Theor Appl Genet (2001) 103:1123–1128

E. Benavente · K. Alix · J.-C. Dusautoir · J. Orellana
J.L. David

Early evolution of the chromosomal structure of *Triticum turgidum* – *Aegilops ovata* amphiploids carrying and lacking the *Ph1* gene

Received: 28 November 2000 / Accepted: 20 March 2001

Abstract After two selfing generations of two different *Triticum turgidum* – *Aegilops ovata* amphiploids carrying the *Ph1* gene, or lacking it (*ph1c* mutant), karyotypes of their offspring were scored by GISH (genomic in situ hybridization). On average, the chromosome number was lower than expected (56 chromosomes) on the basis of the parental constitutions (*T. turgidum*, AABB, 2n=4*x*=28; *Ae. ovata*, $M^oM^oU^oU^o$, 2n=4*x*=28). The lost chromosomes belonged to the wild *Aegilops* species. The two families differed greatly by their number of intergenomic translocations, also detected by GISH. The *ph1c* family showed nine translocations over 12 plants while only one translocation was observed in the *Ph1* family. All exchanges involved either the M^o and U^o chromosomes or the M^o and wheat chromosomes, the size of the exchanged segment ranging from 3% to 36% of the total chromosome length. The results suggest an epistatic effect of the *ph1c* deletion over the genetic diploidizing system that operates in *Ae. ovata* since translocated chromosomes are most-likely derived from homoeologous recombination. The potential of these results for wheat breeding programmes is also considered.

Keywords Wheat-*Aegilops* amphiploids · *Ph1* locus · Karyotypical evolution · Translocations · GISH

Introduction

The use of interspecific hybridization is of primary importance in plant breeding in order to transfer genes of particular agronomic interest from wild species into domesticated crops. The success of such introgression programmes is entirely dependent on, first, the ability to obtain fertile hybrids and, then, the occurrence of intergenomic recombination between the chromosomes of wild and cultivated species. Duplication of interspecific hybrids (which can be spontaneous) leads to amphiploids with two complete sets of homologous chromosomes. While hybrids are often sterile because of the unbalanced segregation of homoeologues, amphiploids possess a relatively good level of fertility since homologous chromosomes can pair in a regular meiosis. Therefore, it can be expected that amphiploids remain stable in their chromosome number and structure after several generations of selfing, and thus are a suitable intermediate material for the further development of distinct introgression programmes with different breeding purposes (e.g. Simonenko et al. 1998). Nevertheless, the chromosomal stability of newly synthesised amphiploids can be questioned since rapid genomic changes at the molecular level, such as elimination or modification of certain DNA sequences from one of the constituent genomes in early selfing generations, have been documented in a number of cases (Song et al. 1995; Feldman et al. 1997; Liu et al. 1998a, b).

The diploid-like meiotic behaviour of wheat is ensured by the *Ph*-pairing homoeologous multigenic system (for a review see Sears 1976), which includes the major-effect *Ph1* locus localised on the long arm of chromosome 5B (Riley and Chapman 1958; Sears and Okamoto 1958). Some studies suggest that *Ph1* could act during interphase by controlling the pre-meiotic alignment of homologous and homoeologous chromosomes and spatially separating the genomes, leading to exclusive homologous pairing (Feldman 1993; Vega and Feldman 1998), while other studies point out that *Ph1* acts at meiotic prophase by affecting the stringency of crossing-over which is thus confined to homologous chromosomes (Holm and Wang 1988; Dubcovsky et al. 1995; Luo et al. 1996). Mikhailova et al. (1998) have proposed that these apparently contradictory functions,

Communicated by B.S. Gill

E. Benavente (✉) · J. Orellana
Unidad de Genética, E.T.S. Ingenieros Agrónomos,
Universidad Politécnica, 28040-Madrid, Spain
e-mail: ebenavente@bit.etsia.upm.es
Tel.: 34-91-336-5714, Fax: 34-91-543-4879

