

The Role of $2n$ Gametes and Endosperm Balance Number in the Origin and Evolution of Polyploids in the Tuber-Bearing Solanums

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ABSTRACT

Polyploidization has played a major role in the origin and evolution of polyploid species. In this article we outline the unique characteristics of $2n$ gametes and implications of their participation in the evolution of polyploid Solanum species. The genetic consequences of $2n$ gametes indicate that sexual polyploidization results in greater variability, fitness, and heterozygosity than does somatic doubling. Further, the mechanisms of $2n$ gamete formation and the frequency of $2n$ gamete-forming genes in present polyploids and their ancestral species provide additional evidence of their involvement. Equally important is the endosperm, via the endosperm balance number (EBN) incompatibility system, in complementing the role of $2n$ gametes. In fact, the EBN system acts as a screen for either $1n$ or $2n$ gametes, depending on the EBN and chromosome numbers of parental species. EBN in combination with $2n$ gametes maintains the ploidy integrity of diploid ancestral species, while providing the flexibility for either unilateral or bilateral sexual polyploidization.

GAMETES with the somatic chromosome number ($2n$ gametes) are the results of modified meiosis affecting specific stages of micro- and megasporogenesis. The incidence of $2n$ gametes is frequent in the plant kingdom, and their occurrence has been reported in species of many families, including Cruciferae, Gramineae, Leguminosae, Rosaceae, Solanaceae, and Vitaceae (VEILLEUX 1985). Several studies have been carried out to allow us to understand the mechanisms of $2n$ gamete production, their genetic control, and usefulness in breeding. In crop species like potato and alfalfa, $2n$ gametes have proven to be essential ingredients for the genetic improvement of cultivated genotypes, allowing the transmission of allelic diversity and of useful genes from wild species to cultivated gene pools through sexual polyploidization crossing schemes. A recent review by PELOQUIN *et al.* (1999) highlights the significance of $2n$ gametes in genetics, breeding, and germplasm transfer in the potato.

Together with the endosperm, $2n$ gametes have also been a major factor in the origin and evolution of polyploids. As early as 1939, BUKASOV (1939) speculated that gametes with an unreduced chromosome complement could have been involved in the origin of polyploids in the tuber-bearing Solanum species. He proposed that the pentaploid *Solanum* × *curtilobum* had probably arisen from the fusion of a $2n$ gamete from triploid

S. × juzepczukii and of a $1n$ gamete from tetraploid *S. tuberosum*, the cultivated potato. The significance of $2n$ gametes in polyploid evolution of plants has also been emphasized in a classic article on the origin of polyploids by HARLAN and DE WET (1975), and more recently by BRETAGNOLLE and THOMPSON (1995). The authors recognized the involvement of $2n$ gametes in the evolution of polyploids and listed several genera in which polyploidization was supposed to involve $2n$ gametes.

Although some plant evolutionists recognized the role of $2n$ gametes in polyploid evolution, a number of authors neglected to indicate their role in polyploidization. STEBBINS (1971), for example, stated that meiotic restitution played only a minor role in the evolution of polyploid complexes and that meiotic anomalies leading to gametes with unreduced chromosome number are rare events that cannot account for the synthesis of new polyploids. More recent treatments indicated lack of knowledge of the role of $2n$ gametes and endosperm in the origin and evolution of polyploids (OTTO and WHITTON 2000; SOLTIS and SOLTIS 2000).

We discuss the occurrence and frequency of $2n$ gametes, their genetic determination, cytological basis, genetic consequences, and role in sexual polyploidization and gene introgression, thereby presenting convincing evidence for the participation of $2n$ gametes in the origin and evolution of polyploids in tuber-bearing Solanum species.

We also outline the fundamental role of the endosperm, via the endosperm balance number (EBN) incompatibility system, in relation to seed formation or

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failure during the polyploid evolution of *Solanum* species. This role is often ignored by plant evolutionists. We provide evidence that both endosperm and $2n$ gametes play a combined role in maintaining and breaking the sexual isolation of sympatric species and in determining the success of interspecific hybridization.

