Theor Appl Genet (1999) 99:1087–1093

K. Anamthawat-Jónsson

Variable genome composition in *Triticum*×*Leymus* amphiploids

Received: 24 November 1998 / Accepted: 12 May 1999

Abstract Three different amphiploid lines originated from crosses between wheat (*Triticum* L.) and lymegrass (*Leymus* Hochst.) were analysed by fluorescence *in situ* hybridization (FISH) using total genomic DNA and 18S.26S ribosomal genes (rDNA). Based on the genomic probes, these lines were the same in that they all were allohexaploids (2n=6x=42) containing 30 wheat and 12 lymegrass chromosomes. The ribosomal gene mapping further identified species origin of the chromosomes, whereby the lymegrass parent was undoubtedly *L. mollis* and the wheat was likely to be a *Triticum* species having the AB genomes. This rDNA mapping was also able to reveal differences in the genome composition among these lines, and such differences were mainly in the wheat nucleolar organizing regions (*Nor*). The first line (M) had two *Nor-B1* (1BS), the second line (G) had one *Nor-B1* (1BS), two *Nor-B2* (6BS) and two *Nor-A1* (1AS), whereas the third line (U) had the same *Nor* loci as the second line but two sites each. The wheat ribosomal genes were variably expressed depending on the lines, but the lymegrass loci appeared inactive. All three lines had the same *Nor* loci belonging to *L. mollis*, two *Nor-m1* and two *Nor-m2*. Analysis of restriction fragment length polymorphism (RFLP) of the rDNA confirmed the identity of *L. mollis* parent in all three lines and verified the differences in the wheat ribosomal genes among them. These amphiploids were, however, similar in their restriction profiles, therefore indicating common origin. The molecular and cytogenetic evidence here suggested that these annual, fully fertile amphiploids that originated from the same crosses 40 years ago became genetically differentiated and fixed in stable forms.

Communicated by J.W. Snape

K. Anamthawat-Jónsson
Department of Biology, University of Iceland,
Grensásvegi 12, Reykjavík 108, Iceland
e-mail: kesara@rhi.hi.is
Fax: +354-525-4069

Key words Amphiploid · *Triticum* · *Leymus* · FISH · RFLP · Ribosomal genes

Introduction

Wide-crosses of wheat (*Triticum* L.) with its wild relatives in the tribe Triticeae (Poaceae), including lymegrass (*Leymus* Hochst.), have been made with the aim of improving wheat crops by alien chromosome transfer via wide-hybrids or by making new hybrid species (Baum et al. 1992; Jiang et al. 1994). Characteristics important to wheat crops have been targeted for transfer from *Leymus*, such as resistance to fungal diseases and viruses, tolerance to drought and salinity (Petrova 1960; Fatih 1983; Mujeeb-Kazi et al. 1983; Plourde et al. 1992). The *Leymus* species themselves, in particular the large-seeded species *L. arenarius* (octoploid 2n=56, North European), *L. mollis* (tetraploid 2n=28, North American/Asian) and *L. racemosus* (tetraploid, Central Eurasian), have been studied for cultivation as perennial grain species and for making amphiploids with wheat (Anamthawat-Jónsson et al. 1997). *Leymus* is known for its agricultural potential (Wagoner 1990; Pimm 1997).

Amphidiploids deriving from the earliest *Triticum*×*Leymus* crosses, independently made in Russia (Tsitsin 1965) and in North America by R.D. Dewey (Shepherd and Islam 1988), have been maintained but only partially characterized (see Table 1). The information about species origin of these amphiploids is limited, and their genome composition is virtually unknown. In addition, it is thought that some of the material has been backcrossed further with wheat. In order to make good use of these materials for *Leymus* breeding or wheat improvement, correct characterization is essential. The aim of the present study was to examine the genome content of these amphiploids using molecular cytogenetic methods, which included genomic fluorescence *in situ* hybridization (FISH), chromosomal mapping of the major ribosomal genes and restriction fragment length polymorphism (RFLP) analysis of the genes.

Materials and methods

Plant materials

Three amphiploid lines (code names in the present study: M, G and U) were kindly provided as seed by their respective breeders (Table 1). They were described as annual, amphidiploids and having breeding material status. Growth in Icelandic climates is not complete to seed set, but under controlled environments (16-h daylength and 15/13°C-day/night scheme) they are fully fertile and generally produce a similar seed yield per plant as wheat. The three lines are somewhat different in their growth and morphology. For example, spikes of the M- and G-lines are awned, but those of the U-line are awnless. These amphiploids were used as seed parents in backcrosses with *L. arenarius* and *L. mollis*, one of which, i.e. G×*L. mollis*, was examined here (Table 1). The backcross progeny are perennial, possess vigorous growth, but are self-sterile. Other plant species (Table 2) were included as references in the RFLP analysis of the ribosomal genes.

