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Establishment and characterisation of wheat genetic mapping populations

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Abstract. Doubled haploid populations from 5 carefully selected wheat (*Triticum aestivum* L.) crosses were established in order to produce genetic maps. The characterisation of the parental material included pedigree analyses to define the extent of the genetic relationships among the lines and to determine the occurrence of alien chromosome segments that may contribute to segregation distortion. The characterisation of the parents also defined the range of grain quality traits that could be examined in the lines derived from each cross. Populations of up to 321 lines were produced using wide cross-mediated doubled haploid production from F_1 plants. Assessment of the lines for heterogeneity was carried out using readily identifiable phenotypic markers and electrophoresis of seed storage proteins, with 2.3–11.6% of the lines being removed from further analysis. Segregation distortion was estimated in several populations where sufficient information from genetic markers was available. In a Sunco/Tasman doubled haploid population, heterogeneity was detected between the first 51 lines and the remainder of the mapping population and this could be traced to F_1 plants that were produced from an earlier set of crosses. χ^2 tests on the mapping data available for the Cranbrook/Halberd, CD87/Katepwa, and Sunco/Tasman doubled haploid populations revealed segregation distortion at rates of 1.8%, 5.1%, and 12.5% respectively. Whereas the wide-cross doubled haploid protocol does not appear responsible for the bulk of the non-Mendelian segregation observed, several potential sources were identified. In particular, clustering of distorted loci at specific chromosome regions appeared to be associated with the presence of alien introgressions in one of the parents. This was especially marked in the Sunco/Tasman population. Providing such distortions are recognised in the models used, these populations provide powerful tools for extensive mapping studies to determine the genetic factors controlling grain quality traits and other wheat characters of interest.

Additional keywords: doubled haploid, wheat × maize, gene segregation, segregation distortion.

Introduction

Haploids have been of great interest to scientists since they were first reported in *Datura stromonium* (Blakeslee *et al.* 1922). To produce the populations analysed in this work, a wheat × maize (*Zea mays* L.) haploid production technique was employed (Laurie and Bennett 1986). The development of doubled haploid populations from crosses between wheat lines has been a major step forward in genetic mapping of wheat. Doubled haploid populations are quicker to generate than conventionally derived inbred populations and have several advantages over F_2 or backcross-derived populations for genetic investigations (Khush and Virmani 1996; Pauls 1996).

An important criterion of doubled haploid production is the stability of derived plants. Kisana *et al.* (1993), when comparing anther culture and wheat × maize systems, observed that wheat × maize-derived plants appeared

genetically stable, whilst aneuploids and sterile plants were observed from anther culture-derived plants. Laurie and Reymondie (1991) found only one aneuploid (a telosomic) in 191 plants derived from wheat × maize crosses. All other aberrations could readily be attributed to the parents. Another issue with doubled haploid production systems is the efficiency of the production protocol in different genotypes. Genotype dependence has been well documented in wheat anther culture (Holme *et al.* 1999; Sadasivaiah *et al.* 1999), but is much less of an impediment to doubled haploid production via the wheat × maize system (Suenaga *et al.* 1991).

One final consideration in any doubled haploid production protocol is the presence of gene selection in resulting populations. In limited studies conducted to date, doubled haploid populations derived via the wheat × maize production system appear to represent an unbiased array of parental genes. Suenaga and Nakajima (1993) studied 6 agronomic traits in 110 doubled haploid lines. Only one trait (glume pubesence) was found to have non-Mendelian segregation, an observation that was attributed to processes following chromosome doubling. Kammholz *et al.* (1998) reported that the segregation of 6 glutenin loci in over 750 doubled haploid lines from 7 doubled haploid populations matched the expected Mendelian frequencies.

The present research has characterised parental lines and established wheat × maize-derived doubled haploid lines from 5 F_1 wheat hybrids for the National Wheat Molecular Marker Project (NWMMP). The study has assessed the levels of non-Mendelian segregation within 3 of these derived populations, discusses possible causes for these non-random processes, and considers the implications of distortion segregation for mapping studies.

Materials and methods

Parental selection

Suitable parents for the mapping populations were selected after consultation with plant breeders representing every Australian wheat improvement program (Table 1). All crosses were made in one direction (i.e. no reciprocal crosses to determine maternal effects). Ten crosses of each combination were made, producing 100–200 F_1 seeds. Seed not maintained within the Leslie Research Centre breeding genebank was obtained from the Australian Winter Cereals Collection, Tamworth, Australia, with the exception of cv. Kukri, which was supplied by Assoc. Prof. Gil Hollamby, Roseworthy Campus, University of Adelaide.

doubled haploid production

The doubled haploid lines from the Cranbrook/Halberd cross were produced by Dr R. Islam (University of Adelaide) and were kindly made available for the current research. All other doubled haploid lines were produced using the scheme outlined in Fig. 1 and the numbers of lines that resulted are shown in Table 2.