DISCUSSION

$2n$ gamete formation in the potato and genetic consequences: A number of meiotic mutations related to spindle formation and cytokinesis have been associated with $2n$ gamete production. In the potato, the main mechanism of $2n$ pollen formation is parallel spindle (ps; WATANABE and PELOQUIN 1993). In normal meiosis during microsporogenesis the metaphase II spindles are oriented so that their poles define a tetrahedron, and following cytokinesis, a tetrad of four $1n$ microspores is produced. When ps is present, the spindles are parallel, and a dyad of two $2n$ microspores is formed at the end of meiosis. MOK and PELOQUIN (1975) demonstrated that this mutation is controlled by a single recessive gene. Parallel spindle is genetically equivalent to a first-division restitution (FDR) mechanism. All loci from the centromere to the first crossover that are heterozygous in the parent will be heterozygous in the gametes, and half the heterozygous parental loci beyond the first crossover will be heterozygous in the gametes (due to small chromosome size, there is normally only one crossover per chromosome arm).

Several mutations leading to $2n$ egg formation have also been found (WERNER and PELOQUIN 1991a). Among them, the most common is the omission of the second meiotic division (os). In normal megasporogenesis, the cell plate forms after the first and second divisions, and four $1n$ megaspores are formed. When os is present, after the first division there is no second division, and the chromatids just fall apart. Of the resulting two $2n$ megaspores, one gives rise to the $2n$ female gametophyte and the other degenerates. Omission of the second division is also a simply inherited Mendelian recessive (WERNER and PELOQUIN 1990) that has been detected in a large number of *Solanum* species (DEN NIJS and PELOQUIN 1977; STELLY and PELOQUIN 1985; WERNER and PELOQUIN 1991a). Omission of the second division is genetically equivalent to a second-division restitution (SDR) mechanism. All the loci from the centromere to the first crossover will be homozygous in the gametes, and all loci past the first crossover, which are heterozygous in the parent, will be heterozygous in the gametes. On the basis of chromosome structure, position of the centromere, and chiasmata frequencies, it has been estimated that FDR $2n$ gametes transmit $\sim 80\%$ of heterozygosity and large fractions of epistasis from the parent to the offspring (PELOQUIN 1983). On the

other hand, SDR $2n$ gametes transmit a smaller amount of heterozygosity ($\sim 40\%$) and of epistatic interactions.

The frequency at which $2n$ gametes are formed is also very important, in that it may affect the rate of new polyploid formation. RAMSEY and SCHEMSKE (1998) suggested that the high frequency of polyploid species in harsh environments can be related to the impact of environmental conditions on $2n$ gamete production. The frequency of $2n$ gametes may be highly variable both between and within species. WATANABE and PELOQUIN (1993) found that $2n$ pollen frequency in diploid, tetraploid, and hexaploid *Solanum* species ranged from 1.9 to 36.3%. A significant variation in the frequency of $2n$ eggs in *Solanum* species (from 4.9 to 22.6%) has been also reported by WERNER and PELOQUIN (1991a). The variability found in *Solanum* is consistent with that reported in other species like *Dactylis glomerata*, *Trifolium nigrescens*, Manihot, and Medicago (see BRETAGNOLLE and THOMPSON 1995) and has been attributed mainly to the incomplete penetrance and variable expressivity of the mutant alleles responsible for $2n$ gamete production, as well as to environmental factors. Generally, available data indicate that the range of $2n$ gamete frequency is between 1 and 40%. This suggests that plants tend to hold high frequencies of n gametes, probably to maintain their diploid level while allowing polyploidization.

