Theor Appl Genet (2002) 104:1164–1172
DOI 10.1007/s00122-001-0827-7

H. Guyomarc'h · P. Sourdille · G. Charmet
K. J. Edwards · M. Bernard

Characterisation of polymorphic microsatellite markers from Aegilops tauschii and transferability to the D-genome of bread wheat

Received: 7 March 2001 / Accepted: 8 October 2001 / Published online: 20 February 2002

Abstract Microsatellites were isolated from a *Aegilops tauschii* (the D-genome donor of bread wheat) library enriched for various motifs. Primers generated from the flanking region of the microsatellites were used successfully to amplify the corresponding loci in the D genome of bread wheat. Additional amplification sometimes also occurred from the A and B genomes. The majority of the microsatellites contained $(GA)_n$ and $(GT)_n$ motifs. GA and GT repeats appeared to be both more abundant in this library and more polymorphic than other types of repeats. The allele number for both types of dinucleotide repeats fitted a Poisson distribution. Deviance analysis showed that GA and GT were more polymorphic than other motifs in bread wheat. Within each motif type (di-, tri- and tetra-nucleotide repeats), repeat number has no influence on polymorphism. The microsatellites were mapped using the *Triticum aestivum* Courtot × Chinese Spring mapping population. A total of 100 markers was developed on this intraspecific map, mainly on the D genome. For polyploid species, isolation of microsatellites from an ancestral diploid donor seems to be an efficient way of developing markers for the corresponding genome in the polyploid plant.

Keywords Microsatellites · Wheat · Transferability · Polymorphism · *Aegilops tauschii*

Introduction

A saturated map is required for mapping many agronomically important genes and QTLs (quantitative trait loci). Compared to other grass species, genetic mapping in bread wheat (*Triticum aestivum* L.) is more difficult because of its low level of polymorphism, the large size of its genome (16×10^9 bp) and its polyploid structure. Wheat is an allohexaploid species made up of three diploid genomes, A, B and D. It results from two successive intercrossings, which involved at least three different species (McFadden and Sears 1946; Kihara 1950). The D genome was introduced during the last intercrossing between the amphiploid AABB and *Aegilops tauschii* (DD), involving probably only a limited number of genotypes from both species (Breiman and Graur 1995). Genetic mapping in bread wheat started with RFLP markers (Chao et al. 1989; Liu and Tsunewaki 1991; Gale et al. 1995; Cadalen et al. 1997; Gupta et al. 1999), but these show a rather low level of polymorphism. By the end of the 1980s, a new class of molecular markers (microsatellites) was developed (Litt and Lutty 1989; Weber and May 1989; Beckmann and Soller 1990). The first mapping studies using microsatellites were carried out on animal species (cattle, Bishop et al. 1994; human, Dib et al. 1996) and more recently on plants (rice, Wu and Tanksley 1993; barley, Saghai-Maroof et al. 1994; wheat, Röder et al. 1998). Microsatellites are sequences consisting of tandem repeats each with 1 to 6 nucleotides in length. They can be amplified using PCR with specific primer pairs designed from the flanking regions of the repeat motif. Microsatellites appear to be very polymorphic in length, due mainly to polymerase slippage during replication (Levinson and Gutman 1987). Weber (1990) divided microsatellites into three categories: perfect repeats, imperfect repeats with one or more interruptions, and compound repeats with adjacent tandem simple repeats of different motifs. Microsatellites combine a number of advantages. They are codominant and show Mendelian inheritance (Lagercrantz et al. 1993; Morgante and Olivieri 1993). Their generally high level of polymorphism makes them suitable for the genetic mapping of species which show little intraspecific polymorphism, such as most inbreeding species. In addition, microsatellites have good potential for automation and require only a small amount of DNA.

Communicated by B. Friebe

H. Guyomarc'h · P. Sourdille · G. Charmet · M. Bernard (✉)
UMR Amélioration et Santé des plantes, INRA-UBP,
63039 Clermont-Ferrand, Cedex 2, France
e-mail: michel.bernard@clermont.inra.fr
Fax: +33-4-73-62-44-53

K. J. Edwards
IACR-Long Ashton Research Station, University of Bristol,
Bristol, BS41 9AF, UK

In wheat, the D genome is known to be less polymorphic than the A and B genomes (Nelson et al. 1995a, b, c; Cadalen et al. 1997). Thus, genetic maps of the D genome have poor coverage despite the fact that microsatellites have been shown to be twice as polymorphic as RFLP markers (Röder et al. 1995). Only 25% of the wheat microsatellite loci developed by Röder et al. (1995) mapped to the D genome while 30% and 45% were mapped to the A and B genomes, respectively. Previous results obtained in our laboratory (Tavaud 1998) showed that microsatellites, which mapped onto the D genome of bread wheat, also amplified the D genome sequences in the ancestral diploid donor (*Ae. tauschii)*. To enhance the probability of developing microsatellites which map onto the D genome, we explore the possibility of using a library enriched for various microsatellite motifs, constructed from *Ae. tauschii* genomic DNA.