K. Alix · J.-C. Dusautoir · J.L. David
UMR 1097 Diversité et Génome des Plantes Cultivées,
INRA Domaine de Melgueil, 34130 Mauguio, France

as well as the altered centromeric structure shown in *ph1b* material (Aragón-Alcaide et al. 1997), are actually the pleiotropic consequences of some primary effect of *Ph1* on chromosome condensation and organization. Concerning allotetraploid *Aegilops* species, the mechanisms controlling meiotic chromosome pairing have been proposed to act on initial synapsis, leading to the achievement of exclusive bivalent formation at zygotene of prophase-I (Cuñado et al. 1996). In wheat, the loss of the *Ph1* locus because of a deletion in the 5BL chromosome arm, namely mutation *ph1b* for hexaploid wheat (Sears 1977) and *ph1c* for tetraploid wheat (Giorgi 1978), induces homoeologous recombination. These mutants have been widely exploited for breeding purposes because they allow the wheat chromosomes to pair with homoeologues from wild related species in monosomic hybrids and/or aneuploid backcrosses (see Jauhar and Chibbar 1999, and references therein).

This paper deals with the early genomic evolution of *Triticum turgidum* ssp. *durum* - *Aegilops ovata* amphiploids. *Ae. ovata* is widespread around the Mediterranean Basin and can spontaneously cross with durum wheat. Moreover, it represents a reservoir of interesting genes for breeding wheat in the western part of Europe involving agronomic traits related to disease and abiotic stress resistances (e.g. Rekika et al. 1997). *Ae. ovata* is considered to be an allotetraploid of *Aegilops umbellulata* and *Aegilops comosa* with modified U and M genomes (Kimber et al. 1988). Thus wheat and *Ae. ovata* constitute a model of interest to study the evolution of amphiploids and, in particular, to exploit their properties in introgression programmes for wheat breeding. The aim of the present study was to compare the karyotypical constitution of the descendants of two *T. turgidum* ssp. *durum* – *Ae. ovata* amphiploids which differ from each other by the presence either of the active *Ph1* gene or of the *ph1c* mutation after two selfing generations. We have used genomic in situ hybridization (GISH) to examine the chromosomal composition for each of the constituent genomes, and to characterise the number, genomic origin and length of the intergenomic translocations which have eventually occurred.

Material and methods

Plant material

Two *T. turgidum* ssp. *durum*×*Ae. ovata* hybrids (2n=28; genome constitution ABU^{o}M^{o}) obtained in 1995 gave rise to two amphiploids (2n=56) in 1996 either by spontaneous doubling (P21) or by colchicine treatment (C45). Primadur, a french commercial cultivar homozygous *Ph1/Ph1*, was the wheat parent of P21; the mutant line Creso *ph1c/ph1c* (kindly provided by J. Jahier, INRA, Rennes) was the wheat parent of C45. The corresponding *Aegilops* parents were two different accessions both of Moroccan origin. The original amphiploids were selfed, S_1 grains were bulked and nine P21 and 12 C45 individuals of the second generation of selfing have been cytologically examined in this study.

For GISH on the parental *Aegilops* species (see Results section) a Bulgarian accession of *Ae. ovata* was used.

Preparation of cells

Plants were grown in the glasshouse and pots were laid on sand to allow good draining. Once roots were out of the pot, they were cut off between 1 and 2.30 p.m. (solar zenith) and immediately placed in melting ice until the following morning to synchronise cell divisions. Tips were then fixed in ethanol:acetic acid (3:1) and stored at 4°C for 2–4 months. Then root tips were heated in 1% acetic carmine and squashed in 45% acetic acid. Slides were stored at 4°C prior to genomic in situ hybridization.

