Genetic recombination in a diploid synaptic mutant and a *Solanum tuberosum* × *S. chacoense* diploid hybrid

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A diploid synaptic mutant, M6 with the genotype *sy3/sy3,ps/ps*, was identified as heterozygous for four enzyme-coding loci, and for the tuber flesh colour locus (*Y*). Through $4\times-2\times$ crosses, half-tetrad analysis was applied to determine the ability of the M6 clone to transmit its heterozygosity to tetraploid offspring. On the average, an 89.03 per cent reduction in recombination was found within the chromosome segments sampled, resulting in 98 per cent transmission of heterozygosity. Cytological observations of microsporogenesis revealed frequent pachytene associations and a high proportion of univalents at diakinesis. These findings show that chromosome pairing in M6 is mostly desynaptic. Diploid and $4\times-2\times$ testcrosses were made to study recombination in a $2n$ -pollen producing diploid hybrid T704 (*ps/ps*); *S. tuberosum* L. × *S. chacoense* Bitt. For the loci compared, recombination was reduced 34.56 per cent on the average. This hybrid transmitted on the average 89.2 per cent of its heterozygosity which was significantly higher than the value previously observed in non-hybrid individuals. Unlike M6, T704 produced both viable n and $2n$ pollen. In addition, cytological examination of PMCs indicate normal bivalent associations. It is suggested that genomic differentiation between *S. tuberosum* and *S. chacoense* could account for the reduced recombination levels.

INTRODUCTION

Meiotic abnormalities which lead to the formation of $2n$ gametes offer unique opportunities to hybridise diploid *Solanum* species with the cultivated tetraploids for the purposes of increasing genetic diversity and ultimately yield. The benefits of such hybridisations aimed to capture novel genetic diversity are directly related to the meiotic mechanism responsible for diplandroid formation. At present, three meiotic mechanisms have been identified that lead to male $2n$ gametes in the diploid potato species: parallel and/or fused spindles at metaphase II (First Division Restitution or FDR), premature cytokinesis I, and premature cytokinesis II (Second Division Restitution or SDR) (Mok and Peloquin, 1975; Ramanna, 1979). The discovery of synaptic genes (Iwanaga and Peloquin, 1979) within the diploid potato species has expanded the potential of the FDR mechanisms. The combination of synaptic chromosomes with the parallel spindles gene has opened the possibility of transferring 100 per cent

of the diploid parent's heterozygosity intact to the tetraploid offspring (Okwuagwa and Peloquin, 1981).

Since Iwanaga and Peloquin (1979) reported the first synaptic mutant (*sy1*) affecting megasporogenesis in a diploid potato clone, three new synaptic mutants have been identified: *sy2* (Johnston *et al.*, 1981); *sy3* (Okwuagwa and Peloquin, 1981); and *sy4* (Iwanaga, 1984), all affecting microsporogenesis. In these new synaptic mutants, general cytological abnormalities are common. Lack of pairing was noted during diplotene and diakinesis stages, followed by random distribution of the chromosomes during the first division leading to pollen sterility. Okwuagwa and Peloquin (1981) identified the parallel spindle mechanism operating during metaphase II within the *sy3sy3* genotype. This resulted in the fusion of unbalanced nuclei, ultimately leading to balanced and viable dyads.

In asynaptic mutants, homologous pairing fails completely or is incomplete. On the other hand, the desynaptic condition can alter the maintenance of chromosome pairing during pachytene, ultimately reducing fertility as a consequence of

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univalents at diakinesis (Hermesen, 1984a). Definitive cytogenetic characterisation of these synaptic mutants has not been possible. Difficulties lie in the inability to differentiate cytologically between the asynaptic and desynaptic condition. The utilisation of synaptic mutants for the purpose of transferring intact genotypes from parents to offspring rests solely on a theoretical basis, since recombination has been known to occur in synaptic phenotypes of other higher plants (as reviewed by Koduru and Rao, 1981). A study of recombination within chromosome segments of these synaptic mutants should provide experimental data to test the utility of these mutants.

