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Molecular and morphological evaluation of doubled-haploid lines in maize. 2. Comparison with single-seed-descent lines

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Abstract. Doubled-haploid (DH) and single-seed-descent (SSD) lines in maize have been compared for quantitatively inherited traits and for RFLP markers. The comparisons of the distributions for agromorphological traits do not allow definite conclusions to be drawn on the similarity of the two reproductive systems. We have used more than 100 RFLP markers to provide a precise description of the parental allele frequency and the recombination fractions. A comparison of two DH populations shows that non-random meiotic reassortment is influenced by differences in the anther culture capacities of the two parental lines. For the DH lines derived from the cross DH5 × DH7, involving two responsive lines in anther culture, the distortion in segregation ($P < 0.05$) affected less than 20% of the genome with half of the deviations towards each parent. DH lines derived from the cross A188 × DH7, where A188 is a non-responsive line, showed more than twice this level of distortion and an excess of DH7 alleles was found for almost all of the skewed loci. The recombination fractions were homogeneous between the two DH populations for most of the genome. The genome sizes calculated with the DH and the SSD lines derived from the same cross, A188 × DH7, were also similar, which suggests that no selection against recombinant gametes occurs during anther culture. The observed recombination fraction after five meioses (SSD) is on average twice as large as after one meiosis (DH). No difference is observed for recombination fractions greater than 20%. Despite a precise description of the material at the molecular level, it has not been possible to make a definite conclusion as to whether or not the differences in some morphological characters are the consequences of differences in the segregation ratio and/or the recombination frequency. However, the agromorphological evaluation shows a narrow range in differences between the two types of lines and suggests that the use of DH lines is possible in breeding programmes.

Key words: Maize – RFLP – Recombination – Disturbed segregation – DH/SSD lines

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Introduction

Doubled haploids (DHs) are a powerful tool for plant breeding and genetic analysis. The production of desired gene combinations in the homozygous state can be achieved very quickly from segregating material: for instance two-rowed BaYMV-resistant barley lines (Foroughi-Wehr and Friedt 1984) and yellow-seeded canola lines (Henderson and Pauls 1992). DH lines have also been shown to be of great utility for the analysis of the inheritance of agronomic traits such as the fatty acid content of rapeseed (Chen and Beversdorf 1990a). Nevertheless, one of the main purposes of using the doubled haploid method in breeding programmes is to accelerate the recovery of complete homozygosity, providing inbred lines for replicated evaluation.

However, to be incorporated successfully in breeding programmes, doubled haploids have to be comparable to conventionally-derived lines. The homogeneity within maize DH lines derived from anther culture has been checked with field observations and the homozygosity has, with few exceptions, been confirmed using a large set of RFLP markers (Murigneux et al. 1993). Somaclonal variation has been shown to be very infrequent in these DH lines produced with a fast regenerating procedure.

Therefore, the most important questions to be answered are whether androgenesis induces selection pressure, leading to significant segregation distortion, and

what the consequences are of one versus several meioses, in respectively DH and single-seed-descent (SSD) lines. Studies on theoretical populations have shown that the distribution of a quantitative character differs if the genes involved in the expression of the trait are linked (Riggs and Snape 1977).

Most of the studies on quantitative traits have concluded that no significant differences occurred in DH populations in barley (Choo 1982), maize (Lashermes et al. 1988), brussels sprouts (Kubba et al. 1989), or oilseed rape (Chen and Beversdorf 1990 b). Using morphological characteristics, isozymes, protein or RFLP markers, segregation distortion was found in microspore-derived plants of broccoli (Orton and Browers 1985) and rice (Guiderdoni et al. 1989; Guiderdoni 1991). In barley, distortion was found more frequently in microspore-derived lines (Powell et al. 1986; Graner et al. 1991; Thompson et al. 1991; Zivy et al. 1992) than in lines derived using the *Hordeum bulbosum* technique (Powell et al. 1986; Schön et al. 1990). Recently, a comparison in maize between DH lines and F_2-generation progenies derived from the same cross, showed 72% segregation distortion of the RFLP markers in DH lines (Bentolila et al. 1992).

Our objective was to compare different DH populations, as well as DH and SSD populations derived from the same cross, using quantitative traits and molecular markers. The comparison of DH lines derived from crosses involving either one or two anther-culture-responsive parental lines, made it possible to evaluate the consequences of differences of parental responsiveness on segregation ratio. The comparison of SSD and DH lines derived from the same hybrid allowed us to reach some conclusions concerning the influence of the reproductive system on the reassortment of the genetic variability. The construction of complete linkage maps facilitated an evaluation of the level of genetic recombination in the three populations. The uniformity of genetic recombination between the two different DH populations was established, as well as the increase in recombination observed when comparing DH and SSD lines derived from the same hybrid.

