

POLYPLOID EVOLUTION VIA 2N GAMETES<sup>1</sup>Ton P.M. Den Nijs and S.J. Peloquin<sup>2</sup>**Abstract**

Polyploid evolution is evident in several important taxonomic series of the tuber-bearing Solanums. Polyploids can result from the functioning of 2n gametes following both intra- and inter-ploidy matings. The bilateral and unilateral sexual polyploidizations (BSP and USP, respectively) are greatly facilitated by the existence of genetically determined 2n gametes. Many species, in series containing polyploids, have individuals with 2n gametes. The genetic basis of 2n pollen formation has been established in Phureja-haploid Tuberosum hybrids, and the genetic basis of 2n egg formation is being investigated in these hybrids and *S. chacoense*. Preliminary results indicate that relatively few genes may be involved. FDR and to a lesser extent SDR 2n gametes avoid the inbreeding in the polyploid that is maximal with somatic doubling. The vigor, fertility and competitive ability of a founder polyploid are dependent on minimizing inbreeding depression. Even more important, polyploids, particularly from FDR 2n gametes, inherit the beneficial epistatic interactions of the parent(s). Genetically determined 2n gametes make systematic sexual polyploidization a recurring phenomenon in two ways. For example, initial BSP of two diploids gives rise to a tetraploid, and thereafter USP ensures continuing contact between ploidy levels resulting in unidirectional introgression. Triploids can also be involved in polyploid evolution if they produce 2n gametes by FDR. Artificial hybridizations indicate that 2n gametes, by overcoming crossing barriers between various ploidy levels, provide the opportunity for continuous gene flow.

**Resumen**

La evolución poliploide es evidente en diversas series taxonómicas importantes de los Solanum tuberosos. Los poliploides pueden resultar del funcionamiento de gametos 2n como consecuencia de apareamientos intra e inter niveles de ploidía. La existencia de gametos 2n genéticamente determinados facilita grandemente la poliploidización sexual unilateral y bilateral (U.S.P. y B. S. P.), respectivamente. Muchas especies, en series que contienen poliploides tienen individuos con gametos 2n. La base genética de formación de polen 2n ha sido determinada en

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híbridos Phureja-tuberosum haploide y la base genética de la formación de huevos  $2n$  está siendo investigada en dichos híbridos y en *S. chacoense*. Los resultados preliminares indican que relativamente pocos genes pueden estar involucrados. Los gametos  $2n$  producidos por FDR y en menor extensión SDR evitan la endocría en el poliploide mientras que es máxima con un doblamiento somático. El vigor, la fertilidad y habilidad competitiva del poliploide original dependen de la minimización de la endocría. Aún más importante, los poliploides particularmente aquellos provenientes de gametos  $2n$  FDR, heredan las interacciones epistáticas beneficiosas del progenitor o progenitores. Los gametos  $2n$  genéticamente determinados hacen de la poliploidización sexual sistemática, un fenómeno recurrente en dos maneras. Por ejemplo, una poliploidización sexual bilateral inicial de dos diploides dá lugar a un tetraploide, y en adelante la poliploidización sexual unilateral asegura el contacto continuo entre niveles de ploidía dando como resultado una introgresión unidireccional. Los triploides pueden también estar involucrados en la evolución de los poliploides si es que producen gametos  $2n$  via FDR. Las hibridaciones artificiales indican que los gametos  $2n$  al superar barreras de cruzamiento entre varios niveles de ploidía, proporcionan oportunidad para un contínuo flujo de genes.

### Introduction

The wild relatives of the commercial potato form a polyploid series with diploids, triploids, tetraploids, pentaploids and hexaploids. Although the majority of the recognized species are diploids, several of the taxonomic series of the subsection *Hyperbasarthrum* (which includes all tuber bearing *Solanums*) exhibit a high degree of polyploidization.