DNA extraction, probe labelling and GISH

U, M and AB genomic DNAs were isolated from young leaves of *Ae. umbellulata* (2n=2*x*=14, genome constitution UU), *Ae. comosa* (2n=2*x*=14, genome constitution MM) and *T. turgidum* ssp. *durum* cv. Langdon (2n=4*x*=28, genome constitution AABB), respectively, following standard protocols. Then U- and M-genome DNAs were mechanically sheared into 10–12-kbp pieces and labelled with digoxigenin-11-dUTP (U_{digo} and M_{digo} probes) or biotin-16-dUTP (U_{bio} and M_{bio} probes) by nick translation (Boehringer Mannheim).

Hybridization mixtures contained differentially labelled *Ae. umbellulata* and *Ae. comosa* (U_{digo} and M_{bio} or U_{bio} and M_{digo}) genomic probes, to a final concentration of 8 ng/µl each. Unlabelled sonicated durum wheat genomic DNA (60-fold in excess of the labelled probe concentration) was also added in order to block the DNA sequences common to the A and B genomes. The GISH protocol was as described in Sanchez-Moran et al. (1999).

Immunological detection and visualisation

Digoxigenin-labelled probes were revealed with 5 ng/µl of goat anti-digoxigenin antibody conjugated with fluorescein isothiocyanate (FITC, Boehringer Mannheim) whereas biotinylated probes were detected with 5 ng/µl of avidin conjugated with Cy3 dye (Amersham). Slides were screened using an Axiophot epifluorescent microscope (Zeiss) equipped with different sets of filters and photographed with Kodak Ektachrome film ASA/ISO 400. The films were scanned and processed for brightness before printing.

Phenotypical study of S_2 individuals

After 8 weeks of vernalization (4°C, 12 h·day^{-1} light), young plantlets were grown in 3-l pots in a glasshouse (16°C/25°C, 16 h·day^{-1} light). The plant height was noted on the tallest tiller. The number of spikes per plant as well as their spikelet number were recorded. Fertility was computed as the number of kernels per spikelet which was recorded only on bagged spikes (3–5 per plant). The two families (P21 and C45) were compared for their variance (*F*-test) and their average (Student *t*-test with a different variance).

Results

Hybridization mixtures containing differentially labelled *Ae. umbellulata* and *Ae. comosa* DNA probes and unlabelled durum wheat genomic DNA allowed identification of U^{o}, M^{o} and AB chromatin in mitotic cells of P21 (*Ph1/Ph1*) and C45 (*ph1c/ph1c*) S_2 plants (Fig. 1). As shown in Table 1, chromosomes were lost in both *Ph1* and *ph1c* families. In P21 the chromosome number was between 53 and 55, while in C45 the range was 54–56. Chromosome elimination seemed not to be random but genome-dependent. Thus, only two out of the 21 plants

