

Homoploid hybridization in the origin and evolution of wild diploid potato species

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Abstract The potatoes, *Solanum* L. section *Petota* Dumortier, are a group of species that possess a very broad range of biological diversity, and a wide geographical distribution in the Americas. These species constitute euploid series with somatic chromosome numbers ranging from $2n = 2x = 24$ to $2n = 6x = 72$. Although special attention has been given to the origin of polyploid potato species, principally the cultivated forms, that are major

food crops, and hybridization has been accepted as an important evolutionary force in the section, the mechanisms involved in the origin and evolution of the diploid species have not been elucidated. Herein, we propose that homoploid hybridization is the main mechanism involved in the origin and evolution of the diploid potato species, and discuss the evidences that support our proposal.

Keywords Wild potato species · Hybridization · Introgression · Speciation

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Introduction

Natural hybridization and polyploidization are two of the most important forces in the evolution of flowering plants. Specifically, interspecific hybridization is a source of adaptive variation and functional novelties, and hybrid speciation is the major outcome of this process (Lewontin 1966). Also genetic material can be transferred from one species into the gene pool of another through hybridization and backcrossing (Anderson 1949), leading to introgressed forms and, eventually, to the formation of semispecies (Grant 1981). Interspecific hybridization may play a role in evolution as (a) a way of perfecting isolating mechanisms if hybrids are rare and inviable, (b) a source of new species, and (c) a means of increasing genetic variability (Mayr 1963).

Two models of hybrid speciation have been proposed (Ferguson and Sang 2001): (1) allopolyploid hybrid speciation, which is the production of a new entity of specific rank by the union of diverged genomes, and the subsequent chromosome doubling of the sterile hybrid that gives rise to a fertile allopolyploid, and (2) homoploid hybrid speciation, in which the newly formed hybrid is fertile and has the same chromosome number as the parental species.

The potatoes, *Solanum* L. section *Petota* Dumortier, are unique among crop plants and their close relatives in possessing a very broad range of biological diversity and a wide geographical distribution (Hawkes 1990). They have been arranged into two subsections, *Estolonifera* Hawkes and *Potatoe* G. Don, the first of which includes species that produce only stolons, whereas the second includes species that produce both stolons and tubers. Species in subsection *Potatoe* are arranged into two taxonomic superseries and 19 series. Their range of ecological adaptation is notorious, since there are species adapted to very high altitudes (3,000–4,500 m.a.s.l.), coastal plains, semidesertic and humid micro- and macro-environments, among other contrasting habitats (Hawkes 1994).

Hawkes (1990) described 235 species in section *Petota*, which are widely distributed along the Americas from Southwestern United States to Southern Chile and, to the East, in Argentina, Paraguay, Uruguay and Brazil, and have their centers of diversity in Central Mexico and the high Andes from Peru to Northwest Argentina. More recently, Spooner and Castillo (1997) reexamined the taxonomy of the South American wild species analyzing chloroplast DNA, and concluded that the number of species described by Hawkes (1990) is an overestimation of the species diversity in the section. Moreover, Spooner et al. (1995), Spooner and Castillo (1997) and Van den Berg et al. (2002), based on morphological and molecular studies, have stressed the necessity of a reevaluation of the taxonomic series and superseries in the section.

The somatic chromosome numbers of the wild and cultivated potato species range from $2n = 2x = 24$ to $2n = 6x = 72$. Most of them are diploid (73%), although there are also tetraploid (15%) and hexaploid (6%) species. Fifteen out of the 21 taxonomic series have exclusively diploid species; in series *Tuberosa*—in which the cultivated potatoes are included—83% of the species are diploid (Hawkes 1990).

Hawkes and Hjerting (1969) have stated that potato species are principally isolated by external barriers. However, both pre- and post-zygotic internal barriers have been identified in section *Petotae*, the first acting at the pollen–pistil level (unilateral and bilateral pollen–pistil incompatibility), and the second acting mostly at the endosperm level, although embryo abortion, male sterility and breakdown of F2 and other segregating progenies have been reported (see Camadro et al. 2004).

