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Pitfalls of genetic analysis using a doubled-haploid backcrossed to its parent

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Abstract A population derived from a doubled-haploid backcrossed to its parent has a unique genetic structure, which affects the mapping and genetic analysis of molecular-marker data. In such a cross: (1) repulsion linkages are reduced dramatically and can be detected only in restricted chromosome sections; each repulsion-linkage group represents a section of a chromosome carrying a crossover that occurred in the meiosis that produced the gamete from which the doubled-haploid was derived; (2) the number of coupling-linkage groups observed depends on how many crossovers occurred during the meiosis; the observed size of a linkage group will be only a fraction of a total chromosome if the chromosome carried in the doubled-haploid resulted from crossover exchanges; (3) the size covered by all the observed linkages is only equivalent to the haploid genome; and (4) the ratio of single-dose to multi-dose markers is inflated. These features have not been recognized in previous reports, resulting in misinterpretations in genetic mapping and analysis of the molecular-marker data. The ratio of single- to multi-dose markers has been used inappropriately to distinguish polyploid type.

Keywords Genetic analysis · Genetic mapping · Doubled-haploid · Polyploidy

Introduction

Genetic mapping has recently been conducted in octoploids using populations generated by backcrossing a doubled-haploid to its parent (Al-Janabi et al. 1993; Da Silva et al. 1993). Populations with such a construction are thought to facilitate genetic mapping (Wu et al. 1992). However, besides the reduced production of polymorphic markers from the parent [for example, approximately 50% reduction of single-dose markers (Al-Janabi et al. 1993; Da Silva et al. 1993)], neither repulsion nor coupling linkages in such a cross can be analyzed similarly to other crosses. The unique properties of such a cross have not been recognized in previous studies (Al-Janabi et al. 1993; Da Silva et al. 1993), resulting in misinterpretations in the genetic mapping and analysis of molecular-marker data, and the development of an incorrect method that uses the ratio of single- to multi-dose markers to distinguish polyploid types.

Haploids are meiotic products that are produced artificially through anther culture or spontaneously during sexual reproduction (Guha and Maheshwari 1964; Burk et al. 1979; Maheshwari et al. 1982). The chromosome numbers of haploids are usually doubled in vitro using chemical agents such as colchicine, but spontaneous production of doubled-haploid plants also occurs during anther culture. The resulting doubled-haploid contains duplicated genes at each locus (identical alleles) which will not segregate. When a doubled-haploid is backcrossed to its parent, meiotic crossover-exchanges that are carried by the gamete producing the doubled-haploid will play an important role in the genetic mapping and analysis of the population. In this paper, we describe the genetic ramifications of crossing with doubled-haploids and outline the precautions that must be considered in analyzing the molecular-marker data.

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Special genetic considerations associated with doubled-haploid backcrosses

The number of repulsion linkages is dramatically reduced in crosses with doubled-haploids, and the linkages can be found only in restricted chromosome sections

It has previously been demonstrated that the detection of repulsion-phase linkages in a polysomic polyploid re-

quires a much larger population size than in a disomic polyploid, because the recombination fraction is expanded due to independent assortment (Wu et al. 1992; Qu and Hancock 2001). In crosses of species with disomic inheritance, the frequency of coupling and repulsion-phase linkages is equal regardless of population size. However, if a doubled-haploid is crossed with its parent, the repulsion-phase linkages are reduced dramatically and are found only on restricted chromosome sections. A repulsion-phase linkage can be observed in a backcross population of a doubled-haploid only if the doubled-haploid carries chromosomes containing crossover exchanges that occurred during its parent's meiosis. If there were no crossovers, the doubled-haploid contains only a duplication of entire parental homologues, and therefore no repulsion linkages will be detected in the cross.

A diagram of this can be seen in Fig. 1 which depicts the meiotic products from a homologous group in a plant with disomic inheritance. Only the crossover products found in gametes #2 and #3 will result in the detection of repulsion-phase linkages in this group, if doubled-haploids are produced from the gametes and backcrossed to its parent. This will greatly limit the number of the original repulsion-phase linkages that can be detected in the parent. Even if a crossover occurs in every bivalent, only $n/2$ of the total homologous groups will carry detectable repulsion-phase linkages since in each homologous group the chromatids without carrying exchanges have an equal probability to be included in a gamete (Fig. 1), where n is the number of homologous groups and 1/2 is the probability of recovering a gamete containing exchanges.