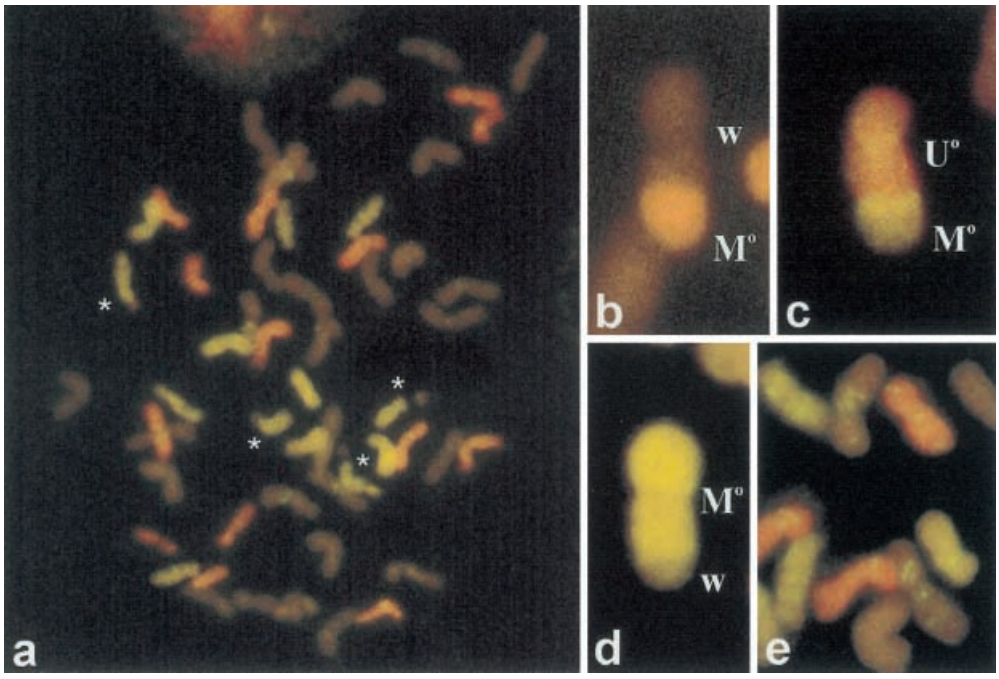

Fig. 1a–e GISH identification of U°, M° and wheat (*w*) parental genomes in mitotic cells (**a, e**) and intergenomic exchanges (**b–d**) from *T. turgidum –Ae. ovata* amphiploid descendants. The hybridization mix contained U_{digo} and M_{bio} (**a, b, e**) or U_{bio} and M_{digo} (**c, d**) genomic-DNA labelled probes and unlabelled durum wheat genomic DNA. **a** Mitotic metaphase of individual C45#5 (2n=55). *Asterisks* indicate U° NOR chromosomes with unlabelled satellited chromatin (see text). **b** Wheat/M° translocation in individual C45#2. **c** U°/M° translocation in individual C45#4. **d** M°/wheat translocation in individual C45#3. **e** Partial mitotic metaphase illustrating biotinylated (M°) and unlabelled (*w*) partners with conspicuous FITC dot signals

(9.5%) counted less than 28 wheat chromosomes whereas 17 plants (81%) showed an incomplete *Ae. ovata* chromosome complement. Comparison between the U° and M° genomes revealed no differences in C45 descendants, while a greater loos of M°-genome chromosomes was observed in P21 (13.9 U° vs 12.3 M° chromosomes per plant; Student test t=5.96, df=11, P<0.001). This indicates that, on average, P21 had less chromosomes than C45 (54.2 vs 55.2; Student test t=3.4, df=16, P<0.05). The reason for such a genotype-dependent selective elimination of M° chromosomes will be considered later.

GISH revealed several intergenomic translocations (Fig. 1b–d). The frequency of chromosomal exchanges was remarkably different between the two progenies examined (Table 1). Thus, only one out of the nine P21 plants showed a small translocation whereas nine translocated chromosomes were found in the 12 C45 plants examined (0.11 and 0.75 exchange events per plant, respectively). According to their size and genomic structure, all exchanges seemed to be different from each other. Six of them had occurred between the two genomes of *Ae. ovata*, the remaining four involving wheat and M° genomes. No wheat-U° translocation was detected in the sample examined. All exchanges were terminal and none was in a homozygous state. Estimations on the length of the exchanged segments relative to the total length of the translocated chromosome, based on measurements of 6–8 mitotic figures, have been included in Table 1. Most translocations did not exceed 15% of the total chromosome length. There did not seem to be any relation between the relative size of the exchanged segment and the genomic origin of the chromosomes involved. Hence, one of the two large exchanges, representing more than 30% of the translocated chromosome, involved wheat and M° genomes whereas the two *Ae. ovata* genomes were involved in the other one.

Either with digoxigenin-labelled U (U_{digo}) and biotin-labelled M (M_{bio}) genomic probes or with the opposite combination of labelled probes (U_{bio} and M_{digo}), small green-yellow fluorescein signals were observed on several biotinylated and unlabelled chromosomes in some experiments (Fig. 1e). Whereas on wheat unlabelled chromosomes these dots were quite abundant and mainly pericentromeric, on *Ae. ovata* chromosomes they also appeared at interstitial and telomeric locations. Such patterns resembled the heterochromatin distribution reported in the wheat B genome (Gill et al. 1991) and in *Ae. ovata* (Friebe et al. 1999), respectively. It is likely that these signals reveal hybridization sites of *Ae. umbellulata* or *Ae. comosa* repeated DNA sequences present in both the *Ae. ovata* genomes as well as in wheat. This is why the observation of such fluorescein dots was not considered in the scoring of translocations.

