Theor Appl Genet (1998) 96: 676–682

G.-L. Sun · B. Salomon · R. V. Bothmer

Characterization and analysis of microsatellite loci in *Elymus caninus* (*Triticeae: Poaceae*)

Received: 8 September 1997/Accepted: 6 October 1997

Abstract Microsatellites are highly variable DNA sequences that can be used as markers for the genetic analysis of plants. The potential of microsatellite markers for use in a genetic diversity study in *Elymus* species was evaluated. Genomic libraries of *Elymus caninus* were constructed. The libraries were screened with two dinucleotide, (GA)n and (GT)n, and two trinucleotide repeats, (TCT)n and (CAC)n. A total of 19 positive clones were found for the two dinucleotide repeats; no positive clone was found for the trinucleotide repeats. Positive clones were sequenced to confirm the presence of microsatellites and to generate polymerase chain reaction (PCR) primers based on the sequences flanking the microsatellite. All sequenced (GA)n clones have repeats of $n > 10$; over half of the (GT)n microsatellites have $n < 10$ repeats. Primer pairs were designed and evaluated for 8 selected microsatellites. PCR products were amplified from 15 *Elymus caninus* accessions. The number of alleles found for the eight loci varied from 1 for *ECGA89* and *ECGT35* to 13 for *ECGA22*, as determined by non-denaturing polyacrylamide electrophoresis. Six microsatellite loci were found to be polymorphic in *E. caninus*. The eight primer pairs were tested on three other species; seven were successful in amplifying DNA from *Elymus alaskanus* and *E. mutabilis*, and four amplified DNA from *E. caucasicus*. Based on these results, microsatellites appear to be useful markers in detecting variation in *E. caninus*.

Key words *Elymus caninus* · Microsatellites · Simple sequence repeats (SSRs) · Polymorphism

Communicated by P. M. A. Tigerstedt

G.-L. Sun (✉) · B. Salomon · R. V. Bothmer
Department of Plant Breeding Research, The Swedish University of Agricultural Sciences, S-268 31, Svalöv, Sweden
Fax: + 46 418 667081
E-mail: genlou.sun@vf.slu.se.

Introduction

DNA molecular markers are used extensively to create genetic and physical genome maps, in taxonomy and population variation studies, as well as to identify individual genotypes. DNA sequences with short repeated motifs (less than 6 bp) are termed microsatellites (Litt and Luty 1989), simple sequence repeats (SSRs) (Jacob et al. 1991), or short tandem repeats (STRs) (Edwards et al. 1991). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeated units. Variations in repeat number accumulate in populations more rapidly than point mutations and the larger insertion/deletion events responsible for most restriction fragment length polymorphisms (RFLPs) (Brown et al. 1996). The DNA sequences flanking microsatellites are conserved, allowing the selection of polymerase chain reaction (PCR) primers that will amplify the microsatellite locus and enable the detection of different alleles of a locus.

Microsatellites offer an attractive combination of features that are useful as molecular markers (Brown et al. 1996) and are regarded as a general and novel source of genetic markers. Microsatellite analysis has been successfully used for linkage mapping in humans (Weissenbach et al. 1992), mouse (Cornall et al. 1991; Love et al. 1990), other mammalian genomes (e.g., Moore et al. 1991; Bishop et al. 1994; Rohrer et al. 1994), and mosquito (Zheng et al. 1993). In the past several years, surveys of DNA sequence databases have revealed an abundance of microsatellite loci in plants, and subsequent studies have demonstrated the informativeness of these markers in several genera (Condit and Hubbell 1991; Akkaya et al. 1992; Morgante and Olivieri 1993; Wu and Tanksley 1993; Thomas and Scott 1993; Kresovich et al. 1995; Röder et al. 1995; Brown et al. 1996; Katzir et al. 1996; Provan et al. 1996; Taramino and Tingey 1996).

Condit and Hubbell (1991) were the first to state that microsatellite analysis has great potential for the study of plant population genetics with respect to parentage, gene flow, and the extent and maintenance of genetic diversity in natural plant populations. The study reported here forms part of a larger investigation to map species and genetic diversity among the Eurasian *Elymus* species, with the aim of establishing the prerequisites for making efficient conservation strategies for the genetic diversity in *Elymus* and related genera (Salomon 1995; Salomon et al. 1996). The means to achieve this goal are by using a multi-methodological approach including studies of alpha-taxonomy, autecology, genetic variation within populations and between geographically widely separated populations, and the mode of reproduction of the different species. The genetic variation is estimated by screening for isoenzyme, prolamine, randomly amplified polymorphic DNA (RAPD), and microsatellite variation.

