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Variation Patterns of Parthenogenetic Plants Derived from "Unreduced" Embryo-Sacs of *Solanum tuberosum* Subspecies *andigena* (Juz. et Buk.) Hawkes

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Summary. Parthenogenetic seed induction was performed on one clone of *Solanum tuberosum* subspecies *andigena* (2n=4x=48) using *S. phureja* (2n=2x=24) marker inducer clones. The parthenogenetic population when grown was found to contain both diploid and tetraploid individuals presumably arising from reduced and unreduced gametes, respectively. Variation patterns in the diploid and tetraploid sub-populations, as well as a population obtained by selfing the parental clone, were compared to try and elucidate the origin of the tetraploid parthenotes. From the results of this one generation it appeared that the tetraploid parthenogenetic plants had been produced by a mechanism equivalent to second division restitution (SDR).

Key woods: *Solanum tuberosum* – Second division Restitution – Parthenogenetic seed induction

Introduction

It is well known that potato species produce numerically unreduced or 2n-gametes which become manifest especially in tetraploid (2n=4x=48) × diploid (2n=2x=24) crosses, where most of the offspring are tetraploid (Hanneman & Peloquin 1968; Swaminathn & Howard 1953).

The mechanisms of 2n microspore formation in diploid potatoes have recently been studied by Ramanna (1974) and Mok & Peloquin (1975). Their results indicate that 2n gametes can be highly heterozygous when they are produced by a mechanism equivalent to first division restitution (FDR) and highly homozygous when they are produced by a mechanism equivalent to second division restitution (SDR). No similar cytological work has been reported on the origin of unreduced embryo-sacs in potatoes but genetical studies on the tetraploid segregating F_2 progenies from diploid × tetraploid crosses indicated that unreduced embryo-sacs in diploid potatoes could be produced by a mechanism equivalent to FDR (Ross & Langton 1974). A study of unreduced embryo-sacs in tetraploid potatoes is not feasible using hybrid progeny for reasons such as difficulty of production, detection and genetic analysis at the resulting high ploidy level. In potatoes the orientation of the products of the meiotic divisions in macrospores is different to that found in microspores. Normal female meiosis results in the production of a linear tetrad (Walker 1955). It is therefore mechanically impossible for parallel spindles to occur. The only way FDR could take place is for the restitution of the two central products of meiosis to form a triad, one gamete being unreduced and two being reduced. The frequency of crossing-over in macrospore production is not known but in certain plant species it is greater than in microspore production (Fogwill 1958; Vosa 1972). This frequency may alter the variation patterns of populations consisting of plants produced from either FDR or SDR.

Parthenogenetic seed production in autotetraploid potatoes has become a routine technique since the discovery of three superior diploid pollinators of *Solanum phureja* (Hougas et al. 1964). The production of parthenogenetic seeds is usually induced to gather dihaploids (2n=2x=24) from tetraploids or monoploids (2n=x=12) from diploids. In a parthenogenetic seedling population from a dihaploid × *S. phureja* mating both monoploid and diploid offspring were found indicating that plants were produced from unreduced and reduced embryo-sacs (Hermsen, pers. comm.). There have been no direct reports of parthenogenetic tetraploids arising from tetraploid × diploid crosses. This may be due to the fact that most workers are usually interested in dihaploid production. Hermsen and Verdenius (1973) proposed their formation to explain the production of non-spotted polyploid seeds from tetraploid × diploid *S. phureja* (homozygous for seed spot) crosses.

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Using a tetraploid clone we have the unique opportunity of sampling one zygote population (self) and two gamete populations (n + 2n). By comparing the variation patterns of continuous and discontinuous characters it should be possible to predict the origin of the 2n gametes. If the tetraploid parthenogenetic population is similar to the zygote sample, i.e., the self population, then it is likely that the plants originated from 2n gametes produced by FDR. If the tetraploid parthenogenetic population is similar to the dihaploid sample it is likely that they were produced by SDR.

Materials and Methods

The original parental tetraploid clone was selected from a seedling population of *S. tuberosum* subspecies *andigena* C.P.C. 2154, a Columbian cultivar. The diploid *S. phureja* marker inducer clones were all received from the Institute of Plant Breeding, Wageningen, Netherlands. They consisted of various crosses of homozygous embryo spot lines. All material was grown in 5 inch clay pots in an insect-free glasshouse. The three populations were grown in a randomised block arrangement.

