

## REVIEW ARTICLE

# A genetic approach to the species problem in wild potato

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## ABSTRACT

Wild potatoes are native to the Americas, where they present very wide geographical and ecological distribution. Most are diploid, obligate out-crossers due to a multiallelic gametophytic self-incompatibility (S) locus that prevents self-fertilisation and crossing between individuals carrying identical S-alleles. They have two alternative modes of reproduction: sexual (by seeds) and asexual (by stolons and tubers), which provide, respectively, for genetic flexibility in changing environments and high fitness of adapted genotypes under stable conditions. Since the early twentieth century, their taxonomic classification has been mostly based on morphological phenotypes (Taxonomic Species Concept). More recently, attempts have been made to establish phylogenetic relationships, applying molecular tools in samples of populations (accessions) with a previously assigned specific category. However, neither the reproductive biology and breeding relations among spontaneous populations nor the morphological and genetic variability expected in obligate allogamous populations are considered when the taxonomic species concept is applied. In nature, wild potato populations are isolated through external and internal hybridisation barriers; the latter, which are genetically determined, can be either pre-zygotic (pollen–pistil incompatibility) or post-zygotic (abortion of embryo, endosperm or both tissues, sterility, and hybrid weakness and breakdown in segregating generations). The internal barriers, however, can be incomplete, providing opportunities for hybridisation and introgression within and between populations and ploidy levels in areas of overlap. The widespread occurrence of spontaneous hybrids in nature was recognised in the mid-twentieth century. Using genetic approaches, results have been obtained that provide strong support to the assertion that populations are at different stages of genetic divergence and are not at the end of the evolutionary process, as presupposed by the Taxonomic Species Concept. Furthermore, since wild potatoes have uniparental and biparental overlapping generations, the Biological Species Concept – developed for sexually reproducing biparental organisms – cannot be applied to them. In this paper, morphological, genetic, molecular and taxonomic studies in wild potato are reviewed, considering the genetic consequences of their reproductive biology, in an attempt to shed light on the species problem, because of its relevance in germplasm conservation and breeding.

## WILD POTATO: CLASSIFICATION AND GEOGRAPHIC DISTRIBUTION

The genus *Solanum* Linnaeus – to which both wild and cultivated potatoes belong – is very large, containing 1000–2000 species, according to various authors (Correll 1962; Seithe 1962; D’Arcy 1979; Nee 1993; Knapp 2002). Most of these species are herbs or shrubs, often clothed with spines and thorns, and only around 10% of them produce tubers. Wild potatoes are widely distributed along the Americas, from the southeast USA to southern Chile and, to the east, in Argentina, Paraguay, Uruguay and Brazil, with their centres of diversity in central Mexico and the high Andes from Peru to northwest Argentina. These species – which form ploidy ser-

ies with  $2n = 2x, 3x, 4x, 5x$  and  $6x$  chromosomes ( $x = 12$ ) – are adapted to a multiplicity of macro- and micro-environments along their geographical distribution (Hawkes 1990).

## REPRODUCTION AND BREEDING BARRIERS

Although the predominant mode of reproduction in wild potato populations has not been ascertained, two alternative means are available: sexual by seeds, and asexual by stolons and tubers. Sexual reproduction provides genetic flexibility by generating new gene combinations during meiosis and upon fertilisation; some of these combinations, by chance, can be better adapted to changing environments. Asexual reproduction, in contrast, provides for high fitness of adapted

genotypes in stable environments; in this way, those genotypes can be preserved over time by mitosis, regardless of their fertility, and, if not completely sterile, can participate in sexual crosses, generating new genetic variability.

Potatoes possess a gametophytic self-incompatibility system controlled by the multiallelic S-locus, which prevents self-fertilisation and crossing between closely related –otherwise fertile – individuals. Most wild potatoes are diploid, with obligate allogamy (cross-pollination) because the S-system is very strict. In polyploids, however, the interaction between different S-alleles in pollen results in compatibility. This phenomenon – known as ‘competition interaction’ (see Frankel & Galun 1977) – allows crossing and self-fertilisation by S-heterozygous male gametes in otherwise incompatible genotypic combinations. Regarding the cytogenetic behaviour of diploids and polyploids, Matusbayashi (1991) concluded that, irrespective of their ploidies, potato species are phylogenetically related because they possess a common (basic) A genome, which, modified to different degrees, originated four additional genomes: B, C, D and E.

Wild potatoes are separated in nature by external geographical and ecological hybridisation barriers (Hawkes & Hjerting 1969), but also by internal barriers that reside in the plant tissues themselves. The internal barriers, which can either hinder or prevent inter-crossing, are divided into two types: pre- and post-zygotic; the first acting at the pollen–pistil level (known as either cross-incompatibility or incongruity) and the second acting mostly at the endosperm level, although embryo abortion, male sterility and breakdown of F<sub>2</sub> and advanced segregating hybrids have been reported (see Camadro *et al.* 2004). Pre-zygotic barriers can be either unilateral or bilateral. Although their molecular bases have not been elucidated, it has been proposed that the S-locus is not involved in their determination and that genes controlling the incompatibility reaction in both pollen and pistil can segregate (Camadro & Peloquin 1981; Camadro *et al.* 2004; Fig. 1a). Thus, in largely incompatible pollen–pistil populations, some genotypic combinations can be compatible. The post-zygotic barriers acting at the endosperm level are also under genetic control. According to the Endosperm Balance Number (EBN) theory (Johnston *et al.* 1980), potatoes have a real ploidy (given by their actual chromosome numbers) and an ‘effective’ ploidy resulting from the action of hypothetical genetic factors, denominated EBN. On the basis of controlled crosses with a species taken as standard, potato species were assigned one, two or four EBN (Johnston & Hanneman 1980). For normal endosperm development, a two (female) to one (male) EBN ratio in the endosperm is necessary. The endosperm barriers in inter-EBN crosses can be circumvented either by functioning of 2n gametes (gametes or gametophytes with the sporophytic chromosome number) from the parent with the lowest EBN (Johnston *et al.* 1980; Masuelli & Camadro 1997) (Fig. 1b) or, as speculated, by reduction by haploidisation of the ploidy level of the parent with the higher EBN, as reported in certain common potato × wild species genotypic combinations (Peloquin & Hougas 1959). Incomplete breeding barriers in wild potatoes provide an opportunity for hybridisation within and between populations and ploidy levels and, since genome differentiation is not large in the group (Ramanna & Hermsen 1979; Matusbayashi 1991; Masuelli & Camadro 1992), vigor-

ous and fertile interspecific hybrids can be formed. Thus, it can be speculated that – at a given time – a wild potato population may be composed of parental genotypes, first and advanced generation hybrids, backcross generations and introgressed forms, in a manner similar to that described for American species of *Iris* (Hodges *et al.* 1996), as will be discussed and illustrated.