***Solanum* species and the EBN:** Potato species form a polyploid series with a basic chromosome number (x) of 12 and a minimal chromosome differentiation among taxa. Most of them ($\sim 70\%$) occur as diploids ($2n = 24$), and the others are triploids ($2n = 36$), tetraploids ($2n = 48$), pentaploids ($2n = 60$), and hexaploids ($2n = 72$). The main cultivated potato is a tetrasomic tetraploid originating from South America. *S. tuberosum* Group Andigena is cultivated mainly under short days and is now restricted to South America. *S. tuberosum* Group Tuberosum is the potato cultivated worldwide under long-day conditions. Also, some diploids, triploids, and pentaploids are still cultivated in South America, as well as a large number of wild tuber-bearing species (~ 160). They grow in a wide range of habitats from the southern part of the United States through Mexico and Central America down to southern Chile.

Besides having different ploidy levels, *Solanum* species also differ in the EBN (JOHNSTON *et al.* 1980). The EBN represents their "effective ploidy" and is not always in accordance with the actual chromosome number. It varies from 1 to 4: diploid species can be either 1 or 2EBN, triploids 2EBN, tetraploids 2 or 4EBN, pentaploids and hexaploids 4EBN (HANNEMAN 1994). The cultivated *S. tuberosum* is 4EBN. The EBN deserves special attention in the context of the evolution of tuber-bearing *Solanum* species. It acts as an effective mechanism to regulate endosperm development after interspecific/interploidy crosses and thus to determine their success or failure. According to the model hypothesized, nor-

mal endosperm development occurs only when there is a 2:1 maternal-to-paternal EBN ratio in the hybrid endosperm. In all the other cases the endosperm degenerates. Clearly, EBN differences between parents prevent hybridization between different ploidies within a species and between species. For this reason, for example, tetraploid *S. acaule* (2EBN) cannot be crossed with *S. tuberosum*, which is also tetraploid but has an EBN of 4. On the other hand, *S. acaule* hybridizes with diploid (2EBN) species. A number of studies have demonstrated that the EBN is under the control of genes with additive effects. EHLENFELDT and HANNEMAN (1988) and CAMADRO and MASUELLI (1995) reported that three and two genes were involved in the determination of the EBN of Solanum species, respectively. Evidence for EBN-like systems has been reported in several other species. In Impatiens, ARISUMI (1982) assigned EBNs to several species according to their crossability with 2EBN testers. PARROTT and SMITH (1986) provided evidence that EBN is operating in Trifolium and assigned EBN values from 2 to 8. EHLENFELDT and HANNEMAN (1992) classified the species belonging to the *Lycopersicon esculentum* crossability group as 2EBN, whereas higher EBN values were assigned to the *L. peruvianum* complex. KATSIOSIS *et al.* (1995) grouped oat species into four EBN groups from 1 to 4. For a review of the EBN, its genetic control, and its usefulness, see CARPUTO *et al.* (1999).

Origin of the cultivated potato: According to the evolutionary pathway proposed by HAWKES (1990), tuber-bearing wild species evolved first in Mexico and then spread throughout Central and South America. The point of origin of the cultivated potato was somewhere between central Peru and central Bolivia, but the exact nature of the original wild parent is still uncertain. The most primitive form of the cultivated tetraploid potato is the diploid *S. tuberosum* group Stenotomum from which all the cultivated forms have arisen either directly or indirectly. Chloroplast DNA analysis confirmed very close relationships among 2x cultivated potatoes and thus the idea of the common origin of the cultivated 2x species from *S. tuberosum* group Stenotomum (HOSAKA and HANNEMAN 1988). *S. tuberosum* group Phureja is believed to have originated by mutation and selection from *S. tuberosum* group Stenotomum after the Andean farmers had taken it to lower and warmer valleys. Among cultivated triploids, *S. tuberosum* group Chaucha is made up of a series of natural triploids between group Stenotomum and group Andigena. *S. × juzepczukii* is a natural hybrid between *S. acaule* and group Stenotomum. The pentaploid *S. × curtilobum* was formed following hybridization between *S. × juzepczukii* and group Andigena. HAWKES (1990) also proposed that the cultivated *S. tuberosum* group Andigena was derived from group Stenotomum and the diploid weedy species *S. sparsipilum* through polyploidization. On the other hand, MATSU-