Fluorescence *in situ* hybridization (FISH)

Mitotic chromosomes were isolated from seedling root tips of the M-, G- and U-amphiploids after pre-treatment in ice-water for 24 h, and they were used in FISH experiments according to Anamthawat-Jónsson et al. (1996). Meiotic chromosomes were isolated from pollen mother cells of the backcross material G01 without pre-treatment. Three types of FISH were performed. (1) FISH using digoxygenin-labelled total genomic DNA of *L. arenarius* as a probe and unlabelled genomic DNA of *T. aestivum* (5× the probe amount) as block (Fig. 1a). Probe hybridization was detected with antidigoxygenin-FITC (green fluorescence) and the chromosomes were counter-stained with propidium iodide (red fluorescence). (2) FISH using green-fluorescing FITC-labelled total genomic DNA of *L. mollis* and red-fluorescing rhodamine-labelled total genomic DNA of *T. aestivum* as simultaneous probes, without blocking DNA (Fig. 1b). (3) FISH using a digoxygenin-labelled rDNA probe (18S.26S ribosomal genes, clone pTa71 from wheat, Gerlach and Bedbrook 1979), and detection of hybridization as in A (Fig. 1c–e). Some samples were hybridized with the rhodamine-labelled rDNA probe and digoxygenin-labelled 5S-rDNA probe (clone pTa794 from wheat, Gerlach and Dyer 1980) and fluorescein detection. Identification of the 18S.26S ribosomal gene loci in wheat followed the procedure of Jiang and Gill (1994); in *Leymus* it followed Anamthawat-Jónsson et al. (1997). The 5S-rDNA loci in wheat was identified after Mukai et al (1990).

Southern hybridization of the rDNA

Total genomic DNAs of the plant materials listed in Table 1 and 2 were isolated using the CTAB-method as in Anamthawat-Jónsson and Heslop-Harrison (1995). For Southern analysis, the DNAs were digested with restriction enzymes *Bam*HI, *Eco*RI and *Dra*I separately, sized-fractionated by gel electrophoresis on 0.7% agarose and transferred to Hybond N+ nylon membrane. The blot was then hybridized with the purified rDNA probe (clone pTa71) according to the standard ECL chemilluminescence protocol (Amersham). Hybridization and washing stringencies were 78% (based on 0.5 *M* NaCl) and 86%, respectively, and exposure time on film was from 5 to 45 min. The polymorphic fragments from *Bam*HI and *Eco*RI digestions, 16 in total, were scored from each of the nine plant samples as present (1) or absent (0). The *Dra*I digestion produced too few bands and hence was excluded from the analysis. These scores were used to calculate the genetic distance matrix according to Rogers and Tanimoto (1960), from which a phenogram (neighbour-joining tree) was constructed using the RAPDISTANCE program of Armstrong et al. (1996).

Results

Genomic *in situ* hybridization

FISH using *Leymus* genomic DNA as probe (common sequences blocked by wheat DNA) revealed all three amphiploid lines to be karyotypically stable in that they were allohexaploid (2n=42) containing 30 wheat and 12 *Leymus* chromosomes (G-line, Fig. 1a). This was con-

Table 1 The three amphiploids derived from crosses between *Triticum* and *Leymus*, and one backcross progeny

Code name	Original identification	Parental species	Cytogenetic status	Source of the material
M	AD99	(*T. durum*×*L. mollis*)×*T. aestivum*	2n=42	Swedish University of Agriculture, Svalöv (Prof. A. Merker)
G	PI-442574	*T. aestivum*×*L. arenarius*	2n=98	Wheat Genetics Resource Center, Kansas (Prof. B.S. Gill)
U		*T. aestivum*×*Leymus* sp.	No data	USDA, Northern Plains Area, Utah (Dr. K.B. Jensen)
G 01	G94BC1–01	G×*L. mollis*	1n=35	The present work

Table 2 Plant species used for DNA isolation and RFLP analysis of the ribosomal genes. Genome designations follow Wang et al. (1994)