Genetic analysis

Map Manager QT (Manly and Olson 1999) was used to develop the genetic maps (see Chalmers *et al.* 2001, this issue). A chi-square

Table 1. Parental crosses selected for the current study

Cross	♀	Origin	♂	Origin
1	Cranbrook	Western Australia	Halberd	South Australia
2	CD87	Victoria	Katepwa	Canada
3	Sunco	New South Wales	Tasman	Queensland
4	Egret	New South Wales	Sunstar	New South Wales
5	Kukri	South Australia	Janz	Queensland

analysis was used ($P < 0.01$) to test for marker deviation from the expected Mendelian segregation on QGENE (Nelson 1997).

Results and discussion

Characterisation of the parents used in the crosses

The Cranbrook/Halberd cross was originally established to study the genetics of flour mixing characteristics, starch pasting characteristics, grain protein, dough strength, and extensibility. Dough extensibility is crucial for a balanced end product. Halberd is typical of the lower extensibility in many Australian wheat lines and there have been several examples where potentially Australian prime hard (APH) breeding lines are assigned only the Australian hard (AH) classification due to poor extensibility. This poor extensibility results in such lines being discarded. The Cranbrook/Halberd cross provides detailed information regarding the effects of particular glutenin subunits in the seed storage proteins on flour processing traits (Payne *et al.* 1981; Gupta *et al.* 1994). The cross segregates at all 3 loci for both the high and the low molecular weight glutenin subunits (see Cornish *et al.* 2001*a*, this issue). This marker system is already widely used for the early generation screening of breeding material for wheat grown in most regions of Australia.

Both Cranbrook and Halberd are hard-grained, with Halberd carrying a dominant gene for awnlessness, which is a useful phenotype for detecting plants in the F_1 that result from self-fertilisation of the Cranbrook parent. This cross is also polymorphic for plant height, maturity (shows considerable transgression), and several rust genes. Although both Cranbrook and Halberd possess brown or red glumes, there is a range in the depth of brownness in this population, which may be linked to maturity class. Although many of their rust resistance genes are no longer effective against current races, the resistance loci provide useful landmarks in genetic maps (see Bariana *et al.* 2001, this issue). Other characteristics of interest in the Cranbrook/Halberd cross include: flour yield, water absorption, aluminium tolerance (Halberd intermediate, Cranbrook sensitive), boron tolerance (Halberd highly tolerant, Cranbrook intermediate), and sprouting susceptibility (Halberd less susceptible). The principal interest in the cross for southern New South Wales arises from differences in competitive ability between these parents (see Coleman *et al.* 2001, this issue).
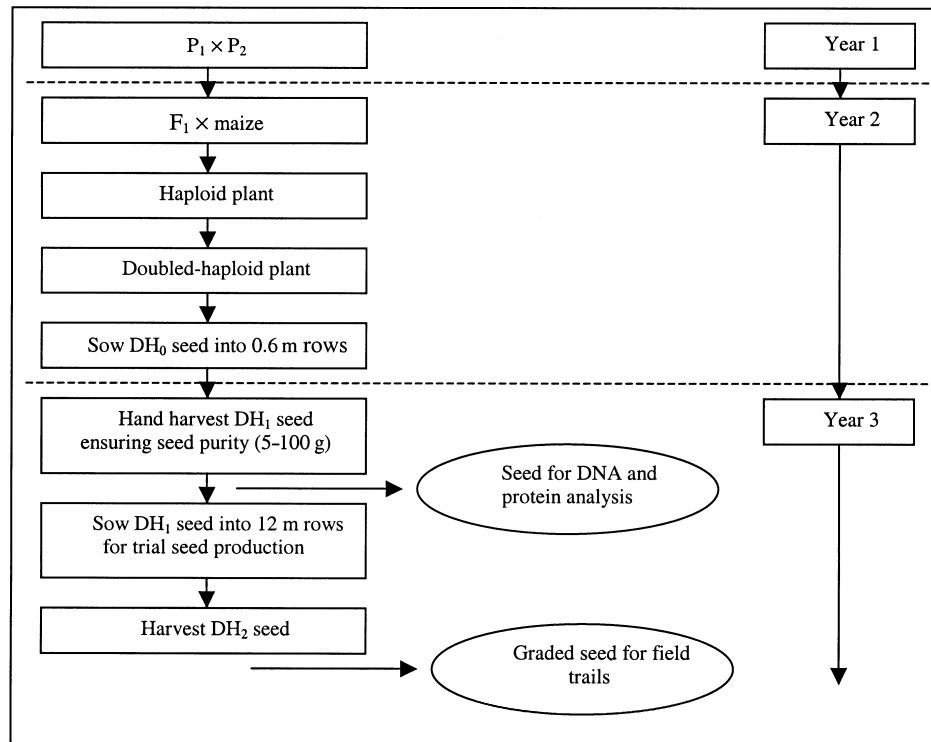
Fig. 1. Timeline of operations involved in population development.