In some cells of P21 and C45 individuals, the chromatin beyond the secondary constriction of the nucleolus organiser region (NOR)-chromosomes 1 U and 5 U either appeared less intensely labelled or was even visualised as the wheat unlabelled chromatin (Fig. 1a). This observation was particularly remarkable in cells with long chromosomes and when the U°-genome chromatin

Table 1 Chromosomal constitution of the descendants of the two amphiploids examined

Amphiploid	Individuals	2n	U^o	M^o	Wheat	Translocations[a]	
P21							
(*Ph1* family)	1, 9	55	14	13	28		
	2, 4, 5, 8	54	14	12	28		
	3	53	14	11	28		
	6	54	13	13	28		
	7	55	14	13	28	M^o/U^o (L, 5%)	
C45							
(*ph1c* family)	1	55	14	13	28	M^o/U^o (L, 3%)	
	2	56	13	14	29[b]	Wheat/M^o (L, 36%)	
	3	55	13	14	28	M^o/wheat (L, 12%)	
	4	56	14	14	28	U^o/M^o (L, 31%)	M^o/wheat (L, 11%)
	5, 9	55	13	14	28		
	6	55	14	13	28	U^o/M^o (L, 3%)	M^o/U^o (L, 4%)
	7	56	14	14	28	M^o/wheat (L, 5%)	
	8	55	14	13	28		
	10	55	13	14	28	U^o/M^o (S, 7%)	
	11	54	14	14	26		
	12	55	14	14	27		

[a] The centromeric genome is noted first. The chromosome arm affected (L: long arm; S: short arm) and the relative size of the exchanged segment are in brackets

[b] Including the wheat/M^o translocated chromosome

Table 2 Morphological description of the *Ph1* and *ph1c* amphiploid descendants

Trait	Plant height (cm)	Number of spikes	Spikelets/ spike	Kernels/ spikelets
P21 (*Ph1*)				
Mean (n=15)	56.5	12.3	6.13	1.73
SE	4.5	3.6	0.39	0.22
C45 (*ph1c*)				
Mean (n=19)	55.8	21.9	5.80	0.29
SE	9.1	5.9	0.32	0.22
P values[a]				
Mean	0.80	3.10^{-6}	0.013	3.10^{-19}
SE	$4\ 10^{-3}$	0.03	0.22	0.17

[a] The Student test and the *F*-test give the *P* values for the null hypothesis of equality in mean and variance

was detected by means of FITC. To determine whether this pattern of hybridization corresponded to U^o/wheat translocations, we hybridized root cells of *Ae. ovata* with a mix containing U_{digo} and M_{bio} probes as well as durum wheat blocking-DNA. It was then noted that the U^o NOR chromosomes in *Ae. ovata* also showed the labelling pattern occasionally found in the amphiploids P21 and C45. Whatever the molecular or technical reason for such a special GISH behaviour of the U^o-genome satellited chromatin, none of these differentially labelled U^o NOR chromosomes was taken as evidence of a U^o/wheat chromosomal exchange.

The phenotypic analysis revealed that the two families significantly differed by a number of morphological traits, except for height (Table 2). This was surely due to genetic differences between the parents of the two progenies. But, the *ph1c* allele could be directly responsible for the lower fertility level observed in C45 (0.29 kernels per spikelet in C45 vs 1.73 in P21). The variances between plants within each family also differed, C45 seeming to be more variable than P21. This trend was apparent for plant height and the number of spikes per plant.

