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Comparative genetic analysis of the *Aegilops longissima* and *Ae. sharonensis* genomes with common wheat

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Abstract *Aegilops longissima* Schw. et Musch. (2n=2*x*=14, S^lS^l) and *Aegilops sharonensis* Eig. (2n=2*x*=14, S^lS^l) are diploid species belonging to the section *Sitopsis* in the tribe Triticeae and potential donors of useful genes for wheat breeding. A comparative genetic map was constructed of the *Ae. longissima* genome, using RFLP probes with known location in wheat. A high degree of conserved colinearity was observed between the wild diploid and basic wheat genome, represented by the D genome of cultivated wheat. Chromosomes $1S^l$, $2S^l$, $3S^l$, $5S^l$ and $6S^l$ are colinear with wheat chromosomes 1D, 2D, 3D, 5D and 6D, respectively. The analysis confirmed that chromosomes $4S^l$ and $7S^l$ are translocated relative to wheat. The short arms and major part of the long arms are homoeologous to most of wheat chromosomes 4D and 7D respectively, but the region corresponding to the distal segment of 7D was translocated from $7S^lL$ to the distal region of $4S^lL$. The map and RFLP markers were then used to analyse the genomes and added chromosomes in a set of 'Chinese Spring' (CS)/*Ae. longissima* chromosome additions. The study confirmed the availability of disomic CS/*Ae. longissima* addition lines for chromosomes $1S^l$, $2S^l$, $3S^l$, $4S^l$ and $5S^l$. An as yet unpublished set of *Ae. sharonensis* chromosome addition lines were also available for analysis. Due to the gametocidal nature of *Ae. sharonensis* chromosomes $2S^l$ and $4S^l$, additions $1S^l$, $3S^l$, $5S^l$, $6S^l$ and $7S^l$ were produced in a (4D)$4S^l$ background, and $2S^l$ and $4S^l$ in a euploid wheat background. The analysis also confirmed that the 4/7 translocation found in *Ae. longissima* was not present in *Ae. sharonensis* although the two wild relatives of wheat are considered to be closely related. The phenotypes of the *Ae. sharonensis* addition lines are described in an Appendix.

Keywords Chromosome addition lines · *Aegilops longissima* · *Ae. sharonensis* · Colinearity · Comparative mapping · Wheat

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Introduction

Aegilops longissima (S^lS^l) and *Aegilops sharonensis* (S^lS^l) are diploid S-genome members of the section *Sitopsis* Zhuk., which also includes the species *Aegilops searsii* (2n=2*x*=14, S^sS^s), *Aegilops bicornis* (2n=2*x*=14, S^bS^b) and *Aegilops speltoides* (2n=2*x*=14, SS). The genomes of these species are closely related to the B genome of wheat, and it is generally accepted that an unknown *Sitopsis* species was the B-genome donor of present-day tetraploid and hexaploid wheats (Sarkar and Stebbins 1956).

Species of the *Sitopsis* group are a useful source of novel genes for wheat improvement. Evaluation of a range of *Aegilops* species has led to the identification of accessions with high levels of resistance to wheat leaf rust (Gill et al. 1985; Manisterski et al. 1988), *Septoria tritici* blotch (McKendry and Henke 1994), wheat powdery mildew, Hessian fly and greenbug (Gill et al. 1985). Examples of *Sitopsis* genes that have been transferred into bread wheat include the powdery mildew resistance gene *Pm12* (Miller et al. 1988), the stem rust resistance genes *Sr32* and *Sr39* (Friebe et al. 1996) and the leaf rust resistance genes *Lr28* (McIntosh et al. 1982), *Lr35* (Kerber and Dyck 1990), *Lr36* (Dvorák and Knott 1990) and *Lr47* (Dubcovsky et al. 1998) from *Ae. speltoides*, and the powdery mildew resistance gene *Pm13* from *Ae. longissima* (Ceoloni et al. 1992).

Within the Triticeae tribe, comparative genetic maps have been constructed of wheat and rye (Devos et al. 1993), *Triticum monococcum* and barley (Dubcovsky

