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Mapping components of flour and noodle colour in Australian wheat

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Abstract. Flour and noodle colour influence the value of wheat (*Triticum aestivum* L.) and are obvious targets for breeders seeking to improve quality, end-product range, and marketability of wheat. The objective of this investigation was to identify quantitative trait loci (QTLs) associated with flour and noodle colour traits and with individual components of colour. One hundred and sixty-three doubled haploid lines derived from Sunco × Tasman, white-grained, prime hard, and hard wheats adapted to the north-eastern region of Australia were used for the bulk of this study and were supplemented by doubled haploid populations derived from CD87 × Katepwa and Cranbrook × Halberd for comparisons of flour colour. Samples of Sunco × Tasman, together with parental lines, were grown at Narrabri, NSW, in 1998 and 1999 and at Roma, Qld, in 1998 and used for visible light reflectance measurements of flour brightness (CIE L*) and yellowness (CIE b*), and white salted noodle (WSN) and yellow alkaline noodle (YAN) brightness, yellowness, and colour stability. Xanthophyll content and polyphenol oxidase (PPO) activity were measured spectrophotometrically.

No consistent QTLs were identified for flour L* or initial L* of WSN and YAN. Xanthophyll content was very strongly associated with QTLs located on chromosomes 3B and 7A and these QTLs also had a major influence on flour b*, WSN b*, and YAN b*. Noodle brightness at 2, 24, and 48 h and the magnitude of change in noodle L* and b* with time were affected by QTLs on 2D, contributed by Tasman, and, to a lesser degree, 2A. The QTL on 2D was clearly associated with control of grain PPO, an enzyme implicated in darkening of Asian style noodles. QTLs located on 2B, 4B, and 5B and associated with control of grain size or flour protein content also appeared to influence a number of colour traits.

Additional keywords: colour stability, xanthophyll, polyphenol oxidase.

Introduction

Flour and end-product colour are important criteria of wheat, *Triticum aestivum* L., quality and play a critical role in determining the suitability of wheat for particular products and markets. Flour colour is affected by two main components: (*i*) the presence of bran flakes resulting in speckiness, which is related to how easily the outer seed coat layers and aleurone can be separated from the endosperm during milling and sieving, and (*ii*) the actual colour of the starchy endosperm. In turn, end-product colour depends on the colour of the flour together with reactions and interactions that occur during the mixing of flour with water and other ingredients and subsequent processing and cooking. Different end products of wheat have different colour requirements, for example, white flour is important for bread production in many regions, a bright white to creamy colour is desirable for white salted noodles (WSN), whereas development of a bright yellow colour is required for yellow alkaline noodles (YAN). Some products are sold in a raw form and both initial dough/product colour and the maintenance of colour and brightness are important.

Flour colour has been shown to be genetically controlled in part, and factors such as brightness, yellowness, and the degree of bran and germ contamination of the flour have long been targets for selection by many wheat breeders (Labuschagne *et al.* 1996; Parker *et al.* 1998; Mares and Panozzo 1999; Parker and Langridge 2000). The constituents and reactions involved in brightness of flour and the initial brightness of noodles are still to be characterised and quantified. In WSN, as in flour, the important components of initial colour include brightness, measured as a CIE (Commission Internationale l'Eclairage) L* value, and the whiteness/creaminess, measured as a CIE b* value,

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which is determined by the level of xanthophylls (lutein and its fatty acid esters, which are retained in the flour) (Mares *et al.* 1997; Ward *et al.* 1997, 1998). Colour stability in WSN is determined by factors that degrade brightness, i.e. cause darkening, or bleach the xanthophyll pigments. In YAN, the important components of initial colour include brightness and xanthophyll content, as in WSN, and also specific flavonoid compounds that are colourless at neutral pH and in WSN and bread doughs but which turn yellow at the high pH resulting from the use of alkaline salts (Mares *et al.* 1997). Mares *et al.* (1997) reported the extraction, separation, and quantitation of the xanthophylls and flavonoids involved in the yellow colour of YAN and demonstrated that there was a strong correlation between CIE b* and the sum of the xanthophyll and flavonoid contents of flour. Colour stability in YAN will consequently be determined by factors that cause darkening or degrade either xanthophylls or the specific flavonoids that turn yellow at high pH. Noodle darkening results from the additive effects of polyphenol oxidase (PPO) and non-PPO reactions (Mares and Panozzo 1999). PPO catalyses the oxidation of some phenolic compounds, which leads to the production of black or dark brown pigments causing noodles to darken over time. Variation in PPO, derived from the seed coat, explains much of the difference in darkening between genotypes and this appears to be superimposed on a background of non-PPO darkening that shows only limited genetic variability (Mares and Panozzo 1999). Darkening is obviously more critical in noodles that are often stored raw for several days prior to cooking ready for consumption and thus PPO levels are particularly relevant in the production of YAN.

Most of these factors are thought to be under independent genetic control (Mares and Panozzo 1999; Mares *et al.* 2001); however, flour colour can also be influenced by environmental factors during grain formation, the milling process, and, in the case of noodles, the particular recipe, ingredients, and preparation conditions used in the manufacturing process (Mares and Panozza 1999).

Flour and noodle colour are expressed as quantitative traits. Parker *et al.* (1998) found quantitative trait loci (QTLs) for flour colour (b*) in an Australian single seed descent population, Schomburgk × Yarralinka, on chromosomes 3A and 7A. Ma *et al.* (1999) located QTLs for flour colour (L*-b*) on homoeologous group 1 linkage groups and chromosome 7B. The QTL found by Parker *et al.* (1998) on chromosome 7A was later converted to an STS marker (Parker and Langridge 2000). This marker has been further validated and shown to be polymorphic over a wide range of Australian germplasm (Sharp *et al.* 2001, this issue).

The aim of this study was to identify QTLs associated with various measures and components of flour and noodle colour by evaluating flour colour data for 3 Australian doubled haploid populations and noodle colour data for the Sunco × Tasman doubled haploid population (DH), respectively (Chalmers *et al.* 2001, this issue). In doing this it should be possible to identify potential molecular markers for flour and noodle colour, which represent a wide range of Australian germplasm. Xanthophyll content and PPO levels will also be examined. Flavonoids, whilst very important in YAN (Mares *et al.* 1997), were not included in this investigation since biochemical studies on their role in colour and genetic variation in colour are still in progress.

Materials and methods

Plant material

This study was based on 3 doubled haploid populations, Sunco × Tasman, Cranbrook × Halberd, and CD87 × Katepwa (163, 180, and 163 lines, respectively). Sunco, a hard-grained wheat with exceptional YAN quality, Tasman, a hard-grained wheat with similar phenology but poor noodle quality, and the Sunco × Tasman doubled haploid population were of particular interest in this investigation. Population details, parental lineage, and trait segregation details are discussed in Kammholz *et al.* (2001, this issue).

Field trials

Flour colour data were collected from trials grown at 3 sites for the Cranbrook × Halberd population over 2 years [Roseworthy, SA (1997); Roma, Qld (1997); Stow, SA (1997, 1998)], at one site for 2 years for the CD87 × Katepwa population [Roma (1998 and 1999)], and at 2 sites for 2 years for the Sunco × Tasman population [Roma (1998 and 1999) and Narrabri, NSW (1998 and 1999)]. Details of trials conducted at Roseworthy, Roma, and Stow are described in Kammholtz *et al.* (2001, this issue). Noodle colour data were collected for the Sunco × Tasman population grown at Narrabri in 1998 and 1999, where effort was made to optimise soil fertility, uniformity, plant growth, and harvest and to exclude any confounding effects due to moisture stress or weather damage. DH lines were sown as 5-m × twin-row plots in a block 20 plots wide × 10 plots deep and with Sunco and Tasman at intervals of 20 plots.