The prime interest in the CD87/Katepwa cross is dough extensibility. The line CD87 has been found to exhibit high dough extensibility in breeding programs in Victoria and Queensland. In Queensland trials, the variety Katepwa exhibited poor dough extensibility and hence was chosen as the other parent in the cross. CD87 represents a substantial increase in the dough strength and extensibility of lines available for breeding, and has been used as a parent in the recently released cv. Chara for southern NSW and Victoria. Other progeny, with similar dough properties and higher yield than CD87, have been used extensively in Agriculture Victoria, NSW Agriculture, and Queensland Department of Primary Industries breeding programs in an attempt to increase dough strength and extensibility. The CD87/ Katepwa cross is important for the purpose of uncovering markers for the exceptional dough properties of CD87.

These markers would be used widely if they proved to be effective. Katepwa is also red grained, awnless, and much later maturing than CD87.

The main feature of interest in the Sunco/Tasman cross is flour colour. This trait has a major bearing on customer perception of the end product. The Sunco/Tasman cross targeted the colour and texture requirements for yellow alkaline noodles (YAN) that provide significant advantages for Australian wheat varieties in Japanese and South East Asian markets. Although these requirements are of principal concern for wheat grown in north-eastern Australia, there is a strong desire to extend the source area for YAN wheat and improve the consistency of supply for this major end use of Australian grain. Consequently, molecular markers for YAN colour have broad breeding implications. Sunco is noted for its yellow colour development, yellow colour stability, and the brightness of this colour. It is also considered by tasting panels to have excellent 'mouth feel' properties. The determination of the control of YAN colour and its stability would enable not only more targeted crosses to be made, but allow more specific screening to be carried out on breeding populations. Although consumer texture preference for the finished noodle varies with different markets, Sunco has many of the desirable attributes, which Tasman generally lacks. The low flour water absorption and longer extensibility of Tasman, relative to that of Sunco, is also of

Table 2. Details of doubled haploid (DH) population production

Cross	Pedigree	Haploids produced	DHs produced
1	Cranbrook/Halberd	–	172[A]
2	CD87/Katepwa	628	225
3	Sunco/Tasman	607	269
4	Egret/Sunstar	350	321
5	Kukri/Janz	350	321