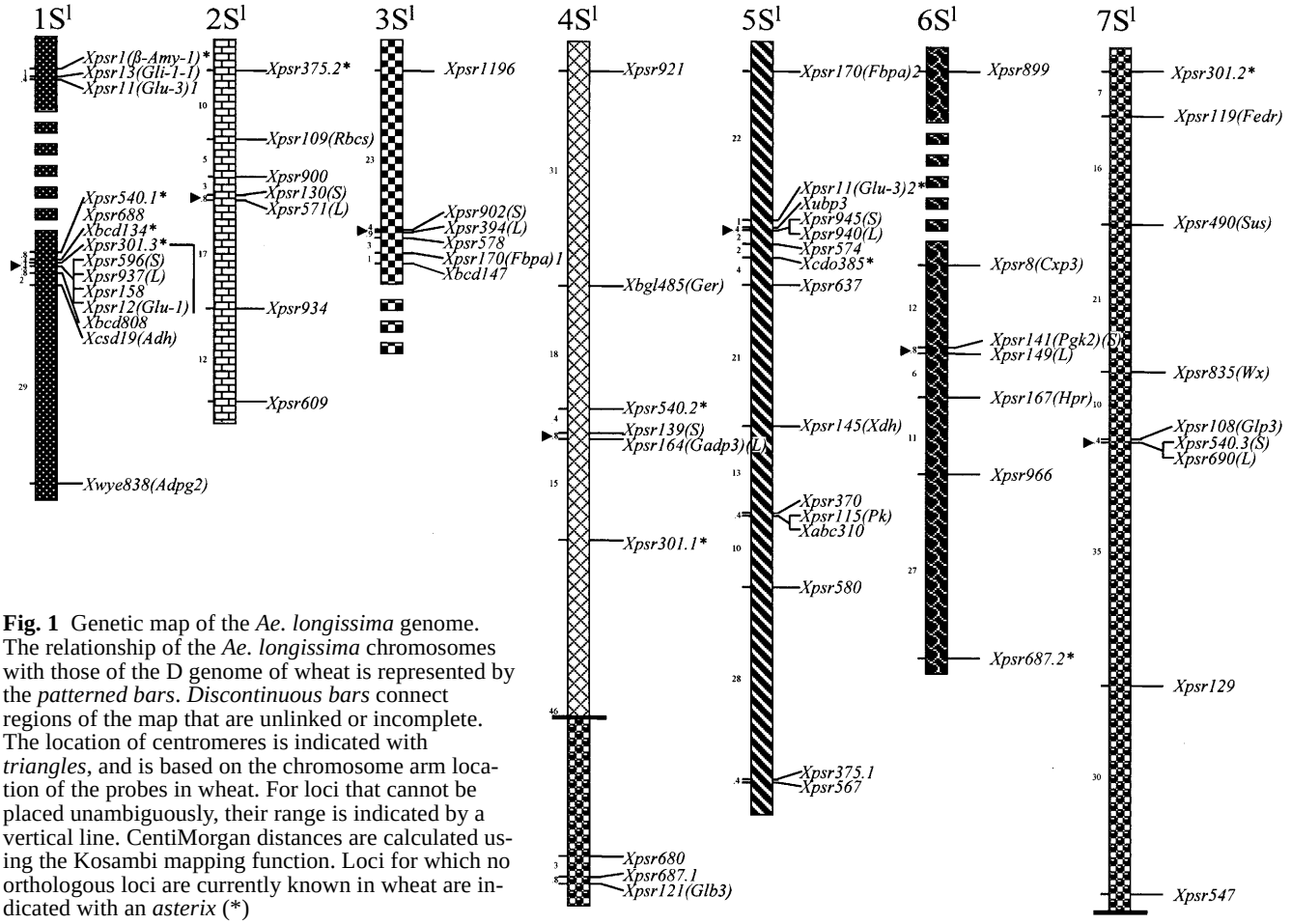

Fig. 1 Genetic map of the *Ae. longissima* genome. The relationship of the *Ae. longissima* chromosomes with those of the D genome of wheat is represented by the *patterned bars*. *Discontinuous bars* connect regions of the map that are unlinked or incomplete. The location of centromeres is indicated with *triangles*, and is based on the chromosome arm location of the probes in wheat. For loci that cannot be placed unambiguously, their range is indicated by a vertical line. CentiMorgan distances are calculated using the Kosambi mapping function. Loci for which no orthologous loci are currently known in wheat are indicated with an *asterix* (*)

et al. 1996) and wheat and *Aegilops umbellulata* (Zhang et al. 1998). These studies have demonstrated that the genomes of some species, such as *Ae. umbellulata* and rye, display considerable chromosomal rearrangements, both inversions and translocations, relative to the cultivated wheat genome. Marker orders within the rearranged segments, however, have remained highly conserved. It is clear, therefore, that in order to evaluate the integrity of chromosomes transferred in the production of alien-wheat chromosome addition and substitution lines it is necessary to first understand the relationship between the chromosomes of the alien and wheat genomes. The relationships of the *Ae. speltoides* (Maestra and Naranjo 1998), *Ae. longissima* (Friebe et al. 1993; Naranjo 1995) and *Ae. sharonensis* (Maestra and Naranjo 1997) genomes with that of wheat have previously been examined by cytogenetic analysis. These studies have indicated that the *Ae. speltoides* and *Ae. sharonensis* genomes are colinear with the D genome of wheat, while *Ae. longissima* has undergone a 4L/7L translocation.

In this paper we describe the construction of the first comparative genetic map of the wheat and *Ae. longissima* genomes, and application of this map in the analysis of a set of CS/*Ae. longissima* addition lines. We also describe the morphological characteristics and composition of a set of CS/*Ae. sharonensis* addition lines.