Code name	Plant species	Collection number	Genomes	Origin (Keeper)
Th	*Thinopyrum junceum* (L.) Á. Löve	PI-234708	2n=42 EEE	France (National Germplasm Resources, USDA-ARS)
Ta	*Triticum aestivum* L. em. Thell.	cv. Chinese Spring	2n=42 ABD	
Td	*Triticum durum* Desf.		2n=28 AB	
Lm	*Leymus mollis* (Trin.) Pilger	Múlakot, Iceland	2n=28 NX	Alaska
La	*Leymus arenarius* (L.) Hochst.	Reykjavík	2n=56 NNXX	Iceland
Lr	*Leymus racemosus* (Lam.) Tzvelev	H-5053	2n=28 NX	Romania (KVL, Royal Veterinary and Agricultural University, DK)

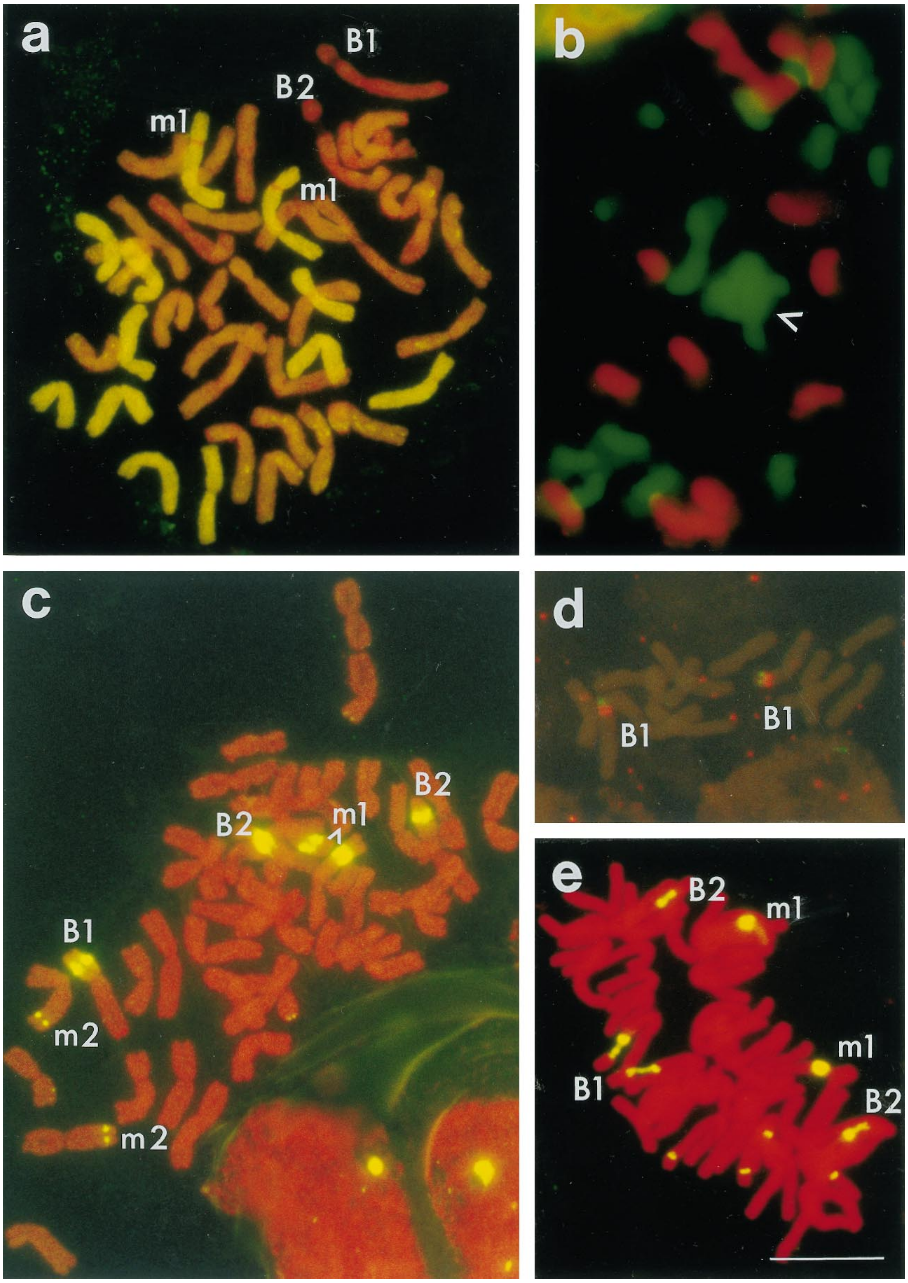

Fig. 1a–e Photomicrographs of chromosomes after fluorescence *in situ* hybridization (FISH). Bar represents 10 µm. **a** Mitotic chromosomes from root tip cells of the G-amphiploid after FISH using the *Leymus arenarius* genomic probe and *Triticum aestivum* block. The cell shows 12 yellow-green fluorescing *Leymus* chromosomes and 30 of the propidium iodide-stained wheat (*Triticum*) chromosomes. The rDNA loci B1 and B2 belonging to the B genome of wheat are evident as extended *Nor*, whereas the homologous pair of *L mollis Nor-m1* loci can be seen as *in situ* negative bands due to the blocking of common sequences between wheat and *Leymus*. **b** Meiotic chromosomes at metaphase I of the G01 backcross progeny of the G-amphiploid×*L. mollis* after simultaneous FISH using the red-fluorescing *T. aestivum* genomic probe and green-fluorescing *L. mollis* genomic probe. The cell shows 15 wheat chromosomes which are mainly univalents and 20 *Leymus* chromosomes as univalents, rod-bivalents and a multivalent (indicated with *arrow*). **c** Mitotic chromosomes of the G-amphiploid showing *in situ* mapping of 18S.26S ribosomal genes (pTa71) in which the wheat loci *Nor-B1* (1BS) and *Nor-B2* (6BS) and the *L. mollis* loci *Nor-m1* and *Nor-m2* are indicated. The wheat telomeric loci *Nor-A1* (1AS) can be seen on two homologous chromosomes in this preparation. **d** Mitotic chromosomes of the M-amphiploid showing red-fluorescing wheat *Nor-B1* loci of the 18S.26S ribosomal genes and green-fluorescing 5S-rDNA loci on the same chromosome. **e** Mitotic chromosomes of the U-amphiploid showing localization the of 18S.26S ribosomal genes consisting of two *Nor-B1* and two *Nor-B2* loci of wheat and two *Nor-m1* of *L. mollis* (indicated). The unmarked loci are one pair of the sub-telomeric *Nor-m2* and one pair of the wheat telomeric *Nor-A1*