Discussion

Genomic changes in early generations after chromosome doubling have been reported in newly synthesized amphiploids of *Triticum* (Feldman et al. 1997) and *Brassica* (Song et al. (1995). The former authors found non-random elimination of certain DNA sequences at the S_5 generation which could not be related to chromosome loss as deduced from RFLP pattern analysis, although no karyological analysis was attempted. In the present study we have cytologically demonstrated chromosomal instability in S_2 plants which seems to affect mainly the *Ae. ovata* chromosomes. In both P21 and C45 amphiploids the female parent was *T. turgidum* which suggests the possibility of a cytoplasmic influence on chromosome loss. Nuclear-cytoplasmic interaction resulting in greater genomic changes of the paternally donated nuclear genome has been documented in *Brassica* reciprocal synthetic amphiploids (Song et al. 1995). However, Liu et al. (1998b) could not find differences between *T. aestivum – Ae. longissima* reciprocal amphiploids. As *T. turgidum – Ae. ovata* amphiploids on *ovata* cytoplasm are

not yet available this question remains open to further investigation.

Within the *Ph1* family, M^o-genome chromosomes have been lost at a higher rate than U^o chromosomes. In fact, none of the P21 individuals examined counted for the expected number of 14 M^o-genome chromosomes (Table 1). Xu and Dong (1992) reported meiotic abnormalities in *Triticum* × *Aegilops* hybrids leading to unbalanced unreduced gametes which could result in aneuploid amphiploid descendants. Therefore, it cannot be excluded that one chromosome belonging to the M^o genome was lost during the spontaneous doubling process which produced the initial P21 seed.

Song et al. (1995) had suggested that intergenomic recombination could be a major factor contributing to genome change in newly synthetic allopolyploids. However, Feldman and coworkers (Liu et al. 1998a,b) rejected this possibility since they found a similar variation ratio between *T. aestivum – Ae. kotschyi* amphiploids carrying and lacking the wheat homoeologous pairing-control gene *Ph1*. Their results are in major disagreement with those presented here, where the frequency of intergenomic translocations is remarkably different in both amphiploid offspring (Table 1) and a trend towards higher variation for morphological traits has been detected within the *ph1c* family (Table 2). Furthermore, the low fertility level of C45 amphiploids is surely due to meiotic irregularities either directly induced by the *ph1c* mutation or as a consequence of their level of chromosomal rearrangement. Song et al. (1995) and Liu et al. (1998a, b) inferred genomic changes occurring during the earlier evolution of amphiploids from a comparison of RFLP patterns between S_5 progenies and the corresponding parental species. The cytological approach followed in our study provides very confident information on gross genome changes, i.e. chromosome elimination or intergenomic exchanges. Molecular markers such as RFLPs are undoubtedly more accurate for monitoring genome variations at the DNA sequence level but they are restricted to cover only a small fraction of the genomic content and, at least to some extent, this could account for the discrepancies noted here with the *Triticum – Aegilops* amphiploids examined by Feldman and coworkers. Cytological analysis should be routinely carried out to complement and contrast results obtained by molecular approaches on the genomic evolution of complex genetic structures such as amphiploids.

By C-banding Fernández-Calvín and Orellana (1992) analyzed homoeologous meiotic pairing at metaphase-I (MI) in *T. aestivum* × *Ae. ovata* hybrids (2n=35, genome constitution $ABDU^oM^o$) carrying the *ph1b* mutation. These authors distinguished neither A- from D-, nor U^o- from M^o-genome chromosomes, but were able to detect differential MI pairing affinities among the genomes in competition. They found that MI homoeologous pairing between both *Ae. ovata* genomes was slightly lower than that between the A and D wheat genomes. The least-frequent types of MI association were those involving B-genome chromosomes, i.e. A or D with B and U^o or M^o with B. Homoeologous pairing-results in the *ph1b* hybrids support the conclusion that translocated chromosomes in the *ph1c* amphiploids arose from homoeologous recombination and that A-genome chromosomes are more-frequently involved in wheat-M^o exchanges (e.g. Fig. 1b, d) than B-genome homoeologues. The absence of U^o-wheat translocations suggests that U^o chromosomes paired rarely with wheat chromosomes or that this type of combination was lethal for the gametes or the zygotes. On this point, it must be noted that the genomes of wheat and *Ae. umbellulata* differ by a minimum of 11 chromosomal rearrangements (reciprocal translocations and inversions) which affect all the seven groups of homoeology (Zhang et al. 1998) and that structural differences hinder homoeologous pairing between chromosomes of wheat and related species (see Maestra and Naranjo 2000, and references therein). If chromosomal modifications are maintained in the U^o genome of *Ae. ovata* from its diploid ancestor, such a high degree of structural differentiation could account for the lack of recombinants involving wheat and U^o genomes in the amphiploid.