Several years ago the importance of genetically determined  $2n$  gametes was recognized for use in experimental potato breeding. The genetic basis of  $2n$  pollen (diplandroid) production has been established in Phureja-haploid Tuberosum hybrids, where two distinct cytological irregularities affecting meiosis in microsporocytes were identified; each controlled by recessive alleles at different loci (8). Also, there are indications for a rather simple inheritance of  $2n$  egg (diplogynoid) production in the same hybrid families, but here more work is needed to establish firm conclusions. The prospects of experimental sexual polyploidizations of promising diploids into vigorous and high yielding tetraploids are excellent, as results and analyses point out (6, 9).

The powerful capacity, especially of First Division Restitution (FDR)  $2n$  pollen produced by clones homozygous for the gene parallel spindles, to transmit a large part of the heterozygosity and epistasis intact to the accordingly vigorous polyploid offspring has spurred the concept that these  $2n$

gametes have played an important part in the evolution of the polyploid complex of the wild and cultivated potatoes. Since Bukasov's (1) original suggestion in this respect in 1939, the only investigation of the function of  $2n$  gametes in speciation of the *Solanums* has been attempted by Marks (4). He illustrated beautifully in *S. chacoense* that such gametes can be responsible for sexual polyploidizations. Throughout the literature, however, occasional mention is made of  $2n$  gametes. Further, recent screenings of introductions of a large number of species for both  $2n$  pollen and  $2n$  eggs have revealed that  $2n$  gametes do indeed occur widely throughout the subsection (12, and unpublished data of the authors).

One diploid species, *S. chacoense*, stood out in these screenings because of  $2n$  gamete production in several clones, as well as vigor, flowering and tuberization in the field and greenhouse. It is an abundant wild and weedy species in Argentina, with excellent vigor and wide adaptation. It is also fertile over a wide range of environments and is easily crossed with the cultivated potatoes. Equally important, *S. chacoense* is considered to represent a divergent gene pool from those of the cultivated diploid and tetraploid potatoes. This makes it a favorable source for introducing into the *Tuberosum* tetraploids additional diversity and with it heterotic yield potential. Accordingly, *S. chacoense* was chosen for a study of the inheritance of  $2n$  egg production, while at the same time a base population of clones with high diplogynoid production could be accumulated.

This report is concerned with the detection, frequency, distribution, and inheritance of  $2n$  eggs in *S. chacoense*. These findings are discussed in relation to their role in polyploidy. It is hoped that a better understanding of the polyploidization process and the function of  $2n$  gametes therein will open up more possibilities for making use of the rich variability present in the wild potatoes for the breeding of future cultivars.

### Materials and Methods

Clones of *S. chacoense* which differed in  $2n$  egg production were identified and selected from a large number of plant introductions obtained from the Interregional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin. Six families were chosen from intercrosses between these clones and grown out in the field at Hancock Experimental Station in 1974. Fruit and seed set were, however, depressingly low, so tubers of up to 35 clones per family were harvested and planted in the greenhouse (16 hr days) after tuber dormancy had been broken with Rindite. Three tubers from each hill were planted to ensure that enough inflorescences for pollination would be available and certain promising clones would be present. Because stolons in *S. chacoense* can be up to 2 meters long, tubers found directly under a certain hill do not necessarily belong to that individual, at the spacing used (40 cm.).

The six families with their parentage are listed in Table 1, together with the number of progeny per family and the total pollinations for each family. On the average each clone was pollinated from 21 (FAM 2) to 35 (FAM 5) times. This seems superfluous, but environmental factors cause a considerable flower and berry drop, and some pollination dates gave only a few berries while others were quite fruitful.

TABLE 1. — *Families of S. chacoense for characterization of 2n egg production.*

Family	Type of Cross	Parentage Code* (S/F)	Progeny	Pollinations	Fruits/ Pollination	% Parthenocarpic Fruits
1	H x H	14-7 (17) x 14-1 (9)	35	928	.33	42
2	H x L	14-1 (9) x 27-13 (1)	23	466	.38	55
3	L x H	21-19 (1) x 14-7 (17)	28	602	.34	46
4	H x O	14-1 (9) x 5-14 (0)	19	429	.21	56
5	O x H	5-17 (0) x 14-1 (9)	21	735	.23	58
6	L x L	27-15 (2) x 21-19 (1)	21	595	.07	76

\*Parentage Codes: 5:P.I. 320293; 14:P.I. 275138; 21:P.I. 189217; 27:(230582 x 265576) 14-1, 7 are selections from that P.I.