Johnston et al. (1980) have proposed that, in the absence of pre-zygotic barriers, the success of a cross in potato does not depend on the real ploidy (chromosome number) of the parents; instead, it depends on the presence of genetic factors (EBN = Endosperm Balance Numbers) in the hybrid endosperm in a 2:1 proportion, provided by the female and male parents. The EBN or “effective ploidy”

and the real ploidy do not necessarily coincide. Potato species have been assigned 1, 2 or 4 EBN on the basis of their crossing behavior (number of normal seeds/fruit and chromosome number of the progeny) in intra- and inter-ploid crosses (Johnston and Hanneman 1980; Hanneman and Bamberg 1986; Chavez et al. 1988). Thus, and provided that pollen–pistil interactions are normal, $4x$ species with 4 EBN can readily cross only with species with the same EBN, independently of their real ploidy (i.e. $4x$ or $6x$) and with species with 2 EBN only if the latter produce functional $2n$ gametes. The taxonomic and evolutionary relevance of the EBN hypothesis has been pointed out by Hawkes and Jackson (1992).

The integrity of the species in areas of sympatry is, therefore, maintained by internal hybridization barriers that reinforce ecological and other external barriers. However, these internal barriers can be incomplete, allowing intercrossing and the formation of fertile hybrids; in addition, the functioning of n and $2n$ gametes provides the opportunity for gene flow among ploidy levels (den Nijs and Peloquin 1977; Carputo et al. 2003; Camadro et al. 2004) as has been also described for *Dichantium agamo* species (de Wet 1968), broadening the genetic base of the hybridizing populations.

Although special attention has been given to the origin of polyploid potato species (Peloquin et al. 1999; Carputo et al. 2003) and hybridization has been accepted as an important evolutionary force in the section *Petota* (Hawkes 1962; Hawkes and Hjerting 1969; Ugent 1970), the mechanisms involved in the origin of the diploid species have not been elucidated. Spooner et al. (1991) found no support for the hybrid origin of *S. raphanifolium* Cárdenas and Hawkes by diploid hybrid speciation, and proposed to reinvestigate the other hybridization hypotheses as well. Herein, we propose that homoploid hybridization is the main mechanism involved in the origin and evolution of the diploid potato species, and discuss the evidences that support our proposal.

Natural interspecific hybrids

The German taxonomist Bitter (Bitter 1912), working almost exclusively with dried herbarium material, thought of *Solanum* species as very narrow units with little intraspecific variability. Even the slightest variants were given specific, subspecific or varietal rank, and his species were generally based on only one or, at the most, few specimens. The Russian botanists and plant breeders who worked with living material, especially Juzepczuk and Bukasov (Bukasov 1930, 1933), followed Bitter’s methods fairly closely. However, the British taxonomist J. Hawkes (Hawkes 1962), after working 1 or 2 years with potato collections, considered that

the species concept in tuber-bearing *Solanum* species had to be drastically revised if taxonomists were not to arrive at a state in which nearly every collection was given a distinctive specific or varietal name. This was due to the fact that the phenotypic plasticity in potato species is great, and the environmental effects on the phenotype are of such a magnitude as to obscure almost completely the expression of essential genotypic characters that point out the differences between species.