Plant and grain characteristics

Flowering times were collected from the field trials at Roma. Plant height (distance from the base of the plant to the tip of the spikes on primary tillers) and kernel weight (mean weight of replicate samples of 20 grains) were measured at the Narrabri site in 1998 and 1999. Plant height data were confirmed using a GA-seedling test under controlled glasshouse conditions. Twenty seedlings per line were raised in potting mix and irrigated with 20 μM gibberellic acid. Heights from the seedling base to the tips of fully expanded first and second leaves were recorded.

Flour protein determination

Flour protein was determined by NIR using an InfraAlyser 260 calibrated against Kjeldahl nitrogen and expressed as percent flour weight.

Flour colour evaluation

All samples were conditioned to 15% moisture content prior to milling. Harvested seed (100 g) from each line was milled on a Quadrumat Junior Mill (Narrabri sites), whilst for all other sites 1 kg was milled on a Buhler Mill. Flour samples were scored for colour using a Spectroflash 300 reflectance spectrometer and an 18-mm measuring aperture (Narrabri sites) and a Minolta CR–200 Chroma Meter equipped with a 50 mm measuring head (all other sites). Colour was

Table 1. Mean values, standard errors (parental lines), and range (doubled haploid lines) for grain, flour, and noodle trait measurements
Standard errors and range in parentheses after the corresponding means. PPO, polyphenol oxidase; WSN, white salted noodle; YAN, yellow alkaline noodle

	Sunco		Tasman		Sunco × Tasman	
	1998	1999	1998	1999	1998	1999
			Roma			
Grain PPO	0.11		0.34		0.25 (0.095 – 0.605)	
			Narrabri			
Grain PPO	0.114 (0.004)	0.209 (0.056)	0.28 (0.018)	0.45 (0.029)	0.20 (0.102 – 0.36)	0.38 (0.19 – 0.73)
Flour protein (%)	12.64 (0.13)	12.70 (0.03)	13.80 (0.05)	12.90 (0.12)	12.9 (11.6 – 15.1)	13.5 (11.8 – 15.8)
Flour L*	93.2 (0.022)	92.8 (0.05)	93.6 (0.074)	93.3 (0.07)	93.3 (92.3 – 94)	93.0 (92.3 – 93.5)
Flour b*	8.9 (0.05)	8.5 (0.06)	8.7 (0.07)	8.4 (0.05)	8.9 (7.2 – 10.9)	8.4 (6.95 – 10.0)
Xanthophyll	0.075 (0.003)	0.072 (0.003)	0.085 (0.002)	0.084 (0.003)	0.081 (0.061 – 0.11)	0.081 (0.052 – 0.124)
WSN L* 0 h	87.6 (0.27)	87.4 (0.14)	86.8 (0.29)	87.0 (0.26)	87.0 (84.8 – 88.9)	87.0 (84.5 – 88.7)
WSN L* (0–24 h)*	6.61 (0.28)	6.4 (0.22)	9.04 (0.41)	7.6 (0.21)	7.87 (3.42 – 14.1)	7.05 (4.0 – 11.6)
YAN L* 0 h	87.3 (0.28)	85.4 (0.20)	86.7 (0.19)	85.4 (0.21)	87.1 (84.44 – 88.78)	85.45 (82.47 – 87.32)
YAN L* (0–24 h)	6.52 (0.17)	5.65 (0.17)	12.2 (0.21)	9.52 (0.32)	9.96 (5.56 – 14.1)	8.16 (4.16 – 13.1)
WSN b* 0 h	14.5 (0.37)	13.64 (0.16)	15.6 (0.42)	14.34 (0.22)	15.7 (11.9 – 20.1)	13.9 (11.5 – 16.8)
WSN b* (0–24 h)	–4.02 (0.57)	–3.33 (0.23)	–2.34 (0.23)	–1.86 (0.18)	–3.36 (–6.05 to –1.17)	–2.8 (–5.03 to –1.01)
YAN b* 0 h	20.95 (0.21)	20.1 (0.21)	20.6 (0.27)	20.2 (0.33)	20.6 (18.2 – 23.8)	19.7 (17.9 – 22.3)
YAN b* (0–24 h)	–5.55 (0.27)	–5.04 (0.13)	–1.76 (0.25)	–1.55 (0.09)	–3.5 (–7.2 to –0.08)	–3.42 (–6.12 to –0.3)

measured in L* a* b* colour space (CIELAB) and expressed as CIE (Commission Internationale l'Eclairage) L* (brightness) and b* (yellowness) values.

Noodle colour evaluation

Noodle colour evaluation was only done on the Sunco × Tasman population. Colour was measured at 0, 2, 24, and 48 h (Narrabri sites), and 2 and 24 h (Roma sites). For the samples produced at Narrabri in 1998 and 1999, small scale WSN (10 g flour + 3.6 mL 2% w/v sodium chloride solution) and YAN (10 g flour + 3.6 mL 2% w/v sodium carbonate solution) sheets were prepared in duplicate. Flour and liquid were combined in a stainless-steel bowl using a butterfly-shaped mixing paddle driven by a commercially available drill press. The noodle dough was then pressed into a block and passed through the rolls of a small domestic pasta machine, progressively reducing the roll gap to finish with a sheet 1.5 mm thick. The sheet was cut to a standard rectangle and stored in a resealable plastic bag between colour measurement readings. In addition to the recorded colour measurements, changes in L* and b* were calculated by subtracting measurements at 2, 24, and 48 h from the initial readings. Mean values from duplicate noodle sheets were used in all the analyses.

PPO evaluation

PPO evaluation was only done on the Sunco × Tasman population. Grain PPO was determined on 20 individual grains from the Narrabri (2 years) and Roma (1 year) sites, according to the microplate method described by Bernier and Howes (1994) and activities expressed as simple $OD_{415\ nm}$ units. The assay procedure was standardised by using samples from a bulk of a low PPO cultivar, Lark, on every assay plate.

Xanthophyll evaluation

Xanthophyll evaluation was only done on the Sunco × Tasman population. Duplicate flour samples (3 g) were extracted with 15 mL absolute methanol for 30 min on a gentle rocker table at room temperature. Samples of the extracts were centrifuged in a microfuge at 14000 rpm then filtered through a 0.45 µm syringe filter prior to measurement of absorbance at 436 nm. Xanthophyll content was expressed simply as $OD_{436\ nm}$. The assay was standardised by including a sample of a bulk flour, cv. Sunvale, with each batch of lines.

Statistical analysis of data

Transgressive segregation among doubled haploid lines, Sunco, and Tasman was tested by the l.s.d. test at $P = 0.05$. Where the mean trait value for a line exceeded that of the nearest parent, it was considered a transgressive segregant. Simple linear regression coefficients and standard errors were calculated in Excel. Broad sense heritability estimates were calculated from ANOVA results using Agrobase.

Genetic maps

The genetic maps used to evaluate phenotypic data are described in Chalmers *et al.* (2001, this issue).