The taxon *Elymus caninus* was chosen as a model species during the development and modification of appropriate tools for the screening of genetic variability. This species has a wide distribution area ranging from Iceland and the British Isles in the west to southern Siberia in the east and from the subarctics in the north to the Mediterranean in the south. It shows considerable variation in morphology, isozyme, and prolamine variation patterns (Díaz et al. 1996, 1998; Kostina et al. 1998). This made *E. caninus* a suitable candidate for studying genetic variation, at the intrapopulational level as well as between geographically widely separated populations, by analysing the microsatellite variation.

The objectives of the present study were (1) to develop microsatellite markers for *Elymus caninus* from a genomic DNA library, (2) to determine the frequencies of (GA)n, (GT)n, (CAC)n, and (CTC)n repeats in the *E. caninus* genome, and (3) to discuss their potential for applications in population genetics.

Materials and methods

Plant materials and DNA isolation

Plant materials used for DNA extraction originated from the Triticeae collection in the Department of Plant Breeding Research, The Swedish University of Agricultural Sciences, Svalöv, Sweden. Fifteen accessions of *Elymus caninus* and 1 accession of *E. alaskanus*, *E. mutablis*, and *E. caucasicus*, respectively, were used for investigation of the polymorphism detected by microsatellites (Table 1). Total DNA was isolated from leaf tissue using a modified method of Junghans and Metzlaff (1990).

Construction of *Elymus caninus* genomic library

Two size-selected *E. caninus* genomic libraries were constructed in the vector LambdaZap Express™ (Stratagene). DNA isolated from *E. caninus* no. H7550 was digested with the restriction enzyme *Mbo*I. Digested DNA was electrophoresed through 2% agarose gel (Agarose DNA grade, AB Kemila-Preparat), and DNA products 200–500 bp (library A) and 500–900 bp (library B) in size were excised and purified from the agarose using a GenElute™ Minus EtBr Spin Columns (Supelco). The selected DNA fragments were ligated with ZAP Express™ DNA and packaged with the Gigapack III Gold packaging extract according to the protocol described by Stratagene. Libraries were checked on 5-bromo-4-chloro-3-indolyl-D-galactoside (X-gal) and isopropyl-D-thiogalactopyranoside (IPTG) after packaging to determine the proportion of phage carrying plant inserts, followed by transfection of XL1-blue MRF′ cells (Stratagene).

Library screening for clones containing microsatellites

Colony lifts were performed according to the procedure supplied with the nylon membrane (Amersham). Synthetic oligonucleotides of

Table 1 *Elymus* species used in the study with their genomic constitution, accession numbers, and origin

Species	Accessions	Genomes	Origin
E. caninus (L.) L.	H3169	SSHH	Sweden, Västmanland, Kungsör
E. caninus (L.) L.	H10338	SSHH	Finland, Sodankylä, 4 km N of Sodankylä
E. caninus (L.) L.	H10346	SSHH	Finland, Lapin Province, Savukoski, in Kuosku
E. caninus (L.) L.	H10353	SSHH	Norway, Nordland Province, Narvik
E. caninus (L.) L.	H10359	SSHH	Iceland, Eyjafjardarsysla, Akureyri
E. caninus (L.) L.	H3698	SSHH	Italy, PI 252044
E. caninus (L.) L.	H10404	SSHH	Russia, Siberia, Krasnojarskij kraij
E. caninus (L.) L.	H10096	SSHH	Russia, Altai, Korgonskij mts, Ku Mir river
E. caninus (L.) L.	H3339	SSHH	Kazakhstan, 30 km SW of Alma-Ata
E. caninus (L.) L.	H3915	SSHH	Slovakia, Sobotiste
E. caninus (L.) L.	H3916	SSHH	Czech Republic, Krkonose, Alberickelomy
E. caninus (L.) L.	H4111	SSHH	Pakistan, NWFP, Swat, Mahodan
E. caninus (L.) L.	H8723	SSHH	China, Xinjiang, Altai County, Hua Ling Park
E. caninus (L.) L.	H8745	SSHH	China, Xinjiang, Habahe County
E. caninus (L.) L.	H7550	SSHH	China, Xinjiang, Habahe, Teilike Town
E. alaskanus (Scrib. ex Merr.) Löve	H3669	SSHH	USA, Alaska
E. mutabilis (Drob.) Tzvel.	H10334	SSHH	Finland, Sodankylä
E. caucasicus (C. Koch) Tzvel.	H3207	SSYY	Armenia, