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Substitutes for genome differentiation in tuber-bearing *Solanum*: interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and endosperm

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Abstract The cultivated potato, *Solanum tuberosum* L. (2*n*=4*x*=48), has a very large number of related wild and cultivated tuber-bearing species widely distributed in the Americas. These species, grouped in 16 taxonomic series, range from the diploid to the hexaploid level. Polyploid species are either disomic or polysomic, and sexual polyploidization via genetically controlled 2*n* gametes has played a major role in their evolution. Species are separated in nature by geographical and ecological barriers. However, there are several examples of sympatric species that share the same niches but do not readily cross (i.e., the diploids *S. commersonii* and *S. chacoense* in certain areas of Argentina). External barriers alone are, therefore, not sufficient to explain species integrity. In addition, there is no strong evidence indicating that genome differentiation is important in the group. In this review we present evidence supporting the assertion that interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and the endosperm are major forces that strengthen the external hybridization barriers allowing, at the same time and under specific circumstances, a certain amount of gene exchange without jeopardizing the integrity of the species.

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Introduction

The cultivated potato, *Solanum tuberosum* L. (2*n*=4*x*=48), has approximately 220 wild and cultivated relatives, which is probably more than any other food or feed crop. This large number of species is grouped in the section *Petota Durmont*, which includes subsections *Estolonifera* and *Potatoe* (Hawkes 1994). The species of the subsection *Estolonifera* are strictly diploid and typically lack stolons and tubers. Species of subsection *Potatoe* range from the diploid to the hexaploid level. Most are diploid and the remaining are triploid, tetraploid, pentaploid, and hexaploid; a few are mixtures of cytotypes. They possess underground stolons and tuberize under various environmental conditions. Taxonomically, they are grouped in 16 series. Series Tuberosa contains the seven cultivated species (the diploid *S. stenotomum*, *S. phureja* and *S. ajanhuiri*; the triploid *S. chaucha* and *S. juzepczukii*; the tetraploid *S. tuberosum* ssp. *andigenum* Hawkes and ssp. *tuberosum*; the pentaploid *S. curtilobum*) as well as the wild species most closely related to them (Hawkes 1994). It has been recently proposed to place all of the cultivated populations into cultivar-groups of a single denomination class, *S. tuberosum* (Huamán and Spooner 2002).

Potato species are distributed from the southwestern USA through Mexico and Central America to southern Chile and central Argentina, along the Andes mountains, and towards the east, in Brazil, Paraguay, and Uruguay. The highest number of species per degree latitude is found from northcentral Peru to central Bolivia and in the central Mexican highlands (Hijmans and Spooner 2001). The species grow under a wide range of environmental conditions and, consequently, possess a wide ecological adaptation that is important for the genetic improvement of cultivated genotypes.

Polyploidy in the tuber-bearing *Solanum* is very unique in that polyploid species can be either polysomic or disomic. Cultivated *S. tuberosum* is a typical tetrasomic polyploid, with tetravalent pairing at meiosis and tetrasomic inheritance. In contrast, several tetraploid and hexaploid species behave as disomic polyploids, with regular bivalent pairing at meiosis (see Dvorák 1983).

Cytological studies and crossing results represent the key to understanding polyploid evolution in these species. Such studies have provided strong evidence that many species produce 2*n* gametes (Peloquin et al. 1999) and revealed the role of sexual polyploidization in generating great genetic variability, fitness, and heterozygosity in the populations (Carputo et al. 2003a). Also, the pattern of variability in these species is strongly influenced by the two alternative means of reproduction available to them (sexual and asexual) and by the fact that most diploid species are obligate outcrossers because they possess a one-locus self-incompatibility system. Therefore, the breeding system, in general, provides for success and opportunism, both in maintaining superior genotypes in stable environments and for the rapid evolution of new forms under varying environmental conditions. Hybridization and subsequent gene flow within and between ploidy levels often result in exceedingly complicated patterns of variation, with an extensive assemblage of inter-related plants within which lines have to be drawn somewhat arbitrarily in order to delineate taxonomic units (Ugent 1966). In fact, many of the taxonomic treatments are discordant due to these particularities and the different taxonomic concepts and practices of leading potato taxonomists (Spooner and Van den Berg 1992).