With the production of $2n$ pollen through the parallel or fused spindle mechanisms, gene-centromere mapping is possible through $4\times-2\times$ crosses. Mok and Peloquin (1982) proposed the use of electrophoretic variants for mapping while Iwanaga (1984) suggested their application to analyse the genetic consequences of the desynaptic gene *sy4*. Recently, Douches and Quiros (1987b) determined gene-centromere relationships for 10 enzyme-coding loci. Positioning of electrophoretic markers on the chromosomes enables the identification and characterisation of meiotic mutants by genetic analysis in the potato.

In this paper, M6, a diploid synaptic mutant with the ability to generate FDR $2n$ pollen through the parallel spindles mechanism (*sy3/sy3,ps/ps*), was analysed through $4\times-2\times$ crosses and subsequent half-tetrad rationale, in conjunction with cytological observations of microsporogenesis. The resulting recombinational frequencies were compared with recombinational values obtained in non-mutant plants to quantify the reduction in recombination. The same approach was applied to determine the level of recombination in an interspecific hybrid of *S. tuberosum* L. and *S. chacoense* Bitt. as a means to detect genomic differentiation between these two species. This work provides experimental data on the extent of transmission of heterozygosity for synaptic mutants and interspecific hybrids to their progenies.

MATERIAL AND METHODS

Plant material

The diploid synaptic, parallel spindles, double mutant, M6 (*sy3/sy3,ps/ps*) ($2n=2\times=24$), and the interspecific hybrid, T704 (*ps,ps*) ($2n=2\times=24$), were kindly supplied by Dr S. J. Peloquin

(University of Wisconsin, Madison). M6 was selected from the progeny of the cross W5295.7 (I) $\times$ W5337.3 (J), both of which were species hybrids between *S. tuberosum* dihaploids ($2n\times2\times=24$) and *S. phureja* Juz. et Buk. ($2n=2\times=24$). T704 is a diploid species hybrid between a *S. tuberosum* dihaploid and *S. chacoense*. These diploids were crossed to tetraploid clones. The tetraploid parents used in the $4\times-2\times$ and $2\times-4\times$ crosses were Lemhi Russet, NDD277-2, BC8370-4, T245.7, and PAS3006-7. These clones were kindly supplied by Dr R. E. Voss (University of California, Davis). Diploid crosses were made in the direction 84S10 $\times$ T704 and T704 $\times$ 85SD56. 84S10 and 85SD56 are diploid clones of *S. phureja* origin.

Electrophoresis

Employing horizontal starch gel electrophoresis, eight enzyme systems were studied which revealed nine enzyme-coding loci: *Dia-1* (diaphorase), *Got-1* (glutamate oxaloacetate transaminase), *Idh-1* (isocitrate dehydrogenase), *Mdh-1* (malate dehydrogenase), *6-Pgdh-3* (6-phosphogluconate dehydrogenase), *Pgm-2* (phosphoglucomutase), *Prx-2*, *Prx-3* (peroxidase), and *Sdh-1* (shikimic acid dehydrogenase). Inheritance data and allozyme patterns for these loci are described by Quiros and McHale (1985) and Douches and Quiros (1987a). Excluding *Dia-1* and *Prx-2*, gene-centromere map distances were previously estimated (Douches and Quiros, 1987b). Specific electrophoretic procedures are described by Quiros (1981). Enzyme stains used are according to Vallejos (1983).

To test for recombination, $4\times-2\times$ (FDR) testcrosses were made for the enzyme loci listed above and *Y* determining tuber flesh colour (Howard, 1970). Since a single $4\times$ parent was not identified to be nulliplex for all the loci tested, a series of crosses were made using Lemhi Russet, NDD277-2, BC8370-4, Y245.7, and PAS3006-7. The genotypes of these clones are shown in tables 1 and 2. Gene-centromere map distances for these loci including *Dia-1* and *Prx-2* were previously estimated by Douches and Quiros (1987b) using diploid stocks from the *S. tuberosum* group. These values were used as standards to compare recombinational frequencies in M6 and T704. Gene-centromere map distances are simply another way of expressing the recombination frequency for a chromosome segment between a locus and its centromere.