A wide range or characters were studied (refer to Tables 1, 2, 3, & 4). Mitotic chromosome counts were undertaken on all parthenogenetic plants and a random sample from the self population. Root tips were pre-treated with 0.002M 8-hydroxyquinoline for 5 hours and then fixed in 3 : 1 ethanol: acetic acid before being stained in lacto-propionic orcein.

Plastids were stained in leaf material with 1% iodine in potassium iodide after removal of the chlorophyll with absolute ethanol.

Pollen stainability was carried out according to the method of Marks (1954).

Flower and tuber pigments were identified from their anthocyanidin derivatives using paper chromatography with forestal as the solvent. The absorption maxima in visible light were also recorded with the use of a scaning spectrophotometer. Reference compounds were extracted from various plant materials according to Harborne (1967). Expected rf values and colours are given by Harborne (1958)

Results

The cross 2154 × 2x *S. phureja* produced a total of 52 berries containing 285 seeds of which 205 were of parthenogenetic origin and 80 were of hybrid origin. There was a frequency of 3.9 parthenogenetic seeds per berry. From the 205 parthenogenetic seeds sown, 169 germinated but not all survived to be scored later in the experiment.

Continuous Characters

Initially all parthenogenetic plants were screened by counting the number of chloroplasts per pair of guard cells as this gives a good indication of the ploidy level of the plant (Frandsen 1968). The results of the number of chloroplasts per stoma in both the self and parthenogenetic populations are presented in Fig. 1. The histograms show two peaks for the parthenogenetic population and only one for the self population. When root tip chromosome counts were made on the parthenogenetic plants it was found that the diploids ranged from 10.2 to 18.1 and the tetraploids from 16.6 to 28.6 chloroplasts/stoma. This gave two sub-populations which slightly overlapped in their ranges of variation.

The frequency of dihaploids in the surviving parthenogenetic population was 38.6%. If this percentage is considered to be a true estimate of the frequency of dihaploids in the original parthenogenetic seed population then the expected frequencies of dihaploids and tetraploid parthenotes can be calculated. This results in an expected frequency of 1.5 dihaploids/berry and 2.4 tetraploids parthenotes/berry. The means and variances of 11 continuously varying characters from the diploid and tetraploid parthenogenetic populations along with those from the self population of clone 2154 are given in Table 1.

It is expected that plants produced by FDR should be highly heterozygous and therefore a population consisting of such plants should show smaller ranges of variation than one consisting of SDR produced plants.

Leaf shape in the three populations was similar, there being no significant differences between the means of the length/breadth ratios of the terminal and uppermost primary lateral leaflets. The range of variation for the terminal leaflet was greatest in the dihaploid population and least in the parthenogenetic tetraploid group, whereas the range of variation for the uppermost primary lateral leaflet was least in the dihaploid population but greater and similar in the two tetraploid populations. Few conclusions can be drawn from the results of leaflet shape. In general

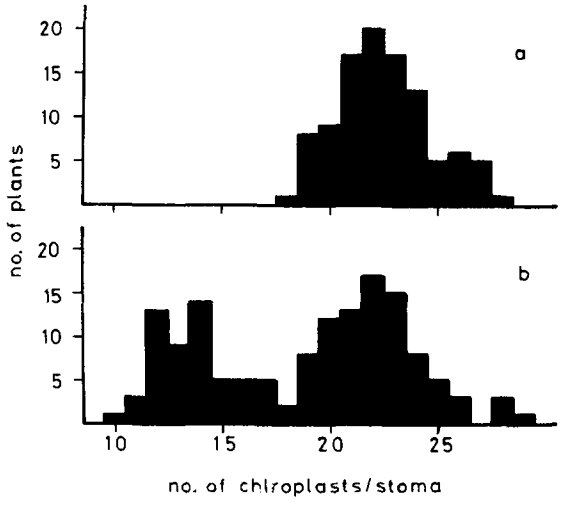

Fig. 1. Chloroplast number/stoma in the offspring from clone 2154 **a.** selfed population **b.** parthenogenetic population

Table 1. Population results and comparisons of continuously varying morphological characters of clone 2154