Materials and methods

Plant material

For the evaluation of Nei's diversity index, also referred to as polymorphism information content (PIC), seven wheat lines were used: W-7984 [synthetic wheat (Van Deynze et al. 1995)], and cultivars Opata, Courtot, Chinese Spring, Renan, Arche, Récital. *Ae. tauschii* accession # 15 (INRA Le Rheu, France) was used as an amplification control.

A subset of 94 androgenetic derived doubled-haploid lines issued from the cross between Courtot and Chinese Spring (for a complete description see Cadalen et al. 1997) was used for segregation analysis. DNA extraction was performed from fresh leaves by a modified CTAB protocol (Murigneux et al. 1993).

Isolation of microsatellite markers

A microsatellite-enriched library was constructed as described in Edwards et al. (1996) using total DNA from *Ae. tauschii* no 15. The enrichment procedure involved the following microsatellite motifs: $(GA)_{15}$, $(GT)_{15}$, $(AT)_{15}$, $(GC)_{15}$, $(CAA)_{10}$, $(ATT)_{10}$, $(GCC)_{10}$, $(CATA)_{10}$, $(GATA)_{10}$ and $(ATAG)_{10}$.

Plasmids containing the enriched fragments were introduced into *Escherichia coli* DH5α competent cells, which were then plated onto L-agar plates containing 100 μg/mL of ampicillin, X-Gal and IPTG (800 ng and 750 ng per Petridish). Colourless colonies were grown overnight in LB containing 100 μg/ml of ampicillin and used to prepare glycerol stocks.

A total of 2,500 clones were sequenced on a 377 ABI Prism sequencer (Perkin Elmer) at the Centre National de Sequençage (Evry, France). Primers flanking the microsatellites were designed with the Primer3 program release 0.6 with an optimal annealing temperature of 60 °C and an optimal length of 20 bases for the amplification of products between 150 and 300 bp in size. Following sequencing, the microsatellites were divided into useful and non-useful. A useful microsatellite was defined as a stretch containing a minimum of three repeats of a motif comprising from one to six nucleotides, with a total length of at least 12 nucleotides, and matching at a minimum level of 85% with a perfect microsatellite.

Screening with different microsatellite motifs

Minipreparation of plasmidic DNAs were prepared from glycerol stocks as described in Zhou (1990). PCR amplification with M13 primers were performed and products were loaded on a 1% agarose gel and separated at 100 V. DNAs were blotted onto nylon membranes (Hybond TM, Amersham Pharmacia Biotech) according to the standard procedure (Sambrook et al. 1989). Hybridization was performed using TMAC1 buffer as described in Depeiges et al. (1995). Because of the addition of TMAC1 (tetramethyl acetate chloride, Sigma) in the hybridisation buffer, annealing temperature has been calculated using probe length only, without giving attention to the G-C content and for all probes annealing steps were performed at 65 °C. Probes consisted of each microsatellite oligonucleotide previously used for the enrichment procedure but 3′ end labelled with α P^{32} (3′ end labelling kit, Amersham Pharmacia Biotech).

PCR amplification and product analysis

PCR reactions were performed as described by Tixier et al. (1998) with an annealing temperature of 60 °C. Microsatellite polymorphisms were visualised by the silver staining method (Tixier et al. 1997). Enzymes and buffers were provided by Roche Diagnostics. Primer pairs were synthesised by Genosys (Cambridge, UK).

Estimation of polymorphism information content

The polymorphism information content, described by Botstein et al. (1980) and modified by Anderson et al. (1993), refers to the ability of a given marker to detect polymorphism within a population, depending on the number of detectable alleles and their frequency. In this study we used a simplified version according to Lagercrantz et al. (1993), which assumes that the wheat lines are homozygous, namely:

$$PIC_i = 1 - \sum_j p_{ij}^2$$

where p_{ij} is the frequency of the jth pattern for marker i and the summation extends over *n* patterns.

Mapping

The polymorphic microsatellites were genotyped with the 94 DH lines and the data integrated into an existing framework map (consisting of both RFLPs and microsatellites; Cadalen et al. 1997). Map construction was carried out using Mapmaker 3.0 b (Lander et al. 1987) employing a threshold of LOD 3 and 50 cM for the ordering. Genetic distances were calculated using the Kosambi function (Kosambi 1944).

Chromosome-arm assignments were done when there was a doubt about the mapping results. In this case, PCR amplifications were performed on nullisomic-tetrasomic stocks of wheat (Sears 1964): the microsatellite loci were then assigned to the chromosomes.

Statistical methods

Chi-square tests for the detection of biased markers were performed with Excel 97.

