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Detecting and mapping repulsion-phase linkage in polyploids with polysomic inheritance

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Abstract It has been suggested that ratios of coupling- to repulsion-phase linked markers can be used to distinguish between allopolyploids and autopolyploids, because repulsion-phase linkages are much more difficult to detect in autopolyploids with polysomic inheritance than allopolyploids with disomic inheritance. In this report, we analyze the segregation pattern of repulsion-phase linked markers in polyploids without complete preferential pairing. The observed repulsion-phase recombination fraction (R) in such polyploids is composed of a fraction due to crossing-over (R_c) and another fraction due to independent assortment (R_i). R_i is the minimum distance that can be detected between repulsion-phase linked markers. Because R_i is high in autopolyploids (0.3373, 0.4000, 0.4286 and 0.4444) for autopolyploids of 2n=4*x*, 6*x*, 8*x* and 10*x*), large population sizes are required to reliably detect repulsion linkages. In addition, the default linkage used in mapping-programs must be greater than the corresponding R_i to determine whether a polyploid is a true autopolyploid. Unfortunately, much lower default linkages than the R_is have been used in recent polyploid studies to determine polyploid type, and markers have been incorporated into polyploid maps based on the R values. Herein, we describe how mapping repulsion linkages can result in spurious results, and present methods to accurately detect the degree of preferential pairing in polyploids using repulsion linkage analysis.

Keywords Polyploid genetics · Repulsion linkage · Preferential chromosome pairing

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Introduction

As many as 60% of all plant species are polyploids with multiple sets of chromosomes, and polyploidy is a major mechanism of plant adaptation and speciation (Stebbins 1971; Grant 1981; Levin 1983; Masterson 1994). One of the most fundamental questions about a polyploid species is whether it is an autopolyploid with polysomic inheritance or an allopolyploid with disomic inheritance. The determination of polyploid type is critical in elucidating phylogeny and developing appropriate breeding strategies. Unfortunately, the genetic constitutions of many natural polyploid species are unknown, especially those with high chromosome numbers.

Classically, polyploids with multivalent pairing were considered to be autopolyploids, while those with bivalent chromosome pairing were thought to be allopolyploids. This has proven to be an unreliable method, however, as homologues of autopolyploids often associate randomly into bivalents rather than multivalents (Crawford and Smith 1984; Soltis and Rieseberg 1986; Qu and Hancock 1998). Segregation ratios of molecular markers (isozyme and DNA) are now thought to be a more-reliable method of determining polyploid type, with polysomic ratios indicating autopolyploidy and disomic ratios signalling allopolyploidy (Soltis and Rieseberg 1986; Krebs and Hancock 1989; Qu and Hancock 1995).

Recently, two other methods have been proposed to distinguish between auto- and allo-polyploids, (1) comparing the number of loci linked in coupling vs repulsion-phase (Sorrells 1992; Wu et al. 1992), and (2) comparing the proportion of single- to multiple-dose markers (Da Silva et al. 1993). Low frequencies of multi-dose or repulsion-phase linked markers are thought to identify autopolyploids. These methods have been accepted but, as far as we know, there has been little critical assessment of the problems associated with the detection of repulsion-phase linkages and their application in determining polyploid type.