Character	Population	Mean	*	Variance	*
	⊕	22.5 ± 0.4	a	4.8	a
chloroplast	4x	22.2 ± 0.5	ab	5.9	ab
numbers/stoma	2x	13.6 ± 0.1		3.1	
	⊕	1.72 ± 0.07	a	0.11	a
l/b terminal	4x	1.72 ± 0.09	ab	0.06	b
leaflet	2x	1.87 ± 0.19	abc	0.20	c
l/b uppermost	⊕	1.80 ± 0.07	a	0.13	a
primary lateral	4x	1.80 ± 0.13	ab	0.12	ab
leaflet	2x	1.93 ± 0.09	abc	0.04	c
length terminal	⊕	2.82 ± 0.11	a	0.25	a
leaflet/length	4x	2.91 ± 0.18	ab	0.29	ab
to broadest point	2x	2.73 ± 0.20	abc	0.20	abc
angle between	⊕	136 ± 5	a	525	a
uppermost primary	4x	136 ± 8	ab	479	ab
lateral leaflet pair	2x	136 ± 9	abc	645	abc
(degrees)					
height of	⊕	151 ± 8	a	1119	a
flowering	4x	140 ± 11	ab	829	ab
plants (cm)	2x	122 ± 16	bc	791	abc
	⊕	147 ± 6	a	522	a
number of days	4x	128 ± 4	b	131	b
to flowering	2x	136 ± 11	ab	451	a c
length of pedicel to	⊕	0.61 ± 0.03	a	0.01	a
articulation against	4x	0.62 ± 0.04	ab	0.01	ab
length of pedicel	2x	0.60 ± 0.08	abc	0.01	abc
radius of corolla	⊕	1.59 ± 0.06	a	0.07	a
to acumen tip against	4x	1.55 ± 0.1	ab	0.07	ab
radius of corolla to	2x	1.58 ± 0.1	abc	0.03	abc
interpetalar notch					
ratio of style	⊕	1.72 ± 0.07	a	0.07	a
length to	4x	1.70 ± 0.10	ab	0.08	ab
anther length	2x	1.70 ± 0.12	abc	0.03	abc
	⊕	30.3 ± 0.5	a	4.1	a
pollen grain	4x	27.7 ± 0.9	b	6.3	ab
diameter (μ)	2x	25.4 ± 1.8	c	9.1	bc

* Comparison of means and variances ($p = 0.05$). Populations without a common letter are significantly different from each other. Populations: ⊕ = self, 4x = tetraploid parthenogenetic, 2x = diploid parthenogenetic

the diploids had narrow leaflets, but not significantly so. This is often found in comparisons between dihaploids and their tetraploid parental clones (Kawakami & Matsubayashi 1960; Peloquin & Hougas 1960).

There were no significant differences between the population variances for the height of plants that flowered but there were differences between their means. The percentage of surviving plants that flowered in each population varied from 58% in the dihaploid through 71% in the selfed to 80% in the tetraploid parthenogenetic population. These percentages suggest that fewer deleterious alleles were expressed in the tetraploid parthenotes indicating that they were still highly heterozygous. The flowering period of the tetraploid parthenotes was also more conservative than that in the other two populations.

It is known that pollen grain size is a reflection of the ploidy level of the plant (Howard 1960). It is therefore expected that the diploid population should show a significantly lower mean pollen grain diameter than the two tetraploid populations. The difference between the two tetraploid populations may be due to different frequencies of 2n microspore production. Two dihaploid plants had average pollen grain diameters within the tetraploid range and it is thought that these plants produced mainly

Table 2. The percentages of each population in three pollen stainability classes

Population	Stainability percentage classes		
	> 10	> 20	> 30
+	42	12.3	2
4x	51	43	30
2x	33	26	13

2n microspores. Pollen viability was scored as the percentage of stainable pollen; the results are represented in Table 2.

The parthenogenetic populations appear to be fitter than the self population in respect to pollen stainability with the tetraploid parthenotes being on average superior to the diploids.

All other continuous characters represented in Table 1 showed no significant differences between the three populations and were therefore of little use in determining the mechanism involved in the production of the tetraploid parthenotes.

Discontinuous Characters

The discontinuous characters, mainly scored as two state phenotypes, were thought to be more likely to indicate the make-up of the tetraploid parthenotes than continuous characters. Genetic ratios in the dihaploid population should be equivalent to gametic ratios from the parental clone and genetic ratios in the selfpopulation should be equivalent to zygote ratios. From these two populations it may be possible to elucidate the genotype of the parental clone for certain characters, and to know whether there is random, chromosome or chromatid assortment. Using this information it may be possible to draw conclusions from the genetic ratios found in the tetraploid parthenogenetic population.