firmed several times with seed-derived plants after three more generations of selfing during the present study. As this FISH method depicted species-specific DNA, in this case *Leymus*-specific, the uniformity of the painting signal on the chromosomes clearly indicated that no major structural exchanges (e.g. translocations) had occurred between the wheat and the *Leymus* chromosomes. The negative bands (sites without a positive signal) on *Leymus* chromosomes (Fig. 1a, indicated m1) were their nucleolar organizing regions (*Nor* loci), due to the highly repeated tandem arrays of the ribosomal genes being conserved between species and hence totally blocked by the wheat DNA. This *Nor-m1* map site was the first evidence in this study revealing the *Leymus* origin of all these amphiploids to be *L. mollis*, contradictory to some of the information on record (Table 1). The genomic FISH method as used here was not able to differentiate between species of *Leymus* and therefore could not verify the identity of *L. mollis* directly.

FISH using wheat and *Leymus* genomic probes simultaneously on meiotic chromosomes of the backcross progeny (e.g. Fig. 1b) confirmed the results obtained from the mitotic studies, that the haploid set of the amphiploids consisted of 15 wheat and 6 *Leymus* chromosomes and that the amphiploids behaved as stable diploids. In this backcross material there were 14 *L. mollis* chromosomes from the pollen donor in addition to the 6 *Leymus* chromosomes of the amphiploid, and therefore *Leymus* multivalents were observed at metaphase I (Fig. 1b) confirming that the *Leymus* parent in the amphiploid was *L. mollis*. If the origin were *L. arenarius* as recorded (Table 1), such high pairing would have been unlikely, as the two *Leymus* species, *L. arenarius* and *L. mollis*, are phylogenetically diverse (unpublished results). Most wheat chromosomes remained univalents as expected.