Table 2. Broad sense heritability estimates for grain polyphenol oxidase (PPO), flour traits (protein content, xanthophyll content, L*, and b*), and noodle colour traits (L* 0 h, b* 0 h, L*0–24 h)
YAN, yellow, alkaline noodles; WSN, white, salted noodles

Trait	Broad-sense heritability
Grain PPO	0.77
Flour protein content	0.47
Flour xanthophyll content	0.93
Flour L*	0.74
Flour b*	0.89
WSN L* 0 h	0.77
WSN b* 0 h	0.92
WSN L*(0–24 h)	0.90
YAN L* 0 h	0.76
YAN b* 0 h	0.88
YAN L*(0–24 h)	0.96

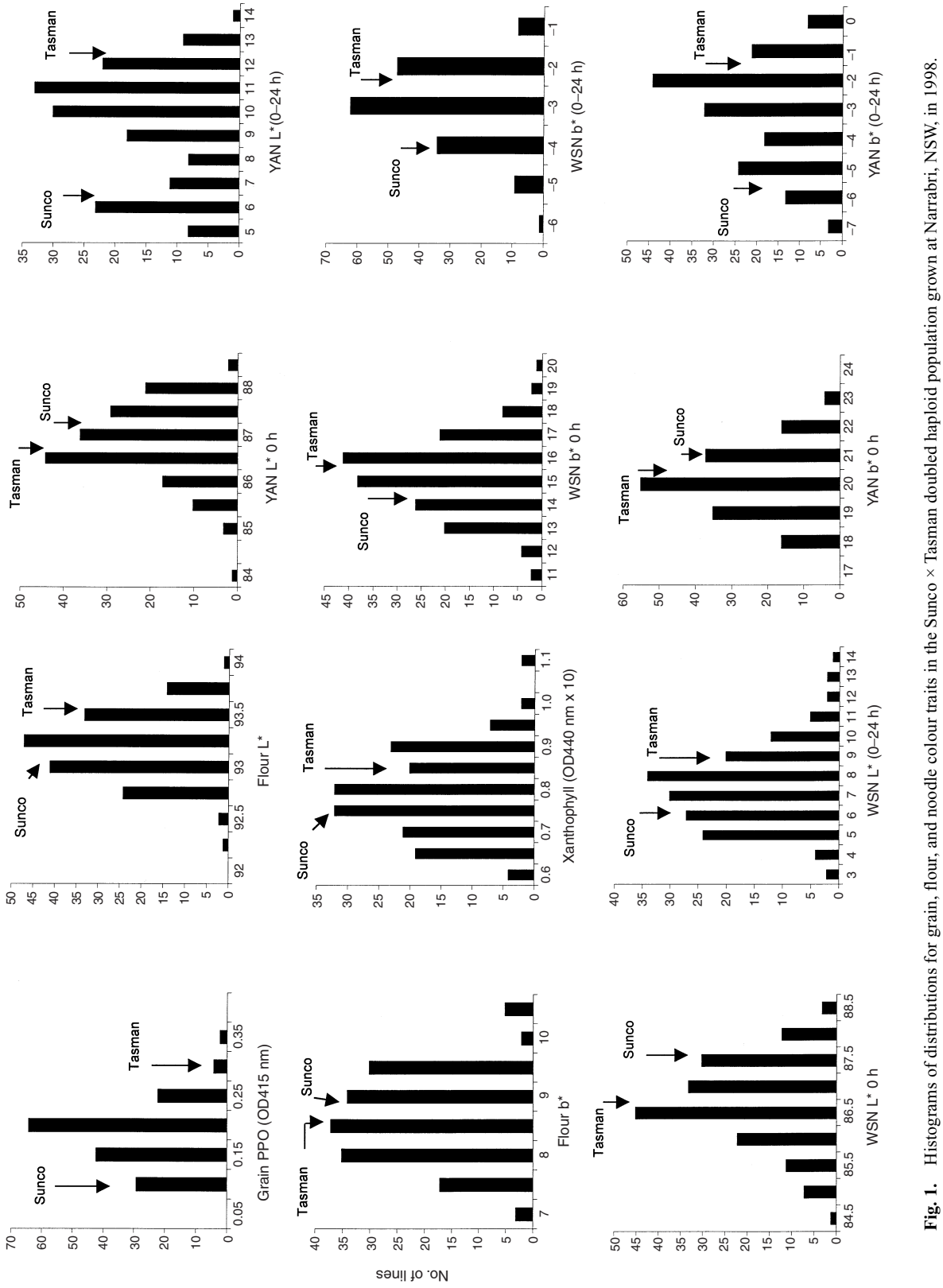

Fig. 1. Histograms of distributions for grain, flour, and noodle colour traits in the Sunco × Tasman doubled haploid population grown at Narrabri, NSW, in 1998.

Genetic analysis

The associations between markers and trait data were tested using a single-point regression analysis in Map Manager QTb29ppc (Manly 1997) based on the likelihood ratio statistic (LRS). Permutation tests using Map Manager were run for each set of trait data compared with the marker data for that population to set a level of significance for that trait. The permutation tests were run 1000 times at a significance level of $P < 0.001$. QTLs identified by a combination of single point analyses and permutation tests were further analysed by interval mapping [using the software Qgene, version 3.04 (Nelson 1997)], which searches for the effects of QTLs using sets of linked markers (Lander and Botstein 1989). A chromosomal display of the significant QTL associations after interval analysis was generated using Qgene.

Results

Field observations on the Sunco × Tasman population grown at Narrabri

In both 1998 and 1999, the trials were disease-free, uniform in appearance, and produced well-filled grain with high protein content (Table 1). Broad sense heritability for protein content in this population was 0.47 (Table 2). Protein contents for the parental lines were similar to one another and to the means of the doubled haploid population. Variation in protein content was associated with QTLs on chromosomes 1B (LOD 5.07), 2B (LOD 7.09), and 5B (3.75). Small differences in maturity were controlled by QTLs on chromosomes 1D (LOD 4.07) and 7B (LOD 4.54). The population contained 3 distinct classes based on plant height: tall (35 lines), semi-dwarf (86 lines), and extreme dwarf (42 lines). Plant height measured on mature field-grown plants or on GA-treated seedlings was associated with QTLs on chromosomes 4B (LOD scores ranging from 12.8 for mature plant height to 8.76 for the GA seedling test) and 4D (LOD score for mature plant height of 22.66). Mean grain size was significantly different between these classes and decreased as plant height decreased (mean 1000-kernel weights of 42.5, 36.9, and 30.1 g in 1998 and 44.35, 40.73, and 33.7 g in 1999 for the tall, semi-dwarf, and extreme dwarf groups, respectively). Grain weight was associated with QTLs on chromosomes 2B (LOD 5.42), 4B (LOD 9.57), and 4D (LOD 5.68). The group of lines with extreme dwarf growth habit also appeared to have a higher proportion of shrivelled or misshapen grains, although no attempt was made to quantify this effect.

Colour traits

Mean values and ranges for all traits for both years are shown in Table 1 together with mean values and standard errors for Sunco and Tasman. All trait distributions were normal except darkening of YAN ($L^*_{(0–24h)}$) and were similar in both years (Fig. 1, data for 1998 only). Significant transgressive segregation occurred in both directions for all traits except grain PPO. For this trait, there was no transgressive segregation towards lower values. With the exception of grain protein content, broad sense heritability estimates for grain, flour, and noodle colour traits were generally very high (Table 2).

Flour colour

Comparisons of flour L* over the 3 populations revealed no chromosome areas that were significant for all crosses. All

Table 3. LOD scores for flour L* and flour b* for 3 doubled haploid populations grown at 1–3 sites for 1 or 2 years

Only chromosomes that had QTLs with LOD scores > 3 are reported. Sites used were Roseworthy, SA (Ros97), Roma, Queensland (Rom97, Rom98, and Rom99), Stow, SA (Sto97 and Sto98), and Narrabri, NSW (Nar98 and Nar99)

	Cranbrook × Halberd				CD87 × Katepwa		Sunco × Tasman		
Site:	Ros97	Rom97	Sto97	Sto98	Rom98	Rom99	Rom98	Nar98	Nar99
					*Flour L**				
1A		4.73	3.51						
1B						3.31		4.55	5.41
2D					3.88	3.78			
4B							3.97	6.63	
5B						3.97	3.62		
5D	5.43		3.99						
Unknown						4.03			
					*Flour b**				
2D	3.55			3.99	5.23	5.17			
3A					3.02	5.46			
3B		3.98					3.81	4.78	3.40
4B							3.59	3.15	
5B							4.48	3.77	4.08
5D	3.43	3.35	5.94						
6A						3.79			
7A	3.41	5.89	4.08	3.29			7.56	10.61	12.08
7B						4.38			
Unknown	4.43	4.00	4.33	3.75					

