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Gene-Centromere Mapping by 4x-2x Matings in Potatoes

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Summary. Two of the four strands of a bivalent are recovered together in tetraploid progeny arising from 4x-2x matings. This provides a method for gene and centromere mapping. The cross pppp × Pp produced 62 nulliplex tetraploid individuals in a total of 951, i.e., 6.5%. The diploid clone was found to produce diplandrous gametes through first division restitution (FDR). The map distance P-centromere was estimated as 13.0 units, the limiting values at the 95% binomial confidence interval being 10.1 and 16.3. The mapping method is explained in detail and a formula is deduced to obtain genotypic series of 2n gametes under particular assumptions. The direction 4x × 2x is advantageous, since high seed set diploid clones which give 99% tetraploid progeny, and whose method of diplandroid formation is known are already available. Diploid clones heterozygous for many markers and tetraploids nulliplex for the same markers are needed to fully exploit this method.

Key words: Half-tetrad analysis – 2n gametes – Potato – Gene-centromere mapping

Introduction

Chromosome mapping needs to be expanded if significant progress is to be achieved in important areas of genetic research concerning tuber-bearing Solanums. The pace of advancement has no doubt been negatively influenced by the complexities associated with tetrasomic inheritance in the common potato. Furthermore, homozygous diploid clones, which suffer fairly drastic inbreeding effects (De Jong and Rowe 1971), are difficult to obtain because of self-incompatibility at the diploid level.

Diploid clones that produce 2n gametes (i.e., gametes with the unreduced number of chromosomes) afford a distinctive opportunity to circumvent some of these difficulties. When these clones are used in 4x-2x matings, the ensuing progeny is almost exclusively tetraploid, provided the frequency of 2n-gametes produced is high (Hanneman and Peloquin 1968). The significant event is that two strands of a bivalent in the diploid parent are recovered together in the tetraploid progeny. Consequently, a half-tetrad analysis, leading to gene-centromere mapping, can in principle be performed.

Since the early work with the attached X chromosomes of *Drosophila melanogaster* (Anderson 1925; Beadle and Emerson 1935), analyses of this sort have been performed in several higher organisms, notably by Rhoades and Dempsey (1966) in maize. More recently, half-tetrad analysis for an autosomal chromosome has been reported in *Drosophila melanogaster* (Baldwin and Chovnick 1967). The same principle has been used in the leopard frog (Volpe 1970), the Mexican axolot (Lindsley, Frankhauser and Humphrey 1956) and proposed to identify genes as being in a particular chromosome in man (Crow 1967).

Materials and Methods

Crosses of the form pppp × Pp were done between tetraploid cultivars and the diploid parent *Solanum tuberosum* Group Phureja, clone 1 of P.I. 225682. The gene *P* determines blue pigmentation of the hypocotyl, stem, flower and tuber. This diploid clone produces abundant diplandroids (2n sperms) thus giving rise to a high proportion of diplandrous tetraploids. The progeny was classified into triploids and tetraploids by means of chromosome counts in root tips, the former were discarded and the latter scored to determine two genotypic categories: pppp and P-pp.

The mapping method may be illustrated by considering the consequences of 2n sporogenesis in the heterozygous (Pp) diploid parent. Meiosis in the tetraploid parent is regarded to be normal, giving rise to diploid n gametes, as expected. Genetic segregation in the tetraploid would have greatly complicated the situation, but it was avoided by using nulliplex stocks.

It will be assumed that 2n gametes originate from failure of the first meiotic division to proceed until completion, the second

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meiotic division being normal or from suppression of the second meiotic division, the first having been normal. These two situations will be referred to as FDR (first division restitution) and SDR (second division restitution), respectively. The possibility that the 2n gamete output for a given clone and sex is made up of varying proportions of FDR and SDR will also be considered.