Stem wing type was scored as either straight or crenulate, the parental clone showing a crenulate wing. The results from the three populations are given in Table 3. The observed ratios from the self and dihaploid populations indicate that wing type is controlled by one gene with crenulate dominant to straight, the genotype of the parent being simplex. It may be possible to postulate the type of unreduced gamete formation from the above conclusions. If we consider that *C* represents crenulate and is dominant to *c*, which represents straight, then the parental clone had the genotype *Cccc*. At meiosis with random chromosome assortment *C* must align with *c* and *c* & *c* must also align. If we consider the *Cc* pair and there is no crossing over, both *C* chromatids will go to one pole. If there is SDR then the gametes must be *CCcc* and *cccc* which gives a 1 : 1 phenotypic ratio. If there is FDR, then at the second division one *C* chromatid will go to each pole and therefore all the unreduced gametes would have the *C* phenotype. If there were chiasma formation between the centromere and the *C* locus at the first division one *C* chromatid would go to each pole. If SDR occurred, all the gametes would possess the *C* locus. If FDR occurred, half of the time the two *C* chromatids would end up in the same nucleus and half the time they would be in different nuclei, therefore a 3 : 1 ratio of *C* to *c* phenotypes would result. Assuming that some crossing-over takes place then the production of unreduced gametes by FDR should give a ratio of not less than 3 : 1 and no more than 1 : 0 for crenulate to straight. SDR produced gametes should give a ratio of not less than 1 : 1 and no more than 1 : 0 for crneulate to straight. The observed ratio in the parthenogenetic tetraploids of 4.9 : 1 indicates that there is chiasma formation between the locus and the centromere but it does not indicate which method of unreduced gamete formation had taken place.

Although different inflorescence types can be seen in potato species it is not known how many genes control this character or whether it is continuous or discontinuous. Initially it was thought that the populations from clone 2154 showed nine different inflorescence types. When successive inflorescences were scored, it was obvious that each type showed more variation than was first thought. Finally, most inflorescences fell into two main groups which were constant on any one plant. There were two plants, one from each tetraploid population, which could not be classified into either group. Group one showed two flower clusters (type 1) and group two showed three flower clusters (type 2).

The results (Table 3) indicate a one gene system with random chromatid assortment where type 1 is dominant to type 2, the parental clone having a duplex genotype. The expected ratios for the parthenogenetic tetraploids are more difficult to estimate due to random chromatid assortment and the fact that there are two doses of the dominant allele. If there were no chiasma produced between the centromere and the locus in question then the SDR produced gametes should show a phenotypic ratio of

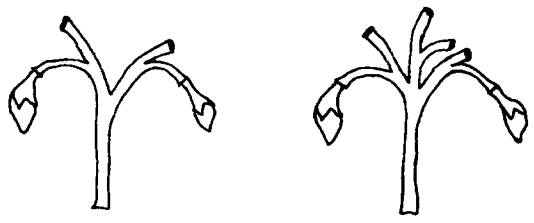

Fig. 2. Diagramatic representation of the different inflorescence types found in the offspring from clone 2154

Table 3. Results of discontinuous characters in the three populations from clone 2154 (excluding anthocyanin pigmentation)

Expected ratio assumptions:
A_1 simplex parent showing random chromosome assortment
A_2 duplex parent showing random chromatid assortment
A_3 simplex parent showing random chromatid assortment
for random chromatid assortment $\alpha = 1/6$

Character	Population	Observed frequencies			Expected ratio		P
	⊕	69		23	3 : 1		> 0.99
stem wing type	4x	34		7		A_1	
crenulate:straight	2x	14		13	1 : 1		> 0.8
	⊕	58		3	19.3 : 1		> 0.99
inflorescence type	4x	26		4		A_2	
1 : 2	2x	14		3	3.5 : 1		> 0.6
(refer to fig. 2)							
calyx length/	⊕	43		19	2.4 : 1		0.8
length calyx tube	4x	22		9		A_3	
short : long	2x	8		9	0.85 : 1		0.3
(≤ 2.2) (> 2.2)							
	⊕	31		31			
calyx shape	4x	24		7			
regular : irregular	2x	5		12			
	⊕	63		26	2.4 : 1		0.99
tuber shape	4x	23		17		A_3	
round : oval	2x	13		14	0.8 : 1		0.8
	⊕	84		5	19.3 : 1		0.7
tuber flesh colour	4x	33		7		A_2	
yellow : white	2x	20		7	3.5 : 1		0.6
	⊕	31	55	3			
tuber eye depth	4x	13	23	4			
shallow:medium:deep	2x	8	16	3			

Table 4. Results of the anthocyanin characters in the three populations from clone 2154