Hybridisation and subsequent gene flow and introgression in sympatric populations, within and between ploidy levels, often results in exceedingly complicated patterns of variation. The net effect of this inter-gradation, as Ugent (1966) clearly pointed out, is an extensive assemblage of interrelated plants, among which lines must be drawn somewhat arbitrarily to delineate the taxonomic units.

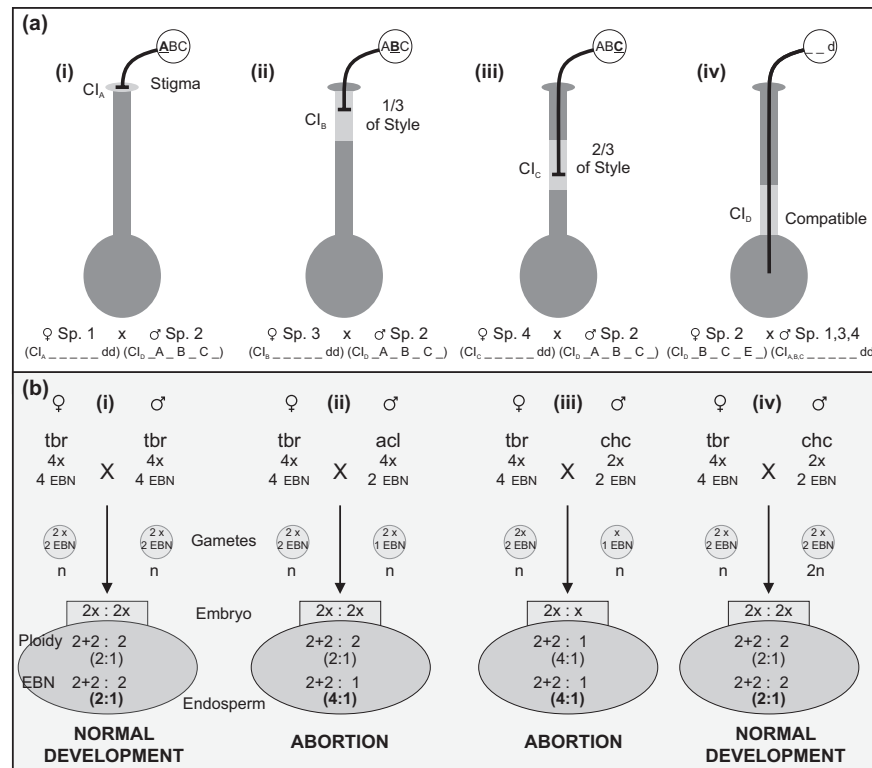
## POTATO ACCESSIONS

Potato accessions are samples of natural populations that are *ex situ* conserved in germplasm banks as either original collections or multiplications of those collections. The primary concern when germplasm banks were created in the last century was to conserve samples of the natural variability of a given genus/species. Later on, and with the need to incorporate genetic variability for breeding, especially in crops with a narrow genetic base such as potato, germplasm banks were established (see Ross 1986) and the need for germplasm characterisation and evaluation was recognised.

Passport information for potato accessions in germplasm banks usually includes collection date, locality, latitude, longitude and altitude above sea level (see <http://www.ars-grin.gov/npgs/holdings.html> and [http://www.anterior.inta.gov.ar/balcarce/banco\\_germopl/catalogo\\_herbario.pdf](http://www.anterior.inta.gov.ar/balcarce/banco_germopl/catalogo_herbario.pdf)). However, information on the number of plants in the sampled populations, number and spatial distribution of the sampled plants at the collection sites, number of sampled fruits and/or tubers per sampled plant or, if only plantlets are sampled, their number and spatial distribution, is usually unavailable or absent. Similarly, for *ex situ* seed or tuber multiplication of the original field collections, there is no available information on the effective number of parental plants, number of fruits or tubers harvested per plant and number of seeds harvested per fruit, as well as any other information that would indicate if genetic drift has been either avoided or reduced to a minimum (providing that unwanted mechanical and/or seed contamination could be disregarded), conserving the gene frequencies of the sampled populations. Thus, when working with *ex-situ* conserved germplasm, it cannot be known with any certainty whether the accessions are representative samples of the genetic diversity of the originally sampled populations. Notwithstanding, it is highly unlikely that a researcher could actually work with his/her own collections, given the complexities and high costs entailed in the exploration and collection processes.

## CONCEPTS OF SPECIES

Natural history, as nowadays understood, started in Classical Greece, with various schools of thought that considered that physical elements were universal and immutable, an idea that permeated the scientific field for several centuries (see Huxley 2007 for a detailed treatment). The first concept of species



**Fig. 1.** Internal pre-zygotic (a) and post-zygotic (b) hybridisation barriers. a: Representation of the cross-incompatibility model of Camadro & Peloquin (1981), in which dominant CI genes expressed in pistils prevent fertilisation by pollen carrying complementary dominant genes. (i) 'Cl<sub>A</sub>' gene, expressed in the stigma, arrests growth of pollen tubes carrying the complementary 'A' gene; (ii) 'Cl<sub>B</sub>' gene, expressed in the first third of the style, arrests growth of pollen tubes carrying the complementary 'B' gene; (iii) 'Cl<sub>C</sub>' gene, expressed in the second third of the style, arrests growth of pollen tubes carrying the complementary 'C' gene; (iv) in reciprocal crosses, pollen tubes carrying the 'd' allele are not arrested by the 'Cl<sub>D</sub>' gene and thus fertilisation takes place. Sp.1, 2, 3 and 4 are different species. b: Representation of the Endosperm Balance Number (EBN) model (Johnston *et al.* 1980), in which a 2:1 (maternal:paternal) ratio of genetic factors must be present in the endosperm for its normal development. For each species, parental ploidy and EBN are given. In (iv), the pollen parent produces 2n gametes. The maternal:paternal ratio in the endosperm is reported in bold, in parenthesis. Number of genomes (x) and sporophytic (2n) and gametophytic (n) chromosome numbers represent real ploidies, whereas EBNs represent 'effective' ploidies. tbr = *Solanum tuberosum*, acl = *S. acaule*, chc = *S. chacoense*.

was developed by John Ray (1627–1705) who, similar to his contemporary Laurent de Jussie (1748–1836), proposed a 'natural' classification approach based on the largest possible number of characters. Further, Carl von Linnæus (1707–1778) proposed an 'artificial' classification, based on morphological similarities and differences that, with few changes, is still in use. This Taxonomic Species Concept makes use of holotypes, which are single physical examples or illustrations of organisms known to have been used when the species or lower-ranked taxa were formally described.