Under normal synaptic conditions, a $4\times-2\times$ (FDR) testcross would generate progeny that

can be divided into three classes (assuming diallelic loci): simplex, nulliplex, and duplex genotypes, with the second and third classes indicating a recombinational event. In case of dominance, only the nulliplex progeny would be diagnostic; however, with these co-dominant electrophoretic markers, the duplex genotype can be distinguished from the simplex on the basis of asymmetric banding intensities (Martinez-Zapater and Olivier, 1985). Under asynaptic conditions assuming zero recombination, these $4 \times -2 \times$ test-crosses should yield exclusively simplex genotypes. If crossing-over does occur, nulliplex and duplex progeny should be recovered. The greater the distance of a gene from its centromere, the better the opportunity for detecting crossing-over in M6 and T704.

All crosses were made in the greenhouse. Offtypes observed in $4 \times -2 \times$ offspring were subjected to chromosome counts in either root-tip or another squashes to rule out triploids. No triploids were observed, presumably due to a strong triploid block (Marks, 1966; Hanneman and Peloquin, 1968; Hermesen, 1984b). Germinated seedlings

were transplanted into trays (50 plants per tray). In 4 to 6 weeks, root or leaf samples from healthy and vigorously growing seedlings were sampled for electrophoretic analysis. To determine tuber flesh colour (*Y* locus), the seedlings were allowed to tuberise in the trays. Small mature tubers were harvested in three to four months. Tubers were then visually compared with parental tubers for flesh colour determination.

To cytologically study diplandroid formation, flower buds were collected and immediately fixed in a freshly prepared ethanol/propionic acid solution (3:1) with ferric chloride added as a mordant (Swaminathan *et al.*, 1954). After 24 hours, the buds were removed from the fixative, rinsed, and stored in 70 per cent ethanol at 4°C. Anthers were dissected and squashed in 1 per cent acetocarmine. All meiotic stages of the pollen mother cells were examined. The occurrence of parallel and/or fused spindles during Metaphase II, followed by dyad formation identified the FDR mode of $2n$ pollen formation (Mok and Peloquin, 1975; Ramanna, 1979). Pollen staining was in accordance with Quinn *et al.* (1974).

Table 1 Half-tetrad analysis of electrophoretic markers in $4 \times -2 \times$ crosses with M6, a synaptic/parallel spindles mutant

Record No.	Cross	Parent	Genotypes	Simplex	Duplex	Nulliplex	Observed recombination frequency (per cent)	Expected recombination frequency ^a (per cent)	χ^2	Percent reduction in recombination
1	86DS32	Lemhi Russet $\times$ M6	<i>Prx-3^1 3^1 3^1 3^1 \times 3^1 3^2</i>	208 (178) ^b	5 (19.5)	4 (19.5)	4.15	18.00	26.6***	76.90
2	86SD16	NDD277-2 $\times$ M6	<i>yyyy \times Yy</i>	357		3				
3	86SD32	Lemhi Russet $\times$ M6	<i>yyyy \times Yy</i>	52		3				
4	Total			409 (375)		6 (35)	2.89	16.84	27.1***	82.89
5	86SD16	NDD277-2 $\times$ M6	<i>6-Pgdh-3^2 3^2 3^2 3^2 \times 3^1 3^2</i>	401	7	0				
6	86SD60	BC8370-4 $\times$ M6	<i>6-Pgdh-3^2 3^2 3^2 3^2 \times 3^1 3^2</i>	60	0	0				
7	Total			461 (328)	7 (72)	4 (72)	2.33	30.50	182.4***	92.36
8	86SD16	NDD277-2 $\times$ M6	<i>Idh-1^1 1^2 1^2 1^2 \times 1^1 1^2</i>	412		0				
9	86SD60	BC8370-4 $\times$ M6	<i>Idh-1^2 1^2 1^2 1^2 \times 1^1 1^2</i>	58	2	0				
10	Total			470 (385)	2 (43.5)	0 (43.5)	0.21	18.40	100.9***	98.86
11	86SD16	NDD277-2 $\times$ M6	<i>Sdh-1^2 1^2 1^5 1^5 \times 1^1 1^5</i>	411 (395)		1 (17)	0.49	8.30	15.7***	94.10