rDNA mapping on chromosomes

FISH localization of the major (18S.26S) ribosomal genes differentiated the three amphiploid lines by both the number of wheat rDNA sites and chromosomal identity of the *Nor* loci (Table 3, Fig. 1c–e). The M-amphiploid had one pair of *Nor-B1*, none of the *Nor-B2* or the *Nor-A1* loci, whereas the G- and U-lines had all these sites except that the G-line had only one locus of *Nor-B1*. These results were consistent between FISH experiments and between plants within each amphiploid line (data not shown). The major and minor loci of *L. mollis* were in this respect unvarying; all amphiploids had two sites of each *Nor-m1* and *Nor-m2* loci. The rDNA-FISH did not detect the presence of any *Nor*-bearing chromosomes from the D genome of wheat in these amphiploids, in contrast to the rDNA mapping of the F_1 hybrids deriving from bread wheat which had the *Nor-D3* locus (Table 3). Particular *Nor* loci in these amphiploids appeared to be actively expressed as the sites were clearly extended across the secondary constriction, for example all four *Nor-B1* and *Nor-B2* loci in the U-line (Fig. 1e), similarly to that observed in the G-line (Fig. 1a). On the contrary, the major *Leymus* loci *Nor-m1* in all the amphiploids studied here appeared to be inactive, similar to the situation in F_1 hybrids between *Triticum* and *Leymus* (Anamthawat-Jónsson et al. 1997).

rDNA restriction fragment analysis

RFLP profiles of the ribosomal genes fractionated by restriction enzymes *Bam*HI and *Eco*RI (Fig. 2) were species-specific, and therefore we were able to verify the chromosome mapping results regarding the identity of parental species and of the amphiploids themselves. The *Bam*HI profiles of the species studied here consisted of three size classes (Fig. 2a): 9.4–11 kb; 5.4–8.6 kb; 3–4 kb; and the *Eco*RI profiles also had three size classes (Fig. 2b): 8–11 kb; 6.6 kb; 3.6–4.4 kb.

The three amphiploid lines evidently had the *L. mollis* fragments, and none of the fragments of other *Leymus* species (*L. arenarius* and *L. racemosus*) or *Thinopyrum*, a genus that was thought for many years to contribute to *Leymus* one of its two genomes (Löve 1984). The genetic distance analysis (Fig. 3) confirmed the *L. mollis* origin of the amphiploids, and other *Leymus* species were clearly not involved. The wheat parent of these amphiploids, however, could not be related with certainty to any of the two wheat species included here, *Triticum durum* and *T. aestivum*, (Figs. 2 and 3). Furthermore, there were unique fragments in the amphiploids, i.e. the 8-kb *Bam*HI fragment common to all three amphiploids (Fig. 2a) and the 3.6-kb *Eco*RI fragment (Fig. 2b), and these must have come from their unknown wheat parent. As the mapping results did not detect *Nor-D3*, this wheat parent is likely to be a tetraploid species other than *T.*

Table 3 FISH localization of 18S.26S rDNA in the amphidiploids (the present study) and amphihaploid F1 hybrids between *T. aestivum* and *L. mollis* from Anamthawat-Jónsson et al. (1997). *Nor* loci in wheat: B1 (1BS), B2 (6BS), A1 (1AS) and D3 (5DS, hexaploid wheat only). *Nor* loci in *L. mollis*: m1, m2 and m3

Amphiploid	Number of major sites	Major *Nor* loci in wheat (*active)	Major loci in *Leymus*	Number of minor sites	Minor loci
M (2x=42)	4	2x B1	2x m1	2	2x m2
G (2x=42)	5	1x B1*^{1}, 2x B2*^{1}	2x m1	4	2x A1, 2x m2
U (2x=42)	6	2x B1*^{2}, 2x B2*^{2}	2x m1	4	2x A1, 2x m2
F1 hybrids (1x=35)	3	1x B1, 1x B2*^{1}	1x m1	4	1x D3, 1x A1 1x m2, 1x m3