For a repulsion linkage to be detected in crosses with doubled-haploids, the two linked markers in the parent must both be absent in the chromosomes recovered in the doubled-haploid. This occurs only if there was a crossover between the two markers (e.g., c and d, Fig. 1). Therefore, the larger the distance between the two markers, the higher is the frequency that both markers are absent in the doubled-haploid. For example, if two repulsion-phase markers are 25 cM apart without double crossovers, the frequency of a gamete without either of the markers is 1/8 (the frequency of a crossover occurring between the two markers is 1/2, and the frequency of recovering the gamete is 1/4). For two markers 10-cM apart, the frequency of chromosomes without markers is 1/20 (the frequency of a crossover occurring between the two markers is 1/5, and the frequency of recovering the gamete is 1/4).

The number of repulsion-phase linkages that can be detected in crosses with doubled-haploids also depends on the default linkage used in mapping (maximum recombination fraction). The default linkage not only sets a limit on the linkage distance between two markers, but also determines the size of the chromosome sections available for repulsion-phase mapping. The only markers that can be mapped are those found within the default-linkage distance measured from the crossover point (Fig. 2C).

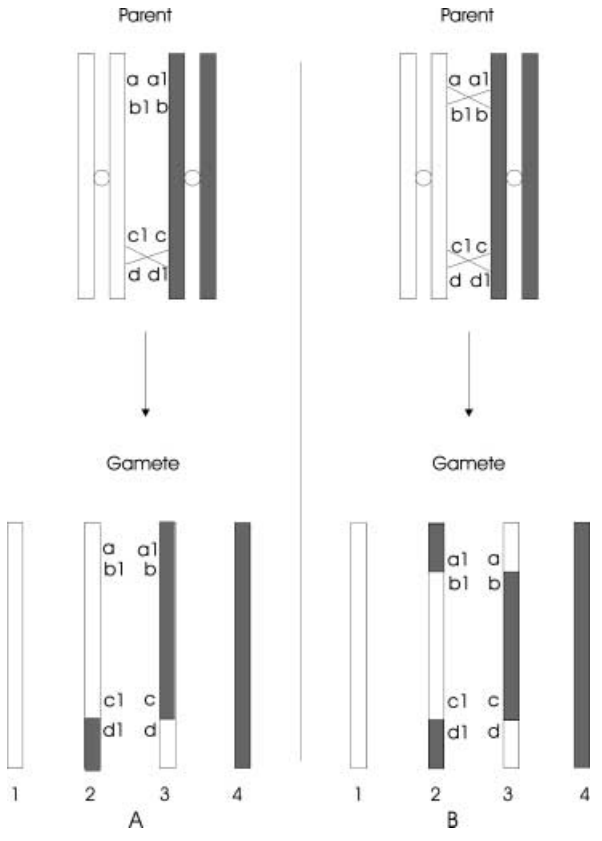

Fig. 1 The constitution of four homologous chromosomes after meiosis. One crossover is shown in bivalent (*A*), and two crossovers in bivalent (*B*). In both cases, only chromosomes 2 and 3 will reveal repulsion linkages, if doubled and backcrossed to the original parent

The number of coupling linkage groups detected depends on the crossover frequency

In backcrosses of doubled-haploids, the number of coupling-phase linkage groups is also dependent on the number of crossovers per bivalent in the meiosis that produced the haploid. This is illustrated in Fig. 2. For each homologous group, one crossover recovered in the doubled-haploid results in two linkage groups, and two crossovers result in three groups, as long as the distance between the crossovers is longer than the default linkage. When the distance between crossovers is shorter than the default linkage, the two coupling-linkage groups can join into one. If there were no crossovers recovered in the doubled-haploid, a homologous set of chromosomes would be represented by only one linkage group. Overall, the number of coupling-phase linkage groups available for detection are $n/2 + n(a + 1)/2$, where a is the number of crossovers per bivalent and n is the number of homologous groups. It is important to keep in mind that only those chromosome sets without crossovers ($n/2$) will be represented by a single linkage group from intact chromosomes. All the other linkage groups will be from sections of chromosomes where there have been crossover exchanges (Fig. 2).