Three arbitrary classes of 2n egg production were defined: High 2n egg production (H,  $\geq 5$  2n eggs detected per fruit); low (L, .5-5); and none (0,  $< .5$ ). The seven parents of the six selected families range from 0 to 17 2n eggs per fruit. Some parents are in more than one cross and others are from the same P.I..

The number of 2n eggs produced is indicated by the seed set per fruit following pollination with n pollen of a tetraploid male parent ( $2x \times 4x$  cross). This interploidy cross yields virtually only tetraploid offspring due to a particularly effective "triploid block" which eliminates almost every triploid embryo, presumably due to endosperm imbalances (13).

A number of the progenies were grown to determine ploidy levels of clones: some were checked by root tip chromosome counts; others by numbers of chloroplasts in the guard cells of stomata. Most were judged by examining the pollen and general morphology. In a few suspect clones microsporocytes were used for meiotic determination of ploidy level.

Crosses were carried out on 15 dates during February and March, with emasculation immediately or about a day prior to pollination; n pollen used in the  $2x \times 4x$  crosses was obtained from two advanced selections of the U.W. Potato Breeding Program (W 231 and W 639) and several cultivars. Tetraploid *S. chacoense* families generated by repeated sibmating of several colchicine-induced  $4x$  clones maintained by IR-1 were the other important source of n pollen.

The seed set of a particular clone in crosses with different pollinators is remarkably consistent and encourages the belief that the  $2x \times 4x$  cross indeed detects (all) the  $2n$  eggs produced. Table 2 provides the total number of fruits and seeds in each of three representative clones, after pollination with three tetraploids on eight dates. While the differences between the clones are substantial, within-clone variation is slight. A similar constancy in seed set was observed in  $2x \times 4x$  crosses in different years in Phureja-haploid *Tuberosum* hybrids (7).

TABLE 2. — *Seeds per individual fruit following  $2x \times 4x$  crosses of three *S. chacoense* clones*

Cross $2x \times 4x$	Date Pollination	Seeds/Fruit
3-13 x CH4	2/20	18;10
3-13 x W639	3/1	7
3-13 x W231	2/10	18;10;17
3-13 x CH4	2/23	15
1-19 x W231	2/10	4;2;6;2
1-19 x W639	2/26	2
1-19 x W639	2/26	6;6;3
1-19 x CH4	3/4	2;4;0
3-2 x W231	2/5	2;1;1;0;0
3-2 x CH4	2/6	1;0
3-2 x CH4	2/16	1;0;0
3-2 x W231	2/3	1;1;0

## Results and Discussion

### *Diplogynoids in *S. chacoense**

Some of the general data of interest for the six families are included in Table 1. The fruits per pollination measures the efficiency of pollination, which ranges from 7% in FAM 6 to 38% in FAM 2. It should be noted that the efficiency is similar in the top three families, while FAM 4 and FAM 5 are much lower. Extremely inefficient were the pollinations in FAM 6.

The column on the right in Table 1 lists the percentage of parthenocarpic fruits obtained in the  $2x \times 4x$  crosses. These on the plant pollination figures are disappointingly high, effectively reducing the pollination efficiency by about one half. The distribution of the parthenocarpic tendency within families varies. Clones that produce high amounts of  $2n$  eggs are in general not likely to have parthenocarpic fruits. On the other hand, clones that produce low numbers or even no diplogynoids have more and sometimes exclusively parthenocarpic fruits.

Table 3 gives some characteristics of the distribution of the seed set per fruit in the six families. The family means of the first three progenies are distinctly different from the latter three. Moreover, the ranges of the distributions clearly reinforce this distinction. While the H x H, H x L and L x H families do contain a few clones with no seed set, they also include individuals with very high 2n egg production. The H x O, O x H and L x L families have more zero seed set clones, and the highest detected number of 2n eggs is only 4.5 s/f in one clone of FAM 6.

TABLE 3. — *2n* egg frequency in *S. chacoense* families.