With such a high number of species, there must be factors that guarantee the maintenance of species integrity by restricting or preventing gene exchange. As in several other genera, geographical and ecological isolation has certainly contributed to species integrity. However, there are several examples of diploid sympatric species that do not readily cross: *S. commersonii* and *S. chacoense*, of series Commersoniana, that are sympatric in certain areas of Argentina (Summers and Grun 1981); *S. jamesii* and *S. stoloniferum* in the USA; *S. stenophyllidium* and *S. stoloniferum*, *S. verrucosum* and *S. bulbocastanum*, and *S. cardiophyllum* and *S. polyadenium* in Mexico (Spooner et al. 2004), among others. Thus, separation in time and space as well as the adaptation to ecological niches are not sufficient to explain species differentiation.

Since 1950, genome formulations for species in the subsection *Potatoe* based on cytological observations and genetic data have been attempted by several researchers (see Matsubayashi 1991). Three types of structural differences between genomes were considered: cryptic, definite, and gross. Following a review of the literature, Matsubayashi (1991) concluded that: (1) species in all of the 16 tuber-bearing series, irrespective of their ploidy levels, are phylogenetically related to one another by possessing a common genome, A, regarded as the basic genome; (2) this genome is modified to different degrees due to cryptic structural differences in the chromosomes; (3) polyploid species differ from one another by virtue of their additional genomes, which are structurally differentiated to a greater or lesser extent and are given different genome formulae; (4) five different genomes, A, B, C, D, and E, are recognized.

The difficulties encountered in the characterization of the small chromosomes of *Solanum*, the results of chromosome pairing analyses, and the success or failure of interspecific crosses have generated controversial information in relation to genome differentiation in the subsection. For example, the A genome and two partial homologous genomes, D and D^d, have been assigned to hexaploid *S. demissum* (Matsubayashi 1991). Chromosome affinities, nevertheless, were found to be higher in the hybrids of this species with diploid species than in its own haploids, which carry three chromosome sets. Similar results led Dvorák (1983) to suggest that heterogenetic pairing is genetically suppressed in the polyploids but occurs in the hybrids due to the genotypes of the diploid species, as suggested for other taxonomic families in addition to the *Gramineae*. Also, in 14 F_1 hybrids among 11 diploid species, Singh et al. (1989) observed mostly bivalents in Metaphase I and, in the majority of them there was an evident kind of heterotic effect on chiasma frequencies, demonstrating that heterogenetic pairing is active. A second example, based on chromosome pairing behavior, is that given the frequency of different configurations at Metaphase I and type of inheritance, *S. tuberosum* sp. has been proposed to be, alternatively, an auto- and an allo-polyploid (see Matsubayashi 1991). Thus, it is clear that there is no strong evidence with respect to important chromosome differentiation in the tuber-bearing species and, thus, other mechanisms must operate to maintain their diversity and integrity.

In nature, external and internal barriers can hinder or prevent gene flow between related taxa (Hadley and Openshaw 1980). Examples of external barriers to genetic exchange are the physical separation of populations in time or space, the adaptation of populations to specific ecological niches, or combinations of barriers that produce discontinuity among populations. External barriers are usually reinforced by internal ones, which are those that reside within plant tissues and can prevent hybrid formation. They act at the pollen-pistil (pre-zygotic) and/or the embryo and/or the endosperm levels (post-zygotic). If hybrids are formed, they can cause hybrid weakness, sterility, or a breakdown in segregating generations.

This paper provides evidence that strong internal reproductive barriers in tuber-bearing *Solanum* species, rather than genome differentiation, reinforce the external hybridization barriers, thereby guaranteeing the integrity of the species in the evolutionary perspective. These barriers are interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and the endosperm.

Interspecific pollen-pistil incompatibility barriers

In sexually reproducing plants, a series of very complex events takes place upon pollination. These events together constitute a system of subsystems that are considered to have, at least, some degree of independent control (Williams et al. 1982; de Graaf et al. 2001).