In 1858, Charles Darwin (1809–1882) proposed the Theory of Natural Selection in evolution (or 'the survival of the fittest'). In the early twentieth century, and based on Darwin's evolutionary ideas and subsequent discoveries in classical and molecular genetics, new interpretations of the diversity encountered in plants and animals arose from a more natural/biological standpoint (*Modern Evolutionary Synthesis* or *Neo-darwinism*). Some twentieth century scholars, in particular Dobzhansky (1935) among others (see Wilkins 2003), proposed definitions of species that incorporated the notion of reproductive isolation. These definitions can be regarded as early approaches to the Biological Species Concept devel-

oped and perfected by Ernst Mayr over many years (Mayr 1940, 1957, 1970). According to this concept, species are groups of interbreeding natural populations, reproductively isolated from other such groups; therefore, the concept does not apply to organisms with either asexual or both sexual and asexual reproduction, with generations separated over time, as is a common feature of many plant groups (Grant 1971), including potato. Various other species concepts have been developed in modern biology, and are thoroughly discussed in Mayr (2000).

## POTATO TAXONOMY

As conceived by early taxonomists, the genus *Solanum* included many taxa, later on raised to separate generic status. Various systems were proposed at early times, which divided the genus into a number of sections (Dunal 1813, in Hawkes 1944) or sub-genera (Wettstein 1895 and Heigi 1907, in Magoon *et al.* 1962; Rodríguez *et al.* 2009).

The German botanist Bitter (1911–1913), using the position of the pedicel articulation and the structure of cell hairs as criteria for his classification, divided section *Tuberarium*

into two subsections: *Basarthurum* and *Hyperbasarthurum* (which included the majority of the tuber-bearing species and a few without stolons or tubers because of their morphological resemblance). Working on almost exclusively dried herbarium material, one or a few specimens per collection and considering only a few characters, this botanist conceived the species in the section as very narrow units with little intraspecific variability; thus, he assigned specific, subspecific or varietal rank to even the slightest morphological variants, in an approach that was of little practical use. The early Russian botanists and potato breeders, especially Juzepczuk and Bukasov (Bukasov 1933, 1939), followed Bitter's methods fairly closely, although they worked mostly with fresh material.

By the mid-twentieth century, there was a change in the species concept in the group: potato species were now interpreted as large units with a wide range of intraspecific variability (Correll 1962; Hawkes 1963). In fact, Hawkes (1963) considered that the species concept in potato had to be drastically revised if taxonomists were not to be confronted with a state in which nearly every new collection was given a distinct specific or varietal name. This was due to the high phenotypic plasticity and large environmental effects that frequently obscured almost completely the expression of essential heritable characters that helped to delineate the differences between species. A few years later, and following a similar line of thought, Hawkes & Hjerting (1969) published a biosystematic and ecological study that took into consideration the pattern of morphological variability, the presence of extreme phenotypes and the huge phenotypic plasticity that was observed in their objects of study. They combined classical taxonomic approaches with observations of phenotypic variability in a wide range of environments. In addition, they considered the presence of geographical reproductive isolation barriers and proposed that (i) hybrids could easily originate between sympatric species that had moderate to high fertility, and (ii) introgressed forms could be successful in eroded environments. They described reproductive systems, chromosome numbers and homologies, affinities of species and taxonomic series, species habitats and geographical distributions, profusely illustrating their study with drawings and photographs. Notwithstanding, in their extensive work, Hawkes & Hjerting (1969) mainly used a morphological approach, although with interpretations of the variability observed in nature.

In the last decade of the twentieth century, potato species were assigned to section *Petota* Dumortier, subsections *Estolonifera* Hawkes (which contains only stolon-bearing species) and *Potatoe* G. Don (which contains both stolon- and tuber-bearing species). Hawkes (1990) recognised 227 tuber-bearing species (including seven cultivated ones) and nine non-tuber-bearing species in section *Petota* Dumortier (formerly *Tuberrarium*). Species in subsection *Potatoe* Don (formerly *Hyperbasarthurum*) were arranged into two super-series and 19 taxonomic series.

Spooner & van den Berg (1992) were critical of Hawkes (1990) proposal, pointing out that this researcher had not rigorously tested the origin of presumed natural spontaneous hybrids and affiliations of species in taxonomic series. Also, van den Berg *et al.* (1998) considered that Hawkes (1990) proposal presented an extensive overlap of state characters

among different species (e.g., in the *Brevicaule* Bitter complex) and that there was an overestimation in the number of species described. Spooner & Hijmans (2001) recognised 203 tuber-bearing species (also including the seven cultivated ones). Spooner & Salas (2006) further reduced the number to 189 species (including one cultivated), and Spooner (2009) suggested that a taxonomic decision in his laboratory was converging on around 110 species. Notwithstanding, the most accepted classification (with minor changes) among potato researchers is that of Hawkes (1990).

## ADVENT OF MODERN MOLECULAR AND STATISTICAL TOOLS

Spooner *et al.* (2010) and Jacobs *et al.* (2011) pointed out that the taxonomy of section *Petota* is complicated by introgression, interspecific hybridisation, auto- and allopolyploidy, sexual compatibility, a mixture of sexual and asexual reproduction, possible recent species divergence, phenotypic plasticity and, consequently, a great morphological similarity among species. These observations are in agreement with those of Hawkes & Hjerting (1969) and Hawkes (1990), and are scientifically supported by the results of Erazzú *et al.* (1999, 2009), Raimondi *et al.* (2003), Camadro *et al.* (2008), Bedogni & Camadro (2009), Masuelli *et al.* (2009) and Larrosa *et al.* (2012), who applied morphological, genetic, cytogenetic and/or molecular tools in the characterisation of wild potatoes for their eventual use in breeding.

Various molecular studies have been carried out in the past 20 years, trying to establish phylogenetic relationships and/or assign taxonomic status of wild potatoes, in an attempt to solve the species problem in the group. Spooner & Castillo (1997), van den Berg *et al.* (2002) and Álvarez *et al.* (2008), among others, were critical of the classification approaches of Hawkes & Hjerting (1969), Hawkes (1990) and Ochoa (1990), which were based on quantitative and qualitative morphological data. Considering that the number of different species had been overestimated, they gave different treatments to potato that led to successive reductions in the number of species described. For their proposals, and in accessions from germplasm banks, they analysed morphological characters, proteins, chloroplast and nuclear DNA, among other markers, in a large number of accessions with previously assigned specific category. In their analyses with modern taxonomic and statistical techniques, they included numerous species, each of them represented by a few accessions and each accession represented by one or a few plants, making use of various types of molecular marker, with a limited exploration of primers/primer combinations.