Materials and methods

Plant material

Anther-culture-derived plants were regenerated from two single hybrids DH5 × DH7 and A188 × DH7 (called 5 × 7DH and 8 × 7DH respectively). A single-seed-descent population (called 8 × 7SSD) was also produced from the A188 × DH7 hybrid by successive selfing, up to the F_6 generation. (Murigneux et al. 1993). A188 is a non-responsive line for androgenesis and DH5 and DH7 are highly androgenetic lines.

Field observation and statistical comparisons

Eighty-one SSD lines and 120 DH lines derived from the A188 × DH7 cross were grown in field conditions for agronomic evaluation. A188 and DH7 lines were included as controls. Seven different characteristics were analysed: plant height, height of ear insertion, number of branches per tassel, length of tassel branches, number of spikelets on the main branch, ear leaf area, and number of leaves. Plant-to-plant measures were carried out on 5–10 plants per nursery row, in two replications at Clermont-Ferrand for 1 year. The DH and the SSD populations were compared with the Wilcoxon test for the comparison of the means, with the median test for the comparison of the medians, and with the Kolmogorof-Smirnof test for the comparison of the distributions. Data from the experiment were analysed using NPAR1WAY, SAS® non-parametric statistical comparisons (SAS Institute Inc. 1988).

RFLP analysis

The 71 5 × 7DH lines, the 109 8 × 7DH and the 60 8 × 7SSD lines used in the RFLP analysis are those described in a previous report (Murigneux et al. 1993). The 109 8 × 7DH and the 60 8 × 7SSD lines are included in the lines used for the agro-morphological traits evaluation. Between 102 and 120 evenly spaced polymorphic probes, obtained from the University of Missouri (USA) and the Brookhaven National Laboratory (USA), were tested on each population. DNA extraction and digestion, Southern blotting and probe hybridization were performed as described earlier (Murigneux et al. 1993).

Linkage analysis and comparison of recombination

For the three populations, a 1:1 single factor segregation was tested at each locus using a chi-square analysis (χ^2). Many loci showed skewed segregations and the detection of linkage was done using two methods: a chi-square test of independence on a 2 × 2 contingency table (Mather 1951) and the method of maximum likelihood using the Mapmaker computer program V 2.0 (Lander et al. 1987). Mapmaker was also used for the ordering of the loci and the estimation of the recombination fraction. For intervals where both loci showed segregation distortion, the estimation of linkage was also evaluated with the product formula (Bailey 1949). DH lines can be analysed as back-cross plants since in both these population types the recombination of one gamete is contained in each individual. The SSD lines were first analysed as DH lines (the heterozygous loci were dropped), then the estimated recombination fraction R was transformed into a single-meiosis-equivalent fraction r by the equation $r = R/(2-2R)$ (Haldane and Waddington 1931). For all populations the map distance in centimorgans was derived from the recombination fraction using the Haldane function $x = -\ln(1-2r)/2$, where x is the estimated distance in Morgans (Haldane 1919).

For the two DH-line populations, the homogeneity of recombination was assessed using the test proposed by Morton (1956) and described more recently by Beavis and Grant (1991): $(2.\ln 10).[\Sigma z_i(r) - \Sigma z_p(r)]$, where z_i and z_p are the respective logs of the odds ratio scores (lodscores) given the maximum likelihood estimates of recombination for population i and the composite population constructed with the pooled data of all N populations. This test is asymptotically distributed as a χ^2 with N-1 degree of freedom. Seventy-seven intervals flanked by the same markers in the two DH populations were compared. The determination of a nominal significance level was made as proposed by Lander and Botstein (1989). For a significance level of 0.05 on the whole experiment the nominal significance level is derived with the equation: $1 - e[\ln(1-0.05)/77] = 0.0006$.

For a comparison between the DH and SSD lines from the same cross, the observed recombination fraction between pairs of adjacent markers in both populations were used. Only chromosome segments having markers mapped in both populations were taken in account, and observed recombination fractions

higher than 0.3 were not considered since in these cases the recombination is not evaluated precisely. The ratio $R_{(SSD)}/R_{(DH)}$ was calculated for seventy nine intervals.

Segregation ratio

For this part of the study, probes covering most of the genome, but avoiding clustered loci, were used. The distribution of these loci is similar to those shown in Fig. 1, except that a few of them were not used because they were too close together. Others (data not shown) were used to allow a better genome coverage with evenly-spaced loci. Segregation ratios have been evaluated at three different levels:
(1) The overall ratio of A188 or DH5 and DH7 RFLP alleles on all lines at all loci for each population.
(2) The segregation at each locus individually. The percentage of the length of the parental genome that is not evenly represented in the progenies has also been calculated: an interval is given with the lower limit taking into account only intervals with both flanking markers skewed. For the upper limit, adjacent intervals with only one skewed extremity are added.
(3) At the plant level, the proportion of each parent genome was also evaluated. The distribution of lines according to their percentage of DH7 alleles at RFLP markers was then analysed as a quantitative trait.