Deviance analyses of GLM (generalised linear model) using the Poisson law family were performed to test the effect of both factors, motif length and the number of tandem repeats for polymorphism. The deviance is a goodness-of-fit statistic based on the log likelihood function (SAS User's Guide) adapted for a comparison of proportions. The generalised linear model procedure assumed that the different alleles appeared randomly and independently, and is more appropriate than the classical linear model based on Gaussian law. Calculations were carried out using the Splus 3.4 statistical package.

Results

Characterisation

In a first step, colony hybridisation was used to identify clones containing microsatellite motifs. Following colony hybridisation, 321 (65%) of the 490 clones tested contained at least one of the microsatellite motifs used in the enrichment procedure. Of these, 25% contained $(GA)_n$ repeats, 25% $(GT)_n$ repeats and 24% $(GCC)_n$ repeats (compound microsatellites may have been counted several times). A set of 1,000 randomly chosen clones (i.e. not subjected to screening), were sequenced. Of these, 75% of clones were shown to contain at least one microsatellite motif. The extent of redundancy within these clones was tested using a cut-off level of 75% homology over 50 nucleotides. This produced a total of 566 different families, which resulted in 378 primers pairs. Following further characterisation 293 primer pairs were used for polymorphic tests. Among the 293 clones used, four types of microsatellites were detected according to Weber (1990): simple and perfect (48%) (type I), simple and imperfect (30%) (type II), compound and perfect (10%) (type III), compound and imperfect (12%) (type IV). Within type I+II sequences, the isolated microsatellites could be grouped in six categories: mono-, di-, tri-, tetra-, penta- and hexa-nucleotides. Di- and tri- nucleotides together represented 70% of the isolated microsatellites (Table 1). Within simple microsatellites (type I+II), $(GCC)_n$ was the most abundant (28%), followed by $(GT)_n$ (22%) and $(GA)_n$ (21%). However, surprisingly, other motifs than those used in the enrichment were also present in the library (Table 2).

Microsatellite transferability from *Ae. tauschii* to bread wheat

Among the 293 primer pairs tested, 270 gave amplification products on bread wheat. From them, 142 (45%) of the primer pairs did not detect any polymorphism between the genotypes used. Among the remaining 138 primer pairs, 84 were polymorphic between Courtot and Chinese Spring and 130 between W-7984 and Opata (parents of the ITMI population). Seventeen failed to amplify products in all genotypes including the control (*Ae. tauschii*). Six primer pairs amplified only the *tauschii* control and W-7984 samples.

Table 1 Occurence and polymorphism of simple microsatellites by repeat-unit type

Type	Occurence	Polymorphic
Mono-	3	0
Di-	109	86
Tri-	96	23
Tetra-	17	5
Penta-	6	2
Hexa-	2	1
Total	233	117

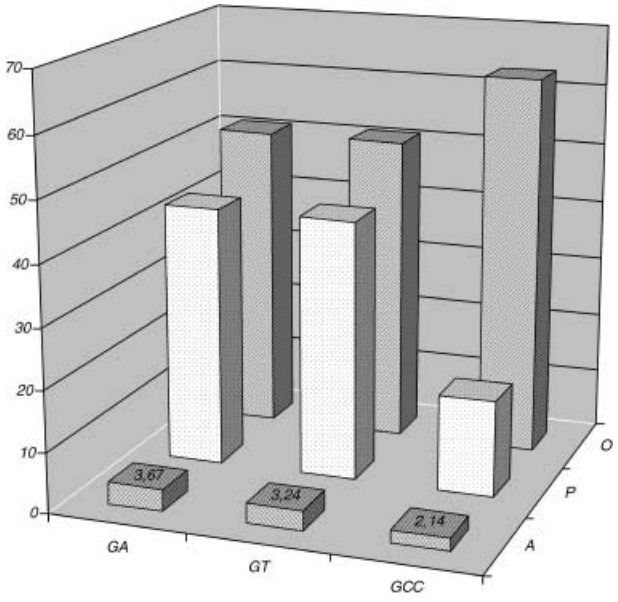

Fig. 1 Wheat D-genome molecular linkage map of Courtot × Chinese Spring population. Kosambi distances are expressed in centimorgans and are written on the left of chromosomes for anchor markers. Distances written on the right of chromosomes are distances between the markers and the anchor marker above. Double bars mean a lack of linkage between two linkage groups with threesholds of LOD3 and 50cM using Mapmaker

Influence of the type of motif on polymorphism detection of simple microsatellites (type I)

The number of alleles per locus ranged between one and six in the seven wheat cultivars used. PIC values ranged between 0.245 and 0.816 for the polymorphic loci. As shown in Table 1, dinucleotide motifs were more polymorphic (79% rate of polymorphism) than the other motif types. Deviance analysis in the generalised linear model showed that the dinucleotides $(GA)_n$ and $(GT)_n$ had a significantly higher number of alleles than the trinucleotides. The $(GA)_n$ and $(GT)_n$ average numbers of alleles (3.67 and 3.24 respectively) do not differ statistically (at $\alpha = 0.05$) and their distribution fitted a Poisson law. Among all tri-nucleotide motifs, 25% were polymorphic. $(GCC)_n$ microsatellites represented 74% of the trinucleotide motifs and showed only 23% polymorphism. For the $(GA)_n$, $(GT)_n$ and $(GCC)_n$ microsatellite motifs polymorphism is summarised in Fig. 1, and the average repeat number and standard deviation in Table 3. If the proportion of polymorphic markers was different between classes, the PIC values were close to each other for the polymorphic markers (Table 4).