The pattern of variability in the tuber-bearing *Solanum* species cannot be adequately understood without a realization of the importance of the two alternative means of reproduction available to them: sexual and asexual. Although the cultivated tetraploid potato commonly reproduces vegetatively, the reverse is largely true for the wild diploid and even-numbered polyploid species. Clonal reproduction, by means of tubers and stolons, is of great evolutionary significance not only for the preservation of hybrid genotypes which would not otherwise survive beyond a single generation, but also for rapidly establishing new gene combinations which, by chance, are adapted to particular environments (Camadro 1981). Most wild diploid potato species are obligate outbreeders, because they possess a one-locus (S) gametophytic self-incompatibility system that ensures allogamy and, as the majority of the polyploid species, are insect-pollinated. Their breeding system, therefore, provides for success and opportunism both in maintaining superior genotypes in stable environments, and in allowing rapid evolution of new forms under varying environmental conditions. Hybridization and subsequent gene flow within and between various ploidy levels often results in exceedingly complicated patterns of variation. The net effect of this intergradation, as Ugent (1966) pointed out, is an extensive assemblage of inter-related plants within which lines must be drawn somewhat arbitrarily in order to delineate taxonomic units. Contributing to the difficulty of hybrid analysis is the fact that the initial morphological differences between parental taxa are not generally of such a magnitude as to furnish characters which can be conveniently measured. Consequently, most of the specific taxa are recognizable taxonomically only on the basis of their degree of development of common sets of characters, and the expression resulting from their particular associations. In fact, the tuber-bearing *Solanum* species have been interpreted as a large pool of morphologically similar species, partially isolated by geographical and ecological barriers (Ugent 1966; Hawkes and Hjerting 1969). These external barriers are reinforced by strong, although sometimes incomplete, internal reproductive barriers (Camadro et al. 2004). Thus, the biological species concept defined as groups of natural populations that intercross and are reproductively (genetically) isolated from other similar groups (Mayr 2004), cannot be applied

to these species. In fact, Grant (1981) grouped the tuber-bearing *Solanum* with species of the genera *Geum*, *Iris* and *Silene* on the basis of life form and breeding system. Species within this group share a similar pattern of inter-specific relationships, known as the *Geum* pattern: they are outcrossers, have floral mechanisms with only moderate species to species differences, and are intercompatible within wide limits, but strong incompatibility barriers have developed between sections or subgenera. Species belonging to the same species group are interfertile and closely related cytogenetically. Related species are isolated in nature by ecological and other external factors. The evolutive species concept proposed by Simpson (1961), a lineage that evolves separately from others and has its own role and unitary evolutive tendencies, seems to accommodate the group. However, it has been criticized by Mayr (2004), because each geographically or chronologically isolated population would fulfill the requisite and, furthermore, it is not possible to determine if a population has its own role and unitary evolutive tendencies.

Potato taxonomists have described variable numbers of natural hybrids among the specimens examined (see Spooner and van den Berg 1992). Hawkes and Hjerting (1969) hypothesized that there were 72% natural inter-specific hybrids among 32 species studied (Table 1). The same authors, in referring to their collection expeditions to Argentina, Brazil, Paraguay and Uruguay, wrote that: "...at first sight it will be thought that we have seen hybrids and introgressed forms everywhere!" (p. 119). In spite of this comment and the fact that they made emphasis on the wide range of morphological variability observed within species, a likely evidence of introgression, they designated as hybrid only 9.5% out of 1,300 specimens examined. More recently, van den Berg et al. (1998) reported an important morphological variation in the *S. brevicaulle* Bitt. complex, formed by the putative ancestors of the cultivated potatoes, due to causes that they could not establish. The phenetic analysis that these authors carried out, based on 53 morphological characters, failed to support the 30 distinct taxa of the complex. They observed an extensive overlapping of character states and weak morphological and geographical separation, and conjectured that it was possible that either former taxa in the *S. brevicaulle* complex were the result of recent hybridization and introgression events, or that the ranges had been disrupted by recent dispersal of weedy taxa, or that the taxa were never well differentiated into species. Regarding the first possible cause, high levels of gene flow between wild species, and the cultivated diploid *S. tuberosum* ssp. *stenotomum* in experimental fields were reported by Huamán (1975) and Rabinowitz et al. (1990). They also claimed that 27 out of 232 species recognized by Hawkes (1962, 1990) were of putative hybrid origin and that 12% of the

Table 1 Natural hybrids and their putative parents among wild and cultivated potato species according to Hawkes (1990)