Results

Field observations

A comparison of the distribution of agromorphological characteristics for DH and SSD lines is reported in Table 1. For three traits – plant height, length of tassel branches, and number of spikelets on the main branch – the mean, median and the distribution showed no difference between the DH and the SSD populations. For the two

Table 1. Comparison of seven agromorphological characters and the parental alleles frequency between DH and SSD lines derived from the cross A188 × DH7 and called 8 × 7DH and 8 × 7SSD respectively. The minimum (Min), maximum (Max) value of the extreme lines and the average value (Mean) for the two populations are given. The test of comparison of the mean, median and the distribution (Distrib.) are shown for every characteristic. NS = not significant; S*, S**, S*** = significant at the 0.05, 0.01, 0.001 probability level respectively

Character	Lines	Min	Mean	Max	Comparison of		
					Mean	Median	Distrib.
Height of plants (cm)	A188		123.2		NS	NS	NS
	DH7		125.5				
	8 × 7DH	89.4	127.7	159.4			
	8 × 7SSD	97.0	128.8	165.8			
Length of tassel branches (cm)	A188		336.0		NS	NS	NS
	DH7		341.0				
	8 × 7DH	126.5	313.2	556.0			
	8 × 7SSD	148.0	305.7	566.7			
Number of spikelets on the main branch	A188		159.0		NS	NS	NS
	DH7		169.0				
	8 × 7DH	72.0	128.6	210.5			
	8 × 7SSD	84.0	135.1	251.5			
Height of ear (cm)	A188		49.2		S*	S*	NS
	DH7		75.6				
	8 × 7DH	35.8	62.4	91.7			
	8 × 7SSD	31.2	59.0	95.2			
Number of branches per tassel	A188		28.5		S*	S*	NS
	DH7		27.9				
	8 × 7DH	13.5	26.6	47.0			
	8 × 7SSD	9.5	24.3	44.0			
Ear leaf area (cm^2)	A188		393.9		S**	S*	S**
	DH7		377.2				
	8 × 7DH	194.4	361.4	581.0			
	8 × 7SSD	271.4	399.2	564.9			
Number of leaves	A188		18.3		S***	S***	S***
	DH7		22.2				
	8 × 7DH	16.6	20.1	23.0			
	8 × 7SSD	16.9	19.2	22.1			
Genome ratio (% DH7)	A188		0.0		S***	S***	S***
	DH7		100.0				
	8 × 7DH	23.6	57.0	83.6			
	8 × 7SSD	29.0	48.9	73.1			

following traits, height of ear and number of branches per tassel, a small difference appeared in both the mean and the median of the distribution. Only for the two last traits, ear leaf area and number of leaves, were differences detected with all three statistical parameters. One factor that could contribute to differences between lines is the level of heterozygosity, which is almost absent in the DH population and is at a value of 8.5% in the SSD population (Murigneux et al. 1993). However, in most cases (for all traits except those related to leaf characteristics) the comparison of the distributions for agromorphological traits reveals no major differences between the DH and the SSD populations. These results confirm that the different reproductive systems have no influence on the reassortment of the genetic variability that is strong enough to be easily observed by comparing polygenic traits.

In most cases, the parental lines A188 and DH7 are very similar for the agromorphological characters, but reassociation during the process of fixation produced extreme DH and SSD lines very different from the parents. Depending on the characters, the more extreme lines are either DH or SSD and, according to the analysis of several traits, no depression in vigour or performance of the DH lines is apparent. For breeding purposes, these results imply that the DH method will be of use for the production of lines in a breeding programme.

Map construction

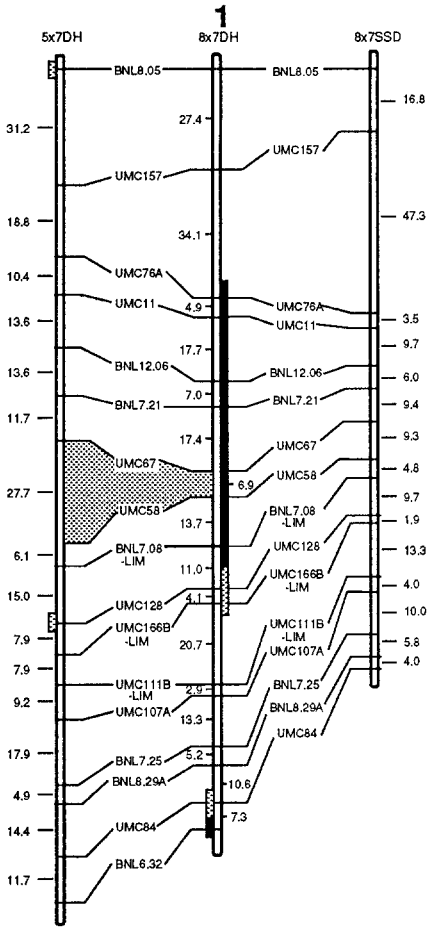
Non-Mendelian segregation was observed at many marker loci in the 8 × 7DH population (Fig. 1). The detection of linkage was therefore done first using the χ^2 test of independence. Because the position of most of the loci is known a *priori* (Coe et al. unpublished data; MNL 64: pp 154–163), it was easy to discriminate between spurious linkage involving two loci located on two different chromosomes and loose linkage between loci mapped on the same chromosome. Table 2 shows that for the 8 × 7DH population, a χ^2 value of 13.5 was found between two loci mapped on chromosomes 7 and 10. The loosest linkage between two adjacent loci on the same chromosome was found on chromosome 4 with a χ^2 of 20.8 (BNL10.05–BNL15.07A). In comparison, the use of the maximum likelihood method for the detection of linkage was found to be less powerful. The lodscore is indeed reflecting linkage and distortion. The lodscore value for the loosest linkage (9.7 for BNL10.05–BNL15.07A) is very close to the strongest spurious linkage (9.6 for BNL7.21–UMC89). However, using either the lodscore or the χ^2 led to the construction of a similar number of linkage groups (ten groups and one isolated locus: BNL3.04A).