Two different kinds of relationships exist between pollen and pistil, both leading to a restriction in hybridization: (1) self-incompatibility, which results either from the identity of alleles at one or more specific loci in both the pollen and pistil, or from the existence of variations in the floral structure—associated or not to an incompatibility locus—that occurs within and between closely related cross-fertilizing species (Frankel and Galun 1977); (2) incongruity, which results from the lack of information in one partner on some of the characters in the other (Hogenboom 1973). This latter type of relationship, also referred to as cross-incompatibility (Camadro and Peloquin 1981), is a corollary of evolutionary divergence.

Most diploid potato species are self-incompatible because they possess a multiallelic S-locus with gametophytic expression; notwithstanding, self-compatible variants have been described among genotypes of some of these species (Cipar et al. 1964). Polyploid species, on the other hand, are self-compatible due to a phenomenon referred to as "competition interaction" because certain interactions that either weaken or suppress the incompatibility reaction take place in pollen grains carrying different S-alleles (Frankel and Galun 1977). Fixed heterozygosity at the S-locus, as observed for the aspartate aminotransferase locus (Camadro et al. 1992), would explain self-compatibility in *S. acaule*, an autogamous disomic tetraploid, and other disomic polyploids that carry partially homologous genomes.

The style determinant of gametophytic self-incompatibility in Solanceous species is a secreted ribonuclease codified by the S-locus (Dodds et al. 1996). S-RNAase genes have been mapped on chromosome 1 of *S. tuberosum* (Gebhardt et al. 1991) and *S. chacoense* (Rivard et al. 1996). S-gene products in pollen have not been identified to date (Thompson and Kirch 1992; Stone and Goring 2001). However, an S-locus inhibitor (Sli) gene, which results in self-compatible plants and whose function has not yet been ascertained, has been mapped on chromosome 12 (Hosaka and Hanneman 1998).

Numerous interspecific crosses in tuber-bearing *Solanum* species, both within and between ploidy levels, have been reported in the literature. Many of these were attempted by plant breeders in the mid-1950s and early 1960s to obtain the F_1 progenies. However, few researchers at that time investigated the site of the incompatibility reactions and only speculated on the possible genetic basis of this hybridization barrier (see Camadro 1981 for a review).

Both unilateral (UI) and bilateral (BI) incompatibility are found in tuber-bearing *Solanum* species, as in other genera and families. Fluorescence microscopy has revealed up to four sites of incompatibility in reciprocal interspecific crosses between and within ploidy levels; for example, tetraploid *S. gourlayi* and hexaploid *S. oplocense* and backcrosses (Camadro and Peloquin 1981), tetraploid *S. tuberosum* ssp. *tuberosum* and diploids *S. kurtzianum* and *S. ruiz-lealii* as well as between these two species and diploid *S. chacoense* (Raimondi and Camadro 2003; Raimondi et al. 2003), diploid *S. maglia* and diploid *S. kurtzianum* (Ispizúa et al. 1999), and even between morphological groups of a diploid species, *S. spegazzinii* (Erazzú et al. 1999). These incompatibility sites are: the stigma, and the first, second and last third of the style. Certain genotypic combinations in the previous examples exhibited more than one incompatibility site, and a few compatible pollen tubes in otherwise incompatible combinations also grew until the base of the style and among the ovules. A reduced number of seeds were obtained from the latter combinations, revealing that pollen-pistil incompatibility barriers can be incomplete. Uniform pollen-tube growth arrest in the upper third of the style has also been observed in certain non-reciprocal interspecific tuber-bearing × tuber-bearing and tuber-bearing × non-tuber-bearing crosses involving other diploid species (Fritz and Hanneman 1989).

In a first approach to the problem, some authors proposed that the S-locus was involved in the cross-incompatibility reaction and assumed that this locus had a dual function: prevention of self-fertilization when the individuals involved in a cross were genetically close and prevention of cross-fertilization when they were genetically distant. A second approach discounts the possibility of the S-locus being involved in the cross-incompatibility reaction because its structure would have to be very complex to account for its dual function. Recently, Stone and Göring (2001) proposed a rather simple structure for this locus: it consists of at least two closely linked loci, one controlling the reaction in the style and the other in the pollen grain. In addition, the arrest of pollen-tube growth does not occur at a single site in the style, as in the self-incompatibility reaction, but rather at various sites along it, and also in the stigma—where pollen germination can also be inhibited—and both UI and BI are observed in species with and without a self-incompatibility system.