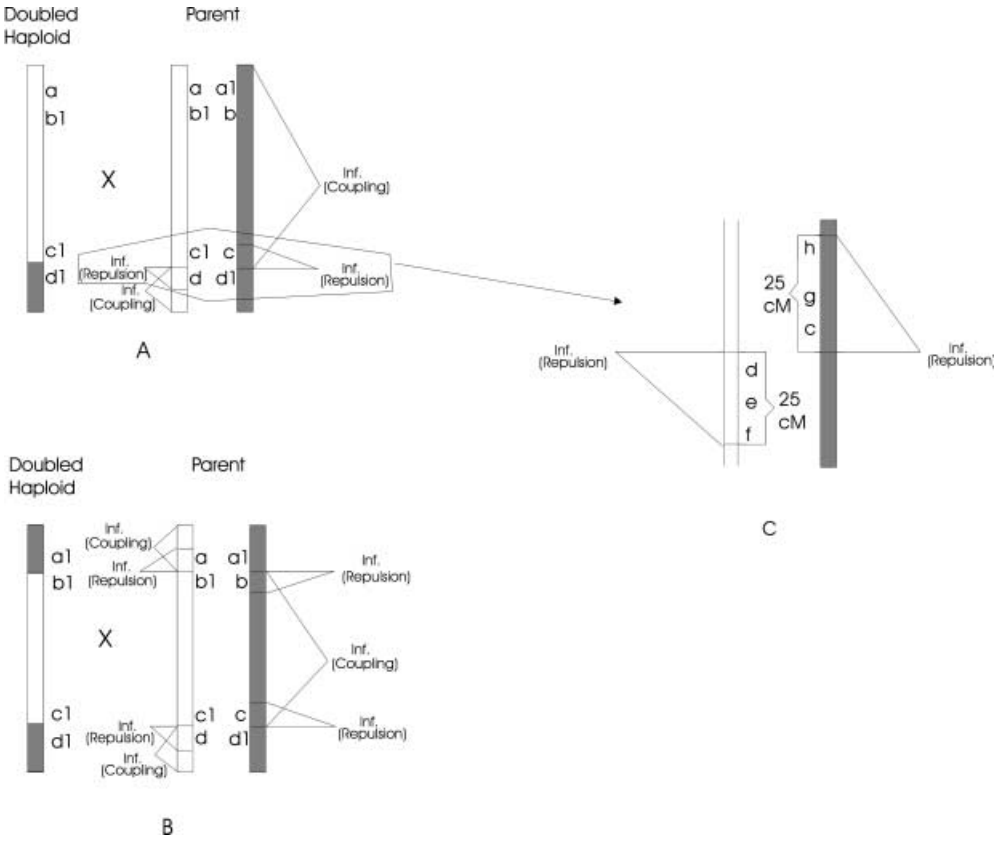

Fig. 2 A doubled-haploid with a chromosome that will reveal repulsion-phase linkages when crossed with its parent. Two (*A*) or three (*B*) coupling linkage groups are available for detection. The chromosome sections that are informative (*Inf.*) are indicated. The enlarged portion (*C*) shows that only markers within the default linkage can be linked to markers in the other section, if the distances are within the default linkage. For example, marker e will not link to markers c, g, and h if the distances between them are larger than the default linkage (25 cM), even though markers e and c, and g and h are within the default linkage within their own chromosome sections

The size covered by all the observed linkages is only equivalent to the haploid genome

This can be seen in Fig. 2, since no matched sections from two homologous chromosomes are mapped. Therefore, when repulsion-phase linkages are detected in such a cross, they must be between markers on two unmatched sections of two homologous chromosomes. As a result, the detection of repulsion-phase linkages does not expand genome coverage, and it is incorrect to expect that there are equal numbers of repulsion and coupling linkages when no repulsion linkages are detected.

Repulsion-phase linkage analysis can be used to calculate the number of exchanges (effective crossovers) per bivalent in such a cross

The number of crossovers per bivalent per meiosis is usually estimated cytologically by the number of chiasmata at the diplotene stage. The accuracy of such an estimation is limited by chromosome-slide quality. For species with small chromosomes, such limitations are particularly great due to the difficulty in examining individual diplotene chromatids. Säll and Nilsson (1994) estimated the crossover distribution in barley by analyzing RFLP (restriction fragment length polymorphism) linkage data generated from doubled-diploid populations.