Material and methods

Plant material

The mapping population consists of 120 F_2 progeny derived from a cross between *Ae. longissima* accessions Y154–1 and Y431–1 from the Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, People's Republic of China. The wheat variety 'Chinese Spring' (CS), the CS/*Ae. longissima* acc. TL01 amphiploid (Feldman 1975), a set of CS/*Ae. longissima* addition lines designated A-G (Feldman 1975), a CS/*Ae. sharonensis* amphiploid (JIC7010087) and a CS/*Ae. sharonensis* $2S^l$ addition line 'Kyoto', provided by T.R. Endo, Kyoto University, Japan, were obtained from the John Innes Centre collection. Also available was an unpublished set of CS/*Ae. sharonensis* addition lines previously extracted by T.E.Miller and S.M. Reader, John Innes Centre. These lines are described in detail in Appendix 1 and are available from S.M.Reader.

Restriction fragment length polymorphism (RFLP) probes

Probes previously mapped in wheat and barley were obtained from the John Innes Centre collection (prefix 'PSR'), Cornell University ('BCD': barley cDNA clone; 'CDO': oat cDNA clone), North American Barley Genome Mapping Project ('ABC'),

University of Bari ('UBP'), C. Ainsworth [pCSS22 – locus *Xwye835(Wx)*; pSh2.25 – locus *Xwye838(Adpg2)*], B. Lane [Germin – locus *Xglb485(Ger)*], P. Carbonero [pST8 – locus *Xpsr490(Sus)*], L. Dennis [3′Adh – locus *Xcsd19(Adh)*], M. Kreis [pcβC51 – locus *Xpsr1(β-Amy-1)*], J. Scandalios (pCat2.1c), N. Raikhel (pNVR1), G. Fincher (G5) and A. Breiman (ATP β 4/A). Loci names follow the rules for wheat gene nomenclature (McIntosh et al. 1998).

Marker analysis and mapping

Isolation of genomic DNA, restriction enzyme digestion with *Eco*RI, *Eco*RV, *Dra*I and *Hind*III, agarose-gel electrophoresis, alkaline Southern blotting to Hybond N^+ (Amersham) nylon membranes, and labelling and hybridization of DNA inserts were performed as described in Devos et al. (1992). The genetic map was constructed using the program 'Mapmaker v.3.0', supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA. Map positions were verified manually by examining recombination events. Genetic distances are expressed in cM.

Results

Comparative mapping of *Ae. longissima* with wheat

RFLP probes, spanning the seven homoeologous chromosome groups of wheat, were tested for their ability to reveal polymorphism between the *Ae. longissima* accessions Y154–1 and Y431–1, the parents of the mapping population. Sixty two percent of the probes revealed polymorphism with at least one of the four restriction enzymes tested and a total of fifty nine probes, detecting 67 loci, were mapped in *Ae. longissima*. A comparative map between the genomes of *Ae. longissima* and wheat was constructed (Fig. 1).

Analysis of *Ae. longissima* and CS/*Ae. sharonensis* chromosome addition lines

Twenty three of the mapped probes, and an additional 20 probes with known chromosomal location in wheat, were hybridised to the DNA of CS/*Ae. longissima* addition lines and the CS/*Ae. longissima* amphiploid. Thirty one probes provided information on the composition of the CS/*Ae. sharonensis* addition lines. The location of the probes used in wheat, *Ae. longissima* and the *Ae. longissima* and *Ae. sharonensis* addition lines is presented in Table 1.

Discussion

Relationship between *Ae. longissima* and wheat chromosomes

Chromosome 1S^l: the 1S^l map comprises 14 loci, spans a genetic distance of at least 85 cM (Fig. 1) and covers most of the wheat homoeologous group-1 map. In wheat, the probe pcβC51, which encodes *β-Amy-1*, detects loci on the group-2 chromosomes and on 5A, 4B and 4D. In *Ae. longissima*, in addition to the *β-Amy-1* locus on 4S^l (Table 1), a second locus was identified that was linked to the loci *Xpsr11(Glu-3)1* and *Xpsr13(Gli-1–1)*, which are located on the short arms of the group-1 chromosomes in wheat. As the *Xpsr11(Glu-3)* locus was shown to be located on chromosome 1S^l using the CS/*Ae. longissima* addition lines (Table 1), and chromosome 1S^l appears to be colinear to the wheat group-1 chromosomes (Fig. 1), this cluster of three loci was placed distally on the short arm in accordance with the known locations of *Glu-3* and *Gli-1* on the wheat genetic map.

Chromosome 2S^l: the map of chromosome 2S^l contains seven loci. Chromosome 2S^l is homoeologous to the wheat group-2 chromosomes and no rearrangements relative to wheat are apparent.