Expected ratio assumptions:
A_2 duplex parent showing random chromatid assortment
A_4 two gene system, both duplex in parent showing random chromatid assortment
for random chromatid assortment $\alpha = 1/6$

Character	Population	Observed frequencies		Expected ratio		P
nodal band	⊕	134	7	19.3 : 1		> 0.95
pigmentation	4x	83	11		A_2	
present : absent	2x	31	10	3.5 : 1		> 0.8
corolla	⊕	54	8	9.2 : 1		> 0.4
pigmentation	4x	25	6		A_4	
present : absent	2x	11	6	1.5 : 1		> 0.5
tuber periderm	⊕	85	4	19.3 : 1		> 0.8
pigmentation	4x	36	4		A_2	
present : absent	2x	21	6	3.5 : 1		> 0.99

5 dominant to 1 recessive. All the offspring produced by FDR in the absence of crossing over would show the dominant phenotype. If there were one cross-over so that there was an interchange between *A* and *a*, then all the offspring from SDR and FDR would have the dominant phenotype. If there were two interchanges of *A* and *a*, each centromere carrying a dominant and a recessive allele to each pole, then the expected ratio of dominant to recessive would be considerably higher than the observed ratio of 6.5 : 1. The only conclusion that could be drawn regarding the origin of these unreduced tetraploids is that they had been produced by SDR with very little chiasma formation between the controlling locus and the centromere.

Calyx type was scored as a continuous and discontinuous character. The calyx was measured from the base of the tube to the acumen tip and the character was expressed as a ratio of this measurement against the length of the calyx tube. It was noticed that the range of variation fell into two groups and therefore it was decided to analyse the range of variation as a two-state character. The observed frequencies (Table 3) appear to fit a one-gene system where short is dominant to long with the parental genotype being in the simplex condition (short). The observed ratio of 2.4 : 1 in the tetraploid parthenogenetic population could be due either to FDR or SDR with some crossing over between the centromere and the locus in question.

Calyx shape was scored as regular or irregular. If this character is considered to be controlled by one gene with dominance then the observed ratios do not correspond to any expected ratios (Table 3). The parental clone possessed a regular calyx on all flowers, therefore it can be assumed that regular is in some way dominant to irregular. The selfed population gave a ratio of 1 : 1 while the parthenogenetic tetraploids gave a ratio of 3.4 : 1. If the parthenotes had been produced by SDR then why had they all a greater ratio of regular offspring than the selfs? Howard (1973) suggested that the gene controlling calyx shape was closely linked to the *S*-incompatibility locus. Woodcock & Howard (1975) found no evidence to support this theory. There may be different states of irregularity and more than one gene may be involved.

Tuber shape was scored as a two-state character either round or oval. The observed frequencies (Table 3) indicate that round is dominant to oval and possibly under the control of one gene, with the parent having the genotype *Rrrr*. The expected phenotypic ratios assuming random chromatid assortment for SDR-produced plants would be 5*R* : 1*r* and 1*R* : 1*r* with and without effective cross-overs, respectively. FDR-produced plants would show a ratio of between 3*R* : 1*r* and 1*R* : 0*r* depending on the frequency of chromosome cross-overs. The observed ratio of 1.4*R* to 1*r* is closest to the 1 : 1 ratio obtained from SDR plants without chiasma formation between the centromere and the *R* locus. It therefore appears from the results of this character that the parthenogenetic plants were produced by SDR.

Tuber flesh colour was recorded as either white or yellow. There appeared to be a great amount of variation in the intensity of the yellow colour from whitish yellow to a really deep yellow colour. Other workers studying the inheritance of tuber flesh colour in tetraploid potatoes have suggested that yellow is dominant to white and controlled by one major gene with modifying genes controlling the degree of yellowness. The parental clone possessed yellow flesh and the results (Table 3) suggest that yellow is dominant to white with the parental clone having a duplex constitution. The system controlling this character is comparable to the one controlling the number of flower clusters/inflorescence. The ratio of 4.7 yellow to 1 white produced by the parthenogenetic tetraploids suggests that they were produced by SDR.

Tuber eye depth was scored visually as a three state-character: shallow, medium or deep. The parental clone showed medium-depth eyes. The results from the three offspring populations are given in Table 3. The largest class in all three populations is medium-depth eyes, followed by shallow eyes, deep eyes being the smallest. It would be expected that the selfs should show higher frequencies of the dominant types to the recessives than would the dihaploids. The selfed population should be more heterozygous than the dihaploid population thus suppressing recessive phenotypes. The observed ratios for the dihaploids are much smaller than for the selfs indicating a reduction in heterozygosity. The tetraploid parthenogenetic population shows similar ratios to the dihaploid group indicating that it possessed more homozygosity for this character than the selfed group.