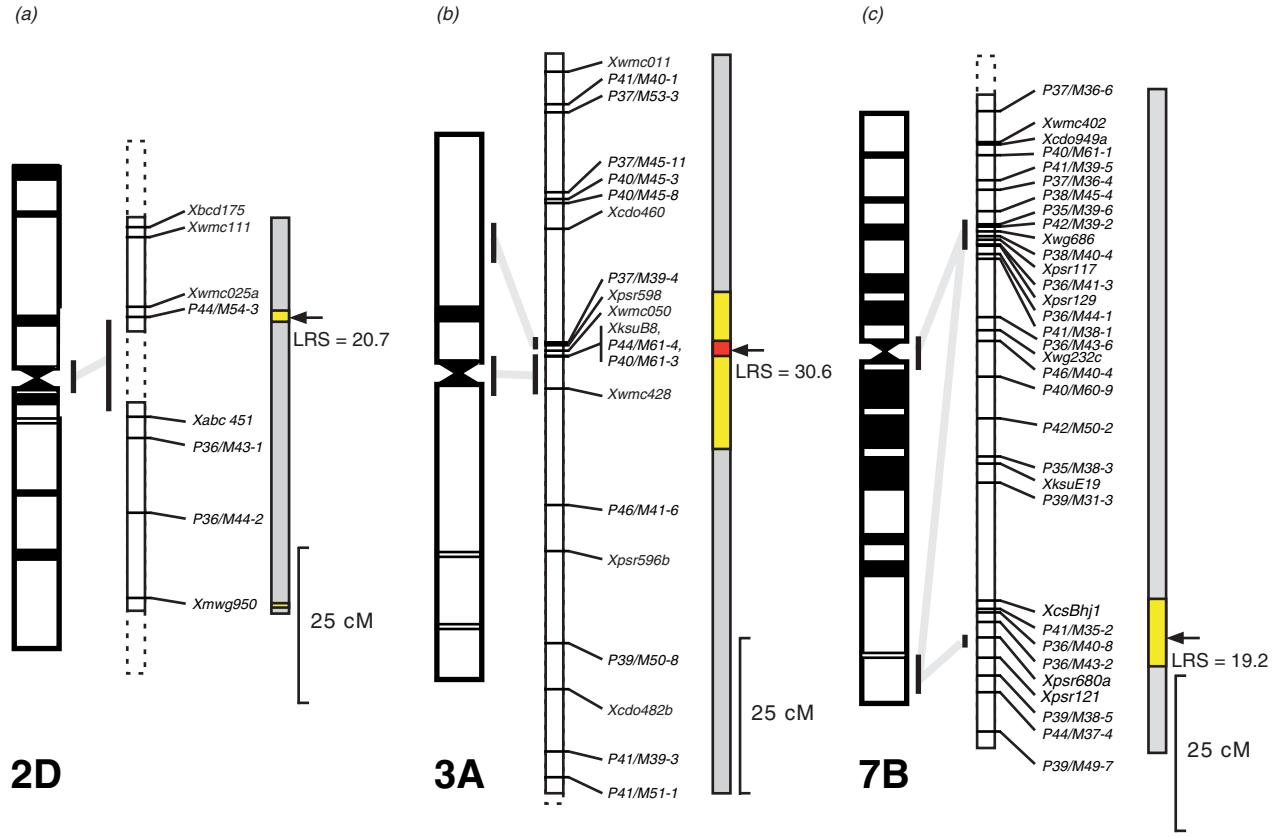

Fig. 2. QTLs on chromosome 2D, 3A, and 7B associated with flour b* in CD87 × Katepwa. The left part of each section in the figure illustrates the standard C-banded karyotype for the chromosome. The accompanying genetic map has been aligned to the C-banded karyotype using published literature (Chalmers *et al.* 2001, this issue), and a scale of genetic distance (cM = centimorgans) is provided. The profile on the right of each chromosome is the result of a regression analysis scan for QTLs (interval analysis, Map Manager). The location of the marker with the highest likelihood ratio statistic (LRS) is marked with an arrow in each case. Red regions indicate highly significant (HS, 99.9%, corresponding to LRS >22.5) and the yellow, significant (S, 95%, corresponding to LRS >14.1), associations as determined using permutation tests.

those with LOD scores >3 (after interval analysis) are reported in Table 3. For the Sunco × Tasman population, flour L* was affected by a QTL on 2B, Narrabri sites only, and a QTL on 4B at 2 sites in 1998; however, this QTL was not significant in the 1999 samples. Flour L* in 1998 was significantly correlated with flour L* in 1999 ($r = 0.57$) and the broad sense heritability for this trait was 0.74 (Table 2). A large number of QTLs affecting flour b* were identified, although again there were significant differences between populations (Table 3). For the CD87 × Katepwa population, 4 QTLs on chromosomes 2D, 3A, 6A, and 7B (Fig. 2) accounted for 12%, 17%, 13%, and 10% of the variation respectively. Cranbrook × Halberd had QTLs for flour b* on 3B, 5D, 7A, and an unknown location that were generally consistent across sites and years (Table 3, Fig. 3). Finally, Sunco × Tasman had QTLs for flour b* on 3B, 4B, 5B, and 7A that were also consistent across sites and years and which accounted for 20%, 7%, 12%, and 27% of the variation (Table 3, Fig. 4). The same regions of chromosomes 3B and 7A were significant for both Cranbrook × Halberd and Sunco × Tasman populations (Figs 3 and 4). In the Sunco × Tasman population, the 3B QTL was contributed by Sunco, whereas the 5B and 7A QTLs were contributed by Tasman. Flour b* was highly heritable (Table 2) and showed the greatest transgressive segregation in both directions, and flour b* data from 1998 were strongly correlated with corresponding data from 1999 ($r = 0.82$). In addition, flour b* QTLs on chromosomes 3B and 7A appeared to be additive. Means of flour b* for progeny genotype combinations of the Sunco 3B QTL with the Tasman 7A QTL (mean flour b* = 9.5) were significantly different from all other combinations (mean flour b* = 8.5–8.7) and the parents at $P < 0.01$.

The QTL located on chromosome 4B that was associated with both flour L* and flour b* appeared to correspond to a QTL controlling plant height and grain size but had no significant effect on protein content (Fig. 5). Similarly, the regression analysis scans for the QTL on chromosome 5B for flour b* appeared to correspond to the scan for a QTL for