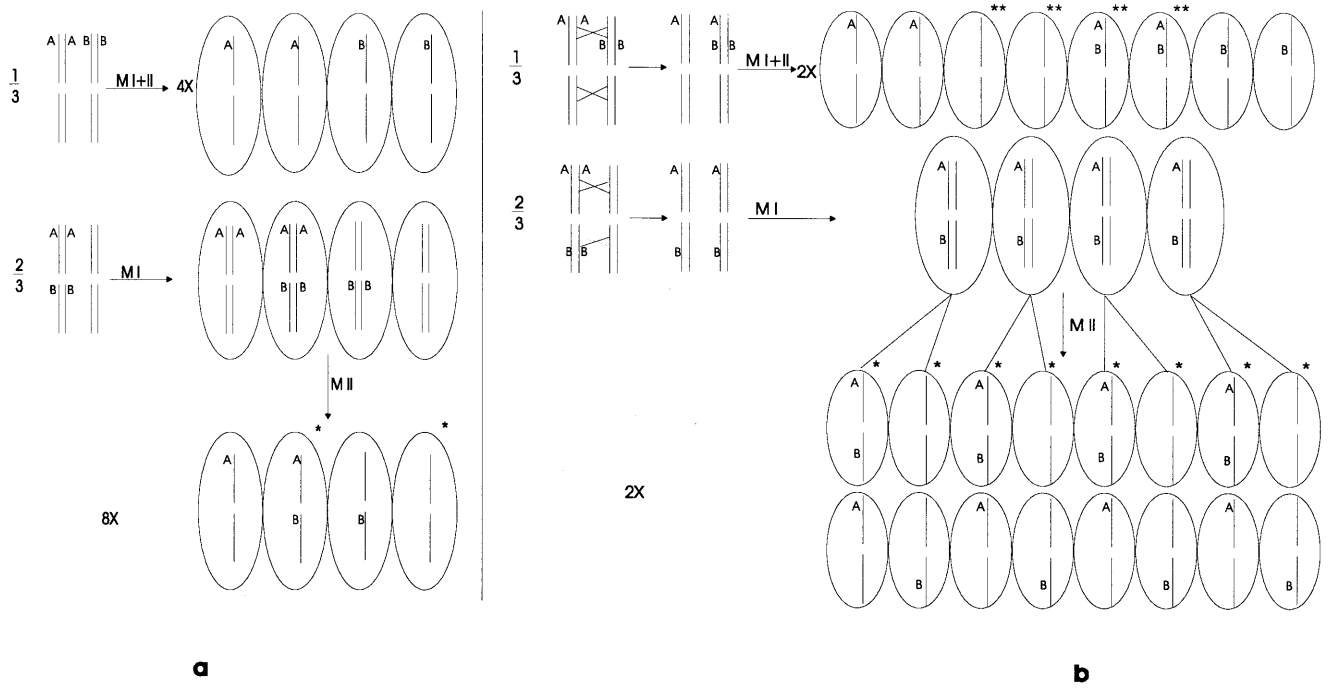

Fig. 1a, b Chromosome pairing patterns and gamete types of repulsion-phase linked markers in an autotetraploid. The genetic distance between markers A and B on two of the homologous chromosomes is 0 (**a**) and 50 cM (**b**). Gametes with one asterisk are the products of independent assortment, while those with two asterisks represent cross-over products. In both cases, 50% of the gametes resulting from independent assortment are recombinants, and the recombination fraction (R_i) is 0.3333 [(16/48)×100]

Mapping in polyploids is typically conducted by searching for 1:1 segregation markers, utilizing either single-dose restriction fragments (SDRF) (Sorrells 1992; Wu et al. 1992) or single-dose amplified fragments (SDAF) (Qu and Hancock 1997). To-date, in cross-pollinated species most of the maps have been based on a "pseudo-testcross", where heterozygous parents were hybridized rather than inbred lines. These hybridizations are analogous to test crosses, as one parent can be considered as the other's tester and vice versa, even though the actual mating configuration of the parents is not known (Ritter et al. 1990; Grattapaglia and Sederoff, 1994). Repulsion-phase linkages have been located by inverting (re-coding) the presence and absence data set for each single-dose marker and adding this inverted set to the original, and then searching for the linkages within the combined data set (Al-Janabi et al. 1993; Da Silva et al. 1993; Grattapaglia and Sederoff 1994).

The theoretical aspects of mapping polyploid species are only now beginning to emerge. Wu et al. (1992) discussed the problems associated with the detection of repulsion-phase linked markers in autopolyploids. They reported that, only with very large population sizes, repulsion-phase linkage can be detected in autopolyploids of high ploidy levels (≥6*x*, 8*x* and 10*x*) and suggested that the ratio of marker pairs linked in repulsion versus coupling phase would 0.25:1 in a population of 75 for an autotetraploid, but at higher ploidies would be effectively 0:1. In this report, we describe what precautions should be taken in detecting and mapping repulsion linkages in autopolyploids, and indicate how map distances between repulsion-phase linked markers can be misleading if calculated using observed recombination fractions. We also discuss how degrees of preferential pairing can be estimated in diploidizing polyploids using repulsion-phase linkage analysis.

Components of repulsion-phase recombination

In diploids and true allopolyploids which have strict disomic inheritance, recombination between markers on homologous chromosomes can occur only by crossing-over. In this pairing pattern, the number of markers linked in coupling and repulsion-phase is in a 1:1 ratio, and the level of recombination between both types of markers is an accurate representation of genetic distance. In autopolyploids**,** while recombination in coupling phase is the same as in allopolyploids, recombination in repulsion-phase can be produced both through independent assortment and crossing-over within a homologous group, depending on whether the two marker-bearing chromosomes pair with each other or with the non-marker-bearing homologues (Fig. 1). Recombinant genotypes are produced by crossing-over when a chiasma forms between repulsion-phase markers on two paired chromosomes. Recombinant genotypes are also formed by independent assortment, when the chromosomes carrying the repulsion-phase markers pair with the homologues not carrying the markers. Stated another way, the observed fraction of repulsion-phase recombi-