No significant differences were observed between the χ^2 and the maximum likelihood methods on the two other populations, which is in agreement with the fact that the single factor distortions were less frequent and of lower magnitude. Working with three populations each having at least one parent (DH7) in common proved to be invaluable for the construction of linkage groups. For example, on chromosome 6, linkage was highly significant for all adjacent markers in the 8 × 7DH population (Fig. 1). Since DH7 is held in common, and because we used the same enzyme when testing the segregating pop-

Table 2. Spurious linkage found in the 8 × 7DH population using χ^2 or lodscore test. Theta (pf) and Theta (ml) are the estimated recombination fraction using respectively the product formula and the maximum likelihood method. The probes in bold character are showing a non-Mendelian segregation at the 0.01 probability level

Probe 1	Probe 2	χ^2	Lod-score	Theta (pf)	Theta (ml)
chromosome	chromosome				
BNL7.21 1	**UMC89** 8	0.03	4.80	0.51	0.28
UMC34 2	**UMC7** 8	4.84	4.65	0.35	0.27
BNL12.06 1	**UMC34** 2	6.45	3.88	0.35	0.30
BNL10.05 4	**UMC164A** 8	1.86	3.57	0.40	0.30
UMC116 7	UMC130 10	13.5	2.46		
UMC132 6	**UMC164A** 8	11.88	0.0		
UMC128 1	**BNL8.45C** 8	11.81	3.71		
UMC67 1	BNL7.26 3	11.43	1.30		