Taxonomic series	Species	Ploidy	Putative parents
Pinnatisecta	<i>S. × sambucinum</i> Rybd.	$2n = 2x = 24$	<i>S. pinnatisectum</i> and <i>S. cardiophyllum</i>
	<i>S. × michoacanum</i> (Bitter) Rybd.	$2n = 2x = 24$	<i>S. bulbocastanum</i> and <i>S. pinnatisectum</i>
Yungasensa	<i>S. × litusinum</i> Ochoa	$2n = 2x = 24$	<i>S. tarijense</i> and <i>S. berthaultii</i>
	<i>S. × trigalense</i> Cárdenas	$2n = 2x = 24$	<i>S. chacoense</i> and <i>S. tarijense</i>
	<i>S. × zudaniense</i> Cárdenas	$2n = 2x = 24$	<i>S. tarijense</i> and <i>S. berthaultii</i>
Tuberosa	<i>S. ajanhuiri</i> Juz. & Bukasov	$2n = 2x = 24$	<i>S. stenotomum</i> and <i>S. megistacrolobum</i>
	<i>S. juzepczukii</i> Bukasov	$2n = 3x = 36$	<i>S. acaule</i> and <i>S. stenotomum</i>
	<i>S. curtilobum</i> Juz. & Bukasov	$2n = 6x = 72$	<i>S. juzepczukii</i> and <i>S. tuberosum</i> ssp. <i>andigena</i>
	<i>S. chaucha</i> Juz. & Bukasov	$2n = 3x = 36$	<i>S. tuberosum</i> ssp. <i>andigena</i> and <i>S. stenotomum</i> or ssp. <i>andigena</i> and <i>S. phureja</i>
	<i>S. × bruecheri</i> Corell	$2n = 2x = 24$	<i>S. acaule</i> and <i>S. megistacrolobum</i>
	<i>S. × doddsii</i> Corell	$2n = 2x = 24$	<i>S. chacoense</i> and <i>S. alandiae</i>
	<i>S. microdontum</i> var. <i>montepuncoense</i> Ochoa	$2n = 2x = 24$	<i>S. violaceimarmoratum</i> and <i>S. microdontum</i>
	<i>S. × mollepujroense</i> Cárdenas & Hawkes		<i>S. gourlayi</i> ssp. <i>pachytrichum</i> and <i>S. sparsipilum</i>
	<i>S. × rechei</i> Hawkes & Hjert.	$2n = 3x = 36; 2n = 2x = 24$	<i>S. microdontum</i> ssp. <i>gigantophyllum</i> and <i>S. kurtzianum</i>
	<i>S. × ruiz-lealii</i> Brücher	$2n = 2x = 24$	<i>S. chacoense</i> and <i>S. kurtzianum</i>
	<i>S. × setulosistylum</i> Bitter	$2n = 2x = 24$	<i>S. chacoense</i> and <i>S. spegazzinii</i>
	<i>S. × subandigena</i> Hawkes	$2n = 4x = 48$	<i>S. tuberosum</i> ssp. <i>andigena</i> and <i>S. sucrense</i>
	<i>S. × sucrense</i> Hawkes	$2n = 4x = 48$	<i>S. oplocense</i> and <i>S. tuberosum</i> ssp. <i>andigena</i>
	<i>S. juzepczukii</i> Bukasov	$2n = 3x = 36$	<i>S. acaule</i> and <i>S. stenotomum</i>
	<i>S. stenotomum</i> Juz. & Bukasov	$2n = 2x = 24$	<i>S. chaucha</i> , <i>S. curtilobum</i> , <i>S. juzepczukii</i> , <i>S. ajanhuiri</i>
	Acaulia	<i>S. × indunii</i> Okada & Clausen	$2n = 3x = 36$
<i>S. × viirsooi</i> Okada & Clausen		$2n = 3x = 36$	<i>S. acaule</i> and <i>S. infundibuliforme</i>
Longipedicellata	<i>S. × vallis-mexici</i> Juz.	$2n = 3x = 36$	<i>S. stoloniferum</i> and <i>S. verrucosum</i>
Demissa	<i>S. × edinense</i> Berthault	$2n = 5x = 60$	<i>S. tuberosum</i> and <i>S. demissum</i>
	<i>S. × semidemissum</i> Juz. & Bukasov	$2n = 5x = 60$	<i>S. demissum</i> and <i>S. verrucosum</i>

specimens listed in Hawkes and Hjerting's (1969) taxonomic treatment of the potatoes of Argentina, Brazil, Paraguay and Uruguay were putative hybrids. Comparatively, in the wild potatoes that grow in Bolivia, 72% (Hawkes and Hjerting 1969) and 32% (Ochoa 1990) were cited as hybrid species.