Chromosome 3S^l: the 3S^l map consists of six loci and spans a genetic distance of 27.6 cM. Markers from the distal region of the long arms of the wheat group-3 chromosomes were shown to be located on chromosome 3S^l using the CS/*Ae. longissima* addition lines (Table 1). However, none of these markers could be mapped, either because of lack of polymorphism or faintness of the signal. The synteny data nevertheless suggest that chromosome 3S^l is homoeologous and probably un-rearranged relative to the wheat group-3 chromosomes.

Chromosome 4S^l: the 4S^l map consists of nine loci and spans a genetic distance of 118 cM . The short arm and the proximal part of the long arm of chromosome 4S^l are homoeologous to the short arms and most of the long arms of wheat chromosomes 4B and 4D. All of the markers that map to chromosome arms 5AL, 4BL and 4DL in wheat are located on 4S^l (Table 1), confirming the results of pairing studies (Naranjo 1995) that *Ae. longissima* does not carry a 4L/5L translocation which is present in many Triticeae species. The distal part of the long arm of chromosome 4S^l, including the loci *Xpsr680, Xpsr687.1* and *Xpsr121(Glb3)*, is homoeologous to the distal regions of the long arms of the wheat group-7 chromosomes. This indicates that a translocation occurred between the long arms of 4S^l and 7S^l. This translocation has been observed in all *Ae. longissima* accessions analysed to-date (Friebe et al. 1993; Naranjo 1995).

The close linkage of the *Xpsr164(Gadp3)* locus with the centromere on 4S^{l}L indicates that considerable variation exists in the distribution of recombination in *Ae. longissma* relative to wheat, because the equivalent locus is located distally on 4L in wheat. The low recombination frequency in the *Xpsr164(Gadp3)* – centromere interval in *Ae. longissima* may be a consequence of the presence of the translocated 7L segment. A comparison of the recombination rates in wheat chromosome arms 4AS, from which the distal segment has been translocated, and 4DL, and in 4AL, which carries translocated 5AL and 7BS segments, and 4DS, had previously dem-

Table 1 Chromosomal locations in *Ae. longissima* based on map position, and in the *Ae. longissima* and *Ae. sharonensis* chromosome addition lines of probes with known map location in wheat

Chromosomal location in wheat	Chromosomal location in *Ae. longissima*[a]	Location on CS/*Ae. longissima* addition lines	Location on CS/*Ae. sharonensis* addition lines	Probes[b]
1AS 1BS 1DS	$1S^l$	B&C		PSR11, PSR688
	$1S^l$			PSR13, PSR596
1AL 1BL 1DL	$1S^l$	B&C		PSR12
	$1S^l$			PSR158, PSR937, BCD808, 3'Adh, pSh2.25
		B&C		PSR162, ATP β 4/A[b], pNVR1
2AS 2BS 2DS	$2S^l$	A&E		PSR109[b]
	$2S^l$			PSR130, PSR900
		A&E		PSR108[b]
		A&E	Kyoto	BCD348[b], BCD855
2AL 2BL 2DL	$2S^l$	A		PSR571
	$2S^l$	A	Kyoto	PSR609
	$2S^l$			PSR934
		A	Kyoto	PSR151
3AS 3BS 3DS	$3S^l$			PSR902, PSR1196
		G	$3S^l$	PSR598
			$3S^l$	PSR903[b]
3AL 3BL 3DL	$3S^l$	G	$3S^l$	PSR170[b], PSR394, PSR578
	$3S^l$			BCD147
		G		CDO455
		G	$3S^l$	BCD828
3AL 3DL		G		PSR1203
3DL		G	$3S^l$	PSR1067
3BL 3DL			$3S^l$	G5
4AL 4BS 4DS	$4S^l$	D		Germin
	$4S^l$			PSR139, PSR921
4AS 4BL 4DL		D		PSR39, CDO38[b]
		D	$4S^l$[c]	PSR163
5AL 4BL 4DL	$4S^l$			PSR164
		D	$4S^l$[c]	pcβC51[b], pCat2.1c[b], CDO20
			$4S^l$[c]	PSR375[b]
5AS 5BS 5DS	$5S^l$			PSR945, pTubp3
	$5S^l$		$5S^l$	PSR170[b]
5AL 5BL 5DL	$5S^l$	F		PSR574
	$5S^l$	F	$5S^l$	PSR145, PSR637
	$5S^l$			PSR370, PSR940
			$5S^l$	PSR109[b]
4AL 5BL 5DL	$5S^l$	F	$5S^l$	PSR115, ABC310[b]
	$5S^l$			PSR567[b], PSR580
5DL	$5S^l$	F	$5S^l$	PSR375[b]
6AS 6BS 6DS	$6S^l$	B&E[d]	$6S^l$(1)&(2)	PSR8, PSR141, PSR167[b]
		B&E[d]		pCat2.1c[b]
6AS 2BS 6DS	$6S^l$	B&E[d]	$6S^l$(1)&(2)	PSR899
6AL 6BL 6DL	$6S^l$			PSR149, PSR966[b]
		B&E	$6S^l$(1)&(2)	PSR605
6BL 6DL		B&E		PSR546
7AS 4AL 7DS	$7S^l$			PSR119, pST8[b], pCSS22
7AS 7BS 7DS	$7S^l$		$7S^l$	PSR108[b]
7BS	$7S^l$			PSR540[b]
7AL 7BL 7DL	$7S^l$	E	$7S^l$	PSR129, PSR690
	$7S^l$			PSR547[b]
7AL 7BL 7DL	$4S^l$	D	$7S^l$	PSR687[b]
	$4S^l$			PSR121[b], PSR680
7DL		D		PSR548
7AL 7BL			$7S^l$	ABC310[b]