Fig. 1. Genetic maps of RFLP markers constructed with 71 DH lines derived from DH5 × DH7 (5 × 7DH, left chromosome), 109 DH lines derived from A188 × DH7 (8 × 7DH, central chromosome) and 60 SSD lines derived from A188 × DH7 (8 × 7SSD, right chromosome). Marker designation are UMC = University of Missouri, BNL = Brookhaven National Laboratory. *NOR* is a sequence of the nuclear organisator region, *ADH2* a cDNA of maize dehydrogenase 2. The probe name is followed by a letter when several loci are mapped for a probe, and by "*LIM*" when the locus has not been described earlier or when the position differs from the current map (Coe et al. unpublished data, MNL 64: pp 153–164). Segregation distortions are indicated with *black bars* ($P < 0.01$) and *shaded bars* ($P < 0.05$). *Bars on the left side* of the chromosome indicate an excess of alleles of the female parent (DH5 or A188); *bars on the right side* of the chromosome indicate an excess of alleles of the male parent (DH7). *Shaded zones* between the chromosome of the two DH populations indicate the intervals with a significant difference in recombination fraction between these two populations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8x7DH
8x7SSD
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BNL8.05
16.8
31.2
27.4
UMC157
UMC157
47.3
18.8
34.1
UMC76A
10.4
UMC11
4.9
UMC76A
3.5
13.6
UMC11
17.7
9.7
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13.6
7.0
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9.4
11.7
17.4
UMC67
9.3
UMC67
UMC58
4.8
6.9
27.7
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9.7
13.7
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6.1
BNL7.08
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UMC166B
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UMC128
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7.9
20.7
UMC107A
5.8
7.9
UMC111B
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2.9
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9.2
UMC107A
13.3
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UMC84
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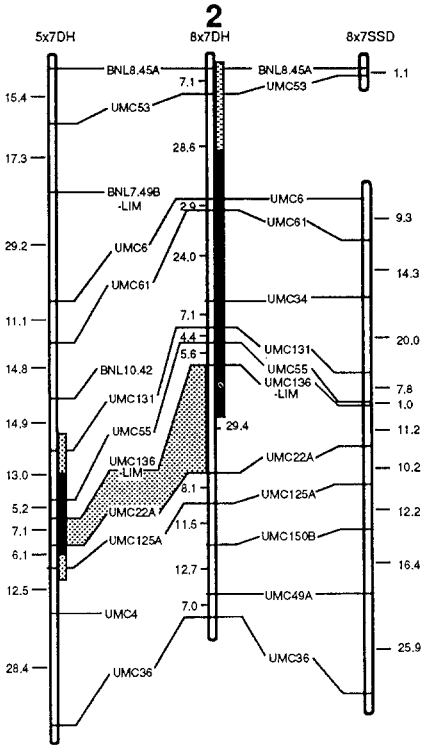
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8x7DH
8x7SSD
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BNL8.45A
1.1
UMC53
15.4
UMC53
28.6
17.3
BNL7.49B
-LIM
2.9
UMC6
UMC61
9.3
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UMC6
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UMC34
7.1
11.1
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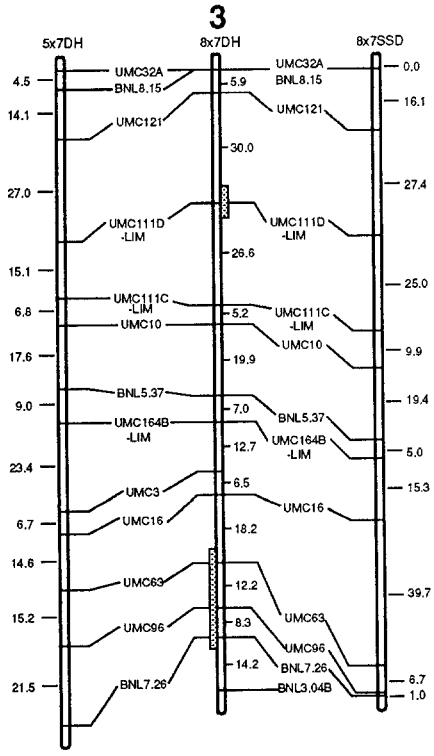
UMC36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UMC3
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UMC16
6.7
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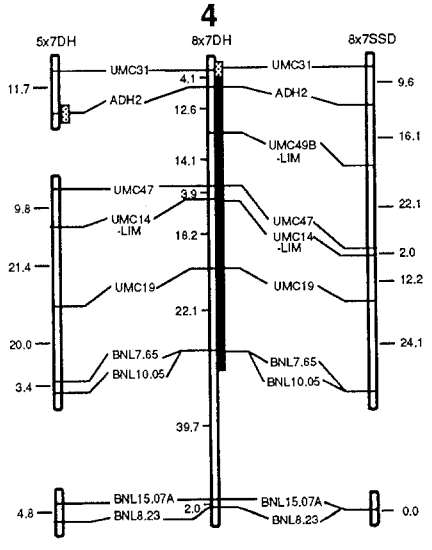
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8x7SSD
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UMC31
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ADH2
ADH2
12.6
16.1
UMC49B
-LIM
14.1
UMC47
3.9
22.1
9.8
UMC14
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UMC14
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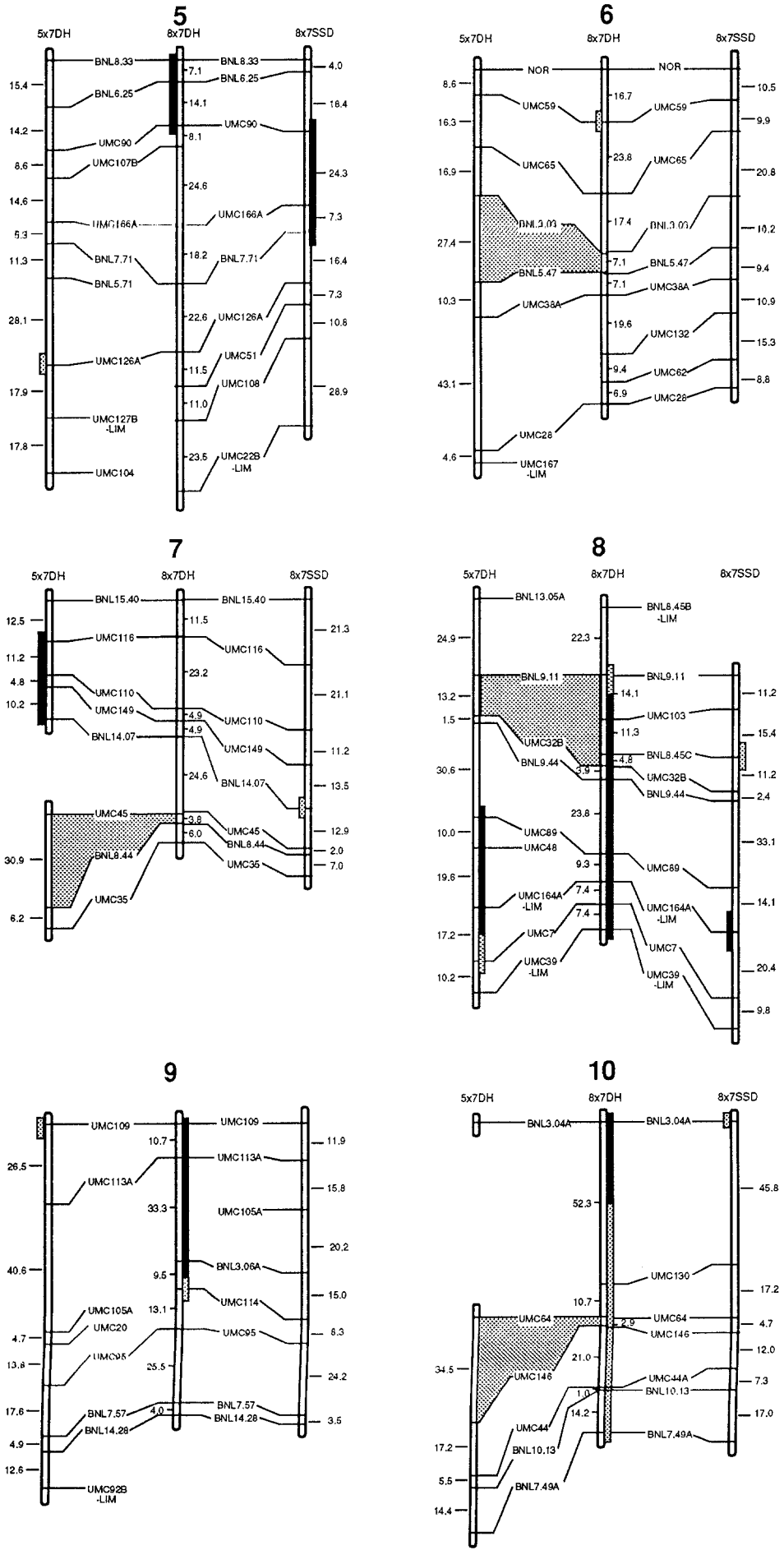
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UMC126A
UMC127B -LIM
UMC104
UMC51
UMC108
UMC22B -LIM
6
NOR
UMC59
UMC65
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UMC38A
UMC132
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UMC103
UMC32B
BNL9.44
BNL8.45C
UMC89
UMC48
UMC164A -LIM
UMC7
UMC39 -LIM
9
UMC109
UMC113A
UMC105A
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UMC64
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UMC44A
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UMC44
BNL7.49A