BAYASHI (1991) hypothesized that it is mainly a group Stenotomum-group Phureja derivative and that *S. sparsipilum* had only a secondary role in the origin of group Andigena. The potato that is cultivated worldwide, *S. tuberosum* group Tuberosum, derived from group Andigena as a result of artificial selection for tuberization under long-day conditions.

In this complex pattern of species, hybrids, and chromosome numbers, polyploidization has been a major mechanism of speciation. But how did polyploidization occur?

Sexual vs. asexual polyploidization: The two main mechanisms of polyploid formation are somatic doubling of chromosomes in meristem tissues of sporophytes, zygotes, or embryo (somatic polyploidization) and meiotic nuclear restitution leading to 2n gamete production (sexual polyploidization). In somatic polyploidization chromosome restitution takes place during mitosis and all the chromosomes of a somatic cell are included in one daughter nucleus, giving rise to a cell with a doubled chromosome number. The most common example of somatic doubling is *Primula kewensis*, a disomic polyploid, which arose by somatic doubling of some flowering branches in the sterile diploid hybrid *P. floribunda* × *P. verticillata* (NEWTON and PELLEW 1929). Somatic doubling is also known to occur in Solanum (JORGENSEN 1928) and Mimulus (HIESEY *et al.* 1971). Little is known about either the natural frequency of somatic doubling in plants or the effects of interspecific hybridization on its occurrence. From the literature available, HARLAN and DE WET (1975) concluded that somatic doubling is a relatively unimportant phenomenon in the production of polyploids.

Sexual polyploidization involves the function of 2n gametes. The union of two 2n gametes, as well as of 1n and 2n gametes, produces polyploid embryos, which can give rise to a new polyploid population, depending on the genotypes involved and the environmental conditions. Compared to somatic polyploidization, more information is available on the genetic and environmental factors influencing the frequency of 2n gamete production. There is also stronger circumstantial evidence that 2n gametes have contributed to polyploid evolution. However, further quantitative assessments of the frequencies of somatic doubling and 2n gametes in natural populations will allow better evaluation of the contribution of somatic and sexual polyploidization in plants. The availability of genetic markers and a detailed knowledge of mechanisms involved in 2n gamete production will be extremely useful for this purpose.

Although both sexual and somatic polyploidization result in chromosome doubling, the genetic consequences of the two modes of polyploidization are very different. Three features of 2n gametes and sexual polyploidization should be emphasized in this context.

1. It is well known that heterozygosity is very important in a polysomic polyploid like the potato in that maximum heterosis for polygenic traits is expected with maximum heterozygosity (WERNER and PELOQUIN 1991b). Heterozygosity refers to the possibility of more than two alleles per locus, and diversity at tetra-allelic loci ($A_1A_2A_3A_4$) gives maximum heterozygosity with six first-order, four second-order, and one third-order interaction. $2n$ gametes are the result of modified meiosis and, as already pointed out, they transmit high levels of parental heterozygosity and epistasis, circumventing the disruptive effects of meiosis on intra- and interlocus interactions. Somatic doubling transmits all the parental heterozygosity, but if a heterozygous individual A_1A_2 is doubled, a balanced diallelic tetraploid is formed, which has only one first-order interaction as its progenitor and high levels of inbreeding.
2. The genetic determination of $2n$ gamete formation ensures repeated events of sexual polyploidization and the incorporation of genetic diversity of more than one diploid species. This "progressive introgression," besides having implications for the taxonomy of polyploids, constitutes the basis for especially high levels of heterozygosity. Newly arisen polyploids may thus possess a wide range of novel phenotypic, ecological, and physiological characteristics upon which natural selection can act. In recent years, use of various molecular techniques allowed detailed analyses of the genomic or DNA sequence constitution of several polyploids, including angiosperms, pteridophytes, and bryophytes (LEITCH and BENNETT 1997; SOLTIS and SOLTIS 2000). All these studies confirmed that nearly all polyploids were polyphyletic and that polyploidy is a dynamic and reversible process. Although the authors recognized this as a significant source of genetic diversity, none of them discussed sexual polyploidization as the cause of the phenomenon.
3. The genetic determination of $2n$ gametes also allows the reciprocal origin of polyploids when diploid progenitors produce both $2n$ pollen and $2n$ eggs. This can result in differing morphological, biochemical, and physiological characteristics, as well as differing nuclear-cytoplasmic gene interactions. As a result, further genetic variability is created.