Notwithstanding, most accessions in potato germplasm banks have been assigned specific ranks. In fact, in biosystematic studies on the origin of *S. sparsipilum* (Bitt.) Juz. et Buk., which has been considered a putative wild ancestor of the cultivated Andean potato *S. tuberosum* ssp. *andigenum* (Hawkes 1956, 1967), Ugent (1970) suggested that this highly polymorphic species arose independently in different locations in Bolivia and Peru, probably due to the stabilization of a large number of diploid hybrid variants formed by natural crosses involving the cultivated diploids *S. tuberosum* ssp. *stenotomum* and ssp. *phureja* and either one or more of the closely related species *S. canasense* Hawkes, *S. brevicaule* Bitt. and *S. raphanifolium* Cárden. et

Hawkes. This author also proposed to maintain the stabilized hybrid derivatives under the single species *S. sparsipilum* instead of giving them specific or subspecific ranks. Concerning other members of this diploid chain in series *Tuberosa* (i.e., *S. brevicaule*, *S. bukasovii*, *S. ochoae*, *S. multidissectum*), Correll (1962) stated that "If all of these were placed together as one highly variable species it would probably be to the best interest of science." This statement, in our opinion, summarizes the difficulties in the taxonomic study of the subsection. It is likely, then, that some plants or seeds collected in the field and conserved in germplasm banks are actually of hybrid origin, and will show segregation upon sexual reproduction (see Clausen et al. 2006 for an example).

Frequent examples of hybridization among sympatric diploid potato populations are observed in nature, providing an indication that interspecific hybridization has to be a strong force in their evolution (see Table 1). Hawkes (1990) and Hawkes and Hjerting (1969) hypothesized that

a likely cause of taxonomic confusion is the hybridization of the habitat, that is, an alteration of the environment due to which hybrids have a greater chance of survival than the parental species. In a recent study, Jacobs et al. (2008) analyzed the phylogenetic structure within section *Petota* using AFLP markers. They found that the taxonomic structure of the section is highly unbalanced, and the majority of the South American species cannot be structured showing polytomies. High levels of hybridization, rapid speciation and geographic conditions in the Andes like dissected topography or habitat heterogeneity may explain the lack of structure in section *Petota*. In spite of these observations, potato taxonomists—in general—have been reluctant to mention hybrid forms in nature, clearly underestimating their numbers and, therefore, their possible role in introgression and speciation events. This could be due to the fact that homoploid hybrid origin is more difficult to ascertain than polyploid hybrid origin. Also, interspecific hybridization and introgression is perceived as a disturbance or a challenge to the biological species concept and as a breakdown of isolating mechanisms (O'Brien and Mayr 1991; Mallet 2005).

Morphological and genetic variability

The remarkable morphological variability observed in potatoes can be attributed to one or more of the following causes: (a) heterogeneity of the habitat and sampling strategies, (b) hybridization and introgression, and (c) genomic instabilities. Examples of these causes are given herein focusing, mainly, in Argentinean species.

Heterogeneity of the habitat and sampling strategies

del Río et al. (2001) collected populations of the allogamous diploid species *S. jamesii* Torr. in the USA that were very homogeneous; they concluded that when natural populations are sampled at particular times and conditions, these samples are subsets of heterogeneous populations. This conclusion was supported by the comparison of samples taken at the same sites but at different times, that differed in their genetic composition. Hamrick (1987) pointed out that habitats can be very heterogeneous even within small areas, so that genetic differentiation among populations can be independent of geographical distances. del Río et al. (2001) also found that populations from a particular area of another North American species, the autogamous tetraploid *S. fendleri* A. Gray, were genetically more diverse than populations from other geographical areas, and that the pattern of genetic diversity was associated to climatic variants. Lamboy et al. (1996), working with wild apple species and Ghislain et al. (2006), del Río

et al. (2001), del Río and Bamberg (2002), and McGregor et al. (2002) working with wild and cultivated potato species, showed a lack of association between geographical distances and genetic diversity.