[A]Produced by Dr R. Islam, University of Adelaide, Australia.
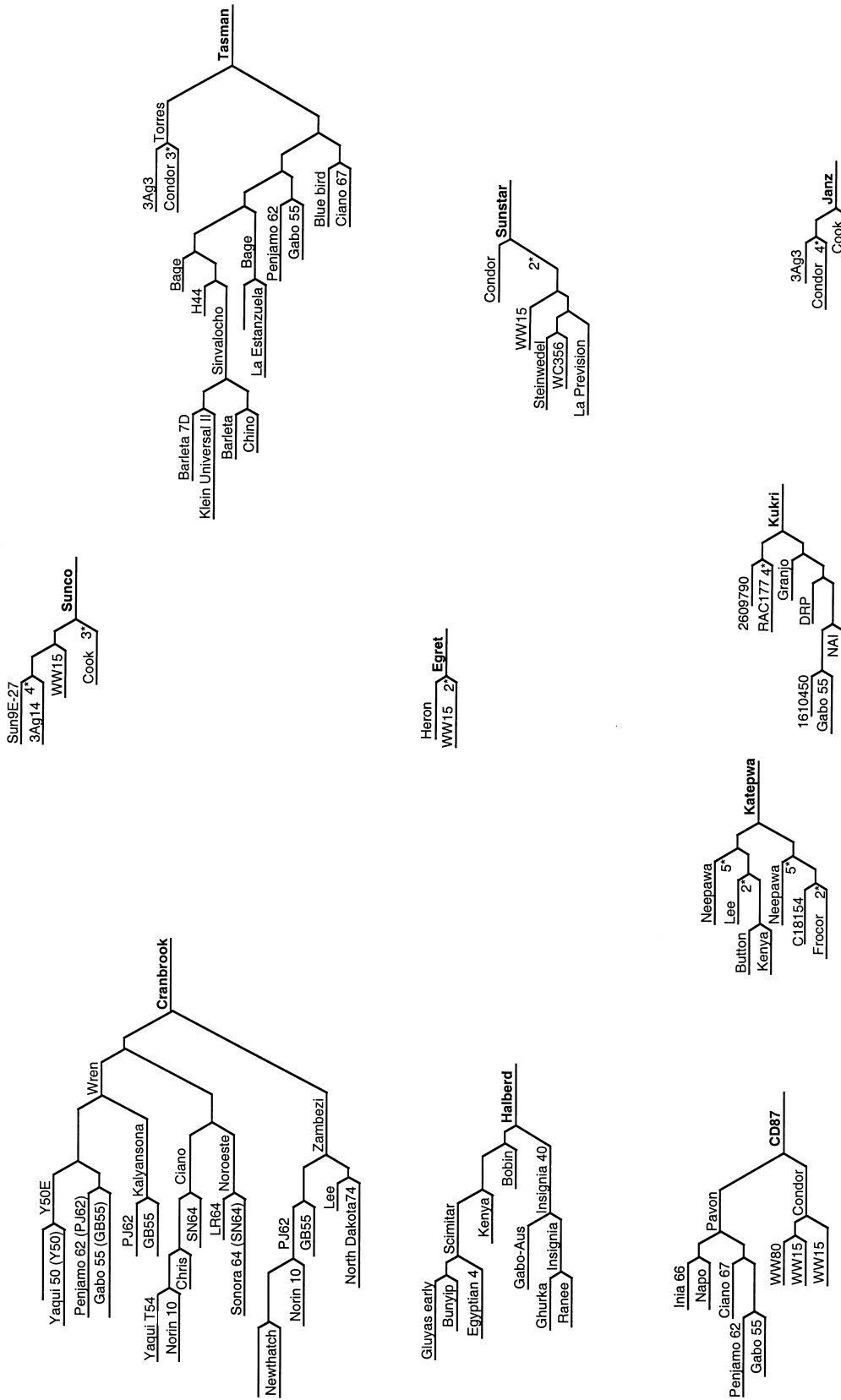
Fig. 2. Pedigree of parents selected for mapping populations (numeral with asterisk indicates number of backcrosses). Based on O'Brien *et al.* (2001).

interest. Several agronomic traits of interest segregating in the Sunco/Tasman cross include dwarfing (*Rht*) genes, glume colour, rust genes, black point resistance, and the fact that Tasman is prone to shattering in southern Australia, a feature that is common among crosses with material introduced from The International Wheat and Maize Improvement Centre (CIMMYT) in Mexico.

The Egret/Sunstar cross is of interest because the parents differ substantially in both dough extensibility and dough strength, yet do not segregate for any of the high molecular weight glutenin subunits and for only one of the low molecular weight glutenin subunits. Detailed knowledge of seed storage proteins and their combinations has only become a breeding focus in recent times (Cornish *et al.* 2001*a*) and is now a determinant in cross selection and will continue to be for the foreseeable future. Consequently, this cross was targetted for the discovery of new markers for chromosomal regions implicated in important dough rheological properties that are not primarily determined by the high and low molecular weight glutenin subunits. An agronomic interest in the cross derives from the well adapted nature of Egret and the possibility of identifying associated quantitative trait loci (QTLs).

The choice of the Kukri/Janz cross derives from the unique high dough strength of Kukri. This genetic material, and the high yielding lines derived from CD87 progeny, are of interest for wheat lines designed to be blended with poorer quality wheat, for use in sponge and dough bread baking, for frozen dough, and aqua-culture pellets. In conventional wheat markets the long mixing time of Kukri and CD87 is undesirable. Janz is a good contrast to Kukri in that it is the only variety in Australia that has been recommended for sowing in every wheat-growing State and so is considered to have genes for 'wide adaptation' and high yield. The cross is therefore of interest both to breeding programs in Western Australia and to those based in the eastern States, with approximately 70 of the doubled haploid lines from this cross being chosen as good breeding lines by the University of Adelaide (Roseworthy) program, with potential for varietal release. Other features of interest in the Kukri/Janz cross include: segregation for different leaf (*Puccinia recondita*), stem (*F. graminis* f.sp. *tritici*), and stripe rust (*F. recondita*) and yellow spot (*Pyrenophthora tritici-repentis*) resistance genes and differences in flour water absorption.

The expanded pedigrees of the parents used in crosses for doubled haploid production are listed in Fig. 2. The striking feature that is evident from this analysis is that the crosses cover a wide range of wheat germplasm used in Australian breeding programs. In particular, the use of diverse germplasm from durum wheat and wheat relatives (e.g. *Agropyron* 'Ag') to introduce new disease-resistance genes has been widespread and is of interest from the point of view of possible sources of segregation distortion (discussed below). The matrix in Table 3 indicates that based on their parentage, degrees of similarity between the parents selected for each population in this study range from 0.047 (Kukri/Janz) to a maximum of 0.43 (Egret/Sunstar).