^a From Douches and Quiros, 1986b

^b Expected values in parentheses

Table 2 Half-tetrad analysis of electrophoretic markers in 4×-2× testcrosses with T704, an interspecific hybrid of *S. tuberosum* × *S. chacoense*

Cross	Parent	Locus	Nulliplex progeny	Progeny size	Observed recombination frequency (per cent)	Expected ^a recombination frequency (per cent)	χ^2	Reduction in recombination (per cent)
86SD19	Y245.7	GOT-1	2/0	341	1.17	—		
86SD46	PAS3006-7	GOT-1	3/0 ^b	224	1.34	—		
86SD54	BC8370-4	GOT-1	1/1	59	3.39	—		
86SD19	Y245.7	PGM-2	4/3	624	1.12	—	—	—
86SD46	PAS3006-7	PRX-2	4 (2) ^c	223 (221)	3.59	2.0	NS	0.00
86SD19	Y245.7	IDH-1	28 (21)	224 (203)	25.0	18.0	NS	0.00
86SD46	PAS3006.7	IDH-1	3	220	5.38			
86SD46	PAS3006.7	IDH-1	10	224	8.93			
86SD54	BC8370-4	IDH	4	57	14.04			
86SD19	Y245.7	SDH-1	20 (41)	501 (460)	7.98	18.4	22.05***	56.60
86SD46	PAS3006-7	SDH-1	3	220	2.69			
86SD46	PAS3006-7	SDH-1	6	224	1.25			
86SD54	BC8370-4	SDH-1	1	59	3.39			
86SD19	Y245.7	DIA-1	10 (23)	503 (480)	3.98	8.3	7.70***	52.05
86SD54	BC370-4	DIA-1	8	119				
86SD54	BC370-4	DIA-1	3	59	10.17			
86SD46	PAS3006-7	6-PGDH-3	11	178	12.36			
86SD46	PAS3006-7	6-PGDH-3	15	221	13.50			
86SD54	BC8370-4	6-PGDH-3	5	51	19.60			
86SD46	PAS3006-7	MDH-1	20 (42)	272 (230)	14.65	30.5	12.18***	52.00
86SD46	PAS3006-7	MDH-1	20 (38)	224 (186)	17.86	33.5	9.81***	46.70

^a From Douches, D. S. and C. F. Quiros, 1987b^b Nulliplex/duplex genotypes^c Expected value in parentheses^d Expected value for *Prx-3*-centromere linkage block

*** Probability >0.001

RESULTS

Extent of recombination in the synaptic mutant M6

Electrophoretic analysis of M6 revealed that it was heterozygous for four isozyme loci: *Idh-1*¹*1*², *Sdh-1*¹*1*⁵, *Prx-3*¹*3*³ and *6-Pgdh-3*¹*3*². In addition, M6 was heterozygous for yellow tuber flesh as determined by the genotype of its parents (Masson, 1985). Five chromosome segments of varying lengths were therefore available to study and test for the occurrence of recombination in the synaptic mutant M6 (table 1).

The *6-Pgdh-3* locus was calculated to be 30.1 m.u. from its centromere in crosses between Group *Tuberosum* accessions (Douches and Quiros, 1987b). Thus, this locus provided the best opportunity to detect cross-over in M6 because of

its distal chromosome arm position. A recombination value of 2.33 per cent was obtained for M6 after pooling segregation data from two tetraploid progenies, 86SD16 and 86SD60 ($\chi^2 = 3.071$, $P = 0.07$) (table 1).

Segregation for the *Idh-1* locus, located at 18.4 m.u. from the centromere, was also amenable to half-tetrad analysis in the M6 progenies. In the family 86DS60, two recombinant duplex individuals were observed from 60 plants. However, in the family, 86SD16, the analysis was not straightforward. The tetraploid parent, NDD277-2, as determined by progeny testing, was a simplex heterozygote (*Idh-1*¹*1*²*1*²) rather than homozygous for the locus as required by half-tetrad analysis. As a result, only half of the progeny was amenable to the half-tetrad rationale. Recombination was not detected in this cross as nulliplex genotypes were not found.