The inheritance of anthocyanin pigmentation has been studied by Dodds & Long (1955, 1956), Harborne (1960), Dodds & Paxman (1962) and reviewed by Howard (1970). Most work has been done with diploid potatoes but Howard proposed a general scheme for tetraploid potatoes. The parental clone 2154 had red to purple flowers, deep red periderm pigmentation in the tubers and stem nodal and floral abscission layer pigmentation. From Howards scheme gene *D* is a basic gene for pigmentation throughout the plant. Gene *E* produces red colour in stems and inflorescences; *ED* plants have tubers with a deep red colour in the periderm. Gene *F* is a gene for flower colour, *DF* plants have violet red flowers. Gene *B* in diploid potatoes, but known to occur in tetraploids, controls a concentration of anthocyanin at the base of all plant organs that are homologous to leaves; i.e. in the cotyledonary node of the embryo, at the base of the petiole (nodal band) in the floral abscission layer and on the tuber eyebrow. There are several *B* alleles which restrict their action

to some of the above places. According to Dodds & Long (1956) plants showing pigmented nodal bands and having pigmented floral abscission layers should also show pigmentation on the embryo. From several thousand selfed seeds examined from the parental clone not one showed the character embryo spot. This clone must therefore possess a different *B* allele to those described for diploid potatoes by Dodds & Long (1956). If selfed seed from 2154 had been spotted then the selection of parthenogenetic seeds would have proven very difficult.

The observed frequencies for nodal band pigmentation in the self and dihaploid populations (Table 4) appear to fit a one-gene system with random chromatid assortment ($\alpha = 1/6$) with the parental genotype being duplex. From the hypothesis on overall plant pigmentation this character should be controlled by genes *D* & *B*. Therefore eiher they are very closely linked or one of them is in the homozygous dominant condition. The ratio of 7.6 : 1 in the parthenogenetic tetraploid population indicates the production of a greater number of homozygous recessive offspring than would be expected from selfing and presumably also a greater number of homozygous dominant offspring. Therefore it is likely that these tetraploids were produced by SDR with a limited amount of chromosome crossing over between the centromere and the locus in question.

The parental clone showed a red to purple flower with white acumen tips. All flowers in all offspring had white acumen tips but they segregated for pigmented and non-pigmented flowers. All pigmented flowers when analysed possessed the same two anthocyanidins, cyanidin and peonidin. There was no segregation of the two pigments. The results of corolla pigmentation from the three populations are given in Table 4. The observed ratios do not fit any expected ratios for a one-gene system but this is not surprising since from the original hypothesis we know that at least two if not three genes are involved, i.e. *D, E & F*. There may be epistatic gene action of two genes: gene *D* which controls the ability to produce anthocyanins throughout the plant and gene *F* which controls flower pigmentation, i.e. *F* is epistatic to *D*. If the parental clone had a duplex genotype for each gene, i.e. *DDdd, FFff*, then on selfing with random chromatid assortment, with no linkage, the expected ratios would be 9.2 pigmented to 1 non-pigmented. Subjecting the observed and expected ratios to χ^2, $p = 0.4$. In order to determine the inheritance of flower colour in clone 2154, it was back-crossed to a white flowered white tubered individual from its self population. Out of a total of 39 plants that flowered 10 had white flowers. If we assume the white flowered white tubered individual to be homozygous recessive for *D*, since there was no obvious anthocyanin pigmentation in the whole plant, then gene *F* could have been present in one of 5 different doses. If the genotype had been homozygous recessive for both *D* & *F* the ratio of pigmented to non-pigmented flowers should have been equal to the gametic ratio of clone 2154 which was 1.8 : 1 for random chromatid assortment. If there was one dose of *F* and three of *f* then the expected ratio of the back-cross would be 2.8 : 1. The observed ratio of 2.9 : 1 compares favourably ($p = 0.9$) therefore it is likely that the parental genotype of clone 2154 for flower colour was *DDddFFff*.