Hybridization and introgression

Hawkes (1962) presented evidences of introgressive hybridization between the diploid species *S. chacoense* Bitter and *S. microdontum* Bitter. *S. chacoense* grows in a very wide range of habitats, from Northwest Argentina towards the East and South East of this country, reaching the neighboring countries of Uruguay, Brazil and Paraguay. The distribution of *S. microdontum*, on the other hand, is restricted to the Andean mountains of the Argentinean provinces of Jujuy, Salta and Tucumán, in a wide altitudinal range, from 1,000 to 3,200 m.a.s.l. (Hawkes and Hjerting 1969). Natural hybrids were described from the Western Andean region, where the two species are sympatric, extending as far as Eastern Buenos Aires province and Paraguay. These authors proposed that *S. chacoense* was restricted initially to the plains below 1,000 m.a.s.l, and that once it was “contaminated” with *S. microdontum* pollen, the hybrids colonized extended ecological areas at altitudes where *S. chacoense* is unable to penetrate; this phenomenon would account for the morphological variations observed at different altitudes. Miller and Spooner (1996) revised the hybridization hypothesis involving *S. chacoense* and *S. microdontum* analyzing morphological and molecular data, and they found no support for the introgression hypothesis. However, they recognized difficulties in detecting introgression in potato species, mainly because the low differentiation among *Solanum* species complicates to find species-specific morphological and molecular markers.

Raimondi et al. (2005), in studying the putative diploid ancestors of the Argentinean species of hybrid origin *S. ruiz-lealli* Brücher, observed that the accessions of one of them, *S. kurtzianum* Bitt. et Wittm.—available at the Potato Germplasm Bank in Balcarce—were morphologically very variable. This observation led Bedogni and Camadro (2007) to carry out a morphological, biochemical and molecular characterization of accessions of this species from four Western and Northwestern Argentinean provinces (Mendoza, San Juan, La Rioja and Catamarca) and accessions of three other diploid species from its distribution area, *S. chacoense*, *S. spegazzinii* Bitter and *S. maglia* Schlecht. In a multivariate analysis integrating all the variables studied, a weak separation of the four species was observed. The phenograms, as well as the group analyses for both quantitative and qualitative variables, did not separate the accessions according to their specific ranks; instead, they were separated by geographical areas. These authors concluded that the wide distribution of

S. kurtzianum and the overlapping in some areas with the other three species could account for the morphological variability observed, as the result of hybridization and introgression. Moreover, in Sierra de Famatina, in La Rioja province, *S. microdontum* is sympatric with *S. kurtzianum* and a hybrid between them, *S. × rechei* Hawkes and Hjert., grows in their overlapping distribution area (Okada and Hawkes 1978). An extensive morphological variability in this hybrid was observed in nature by Brücher (1989).

Furthermore, Erazzú et al. (1999), in studying pollen–pistil relations in accessions of *S. spegazzinii* that have been previously grouped according to their morphology by Ispizúa (1994), observed incompatible reactions within two of these groups as well as among the three of them. They speculated that the morphological variability and the prezygotic breeding barriers could be the result of interspecific crosses in nature, probably with the sympatric species *S. chacoense*.