Segregation of the *Sdh-1* locus, located 8.3 m.u. from the centromere, was studied in the family 86DS16. Since the tetraploid parent was a duplex heterozygote (*Sdh-1*²*1*⁵*1*⁵) and M6 was heterozygous with the alleles *Sdh-1*¹*1*⁵, only one of the recombinant progeny classes was observable in the cross. A 0.49 per cent recombination rate was detected for this gene-centromere segment in a progeny of 412 plants.

The cross between Lemhi Russet and M6 (86SD32) estimated a 4.15 per cent recombination rate in the chromosome region between the *Prx-3* locus and its centromere. The expected rate for this locus was 18.0 per cent.

Recombination between the *Y* locus and its centromere was evaluated in the same family, 86SDS32, along with 86SD16. Based upon the frequency of white-fleshed tubers (table 1), pooled families ($\chi^2 = 3.83$, $P = 0.06$) revealed a 2.89 per cent rate for this proximal arm region compared with the normal location of 16.8 m.u. from the centromere.

Cytological analysis of microsporogenesis in M6(sy3/sy3,ps/ps)

General asynchrony was found in the meiotic process. At times, single anther squashes revealed meiotic stages from prophase I to dyad formation. During pachytene, meiocytes were identified with varying degrees of chromosome pairing. Complete synapsis was identified frequently, suggesting that some level of recombination may be occurring. (fig. 1A). Diakinesis was generally characterised by cells showing mainly univalents, hence revealing the desynaptic nature of this clone. Bivalent formation was very low with a range of zero to three bivalents per cell (fig. 1B).

The chromosomes of M6 during metaphase I lacked normal orientation on the equatorial plate, hence anaphase I was not clearly distinguishable from metaphase I. Spindles were elongated with a curved orientation (fig. 1C). Lagging chromosomes were regularly observed during the first division, leading to abnormal chromosome distri-

Figure 1 Various stages of meiosis in pollen mother cells of the desynaptic, diploid clone M6. (A) Pachytene showing mostly bivalents. (B) Diakinesis with cell showing mainly univalents. (C) Metaphase/anaphase I characterised by unbalanced chromosome segregation and curved, elongated spindles. (D) Telophase II with fused spindle orientation (fs). Note tetrad formation.

bution. Bivalent frequencies within these meiocytes ranged from zero to four; however, most cells lacked clear bivalent associations. Chromosome imbalance was noted at the end of the first division. At times bivalent separation was observed during late anaphase (fig. 1C). Precocious chromatid separation of univalents was not evident. Metaphase II was characterised by a strong expression of the parallel/fused spindles mechanism. Anaphase II and telophase II were typical of *psps*. Expression of tripolar spindles and normal spindle orientation with balanced tetrads was evident at low frequencies (fig. 1D). An estimate of pollen fertility, based upon the frequency of plump, stainable pollen, was 36.0 per cent.

Extent of recombination in the interspecific hybrid T704

Electrophoretic analysis of T704, a *S. tuberosum* × *S. chacoense* species hybrid, revealed eight heterozygous enzyme-coding loci: *Dia-1*, *Got-1*, *Idh-1*, *Mdh-1*, *6-Pgdh-3*, *Pgm-2*, *Prx-2* and *Sdh-1*. Unlike M6, T704 yielded progenies in both 4×-2× and 2×-2× crosses because of viable 2*n* and *n* pollen. Recombination frequencies were studied in progenies of both ploidy levels.

The 4×-2× rationale applied to the synaptic mutant was similarly extended to the 4×-2× crosses involving T704 as the pollen parent. A series of three 4×-2× crosses and two 2×-2× crosses were made to estimate the recombination rates for the eight different loci and two linkage blocks (table 2).

The standard recombination rate for the chromosomal segment between *Mdh-1* and its centromere was estimated to be 33.5 per cent. In a 4×-2× cross involving T704, a rate of 17.86 per cent was estimated from a progeny of 224 plants in family 86SDS46 (table 2).

For *6-Pgdh-3*, located at 30.1 m.u. from its centromere, a recombination value of 14.65 per cent was obtained for T704 after pooling the segregating

data from progenies 86SD46 and 86SD54 ($\chi^2 = 0.48$, $P = 0.53$).