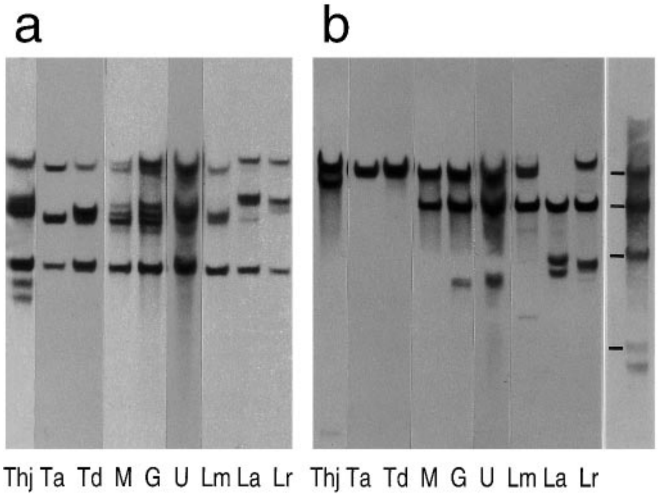

Fig. 2a,b Luminograghs after Southern hybridization of the 18S.26S ribosomal gene probe to restriction-digested genomic DNA of the M-, G- and U-amphiploids together with species of *Thinopyrum*, *Triticum* and *Leymus* (code names according to Tables 1 and 2). The *Bam*HI profiles **a** consist of three size classes: 9.4–11 kb; 5.4–8.6 kb; 3–4 kb, and the *Eco*RI profiles **b** have three size classes: 8–11 kb, 6.6 kb, 3.6–4.4 kb. While the amphiploids have certainly derived from *Triticum* and *Leymus*, this analysis can only confirm the *L. mollis* parent. The lambda size marker shows bands at 9.4, 6.6, 4.4 and 2.3 kb, indicated at *right* from *top to bottom*

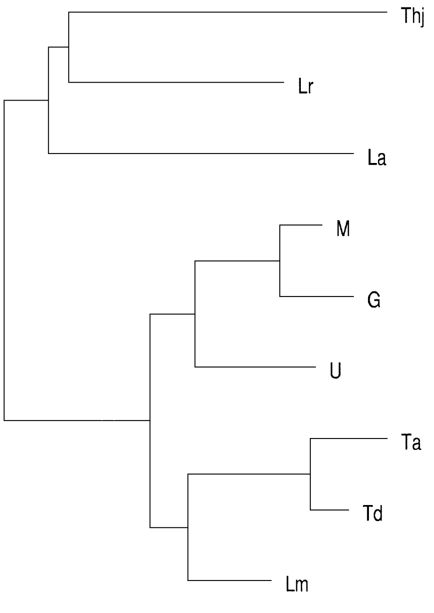

Fig. 3 Phenogram of 18S.26S ribosomal RFLP, based on 16 *Bam*HI and *Eco*RI fragments in Fig. 2, showing genetic distances among the species and amphiploids whose code names are listed in Tables 1 and 2. The amphiploids have clearly originated from a cross between *Leymus mollis* and a *Triticum* species

durum. Regarding the differentiation among the three amphiploids, the rDNA-RFLP analysis here was complementary to the mapping data. For example the 3.6-kb *Eco*RI fragment, which was observed only in the G- and U- lines, revealed a restriction site not existing in the M- line and this could therefore be the site in the *Nor-B2* specific to their wheat parent. Taken all the rDNA-RFLPs together (Fig. 3) the M- and the G-amphiploids were more related to one another than to the U-line, and this seems to agree with their morphology.

Discussion

Species origin of the amphiploids

The present study has revealed the *Leymus* origin of the amphiploids to be *L. mollis*, a tetraploid species having NX genomes, whereas the wheat origin was most likely a tetraploid wheat having AB genomes, therefore indicating misclassification of the breeding materials, some of which are germplasm collections. These amphiploids were presumably derived from crosses made about 40 years ago between *Triticum* and *Leymus* (Petrova 1960; Tsitsin 1965). The crosses involved a number of species including *T. aestivum* (6×), *T. durum* (4×), *T. carthlicum* (4×), *T. dicoccum* (4×), *T. persicum* (4×), *L. arenarius* (8×), *L. mollis* (4×) and *L. racemosus* (4×, 8×), resulting in several female fertile amphiploids having 2n numbers of 56, 70, 84 and 98. The materials might have been distributed and maintained in different places, and after so many generations of controlled or uncontrolled fertilization, only the cross combination discovered here has survived. A particular cross combination can be more successful if, for example, the fertility is efficiently recovered, stability of diploidization is effective and the genome is not too big to maintain. Indeed, our meiotic study of new F_1 hybrids between *Triticum* and *Leymus* has detected bivalent pairing and viable pollen in the hybrids having *L. mollis* as a parent, suggesting that they are probably more fertile than those derived from *L. arenarius* (Anamthawat-Jónsson and Bödvarsdóttir 1998). Similar hybrids were backcrossed to wheat (Merker and Lantai 1997), but only the hybrids deriving from *L. mollis* successfully set seed. An annual life form with good seed set may be convenient for breeders to maintain potential breeding lines.