Family	Seeds/Fruit Parents	Seeds/Fruit Progeny			
		Mean	Range	% over 5 s/f	% over 10 s/f
1	H (17) x H (9)	4.8	0-21	26	14
2	H (9) x L (1)	3.6	0-14	27	5
3	L (1) x H (17)	6.8	0-20.5	36	28
4	H (9) x O (0)	1.9	0-4	0	0
5	O (0) x H (9)	2.3	0-4	0	0
6	L (2) x L (1)	2.3	0-4.5	0	0

The percentages of the total progeny with more than 5 and more than 10 seeds per fruit are given in the last columns of Table 3. The first three families have a considerable frequency of clones with high diplogynoid production as defined in the classification. Somewhat unexpectedly, the H x H FAM 1 is not the best in this respect. The relatedness of the parents of this family may have played some part in this. Although inbreeding depression was not obvious, the tall growing, early maturing family may have been too limited in genetic makeup.

It is interesting to note that the highest seed set clones in both FAM 1 and 3 are very similar, and higher than the High parent. This transgressive inheritance also appears in FAM 2 and at a much lower level in FAM 6. On the other hand, the combination of a High parent with two zero seed set parents (both from the same P.I.) produces progenies with average as well as maximum seed set well below the mid-parental value. The similar behavior of parents and of the same P.I. suggests identical genotypes for this trait. This does not appear to be true for both High parents. FAM 3 represents the best opportunity for creating vigorous high 2n egg producing clones, with as much as 28% over 10 seeds per fruit.

Although the exact genetic basis of the variation in 2n egg production is not yet clear, the striking differences between the families and the independence of environment of the seed set per fruit in any clone indicate that heredity is the major governing factor of this trait. The distributions of the progeny in the top three families are rather discrete suggesting a more

qualitative than quantitative inheritance. More advanced crosses are being tested to further investigate this hypothesis.

The results so far indicate that the most promising way to build up a population of clones of *S. chacoense* with high diplogynoid production for incorporation into an analytic breeding scheme (2, 6) appears to be the intercrossing of unrelated tuberizing clones selected for high or high and low seed set on the basis of the  $2x \times 4x$  cross screening system. Inclusion of clones which have repeatedly shown a lack of  $2n$  eggs severely delays progress, and should be avoided.

#### *Evolutionary Implications*

As the record begins to show more and more species in subsection *Hyperbasarthrum* containing clones capable of  $2n$  gamete production, we can visualize more clearly how these gametes could have played a role in the building of the polyploid complex of this subsection. Clones with  $2n$  pollen and/or  $2n$  eggs have now been observed in more than 20 species. Significantly, almost all these species belong to series in which polyploidization is evident.

Polyploidization can principally be achieved in two ways: mitotic or meiotic. The first method involves deregulation of mitosis at any stage in the vegetative cycle, resulting in a doubling of the chromosome number, with two identical gene-complements. The meiotic mechanism involves  $2n$  gametes (gametes with somatic chromosome number). These gametes can be genetically FDR or SDR, with important differences in the heterozygosity they retain. Several outstanding features of  $2n$  gametes should be emphasized as they function in sexual polyploidization, as opposed to mitotic doubling.

1. Sexual polyploidization minimizes the amount of inbreeding which is necessarily imparted on any novel polyploid. On the other hand this inbreeding is maximal after somatic doubling. Since inbreeding depression is important in potatoes, the avoidance thereof will increase the vigor of a polyploid founder population which may determine whether or not it can compete with its diploid progenitors.

2. The presence of the genes for  $2n$  gamete production in many different species and populations makes recurring sexual polyploidization possible. This makes the establishment of a polyploid population much more likely. Figure 1 illustrates this in a diploid population where one individual has the genetic capacity to form  $2n$  pollen and another  $2n$  eggs. Chance meeting of these two types of  $2n$  gametes results in a tetraploid. This is an example of bilateral sexual polyploidization, giving rise to a diplandrogynous tetraploid, from a diplandroid and a diplogynoid. In the work described above one such tetraploid was encountered, and they have repeatedly been observed in Phureja-haploid *Tuberosum* intercrosses (5, and unpublished results).