Twenty nine percent of the tetra-nucleotide motifs were polymorphic. Penta- and hexa-nucleotides were too scarcely represented to make any conclusions concerning the level of polymorphism they revealed.

Table 2 Description of microsatellite loci. Length/*Ae.t* : length of amplification product on *Ae.tauschii*, imp : imperfect, NA: non-assigned

Primers	Length/*Ae.t*	motifs	Loci (length/Courtot, Chinese Spring)
Xcfd1	222	(GCC)6	6A(222,225)
Xcfd2	288	(CA)2AA(CA)18	5B.1(400,0) 2A(307,305) 4A(292,0) 5B.2(242,0)
Xcfd3	163	(CA)16	5D(173,171)
Xcfd4	262	(TC)37	3B(234,232)
Xcfd5	195	(GT)17	6D(205,179)
Xcfd6	236	(TAGAGA)2(N)103(GCTA)4imp	7A(150,0)
Xcfd7	250	(TC)27	5D(230,234)
Xcfd8	162	(CT)19GCGTTGG(TCCT)4imp	5D(158-156)
Xcfd9	209	(TC)29	3D(205,256)
Xcfd10	288	(CA)16	5D(206,288)
Xcfd11	167	(CA)3AA(CA)27	2D(230,360) 2B(130,134)
Xcfd12	192	(CTT)CT(CTT)4	5D(190,192)
Xcfd13	254	(CT)20CCAGTGCAACT(TGTA)3imp	6B(237–245)
Xcfd14	152	(TC)28	7D(122-120)
Xcfd15	179	(CT)20	1A(175,0)
Xcfd16	241	(GGC)5	4A(238,241)
Xcfd17	205	(CA)18	2D(210-204)
Xcfd18	169	(GA)24CG(AG)2	5D(198,194)
Xcfd19	267	(GA)18	6D(308,310) 5B(304,302) 1D(292,272)
Xcfd20	296	(GGAA)3imp(TGG)2(CTAC)3imp	1B(288,296)
Xcfd21	281	(GA)21	7D(282,280) 1D(268,272)
Xcfd22	254	(GA)21TA(GA)6	4B(216,218)
Xcfd23	187	(AGT)21impCAGTAG(CAG)3(TAG)8	4D(200,202)
Xcfd24	273	(T)4AC(T)10	4A(0,277)
Xcfd25	203	(GA)35	2B(173,175)
Xcfd26	271	(GAGAA)2(GA)37	5D(269,257)
Xcfd27	161	(CA)20	1D(155,153)
Xcfd28	190	(CAA)22	1D(205,214)
Xcfd29	153	(TC)18	5D(169,181)
Xcfd30	236	(GA)33	6A(230,234)
Xcfd31	276	(GA)51	7D(252,246)
Xcfd32	176	(CA)18	1D(176–178)
Xcfd33	170	(CA)13CG(CA)2CG(CA)7CG(CA)8	6D(170,173)
Xcfd34	203	(GGA)5imp(N)16(G)13	3D(193,0)
Xcfd35	191	(TC)20	3D(189,197)
Xcfd36	192	(CA)25(CCCG)3	2D(200,186)
Xcfd37	218	(CT)22	6D(206,210)
Xcfd38	215	(GA)32	6D(223,225)
Xcfd39	175	(GA)26	4B(200,206)
Xcfd40	223	(TAA)5imp	5D(193,208)
Xcfd41	286	(CA)17	7D(280,284)
Xcfd42	177	(GA)24	6D(224,202)
Xcfd43	150	(CA)20	2D(208,174)
Xcfd44	238	(CT)24	2D(217,0)
Xcfd45	187	(GCC)7	6D(189,187)
Xcfd46	175	(GT)29	7D(177,187)
Xcfd47	184	(GT)25	6D(186,192)
Xcfd48	250	(TC)2CC(TC)19	1B(242,240)
Xcfd49	214	(GA)5AA(GA)27	6D(224,218)
Xcfd50	261	(CA)16	2D(239,245)
Xcfd51	172	(GA)45	2D(233,247)
Xcfd52	275	(GT)2GA(GT)3(N)63(CTACTT)2imp	5D(279,281)
Xcfd53	233	(CT)22(CA)10	2D(275,273)
Xcfd54	200	(GA)40	NA(176,168) 4B(150-0)
Xcfd55	263	(GT)23	3D(271,0)
Xcfd56	221	(GA)31	2D(246,238)
Xcfd57	291	(GA)29	5D(271,275)
Xcfd58	179	(CT)5(CA)10	1D(179,185)
Xcfd59	296	(GC)6(GT)2GC(GA)23	1D(296,300) 1B(292,0) NA(282,0) NA(274,0)
Xcfd60	218	(CA)5CG(CA)11	6D(212,206)
Xcfd61	218	(CACAA)2N49(AC)22	1D(232,0)
Xcfd62	220	(CT)27	2D(210,208) 7A(0,190)
Xcfd63	275	(CA)26	1D(293,283)
Xcfd64	263	(CT)9(CA)22	3D(239,241)
Xcfd65	199	(CT)3CG(CT)28	1D(201,185) 1B(149,143)
Xcfd66	202	(GC)9(AG)9+(AG)51	7D(174,156)
Xcfd67	188	(CA)6N21(CA)5CG(CA)15	5D(200,204)