The first to propose the action of specific genes independent of the S-locus in tuber-bearing *Solanum* species were Grun and Radlow (1961) and Grun and Aubertin (1966). Hogenboom (1973, 1979), on the basis of his studies in *Lycopersicon*, proposed the theory of incongruency, according to which the partners do not have a complete potential for functioning together due to the lack of information in one of them with respect to the other. The presence of such genetic systems of cross-incompatibility or incongruity, in which genes interact on a one-to-one basis to hinder or prevent hybridization, could account for the isolation of *Solanum* species and the maintenance of their genetic integrity. It also supports the results obtained in tuber-bearing species of *Solanum*, in both intra-specific and interspecific crosses, by many authors (Grun and Aubertin 1966; Hermsen and Sawicka 1979; Camadro and Peloquin 1981; Sala 1993; Masuelli and Camadro 1997; Camadro et al. 1998; Erazzú et al. 1999, among others). Camadro and Peloquin (1981) proposed a genetic model with dominant CI genes in styles that prevent fertilization by pollen carrying specific dominant complementary genes. This model, which assumes segregation for both type of loci, accounts for the results of both UI and BI incompatibility in inter- and intraspecific crosses in tuber-bearing *Solanum* and also accommodates the results obtained in similar crosses in *Asparagus* between species that do not possess a self-incompatibility system (Marcellán and Camadro 1996). A few genes are similarly involved in cross-incompatibility reactions in maize. In fact, one dominant gene or gene cluster from teosinte has been found to restrict its crossability with maize. This gene is loosely linked to another gene present in many popcorns that confers non-receptivity to the pollen of other maize varieties (Evans and Kermicle 2001). Likewise, Boavida et al. (2001) suggested that the mechanisms involved in the UI observed in interspecific crosses in *Quercus* are distinct from the ones acting upon self-pollinations.

It seems reasonable to assume that complementary genetic systems developed during the evolution of sympatric species at the pollen-pistil level. In fact, their continued existence would have been threatened in proportion to the freedom of interbreeding between them. In this way, a CI gene-specific complementary genetic system would have developed, comparable to the gene-for-gene system proposed by Flor (1955) for host parasite-relations in flax. Segregation at the CI loci allows a certain amount of gene exchange between sympatric populations without threatening the identity of either species because the progenies of backcrosses to the parental adapted populations would most likely have the required ecological characteristics and, thus, would be probably favored by the existing environmental conditions.

Nuclear-cytoplasmic male sterility barriers

The second strong internal reproductive barrier that guarantees species integrity in tuber-bearing *Solanum* species is nuclear-cytoplasmic male sterility. This isolating barrier is not connected with any constitutional weakness of the hybrid endosperm, embryo, or whole organism but rather with specific interactions between the nucleus of one species and the cytoplasm of another (eventually sympatric) species. As a result of this interaction, the progeny is male-sterile. Stebbins (1950) reported that the barriers that act by preventing the growth or the reproduction of F_1 hybrids are the most important and widespread among those that allow species integrity. There is an important reproductive aspect to consider that is strongly related to nuclear-cytoplasmic male sterility and its role in sexual isolation. Bumblebees, the only insects that pollinate tuber-bearing *Solanum* species, do not visit male-sterile plants that are, consequently, isolated.

The existence of nuclear-cytoplasmic male sterility in tuber-bearing *Solanum* is well-documented. In the past, several authors observed male sterility in F_1 hybrids derived from matings involving various wild and cultivated species. In particular, male sterility has been reported to be a result of interactions between *S. tuberosum* cytoplasm and nuclear genetic factors in the wild species *S. sanctae-rosae*, *S. infundibuliforme*, *S. raphanifolium*, *S. curtilobum*, *S. acaule*, *S. commersonii*, *S. chacoense*, *S. spegazzini*, and *S. stoloniferum* (Lamm 1941, 1953; Brown 1984; Hermunstad and Peloquin 1985; Tucci et al. 1996; Santini et al. 2000; Carputo et al. 2003b) and in other cultivated potatoes (Ross et al. 1964; Grun 1973; Hanneman and Peloquin 1981), in *S. verrucosum* cytoplasm and nuclear genes of *S. phureja*, *S. chacoense*, and *S. tuberosum* (Buck 1960; Abdalla and Hermsen 1972), and in *S. demissum* cytoplasm and nuclear genes of *S. tuberosum* (Dionne 1961).