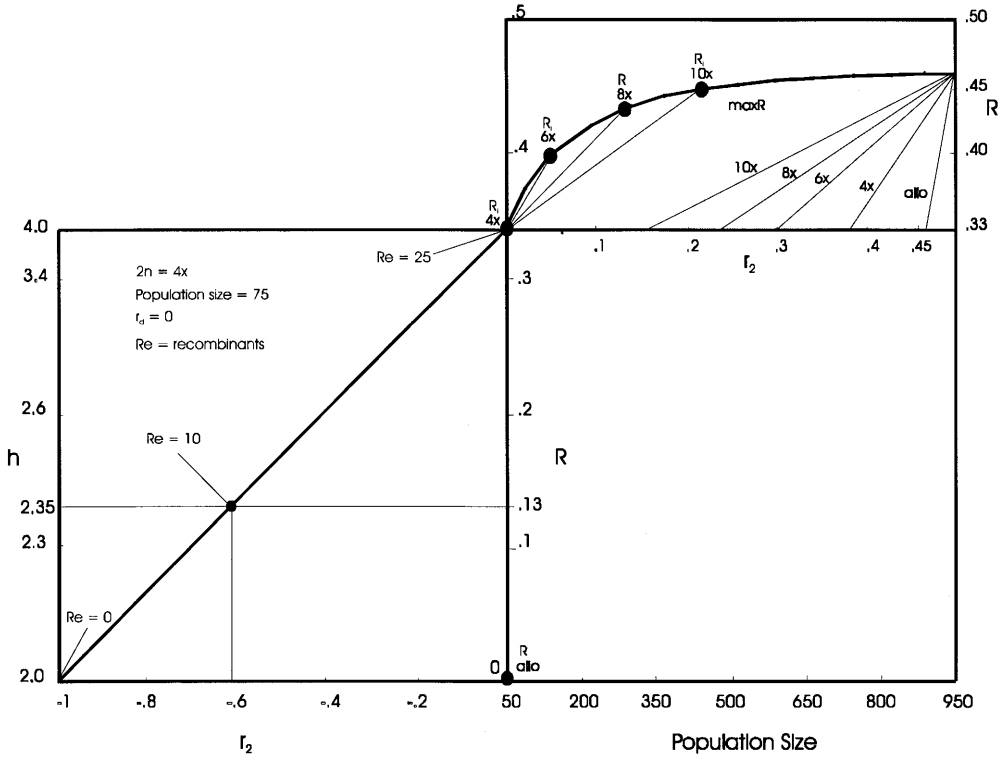

Fig. 2 The maximum recombination fractions (maxR) that can be detected for repulsion-phase linkages in polyploids (allo- and autopolyploids of 4*x*, 6*x*, 8*x* and 10*x*) (right), and the relationship of R with h and r_2 in a tetraploid population (left). *Right*. The curve is based on $maxr_1 = 0.5(1–2.3264\sqrt{1/n})$ (Wu et al. 1992) [$maxR = maxr_2/(h–1)+R_i = [0.5(1–2.3264(h–1)\sqrt{1/n}]/(h–1)+(h–2)/2(h–1) = 0.5(1–2.3264\sqrt{1/n}) = maxr_1$]. The larger population sizes required to detect repulsion-phase linkages in autopolyploids than for allopolyploids result from the large R_is of the autoploids. The R values for allopolyploids and autopolyploids fall on the same curve, but have different starting points (*black circles* ●), because they have different R_i values. In autopolyploids, if the default linkages used in mapping programs are lower than R_i, no repulsion linkages will be detected, regardless of the size of the population and the number of markers used. The maximum values of R and the corresponding r_2 for different ploidy levels are indicated by the *thin lines*. For example, the Max R = 0.45 and r_2 = (4–1) (0.45–0.33) = 0.36 for an autopolyploid. *Left*. The relationship of R with h and r_2 in a tetraploid population produced via 2n gametes from diploid parents. The r_d of a pair of repulsion-phase linked markers was determined by screening a diploid population generated by the parents. Once this r_d is known, R, r_2 and h can be calculated. For example, if r_d = 0 cM, and 25 recombinants are observed in a progeny population of 75 tetraploids, R=25/75 = 0.33 (equation 1), r_2=3×25/75 –1=0 (equation 2), and h = 2(0.33+0–1)/(2×0.33–1) = 4 (equation 4). Therefore, the tetraploid is a true autoploid, since all the recombinants had to be generated by independent assortment. If the observed number of recombinants is 10 (R = 10/75 = 0.13<R_i, r_2 = –0.6<0, and h = 2.35), preferential pairing is indicated. If no recombinants are detected, strict preferential pairing is indicated and the polyploid is an allopolyploid (R = 0, r_2 = –1, and h = 2)

nants (R) is composed of a recombination fraction due to crossing-over (R_c) (cross-overs that bring two repulsion-phase linked markers together on one chromosome) and a recombination fraction (R_i) resulting from independent assortment (independent assortment that brings two repulsion-phase linked markers into one gamete, but the markers are still on two individual homologous chromosomes).

In autopolyploids, the frequency of pairing between two homologous chromosomes carrying the repulsion-phase markers is 1/(h–1), where h = the number of homologues in a group. The recombination fraction due to independent assortment of repulsion markers is $R_i = {}^1/_2[1–1/(h–1)]n/n = (h–2)/2(h–1)$, where n is the total number of gametes. The recombination fraction resulting from crossing-over is $R_c = r_2n/(h–1)/n = r_2/(h–1)$, where r_2 is the genetic distance between two markers linked in repulsion-phase. The total observed recombination fraction is

$$R = a/n = R_i+R_c = (h–2)/2(h–1)+r_2/(h–1),$$

where a is the observed number of recombinants in repulsion-phase linkage. This equation can be rearranged to

$$r_2 = [(h–1)\ a–0.5(h–2)n]/n.$$

A similar equation for calculating r_2 also has been developed by Wu et al. (1992) in another way. For a known autopolyploid the simplest way to calculate r_2 is

$$r_2 = (h–1)(R–R_i).$$

In any autopolyploid with completely polysomic inheritance, R_i is greater than R_c in a repulsion linkage. In fact, while R_c is a variable parameter that is dependent on genetic distance, R_i is a fixed value that depends on ploidy level. Using $R_i = (h–2)/2(h–1)$, these values are 0.3333, 0.4000, 0.4286 and 0.4444 for autopolyploids of 2n = 4*x*, 6*x*, 8*x* and 10*x*. When a pair of repulsion-phase markers is completely linked in an autopolyploid (r_2 = 0, there-