The selected parents have been screened for polymorphisms. The polymorphisms assayed included a range of DNA markers, protein markers, and disease reactions (Chalmers *et al.* 2001; Cornish *et al.* 2001*b*; Bariana *et al.* 2001; this issue). The analyses verified that even though many of the parents were of direct relevance to breeding programs, sufficient differences existed to allow mapping to be undertaken.

Generation of doubled haploid lines used for mapping

The analysis of proteins at the high and low molecular glutenin loci, along with readily identifiable phenotypic characters (awns, plant height, maturity, etc.), were used to remove doubled haploid lines listed in Table 2 that contained foreign alleles or were contaminated at some point during development. The number of lines removed in this way ranged from 2.3% to 11.6%, depending upon the population. The final sizes of the mapping populations were restricted to between 172 and 180 lines. All 172 available Cranbrook/Halberd doubled haploid lines were used for marker analysis. Lines for mapping studies from CD87/Katepwa and Sunco/

Table 3. Degree of similarity between parental lines

The similarity indices were calculated using standard procedures as described in the International Wheat Information System (IWIS) contained in Skovmand *et al.* (1999)

Name	CD87	Cranbrook	Egret	Halberd	Janz	Katepwa	Kukri	Sunco	Sunstar	Tasman
CD87	1.000									
Cranbrook	0.163	1.000								
Egret	0.349	0.086	1.000							
Halberd	0.043	0.062	0.134	1.000						
Janz	0.481	0.092	0.503	0.031	1.000					
Katepwa	0.154	0.131	0.144	0.021	0.154	1.000				
Kukri	0.065	0.057	0.046	0.019	0.047	0.046	1.000			
Sunco	0.416	0.093	0.449	0.040	0.796	0.143	0.046	1.000		
Sunstar	0.313	0.067	0.434	0.032	0.461	0.114	0.036	0.401	1.000	
Tasman	0.343	0.136	0.301	0.037	0.430	0.139	0.065	0.361	0.270	1.000

Tasman were selected in the order they were transferred from culture. Lines from Egret/Sunstar and Kukri/Janz doubled haploid populations for mapping analysis were randomly selected from the available population.

Segregation distortion and marker distribution

A close examination of the segregation patterns of markers assigned to chromosomes in the Cranbrook/Halberd, CD87/ Katepwa, and Sunco/Tasman linkage maps revealed the presence of segregation distortion within these populations (Table 4). All DNA-based marker types in each population showed some level of skewed segregation in each of the 3 populations. The proportion of total markers showing distorted segregation to either parental type within each population ranged from 1.8 to 12.5%. The level of distorted markers was largely consistent with the proportion of each marker type (AFLP, RFLP, microsatellite) for each respective linkage map (see Chalmers *et al.* 2001, this issue) thus, no DNA-based marker type was more susceptible to non-Mendelian segregation than the others. Markers showing distorted segregation were not evenly distributed across the genome (Table 5). The greatest proportion of distorted markers occurred in 'hot spots' where distorted markers grouped together (60–82% over the 3 populations) (Table 6). The chromosomal location of these hot spots varied between the populations.

Overall, the total number of polymorphic markers screened across each wheat population was not randomly distributed across the hexaploid genome. In all 3 populations examined the total number of polymorphisms recorded on the B genome was higher than for the A or D genomes, with the D genome consistently being the least polymorphic. This pattern of differing polymorphism levels between the 3 genomes (B > A > D) is entirely consistent with mapping studies in other wheat populations (Marino *et al.* 1996; Röder *et al.* 1998). The recent demonstration by Pestova *et al.* (2000), that many microsatellite markers developed in *Aegilops tauschii* are also present and polymorphic in the D genome of wheat, should improve the map density of this genome.

Sources of segregation distortion

Gene mapping studies frequently reveal distorted marker segregation (Cadalen *et al.* 1997; Faris *et al.* 1998). The majority of studies conducted on large (>50 DH) populations that use more than 10 markers show some degree of non-Mendelian segregation (Kammholz 2001). There are several reasons for explaining non-Mendelian segregation within wheat × maize-derived doubled haploid populations. These include heterogeneity within the parents, selection associated with the doubled haploid production process, outcrossing and admixture of seed during increase for trials, and errors in polymorphism scoring and map construction.