Table 2 (continued)

Primers	Length/*Ae.t*	motifs	Loci (length/Courtot, Chinese Spring)
Xcfd68	204	(GT)14	7D(210–212)
Xcfd69	240	(AC)5GC(AC)41	7D(216,218)
Xcfd70	172	(CA)19	3D(182,178)
Xcfd71	216	(CA)10(GA)30	4D(206,216) 4A(162,166)
Xcfd72	223	(GA)25	1D(235,231)
Xcfd73	242	(CT)19	2B(248,246)
Xcfd74	338	(CATG)3impN70(CA)15imp	7B(257,255)
Xcfd75	297	(TGGA3impN110(AG)22	6D(315,305)
Xcfd76	166	(GA)23GG(GA)2	6D(154,166)
Xcfd77	209	(GGC)6	2D-1(212,209) 2D-2(209,206)
Xcfd78	182	(GA)19	5D(168,170)
Xcfd79	284	(GA)26	3D(292,278) 3B(272,268)
Xcfd80	179	(GA)27	6D(171,188)
Xcfd81	283	(GT)14(GA)19	5D(275,273)
Xcfd82	242	(TGC)5	6A(227,0)
Xcfd83	233	(TG)14	1D(239,241)
Xcfd84	193	(GA)26	4D(185,187)

Table 3 The average repeat number and standard deviation for $(GCC)_n$, $(GA)_n$ and $(GT)_n$

Motifs	Mean repeat number	Standard deviation
GCC	6.07	1.03
GA	27.7	7.87
GT	22	6.58

Table 4 Polymorphism of microsatellite categories

Type of microsatellite	Number	Polymorphic	Average PIC for polymorphic loci
Simple and perfect	145	83 (57%)	52
Simple and imperfect	85	35 (41%)	53
Compound and perfect	13	8 (61%)	51
Compound and imperfect	44	30 (68%)	53

Influence of stretch length on polymorphism

The influence of the stretch length (i.e. the number of nucleotides forming the microsatellite = nucleotide number of a repeat multiplied by the number of repeats) on polymorphism was tested. When stretch length was introduced as the unique factor of variation, it was highly significant; however, when tested within each motif class, its effect was found not to be significant. This can be explained by the fact that the most-polymorphic microsatellites are the dinucleotidic ones, which also have the greatest average length.

Influence of mutations inside the stretch on polymorphism

When we compared the polymorphism revealed by compound microsatellites versus simple microsatellites, no significant difference was detected either on the polymorphism ratio or on the PIC mean.

Mapping

The 84 microsatellites (Table 2) polymorphic between Courtot and Chinese Spring generated 100 loci. A distorted segregation ratio was observed ($P < 0.01$) for 15 markers. These markers were assigned to regions known for presenting distorted segregation; for instance, on chromosomes 4A and 6B (Cadalen et al. 1997). Among the remainder, 64 mapped to the D genome, 7 to the A genome and 11 to the B genome; three were not assigned. Markers which mapped to the D genome were evenly distributed all over the genome (Fig. 2 with the exception of chromosome 4D, the coverage of which was very poor with only two markers. For primer pairs which produced only one band, polymorphic microsatellites mapped systematically to the D-genome of bread wheat. In the case of multiband patterns, polymorphic bands did not systematically correspond to loci located in the D genome of bread wheat; however, we verified that at least one band was located on this genome. When bands were monomorphic between Courtot and Chinese Spring, we assigned their location using nullitetrasomic lines of Chinese Spring.

Discussion

In order to improve the genetic map of the D genome of bread wheat, microsatellites were isolated from an enriched library constructed using total DNA of *Ae. tauschii*. Seventy five percent of clones from this library contained at least one microsatellite. However, a high proportion of the microsatellites developed (45%) failed to show any polymorphism in the set of lines used. It is possible that our microsatellite definition was too wide and included stretches which are too short or too imperfect to detect polymorphism. Another possibility is that the genetic polymorphism on the D genome is definitely low.