A combined estimate of 7.98 per cent was found for T704 after pooling the three families for segregation of the *Idh-1* locus ($\chi^2 = 2.514$, $P = 0.30$). This rate was significantly reduced from the expected rate of 18.4 per cent. The *Sdh-1*-centromere recombination value in the same three families ($\chi^2 = 0.987$, $P = 0.63$) was reduced to 3.90 per cent from the standard rate of 8.3 per cent.

No reduction in recombination was found for the chromosome region between *Pgm-2* and its centromere. Based upon a fit to chi-square expectations, the present estimate of 3.58 per cent was not significantly different from the expected value of 2.00 per cent.

The highest recombination rate (25 per cent) for any chromosome segments studied in T704 was found within the *Prx-2*-centromere segment in family 86SDS46 of 224 plants.

Segregation of the *Dia-1* and *Got-1* loci were also studied in these 4×-2× testcrosses: however, no standard estimates of the recombination frequencies exist to compare these values. The pooled families 86SD19 and 86SD54 ($\chi^2 = 0.182$, $P = 0.70$) estimated a 12.36 per cent rate for the *Dia-1*-centromere segment in T704. Segregation of the *Got-1* locus was studied in 86SD19, 86SD46, and 86SD54. A pooled estimate of all three families ($\chi^2 = 2.82$, $P = 0.12$) places *Got-1* proximal to its centromere with a 1.12 per cent rate.

The reciprocal 2×-4× cross involving T704 was unsuccessful because of little or no expression of 2*n* egg formation, however, T704 was used as the pistillate parent in a diploid cross (86SD47). A 17 per cent recombination rate was observed for the *Idh-1/Sdh-1* linkage block, which was reduced from the standard value of 36.8 per cent (table 3). An earlier cross between 84S10 and T704 (86SD10) estimated a 10.4 per cent recombination frequency for this same linkage block, while a 32.4 per cent rate was obtained between *6-Pgdh-3* and *Dia-1* (Douches and Quiros, 1987a).

Table 3 Diploid testcross data with T704, an interspecific hybrid of *S. tuberosum* × *S. chacoense*

Family	Cross	Linkage	RF per cent	RF expected ^a	Progeny size	Reduction in recombination (per cent)
86SD31	84S10 × T704	<i>Idh-1/Sdh-1</i>	10.4	46.8	78	71.7
86SD47	T704 × 85SD56	<i>Idh-1/Sdh-1</i>	17.0	36.8	80	53.8
86SD31	84S10 × T704	<i>6-Pgdh-3/Dia-1</i>	32.4	—	78	—

^a From Douches and Quiros (1986a)

Cytological observations of T704

Examination of PMCs confirmed a strong expression of the parallel/fused spindles mechanism leading to $2n$ pollen formation. Analysis of early stages found normal chromosome pairing during pachytene and bivalent formation in diakinesis. Normal chromosome disjunction followed. Pollen fertility was high (>90 per cent).

DISCUSSION

Genetic and cytological analyses of M6

In $4\times-2\times$ testcrosses with M6, five opportunities were available to sample this clone's ability to transmit its heterozygosity. Despite general pachytene pairing, all five chromosome segments of M6 showed severe reductions in crossing-over throughout the genome. The average reduction in crossing-over from expected was 89.03 per cent with a range of 76.94–98.86 per cent. Under normal synaptic conditions, these five regions would have transferred an average of 81.59 per cent of their heterozygosity. Considering the reduction in recombination under the abnormal synaptic conditions conferred by the *sy3* gene, 97.99 per cent of M6's heterozygosity was transmitted to its progeny.

The genotypes combining the *sy3* gene and the *ps* genes were first described by Okwuagwu and Peloquin (1981), however, pachytene stages were not mentioned. In many respects, our cytological observations in M6 agree with those of Okwuagwu and Peloquin. The *sy1*, *sy2*, and *sy3* phenotypes have similar expression, but *sy1* was expressed only in megasporogenesis. Ramanna (1983) observed from 1.1 to 8.3 II per cell during metaphase I in desynaptic plants derived from the parents ($I\times J$) of M6. This range of restricted pairing could suggest a variable expression for the desynaptic genes as influenced by other genotypic factors and interactions.