Most wheat breeding programs in Australia practice modified pedigree methods, which usually involve ceasing single plant selection at much earlier generations to that practiced in Europe. Thus released varieties may be F_4-derived, and whilst they appear homogeneous, still contain segregating loci in regions that have not been under selection pressure by the breeder. In the present study, efforts were made to minimise this source of variation by screening the populations with readily identifiable marker genes. This resulted in 2.3–11.6% of doubled haploid lines per population being excluded from further analysis, due to heterogeneity within the line or the detection of alleles not present in the parent wheats. This screening also removed doubled haploid lines that had become contaminated or mixed during production and subsequent seed increases.

An example of heterogeneity within parental lines was found in the Sunco × Tasman cross, where the first 51 lines were monomorphic for 4 DNA markers that were polymorphic in the remaining mapping population. The markers affected included: two SSRs, one RFLP, and one AFLP. These markers were located on chromosome 1D (RFLP), 2B (SSR), 4D (AFLP), and 7A (SSR), and originated from both parents. Investigations revealed that the 51 lines involved were doubled haploids made from a different F_1 to the remaining lines. This indicates that a relatively low level of heterogeneity is present in both the Sunco and Tasman parents and illustrates the problems that can arise when mapping populations are not made from a single F_1 cross. Although the 51 lines were not removed from

Table 4. Distorted marker segregations in 3 doubled haploid populations

AFLP, amplified fragment length polymorphism; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat (microsatellite); other, protein markers, resistance genes, and phenotypic markers

Pedigree	Marker type	Segregations favouring one parent[A]	
		Female	Male
Cranbrook/Halberd	552 AFLP	8(1.5)	5(0.9)
	168 RFLP	0(0)	1(0.6)
	74 SSR	1(1.4)	0 (0)
	19 other	0(0)	0(0)
	813 total	9(1.1)	6(0.7)
CD87/Katepwa	247 AFLP	6(2.4)	6(2.4)
	112 RFLP	2(1.8)	5(4.5)
	47 SSR	2(4.3)	0(0)
	8 other	0(0)	0(0)
	414 Total	10(2.4)	11(2.7)
Sunco/Tasman	108 AFLP	11(10)	6(5.6)
	77 RFLP	7(9.1)	5(6.5)
	76 SSR	3(3.9)	1(1.3)
	12 other	1(8.3)	0(0)
	273 Total	22(8.1)	12(4.4)

[A]Number of markers for which segregation ratio significantly different from 1:1 ($P < 0.01$); percentages in parentheses.

Table 5. Markers per chromosome, markers per genome and proportion of markers with distorted segregation ($P < 0.01$) in doubled haploid mapping populations

M, male parent; F, female parent; percentages in parentheses

Chromosome	Cranbrook/Halberd		CD87/Katepwa		Sunco/Tasman	
	Total markers	No. of markers favouring one parent	Total markers	No. of markers favouring one parent	Total markers	No. of markers favouring one parent
1A	45	0	15	0	8	M 1(13), F 0
1B	40	0	27	M 1(4), F 0	18	M 2(11), F 0
1D	39	0	17	0	13	0
2A	25	0	13	M 0, F 3(23)	5	0
2B	69	M 0, F 6(9)	24	0	44	M 0, F 17(39)
2D	36	M 1(3), F 2(6)	8	0	12	M 2(17), F 0
3A	31	0	20	M 0, F 1(5)	10	M 1(10), F 1(10)
3B	51	0	39	M 4(10), F 4(10)	17	M 2(12), F 1(6)
3D	5	0	0	0	17	M 2(12), F 0
4A	74	M 1(1), F 0	10	0	20	0
4B	18	0	17	0	8	0
4D	19	M 0, F 1(5)	0	0	5	0
5A	14	0	11	0	12	M 0, F 1(8)
5B	55	M 1(2), F 0	31	0	18	M 1(5), F 0
5D	16	0	17	0	1	0
6A	51	M 1(2), F 0	37	M 5(14), F 2(5)	0	0
6B	28	0	31	M 1(3), F 0	25	M 1(4), F 1(4)
6D	10	0	4	0	0	0
7A	78	M 2(3), F 0	47	0	26	0
7B	81	0	33	0	10	M 0, F 1(10)
7D	28	0	13	0	4	0
A genome	318	M 4(1), F 0	153	M 5(3), F 6(4)	81	M 2(2), F 2(2)
B genome	342	M 1(0.3), F 6(2)	202	M 6(3), F 4(2)	140	M 6(7), F 20(14)
D genome	153	M 1(0.6), F 3(2)	59	0	52	M 4(5), F 0

the mapping population, the markers that showed the unusual segregation were omitted from subsequent QTL analysis. All other populations were generated from F_1 seed from the same series of crosses to reduce the effect of parental heterogeneity on marker segregation.