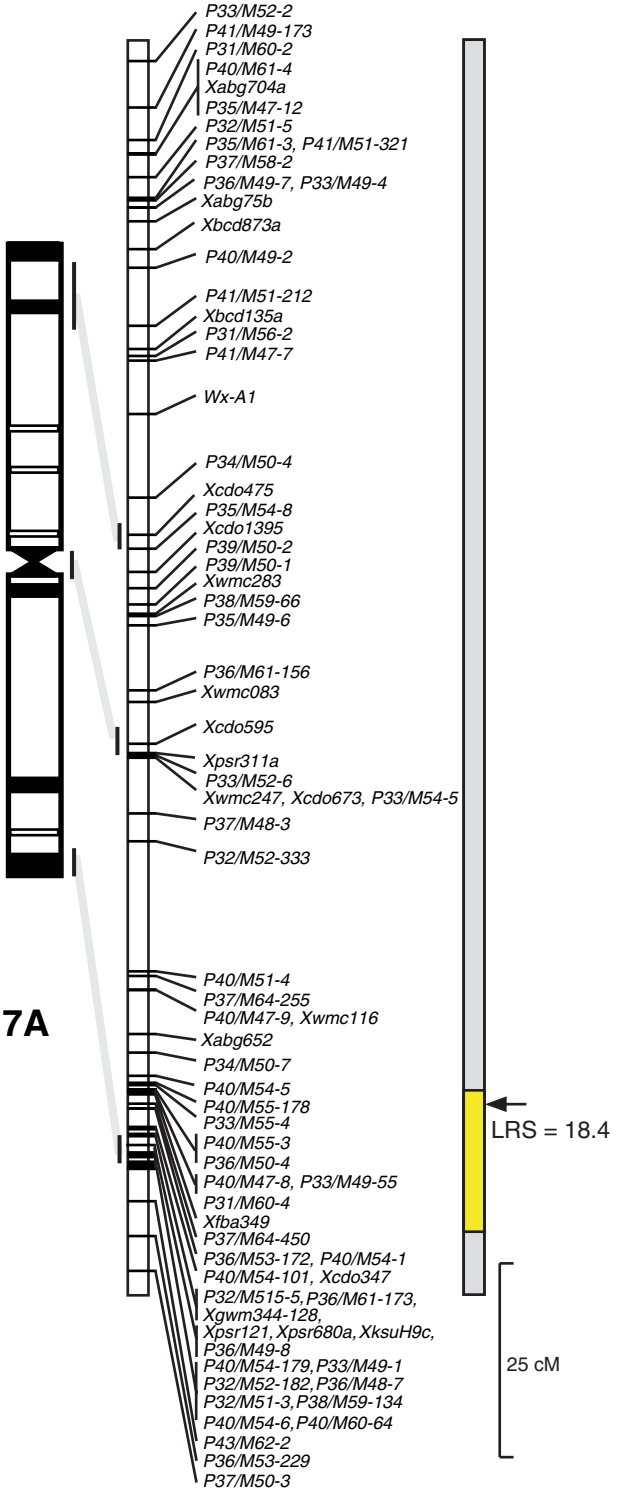

Fig. 3. QTL on chromosome 7A associated with flour b* in Cranbrook × Halberd. The colour system for showing the location and significance of QTLs is described in Fig. 2. HS (99.9%) corresponds to LRS > 21.5 and S (95%) corresponds to LRS > 13.6. The location of the marker with the highest likelihood ratio statistics (LRS) is marked with an arrow.

protein content on 5B (data not shown). There were no significant QTLs for any of the colour traits that corresponded to the QTLs for height and grain size on 4D.

Noodle colour

Initial brightness (L^*_{0h}) of noodle sheets was associated with QTLs on 1D and 2B (YAN) (Table 4), and 1B and 2B (WSN) (Table 5). These QTLs were not significant for measurements of L* at 2, 24, or 48 h or for any of the measures of darkening ($L^*_{0–2h}$, $L^*_{0–24h}$, $L^*_{0–48h}$). Rather these traits were associated with QTLs on 2A, 2D, and 4B with the QTL on 2D, in particular, having a very high LOD score (Tables 4 and 5) and characterised by a substantial increase in LOD score between 2 and 24 h. The QTL on 2D accounted for 43% of the variation in YAN L* at 24 h. The role of the QTL on chromosome 2D in determining darkening in alkaline noodles is graphically illustrated in Figs 6 and 7, having no impact on initial brightness (L^*_{0h}) but a rapidly increasing effect with time. This QTL appeared to correspond exactly to a QTL associated with grain PPO activity (Fig. 6). Rates of darkening in WSN and YAN were highly correlated in both years (r = 0.71 and 0.74) and had a high heritability (Table 2). Initial yellowness (b^*_{0h}) of noodle sheets was associated with QTLs on 2B, 3B, 5B, and 7A (YAN and WSN) (Tables 4 and 5). These QTLs, with the exception of the 5B QTL, were also associated with b* measured at 2, 24, and 48 h but not with calculated changes in b*. Change in YAN b* ($b^*_{0–2h,\ 0–24h,\ 0–48h}$), as with darkening, were controlled by QTLs on 2A and 2D (Table 4), whereas for WSN there appeared to be no consistent pattern.

Xanthophyll content

Two significant regions on chromosomes 3B (contributed by Sunco) and 7A (contributed by Tasman), respectively, were identified as associated with xanthophyll levels (Fig. 4). These QTLs corresponded to 2 of the regions controlling b* in flour and noodle sheets in this population. Xanthophyll content was consistent over 2 years (r = 0.72) and had a very high heritability (Table 2). There were no significant QTLs associated with xanthophyll content on chromosomes 4B and 5B, corresponding to QTLs for flour b*.

PPO

A highly significant region on chromosome 2D was identified as associated with variation in PPO levels over 2 sites and 2 years (Table 6 and Fig. 8), and accounted for 39% of the variation. LOD scores ranged from 12.02 to 17.68 and data between sites and years were strongly correlated (r values 0.60–0.54). This chromosome region had a strong impact on noodle brightness at 2, 24, and 48 h (Fig. 6) and all measurements of darkening ($L^*_{0–2h,\ 0–24h,\ 0–48h}$), but was not associated with flour brightness (L*) or initial noodle brightness (L^*_{0h}). Measurements of grain PPO activity were

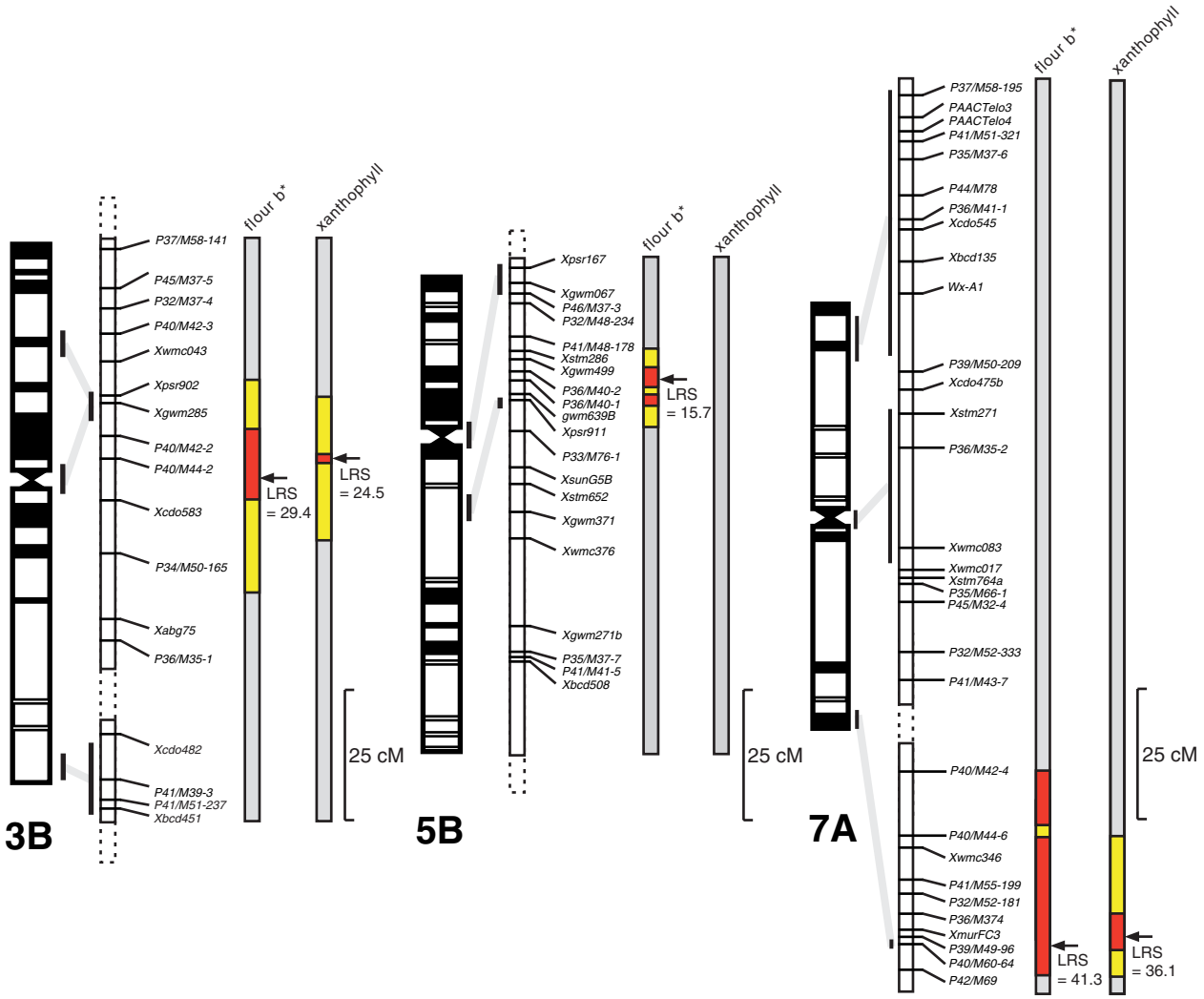

Fig. 4. QTLs on chromosome 3B, 5B, and 7A associated with flour b* and with xanthophyll content in Sunco × Tasman. The colour system for showing the location and significance of QTLs is described in Fig. 2. HS corresponds to LRS >14.1 and >24.4 for flour b* and xanthophyll content QTLs, respectively, whilst S corresponds to LRS >9.5 and >13.7 respectively. The location of the marker with the highest likelihood ratio statistic (LRS) is marked with an arrow in each case.