The parental clone of 2154 showed red tuber periderm pigmentation with no phelloderm or flesh pigmentation. The periderm anthocyanidin was identified as pelargonidin. The tuber colours of the offspring ranged from white through orange and red to dark red but all pigmented tubers possessed only the parental anthocyanidin. The offspring were scored for presence or absence of this pigment; the results are represented in Table 4. The results fit a one-gene system with random chromatid assortment with the parental plant having a duplex genotype. From the original hypothesis, tuber pigmentation should be controlled by genes *D* and *E*. We know from the results of flower colour that *D* is in the duplex state, therefore gene *E* must be in the quadriplex state to give the observed ratios. The parthenogenetic tetraploids give an intermediate ratio between the selfed and dihaploid populations. This is comparable to the observed results on inflorescence type and stem nodal pigmentation, i.e. the parthenogenetic tetraploids must have been produced by SDR.

Tuber colour was also scored in a back cross using a white flowered white tubered individual. Previous results indicate that the parental plant had the genotype *DDddEEEE* and the white tubered individual was in the duplex condition for *D* and must have been in the quadriplex condition for *E*. Therefore the cross must have been *DDddEEEE* × *ddddEEEE*. The expected ratio of the offspring would be the same as for a one-gene system. If we assume random chromatid assortment then the expected ratio is 3.5 : 1. The observed frequency was 39 pigmented and 12 non-pigmented, which is a ratio of 3.25 : 1. Subjecting this to the χ^2-test, $p = 0.8$.

There is further evidence to suggest that the proposed genotype of the parental clone is correct. In the self offspring from clone 2154 all white tubered individuals that flowered had white flowers but not all white flowered individuals had white tubers.

In diploid potatoes there is a gene *R* which controls the production of cyanidin in the flowers and pelargonidin in the tubers. This gene appears to be exactly the same as gene *E* in clone 2154.

Discussion

If we consider the continuously varying characters it can

be seen that very little useful information regarding the mechanism of unreduced gamete formation was revealed from the results. Most characters showed wide ranges of within population variation and in general there were no significant differences between populations, often not even between ploidy levels. The use of continuous characters in this kind of study might be better suited to cases where the parental clones were diploid, in which case homozygosity would probably be more easily detectable. In FDR-produced plants there may be more crossing over than anticipated, in which case the range of variation would be enlarged. Similarly in SDR produced plants, if the frequency of crossing over was high then the differences between populations produced by FDR and SDR would become much less.

Characters controlled by single genes provided much more information on the type of unreduced gametes involved, especially as the expected genetic ratio ranges for populations produced by FDR and SDR can be calculated. Let us consider those characters that were thought to be controlled by one gene with dominance and were in the simplex state in the parental clone. If we consider a character showing random chromosome assortment then an SDR produced population must range from 1 : 1 to 1 : 0 for dominant to recessive, assuming no effective cross-overs and total effective cross-overs, respectively. An FDR-produced population must range from 1 : 0 to 3 : 1 assuming no effective cross-overs and total effective cross-overs, respectively. Therefore any ratio approaching 1 : 1 must be due to plants produced by SDR. Results showing ratios of 3 : 1 or greater are of little use in determining the mechanism of unreduced gamete formation. For characters showing random chromatid assortment the ratios are different. An SDR produced population must range from 1 : 1 to 5 : 1 without and with effective cross-overs, respectively and an FDR produced population must range from 1 : 0 to 2 : 1 without and with effective cross-overs, respectively ($\alpha = 1/6$). Therefore any population showing a ratio significantly higher than 5 : 1 must be comprised of FDR produced plants. Similarly any ratio found to be approaching 1 : 1 should be due to plants produced by SDR. The three characters thought to be in the simplex condition in the parental clone were wing type, giving a 4.9 : 1 ratio which is not indicative of either method; calyx type, giving a 2.4 : 1 ratio which could have been produced by either mechanism and tuber shape which gave a ratio of 1.4 : 1. This latter ratio is less than the minimum 2 : 1 ratio expected for FDR produced plants and therefore it is likely that most if not all of the plants were produced by SDR with a limited frequency of effective crossing over.