Computer simulation has been performed to compare the genetic consequences of sexual and somatic polyploidization (WATANABE *et al.* 1991). The results confirmed that regardless of the position of a locus in relation to the centromere, sexual polyploidization generally produces less inbreeding, more genotypic diversity, and a higher frequency of tri- and tetraallelic genotypes. As the number of alleles at a locus increases, these results become even more prominent. Only when two alleles are possible at a locus can somatic polyploidization

be equal to sexual polyploidization. SDR \times FDR proved the most stable mechanisms for sexual polyploidization against extreme recombination events. Table 1 reports coefficients of inbreeding and the number of genotypes at a locus in tetraploid populations deriving from somatic doubling and an SDR \times FDR crossing scheme.

In brief, there is genetic evidence that while somatic doubling does not change the genotype in any way except that all alleles become redundant and doubled plants become inbred, sexual polyploidization is a creative phenomenon providing new allelic diversity and gene combinations while avoiding inbreeding. It would be extremely interesting to know the rate of fitness of sexual polyploids *vs.* diploid progenitors in natural habitats and the specific genetic mechanisms that may influence fitness. These studies would allow a better understanding of the role of polyploidization in adaptation. Recently, OTTO and WHITTON (2000) suggested that sexual and asexual polyploids can adapt faster than diploids, especially if beneficial mutations appear in relatively small populations and have a partially dominant effect on fitness.

***ps* and evolution:** Most genetic studies of $2n$ gametes concern $2n$ pollen, and results obtained in *Solanum* provide useful information to extend our understanding of the evolution of polyploids. The genetic determination of $2n$ pollen formation and the large number of diploid *Solanum* species in which $2n$ pollen has been found (DEN NIJS and PELOQUIN 1977; WATANABE and PELOQUIN 1993) represent the first clear proof that $2n$ pollen was involved in the evolution of polyploid potatoes. Significantly, $2n$ pollen has not been recovered in species belonging to taxonomic series (*e.g.*, *Circaeifolia*, *Morelliformia*) that are entirely diploid, whereas it is largely present in series (*e.g.*, *Tuberosa*) where a wide range of ploidy levels is found. Studies by CAMADRO and PELOQUIN (1980) and WATANABE and PELOQUIN (1989) demonstrated that the *ps* allele was widely distributed in natural populations of diploid species and that a high frequency of recessive homozygous and heterozygous diploid wild genotypes can be found at the *ps* locus. Table 2 summarizes the results of these researches and gives the estimated gene frequency for a number of wild species from Peru, Chile, and Argentina. It should be pointed out that these values are probably underestimated, given that *ps*, like most meiotic mutations, is characterized by variable penetrance and expressivity, so that plants that do not produce $2n$ pollen may have the *psps* genotype. It is also remarkable that IWANAGA and PELOQUIN (1982), in screening several tetraploid cultivars for *ps* gene frequency, found that most of them were simplex (*Pspspsps*). The high *ps* gene frequency in wild diploid species and related tetraploids is further convincing evidence that sexual polyploidization through genetically determined $2n$ pollen is an important evolutionary pathway in *Solanum* species.