Table 1 Parameters associated with recombination of repulsion-phase linkages in polyploids

Ploidy	R	R_i		r_2		Pairing	
		Auto	Range	Auto	Range[a]	Random	Preferential[a]
Allo	0–0.50	0	0	R	0–0.50	$R = r_2 \geq 0$	–
4*x*	0–0.50	0.3333	0–0.3333	3(R = 0.3333)	–1–0.50	$R \geq 0.3333$ $r_2 \geq 0$	$-1 \leq r_2 \leq 0$
6*x*	0–0.50	0.4000	0–0.4000	5(R = 0.4000)	–2–0.50	$R \geq 0.4000$ $r_2 \geq 0$	$-2 \leq r_2 \leq 0$
8*x*	0–0.50	0.4286	0–0.4286	7(R = 0.4286)	–3–0.50	$r \geq 0.4286$ $r_2 \geq 0$	$-3 \leq r_2 \leq 0$
10*x*	0–0.50	0.4444	0–0.4444	9(R = 0.4444)	–4–0.50	$R \geq 0.4444$ $r_2 \geq 0$	$-4 \leq r_2 \leq 0$

[a] Based on equation 2 or 3

fore, $R_c = 0$), all the recombinant progeny will come from independent assortment (i.e., $R = R_i$); as a result, R_i at each ploidy level is the minimum value that can be detected through segregation analysis between two markers linked in repulsion-phase. Stated another way, to detect a complete linkage ($r_2 = 0$) between a pair of repulsion-phase markers in an autopolyploid, the required population size is equal to a population size needed for detecting the recombination fraction R_i of the polyploid (e.g., 0.4 for an autohexaploid). This is why large population sizes are required to detect repulsion-phase linkages in autopolyploids (Fig. 2, right), as others have noted (Wu et al. 1992).

These principles can be applied to polyploids with different degrees of preferential pairing. Equation $R_i = (h–2)/2(h–1)$ indicates that variation on R_i is dependent on ploidy levels in autopolyploids. R_i is also influenced by preferential pairing when applied to all polyploids. The value of h will change as a polyploid with polysomic inheritance becomes diploidized with disomic inheritance. The value of h would only equal 2 when there was strict bivalent pairing. Therefore, if an autopolyploid gradually evolves to an allopolyploid, the value of h can be thought of as becoming progressively smaller until the completely diploidized level of h = 2 is reached. The value of R_i will begin to drop when the two homologous chromosomes with the repulsion-phase markers pair more frequently with each other than at random. Put another way, the higher the frequency of preferential pairing, the lower the value for h and R_i. In fact, R_i in a polyploid with any degree of preferential pairing will be smaller than the R_i in an autopolyploid of the same ploidy level. Because the minimum observed recombination fraction R for a polyploid is R_i, and R_i reaches the maximum in a true autopolyploid, a R value detected in a polyploid with preferential pairing can be smaller than the R_i that is for a true autopolyploid of the same ploidy level. Without further indication, R_i in the following text will be for an autopolyploid.

Equation 3 indicates that if R is $<R_i$, a negative value for r_2 will arise. This signals preferential pairing in the polyploid. Applying equations 1 and 2, in tetra-, hexa-, octa- and decaploids, r_2 will vary from –1, –2, –3 and –4 to 0.5 and R_i from 0 to 0.3333, 0.4000, 0.4280 and 0.4444, respectively. Therefore, if all R values are $\geq R_i$ ($r_2 \geq 0$) for a polyploid, it has completely random association of homologues, while if R is $<R_i$ ($r_2<0$), preferential pairing is indicated. For example, if all Rs are ≥0.3333 (r_2s≥0 cM) for a tetraploid, it is a true autotetraploid with no preferential pairing among homologous chromosomes. Conversely, if $-1<r_2<0$ cM ($0<R<0.3333$) is detected, preferential pairing is indicated. Unfortunately, the value for R cannot be used to estimate the degree of preferential pairing, as the same R can be produced by different combinations of crossing-over and independent assortment. Likewise, r_2 is a poor indicator of the degree of preferential pairing as it is calculated from R. A strict allopolyploid is most rigorously established if repulsion-phase linkages are found with $r_2=–1$ ($R_i = 0$) for a tetraploid, since this can only arise if two homologous chromosomes pair solely with each other. The parameters associated with recombination of repulsion-phase linkages are outlined in Table 1.