In a mapping analysis of a cross of a doubled-haploid with its parent, the number of repulsion-phase linkages represents the number of effective crossovers in the meiosis producing the gamete, since repulsion linkages can be detected between two coupling linkage groups only where an exchange has occurred. If a saturated map is generated, it could be used to detect all of the effective crossovers in the meiosis recovered by the doubled-haploid, if the distance between crossovers is longer than the default linkage.

The actual ratio of single- to multi-dose polymorphic markers in a parent is inflated in backcrosses of doubled-haploids

Not only is there an overall reduction in the number of polymorphic markers detected in such a cross, but the number of single- and multi-dose markers is also affected differentially. A multi-dose marker has a higher likelihood of being present in the doubled-haploid (Table 1), amplifying the ratio of single- to multi-dose polymorphic markers in the parent, and the increase is larger for autopolyploids than for allopolyploids.

Table 1 Probability that a polymorphic marker in an octoploid parent will be present in its doubled-haploids[a]

Marker dose	Probability (%)	
	Polysomic	Disomic
1	50	50
2	78.6	75
3	92.9	87.5
4	98.6	93.7

[a] Table 1 is developed based on the segregation ratios of the markers. For example, for a double-dose marker in an auto-octoploid the segregation ratio is 3:11 (absent:present) among gametes. Therefore, the probability for this marker being absent in a haploid is 21.4% (3/14) and 78.6% for being present. For an allo-octoploid the segregation ratio is 1 : 3, and the probabilities are 25% and 75%, respectively

Misinterpretations in previous studies

In previous mapping studies in sugarcane using a backcross of doubled-haploids, the features noted above were not considered, leading to some misinterpretations (Al-Janabi et al. 1993; Da Silva et al. 1993). The doubled-haploid was derived from a culture of SES 208 anthers, followed by spontaneous doubling of chromosomes during regeneration (Al-Janabi et al. 1993). Therefore, all the single-dose restriction fragments (SDRF) generated from the population of the doubled-haploid crossed with SES 208 must have come from parental chromosomes or sections of the chromosomes that were not present in the doubled-haploid. The authors recognized that the marker data is skewed against detection of repulsion-phase linkages if SES 208 is an allopolyploid; however, they claimed that only those markers that were completely linked would be undetectable. They stated that "for markers separated by a recombination fraction of 2, the probability of finding repulsion-phase linkages is a function of θ/2…'', and that "we assume that an equal number of linkages in repulsion phase would have been observed if the number of progeny we investigated were sufficiently large." Based on what has been discussed above, in only 16 homologous groups of the sugarcane is there a chance of detecting the rare repulsion linkages, since $n/2 = 32/2$ (the sugarcane has 32 homologous groups, assuming disomic inheritance).

The authors expected to detect 64 linkage groups (the $2n$ number of the plant material) in coupling phase, each representing an intact chromosome. They stated that "we expect to observe 64 ($2n$) linkage groups, rather than 32 (n), as any chromosome can contain a simplex (therefore single-dose) allele." (Al-Janabi et al. 1993). Based on what has been discussed, the number of coupling linkage groups that could be detected were actually dependent on the number of crossovers that occurred in the meiosis that produced the gamete, although an average of two crossovers per bivalent could generate 64 groups [$n/2 + n(a + 1)/2 = 16 + 16 \times 3 = 64$]. More importantly, of the 44 (Al-Janabi et al. 1993) and 42 (Da Silva et al. 1993)] coupling groups that have been identified in sugarcane, only about 16 represented intact chromosomes (the sugarcane has 32 homologous groups, so $n/2 = 32/2$).

In their genome-coverage analysis, the authors assumed that the actual number of repulsion linkages should equal the number of the coupling linkages. Because the authors failed to detect any repulsion-phase linkages, they corrected their initial genome-size estimate (using all the linkages) by dividing it by 2 (Al-Janabi et al. 1993; Da Silva et al. 1993). However, based on what we have discussed, this method of analysis underestimated the genome size by 50%.