The cytological similarities between *sy4* and *sy3* were striking. Iwanaga's (1984) cytological description of the synaptic abnormalities matched closely with the present description of the *sy3* in M6. He noted a consistent low frequency of synapsis during pachytene and diakinesis. Thus, Iwanaga predicted a 97 per cent transmission of heterozygosity for this mutant, based upon a frequency of 3 chiasmata per meiocyte.

Hermesen and Ramanna (1983) suggested that desynaptic genes would be expressed in both micro- and megasporogenesis. No fruit set was obtainable in $2\times-4\times$ crosses with M6. Lack of seed

set may be attributed to either lack of expression of the *sy3* gene in the female side or lack of a restitution mechanism. Cytological examination of megasporogenesis would be needed to discriminate between these two possibilities.

Although the cytogenetic data indicated that M6 is desynaptic; *i.e.* generalised pairing during pachytene and high frequency of univalents at diakinesis, the $4\times-2\times$ genetic data revealed a reduced recombination typical of an asynaptic mutant. This indicated that the *sy3* mutation interferes with chiasma formation leading to a premature separation of the bivalents in diakinesis. The theoretical expectation of 100 per cent transfer of the parent's heterozygosity to the offspring was close to being realised in M6. Thus, this restricted recombination caused the transfer of a significantly greater (81.54 per cent vs. 97.99 per cent) amount of heterozygosity than the *ps* gene alone. The potential value of the *sy3/sy3,ps/ps* genotype for $4\times-2\times$ breeding schemes to develop highly uniform, heterozygous progenies for true seed production (TPS), is demonstrated. However, the utility of the *sy3* gene in the development of an apomictic system would be questionable because of its potential for recombination.

Genetic analysis of T704

In comparison to M6, crossing-over occurs much more frequently in the interspecific hybrid T704. Of the loci sampled, an average recombinational frequency of 2.01 per cent was found in M6, while T704 had 10.82 per cent, a five-fold increase, whereas recombination in normal FDR clones had a 18.55 per cent recombination frequency. In $4\times-2\times$ testcrosses, the recombination rate for six chromosome regions were compared to expected values for their respective regions. Reduction in crossing-over in T704 ranged from 0 to 56.60 per cent, with an average of 34.56 per cent. Considering the eight gene-centromere segments sampled in these $4\times-2\times$ testcrosses, the average heterozygosity transferred was 89.18 per cent. Based upon χ^2 comparisons of observed and expected recombination rates for individual loci, a significantly greater amount of heterozygosity is transferred to the tetraploid offspring (89.18 per cent vs. 81.45 per cent respectively) using T704.

For the four chromosome arm regions where reduction in recombination was significant, the decrease regressed linearly. For each recombination value observed, the reduction averaged 51.84 per cent regardless of the expected recombinational frequency for the respective segments.

The chromosome arm region involving *Pgm-2* did not deviate from the expected value. Based upon chi-square tests, a 3.59 per cent recombination frequency for the segregation of the *Pgm-2* locus was not significantly different from the expected value of 2.0 per cent. It can be argued that a significant reduction in crossing-over would be difficult to detect statistically in small chromosome segments with population sizes of approximately 200.

A pooled recombination frequency of 1.12 per cent was observed for the segregation of the *Got-1* locus in $4\times-2\times$ testcrosses. No additional $4\times-2\times$ crosses have been available using different diploid parents for a comparison. Applying the reasoning set forth for the *Pgm-2* segregation along with $2\times-4\times$ (unpublished) and $4\times-4\times$ data (Douches and Quiros, 1987b), it is likely that the *Got-1* recombination frequency obtained expresses a true proximal position for this locus.