The R values discussed so far are based on the assumption that pairing occurs either randomly among homologues or preferentially only between homologous carrying the repulsion-phase linked markers. In reality, preferential pairing might also occur between one of the chromosome carrying a repulsion-phase marker and a homologous chromosome without the corresponding marker. When this occurs, the R_i will be larger than would be expected due to random pairing and, as a result, some tightly linked loci ($r_2 = 0$) will have larger R (e.g., >0.3333 in a tetraploid) making them appear to be largely random assorting. However, detecting a value of R that is less than R_i ($r_2<0$) is always an accurate indicator of preferential pairing for a polyploid.

Detecting and mapping repulsion-phase linkages in autopolyploids

It has been suggested that the type of polyploid can be distinguished by comparing the expected with the ob-

served number of markers linked in coupling and in repulsion-phase (Wu et al. 1992). As already has been indicated, the detection of repulsion-phase linkages in polyploids has been done by analyzing the combined data set of original markers and its inverse (Al-Janabi et al. 1993; Da Silva et al. 1993; Mudge et al. 1996). The computer mapping program MAPMAKER (Lander et al. 1987) is typically utilized to calculate map distances.

Our discussion above stresses how important it is that the proper default linkage be selected in such an analysis, and these values depend on ploidy levels. For example, if a default linkage less than 0.3333 is used in a autotetraploid population, no matter how large the population size and the number of markers used, it will be impossible to detect any repulsion-phase linkages, because the R_i in autotetraploids is 0.3333.

To accurately detect repulsion-phase linkage in polyploids with polysomic inheritance, it is also necessary to analyze each pair of markers individually rather than in a combined data set. This approach has been used to detect repulsion-phase linkages between specific markers supposedly on homologous chromosomes, but has not been used for mapping whole genome linkages (Grivet et al. 1996; Ming et al. 1998). Pairwise comparisons are necessary because R in a polyploid with preferential pairing is larger than r_2 due to independent assortment and, when R values are placed on the map, they will break most other reasonable linkages between coupling-phase markers and, as a result, will be left out of the linkage group (Fig. 3). Therefore, when analyzing the combined data set of a polysomic polyploid, some repulsion linked markers will be left undetected and the mapped repulsion-phase linkages will have enlarged map distances because R values are used. In addition, the repulsion-phase linkages will map at only one of three positions; (1) between end markers of two coupling groups, (2) as a single repulsion-phase marker linked to an end marker of a coupling group, and (3) as a single linkage group of two markers. These are exactly the orientations of the 12 repulsion-phase linkages identified by Mudge et al. (1996) in sugarcane mapping using a combined data set.

Even if pairwise comparisons are made in polyploids with polysomic inheritance, repulsion-phase markers can still not be accurately placed on a map, unless degrees of preferential pairing are known. This is the case when R is used to calculate map distance, and the R values generated for the repulsion-phase markers are a function of both cross-overs and independent assortment. We suggest that repulsion linkages can only be placed on a polyploid map if the degree of preferential pairing (h′) among chromosomes in the same homologous group is known, so that the real r_2 can be calculated. If R values are used to build a map in polyploids with an unknown degree of preferential pairing, only coupling-phase linkages can be utilized in map construction.

Therefore, to place repulsion-phase linkages accurately on the map of a polyploid with preferential pairing,

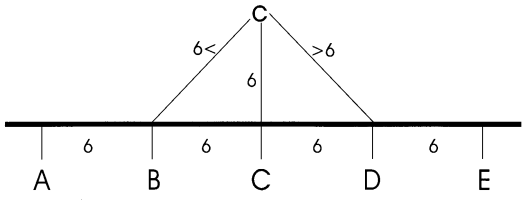

Fig. 3 An illustration that in a polyploid with polysomic inheritance, a pair of repulsion-phase linked markers with a R value can not be linked to each other by an analysis of the combined marker data set, if one of the markers has already been linked to a coupling-phase group and is not located at the ends of the group. Markers A, B, C, D and E are in coupling-phase linkage. Marker c is in repulsion-phase linked to C. The actual distance between c and C is r_2=0 cM. However, because recombinants are also generated by independent assortment, the R between c and C is 6 cM. As a result, the Rs between c and A, B, D and E must be >6 cM. Therefore, marker c can not be linked directly to markers B, C and D in the coupling-phase linkage group. The marker c may be linked to A or E if the distance between them is less than the default linkage set up for the analysis

the following conditions must be met: (1) each pair of markers must be examined individually, (2) the degree of preferential pairing (h′) in the species must be known such as for a true autopolyploid (h′ = 4), and (3) the genetic distance is expressed by r_2, or indicated as such when R is used. These issues have not been considered in most of the repulsion linkage studies conducted to-date on such polyploids (Da Silva et al. 1993; Al-Janabi et al. 1994; Mudge et al. 1996).

As an illustration, we can use our own studies in blueberry. We previously determined that an interspecific tetraploid hybrid US 75 derived from *Vaccinium darrowi* (2x)×*V. corymbosum* (4*x*) is an autotetraploid by analyzing segregation ratios of single-locus double-dose markers (Qu and Hancock 1995). We then developed a linkage map (48 coupling linkage groups) of the hybrid using MAPMAKER (Qu and Hancock 1997). In this study, in order to determine if any degree of preferential pairing exists in US 75, we first combined the original data set (154 markers of 1:1 ratio) and the inverted data set and ran MAPMAKER to detect repulsion-phase linkages. The default linkage was set to 34.66 cM [the maximum detectable recombination fraction (Max R) is 35.1 cM ($p \leq 0.01$) (Wu et al. 1992)], for our population size of 61. In this analysis, we detected only one pair of repulsion linkages located between two end markers of two coupling linked groups (R = 33.8 cM, r_2 = 1.4 cM).