The analysis of synaptonemal complex formation by means of spreading techniques reveals striking differences in the synaptic behaviour between tetraploid wheat and allotetraploid *Aegilops* species, including *Ae. ovata*. In durum wheat carrying the *Ph1* gene, multivalents are quite frequent at zygotene but are corrected to bivalents prior to chiasma formation at the pachytene stage (Martinez et al. 2000), whereas almost exclusive bivalent formation is achieved in *Aegilops* early at the zygotene stage (Cuñado et al. 1996). Our results provide further information on the interaction between the genetic systems controlling the diploid-like meiotic behaviour of these two allotetraploid species. The observation of translocations in C45 amphiploids (*ph1c/ph1c*) confirms that the diploidization mechanism of *Ae. ovata* cannot functionally replace that of wheat (McGuire and Dvorak 1982), and that removing *Ph1* allows homoeologous pairing and recombination to take place not only in hybrids but also when complete homologous pairing is possible, as in amphiploids. Furthermore, the considerable number of M^o–U^o translocations found in the *ph1c* family provides evidence that the mechanism that ensures a diploid-like behaviour in *Ae. ovata* by impeding meiotic pairing between its two constituent genomes is not efficient when in a *ph* mutant background. Such an epistatic effect could give some clue to deciphering the mode and timing of action of *Ph1*.

A final comment can be made on the breeding potential of the findings reported here. If selfing an initial amphiploid for successive generations leads to chromosomal elimination (and translocations, if *ph1* mutant lines are used as parents), an original way of using amphiploids before backcrossing onto the crop species could be to increase the number of generations after the original doubling event in order to create highly outcrossing populations from variants of the amphiploid, thus obtaining many different translocated or substituted

lines. To settle such an introgressive strategy, there is a need to quantify the rate of interspecific exchanges according to the dynamics of the chromosome composition as well as to characterise the translocations events in terms of length and frequencies, in addition to the specific genomes and chromosomes involved. Technical limitations of GISH, such as those described above in terms of the discrimination of small-sized exchanges or artifactual labelling of specific chromatin regions, have to be kept in mind for accurate interpretation of the results but do not invalidate the great advantages of molecular cytogenetic tools for the genomic characterization of amphiploids and complex derivatives.

Acknowledgements The authors thank Julie Goy for technical assistance in the phenotypic study. This work has been supported by grant No. AGF96–0443 from the Comisión Interministerial de Ciencia y Tecnología (Spain) and generous funding from the french Ministère de l'Education Nationale et de la Technologie and Ministère des Affaires Étrangères (Picasso Program).

References

Aragon-Alcaide L, Reader S, Miller T, Moore G (1997) Centromeric behaviour in wheat with high and low homoeologous chromosomal pairing. Chromosoma 106:327–333

Cuñado N, Callejas S, Garcia MJ, Fernandez A, Santos JL (1996) The pattern of zygotene and pachytene pairing in allotetraploid *Aegilops* species sharing the U genome. Theor Appl Genetics 93:1152–1155

Dubcovsky J, Luo MC, Dvorak J (1995) Differentiation between homoeologous chromosomes 1 A of wheat and 1 A(m) of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. Proc Natl Acad Sci USA 92:6645–6649

Feldman M (1993) Cytogenetic activity and mode of action of the pairing homoeologous (*Ph1*) gene of wheat. Crop Sci 33:894–897