The enrichment procedure was not efficient for all motifs and non-selected motifs were present. Twenty two

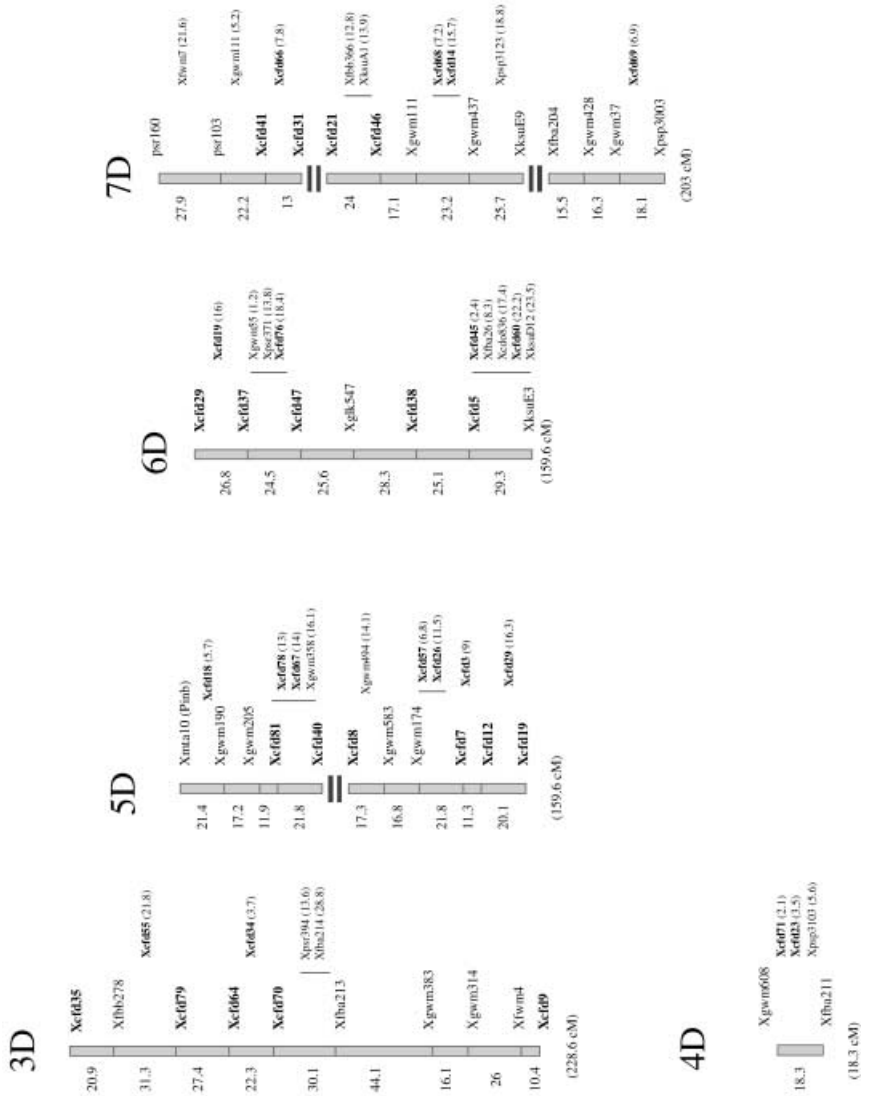

Fig. 2. Polymorphism of GA, GT, GCC microsatellites O: occurrence P: proportion of polymorphic microsatellites A: average mean number for polymorphic microsatellites

percent of the clones contained compound microsatellites. This type of repeat sequence could have been strongly selected by the enrichment procedure, which simultaneously enriched for ten microsatellite motifs. In this library, we found as many $(GT)_n$ as $(GA)_n$ microsatellites (about 28% each). Röder et al. (1995) estimated that there were 1.5-times more $(GA)_n$ repeats than $(GT)_n$ repeats per haploid wheat genome; as they developed microsatellites from an un-enriched genomic library, their results could be more-representative of biological reality.

Our library appeared to be particularly enriched for microsatellite motifs having a high GC content : $(GCC)_n$, $(GA)_n$ and $(GT)_n$. This could be due to the stringency of the washing conditions employed during the enrichment procedure (see Materials and Methods). No enrichment was observed for palindromic microsatellites like $(GC)_n$ or $(AT)_n$, although $(AT)_n$ microsatellites are believed to be very abundant in plants (Lagercrantz et al. 1993, Morgante and Olivieri 1993; Wang et al. 1994), presumably due to self complementation, which prevents hybridisation to the membranes.