TABLE 1
Genetic consequences of asexual and sexual polyploidization

No. of possible alleles	$P = 0.0$		$P = 0.5$	
	Asexual	SDR × FDR	Asexual	SDR × FDR
Two alleles				
Inbreeding	0.333	0.500	0.333	0.458
No. of genotypes	1	2	1	5
Four alleles				
Inbreeding	0.333	0.333	0.333	0.292
No. of genotypes	6	24	6	35
Six alleles				
Inbreeding	0.333	0.278	0.333	0.243
No. of genotypes	15	90	15	126
Eight alleles				
Inbreeding	0.333	0.250	0.333	0.208
No. of genotypes	28	224	28	330

Coefficient of inbreeding and number of genotypes at a locus adjacent to the centromere ($P = 0$) and distal to the centromere ($P = 0.5$) in tetraploid populations originated through asexual polyploidization and bilateral sexual polyploidization (SDR × FDR crosses), assuming various numbers of possible alleles in the original diploid populations (from WATANABE *et al.* 1991).

Comparison between *ps* gene frequency in polyploid species and their original diploid ancestors provides further proof of the major role played by parallel spindle in polyploid evolution. Gene frequency for $2n$ pollen in the tetraploids should be higher if sexual polyploidization through $2n$ pollen is involved in the origin of polyploid potato than if polyploidization occurred through somatic doubling. In fact, in the latter case, the same gene frequency in the tetraploids as in their diploid progenitors is expected. But if polyploids originate from sexual polyploidization, a higher gene frequency for *ps* can be expected in tetraploids because (1) both *ps* alleles are transmitted from the male diploid parent to the tetraploid population that originated through sexual polyploidization and (2) the *ps* gene

frequency in $2n$ eggs is independent of the position of the *ps* locus in respect to the centromere (WATANABE and PELOQUIN 1989). As a result, the *ps* gene frequency in new tetraploids is increased from that in the original diploid populations.

Genetic analysis by CAMADRO and PELOQUIN (1980), WATANABE and PELOQUIN (1989), and IWANAGA and PELOQUIN (1982) supported this hypothesis (Table 2). The *ps* gene frequency in possible progenitors of tetraploid *S. tuberosum* group Andigena and group Tuberosum was 0.39 for *S. sparsipilum*, 0.42 for *S. tuberosum* group Stenotomum, and 0.47 for *S. tuberosum* group Phureja. By contrast, the *ps* gene frequency was much higher in *S. tuberosum* group Andigena (0.85) and group Tuberosum (0.69). Similarly, the *ps* gene frequency of

TABLE 2
Gene frequency at the *ps* locus estimated in diploid ancestors and tetraploid-deriving potatoes

Solanum species	Ploidy	<i>ps</i> gene frequency	Reference
Ancestors			
<i>S. sparsipilum</i>	$2n = 2x$	0.39	WATANABE and PELOQUIN (1989)
<i>S. tuberosum</i> group Phureja	$2n = 2x$	0.47	WATANABE and PELOQUIN (1989)
<i>S. tuberosum</i> group Stenotomum	$2n = 2x$	0.42	WATANABE and PELOQUIN (1989)
Derivatives			
<i>S. tuberosum</i> group Andigena	$2n = 4x$	0.85	WATANABE and PELOQUIN (1989)
<i>S. tuberosum</i> group Tuberosum	$2n = 4x$	0.69	IWANAGA and PELOQUIN (1982)
Ancestors			
<i>S. gourlayi</i>	$2n = 2x$	0.46	CAMADRO and PELOQUIN (1980)
<i>S. infundibuliforme</i>	$2n = 2x$	0.37	CAMADRO and PELOQUIN (1980)
Derivative			
<i>S. gourlayi</i>	$2n = 4x$	0.80	WATANABE and PELOQUIN (1989)