[a] Based on mapping data

[b] These probes are not single copy and detect multiple loci in wheat and/or *Aegilops*

[c] Line $4S^l$ is a disomic addition line; lines $3S^l$, $5S^l$, $6S^l$(1)&(2) and $7S^l$ are disomic (4D)$4S^l$ substitutions; see text for explanation

[d] The wheat 6B fragment is absent

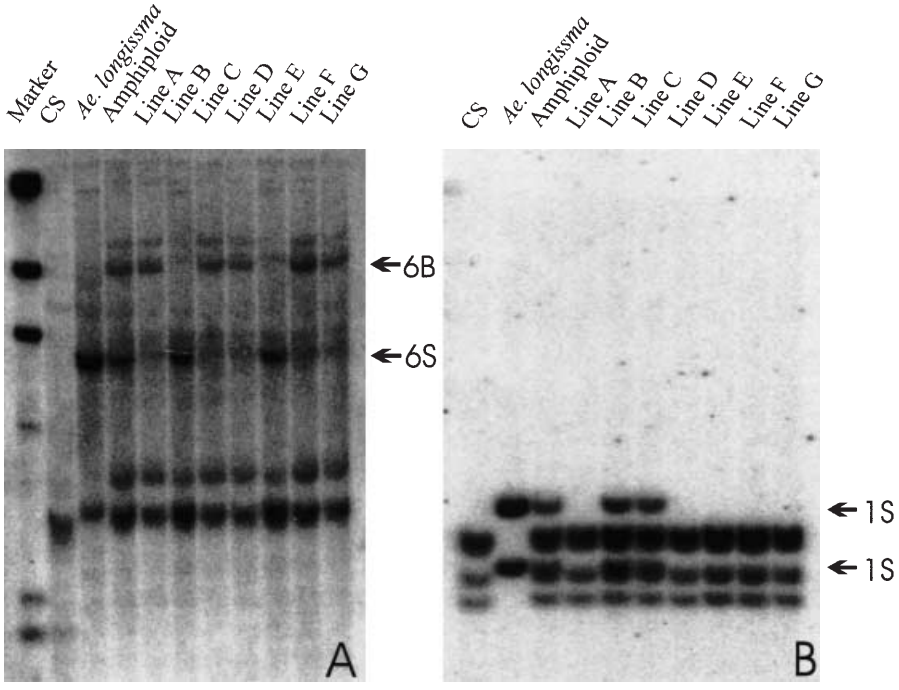

Fig. 2A, B Autoradiographs revealing the hybridisation patterns of the RFLP probes PSR8 and PSR12 to *Hind*III digested DNA of CS, *Ae. longissima*, a CS/*Ae. longissima* amphiploid and the CS/*Ae. longissima* addition lines A–G. **A** Hybridisation with PSR8, a group-6 probe, shows that lines B and E carry a 6S^{l} *Ae. longissima* fragment, but lack the wheat 6B fragment. **B** Hybridisation with PSR12, a group-1 probe, shows that a 1S^{l} fragment is present in both lines B and C

onstrated that relative recombination rates were largely dependent on the distance of these segments from the telomere (Devos et al. 1995). Translocation of the distal 7S^{l}L segment to 4S^{l}L may thus have led to a reduction in recombination in the original 4S^{l}L arm, and an increase in recombination in the non-translocated portion of 7S^{l}L.

Interestingly, the chromosomal segment carrying the group-7 markers that are involved in this 4L/7L translocation has also been translocated during the evolution of rye (Devos et al. 1993) and *Ae. umbellulata* (Zhang et al. 1998), and has been deleted from chromosome arm 7AL in the CS ditelosomic 1BS stock and 7DL in the CS nullisomic 1A-tetrasomic 1D stock (Devos et al. 1999). This supports a previous suggestion that the long arm of the Triticeae group 7 chromosomes may contain a fragile site.

Chromosome 5S^{l}: the 5S^{l} map consists of 15 loci and shows a normal homoeologous relationship to wheat chromosomes 5B and 5D.