If we take the same recombination data and examine each pair of repulsion-phase markers individually (154×154=23 716 pairs), we detected six more pairs of repulsion-phase linkages in four homologous groups (Fig. 4). Three of the seven repulsion linkages are >33.33 cM and the smallest is 27.8 cM (r_2 = –0.17). Since no repulsion-phase linkages were detected that are significantly smaller than the R_i (33.33 cM), our previous conclusion that the inheritance pattern of US 75 is tetrasomic (Qu and Hancock 1995) is strongly supported.

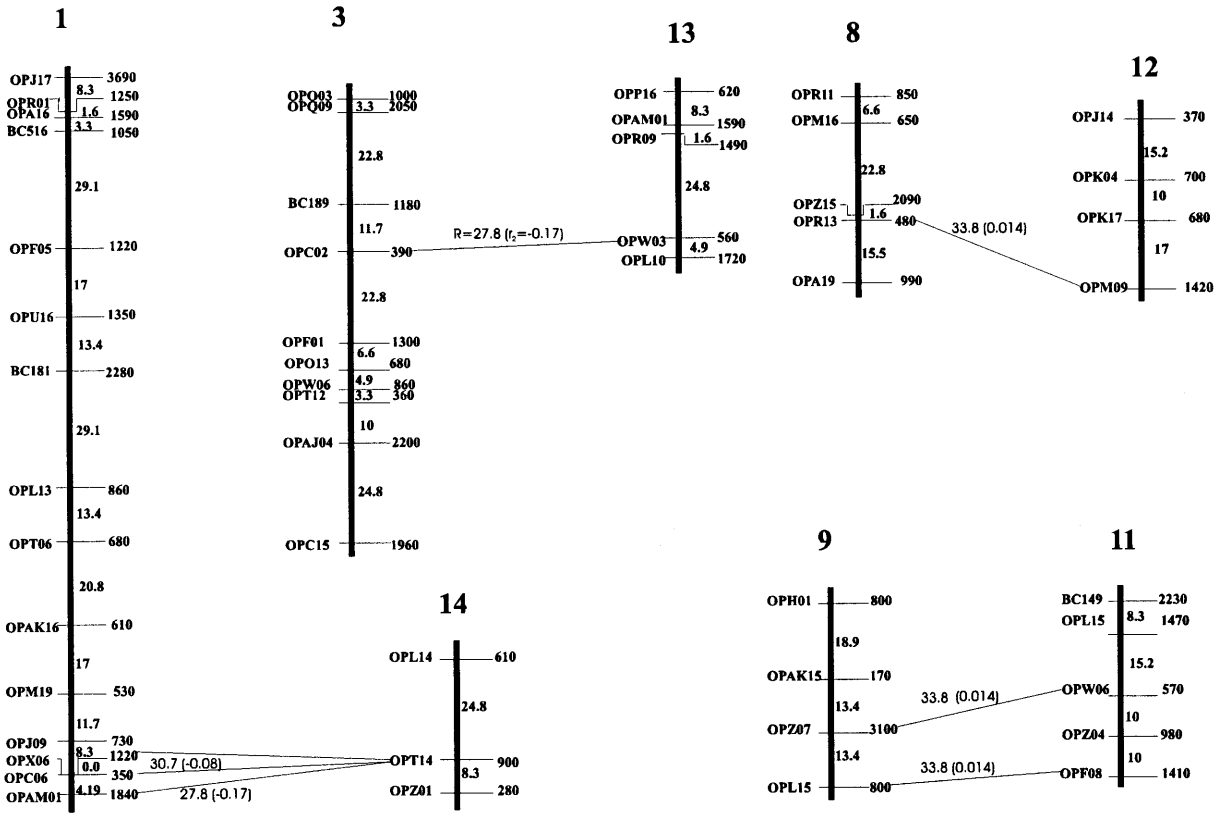

Fig. 4 Results of our repulsion-linkage analysis where the markers were examined in pairwise combinations vs the combined marker set (see text for detail). Utilizing the combined data set, only one repulsion linkage was detected (in groups 9 and 11, between a group-end marker OPL15–800 and a group-end marker OPF08–1410). Using pairwise comparisons, six more repulsion linkages were detected between non-group-end markers. Because of negative values, r_2s were not converted to cM values to avoid confusion