Another misinterpretation in the sugarcane reports concerns how the authors calculated the expected proportion of single-dose vs multi-dose markers to determine the polyploid type. The authors summed the expected segregation ratios for fragments in two, three, and four doses in auto-octoploids (3/14, 1/14, and 1/70, respectively) and suggested that the resulting value 0.3 was the expected proportion of non-SDRF (Al-Janabi et al. 1993; Da Silva et al. 1993). For allo-octoploids they calculated the proportion to be 0.44 (1/4, 1/8 and 1/16, respectively) (Al-Janabi et al. 1993). The authors obtained the expected proportion of SDRF by subtracting their expected proportion of non-SDRF from 1.0, and arrived at a value of 70%. They then did a chi-square test to determine which proportion best fit their data, and on this basis distinguished the polyploid type. However, taking the expected marker segregation ratio as the proportion of the marker category is incorrect.

The expected segregation ratio of a multi-dose polymorphic marker in a population is a function of several fixed factors including the ploidy level, the marker dose and the nature of chromosomal pairing (disomic or polysomic) in the parental material. For each ploidy level, the expected segregation ratio for a marker is dependent on the type of inheritance, and the segregation ratio of a multi-dose marker can be used to determine the polyploid type (Soltis and Rieseberg 1986; Krebs and Hancock 1989; Qu and Hancock 2001). However, the proportion of each marker-dose category is completely independent of inheritance patterns, and the segregation ratio of a marker does not in any way relate to it. For example, if 20% of the total polymorphic loci in an octoploid are in a tetra-dose, no matter whether it has disomic (expected segregation ratio = 1/16) or polysomic (1/70) inheritance, the marker proportion detected will be the same (20%). In backcrosses with doubled-haploids, the lower probability that multi-dose polymorphic markers can be identified than single-dose markers may be a reason why fewer such markers have been detected in the octoploid (Al-Janabi et al. 1993; Da Silva et al. 1993).

In the analysis so far the plant material has been assumed to have disomic inheritance. It should be pointed out that for a polysomic polyploid with bivalent pairing, mapping and genetic analysis of backcrosses with doubled-haploids are no different than disomic inheritance

for the features of reduced repulsion linkages, fragmentations of linkage groups, linkage group numbers being dependent on crossovers, and genome coverage. With polysomic inheritance, repulsion linkages are more common than with disomic inheritance because each chromosome has more than one homologue with which to pair. However, much larger population sizes are required to detect the reduced number of repulsion-phase linkages in polysomic polyploids because of the role of independent assortment in repulsion linkages (Wu et al. 1992; Qu and Hancock 2001).

In conclusion, our analysis indicates that understanding the genetic constitution of the population of a doubled-haploid backcrossed to its parent is essential for correctly analyzing molecular-marker data. Doubled-haploids can be used to construct genetic maps, but fewer repulsion linkages are detected in their segregating populations, and most individual chromosomal maps are fractured. One cannot assume that the ratio of single- to multiple-dose markers is an indicator of polyploid type. However, repulsion linkage analysis in backcrossing with a doubled-haploid can be used to estimate crossover numbers per bivalent. Since such a cross may be used in genetic studies in other crops, our analysis should be very beneficial.

References

Al-Janabi SM, Honeycutt RJ, McClelland M, Sobral BWS (1993) A genetic linkage map of *Saccharum spontaneum* L. (SES 208). Genetics 134:1249–1260

Burk LG, Gestel DU, Wernsman EA (1979) Maternal haploids of *Nicotiana tabacum* L. from seed. Science 206:585

Da Silva JAG, Sorrells ME, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum*. Genome 36:782–791

Guha S, Maheshwari SC (1964) In vitro production of embryos from anthers of *Datura*. Nature 204:497

Maheshwari SC, Rashid A, Tyagi AK (1982) Haploids from pollen grains – retrospect and prospect. Am. J Bot 69:856–879

Krebs SL, Hancock JF (1989) Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity 63:11–18

Qu L, Hancock JF (1995) Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. Theor Appl Genet 91:1309–1315

Qu L, Hancock JF (2001) Detecting and mapping repulsion-phase linkage in polyploids with polysomic inheritance. Theor Appl Genet 103:136–143

Säll T, Nilsson N-O (1994) Crossover distribution in barley analysed through RFLP linkage data. Theor Appl Genet 89:211–216

Soltis DE, Rieseberg LH (1986) Autopolyploidy in *Tolmies menziesii* (*Saxifragaceae*): genetic insights from enzyme electrophoresis. Am J Bot 73:310–318

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor Appl Genet 82:294–300