Transportability of microsatellite markers from *Ae. tauschii* to bread wheat was very good (92%). All primer pairs isolated from *Ae. tauschii* amplified at least one product, which can be located to the D genome of bread wheat. This good transferability, recently confirmed by Pestsova et al. (2000), strongly suggests that the wheat and *Ae. tauschii* genomes are closely related. The apparent random distribution of the mapped loci indicates that microsatellite markers could provide excellent map coverage, except for chromosome 4D. Interestingly, in the case of multiband patterns on bread wheat, products were rarely mapped at homoeologous positions. This suggests the existence of numerous inter- and intra-chromosomal duplications within the wheat genome. We noticed that, in some cases, the *Ae. tauschii* amplification product was either far smaller or larger than any allele of the hexaploid lines. These differences suggest that the allele diversity of *Ae tauschii* might be higher than that observed in wheat.

Only 17 of the 293 primer pairs tested (6%) failed to amplify all genotypes including the control. The lack of amplification could be due to either sequence errors or to problems of primer synthesis. Six other primer pairs amplified only *Ae. tauschii* and W-7984 (synthetic wheat), whose D-genome comes from a *Ae. tauschii* accession, and then appears as '*tauschii*' specific. Three primers were defined in the very close vicinity of the microsatellite sequence. It is known that the proximal regions of microsatellite sequences are highly variable (Karhu et al. 2000), so they might be too different between *Ae. tauschii* and *T. aestivum* to allow common amplification. The three remaining primer pairs contained very short microsatellites (proto-microsatellites) which could confer particular instability in the sequence. Feldman et al. (1997) recently reported that the formation of allopolyploid wheat was accompanied by the rapid non-random elimination of specific low-copy, non-coding DNA sequences. It can be hypothesised that these microsatellite loci were in the vicinity of such low-copy DNA sequences, which could have been rearranged or deleted, thus preventing their amplification by PCR.

Dinucleotides appeared to be more polymorphic than other types of motifs as shown in humans by Chakraborty et al. (1997) or in *Drosophila* by Schug et al. (1998). Microsatellites mapped on the Courtot × Chinese Spring population were mostly either $(GA)_n$ or $(GT)_n$ repeats, or compound microsatellites containing $(GA)_n$ or $(GT)_n$. Average PIC levels of the dinucleotide and trinucleotide motifs were in agreement with those described in Bryan et al. (1997). The proportion of polymorphic markers for $(GA)_n$ and $(GT)_n$ microsatellites was similar, as was the average allele numbers.

Regarding stretch length, some authors found an influence (Weber 1990; Innan et al. 1997) and others did not (Plaschke et al. 1995; Chin et al. 1996). We failed to observe any significant influence of the stretch length on the polymorphism within each class of motifs. However, dinucleotides appeared to be far more polymorphic than other classes of repeats, as well as being longer than the other microsatellite motifs (Table 3). Accordingly, if we consider all microsatellites together, we could say that polymorphic levels are influenced by stretch length.

In simple microsatellites, the presence of interruptions seems to reduce the polymorphism (Table 4) as described by Bell and Ecker (1994) and Taylor et al. (1999). However, we noticed that interrupted compound microsatellites seemed to be more polymorphic than non-interrupted microsatellites. Our classification into the four categories was based on *Ae. tauschii* sequences, and interruptions in microsatellites can change according to taxonomic groups, as already shown by Karhu et al. (2000). Therefore, we are sequencing alleles from hexaploid wheat in order to compare them to *Ae. tauschii* sequences (data not shown).

For polyploid species like wheat, the isolation of microsatellites from a diploid ancestral species coud appear as a powerful method to develop such markers for the corresponding genome at the polyploid level. Further experiments to isolate microsatellites from the ancestral diploid donors of the A and B genomes (*Triticum urartu* and *Aegilops speltoides*) do not completely confirm this result (data not published), underlying the importance of genetic proximity to obtain good portability. Analysis on the specificity and the transferability of these markers on a range of diploid species and polyploid wheats are under investigation and should permit a clearer view on the evolution of microsatellites under polyploidisation events.

Acknowledgements We are grateful to Dr. Philippe Leroy for establishing the collaboration with Dr. Keith J. Edwards and in obtaining the library used in this paper. We thank Sylvie Bernard and André Madéore for providing the double-haploid lines of Courtot × Chinese Spring population. We thank Cécile Baron for genotyping population progenies. This work was supported by a grant from the Institut National de Recherche Agronomique and the Région Auvergne. IACR - Long Ashton receives grant-aided support from the Biotechnology and Biological Sciences Research

Council of the United Kingdom. All the experiments performed complied with the current french law. Microsatellite markers are available as primers pairs and sequences for academic research (Patent 00403659.6). Requests should be addressed to M. Bernard.