BILATERAL SEXUAL POLYPLOIDIZATION

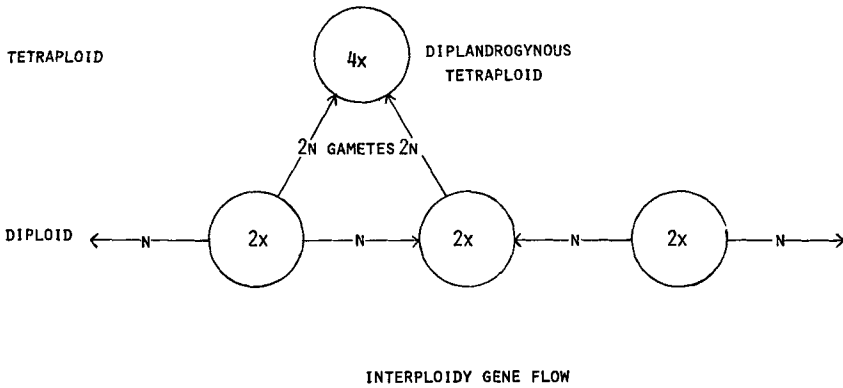


FIG. 1.

3. Interoity hybridization is a typical and unique characteristic of  $2n$  gametes. Diploid progenitors can in this way maintain contact with their tetraploid relatives negating the so called ploidy barrier. Figure 2 illustrates this situation. The gene flow from the diploid individual with  $2n$  eggs to the tetraploid represents a system of unidirectional introgression, broadening the genetic base of the tetraploid population. This is called unilateral sexual polyploidization, which in this instance results in a diplogynous tetraploid.

UNILATERAL SEXUAL POLYPLOIDIZATION

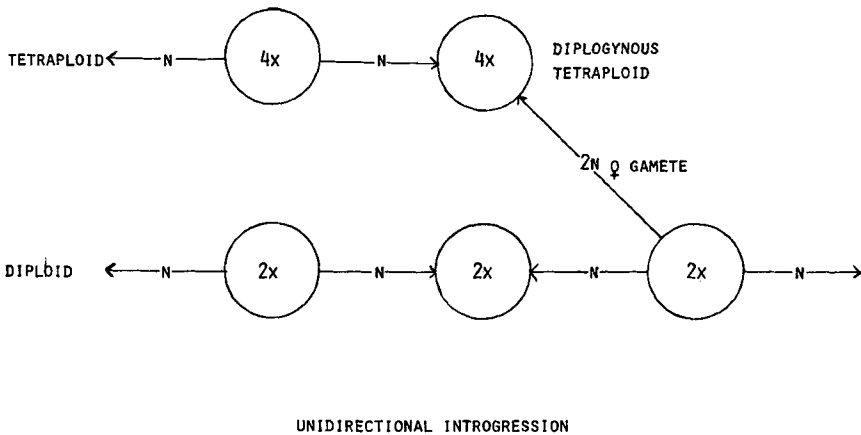


FIG. 2.



Were  $2n$  pollen involved, a diplandrous tetraploid would result. Incidentally, these sexual polyploidizations can occur both within and between species, as they have been encountered in experimental crossings for a long time.

4. Triploids can be functional links in polyploid evolution. They are usually referred to as "evolutionary dead ends" (see e.g. 3). However, triploids with parallel spindles (11) or other FDR gametes are potentially fertile in crosses to hexaploid relatives.

5. Bilaterally polyploidized tetraploids can arise from selfing diploid clones. For this to happen it is necessary to have a clone that produces  $2n$  pollen by FDR and also  $2n$  eggs. Due to competitive interaction of the S-alleles in the heteroallelic  $2n$  pollen grains the incompatibility breaks down, and with triploids rare, tetraploids are obtained almost exclusively (10).

Although understandably no direct evidence as to these mechanisms of polyploidization is to be found in the past evolution of the Solanums, experimental evidence strongly suggests the participation of  $2n$  gametes. The genes determining diplandroid and probably also those governing diplogynoid production appear to be present both in diploids and related polyploids. Thus the participation of both normal and  $2n$  gametes facilitates polyploidization and gene flow throughout the polyploid complex.

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