also not correlated with flour brightness or initial noodle (WSN and YAN) brightness (r = 0.051, 0.08, and 0.01, respectively, in 1998), but were strongly correlated with darkening ($L^*_{0–24h}$) in both WSN and YAN (r = 0.55 and 0.735, respectively), and this trait had a high heritability (Table 2). Selection of lines with low and high PPO, respectively, from the 1998 trial resulted in the identification of doubled haploids with low and high rates of darkening. The performance of these sets of lines was consistent in both 1998 and 1999. The 2D QTL was also associated with changes in YAN b* ($b^*_{0–2\,h}$ etc.). Grain PPO activity was not correlated with yellowness or xanthophyll content; however, when the doubled haploid lines were ranked according to high noodle sheet b* at 24 or 48 h, the lines with highest b* invariably also had low PPO and low darkening (data not shown).

Effect of time on the expression of QTLs

LOD scores for QTLs related to L* measurements on YAN were compared at 0, 2, 24, and 48 h. Scores for QTLs on 1D, 2B, and 4B were constant or decreased over time, whereas the LOD scores for QTLs on 2A, and in particular 2D, increased as noodles aged (Fig. 7). Similarly, with YAN b* measurements, LOD scores for QTLs associated with PPO and darkening, 2A and 2D, both increased with time, whilst scores for QTLs associated with the yellow pigment itself, 3B and 7A, showed little change (Fig. 9).

Discussion

The overall objective of this study was to find markers for flour and noodle colour, which could be applied to a wide range of Australian germplasm. Specific chromosome regions that are very important for different components of

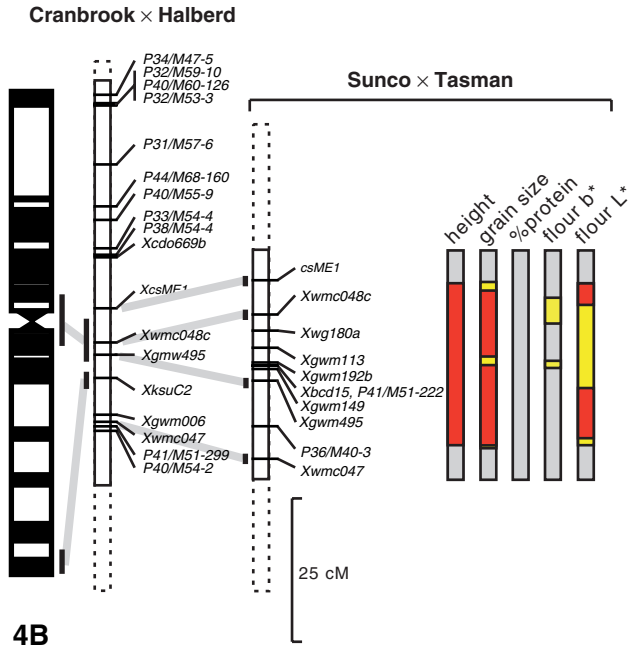

Fig. 5. QTL on chromosome 4B associated with plant height in Sunco × Tasman. The same region of chromosome 4B contained highly significant QTLs for grain size and flour L*, a significant QTL for flour b*, but no significant association with protein content. The map of chromosome 4B from Cranbrook × Halberd, which contains more detail, is included at the left side of the figure for comparison. The colour system for showing the location and significance of QTLs is described in Fig. 2. LRS values corresponding to HS (99.9%) and S (95%) are 19.5 and 13.5 (height), 22.6 and 11.0 (grain size), 16.9 and 11.3 (percent protein), 14.1 and 9.5 (flour b*), and 18.0 and 10.2 (flour L*), whilst the highest LRS values are height (51.4), grain size (35.3), percent protein (4.7), flour b* (10.7), and flour L* (24.5).

flour and noodle colour in Australian wheat were identified and support biochemical studies that suggest that colour is a product of a number of independent components and interactions between these components.

Colour of flour and noodles is measured by visual or instrumental analysis of the light reflected from the surface of the material. In addition to being affected by components that absorb light in specific regions of the visible spectrum, the amount of reflected light is affected by the physical nature of the flour or noodle, in particular the particle size distribution. This latter characteristic is determined during milling and will be influenced by factors such as grain protein content, grain hardness, grain size, and environmental factors. QTLs associated with plant height also affected grain size, and as anticipated, these effects were reflected in flour colour measurements. Grain protein content in the Sunco × Tasman population also affected flour colour and initial noodle colour.

The QTL located on chromosome 7A, which was previously identified by Parker *et al.* (1998) as being with associated flour colour, has been confirmed in this study to be an important QTL for the b* component of flour and noodle colour. Both Cranbrook × Halberd and Sunco × Tasman populations had significant QTLs on the long arm of chromosome 7A. Interestingly, however, the marker identified in the study by Parker *et al.* (1998) was polymorphic in Cranbrook × Halberd but not Sunco × Tasman. The RFLP marker *Xcdo347*, identified as important for flour colour by Parker *et al.* (1998), maps to the long arm of chromosome 7A in Cranbrook × Halberd and is associated with b* flour colour (LOD score 3.28). There are markers closer to this trait on the Cranbrook × Halberd map (P43/M62-2, LOD score 4.17), which is not unexpected given the

Table 4. LOD scores for YAN L* (0, 2, 24, 48 h) and YAN b* (0, 2, 24, 48 h), and changes in YAN L* and b* over 2, 24, and 48 h

All data are for the Sunco × Tasman doubled haploid population grown at Narrabri, NSW, in 1998 and 1999. Only chromosomes that had QTLs with LOD scores >3 are reported.

Time:	0 h		2 h		24 h		48 h		0–2 h		0–24 h		0–48 h	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
							*YAN L**							
1D	3.66		4.97											
2A			4.13		7.39	6.41	6.30	5.86	5.89	8.65	7.04	8.20	6.09	6.92
2B		7.34												
2D			5.58	3.33	18.18	16.42	18.68	17.28	9.81	9.02	21.28	17.28	19.47	18.17
4B			3.86		3.67		4.04		3.50		3.53		3.69	
							*YAN b**							
2A					6.01		5.01		6.16		7.85			
2B		5.33	5.95	3.73										
2D					10.76	6.10	5.87		5.58	7.63	14.42			
3B	3.96	3.12		4.16		3.51		3.13						
5B	4.74	3.12		3.30										
7A	6.85	7.79	9.33	9.15	6.18	7.67	5.86	6.11						

Table 5. LOD scores for WSN L* (0, 2, 24, 48 h) and WSN b* (0, 2, 24, 48 h) and changes in WSN L* and b* over 2, 24, and 48 h

All data are for the Sunco × Tasman doubled haploid population grown at Narrabri, NSW, in 1998 and 1999. Only chromosomes that had QTLs with LOD scores >3 are reported