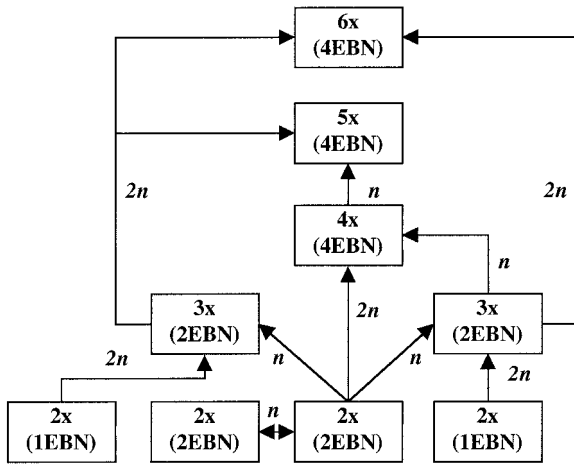


FIGURE 1.—Complementary role of endosperm and $2n$ gametes in maintaining and breaking the sexual isolation of *Solanum* species and in determining the success of interspecific hybridization during the evolution of tuber-bearing *Solanums*. The EBN acts as a screen for either $1n$ or $2n$ gametes, allowing the maintenance of the ploidy integrity of the diploid parental species through $2x(2EBN) \times 2x(2EBN)$ crosses, while permitting polyploidization through interploidy inter-EBN crosses. In the case of $3x(2EBN) \times 2x(2EBN)$ crosses to obtain tetraploids, the functional gametes of the $3x$ parent have a 24-chromosome complement and an EBN of 2.

diploid *S. gourlayi* and *S. infundibuliforme*, possible progenitors of tetraploid *S. gourlayi*, was 0.46 and 0.37, respectively. Again, in tetraploid *S. gourlayi* it was much higher (0.80). In all these studies the *ps* gene frequencies in tetraploid species were almost twice those of the diploid progenitors. Without doubt this indicates that the tetraploids originated from sexual polyploidization.

Although the *os* gene frequency has not been estimated, data on $2n$ egg production support the hypothesis that $2n$ gametes were involved in the origin of tetraploids. WERNER and PELOQUIN (1991a) screened diploid species and group *Tuberosum* haploids for the frequency of plants producing $2n$ eggs through omission of the second meiotic division. About 60% of the group *Tuberosum* haploids produced $2n$ eggs *vs.* only 24% of wild species.

$2n$ gametes and endosperm: The endosperm, together with $2n$ gametes, has been a major factor in the origin and evolution of polyploids. The endosperm dosage system regulates the within-species and between-species congruity in that endosperm development is dependent on the relative proportion of maternal and paternal contributions. Due to endosperm imbalance, for example, a strong triploid block exists in the majority of angiosperms. The development of the EBN system in *Solanum* has shed new light on the role of the endosperm in polyploid evolution. In fact, the EBN in combination with $2n$ gametes maintains the ploidy integrity of diploid ancestral species, while providing the flexibil-

ity for either unilateral or bilateral sexual polyploidization. DEN NIJS and PELOQUIN (1977) proposed an evolution pathway in which $1n$ and $2n$ gametes link all ploidy levels of *Solanum* species, thus overcoming the ploidy barriers and giving opportunity for gene flow throughout sympatric species. In this evolutionary scheme the endosperm, through the EBN incompatibility system, strongly complements the role of $2n$ gametes. It acts as a powerful screen for either $1n$ or $2n$ gametes, depending on the EBN and chromosome number of parents. This guarantees the maintenance of the ploidy level integrity of the two diploid parental species in crossing with other diploids, while allowing polyploidization.