Chromosome 6S^{l}: seven loci were mapped on chromosome 6S^{l}. The locus *Xpsr899*, which is located on chromosome arms 6AS, 2BS and 6DS in wheat, was unlinked, but was shown to be located on chromosome 6S^{l} using the CS/*Ae. longissima* addition lines. Markers were found in the same order on 6S^{l} and the wheat group-6 chromosomes, with the exception of *Xpsr167(Hpr)*, which is located on the long arm in *Ae. longissima* and on the short arm in wheat. However, PSR167 detects multiple fragments in *Ae. longissima*, and it is possible that paralogous loci were mapped in the two species.

Chromosome 7S^{l}: the 7S^{l} map comprises nine loci. The short arm and most of the long arm of chromosome 7S^{l} are homoeologous to wheat chromosome 7D. Markers that map distally on wheat 7L are located on 4S^{l}L. No RFLP markers that mapped to the distal regions of wheat chromosomes 4B and 4D and were located on 7S^{l} were found. This indicates that, if the 4/7 translocation is reciprocal, it involved only a small segment of 4S^{l}L and a relatively large 7S^{l}L segment. The occurrence of an unequal translocation was also suggested by pairing data, which showed that, in wheat-*Ae. longissima* hybrids, chromosome arm 4S^{l}L preferentially pairs with the long arms of the wheat group-7 chromosomes, while 7S^{l}L seldom pairs (Naranjo 1995).

The CS/*Ae. longissima* addition lines

The composition of the CS/*Ae. longissima* addition lines, designated A to G, has previously been analysed using protein studies (Hart and Tuleen 1983; Levy et al. 1985; Hueros et al. 1990) and C-banding (Friebe et al. 1993). The present RFLP data (Table 1) agree that lines A, C, D, F and G carry *Ae. longissima* chromosomes 2S^{l}, 1S^{l}, 4S^{l}, 5S^{l} and 3S^{l}, respectively, in addition to the 42 wheat chromosomes. The presence on 4S^{l} of the group 7L RFLP loci *Xpsr687* and *Xpsr548* (Table 1) and the isozyme locus endopeptidase (*Ep-S^{l}1*) (Hart and Tuleen 1983) confirms the integrity of 4S^{l} in line D. We note that Hart and Tuleen also reported that line D expressed *Lpx–S^{l}2*, which is a Group-5 marker. In the light of the results of Yang et al. (1996) and the present analysis, the presence of a significant fragment of 5S^{l} translocated to 4S^{l} would appear to be most unlikely. Thus the expression of *Lpx–S^{l}2* remains an anomaly.

Line E is a disomic (6B)6S^{l} substitution line (Fig. 2) with an added 2S^{l}S.7S^{l}L translocated *Ae. longissima* chromosome. It should be noted that the arm designa-

tions of the *Ae. longissima* chromosomes used here are based on their homoeology to wheat. For chromosome $7S^l$, the arm that is physically the shortest is homoeologous to the long arms of the wheat homoeologous group 7 chromosomes and *vice versa* (Naranjo 1995). The $2S^lS.7S^lL$ translocation is likely to have occurred during the generation of the addition lines and does not represent a native *Ae. longissima* chromosome.

Line B is also a (6B)$6S^l$ disomic substitution line but the identity of any additional chromosome has been controversial. Jewell and Driscoll (1983) observed an added telocentric chromosome, while Hart and Tuleen (1983) reported the presence of an heteromorphic chromosome pair. No added chromosome was present in the line B analysed by Friebe et al. (1993). The line-B stock maintained at JIC comprises 44 chromosomes, none of which are telocentric, and forms 22 bivalents during meiosis. RFLP results demonstrate that this line contains, in addition to the (6B)$6S^l$ substitution, an added chromosome $1S^l$ (Fig. 2). Probes that hybridised to both the short and long arms of the wheat group-1 chromosomes detected loci in line B, suggesting that a complete $1S^l$ chromosome is present (Table 1). The restriction fragments detected by the group 1 probes were of the same molecular weight in line B and in the CS/*Ae. longissima* $1S^l$ addition line (line C) (Fig. 2).

The hybridisation patterns of two group-6 long-arm probes, PSR605 and PSR546, to the CS/*Ae. longissima* acc. TL01 amphiploid suggested that this line lacks the long arm of chromosome 6B. C-banding of accession TL01, used in the construction of the amphiploid, by Friebe et al. (1993) did not indicate the presence of a telocentric chromosome. As cytological examination of sib lines of the amphiploid analysed by RFLP showed the presence of an heteromorphic satellited chromosome pair, it is likely that the original amphiploid was heteromorphic for chromosome 6B. Progeny would thus be segregating for the 6BS telosome.

The CS/*Ae. sharonensis* addition lines

Chromosome $4S^l$ of *Ae. sharonensis* is preferentially transmitted in the wheat background and functional gametes require the presence of at least one $4S^l$ chromosome (Miller 1982). As a result, attempts to produce disomic CS/*Ae. sharonensis* addition lines led to the generation of only one single-chromosome addition line, which contains chromosome $4S^l$ (Miller 1982). Using a CS (4D)$4S^l$ disomic substitution line rather than a CS euploid as a recipient parent during the construction of the addition lines, Miller (1982) was able to extract a set of CS/*Ae. sharonensis* disomic addition lines. The phenotypic descriptors associated with the added *Ae. sharonensis* chromosomes are described in an Appendix to this paper (See Miller and Reader 1987 for descriptors). RFLP analysis of the $3S^l$, $4S^l$, $5S^l$, $6S^l$ and $7S^l$ addition lines confirmed that these lines had been correctly identified. Addition line $1S^l$ was not analysed by RFLP due to limited seed availability.

A CS/$2S^l$ disomic addition line, designated 'Kyoto' and obtained from T.R. Endo, was also analysed by RFLP. Endo (1985) identified two types of *Ae. sharonensis* gametocidal chromosomes, one with homoeology to the wheat group-4 chromosomes, and one with homoeology to the wheat group-2 chromosomes. The $2S^l$ chromosome carried by 'Kyoto' is of the latter type and its preferential transmission accounts for the availability of this disomic addition line in a pure wheat background. The RFLP data confirmed that 'Kyoto' carried chromosome $2S^l$. However, because of a high level of polymorphism relative to the CS variety maintained at JIC, no conclusions could be drawn on the putative presence of other *Ae. sharonensis* chromosomes. The observed polymorphism is probably due to the use of a different CS biotype by Endo, and Miller and Reader in the production of the addition lines.

Relationship between *Ae. sharonensis* and wheat chromosomes

Analysis of the *Ae. sharonensis* addition lines suggests that this species, although closely related to *Ae. longissima* (Yen and Kimber 1990), does not carry the 4/7 translocation. Chromosome pairing studies in hybrids between *Ae. longissima* and *Ae. sharonensis* showed the formation of five bivalents and one quadrivalent, indicating that the two species differed by the presence of a translocation (Kihara 1954). The lack of a 4/7 translocation in *Ae. sharonensis* is also supported by compensation by chromosome $4S^l$ for the absence of chromosome 4D when substituted into wheat (Miller 1982). The *Ae. sharonensis* genome thus appears, within the limits of this analysis, to be entirely homeologous to the D genome of wheat. This concurs with the conclusions of pairing studies by Maestra and Naranjo (1997).

Conclusions

The RFLP data confirm the conclusions inferred from pairing studies that *Ae. longissima* chromosomes $1S^l$, $2S^l$, $3S^l$, $5S^l$ and $6S^l$ are colinear with wheat chromosomes 1D, 2D, 3D, 5D and 6D, and that *Ae. longissima* chromosome arms $4S^lL$ and $7S^lL$ have been involved in a translocation relative to chromosomes 4D and 7D. The D genome has been considered the basic wheat genome because, in contrast to the A and B genomes of present-day bread-wheat, it has not been involved in inter-genome translocations (Devos et al. 1995). The conserved structural organisation of the *Ae. longissima* and wheat genomes would indicate that, for most of the *Ae. longissima* genome, gene transfers to cultivated wheat through homoeologous recombination would result in genotypes with a balanced genome (see Devos et al. 1993 for a discussion). Care will have to be taken, however, that transfers from the translocated 7L segment on $4S^lL$ are made to wheat group-7 chromosomes, while

transfers from the remainder of $4S^lL$ should be made to wheat chromosomes 4B or 4D. The latter transfers will, however, result in unbalanced genomes, and a second recombination event will be required to reconstitute the genome integrity. As recombination is highly reduced in the original $4S^lL$ segment, the introduction of genes from this region into wheat is expected to be very inefficient. Similar problems will be encountered for the transfer of genes from $7S^lL$ to wheat group-7 chromosomes, although the higher recombination rates in $7S^lL$ should facilitate the alien introgression.

The low number of rearrangements observed in *Ae. longissima*, a *Sitopsis* species, relative to wheat is in stark contrast to the high number of evolutionary rearrangements present in *Ae. umbellulata*, a *Polyeides* species. Considering the close phylogenetic relationship between the *Sitopsis* and *Polyeides* species (Kellogg et al. 1996), it is clear that rearrangements accumulate and are fixed in species at different rates. This is unlikely to be related to the breeding system, as evolutionary translocations are prevalent in both inbreeding species such as *Ae. umbellulata* and outbreeding species such as rye (Zhang et al. 1998; Devos et al. 1993). Others, such as barley (Dubcovsky et al. 1996) and *Ae. longissima* (this paper), which are predominantly inbreeding species, and *Ae. speltoides* (Maestra and Naranjo 1998), another outbreeding species, have largely maintained the basic Triticeae genome structure, as represented by the D genome of wheat.