ulation, it has been possible to check that the segregating bands characterised were the same in both populations. Therefore, it confirms the location of UMC28 on chromosome 6 for the 5 × 7DH population. The number of linkage groups was reduced using this strategy.

Bailey (1949) recommended the use of the product formula for the evaluation of the recombination fraction between loci showing differences in viability factors, when viability factors are independent. The recombination fraction for the spurious linkages involving two skewed loci (Table 2) were found to be lower when using the maximum likelihood, rather than the product, formula. The higher value calculated with the product formula is in agreement with the fact that χ^2 detected no linkage between these loci. The use of χ^2 for linkage detection and the product formula for the estimation of the recombination fraction is therefore to be preferred. On the other hand, for real linkages between loci located in the same skewed chromosome regions (the segregation is influenced by the same viability factor) the recombination fractions estimated by both methods are very similar (data not shown). Therefore the maximum likelihood method is adapted for linkage evaluation between skewed loci when the segregation of both loci is influenced by the same viability factor.

Figure 1 summarises the maps constructed for the three populations. Not all the mapped loci are shown; the probes that mapped in the three populations were chosen, as often as possible, to facilitate a comparison of recombination. Working with homozygous lines proved to be very powerful for mapping several loci for probes showing more than one pair of segregating bands. When mapping 100 probes, an average of 20 extra loci were mapped. Several dominant markers were also mapped.

In all cases the probe order was consistent between maps, and most of the time with published maps (Coe et al. unpublished data, MNL 64: pp153–164). Figure 1, however, shows six cases where the position using our clone differs from the current RFLP map. In some cases the probes show several segregating bands and we have scored different loci (e.g., BNL7.08). In other cases, we probably have, under the same name, a different probe than the laboratory of origin (eg: UMC39).

This mapping experiment suggests that the use of DH populations for map construction may be limited when strong genome distortion occurs. It was not a major problem in the present study, since a saturated genetic map is available for maize but it could induce problems for the constitution of linkage groups in species where no map and only few markers are available.

Segregation ratio

The overall genome ratios estimated with approximately 100 loci are 48.5%/51.5% respectively for the DH5 and DH7 alleles in the 5 × 7DH population, 43.0%/57.0% for A188 and DH7 in the 8 × 7DH population and 51.1%/48.9% for A188 and DH7 in the 8 × 7SSD population. A 1:1 ratio is not observed for both DH populations but less bias is shown in 5 × 7DH ($P=0.0065$) than in 8 × 7DH ($P<10^{-4}$). The SSD population was constructed with considerable care in order to avoid selection and shows a random reassortment of both parental genomes ($P=0.078$).