Consider a character controlled by a single gene with *A* being dominant to *a*. If the parental clone was in the duplex state and showed random chromatid assortment,

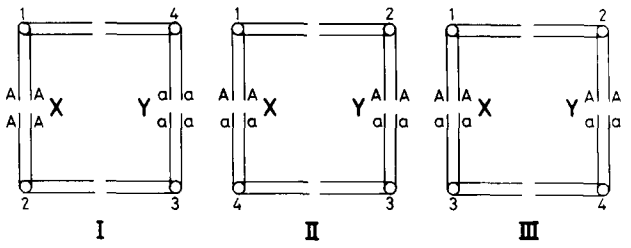

Fig. 3. Diagramatic representation of a quadrivalent at meiosis

what are the expected ratios for populations made up of plants produced by FDR or SDR? (consider a quadrivalent (Fig. 3). If there is no effective chromosome crossing over between the centromere and the locus in question, i.e., at either X or Y, then at first division any two chromosomes can go to any one pole, e.g.;

1 & 4	*AAaa*	*AAaa*	2 & 3
1 & 3	*AAaa*	*AAaa*	2 & 4
1 & 2	*AAAA*	*aaaa*	3 & 4

It can be seen from the above arrangement that if there is SDR then a 5 : 1 phenotypic ratio of *A* : *a* results and if FDR occurs then the phenotypic ratio must be 1*A* : 0*a*. If we consider one cross-over at X, one-third of the time the orientation must be as in 3.I, one-third as in 3.II and one-third as in 3.III. If there is one cross-over at X, in 3.I the ratios for FDR and SDR will be the same as with no crossing over. If we consider 3.II, chromosome 1 will have *Aa* and so will chromosome 4, the other two chromosomes will still be homozygous. At first division any two centromeres can go to any one pole. Since three chromosomes carry at least one *A*, if there is second division restitution there will be a 1 : 0 phenotypic ratio of *A* : *a*. The same ratio also results from 3.III. Therefore, two-thirds of the time there is a 1 : 0 ratio and one third of the time there is a 5 : 1 ratio. Therefore, the overall phenotypic ratio must be 17 : 1. If we now consider FDR we can see that chromosome two is still in the homozygous dominant condition and therefore any FDR nucleus must carry at least one *A* which will result in a 1 : 0 ratio of *A* : *a*. The overall ratio assuming one cross-over with FDR is 1 : 0 for *A* : *a*. We now consider two cross-overs, one at X and one at Y. If the orientation is as in 3.I. then the ratios will be the same as without any crossing over. If the orientation is as in 3.II. or 3.III then after crossing over each chromosome will carry *A* and *a*. If there is SDR then all nuclei will contain *A* which would result in a 1 : 0 ratio of *A* : *a*. The overall expected phenotypic ratio would therefore be the same as with one cross-over which was found to be 17 : 1 for *A* to *a*. If there was FDR then each centromere could send either *A* or *a* to the restitution nucleus. From each pole there would be a ratio of 1*AA* : 2*Aa* : 1*aa* going to the restitution nucleus. Therefore, overall there would be a 15 : 1 phenotypic ratio of

A : *a* in the restitution nucleus. This ratio is produced two-thirds of the time and one-third of the time there is a 1 : 0 ratio. Therefore, overall there must be a 23 : 1 phenotypic ratio of *A* : *a*. For the two types of population considered we now have the expected ratio ranges. For a population consisting of SDR produced plants the ratios must be between 5 : 1 and 17 : 1 and for a population consisting of FDR produced plants between 1 : 0 and 23 : 1.

The characters studied which were found to be in the duplex state in the parental clone and which showed random chromatid assortment were: inflorescence type which showed a ratio of 6.5 : 1 in the tetraploid parthenogenetic population; tuber flesh colour with a ratio of 4.7 : 1; stem nodal pigmentation 7.6 : 1 and tuber periderm pigmentation 9 : 1. All of these ratios only fit into the range expected for plants produced by SDR. The overall trend from all the characters studied leads us to the conclusion that most, if not all, of the parthenogenetic tetraploids were produced by a mechanism equivalent to Second Division Restitution.

The four characters giving the clearest evidence as to the origin of the tetraploid parthenotes all appeared to show random chromatid assortment. This means that there must have been a high frequency of effective crossing over between the centromere and the locus concerned with each character. The ratios observed in the tetraploid parthenogenetic population suggest that there was only a limited amount of effective crossing over for each of the four characters. This apparent conflict cannot be resolved at this time with these data.

From all of the characters scored very few have given direct information as to the composition of the tetraploid parthenotes. It has been demonstrated that the characters controlled by one gene with dominance with the parental plant having a duplex constitution are much more useful in determining the type of unreduced gamete formation than continuous characters which are controlled by several genes.

Once the genotype of a particular parent is known it may be relatively straight forward to choose those characters which will reveal the greatest amount of information regarding the mechanism of unreduced gamete formation. This may become very important in the near future as potato breeders make more use of unreduced gametes in their breeding programmes.

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