One may conceive of a population or pool of tetrads in existence at a certain moment during the modified meiotic process resulting in 2n gametes. This pool will be made up of two alternative types of tetrads: no exchange and single exchange tetrads. These will be referred to as NET and SET, respectively. These two groups will comprise the total population, such that if there is a proportion p of single exchange tetrads, all others, or a proportion 1-p will be no exchange tetrads. The proportion of SET's will be a function of the map distance between the locus in question and the centromere, the closer the gene is from the centromere the lower the probability of exchange.

In order to consider the possibility of varying proportions of first and second division restitution, one may let x be the fraction of 2n gametes resulting from FDR, so that the remaining fraction, 1-x, of 2n gametes have originated through SDR.

The way each of the two types of tetrads behave under each of the two modified meiotic processes to give rise to a 2n gamete, and the gametic series produced, are illustrated in Figure 1.

Putting together the results from Fig. 1, and summing the contributions of the four combinations of events possible, the gametic series may be expressed as follows:

$$\tfrac{1}{4}\,px + \tfrac{1}{2}(1-p)\,(1-x)\,AA \,:\, x + p - \tfrac{3}{2}\,px\,Aa \,:\, \tfrac{1}{4}\,px + \tfrac{1}{2}(1-p)\,(1-x)\,aa$$

So, with a contribution of both first division and second division restitution to the pool of 2n gametes, the equation cannot be solved, since there are two unknowns. Owing to this possible indetermination, one should be sure that either FDR only, or SDR only, is responsible for 2n gamete formation, i.e., either x = 1 or x =0. Segregation ratios from 2x X 4x matings would be subjected to this ambiguous interpretation until sporogenesis is studied in the female side. Clones which are capable of producing both diplandroids and diplogynoids (2n eggs) should be of use in this respect since they afford a situation where segregation ratios in reciprocal crosses could be compared.

The situation is further illustrated in Figure 2, where some arbitrary values have been assigned to p in order to solve the equation $y = x + p - (3/2)\,px$, the frequency of heterozygous 2n gametes. The frequency of each homozygous gamete is thus $(1-y)/2$. The limiting values of p are zero (100% no exchange between the locus and the centromere) and one (100% single exchanges). The zero value should be characteristic of all loci located very near (at) one centromere. The other limit, however, not only requires independence of the gene from its centromere, but also demands complete chiasma interference. It may be observed, for example, that there are two ways of getting all heterozygous gametes: either the gene is very close to the centromere (p = 0) and gametes are all formed through first meiotic division restitution (x = 1), or the gene is far from the centromere, there is complete chiasma interference (p = 1) and all gametes are formed through second division restitution (x = 0). It is interesting to notice in Figure 2 that, when there are twice as many FDR as SDR, the distribution of 2n gametes is always 1/6 *AA* : 4/6 *Aa* : 1/6 *aa*, irrespective of the value of p (the same as for a gene very close to the centromere in a duplex tetrasomic locus). In this situation loci cannot be mapped with respect to the centromere, since all behave alike, no matter how distant they are from the centromere. This particular situation is, then, different from all others, since, when $x \neq 2/3$ and a mixture of FDR and SDR occurs mapping is still possible in principle (it would be inefficient, though, and would require a previous estimation of the value of x for a given clone and sex).

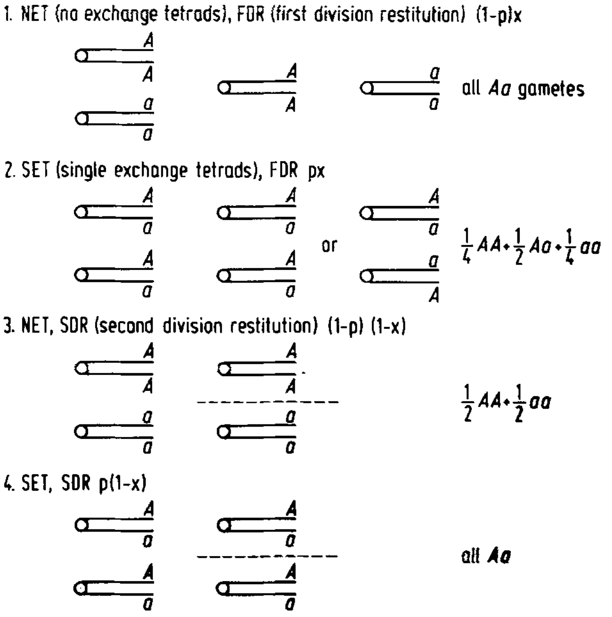

Fig. 1. Gametic series produced by a heterozygous locus under four possible situations