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM (1997) Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147:1381–1387

Fernandez-Calvin B, Orellana J (1992) Relationship between pairing frequencies and genome affinity estimations in *Aegilops ovata×Triticum aestivum* hybrid plants. Heredity 68: 165–172

Friebe BR, Tuleen NA, Gill BS (1999) Development and identification of a complete set of *Triticum aestivum – Aegilops geniculata* chromosome addition lines. Genome 42:374–380

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Giorgi B (1978) A homeologous pairing mutant isolated in *Triticum durum* cv Capelli. Mutant Breed Newslett 11:4–5

Holm PB, Wang X (1988) The effect of chromosome 5B on synapsis and chiasma formation in wheat, *Triticum aestivum* cv Chinese Spring. Carlsberg Res Commun 53:191–208

Jauhar PP, Chibbar RN (1999) Chromosome-mediated and direct gene transfers in wheat. Genome 42:570–583

Kimber G, Sallee PJ, Feiner MM (1988) The interspecific and evolutionary relationships of *Triticum ovatum*. Genome 30: 218–221

Liu B, Vega JM, Feldman M (1998a) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. II. Changes in low-copy coding DNA sequences. Genome 41: 535–542

Liu B, Vega JM, Segal G, Abbo S, Rodova H, Feldman M (1998b) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy noncoding DNA sequences. Genome 41:272–277

Luo MC, Dubcovsky J, Dvorak J (1996) Recognition of homeology by the wheat *Ph1* locus. Genetics 144:1195–1203

Maestra B, Naranjo T (2000) Genome evolution in Triticeae. In: Olmo E, Redi CA (eds) Chromosomes today 13. Birkhäuser Verlag, Basel, pp 155–167

Martinez M, Naranjo T, Cuadrado C, Romero C (2001) The synaptic behaviour of *Triticum turgidum* with variable doses of the *Ph1* locus. Theor Appl Genet 102:751–758

McGuire PE, Dvorak J (1982) Genetic regulation of heterogenetic chromosome pairing in polyploid species of the genus *Triticum sensu lato*. Can J Genet Cytol 24:57–82

Mikhailova EI, Naranjo T, Shepherd K, Eden JW, Heyting C, deJong JH (1998) The effect of the wheat Ph1 locus on chromatin organisation and meiotic chromosome pairing analysed by genome painting. Chromosoma 107:339–350

Rekika D, Monneveux P, Havaux M (1997) The in vivo tolerance of photosynthetic membranes to high and low temperatures in cultivated wheat of the *Triticum* and *Aegilops* genera. J Plant Physiol 150:734–738

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715

Sanchez-Moran E, Benavente E, Orellana J (1999) Simultaneous identification of A, B, D and R genomes by genomic in situ hybridization in wheat-rye derivatives. Heredity 83:249–252

Sears ER (1976) Genetic control of chromosome pairing in wheat. Annu Rev Genet 10:31–51

Sears ER (1977) An induced mutant with homeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Sears ER, Okamoto M (1958) Intergenomic chromosome relationships in hexaploid wheat. Proc 10th Int Cong Genet 2:258–259

Simonenko VK, Motsny II, Sechnyak AL, Kulbida MP (1998) The use of wheat-alien and *Aegilops*-rye amphiploids for introgression of genetic material to wheat. Euphytica 100:313–321

Song KM, Lu P, Tang KL, Osborn TC (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc Natl Acad Sci USA 92:7719–7723

Vega JM, Feldman M (1998) Effect of the pairing gene *Ph1* and premeiotic colchicine treatment on intra- and inter-chromosome pairing of isochromosomes in common wheat. Genetics 150:1199–1208

Xu S, Dong Y (1992) Fertility and meiotic mechanisms of hybrids between chromosome autoduplication tetraploid wheats and *Aegilops* species. Genome 35:379–384

Zhang H, Jia J, Gale MD, Devos KM (1998) Relationships between the chromosomes of *Aegilops umbellulata* and wheat. Theor Appl Genet 96:69–75