To determine whether segregation distortion could arise from selection of a subpopulation for mapping (e.g. the earliest lines to be potted out from haploid embryo culture), the available Sunco × Tasman population of 269 lines was examined for the segregation of major genes controlling plant height. The observed segregation of double dwarfs : semi-dwarfs : tall lines was 78:134:57, a ratio that was not significantly different from the expected 1:2:1 ratio for a 2-gene model. In the subpopulation of 180 doubled

haploid lines used for mapping, a random selection of 145 lines gave a ratio of 34:79:32 (not significantly different from the expected 1:2:1 ratio). The presence of *Rht-B1* and *Rht-D1* in Sunco and Tasman, respectively, based on their pedigrees, was consistent with these observations. Thus the non-random selection of a subpopulation of lines for mapping, did not appear to introduce any segregation distortion with respect to these field traits.

Another possible source of non-Mendelian segregation is selection that arises as part of the doubled haploid process itself. This poses the question of whether apparent segregation aberrations are caused by the doubled haploid production system, or whether these distortions can be put down to a random process such as genetic drift. Genetic drift,

Table 6. Chromosomal distribution of segregation distorted markers ($P < 0.01$) in 3 doubled haploid populations

'Hot spots' are defined as groups (2 or more) of segregation distorted markers; percentages in parentheses

Population	Markers	Markers distorted	On ends of linkage groups	Randomly located (%)	Located in hot spots
Cranbrook/Halberd	813	15	3(20)	3(20)	9(60)
CD87/Katepwa	414	21	1(5)	5(24)	15(71)
Sunco/Tasman	273	34	3(9)	3(9)	28(82)

or changes in allele frequencies due to chance processes, has been widely reported in conventionally derived populations and is expected to occur in doubled haploid populations. For example, Bjornstad *et al.* (1993) found that the same number of distorted loci was present in the single seed descent and doubled haploid population they compared. Thus, in doubled haploid populations, only levels of distortion above those observed in F_2 or single seed descent populations can be reasonably attributed to the doubled haploid production process itself. The levels of segregation distortion identified within populations developed for the NWMMP (Table 4) are comparable with, or lower than, the levels of non-Mendelian segregation identified in mapping studies using conventionally derived inbred populations (Xu *et al.* 1997; Blanco *et al.* 1998).

Of the 2 gametes available for haploid production, current evidence indicates that maternally derived doubled haploids from wide crosses (e.g. wheat × maize) generally produce the most suitable populations, a fact that is highlighted when direct comparisons are made with anther culture (Wang *et al.* 1995). Although distortions have been isolated to specific genomic regions in anther culture populations (Wang *et al.* 1995), no clear linkage with characters of agronomic performance, which might explain the distortion, has been made.

Segregation distortion can also arise from errors in the scoring of markers in the mapping experiments. One marker, located on the short arm of chromosome 2B, showing segregation distortion in the doubled haploid lines from 2 crosses, Cranbrook/Halberd and Egret/Sunstar, has been investigated in detail (W. Keys and R. Appels, unpubl. data). The microsatellite marker (wmc314) showed a segregation of 126:54 (Egret/Sunstar) and 105:52 (Cranbrook/Halberd), which deviates significantly from the expected 1:1 ratio. Since this marker was scored as a dominant marker, the segregation distortion favoured the 'presence' score. The initial agarose gel-based score was re-examined using the high resolution ABI/Genescan analytical system. The high resolution analyses were aimed at removing the possibility of multiple bands, differing in size of only 1–5 bp, behaving independently but being scored as a single band and thus leaving open the possibility of artificially distorting the scoring of the marker on the less discriminating agarose gels, in favour of the 'presence' allele. High resolution analysis (Fig. 3) indicated that 5 bands (size 185–190 bp) comprised the 'presence' allele of wmc314. Significantly, however, the bands always behaved as a group in the doubled haploid lines (2 examples are shown in Fig. 3), indicating that they were probably the result of the stuttering often observed in the analysis of microsatellites (see Rampling *et al.* 2001, this issue) and had not been mis-scored. Similar analyses on other markers argued further against a scoring problem in the assay of segregation. Significantly, only a very low proportion (Table 6) of all the distorted markers were located on the ends of linkage groups. Most polymorphic bands were
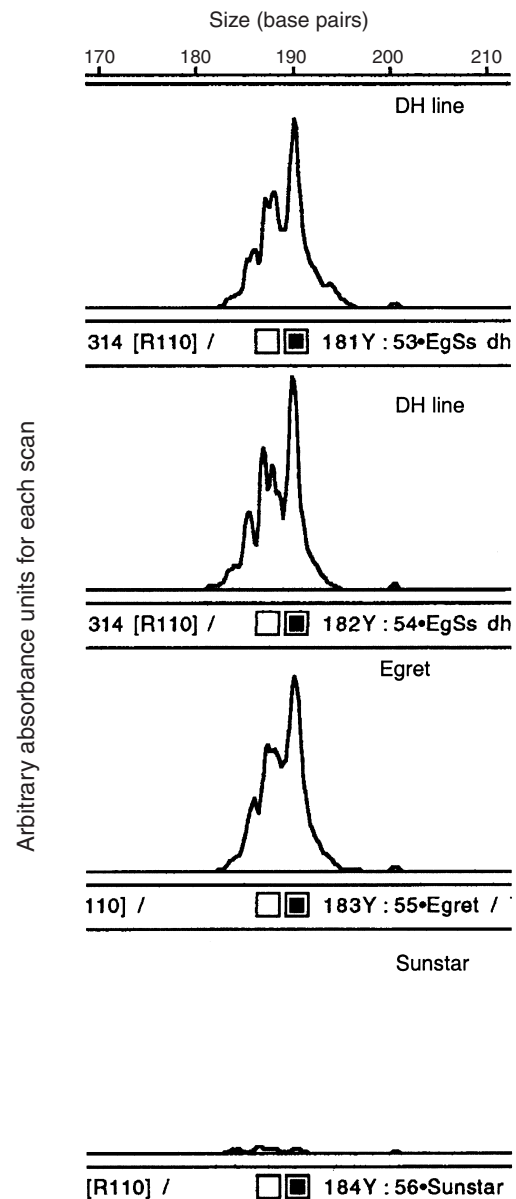
Fig. 3. Sections of traces from an ABI sequencer/Genescan analysis of wmc314 PCR products obtained from the lines Egret and Sunstar and 2 representative examples of the 180 doubled haploid (DH) lines derived from the cross.