Nuclear-cytoplasmic male sterility in *Solanum* species was studied by Koopmans (1959), who demonstrated that the abnormalities observed in flowers of *S. rybinii* × *S. chacoense* hybrids were due to the interaction between the cytoplasm of the female parent and the nucleus of the male one. The importance of this type of sterility in the speciation of *Solanum* species was emphasized by Grun (1979). He demonstrated the presence of cytoplasmic genes ("plasmon factors") as determinants of cytoplasm-affected male sterility. For example, the diploid cultivated species *S. stenotomum* and *S. phureja* contain a recessive cytoplasmic factor (in^r) that conditions resistance to the effects of dominant nuclear *In* genes that are present in biotypes of the two species. *In* genes, therefore, do not have any effect on plants of *S. stenotomum* and *S. phureja*, but when they are introduced into plants having the indehiscence-sensitive cytoplasmic factor (In^s), they lead to indehiscence of anthers and male sterility. Nine of these cytoplasmic factors affecting anther indehiscence, sporad formation, anther style fusion, deformed flowers, and shrivelled microspores have been described (Grun 1979). Two of these interact with nuclear recessive alleles, a third with dominant ones. Similar results were reported by Abdalla and Hermsen (1972), who detected seven male-sterility types in accessions of *S. verrucosum* and their interspecific hybrid derivatives. They proposed that this species possesses at least four plasmons, each of which produces a diagnostic type of male sterility in the presence of the specific plasmon-sensitive genes, which are widely present.

Nuclear genes like the ones previously mentioned might be involved in the control of several steps in the process leading to anther and pollen differentiation. Changes in the plasmon would lead to blockage of the corresponding biosynthetic steps. The establishment of new plasmons in a population would require sympatry with the old as a source of pollen. Likewise, the nuclear genes are also expected to mutate. After the new plasmon becomes established as a pollen-fertile population, it can be envisioned that plasmon differentiation would operate as an isolating mechanism because the hybrids would be a mixture of male-sterile and male-fertile plants, depending on the direction of the cross (Grun and Aubertin 1965).

Information about the genetic bases of nuclear-cytoplasmic male sterility is generally limited. The sterility found in *S. tuberosum* haploids × *S. stenotomum* or *S. phureja* crosses (Ross et al. 1964) was attributed to the interaction of a dominant gene, *Ms*, from the cultivated diploids with *S. tuberosum* cytoplasm. Hanneman and Peloquin (1981) suggested that the variability observed in the frequency of male-sterile progeny in these crosses could be due to the action of dominant male-fertility restorer (*Rt*) genes. Evidence for this was provided by Iwanaga et al. (1991). Kaul (1988) and Ortiz (1998) reported that one or few nuclear genes can interact in incompatible cytoplasms and cause male sterility. However, it is still unknown how *Rt* genes can suppress the expression of the abnormal protein produced by the organellar genome.

Chloroplast (cp)DNA analysis in several tuber-bearing *Solanum* species suggests that the expression of nuclear-cytoplasmic male sterility is not controlled by cpDNA, as was also reported for other species (Buckner and Hyde 1985; Breiman and Galun 1990). Using restriction fragment length polymorphism (RFLP) techniques, potato varieties and haploids have been assigned to four mitochondrial groups and genetic distances among mitochondrial types have been calculated using a large number of species (Lössl et al. 1999). Interestingly, a cytoplasmic divergence was found among cultivated *S. tuberosum* genotypes (Scotti et al. 2003). The characterization of hybrids obtained by somatic fusion between *S. tuberosum* and *S. commersonii* provided evidence that a negative interaction between cultivated cytoplasm and genes from *S. commersonii* occurred (Cardi et al. 1999). Molecular analysis with mitochrondrial (mt)DNA gene probes demonstrated that most of the male-sterile hybrids showed a preferential inheritance of mtDNA fragments from the cultivated species. The fertile hybrids had predominantly mtDNA fragments from the wild species, indicating that the *S. tuberosum* region involved in the nuclear-cytoplasmic interaction had been lost. These data confirmed that the mitochondrial genome is involved in the determination of the trait. The specific region associated to it still remains unknown.