Some of the taxonomic treatments of wild potato carried out in the late twentieth and early twenty-first centuries will be examined in more detail because they are representative examples of the current approaches. In these, accessions from germplasm banks with previously assigned specific status for conservation purposes (Taxonomic Species) have been studied with modern molecular tools. Spooner & Castillo (1997) proposed taxonomic changes of sets and supersets in section *Petota* for 76 taxa of wild potato using a single type of marker (cpADN) and by pooling leaf samples of six plants per accession. Similarly, Spooner & Hijmans (2001) made a



literature review on the systematic treatment of wild potato during a period of 11 years (some from their own group) and new collection data.

With morphological and molecular markers, van den Berg *et al.* (2002) studied 78 accessions belonging to six species of series *Longipedicellata* from 36 geographic regions. Each accession was represented by one plant. The results obtained with chloroplast SSRs did not agree with those obtained with nuclear RAPDs and AFLPs. They concluded that the combined morphological and molecular data supported their proposal of reducing the number of wild potato species of this series and recognising only two out of the six species studied: *S. hjertingii* Hawkes, which included *S. matehualae* Hjerting and T.R. Tarn (one accession analysed), and *S. stoloniferum* Schlechtendal, whose accessions appeared associated with different groups that included *S. papita* Rydberg, *S. fendleri* Van Heurck and Muller and *S. polytrichon* Rydberg. The authors did not discuss the association between *S. papita*, *S. guereorensis* Correll (series *Demissa*) and *S. avilesii* Hawkes and Hjerting (series *Tuberosa*), and the dispersion of accessions of *S. fendleri*, *S. stoloniferum* and *S. polytrichon* in different groups. Also using morphological and molecular markers, Spooner *et al.* (2007) studied the diploid species *Solanum tarijense* Hawkes and *S. berthaultii* Hawkes and spontaneous interspecific hybrids from Bolivia and Salta, Argentina, using one plant per accession/hybrid. They proposed the synonymy of these two species, continuing with the tendency of reducing the number of wild potato species initiated in the late twentieth century.

Various researchers recognised the importance of sample size in population studies. Among them, del Rio *et al.* (2001), del Rio & Bamberg (2002), McGregor *et al.* (2002) and Ghislain *et al.* (2006) studied the genetic diversity of wild potato with molecular markers, using either a small number of plants/accession (one or two) or bulked seedlings. They found a lack of association between geographical distance and genetic diversity that was attributed to the small sample size, which made other stochastic events more important in partitioning the genetic diversity.

More recently, Jacobs *et al.* (2008) compared AFLP and cDNA data from *Solanum* section *Petota* (951 accessions representing 196 species, 15 subspecies and 17 hybrids from various germplasm banks, using five plants per accession and five accessions per species). They found inconsistencies between the results obtained with the two markers and suggested that the taxonomic structure of this section was highly unbalanced because a few species groups had high support and their inner structure also supported the subdivisions, but a large proportion of the species could not be structured and appeared to be all equally related to each other and to the supported groups.

Jacobs *et al.* (2011) used the largest collection of *Solanum* section *Petota* accessions ever analysed simultaneously, in which one genotype that they considered representative of each accession was included. Their conclusion was that due to their thorough sampling, it was possible to propose species groups without too many reservations. In their results, a number of species groups coincided with some of the series recognised by Hawkes (1990); however, most of the series recognised by this author and his predecessors could not be supported as natural groups. They considered it likely that a relatively rapid spread of potato species in South America,

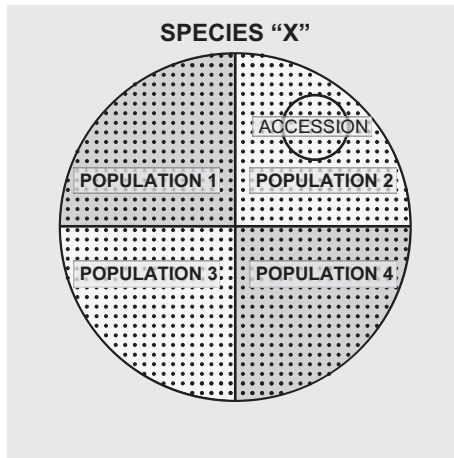
combined with high hybridisation levels, could explain the difficulties encountered in establishing phylogenetic links between species. In their final considerations, they even doubted that it would be possible to obtain more resolution with other methods or more markers, and that it was likely that the polytomy was indicative of the real situation in section *Petota*. They then proposed studying the structure of closely related sympatric species for identifying hybrid zones and obtaining information on how the species boundaries are maintained or eroded.

## MORPHOLOGICAL AND GENETIC VARIATION IN SPONTANEOUS POPULATIONS

Organisms that constitute a species are not immutable; on the contrary, spontaneous populations are subject to natural selection. Since the spatial genetic structure results from numerous genetic and environmental factors that can vary in intensity across populations, generations, years and microhabitats (Wallace 2006), the choice of genetic material and methodological approach for plant classification is of utmost importance.

The high morphological variability and extensive overlap of state characters in wild potatoes has been recognised by various authors in the last 15 years (Castillo & Spooner 1997; Spooner & Hijmans 2001; van den Berg *et al.* 2002; Spooner *et al.* 2007; Jacobs *et al.* 2008, 2011; among others). However, the current classification approaches are based on the Taxonomic Species Concept (using holotypes) and do not take into consideration that (i) natural populations of sexually reproducing allogamous plants are expected to exhibit morphological and genetic variation because each plant is a unique genotype; (ii) the two types of variation can occur both within and between populations (*i.e.*, there could be variation within and between accessions) of the same Taxonomic Species (Fig. 2); and (iii) the breeding relationships within and among wild spontaneous populations can provide for hybridisation and introgression. Knowledge of the natural variability and breeding relationships of populations is of fundamental importance at the moment of choosing genetic material, number of accessions per 'species', number of plants per accessions, and methodologies to establish genetic relations and distances. Spooner & Hijmans (2001) stated that the biggest future changes in wild and cultivated potato classification would come from definitions of species and in-group relationships. However, up to the present, potato taxonomists have not attempted to resolve this crucial point.