independently scored twice (Chalmers *et al.* 2001, this issue) and carefully entered into the linkage maps, with any double cross-over events being re-examined to ensure accuracy. This reduces the possibility of distorted gene segregation resulting from poorly scored markers.

Thus, the observed distortions are unlikely to be artefactual. The fact that they cluster in regions of specific chromosomes in particular crosses is significant. For example, in the Sunco/Tasman cross, distinct sections of chromosome 2B, defined by groups of markers, show

segregation distortion even though homologous sections in other crosses (Cranbrook/Halberd, CD87/Katepwa) do not show the same level of gene selection (Table 5).

Alien introgressions

The 3 crosses analysed in detail contain various segments of alien chromatin, either introgressed from a distant relative (*Agropyron* syn. *Thinopyrum* syn. *Lophopyrum*), or tetraploid wheat (Fig. 2). Alien chromatin segments have the potential to lead to segregation distortion since they may introduce a block of new genes that substitute for chromosome regions bearing loci that determine aspects of whole plant phenotype that influence population development.

Evidence to support this contention can be found in the Sunco/Tasman population. The high level of segregation distortion seen on chromosome 2B in the Sunco/Tasman population favoured the maternal parent Sunco and 50% of the markers showing non-Mendelian segregation ratios observed within the Sunco/Tasman population were located on this chromosome (Table 5). Chromosome 2B in Sunco contains a translocation from *Triticum timopheevii* that includes *Sr36* (McIntosh *et al.*1995.) A large block of distorted markers, which included the *T. timopheevi*-derived genes such as *Sr36*, mapped to this region (Chalmers *et al.* 2001, this issue). Thus, this alien segment in one of the parents is likely to be the cause of the segregation distortions observed, and at the same time almost certainly explains the highly polymorphic nature of chromosome 2B in this population (44 markers mapped to 2B, whereas the chromosome with the next highest marker number is 7A with 27 markers).

Significance of segregation distortion

The consequences of segregation distortions must not be under-estimated (Foisset *et al.* 1996) and are of particular concern when populations are intended for genetic mapping. The majority of software packages used to construct genetic maps, including the very popular Map Maker (Lander *et al.* 1987) and Map Manager QT (Manly and Olson 1999), assume the absence of disturbed segregation ratios. Segregation distortions serve as a source of spurious linkage. Small but significant segregation distortion can easily result in a reduced estimate of the recombination fraction (Cloutier *et al.* 1997). Therefore, it is essential that improvements in mapping software include the possibility to readily correct for non-Mendelian segregation ratios, in particular at the stage of fine mapping of specific chromosome regions.

Conclusions

doubled haploid populations developed for QTL mapping within the National Wheat Molecular Marker Project have been introduced. For the populations studied in this paper, where extensive maps have been established (Chalmers *et al.*

2001, this issue), no consistent pattern of segregation distortion could be determined across doubled haploid populations. Non-Mendelian segregation was generally associated with hot spots within the genome and, in at least one case, was associated with chromosome regions that included alien translocations.

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