When the locus in consideration is located at a map distance from the centromere such that p = 2/3 the gametic series is the same, 1/6 *AA* : 4/6 *Aa* : 1/6 *aa*, irrespective of the proportion of first and second division restitution. Loci that are so located segregate similarly under all types of 2n sporogenesis considered here.

Mapping with Either FDR or SDR

When no mixture of FDR and SDR occurs in 2n sporogenesis the equations describing the gametic series can be expressed in terms of p. With first division restitution the gametic series for a simple

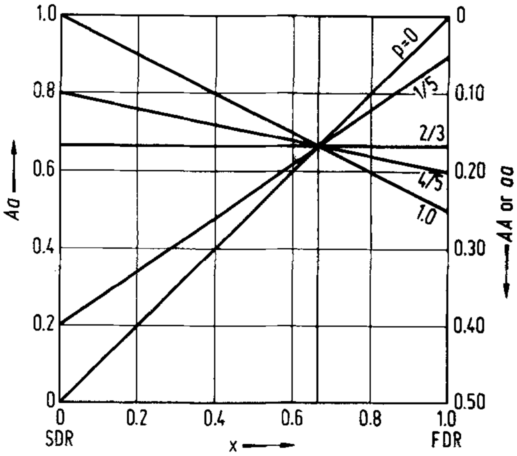

Fig. 2. Expected gametic series from different proportions of FDR (x) and SDR (1-x) under various assumed values of p

Table 1. Gametic series (2n) from *Aa* genotype with all FDR (X = 1) or all SDR (X = 0)

		Gametes		
		AA	*Aa*	*aa*
FDR	NET (1−p)	0	(1−p)	0
	SET (p)	$\frac{1}{4}p$	$\frac{1}{2}p$	$\frac{1}{4}p$
	TOTAL	$\frac{1}{4}p$	$1-\frac{1}{2}p$	$\frac{1}{4}p$
SDR	NET (1−p)	$\frac{1-p}{2}$	0	$\frac{1-p}{2}$
	SET (p)	0	p	0
	TOTAL	$\frac{1-p}{2}$	p	$\frac{1-p}{2}$

heterozygous locus is, (1/4)p *AA* : (1−(1/2)p) *Aa* : (1/4)p *aa*; and, with second division restitution (1−p)/2 *AA* : p *Aa* : (1−p)/2 *aa* (Table 1).

Assuming that double or higher order crossovers do not occur in potato it is possible to equate p to map distance between gene and centromere in the simplest manner. Since a single exchange tetrad is made up of 2 non-crossover strands and 2 single-crossover strands, (1/2)p estimates the map length of the relevant region. The assumption underlying this relation appears justified in potato for most of the chromosomal arms on the basis of chiasma frequencies (Howard 1970) and the short length of most chromosomal arms. The assumption will be incorrect when at least a double crossover occurs in the same chromosomal arm, but, even in this case, only the genes distal to both exchanges will be affected.

As is illustrated in Figure 3, with first meiotic division restitution the map distance between a gene locus and the centromere is estimated by twice the proportion of *aa* gametes, or nulliplex progeny from *aaaa-Aa* matings. Subtracting the observed frequency of aaaa progeny from 0.5 and multiplying by 100 provides an estimation of the corresponding map distance when second meiotic division restitution is responsible for 2n gameto-genesis, as is illustrated in Figure 4.