In the current taxonomic treatments described in previous paragraphs, the natural variability of each 'species' is represented by very small or small numbers of plants (combinations of accessions and plants within accessions). Furthermore, DNA bulking presupposes that all plants in one accession belong to one 'species' (*i.e.*, to a 'pure' entity) with low morphological and/or genetic variation. If an accession actually contains hybrid and/or segregating genotypes (which is highly likely in obligate sexually reproducing allogamous plants, as are the diploid wild potato), these individual genotypes will be masked, underestimating the within-accession variation and, thus, the genetic distances among accessions. Also, the occurrence of non-homologous co-migrating DNA fragments (bands) that could interfere with interpretation of



**Fig. 2.** Natural genetic variation of an allogamous Taxonomic Species ('X') with sexual reproduction. Each dot represents an individual plant (unique genotype). Individual plants belong to four spontaneous populations, one of which is sampled for conservation purposes (accession).

the data, or the efficiency in representation and significance of the molecular markers are not taken into consideration. Attempts to circumvent the difficulties encountered in trying to relate the molecular data to relevant morphological and/or ecophysiological traits are also lacking in the literature, although, as pointed out by McRoberts *et al.* (1999), these relations could be of value to differentiate natural populations (molecular *versus* phenotypic variation). Also, a reduced number of primer combinations is frequently used to represent a large portion of the wild potato genome. In this regard, Milbourne *et al.* (1997) estimated that at least 650 polymorphic RAPD bands would be required to establish a relatively accurate phenetic relationship at the intra-specific level. Moreover, in comparing various types of PCR markers, Milbourne *et al.* (1997) determined a Spearman correlation of 0.39 between RAPDs and AFLPs, which they partially attributed to the algorithm used, which can either underestimate or overestimate true similarities. In this way, a dominant marker present in a single, double, triple or quadruple dose in one tetraploid accession and in the same dose in another accession, will result in an apparent similarity of 100%, when the true similarity could actually be as low as 25%.

McRoberts *et al.* (1999) suggested that, by chance, it is possible to select various sets of primers that may give different estimates of the molecular diversity within and between populations. They also stated that, similarly and for a given set of primers, the variation that can be detected will depend on the particular plant samples that are analysed. To make inferences on the diversity of functional genes, it is necessary to assume that the detected variation levels are representative of the entire genome. The obtaining of samples that are representative of a given plant population, as well as unbiased estimators of the molecular variation of that population, is of utmost importance in order to relate the molecular markers to particular traits, particularly when small populations are analysed (McRoberts *et al.* 1999).

More recently, Spooner (2009) tested the DNA barcoding method in 143 accessions of 63 in-group species and 10

accessions of nine out-group species, using the most variable and frequently suggested plant barcoding regions: the internal non-transcribed spacer of nuclear ribosomal DNA (ITS) and the plastid markers, *trnH-psbA* intergenic spacer and *matK*. These DNA regions failed to provide species-specific markers in sect. *Petota* because the ITS contained too much intraspecific variation and the plastid markers lacked sufficient polymorphisms. He concluded that the method relies on well-defined species in order to function, is based solely on a limited number of DNA sequences that are often inappropriate at the species level, has been poorly tested with geographically well-dispersed replicate samples from difficult taxonomic groups, and discounts substantial practical and theoretical problems in defining species.

### THE TAXONOMIC PROBLEM IN WILD POTATO

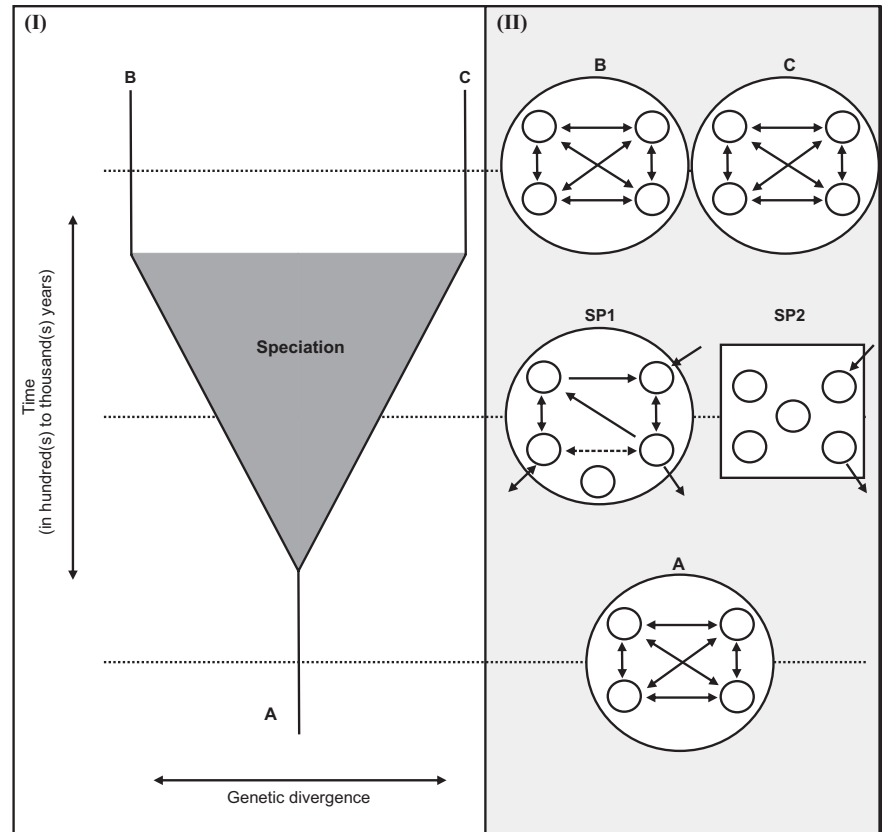
The concept of species and the classification criteria are major issues underlying the taxonomic problem (Mayr 1963). Species are regarded as units that reached a final separation from other entities in their evolutionary divergence; during the process and at each evolutionary stage, there is a variation in the genotypic characteristics of populations (Grant 1981; Fig. 3I).

In many genera of higher plants, allopatric populations living in similar ecological habitats in their respective distribution areas can morphologically differentiate to such an extent that taxonomists usually assign them to different taxa. Some of these populations, however, have proved to be interfertile when experimentally crossed, *e.g.*, various genera of orchids in which the major hybridisation barriers are ethological and mechanical (Stebbins & Ferlan 1956; Lenz & Wimber 1959). These separate population systems are more than races but less than biological species, and are thus considered to be at the semi-species level of divergence.

Natural evolution is a continuous process. However, taxonomic studies in wild potato have been carried out using the Taxonomic Species Concept, mostly with herbarium specimens and/or population samples (accessions) from germplasm banks. But when the number of plants (genotypes) growing at a given site at the collection time is small or, even when the actual number is high but only a few plants can be sampled because they are the only ones at the seedling stage or bearing fruits and/or tubers, the genetic variability of these accessions (and the *ex-situ* multiplications) may not be representative of the natural genetic variation of a given population. In this regard, it is important to note that – with few exceptions – wild potato populations growing at specific sites have been sampled only once. Hence, gene frequencies of spontaneous populations could be strongly changed in the samples for *ex-situ* conservation and more so by genetic drift or through unwanted seed or pollen contamination in the *ex-situ* multiplication process. The current taxonomic approach – in which most accessions are assigned specific status for conservation purposes – presupposes that potato populations are at the end of the speciation process and not at various stages of divergence.