Endosperm barriers

In addition to being a major food and feed source, the endosperm is an extremely important tissue in relation to seed formation. As reviewed by Berger (1999), the endosperm represents a major site of reserve storage and controls nutrient delivery to the embryo. It may also be a source of signals involved in embryogenesis (Lester and Kang 1998). Endosperm breakdown is considered the primary reason for seed failure following double fertilization in angiosperms (Brink and Cooper 1947; Friedman 1998). Genetically, the nuclear constitution of the endosperm differs from the embryo only in having one extra set of maternal chromosomes. Thus, endosperm development is dependent on the same genes as the embyo but in different doses.

Various studies have been carried out to explain the genetic basis for normal endosperm development following interspecific hybridization. Early hypotheses suggested a need for a particular balance of chromosome sets between maternal tissue, embryo, and endosperm (Boyes and Thompson 1937; Valentine 1956). In corn, Lin (1975) demonstrated that a 2:1 maternal to paternal genome dosage in the endosperm was fundamental for normal endosperm growth. To explain failures in interspecific crosses in *Solanum*, Johnston et al. (1980) developed a model which extended this 2:1 genomic ratio. This model relies on a balance of qualitative genetic factors [endosperm balance number (EBN)]—and not genomes—for normal endosperm development. In this model, each species has a genome-specific "effective number" (the EBN), which is not necessarily a direct reflexion of its ploidy. It is the EBN which must be in a 2:1 maternal to paternal ratio in the hybrid endosperm for normal development of this tissue and, consequently, of the normal hybrid embryo. Thus, successful interspecific hybridization occurs only when parents produce gametes with the same EBN. The EBN is an arbitrary value experimentally assigned to each *Solanum* species based on its behavior in crosses with EBN standards and on the assumption that the 2:1 ratio is a prerequisite for normal endosperm development.

Various studies have contributed to the assignment of an EBN value to several *Solanum* species (Johnston and Hanneman 1980, 1982; Ochoa 1992; Hanneman 1994). Following these studies, cultivated *S. tuberosum* was assigned 4EBN. Diploid species have been assigned either 1EBN (e.g., those belonging to *Etuberosa* and *Bulbocastana* series) or 2EBN (e.g., all diploid cultivated species and most wild species, like *S. tarijense*, *S. berthaultii*, *S. verrucosum*). Tetraploid species have been assigned either 2EBN (species of series *Longipedicellata*) or 4EBN. All of the hexaploid species, such as *S. demissum* and *S. oplocense*, have been assigned 4EBN.

Several genetic studies have provided evidence that EBN is under oligogenic control in *Solanum* species (Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995; Johnston and Hanneman 1996). Based on the result of crossability studies, Masuelli and Camadro (1997) hypothesized that the EBN may be part of a more complex system of pre- and post-zygotic barriers to interspecific hybridization. The central role of EBN as a mechanism influencing the evolution of the tuber-bearing *Solanum* has been recognized by Hawkes and Jackson (1992). These authors reported that the evolution of the four corolla types of *Solanum* species (primitive stellata, advanced stellata, primitive rotata, and advanced rotata) is correlated with their EBN values. A general trend was found between ploidy level and EBN that showed an increase from the 2×(1EBN) primitive stellata to the 4×(2EBN), 4×(4EBN), and 6×(4EBN) advanced rotata. According to the proposed evolution pathway, 1EBN primitive stellata represent the original state of wild diploid species in Mexico, and 2EBN advanced stellata arose in South America as an isolating mechanism during the evolution of 2EBN species of series *Yungasense* from 1EBN species of series *Commersoniana*.