Time:	0 h		2 h		24 h		48 h		0–2 h		0–24 h		0–48 h	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
							*WSN L**							
1B		4.80												
2A														3.41
2B		4.58												
2D					13.06	16.90	13.90	24.57			15.72	19.27	15.41	26.89
4B			3.45		3.47	3.50		3.24	3.24			3.51		3.25
							*WSN b**							
1B		3.58		3.18								3.99		5.03
2B		4.33		6.18		3.89								
2D											5.15			
3B	3.07	4.08		3.76		3.49		3.70					3.19	
3D													3.16	
4B						6.18		5.87				3.30		
5B	4.54	4.09		4.34										3.36
6B													5.05	
7A	7.90	10.73		7.62		6.46		4.75						

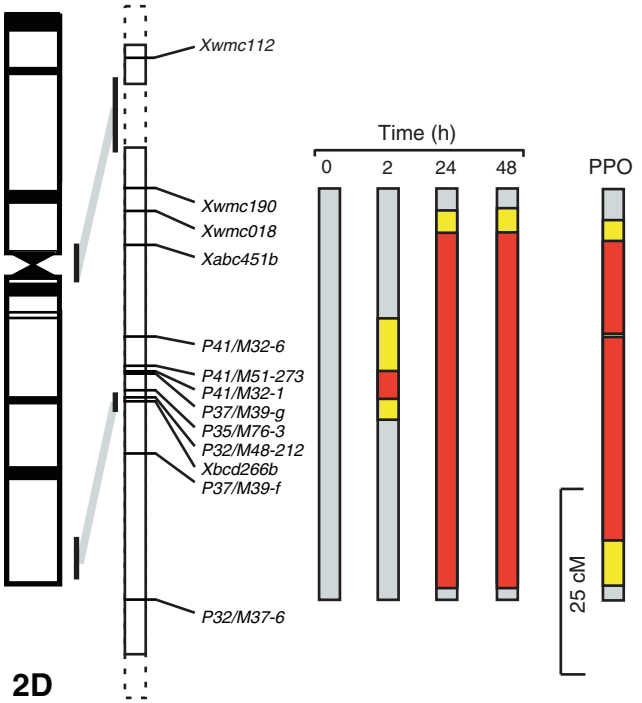

Fig. 6. QTL on chromosome 2D associated with grain PPO and its impact on noodle brightness at 0, 2, 24, and 48 h in Sunco × Tasman. The colour system for showing the location and significance of QTLs is described in Fig. 2. LRS values corresponding to HS (99.9%) and S (95%) are 19.7 and 11.0 (YAN L* 0 h), 20.8 and 11.6 (YAN L* 2 h), 16.2 and 10.2 (YAN L* 24 h), 18.5 and 11.1 (YAN L* 48 h), and 18.8 and 11.7 (PPO), whilst the highest LRS values within the QTLs are YAN L* 0 h (2.2), YAN L* 2 h (25.0), YAN L* 24 h (75.1), YAN L* 48 h (77.9), and PPO (71.2).

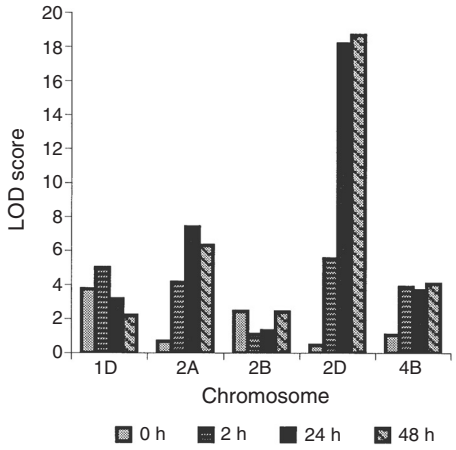

Fig. 7. The effect of time on the expression of QTLs related to YAN L* measurements.

Cranbrook × Halberd map has 900 markers as opposed to the map produced by Parker *et al.* (1998), Schomburgk × Yarralinka, which had 147 markers. Given the position of the QTL identified in the Cranbrook × Halberd (originating from the Halberd parent) population compared with the QTL located on 7A in the Yarralinka × Schomburgk population (originating from the Schomburgk parent), it is probable that this is the same QTL. However, the LOD score of 4.17 in the Cranbrook × Halberd population is considerably lower than that of 10.77 in the Yarralinka × Schomburgk population (Parker *et al.* 1998). This could be due to a number of factors,

Table 6. LOD scores for grain polyphenol oxidase (PPO) in the Sunco × Tasman doubled haploid population grown at Roma, Qld, in 1997 (Rom97), and at Narrabri, NSW, in 1998 and 1999 (Nar98 and Nar99)

Only chromosomes that had QTLs with LOD scores >3 are reported

Site	Rom97	Nar98	Nar99
2A		4.01	
2D	12.02	17.68	15.94

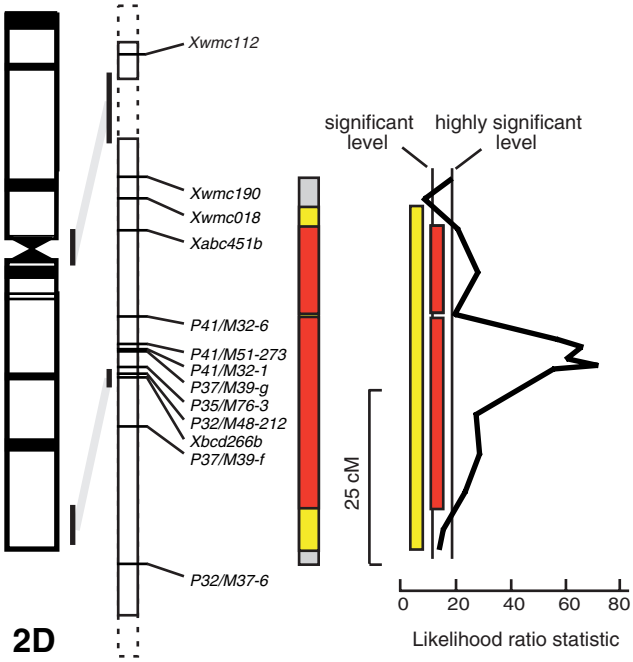

Fig. 8. QTLs on chromosome 2D associated with grain PPO activity together with the Map Manager output showing the LRS profile across the relevant region of the chromosome. Vertical black lines on the LRS profile represent the LRS values indicative of significant and highly significant associations respectively. The colour system for showing the location and significance of QTLs is described in Fig. 2. LRS values corresponding to HS (99.9%) and S (95%) are 18.8 and 11.7, respectively, whilst the LRS at the peak is 71.2.

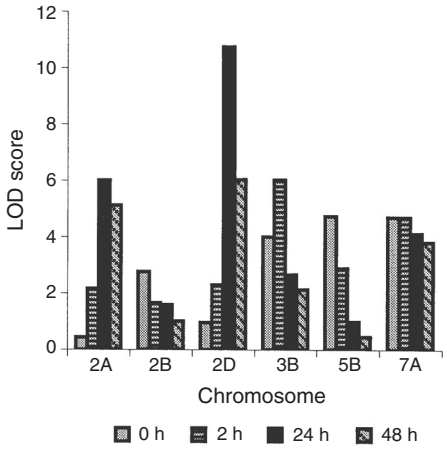

Fig. 9. The effect of time on the expression of QTLs related to YAN b* measurements.

such as the differences in backgrounds in which the QTLs are expressed. In the study by Parker *et al.* 1998, very little transgressive segregation was observed and all of the increased effects on flour colour originated from the Schomburgk parents, whereas in the Cranbrook × Halberd cross, both parents contributed to increased effects on flour colour and transgressive segregation occurred.