Den Nijs & Peloquin (1977) proposed that  $2n$  gametes and haploidisation in potato allow for relationships of plants among all ploidy levels. Their scheme applies to the South American taxonomic series, in which polyploidisation was an

**Fig. 3.** Population divergence over time and breeding relationships in populations sampled at specific moments. Note: (I) Primary divergence process (modified from Grant 1981): A = ancestral species, B and C = derived biological species (BS). (II) Populations (large circles and square) sampled at three different moment of the divergence process, with expected compatibility relationships among individual plants (genotypes) (small circles). ➔ = direction of successful crosses (if dotted = partially compatible crosses). A, B and C populations: individual plants are compatible only with plants of the same BS. SP1 = both sexually and asexually reproducing plants that can exhibit either full or partial compatibility/incompatibility in one or both directions of the cross, depending on the particular genotypic combination, both within and between (arrows entering and/or leaving the circle) populations. SP2 = asexually reproducing plants (clones) that can be either highly sterile or, if fertile, unable to cross due to identity at the S-locus, but able to sexually cross with other populations.



important evolutionary force. But homoploid hybridisation has also played a relevant role in the evolution of the group, as illustrated and discussed in Masuelli *et al.* (2009).

Raimondi *et al.* (2003), Bedogni & Camadro (2009), Erazzú *et al.* (1999, 2009) and Larrosa *et al.* (2012) used morphological, histological, genetic, cytogenetic and molecular tools in attempts to characterise the natural diversity of the wild Argentinean potato germplasm for both basic and applied ends, working with accessions of germplasm banks and/or their own population samples. They detected accessions with specific status having unexpected reproductive behaviour, as will be explained below, with a few examples. From now on, quotation marks will be used to refer to the specific category of accessions from germplasm banks.

For examining the EBN theory (Johnston *et al.* 1980), Masuelli & Camadro (1997) performed histological studies of embryo and endosperm development in 'intra'- and 'inter-specific' crosses, both intra- and inter-EBN, involving two diploid wild 'species' and two cytotypes (diploid and tetraploid) of a third 'species'. Working with nine genotypes of '*S. gourlayi* Hawkes' in 13 'intra-specific', intra-EBN genotypic combinations (five of them within one accession and the remaining between plants of this and a second accession), only one fruit was obtained from each type of combination. Since the most frequent post-zygotic barrier in potato occurs at the endosperm level in inter-EBN genotypic combinations (Johnston *et al.* 1980), the observation that embryos and endosperms in these intra-EBN combinations had retarded growth and early abortion was striking. The unexpected behaviour was attributed to a likely hybrid origin of the accessions.

Erazzú *et al.* (1999) worked with nine accessions and more than 15 plants/accession of diploid '*S. spagazzinii* Bitter', which had been previously assigned to three distinct morphological groups by another author (Ispizúa 1994). They observed strong pollen–pistil incompatibility reactions at various sites of the pistil upon controlled crosses of individual plants within two of the morphological groups and among three of them. They speculated that both the variability for morphological phenotypes and site of expression of the incompatibility reaction (stigma, first, second and last third of the style) could be the result of spontaneous interspecific crosses in nature. Based on the records of Hawkes & Hjerting (1969) and field notes kept at the Potato Germplasm Bank of INTA, Balcarce, Argentina, they further speculated that hybridisation in some accessions could have involved the sympatric diploid '*S. chacoense* Bitter', which has the widest distribution area in the country, overlapping with many 'species' in different, and sometimes contrasting, environments (Hawkes & Hjerting 1969).

Raimondi *et al.* (2003) studied the likely hybrid origin of the diploid wild species '*S. x ruiz-lealli* Brucher' (rzl) from Mendoza Province using, among other accessions, five of this species and 10 of one of its putative ancestors, diploid '*S. kurtzianum* Bitter and Wittman' (ktz) from its distribution area, which also included three other western provinces. Working with 15–20 plants/accessions, and with over 341 genotypic combinations analysed, they detected (i) pollen–pistil incompatibility (mostly in the stigma, which eliminates the possibility of being an S-locus self-incompatibility reaction) of similar magnitudes in both the 'intraspecific' genotypic combinations (56% of ktz–ktz and 84% of rzl–rzl)



and 'interspecific' genotypic combinations (83.6% of  $ktz \times rzl$  and 68.2% of  $rzl \times ktz$ ), with the percentage of incompatible combinations within and between accessions varying from 0% to 100% in both types of cross; and (ii) high morphological variability among the accessions of '*S. kurtzianum*' that were collected along an approximately 750-km transect. Bedogni & Camadro (2009) further investigated the basis of the morphological variability observed in those accessions of '*S. kurtzianum*', using morphological, genetic, biochemical and molecular approaches. They worked with 20–25 plants of each of seven accessions of '*S. kurtzianum*' and, respectively, two, three and five accessions of three sympatric 'species' along that transect, the diploids '*S. chacoense*', '*S. spagazzinii*' and '*S. maglia* Schlechtendal'. In a multivariate analysis integrating all studied variables, a weak separation of the four 'species' was observed. The phenograms and the group analyses for both quantitative and qualitative variables did not separate the accessions according to their specific ranks; instead they were separated by geographic area. These authors concluded that the wide distribution of '*S. kurtzianum*' and its overlap in some areas with the other three 'species' could account for the morphological variability observed, as a result of hybridisation and introgression. Based on morphological observations, Correll (1962) previously noted that *S. kurtzianum* and three other Argentinean species 'form an extremely close alliance' and that these four and another three Argentinean species 'appear to grade into one another'. He also wrote: 'In this complex, recognition of entities entails an arbitrary limitation for each in a sliding scale of taxonomic values'.