The essential role of EBN as an internal reproductive barrier to interspecific hybridization is that it acts as a powerful sexual isolating mechanism. Differences in EBN create effective barriers between sympatric species, subsequently leaving intact their genotypic integrity. For this reason, for example, 2×(1EBN) *S. commersonii* cannot be crossed with *S. chacoense*, which is also diploid but with 2EBN. On the other hand, *S. commersonii* hybridizes with diploid (1EBN) species. Similarly, 4×(2EBN) *S. acaule* plants do not hybridize with 4×(4EBN) *S. tuberosum*. Only mutation events causing the duplications of genes involved in the EBN value control or changes in the level of regulation of these genes can cause the synthesis of a new species without concomitant doubling of the genomes (Hanneman 1999). If fertilization between sympatric species involves gametes with matching EBN, a new genetically isolated but sympatric population arises, and it could eventually form a new species, without any changes in the ploidy, environmental isolation, or population size of the original one. Meiotic mutations leading to 2*n* gamete production can also result in hybridization between incongruent sympatric species; they double both ploidies and EBN values, so that following intraploidy, inter-EBN crosses, the 2:1 EBN requirement favors 2*n* gametes of the parent with the lower EBN. Thus, a 2×(1EBN) species producing 2*n* gametes can cross with a sympatric 2×(2EBN) species, resulting in triploid hybrids.

From an evolutionary standpoint, the EBN represents a powerful screen for 2*n* gametes during sexual polyploidization events that lead to the polyploid evolution of *Solanum* species. It should be pointed out that this complementary role of EBN and 2*n* gametes has an important meaning, not only because it facilitates interspecific gene introgression but also because it maintains the ploidy integrity of the two parental species. The evolution pathway of potato species has been described by den Nijs and Peloquin (1977) and more recently by Carputo et al. (2003a). They proposed a scenario where *n* and 2*n* gametes link together all ploidy and EBN levels, thereby providing an opportunity for gene flow throughout sympatric species with different EBN and chromosome numbers. Inter-EBN crosses can occur, but they are only sporadic events that probably result from non-heritable random events, such as multiple fertilizations of the central cell, mitotic abnormalities in the gametophyte, endomitosis of the polar nuclei in the endosperm, increase in the number of polar nuclei (Hanneman 1999)

Ortiz and Ehlenfeldt (1992) hypothesized that tetraploid *S. acaule* could have originated through bilateral sexual polyploidization after the fusion of 2*n* 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 dysfunction due to an unbalanced EBN ratio. Under the EBN incompatibility system, *S. tuberosum* ssp. *tuberosum* may have arisen from the fusion of 2*n* gametes of 2EBN diploid species. Hawkes (1994) reported that it is likely that the two diploid species involved were *S. stenotomum* and *S. sparsipilium*, both 2EBN. Recent cpDNA analysis by Hosaka (2003) demonstrated the involvement of another species, 2×(2EBN) *S. tarijense*, in the hybrid origin of *S. tuberosum*.

Endosperm barriers based on the EBN concept are not restricted to *Solanum*. Evidence for EBN-like systems has been also reported in other species such as *Trifolium* spp. (Parrott and Smith 1986), *Glycine* spp. (Zhang and Palmer 1990), *Lycopersicon* spp. (Ehlenfeldt and Hanneman 1992), *Datura* (Johnston and Hanneman 1999), *Impatiens* (Arisumi 1982), and *Avena* spp. (Katsiotsis et al. 1995). These findings further support the importance of post-zygotic barriers acting at the endosperm level in contributing to species integrity within the genus *Solanum*.

Conclusions

Tuber-bearing *Solanum* species were grouped by Grant (1971) with species of the genera *Geum*, *Silene*, and *Iris* on the basis of their life form and reproductive system. These species present a comparable pattern of interspecific relations because they are allogamous, possess similar floral mechanisms, are intercompatible within broad limits, and their genomes are closely related. Species in these genera are isolated in nature by ecological and other external factors. Although this description applies to potato species, internal barriers to hybridization may have played a more important role in the evolutionary divergence of sympatric species than has been attributed to date. Interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and EBN are widely present barriers that reinforce geographic and ecological isolation.

Both uniparental and parental lineages occur in the tuber-bearing *Solanum* species and hybrids, and regular meiosis and chiasmata are often observed (Matsubayashi 1991) because genome differentiation has not been extensive in the group. Thus, the biological species concept cannot be applied to them. In contrast, the evolutionary species concept developed by Simpson (1961) provides a perspective on the problem of hybridization and species integrity. As stated by Grant (1971), the important question is not whether two species hybridize or not, but whether two hybridizing species do or do not lose their distinct and evolutionary role. If, despite some hybridization, they do not merge, then they remain separate species in the evolutionary perspective.

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