Sexual polyploidization can occur bilaterally or unilaterally (HARLAN and DE WET 1975; BRETAGNOLLE and THOMPSON 1995). In the former, both parents produce $2n$ gametes and have the same EBN value. This may lead to the direct synthesis of a tetraploid following $2x \times 2x$ crosses. In the latter, one parent produces $2n$ gametes and the ploidy level of the offspring will depend on the EBN of the parents. In a $4x(2EBN) \times 2x(2EBN)$ cross, the required 2:1 ratio between female and male parental EBNs favors $1n$ gametes of the male parent and an intermediate $3x$ progeny is generated. By contrast, a $4x(4EBN) \times 2x(2EBN)$ cross will favor $2n$ gametes of the male parent and a tetraploid offspring is produced. Tetraploid potatoes most likely originated from bilateral sexual polyploidization via fusion of $2n$ gametes of diploids with the same EBN. It is unlikely that diploid ancestors had different EBNs since that would have caused endosperm degeneration. After the emergence of tetraploids, chances for “progressive introgression” between diploids and newly arisen tetraploids through unilateral sexual polyploidization ($4x \times 2x$ crosses) were then possible. Under the complementary hypothesis of $2n$ gametes and endosperm, $4x(4EBN)$ *S. tuberosum* group *Andigena* may have arisen from the fusion of $2n$ gametes of $2x(2EBN)$ *S. stenotomum* and *S. sparsipilum* (HAWKES 1990).

Strong evidence of EBN-like systems exists for several species (*Avena*, *Impatiens*, *Medicago*, and *Trifolium*) in which $2n$ gametes and ploidy series have also been found. Thus, a combined role of $2n$ gametes and endosperm can be hypothesized to explain the polyploid evolution of these species as well. It should be pointed out that in the *Lycopersicon* system $2n$ gametes have not been detected, ploidy series do not exist, but the EBN does operate (EHLENFELDT and HANNEMAN 1992). In this case it acts as an isolating mechanism between the “esculentum group” species and the “peruvianum group” species and has probably evolved through genic changes within a diploid system rather than through polyploidization events, as in *Solanum*.

Recently, RAMSEY and SCHEMSKE (1998) reviewed the role of triploids in the evolution of polyploids, their fertility and meiotic behavior, and the chromosome

number of progeny deriving from triploid parents. Their results indicated that triploids are often semifer- tile, producing 1x, 2x, and 3x gametes, and thus contrib- ute significantly to the formation of both polysomic and disomic polyploids. These findings are consistent with the 2n gamete-EBN-based evolution pathway of the po- tato (Figure 1). In 2x(2EBN) × 2x(1EBN) crosses, the required 2:1 ratio between female and male in the endo- sperm favors 2n gametes of the male parent, resulting in the formation of a viable tetraploid endosperm associ- ated with a triploid embryo. An example is provided by *S. chacoense* and *S. commersonii*, two sympatric diploid species from Argentina. *S. chacoense* is 2x(2EBN) while *S. commersonii* is 2x(1EBN), and the two species do not normally intercross. However, if *S. commersonii* produces 2n gametes, triploids will result. Alternatively, triploids can also be produced following 2x(2EBN) × 4x(4EBN) crosses if endomitosis in the polar nuclei of the 2x par- ent occurs (CARPUTO *et al.* 1999). Newly arisen triploids can function as parents in 3x(2EBN) × 2x(2EBN) crosses to obtain tetraploids. In this case they produce functional gametes with a 24-chromosome complement and have an EBN of 2. They can also self or by sexual polyploidization via 2n gametes from both parents form hexaploids with 4EBN.

Conclusions: Polyploidization is a major factor in the evolution of plants. Somatic doubling and sexual poly- ploidization are two of the possible origins for poly- ploids. In-depth analyses of the *Solanum* polyploid series suggest that 2n gametes and EBN are the mech- anisms leading to the formation of polyploids. This pro- cess of sexual polyploidization maximizes heterosis and genetic diversity, characteristics of polyploids, yet allows interploidy gene flow. The same evolution path may account for polyploid origin and evolution in other plant families.

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