Camadro *et al.* (2008), who investigated the crossability of diploid '*S. okadae* Hawkes and Hjerting' with common potato, reported that the studied accessions or progenies from inter-accession crosses (six from Argentina and four from Bolivia, with 6–20 plants each) could be clearly distinguished through their plant morphological phenotypes, which closely resembled, respectively, the holotype specimen of *S. venatoris* Ochoa (Ochoa 1990) and the holotype specimen of '*S. okadae*' (Hawkes & Hjerting 1989). Previously, Clausen & Ispizúa (2005) had reported 'notable' morphological differences between accessions from both countries using multivariate analyses, grouping techniques and principal components analyses, but they did not speculate on the possible causes. Since the compatibility relations of the Argentinean and Bolivian accessions with the common potato also differed according to country of origin, Camadro *et al.* (2008) highlighted the need to reconsider the current classifications of these accessions, which had been incorporated in germplasm banks as a single taxon for conservation purposes. Furthermore, the production of sexual seeds within '*S. okadae*' accessions is very difficult (Clausen, personal communication.; Camadro, unpublished results) becoming this a clear example of two morphologically very different groups of allopatric accessions that had been, nevertheless, classified as a single taxon, and that also present reproductive constraints both within and between accessions.

Erazzú *et al.* (2009) described the persistence in nature of wild populations of potato 'species' recorded by other researchers more than 20 years before in northwest Argentinean provinces. They recorded plants of diploid '*S. spagazzinii*' and of both diploid and tetraploid cytotypes of

'*S. gourlayi*' growing at the original collection sites or in close proximity. They reported an extensive overlap of 'species' in their collection expedition. For example, '*S. spagazzinii*' was sympatric with the diploids '*S. vernei* Bitter and Wittman', '*S. chacoense*' and '*S. okadae*'; diploid '*S. gourlayi*' was found growing with the tetraploids '*S. acaule*' and '*S. tuberosum* ssp. *andigenum* (Juzepzuck and Bukasov) Hawkes' (a cultivated species); and the tetraploid cytotype of '*S. gourlayi*' was growing proximity to diploid '*S. infundibuliforme* Philip' and tetraploid '*S. tuberosum* ssp. *andigenum*'. Through controlled reciprocal crosses of plants (genotypes) of '*S. spagazzinii*' and the diploid cytotype of *S. gourlayi*, it was demonstrated that hybridisation between them was feasible, since 34% and 16% of the genotype combinations yielded hybrid progeny. In principal components and grouping analyses of AFLP markers, Erazzú *et al.* (2009) detected a lack of consistency in the relations among the studied accessions: plants from distant geographic sites and belonging to collections made in different years were associated without well-defined patterns due to the high variability present within accessions. This became more evident when they performed an AMOVA, because the higher percentage of the molecular variation corresponded to the 'within accession' source of variation. One of these populations contained diploid and tetraploid plants of '*S. gourlayi*' (Erazzú & Camadro 2008), leading to speculation on the occurrence of hybridisation, gene flow and introgression within and between ploidy levels in spontaneous populations.

More recently, Larrosa *et al.* (2012) carried out a comparative study of pollen viability in artificial hybrids between wild diploid 'species' and the common potato ('*S. gourlayi*'–'*S. tuberosum* Linnaeus ssp. *tuberosum*'), and accessions and samples of spontaneous wild populations from northwest Argentina that – for conservation purposes – had been classified as 'species' ('*S. chacoense*') or 'presumed hybrids' ('*S. gourlayi*'–'*S. infundibuliforme*'). Regardless of their classification ('species', 'presumed hybrids', 'artificial hybrids'), many plants had 2n and 4n pollen, in addition to normal pollen, and a high percentage of the pollen grains were non-viable. In a sample of male-sterile plants in which meiosis was studied, frequent and diverse anomalies were observed at all stages. Similar observations were reported for accessions of '*S. okadae*' (Bottini *et al.* 2008; Camadro *et al.* 2008), previously referred to with regard to morphological phenotypes of accessions of Bolivian and Argentinean origin.

In studying the T-type chloroplast DNA, characteristic of the worldwide-cultivated tetraploid potato *S. tuberosum* L. ssp. *tuberosum*, Hosaka (2004) found the same chloroplast DNA in some populations of the wild diploid species *S. tarijense* Hawkes, which is found from central Bolivia to northwest Argentina. To elucidate an evolutionary pathway for this type of chloroplast DNA from the wild species to the cultivated Chilean tetraploid potato, 200 accessions of each of the cultivated Andean diploid *S. stenotomum* Juz. et Buk. and the tetraploid *S. tuberosum* L. ssp. *andigena* Hawkes were examined. T-type chloroplast DNA was not present in accessions of *S. stenotomum* but was present in nine accessions of *S. tuberosum* ssp. *andigena*, mostly from northwest Argentina. He concluded that some populations of *S. tarijense* with T-type chloroplast DNA had spontaneously hybridised, as females,



with *S. tuberosum* ssp. *andigena*, from which the Chilean potato was selected. This is another example of spontaneous hybridisation in nature, in this case between wild and cultivated potatoes.

The preceding results are in agreement with those expected in populations undergoing a genetic divergence process (see Fig. 3) and provide sound scientific support to the observations of early potato taxonomists on the wide occurrence of spontaneous hybrids in nature. In fact, Hawkes (1990) described 24 natural hybrids (and their putative parents) in seven taxonomic series, 14 of them in series *Tuberosa* (to which the cultivated potato belongs). Previously, Correll (1962), in relation to members of that section, stated that it would probably be in the best interest of science if all of these were placed together in one highly variable species. This observation is similar to that of Ugent (1966), who pointed out that wild potato constitutes an extensive assemblage of interrelated plants.

### EVOLUTIONARY DIVERGENCE: A PROCESS SUBJECT TO REVERSION

Regarding the evolutionary process that can lead to speciation, Grant (1963) stated that plant populations are neither good races nor good species, but are interrelated through a reduced amount of random crosses and gene flow, creating complex relationship networks. Population systems at such stages of divergence are considered to be semi-species, but the process of evolutionary divergence is not inexorable (resulting in the formation of new biological species); on the contrary, within wide limits, it can be subject to reversion.

In populations at various stages of evolutionary divergence that are able to hybridise – as frequently observed in potato – the Biological Species is not the most inclusive reproductive group. In analogy to the discussion in Grant (1963) for species and semi-species when there is genetic interchange between populations, the most inclusive group is a number of genotypes ('semi-species', in Grant's concept) that are differentiated to a certain extent but that have incomplete internal hybridisation barriers ('syngameon'). Grant (1963) also stated that syngameons are much more common than well-defined biological species, for example, in *Pinus*, *Quercus*, *Aquilegia*, *Iris*, *Ceanothus* and *Diplacus*, among other genera of higher plants. If hybridisation occurs on a large scale, the speciation process could be reversed.