The methods of FISH used here were effective in identifying the species origin of chromosomes and differentiating similar breeding lines. The genomic *in situ* hybridization in particular, detected species-specific sequences that were dispersed over the whole genome and was therefore useful in differentiating between wheat and *Leymus* chromosomes in these amphiploids. Such chromosome painting is indeed an important method for investigating genome relationships in hybrids and polyploid species (Anamthawat-Jónsson et al. 1996). Meiotic materials are especially valuable for this purpose, for example to identify autosyndetic pairing in *L. mollis* (the present study), intergenomic pairing and recombination in wheat×*Leymus* hybrids (Anamthawat-Jónsson and Bödvarsdóttir 1998). The rDNA markers, in contrast to the genomic probing, are specific as they can differentiate individual *Nor*-bearing chromosomes and are therefore the key markers in identification of parental species of amphiploids in the present study. The combination of genomic and rDNA FISH, especially if used simultaneously, would be a simple and effective way to characterize diverse collections of germplasm materials and breeding lines correctly.

The genome composition of the wheat x lymegrass amphiploids

All the amphiploids examined here are stable as hexaploids (2n=42) consisting of six pairs of *Leymus* and 15 pairs of wheat chromosomes, without any numerical variation either within or between plants. No other stable amphiploids deriving from crosses between *Triticum* and *Leymus* have been described before, although hybrids from several cross combinations have been made and backcrossed to wheat resulting in wheat addition and substitution lines containing *Leymus* chromosomal material (Dong et al. 1986; Qi et al. 1997). The diploid status of these lines is also evident as they are fully fertile and their haploid genome in the backcross progeny consists exactly of 6 *Leymus* and 15 wheat chromosomes. This unique genome composition is probably a result of the stable replacement of one pair of *Leymus* chromosomes by wheat, but which chromosomes are involved can not be determined at this stage. Such replacement is likely to be between closely related (homoeologous) chromosomes, as only then can the genetic content be complemented. Recent molecular evidence has shown for the first time the homoeology between *Leymus* (*L. racemosus*) chromosomes with those of wheat (Qi et al. 1997). Homoeologous chromosomes, as shown by comparative genome mapping across cereal species, are in general similar in their gene content and gene orders, and this has been remarkably conserved (Gale and Devos 1998).

The mapping of ribosomal genes has detected apparent differences among these amphiploids in their rDNA location and number, indicating that such variation is tolerated at the genetic level. The number of rDNA sites, for example, varies from six to ten, including two to four from wheat and four to six from *Leymus* (see Table 3), whereas in the parental species there are six sites in each. It is therefore probable that where the genes are lacking in wheat, those in *Leymus* compensate, and vice versa. But such compensation may not even be necessary as it is known that the cereals have ample excess in the number of genes required to sustain ribosomal synthesis (Leitch and Heslop-Harrison 1993). Indeed the ribosomal genes in these amphiploids appear to be active essentially in the wheat loci, as indicated by extended constriction and the dispersed *in situ* pattern of rDNA expression in interphase nucleoli typical of wheat ribosomal genes (Leitch et al. 1992), whereas the *Leymus* loci do not form nucleoli. A similar expression of ribosomal genes has been observed in triticale, whereby the wheat genes are active but the rye genes are preferentially suppressed (Lacadena et al. 1984), probably due to methylation (Neves et al. 1995). On the other hand, the expression of seed storage protein genes from rye is normal or even enhanced in wheat genetic background (Bittel and Gustafson 1992). For the wheat×lymegrass amphiploids described here, it is undoubtedly important to understand how the different genomes interact to the benefit of the plants themselves, if they are to be domesticated further.

Acknowledgements The amphiploid seed stocks provided by Prof. A. Merker, Prof. B.S. Gill and Dr. K.B. Jensen are most appreciated. The author would like to thank Sigridur Klara Bödvarsdóttir for assistance with the breeding work. I hereby declare that the experiments comply with the current laws of Iceland where they were performed.

References

Anamthawat-Jónsson K, Bödvarsdóttir SK (1998) Meiosis of wheat x lymegrass hybrids. Chromosome Res 6:339–343

Anamthawat-Jónsson K, Heslop-Harrison JS (1995) Molecular cytogenetics of Icelandic birch species: physical mapping by in situ hybridization and rDNA polymorphism. Can J For Res 25:101–108

Anamthawat-Jónsson K, Heslop-Harrison JS, Schwarzacher T (1996) Genomic *in situ* hybridization for whole chromosome and genome analysis. In: Clack M (ed) In situ hybridization, laboratory companion. Chapman & Hall, Weinheim, Germany, pp 1–23

Anamthawat-Jónsson K, Bödvarsdóttir SK, Bragason BTh, Gudmundsson J, Martin PK, Koebner RMD (1997) Wide-hybridization between species of *Triticum* L. and *Leymus* Hochst. Euphytica 93:293–300