The number of probes characterised by a non-random segregation using the χ^2 test ($P<0.05$) is shown in Table 3. Distortions in segregation ratios were found in only seven loci over 99 tested for SSD lines, with half of the deviation towards each parent. This corresponds to a range of 2.4–15.3% in terms of the percentage of the genome. A comparable level of distortion has also been observed in F_2 populations studied in our laboratory. The distortion evaluated in this population can, therefore, be considered as a base-line level for conventional populations and can be the consequence of either sampling effect, inadvertant selection, or a difference in the viability of some genetic combinations. If any viability factor influences the allele frequency in this cross, it does not affect both methods (selfing and androgenesis) of

Table 3. Distortion of segregation at RFLP marker loci ($P<0.05$) for 5 × 7DH lines; 8 × 7DH lines and 8 × 7SSD lines. The percentage of the length of the parental genome showing distortion is given as an interval. All chromosome intervals delimited with two consecutive probes being skewed are added for the lower limit. Adjacent chromosome intervals with only one skewed probe are added for upper limit calculation

Cross	Segregation distortion towards					
	Female parent (DH5 or A188)		Male parent (DH7)		Total	
	Number of loci	Genome percent	Number of loci	Genome percent	Number of loci	Genome percent
5 × 7DH	8/100	1.9–3.9	10/100	5.7–10.8	18/100	7.7–23.9
8 × 7DH	9/103	3.8–11.7	45/103	38 –54.4	54/103	41.7–66.2
8 × 7SSD	3/99	0.0–8.2	4/99	2.4–7.1	7/99	2.4–15.3

plant production equally, since few distortions were found in common in the two populations derived from A188 × DH7 (Fig. 1).

The distortions found in DH lines are more abundant and clearly different in the two populations. In 5 × 7DH lines, derived from a cross between two lines responsive to anther culture, twice as many distortions (18/100 loci) were observed when compared to the SSD lines. However, a roughly equal number of deviations was found towards each parent. Deviations are much larger in the 8 × 7DH population where substantial parts of chromosomes 2, 4, 8 and 9 and the whole of chromosome 10 are skewed; 45/103 loci, and between 38 to 54 percent of the genome are skewed towards DH7, the anther-culture-responsive line. It is noticeable, however, that despite this deviation towards DH7 alleles, several segments show an excess of A188 alleles. Therefore, in maize, as already observed in barley (Graner et al. 1991), gametic selection seems to be related to a difference in the anther culturability of the two parental lines. The distortions were less frequent in the DH5 × DH7 population, where the two parents are from highly androgenetic lines, than in two other populations involving only one androgenetic line, namely 8 × 7DH and the population studied by Bentolila (1992). Concerning these last two crosses the imbalance is larger in the population reported by Bentolila (35/65) than in the 8 × 7DH population (43/57). This might be the consequence of a smaller difference in parental capacity in the 8 × 7DH cross. A188 does not show any response to anther culture, but has been utilised extensively because of its capacity for somatic embryogenesis. Genes might exist in this line which facilitate regeneration and which balance its poor capacity for anther culture.

The proportion of parental alleles has also been studied at the line level. The distribution of the lines is skewed towards a high percentage of DH7 alleles in the 8 × 7DH population (Fig. 2). A statistical comparison of the 8 × 7DH and 8 × 7SSD populations for the mean and median as well as for the distribution shows, in all cases, a significant difference (Table 1). Although the deviation in 8 × 7DH lines is biased towards DH7 alleles, very extreme lines are observed on both sides of the distribution, with lines containing either 23.6% or 83.6% of the DH7 genome. Since the frequency of gametes carrying a large excess of one parent is low, the probability of recovering a plant with a biased genotype is higher using androgenesis involving one gamete than selfing where lines are derived from F_2 plants involving two gametes.

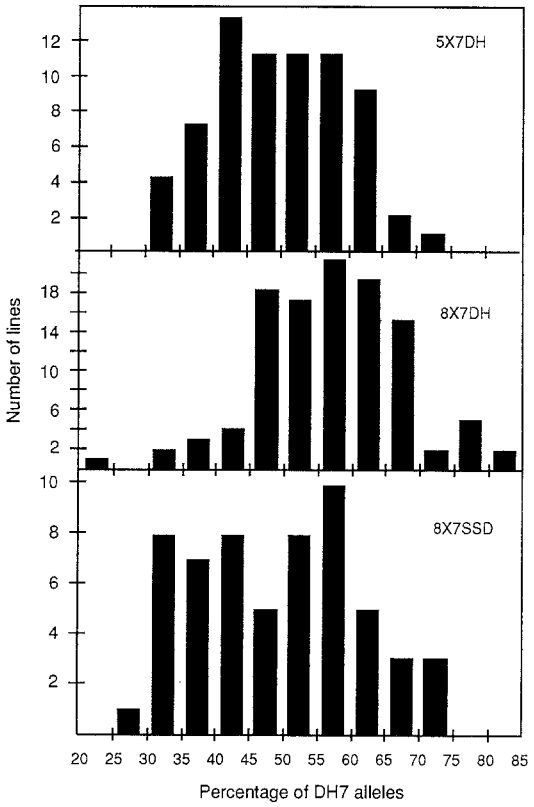

Fig. 2. Distribution of lines in three different populations (71 DH lines derived from DH5 × DH7: 5 × 7DH; 109 DH and 60 SSD lines derived from A188 × DH7: 8 × 7DH, 8 × 7SSD) according to the percentage of DH7 RFLP alleles