The classification approaches in wild potato had not taken into consideration either the genetic structure of the populations or the breeding relationships within them and between them and their neighbouring populations, with the feasibility of gene flow and introgression (Fig. 3b). There are no reports in the potato literature on that type of approach to the species problem, as has been done, for example, for North American species of *Iris*, which share many similarities with wild potato in their reproductive systems (Grant 1971). In fact, Hodges *et al.* (1996) demonstrated that the rate of advanced generation hybrid formation in species of *Iris* can be higher than the rate of initial F<sub>1</sub> hybrid formation, and speculated that although F<sub>1</sub> hybrids could have initial difficulties in their establishment, they can act as bridges for the rapid establishment of hybrid zones. Moreover, Arnold *et al.* (1999) and Taylor *et al.* (2009) provided evidence that the

extremely low fertility or viability of early-generation hybrids (F<sub>1</sub>, F<sub>2</sub>, BCs) in angiosperms does not necessarily prevent extensive gene flow and the establishment of new evolutionary lineages in nature, because they can have equivalent or higher fitness than their parents in certain habitats. Similarly, wild potatoes are known to thrive in disturbed habitats, e.g., as weeds in cultivated fields, as reported in Correll (1962), Hawkes & Hjerting (1969) and Erazzú *et al.* (2009), and apparently revealed in Hosaka's (2004) chloroplast DNA studies. Various authors (e.g., Anderson & Hubricht 1938; Anderson 1949; Arnold 1997) have pointed out that spontaneous hybridisation in higher plants is a common phenomenon, frequently overlooked until close and detailed examinations are carried out at various levels. In fact, hybridisation can have subtle but long-lasting consequences in plant populations and can even facilitate major ecological shifts by generating better-adapted forms (Rieseberg *et al.* 2003; Martin *et al.* 2006). As Knapp (2008) stated, hybridisation might be better viewed as a fact of life, not necessarily a problem.

### CLASSIFICATION APPROACHES IN HIGHER PLANTS

Systematic botanists, in general, have been reluctant to utilise the Biological Species or similar concepts (for discussions, see Ehrlich 1967; Raven 1976; Mayr 1982; Masuelli *et al.* 2009; among others). In fact, the classification systems proposed by Linnaeus and de Jussieu have been in current use since the eighteenth century with only minor modifications, although morphological observations have now been complemented with molecular and biochemical data in studies published from the late twentieth century up to the present.

As Grant (1981) has clearly stated, the current classification approaches do not take into consideration that (i) the hierarchy of the taxonomic units is pre-evolutionary, (ii) the 'Linnean' species was formed based on the creationist model (according to which species are creations of a divinity and, as such, immutable), and (iii) there have been no fundamental changes in the taxonomic hierarchy since the eighteenth century. Augmenting these constraints, two concepts of species are mainly used in plants: (i) the Biological Species Concept, developed for bi-parental organisms and based on reproductive relations and morphological discontinuities; and (ii) the Taxonomic Species Concept, based on morphological similarities and differences of holotypes. Biological Species and Taxonomic Species do not always coincide, perpetuating the confusion when the terms are used indistinctly. One example is the 'sibling species' in *Gillia*, which, being morphologically indistinguishable, are unable to cross and produce fertile progeny (Grant 1971). Thus, two Biological Species are reduced to one Taxonomic Species, a fact that has consequences for basic and applied studies. Using a similar line of thought, Hendry *et al.* (2000) – among other modern authors – recognised the existence of more-or-less distinct clusters of organisms at varying biological scales, and stated that it cannot be assumed that there is a fundamental and universal level of clustering that has greater evolutionary significance than all other levels.

The criteria of morphological similarities and differences applied in the classification of Taxonomic Species have the

disadvantage that the degree of difference that deserves such a category cannot be objectively determined. T. Reagan (in Grant 1971) considered that one species is actually one or various related communities whose distinctive morphological characters are, in the opinion of a competent plant taxonomist, sufficiently clear to assign a specific name. The practicality in the identification and classification has been one of the most important points in favour of the Taxonomic Species concept, which Mayr (1957) judged reasonably satisfactory for inventory-taking of various organisms. However, to make effective and efficient use of the related germplasm in potato breeding, it is necessary to understand breeding relations and genetic distances to maximise heterosis.

In applying statistical tools to their analyses, taxonomists have shown a tendency to over-differentiate species. Knapp (2008) pointed out that, in some studies, more than one species has been differentiated per morphological phenetic cluster, when species differentiation actually depends upon where the clustering line is drawn. Likewise, some morphological studies have uncovered paraphyletic or polyphyletic groupings, precipitating taxonomic rearrangements (see Hendry *et al.* 2000). This arbitrariness in assigning specific categories was one of Mayr's (1992) more powerful arguments against a purely mechanistic phenetic definition of species.

The corollary is that the Taxonomic Species is an artificial category, which in plants, more frequently than not, does not coincide with the natural unit, particularly when behavioural and ecological properties are considered. But most pernicious is the fact that the morphological characters are unreliable for the recognition of Biological Species (Mayr 1957; Hawkes 1963). As Beaudry (1960) so precisely stated, nature has evolved with total disregard to the practical problems that scholars have to deal with.

## FINAL CONSIDERATIONS

A consensus in the classification of potato species or interpretation of the biological complexities of the group has not yet

been reached. To adequately interpret and classify potato populations, it is important to consider the particularities of the group: sexual reproduction that generates new gene combinations, asexual reproduction that provides for the maintenance of adapted genotypes over time, overlap of sexually and asexually reproduced generations that are able to hybridise, genetically controlled meiotic mutants that result in 2n and 4n gametes (pollen and eggs) which are functional in sexual polyploidisation and n eggs that can develop parthenogenetically into haploid plants (as speculated), external hybridisation barriers (spatial, temporal, ecological, among others) and internal (pre- and post-zygotic, such as self- and cross-incompatibility, Endosperm Balance Number, nuclear-cytoplasmic male sterility) that can be incomplete, providing opportunities for gene flow and introgression between and within ploidy levels, within and between sympatric populations. Thus, a better understanding of the reproductive biology of wild potatoes and its consequences in the functioning of natural populations will provide for a better understanding of the morphological and molecular variability encountered in the group.

A crucial point for taxonomic studies in potato (and in other plant species as well) is to use a systemic approach, by considering genetic, reproductive and environmental aspects, among others. The appropriate choice of the genetic materials to be studied and the methodological approaches to be followed (sampling strategies, type and number of molecular markers, statistical methods, among others) are of utmost importance. A set of genetic markers at various exploratory levels, plus sampling strategies that allow manifestation of the dynamics of wild populations will provide more suitable tools than those currently in use to analyze and provide some answers on the taxonomic problem in wild potatoes. As Padial & de la Riva (2010) stated, '...taxonomists should be able to incorporate theory, methods and data from all other disciplines studying the origin and evolution of species, should abandon the plea for the superiority of some lines of evidence and should stop demanding full congruence between lines of evidence.'

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