The set of CS/*Ae. longissima* addition lines, designated A to G (Feldman 1975), was confirmed to contain disomic additions for $1S^l$ (C), $2S^l$ (A), $3S^l$ (G), $4S^l$ (D) and $5S^l$ (F). No addition line was present for $6S^l$ or $7S^l$ and these lines have been isolated by Friebe et al. (1993). Lines B and E were disomic (6B)$6S^l$ substitutions with an added $1S^l$ and translocated $2S^lS.7S^lL$ chromosome pair, respectively.

Disomic addition lines in a Chinese Spring background are available for *Ae. sharonensis* chromosome $4S^l$. Disomic additions for chromosomes $1S^l$, $3S^l$, $5S^l$, $6S^l$ and $7S^l$ are available in a CS (4D)$4S^l$ background. With the exception of CS/$1S^l$, the identity and integrity of these additions were confirmed by RFLP analysis. The line 'Kyoto' was confirmed to contain an added $2S^l$ chromosome, but the precise composition of this line could not be established due to the high level of polymorphism in the CS biotype used as background relative to the CS stock maintained at JIC.

Appendix

Descriptors for the *Ae. sharonensis* chromosome addition lines generated by Miller and Reader

The general descriptors for alien-wheat chromosome addition lines and other aneuploids are listed in Miller and Reader (1987). Unusual phenotypes were observed in some of the addition lines, and it is assumed that these were caused by the (4D)$4S^l$ substitution in the background.

$1S^l$ addition line. This line is maintained as a (4D)$4S^l$ disomic substitution and a $1S^l$ monosomic addition (2n=43; 21″+1′). The disomic addition has low fertility. The $1S^l$ addition line has a short stature and small spikes. The plant is late maturing and tillers profusely.

$2S^l$ addition lines. No plants were identified that contained a complete $2S^l$ chromosome. However a line disomic for a $2S^l$ telosome in the presence of the (4D)$4S^l$ substitution was isolated (2n=42+2t; 21″+t″). These plants are characterised by thin culms and narrow leaves. The spikes are small, dense and narrow, and awnless. Two further group-2 characteristics, tough glumes and twisted flag leaf were not observed, probably because these characters are conferred by genes located on the missing arm.

A CS/$2S^l$ disomic addition line, designated 'Kyoto', was obtained from T.R. Endo. 'Kyoto' has a tall stature, which is rather unusual for a CS group-2 addition, thin culms, narrow leaves and spikes, a twisted flag leaf and short awns. The CS biotype used in the construction of the $2S^l$ addition line is different from the CS background in the *Ae. sharonensis* additions extracted by Miller and Reader.

$3S^l$ addition line. The $3S^l$ addition line (2n=44, 22″) was characterised by its short stature, small spikes, prevalence of backtillers and reduced fertility. Group 3 alien additions are normally quite robust and similar in phenotype to the CS euploid, and the unusual characteristics of the mature $3S^l$ addition is assumed to have been caused by the presence of the (4D)$4S^l$ substitution in the background. The $3S^l$ chromosome confers disarticulation of the spike. The line is also later maturing and is prone to powdery mildew infections.

$4S^l$ addition line. The identity of the $4S^l$ chromosome was established by the erect plant habit, the erect flag leaf, the presence of lax spikes of which the top one-third is sterile, the presence of supernumerary florets, and the brown straw colour. The $4S^l$ addition line (2n=44, 22″) is later-maturing compared to the euploid. This line is a pure $4S^l$ addition line which carries neither the 4L/5L translocation that is present in many Triticeae species, nor the 4L/7L translocation that characterises *Ae. longissima.* A ditelosomic addition line (2n=42+2t, 21″+t″), putatively identified as $4S^lL$, is also available. This line has a phenotype similar to the complete $4S^l$ addition.

$5S^l$ addition line. This line (2n=44, 22″), which was confirmed to be a (4D)$4S^l$ disomic substitution and a $5S^l$ addition line, is characterised by a short stature, thick culms, broad leaves, broad clavate spikes which are lax at the base, coarse textured grains, papery chaff and brown straw. The $5S^l$ addition line also has an increased susceptibility to powdery mildew. A ditelosomic long-arm addition (2n=42+2tL, 21″+t″) is available and is very similar in appearance to the complete $5S^l$ addition.

$6S^l$ addition line. Group-6 alien additions are usually similar to the CS euploid, but taller and with rounded

glumes. Lines that were (4D)4S^{l} disomic substitutions 6S^{l} disomic additions (2n=44; 22″) had rounded glumes, but also had a shorter stature, brown straw and small spikes, characters assumed to result from the (4D)4S^{l} substitution. A ditelosomic addition (2n=42+2t, 21″+t″) is available that displays the same characteristics, but with a spike that is more lax at the base.

7S^{l} addition line. This line (2n=44; 22″) has purple culms, smaller lax spikes, and the tip of the spike is sterile. This line contains a (4D)4S^{l} disomic substitution and carries an added chromosome 7S^{l}.

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