References

Anderson JA, Churchill GA, Sutrique JE, Tanksley SD, Sorrels ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36:181–186

Beckmann JS, Soller M (1990) Toward a unified approach to genetic mapping of eukaryotes base on sequence tagged microsatellite sites. Biotechnology 8:930–932

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics 19:137–144

Bishop MD, Kappes SM, Kecle JN, Stone RT, Sanden SLF, Hawkins GA, Toldo SS, Fries K, Gusz MD, Yov J, Beattie CW (1994) A genetic linkage map for cattle. Genetics 136: 619–639

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 62:1408–1415

Breiman A, Graur D (1995) Wheat evolution. Israel J Plant Sci 43:85–98

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An intervarietal molecular marker map in *Triticum aestivum* L. Em. Thell. and comparison with a map from wide cross. Theor Appl Genet 94:367–377

Chakraborty R, Kimmel M, Stivers DN, Davidson LJ, Deka R (1997) Relative mutation rates at di-, tri-, and tetra-nucleotide microsatellite loci. Proc Natl Acad Sci USA 94:1041–1046

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-base genetics maps of wheat homeologous group-7 chromosomes. Theor Appl Genet 78:495–504

Chin ECL, Senior ML, Shu H, Smith JSC (1996) Maize simple repetitive DNA sequences: abundance and allele variation. Genome 39:866–873

Depeiges A, Goubely C, Lenoir A, Cocherel S, Picard G, Raynal M, Grellet F, Delseny M (1995) Identification of the most represented repeated motifs in *Arabidopsis thaliana* microsatellite loci. Theor Appl Genet 91:160–168

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morisette J, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequences in plants. Biotechniques 20:758–760

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

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jiu J, Li QY, Devos KM (1995) Genetic map of hexaploid wheat. In: Liu ZS, Xin XY (eds) Proc 8th Int Wheat Genet Symposium. Beijing, China, pp 29–40

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Innan H, Terauchi R, Miyashita NT (1997) Microsatellite polymorphism in natural populations of the wild plant Arabidopsis thaliana. Genetics 146:1441–1452

Karhu A, Dieterich JH, Savolainen O (2000) Rapid expansion of microsatellite sequences in pines. Mol Biol Evol 17:259–265

Kihara H (1950) Genome analysis in *Triticum* and *Aegilops*. Concl Rev Cyt 16:101–123

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lagercrantz U, Ellegren H, Anderson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21:1111–1115

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Levinson G, Gutman GA (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol 4: 203–221

Litt M, Lutty JA (1989) An hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Liu Y, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free threshing hexaploid relatives. J Hered 37:81–89, 107–116

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182

Murigneux A, Barloy D, Leroy P, Beckert M (1993) Molecular and morphological evaluation of doubled-haploid lines in maize. 1. Homogeneity within DH lines. Theor Appl Genet 86:837–842

Nelson JC, Sorrels ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Farris JD, Anderson JA (1995a) Molecular mapping of wheat: major genes and rearrangements in homoelogous groups 4, 5, and 7. Genetics 141:721–731

Nelson JC, Van Deynze AE, Autrique E, Sorrels ME, Lu YH, Merlino M, Atkinson MD, Leroy P (1995b) Molecular mapping of wheat. Homoelogous group 2. Genome 38:516–524

Nelson JC, Van Deynze AE, Autrique E, Sorrels ME, Lu YH, Negre S, Bernard M, Leroy P (1995c) Molecular mapping of wheat. Homoelogous group 3. Genome 38:525–533

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. Proc Natl Acad Sci USA 91:5466–5470

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbour, New York

Sears ER (1964) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Chromosome manipulations and plant genetics. In: Riley R, Lewis KR (eds) Oliver and Boyd, Edinburgh, pp

Schug MD, Hutter CM, Wetterstrand KA, Gaudette MS, Mackay TFC, Aquadro CF (1998) The mutation rates of di-, tri- and tetra-nucleotide repeats in *Drosophila melanogaster*. Mol Biol Evol 15:1751–1760

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39:277–287

Tavaud (1998) Estimation de la diversité génétique entre *Triticum aestivum* et les espèces diplo ancêtres par des marqueurs de type microsatellite. Etude préliminaire de la portabilité de ces marqueurs vers d'autres espèces de Poacées. Mémoire de DEA

Taylor JS, Durkin JMH, Breden F (1999) The death of a microsatellite: a phylogenetic perspective on microsatellite interruptions. Mol Biol Evol 16:567–572

Tixier MH, Sourdille P, Röder M, Leroy P, Bernard M (1997) Detection of wheat microsatellites using a non-radioactive silver-nitrate staining method. J Genet Breed 51:175–177

Tixier MH, Sourdille P, Charmet G, Gay G, Jaby C, Cadalen T, Bernard S, Nicolas P, Bernard M (1998) Detection of QTLs for crossability in wheat using a doubled-haploid population. Theor Appl Genet 97:1076–1082

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group-1 chromosomes of Triticeae species and their relation to chromosomes in rice and oats. Genome 38:45–59

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1–6

Weber JL (1990) Informativeness of human (dC-dA)*n*.(dG.dT)*n* polymorphisms. Genomics 7:524–530

Weber JL, May PE (1989) Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241: 225–235

Zhou C (1990) Mini-prep in ten minutes. Biotechniques 8:172–173