The highest recombination rate (25 per cent) for any chromosome segments studied in T704 was found within the *Prx-2*-centromere segment. At this time no direct estimates for this segment exist, but it is known that *Prx-2* and *Prx-3* are tightly linked with 0.6 map units separating the two loci (Douches and Quiros, 1987a; Quiros and McHale, 1985). Hence, the *Prx-3*-centromere linkage (18.0 m.u.) was substituted as the reference. Knowing this relationship, the observed *Prx-2*-centromere recombination rate was deduced to be not significantly different than expected. With this linkage block of 18.0 m.u., a reduction in recombination would have been expected. In comparison, *Idh-1* segregation has a similar rate of 18.4 per cent under normal instances. Within T704, a rate for this locus was reduced to 7.98 per cent. The differential rates for the two loci situated at equivalent distances from their centromeres indicate different degrees of chromosome homology between the two genomes. This conclusion is supported by higher recombination frequencies found in certain chromosome segments of higher exhibiting de- or asynaptic conditions (Rick, 1971; Koduru and Rao, 1981).

In the diploid cross where T704 was used as the pistillate parent, a 53.80 per cent reduction in expected recombination was seen for the *Idh-1/Sdh-1* linkage block. The reduction in crossing-over regressed in a similar fashion as the chromosome segments studied in microsporogenesis. Previously half-tetrad analysis and diploid testcross data using standard diploid stocks positioned the *Sdh-1* and *Idh-1* on opposite chromosome arms (Douches and Quiros, 1987b). The recombination

rate for this linkage block in T704 agrees with the $4\times-2\times$ data for the *Idh-1* and *Sdh-1* gene-centromere segments when T704 was also used as the pollen parent.

Since *Dia-1* and *6-Pgdh-3* are found on the same chromosome it can be assumed that the recombination frequency within the *Dia-1*-centromere segment was reduced at a similar rate as *6-Pgdh-3*. Hence, we can estimate a normal recombination frequency of 25.73 per cent for the *Dia-1*-centromere segment. If the predicted recombination value for the *Dia-1*-centromere segment and the expected value for the *6-Pgdh-3*-centromere are summed, a 56.23 m.u. map distance is then predicted. Under normal synaptic conditions, this linkage block had not been detected. It is possible that in utilising T704, we were able to take advantage of the reduced recombination levels to identify this new linkage block and position the loci in relation to their centromere. The usefulness of this interspecific hybrid in assigning new markers to linkage groups could continue to be realised until more markers saturate the genome.

Douches and Quiros (1987b) reported gene-centromere map distances for ten enzyme-coding loci and the *Y* locus. Their investigation and the current study suggest that these markers are distributed over 11 chromosome arms of the potato genome. With the current $4\times-2\times$ linkage data for *Dia-1* and *6-Pgdh-3*, we assign *Dia-1* and *6-Pgdh-3* to opposite chromosome arms. The genomic distribution of these 12 loci is now expanded to 12 chromosome arms.

Genomic differentiation in T704

Unlike M6, the reduction in recombination in T704 must arise from a different mechanism. Besides the formation of parallel spindles, other meiotic or chromosome abnormalities were not noted in T704. Reduction in crossing over was not expected in this clone since both parental species (*S. tuberosum* and *S. chacoense*) are supposed to have the same genome (Hawkes, 1978).

Our findings, however, raise the question of genomic differentiation between *S. tuberosum* and *S. chacoense*. This is supported by distorted segregations for several genes in *S. tuberosum* $\times$ *S. chacoense* progenies (Quiros and McHale, 1985; Douches and Quiros, 1987a). Hawkes (1978) assigns the genomic formula *A1* to these two species, which belong to the South American subgroup. Most of the diploid species of this subgroup hybridise readily and produce fertile F_1 hybrids.

From pairing studies, Hawkes concluded that genomic differentiation in the conventional sense has not progressed very far among the South American diploid species. Ramanna and Hermesen (1979) suggest differentiation has not resulted in pairing failure at meiosis. However, when Swaminathan (1953) doubled the chromosomes of *S. rybinii* × *S. tuberosum* and *S. chacoense* × *S. tuberosum* species hybrids, quadrivalent frequencies were reduced in the *S. chc* × *tub* hybrids, suggesting low levels of genomic differentiation. To account for observations like these, Dvorak (1983) suggested that the genotypes of the diploid species act in a way to suppress heterogenetic pairing. The segregation data for T704 reported in this paper may supply evidence to support this proposal of genomic differentiation. Further work must be done with other accessions of *S. chacoense* and other diploid species to ascertain the significance of this question.

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