Homogeneity between recombination fraction in different populations

The genome sizes estimated for the three populations 5 × 7DH, 8 × 7DH, 8 × 7SSD are respectively 1351, 1266, and 1297 centimorgans. Genome sizes are, however, underestimated for 5 × 7DH and 8 × 7SSD since the genome is not entirely covered with probes: a few gaps are present on Chromosomes 4, 7 and 10 for the 5 × 7DH lines and on chromosome 2 for the 8 × 7SSD lines.

Although the genome sizes are similar between the different populations, differences were observed when looking at specific pairs of loci. The comparison between the two DH populations shows that when a nominal significance level of 0.0006 is used, only six intervals are significantly different in the two populations. They are indicated by a shaded zone between the two chromosomes in Fig. 1. These intervals correspond to 9.6% of the genome length on the composite map. At the whole-genome level, however, the recombination frequencies observed in the two DH populations are similar and also similar to those observed in our laboratory in other crosses and other types of populations, such as F_2, which suggests that the androgenetic process does not seem to repress recombination by selecting for gametes with low recombination frequency. The genome size of the SSD population is also similar to that of the DH population which suggests a homogeneity of recombination between the two types of population.

Table 4. Ratio of the recombination fraction observed in SSD lines over the recombination fraction observed in DH lines derived from the A188 × DH7 cross (R_{SSD}/R_{DH}). The ratio is given for all 79 comparisons and according to the recombination observed in the DH population, R_{DH}

Recombination fraction in DH lines	$0 \leqq R_{DH} \leqq 0.1$	$0.1 < R_{DH} \leqq 0.2$	$0.2 < R_{DH} \leqq 0.3$	Global
Number of intervals	43	30	6	79
Average value of R_{SSD}/R_{DH}	2.55	1.37	1.13	1.99

The observed recombination fractions after one meiosis in 8 × 7DH (R_{DH}) and after four meioses in 8 × 7SSD (R_{SSD}) have been compared (The F_5 level is evaluated since the DNA is extracted from ten F_6 seedlings). The ratio $R_{(SSD)}R_{(DH)}$ calculated for each interval averages 1.99 and is clearly influenced by the linkage distance between the two markers (Table 4). It varies from 2.55 when considering a recombination fraction in 8 × 7DH lower than 10%, to 1.13 for a recombination fraction higher than 20%. The average value of 1.99 confirms the theoretical expectation: the first meiosis is half effective and after several meioses twice the amount of recombination is observed. No real increase of recombination is observed after one meiosis for a recombination fraction higher than 20%, whereas for small intervals, where the frequency of double crossovers is rare, the recombination frequency is increased by a factor higher than 2. This last result is unexpected since recombination is observable only when loci are heterozygous and since at each generation half of the heterozygous loci are fixed. Before proposing any hypothesis to explain this observation, more experiments on larger populations need to be made.

From a breeding point of view it means that in order to obtain a reassociation between two closely-linked genes, about three times more DH individuals than SSD individuals will be required to be scored. However, as soon as genes are separated by over 20% of recombination, about the same number of plants can be used.

Discussion

DH and SSD populations can differ in gene reassociation when genes are closely linked (recombination fraction lower than 20%), and in segregation ratio when the DH lines are derived from two parents showing different anther culture capacities. Since the genetic determinism of the quantitative traits is unknown, it is not possible to formulate hypothetical relations for the differences observed between the two types of populations for these traits or for the differences in recombination and allele frequencies. More information might be obtained by dissecting the genetic determinism of such traits into Mendelian factors. A QTL (quantitative trait locus) analysis locating chromosomal regions controlling the traits can be used to check for this type of relation. An examination of the distortion in allele frequencies shows additional questions that need to be answered:

(1) Is selection occuring during embryo induction or plant regeneration, or during a later stage such as chromosome doubling?
(2) Is there a relationship between segregation distortion and the QTLs for androgenesis?
(3) Is selection affecting some chromosome regions randomly during successive rounds of haploid production, or is there an accumulation of distortion through the generations of androgenesis?

At the present time, however, the narrow range of variation between the DH and SSD populations should allow the use of DH plant production in breeding programmes. However, in order to be able to recover the same genetic variability as that obtained with conventional methods, the doubled haploid method might not be applied to crosses involving two lines with very different anther culture capacities. Consequently, the strategy used for broadening the androgenetic capacities should probably be focused on a global increase of these capacities in the genetic material used by the breeders, rather than the production of elite lines isogenic for the anther culture response.

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