The region of chromosome 7A identified as important for b* flour colour in the Sunco × Tasman population also appears to be the same region as that seen in the Cranbrook × Halberd cross. However, neither the original RFLP found to be associated with this region in the Yarralinka × Schomburgk population, nor the STS marker (Parker *et al.* 1998; Parker and Langridge 2000) are polymorphic in the Sunco × Tasman cross. Further work on this marker (Sharp *et al.* 2001, this issue) has resulted in the development of a more robust and simple PCR test that is polymorphic in the Sunco × Tasman population and this marker has the highest LRS for b* in this population (Fig. 4). There is no common parent in the pedigree of the 3 cultivars with the chromosome 7A-associated colour QTL, Halberd and Tasman (this study) and Schomburgk (Parker *et al.* 1998)). However, Schomburgk and Tasman have common parentage through WW15 (from CIMMYT, Mexico) and Schomburgk and Halberd have common parentage through Dirk (a line from Roseworthy, South Australia). The significance of this chromosome region in all 3 populations indicates that the marker derived from it will have wide use in Australian germplasm. No QTLs for flour b* were identified on chromosome 7A in the CD87 × Katepwa population; however, a QTL was identified on a similar region of chromosome 7B.

Other chromosome regions were also identified as important for each of the crosses for both L* and b* (summarised in Table 3). Most of these regions were consistent over different sites and different years, although not across the different crosses. Chromosome 3A was important in the CD87 × Katepwa cross and this was also shown to be important in the Yarralinka × Schomburgk cross (Parker *et al.* 1998). The position of the QTLs relative to markers on the Cranbrook × Halberd map of chromosome 3A that were also polymorphic in the CD87 × Katepwa population (Xwmc050 and Xwmc428) or the Schomburgk × Yarralinka population (Xbcd828) (Parker *et al.* 1998) indicated that the QTL was the same in both these populations. Chromosome 3B in Sunco × Tasman was shown to be important in all sites over both years (Queensland and

Narrabri in northern NSW), and this region was also identified as significant in the Cranbrook × Halberd population in Roma 1997, the only Queensland site at which this population was trailed. This may indicate that this QTL is only important for the warmer, summer-dominant rainfall environments of Queensland and northern NSW. The QTLs on 3A (CD87 × Katepwa, Schomburgk × Yarralinka) and 3B (Cranbrook × Halberd, Sunco × Tasman) appeared to map to homoeologous regions of the respective chromosomes.

Xanthophyll content measurements of the Sunco × Tasman population indicated that QTLs were present on chromosomes 3B and 7A. The QTLs identified were in the same region as those identified for flour b* and noodle b^*_{0h} in Sunco × Tasman, discussed above. The increased xanthophyll effect also came from the same parents as the increased b* effect (3B, Sunco; 7A, Tasman). This would indicate that it is likely to be the same QTLs causing each increased effect and there may be xanthophyll genes present on chromosomes 3B and 7A, and by extrapolation from the flour b* analysis for CD87 × Katepwa 3A and 7B, or genes affected by xanthophyll content. In support of our findings, a study by Alvarez *et al.* (1998) showed that carotenoid pigments could be assigned to chromosome $7H^{ch}$ in tritordeum, which in comparative genetic maps shows extensive homologies to wheat chromosomes 7A, 7B, and 7D (Hohmann *et al.* 1995). Within the Sunco × Tasman doubled haploid population there was substantial transgressive segregation for xanthophyll content, flour b*, and noodle sheet b*. When the range of genotypes present in the progeny for the 2 QTLs associated with b* and xanthophyll content (3B-Sunco and 7A-Tasman) were compared, those progeny with both QTLs had a significantly higher mean b* than either parent, and those progeny with one or no QTLs. The presence of either QTL results in a medium level of yellowness. Potentially, further variation could be introduced by using the genes on 3A and 7B. This has significant implications for breeders and the wheat industry since different products require or tolerate different levels of yellowness. Sunco and Tasman flours appear white to creamy to the naked eye and are acceptable for production of a wide range of breads and noodles. Very low b* is unacceptable for WSN in some markets, whereas high b*, whilst advantageous for YAN, could preclude the use of cultivars from bread and other end-product markets. QTLs on 4B and 5B associated with flour b*, but not xanthophyll content, were attributed to the effects of height and subsequently grain size (4B) and protein content (5B) respectively. Presumably these loci influence flour colour via effects on the milling process, flour granularity, and the light absorption/reflection characteristics of flour samples. Interestingly, whilst QTLs on 4B and 4D, that corresponded to the semi-dwarfing genes *Rht1* and *Rht2*, had a marked influence on plant height and grain size, only the 4B QTL had effects on flour and noodle colour (L* and b*).

Sunco is noted for its yellow colour development, yellow colour stability, and brightness of colour (Kammholz *et al.* 2001, this issue). For this reason, Sunco is considered to be the YAN benchmark throughout Australia. Results from this study highlight a large segregation for PPO levels in the Sunco × Tasman population and indicate that the PPO gene(s) was probably located on chromosome 2D of Tasman. LOD scores for PPO levels ranged from 11 to 17 on chromosome 2D. Fine mapping of this region in future would aid in narrowing the range of the region and show whether this effect is due to the action of one or multiple QTLs. The observed distribution of PPO activity in a number of wheat populations is difficult to reconcile with control by a single gene (D. J. Mares, unpublished data). It is possible therefore that multiple QTLs present on the long arm of chromosome 2D and/or QTLs on other chromosome regions (e.g. 1B and 2A), which were significant in some environments (Table 6), are important in determining PPO activity. The 2D chromosome region was also shown to be important in conveying colour stability in both YAN and WAN in L* and b* measurements. The QTL was most significant at the 24 and 48 h measurements (LOD scores ranged between 6.16 and 22.94), peaking at 24 h. This is consistent with the observed time course of browning of noodles. Black or brown pigments resulting from PPO activity are responsible for part of the darkening that occurs over time in raw WSN and YAN (Mares and Panozzo 2001). Because these pigments absorb light throughout the visible spectrum they would be expected to decrease L*, and mask perception of pigments such as xanthophylls that absorb in a specific region of the spectrum. Mares *et al.* (2001) demonstrated that addition of black dye to noodle sheets resulted in a reduction in both L* and b*. Changes in b* during darkening could not be associated with change in xanthophyll or flavonoid compounds. Mares and Panozza (1999) concluded that there is another factor that contributes to noodle darkening, which must be attributed to some other mechanism, possibly another oxidase. Since there is little genetic variation for the non-PPO darkening it was not anticipated that this investigation would identify QTLs for this component of colour stability. There were other chromosomes also shown to be important in noodle colour stability: 1D, 2A, and 4B. The expression of these QTLs was either consistent over time (therefore probably not contributing to darkening), e.g. the b* QTL located on chromosome 7A, or differed over time, e.g. the L* and b* QTLs identified on chromosomes 2A and 2D.

Time-related mapping, such as shown here, demonstrates that in order to understand many important traits it may be necessary to know not only their effects at a given time or stage, but also their ‘expression dynamics’. This also has important implications for detection of QTLs, in that as heritability varies with trait development, we can assume that for most QTLs there is a time or time interval at which the

QTL has maximum heritability and therefore is most likely to be detected (Wu *et al.* 1999). Measurement of noodle brightness and yellowness as soon as practicable after preparation provided information that was obscured by PPO effects at 2, 24, and 48 h.

This study has shown that there are a number of chromosome regions associated with flour and noodle colour in Australian germplasm. Many of these chromosome regions are significant over multiple sites and multiple years, although environmental factors can determine their level of expression. Other QTLs are only present in some environments. For the purposes of selecting markers for use in Australian wheat breeding programs, it would be most efficient to select markers that were significant over a wide range of germplasm and environmental conditions. Given that for most traits more than one QTL is expressed, it may be important to select only one QTL for some traits, such as b* flour colour, in that if two QTLs were selected, the resulting progeny would be too yellow. For other traits, such as colour stability, it may be desirable to select for multiple QTLs, ensuring maximum stability.

The identification of markers for QTLs controlling flour and noodle colour in Australian wheat is a first step in using these markers effectively in breeding programs. A number of the markers identified in this study will be converted to sequence characterised amplified region (SCAR) markers and strictly validated over a wide range of Australian germplasm. The more robust markers will then be able to be implemented in breeding programs throughout Australia to ensure that stringent colour requirements are met through early screening of breeding lines.

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