Results and Discussion

Crosses of the form pppp × Pp were done between tetraploid cultivars and the diploid parent *S. tuberosum* Group Phureja, clone 1 of P.I. 225682. This clone produces diplandrous gametes. These matings gave rise to 951 diplandrous tetraploids, 62 of which were nulliplexes pppp. The frequency of pp gametes produced by the diploid parent is thus estimated as 6.5 percent. Since chromosomes were actually counted in the progeny, there is no error introduced by scoring triploids which have originated from n gametes.

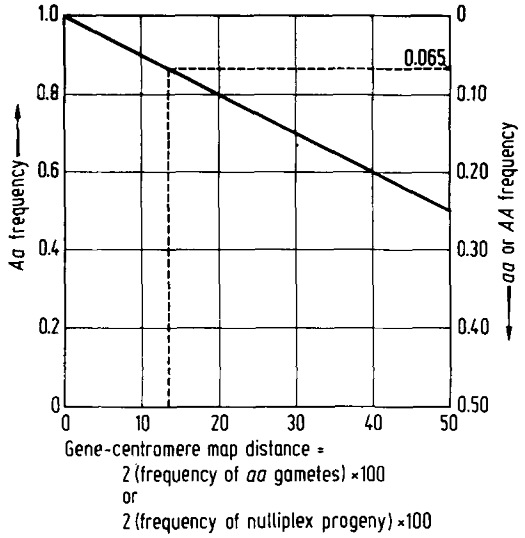

Fig. 3. Mapping relations with only FDR (based on nulliplex progeny from *aaaa* × *Aa* crosses)

The observed value may be accounted for by postulating that the P locus is almost independent from the centromere, located at least 43.5 map units from it, which requires second division restitution. Alternatively, the frequency of pp gametes may be taken to indicate that P is at an estimated 13.0 units of crossing-over from the centromere, providing that diplandrous gametes originated through first meiotic division restitution (Figs. 4 and 5).

The result is interpreted on the basis of first division restitution, since independent evidence from double reduction (Lunden 1937) places P close to its centromere.

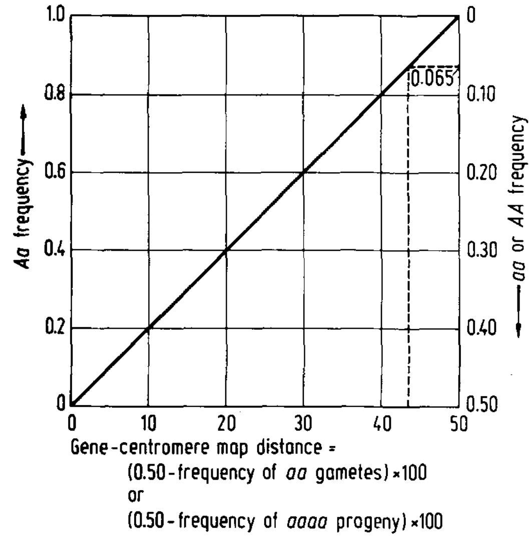

Fig. 4. Mapping relations with only SDR (based on nulliplex progeny from *aaaa* × *Aa* crosses)

(S.J. Peloquin has recently confirmed first meiotic division restitution in microsporogenesis of this clone by cytological methods). The upper and lower values of the 95% binomial confidence interval are 10.1 and 16.3 crossing over units. These values were obtained from tables published by Mainland, Herrera, and Sutcliffe (1956).

A more precise estimation of map distances with the same number of progeny would, of course, be attained by completely classifying them into nulliplexes, simplexes, and duplexes. With a dominant gene this demands progeny testing at the tetraploid level and, therefore, the precision thus gained would hardly compensate the extra effort.

The direction of the cross 4x × 2x is advantageous since many clones which produce diplandroids by only FDR and SDR have been identified (Mok and Peloquin 1975). The method of diplandroid, as compared to diplogynoid, formation is very much easier to ascertain cytologically. If the gene-centromere map distance is known, however, it can be used to deduce the methods of diplogynoid formation. Diplandroid producers heterozygous for many genes are needed to efficiently exploit this method of genetic mapping.

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