Armstrong JA, Gibbs A, Peakall R, Weiller G (1996) RAPDISTANCE programs, Version 1.04 for the analysis of patterns of RAPD fragments. http://life.anu.edu.au/molecular/software/rapd/html

Baum M, Lagudah ES, Appels R (1992) Wide crosses in cereals. Annu Rev Plant Physiol Plant Mol Biol 43:117–143

Bittel DC, Gustafson JP (1992) Dosage response of rye genes in a wheat background. 2. Secalin genes on 1RS. Theor Appl Genet 84:1–5

Dong Y, Zhouzhou Y, Ganyuan Z (1986) Study on hybridization of *Triticum aestivum* with *Leymus multicaulis* and *Leymus racemosus*. In: Li Z, Swaminathan (eds) Proc 1st Int Symp Chromosome Eng Plant. Xian, China, pp 185–187

Fatih AMB (1983) Analysis of the breeding potential of wheat-*Agropyron* and wheat-*Elymus* derivatives. Hereditas 98: 287–295

Gale MD, Devos KM (1998) Plant comparative genetics after 10 years. Science 282:656–659

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885

Gerlach WL, Dyer TA (1980) Sequence organization of the repeating units in the nucleus of wheat which contain 5S rRNA genes. Nucleic Acids Res 8:4851–4865

Jiang J, Gill BS (1994) New 18S.26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma 103:179–185

Jiang J, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73:199–212

Lacadena JR, Cermeno MC, Orellana J (1984) Evidence for wheat-rye nucleolar competition (amphiplasty) in triticale by the silver-staining procedure. Theor Appl Genet 67:207–213

Leitch AR, Heslop-Harrison JS (1993) Ribosomal RNA gene expression and localization in cereals. In: Sumner AT, Chandley AC (eds) Chromosome today, vol. 11. Chapman & Hall, London, pp 91–100

Leitch AR, Mosgöller W, Shi M, Heslop-Harrison JS (1992) Different patterns of rDNA organization at interphase in nuclei of wheat and rye. J Cell Sci 101:751–757

Löve Á (1984) Conspectus of the Triticeae. Feddes Repert Z Bot Taxon Geobot 95:425–521

Merker A, Lantai K (1997) Hybrids between wheats and perennial *Leymus* and *Thinopyrum* species. Acta Agric Scand 47:48–51

Mujeeb-Kazi A, Bernard M, Bekele GT, Mirand JL (1983) Incorporation of alien genetic information from *Elymus giganteus* into *Triticum aestivum*. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Maruzen, Kyoto, Japan, pp 223–231

Mukai Y, Endo TR, Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. J Hered 81: 290–295

Neves N, Heslop-Harrison JS, Viegas W (1995) rRNA gene activity and control of expression mediated by methylation and imprinting during embryo development in wheat x rye hybrids. Theor Appl Genet 91:529–533

Petrova KA (1960) Hybridization between wheat and *Elymus*. In: Tsitsin NV (ed) Wide hybridization in plants. Israel Program for Sci Transl, Jerusalem, pp 226–237

Pimm SL (1997) In search of perennial solutions. Nature 389: 126–127

Plourde A, Comeau A, St-Pierre CA (1992) Barley yellow dwarf virus resistance in *Triticum aestivum*×*Leymus angustus* hybrids. Plant Breed 108:97–103

Qi LL, Wang SL, Chen PD, Liu DJ, Friebe B, Gill BS (1997) Molecular cytogenetic analysis of *Leymus racemosus* chromosomes added to wheat. Theor Appl Genet 95:1084–1091

Rogers DJ, Tanimoto TT (1960) A computer program for classifying plants. Science 132:1115–1118

Shepherd KW, Islam AKMR (1988) Compendium of amphiploids in the Triticeae. In: Miller TE, Koebner RMK (eds) Proc 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp 1351

Tsitsin NV (1965) Remote hybridization as a method of creating new species and varieties of plants. Euphytica 14:326–330

Wagoner P (1990) Perennial grain development: past efforts and potential for the future. Plant Sci 9:381–409

Wang RR-C, von Bothmer R, Dvorak J, Linde-laursen I, Muramatsu M (1994) Genome symbols in the Triticeae (Poaceae). In: Wang RR-C et al. (eds) Proc 2nd Int Triticeae Symp. Utah State University Press, Logan, Utah, pp 29–34