Detecting degrees of preferential pairing in polyploids

Polyploids with known parents

If a 2n gamete from a known diploid species is involved in the formation of a polyploid, repulsion linkage can be used to estimate levels of preferential pairing. The distance between repulsion-phase linked markers (r_d) should first be determined in a segregating 2*x* population produced by the parent, and then the degree of preferential pairing in the polyploid can be estimated by comparing the observed recombination frequencies (R) of the markers in the polyploid population with r_d. The closer the R is to r_d, the stronger is the preferential pairing. The parameter h also can be used to estimate preferential pairing, if we rearrange equation 1 into

$$h' = 2(R+r_d-1)/(2R-1),$$

where $r_d \geq 0$ (because it has been determined in the diploid population) and $2 \geq h' \geq 4$ for a tetraploid, $2 \geq h' \geq 6$ for a hexaploid, $2 \geq h' \geq 8$ for a octaploid and $10 \geq h' \geq 2$ for a decaploid. As has been discussed, the smaller the h, the stronger is the preferential pairing. If h = 2, or $R=r_d$, the polyploid has completely disomic pairing and therefore is an allopolyploid. Figure 2 (left) illustrates how the degree of preferential pairing in a tetraploid can be estimated using such repulsion-phase linked markers that are 0 cM apart in the diploid parent. Table 2 shows the relationship between R, r_2 and h for different ploidy levels.

Polyploids with unknown parents

We have already noted that while preferential pairing is indicated in a polyploid if R between repulsion linked markers is less than R_i ($r_2<0$), the exact degree of preferential pairing is not known. However, when a large number of marker linkages are evaluated (e.g., >150), several pairs of coupling-phase linked markers of 0 cM apart may be found. While we cannot detect repulsion-phase linkages this close due to independent assortment, we can assume that the actual distance between the closest linked repulsion-phase markers would also be 0 cM, since the likelihood of detecting marker pairs 0 cM apart would be similar for coupling- and repulsion-phase linkages. Therefore, the R value of the closest repulsion linkage can be taken as the R_i for the polyploid. The degree of preferential pairing can be estimated by comparing this R_i with the R_i for an autopolyploid of the same ploidy level, or by calculating r_2 and h using equations 2 and 4, respectively.

Table 2 Parameters associated with preferential pairing in polyploids when the actual repulsion linkage is r_2=0 cM[a]

4*x*			6*x*			8*x*			10*x*		
R	r_2	h′	R	r_2	h′	R	r_2	h′	R	r_2	h′
0.3333	0	4	0.40	0	6	0.4286	0	8	0.4444	0	10
0.32	–0.0399	3.7777	0.39	–0.0500	5.5455	0.42	–0.0602	7.2500	0.43	–0.1296	8.1429
0.31	–0.0699	3.6315	0.38	–0.1000	5.1667	0.41	–0.1302	6.5556	0.42	–0.2196	7.2500
0.30	–0.0999	3.5000	0.37	–0.1500	4.8462	0.40	–0.2002	6.0000	0.41	–0.3096	6.5556
0.29	–0.1299	3.3810	0.36	–0.2000	4.5714	0.39	–0.2702	5.5455	0.40	–0.3996	6.0000
0.28	–0.1599	3.2727	0.35	–0.2500	4.3333	0.38	–0.3402	5.1667	0.39	–0.4896	5.5455
0.27	–0.1899	3.1739	0.34	–0.3000	4.1250	0.37	–0.4102	4.8462	0.38	–0.5796	5.1667
0.26	–0.2199	3.0833	0.33	–0.3500	3.9412	0.36	–0.4802	4.5714	0.37	0.6696	4.8462
0.25	–0.2499	3.0000	0.32	–0.4000	3.7778	0.35	–0.5502	4.3333	0.36	–0.7596	4.5714
0.24	–0.2799	2.9231	0.31	–0.4500	3.6316	0.34	–0.6202	4.1250	0.35	–0.8496	4.3333
0.23	–0.3099	2.8519	0.30	–0.5000	3.5000	0.33	–0.6902	3.9412	0.34	–0.996	4.1250
0.22	–0.3399	2.7857	0.29	–0.5500	3.3810	0.32	–0.7602	3.7778	0.33	–1.0296	3.9412
0.21	–0.3699	2.7241	0.28	–0.6000	3.2727	0.31	–0.8302	3.6316	0.32	–1.1196	3.7778
0.20	–0.3999	2.6667	0.27	–0.6500	3.1739	0.30	–0.9002	3.5000	0.31	–1.2096	3.6316
0.19	–0.4299	2.6129	0.26	–0.7000	3.0833	0.29	–0.9702	3.3810	0.30	–1.2996	3.5000
0.18	–0.459	2.5625	0.25	–0.7500	3.0000	0.28	–1.0402	3.2727	0.29	–1.3896	3.3810
0.17	–0.4899	2.5152	0.24	–0.8000	2.9231	0.27	–1.1102	3.1739	0.28	–1.4796	3.2727
0.16	–0.5199	2.4706	0.23	–0.8500	2.8519	0.26	–1.1802	3.0833	0.27	–1.5696	3.1739
0.15	–0.5499	2.4286	0.22	–0.9000	2.7857	0.25	–1.2502	3.0000	0.26	–1.6596	3.0833
0.14	–0.5799	2.3889	0.21	–0.9500	2.7241	0.24	–1.3202	2.9231	0.25	–1.7496	3.0000
0.13	–0.6099	2.3514	0.20	–1.0000	2.6667	0.23	–1.3902	2.8519	0.24	–1.8396	2.9231
0.12	–0.6399	2.3158	0.19	–1.0500	2.6129	0.22	–1.4602	2.7857	0.23	–1.9296	2.8519
0.11	–0.6699	2.2821	0.18	–1.1000	2.5625	0.21	–1.5302	2.7241	0.22	–2.0196	2.7857
0.10	–0.6999	2.2500	0.17	–1.1500	2.5152	0.20	–1.6002	2.6667	0.21	–2.1096	2.7241
0.09	–0.7299	2.2195	0.16	–1.2000	2.4706	0.19	–1.6702	2.6129	0.20	–2.1996	2.6667
0.08	–0.7599	2.1905	0.15	–1.2500	2.4286	0.18	–1.7402	2.5625	0.19	–2.2896	2.6129
0.07	–0.7899	2.1628	0.14	–1.3000	2.3889	0.17	–1.8102	2.5152	0.18	–2.3796	2.5625
0.06	–0.8199	2.1364	0.13	–1.3500	2.3514	0.16	–1.8802	2.4706	0.17	–2.4696	2.5152
0.05	–0.8499	2.1111	0.12	–1.4000	2.3158	0.15	–1.9502	2.4286	0.16	–2.5596	2.4706
0.04	–0.8799	2.0870	0.11	–1.4500	2.2821	0.14	–2.0202	2.3889	0.15	–2.6496	2.4286
0.03	–0.9099	2.0638	0.10	–1.5000	2.2500	0.13	–2.0902	2.3514	0.14	–2.7396	2.3889
0.02	–0.9399	2.0417	0.09	–1.5500	2.2195	0.12	–2.1602	2.3158	0.13	2.8296	2.3514
0.01	–0.9699	2.0204	0.08	–1.6000	2.1905	0.11	–2.2302	2.2821	0.12	–2.9196	2.3158
0	–1	2	0.07	–1.6500	2.1628	0.10	–2.3002	2.2500	0.11	–3.0096	2.2821
			0.06	–1.7000	2.1364	0.09	–2.3702	2.2195	0.10	–3.0996	2.2500
			0.05	–1.7500	2.1111	0.08	–2.4402	2.1905	0.09	–3.1896	2.2195
			0.04	–1.8000	2.0870	0.07	–2.5102	2.1628	0.08	–3.2796	2.1905
			0.03	–1.8500	2.0638	0.06	–2.5802	2.1364	0.07	–3.3696	2.1628
			0.02	–1.9000	2.0417	0.05	–2.6502	2.1111	0.06	–3.4596	2.1364
			0.01	–1.9500	2.0204	0.04	–2.7202	2.0870	0.05	–3.5496	2.1111
			0	–2	2	0.03	–2.7902	2.0638	0.04	–3.6396	2.0870
						0.02	–2.8602	2.0417	0.03	–3.7296	2.0638
						0.01	–2.9302	2.0204	0.02	–3.8196	2.0417
						0	–3	2	0.01	–3.9096	2.0204
									0	–4	2