Overlapping of potato species in their distribution areas has been recorded by different researchers over many years. For example, in the province of Salta, in North West Argentina, K. Okada in 1973 (field notes available at the Potato and Forage Germplasm Bank in Balcarce, <http://www.inta.gov.ar/balcarce>) registered that the accession OL 4911 of *S. spegazzinii* was growing with accessions OL 4907 of *S. chacoense* and OL4908 of *S. okadae* in Chicoana Department, and that accession OL 4841 of 2x *S. gourlayi* Hawkes was the growing with accession OL 4842 of *S. tuberosum* ssp. *andigenum* in La Poma Department. Likewise, in the collection trip of 1983, the same researcher recorded, in the province of Jujuy, a population of 4x *S. gourlayi* (OKA 7547A) in Quebrada de Lipán growing with *S. tuberosum* ssp. *andigenum* (OKA 7547C) and *S. microdontum* (OKA 7537B), and that accession OKA 7588A of 4x *S. gourlayi* was growing with accession 7588B of *S. tuberosum* ssp. *andigenum* and accession OKA 7588C of *S. oplocense* in Quebrada de Jueya. A long list of sympatric wild potato species was described by Hawkes and Hjerting (1969); in particular, populations of *S. chacoense*, *S. tarijense* Hawkes, *S. spegazzinii*, *S. gourlayi*, *S. microdontum* and *S. vernei* Bitt. et Wittm. were described growing with probable hybrids in Cuesta del Obispo, Salta province. These authors also recorded species that overlapped in their distribution area, like *S. gourlayi* and *S. infundibuliforme* Phil. in the proximities of Tilcara, Jujuy province, in typically dry gorges. More recently, in 2004, Clausen and Erazzú (personal communication) collected samples of overlapping potato species, some of them partially intercompatible (Camadro and Peloquin 1981), in various sites of the distribution area of *S. spegazzinii*, and of 2x and 4x *S. gourlayi* also in NW Argentina; these samples were incorporated into the Potato Germplasm Bank of EEA Balcarce, INTA: in the province of Salta, accession CLE

1581 of *S. spegazzinii* from Quebrada de Escoipe was sympatric with accessions CIE 1579 of *S. vernei*, CIE 1585 of *S. chacoense* and CIE 1583 of *S. okadae*; similarly, accession CIE 1566 of 2x *S. gourlayi* from La Poma grew in the same site than accession CIE 1567 and close to accession CIE 1569 of 4x *S. tuberosum* ssp. *andigenum*; in the province of Jujuy, in the collection site of accession CIE 1587 of 4x *S. gourlayi*, in Quebrada de Jueya, a population of 2x *S. infundibuliforme* (CIE 1588) was growing 800 m apart, and in Quebrada de Lipán, accession CIE 1597 of 4x *S. gourlayi* was growing with accession CIE 1594, CIE 1595 and CIE 1596 of 4x *S. tuberosum* ssp. *andigenum*. Clausen et al. (2005) in a collecting expedition in Jujuy, had previously reported that *S. tuberosum* ssp. *andigenum* and *S. acaule* was growing in the vicinity of *S. megistacrolobum* Bitt., *S. infundibuliforme* and *S. × viirsooi*, a natural triploid hybrid of *S. acaule* Bitter × *S. infundibuliforme* (Okada and Clausen 1985). Likewise, Clausen et al. (2006) collected populations of wild potatoes, identified as *S. infundibuliforme*, *S. gourlayi* and probable interspecific hybrids in a narrow gorge in Quebrada de Incacuevas, in Jujuy province. From the cytological, genetic and morphological studies, they concluded that the large variability registered in these populations in their natural habitat was the result of gene flow, probably by sexual polyploidization, and the establishment of hybrid populations with varying degrees of male fertility.

Genomic instabilities

In a recent paper, Raimondi et al. (2005) informed that in *S. ruiz-lealii*, a putative hybrid between *S. kurtzianum* and *S. chacoense*, some plants presented both normal and aberrant flowers. Methylation analysis carried out by Marfil et al. (unpubl. results) in plants of this species with normal flowers and plants with both normal and aberrant flowers showed that: (a) the epigenetic variability was higher than the genetic variability, (b) plants bearing abnormal flowers had similar methylation patterns, that differed from the methylation patterns of plants with normal flowers, (c) chemical demethylation of plants with normal flowers induced the production of aberrant flowers.

