \times 2x$ crosses between 4EBN pentaploids and 2EBN *phu* were possible through the functioning of 2n pollen, which balanced the EBN of the male parent and resulted in a compatible EBN ratio in the hybrid endosperm. Crosses between pentaploid and tetraploid varieties could be easily performed in both directions (Table 2). These crosses can be regarded as intra-EBN crosses, with no restriction on endosperm development. They provide further evidence that EBN is more important than chromosome ploidy in determining the success of crosses.

Conclusions

The EBN represents a single unifying factor controlling the development of the endosperm, and thus of the embryo, after intra- and inter-ploid interspecific crosses. It is a powerful isolating mechanism, and may account for maintaining the genomic integrity of *Solanum* species. It also complements the role of 2n gametes in the polyploid evolution of the cultivated potato. Ehlenfeldt and Ortiz (1995) hypothesized that all the species in which a triploid block occurs may have endosperm dosage relationships. EBN-like systems have already been suggested in several other genera, such as *Impatiens*, *Lycopersicon*, *Avena*, and *Trifolium* (see Ortiz and Ehlenfeldt 1992). The concept of EBN as the effective ploidy of tuber and non-tuber bearing *Solanum* species has also put interspecific hybridization on a more predictable and successful basis. Due to the growing interest in exploiting exotic germplasm for breeding purposes, we believe that an understanding of EBN can be used for interspecific gene transfer in any crop species where an EBN-like system operates.

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