[a] R is the observed repulsion phase recombination fraction; r_2 is based on equation 3; h′ is based on equation 4

Conclusions

Awareness of the contribution R_i to R is fundamentally important to understanding the segregation of repulsion-phase linkages in polyploids with polysomic inheritance. Evidence is emerging that autopolyploidy is much more common than previously thought, is a much more dynamic evolutionary force (Thompson and Lumaret 1992; Sotis and Sotis 1993, Qu and Hancock 1998), and that the rate of autopolyploid formation may be as high as the spontaneous mutation rate for individual genes (10^{-5}) (Ramsey and Schemske 1998).

Clearly, genomic diploidization is thought by many to be an important step in the adaptation of autopolyploids (Stebbins 1974; DeWet 1980). However, With the exception of the *ph* gene in wheat (Riley and Chapman 1958; Gill et al. 1993), there is little direct information on pairing control genes in polyploids, even though they are probably present in natural autopolyploid populations (Jackson and Hauber 1994). It is not known if diploidization is due to the accumulation of minor or major mutations regulating chromosomal pairing relationships, but polyploids with differential levels of preferential pairing have been described, indicating that diploidization can be a gradual process. Qu and Hancock (1998) has suggested that there may even be an alternate type of diploidization in the evolution of polysomic inheritance where genes arise that lead to bivalent rather than quand-

rivalents. In the methods described above, the diploidization process could be monitored by following r_d.

In conclusion, great care must be taken in detecting repulsion-phase linkages in autopolyploids and placing them on genetic maps. It must be recognized that repulsion recombinant progeny result from both independent assortment and crossing-over, and that the selection of proper default linkages is extremely critical to identifying repulsion-phase linkages in the first place. Repulsion linkages cannot be placed directly on a genetic map unless three conditions are met: (1) each pair of markers is examined individually, (2) the degree of preferential pairing in the species is known, and (3) the genetic distance is expressed by r_2 not R. The degree of preferential pairing can be estimated if r_2 in the progenitor diploid can be accurately determined, or by a search for the closest linked repulsion markers in a large number of segregating markers in a population of an unknown polyploid type.

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