Aberrant flowers had also been observed in plants of *S. chacoense* (Roig 1956), and in synthetic diploid hybrids between *S. tuberosum* and *S. kurtzianum* Marfil et al. (2006). In the latter, it was shown that the synthetic hybrids were demethylated in comparison with the parental species, and presented genomic instabilities with the appearance of novel AFLPs fragments (Marfil et al. 2006). Clausen and Spooner (1998) had previously reported the presence of unique RFLP bands in the hybrid *S. × rechei*, that were not present in the parental species *S. microdontum* and *kurtzianum*. More recently, Ercolano et al. (2004) observed

novel AFLPs fragments in a haploid of *S. tuberosum* ssp. *tuberosum* obtained in crosses with *S. tuberosum* ssp. *phureja*. Likewise, rapid genomic changes and appearance and disappearance of RFLP fragments were observed in interspecific and intergeneric hybrids in wheat (Liu et al. 1998), and Cheng et al. (2002) detected new RAPD fragments in F1 intergeneric hybrids of *Brassica napus* × *Orychophragmus violaceus*. These results are relevant for three reasons: (1) if interspecific crosses are inducing genomic changes and novel AFLP or RFLP fragments are generated in the hybrids, additivity supporting the hybridization hypothesis will not be observed, (2) the novel fragments and the epigenetic modifications represent additional sources of variation as a result of natural homoploid hybridization, increasing the variability of the wild species under investigation, (3) epigenetic alterations in the hybrids inducing flower aberrations could affect their fertility, isolating them reproductively from the parental species. The significance of epigenetics for the induction of morphological changes was reviewed by Rapp and Wendel (2005), who cited the observation of Pires et al. (2004) on the striking amount of polymorphism for flowering time generated in genetically homogeneous resynthesized allopolyploids lines of *Brassica rapa*.

Consequences of homoploid hybridization

Based on the evidences presented and previously discussed, we propose that recurrent hybridization, in particular interspecific hybridization, acts as a creative evolutionary force in diploid potatoes by releasing variation in natural populations, because it can induce both genetic and epigenetic changes (the latter as the result of genomic stresses). This heritable variation can provide colonizing advantages when stabilized by clonal propagation, until the fittest genotypes are established in nature, and eventually can lead to homoploid hybrid speciation.

Hybridization events may have various results: introgression, semispeciation and speciation (Grant 1981). It is likely that more than one of these events had taken place during the evolution of diploid potato species. For example, and as previously mentioned, hybrids between *S. chacoense* and *S. microdontum* have colonized intermediate habitats (Hawkes and Hjerting 1969). But introgressed populations and hybrid swarms can also be formed by interspecific hybridization. Introgressed populations will resemble the recurrent parent but will vary in the direction of the other parental species. Hybrid swarms, on the other hand, are a complex mixture of parental forms, F1 hybrids, backcross and segregating progenies. A different phenomenon can take place once these hybrid swarms are formed: in the original habitats, hybrids may be less adapted than their parents and

segregating genotypes may have a lower fitness and be eventually eliminated from the population for a number of generations. Thus, after a certain number of years, only the parental species and the genotypes that more closely resemble them will remain due to their higher reproductive success. This phenomenon, known as the Wallace effect, has also been observed in potatoes (Hawkes and Hjerting 1969; Summers and Grun 1981). Notwithstanding, if hybridization events take place in a large scale, they can lead to the formation of semispecies and, eventually, to homoploid hybrid species if the new genotypes have either the capacity to colonize new habitats or an important adaptation capacity in comparison with the parental species. Computer simulation of recombinational chromosomal speciation model shown that homoploid hybrid speciation may occur in outcrossing species without the local extinction of parental species. In addition, these studies confirm the importance of ecological and geographical isolation in hybrid speciation (Buerkle et al. 2000; Gross and Rieseberg 2005). Thus, ecological selection could be a major factor promoting homoploid hybrid speciation in section *Petota*. Hybrids may have intermediate trait values, combine traits from both parents, and/or exhibit extreme trait values. All three possibilities might allow for high fitness of hybrids in a new environment.

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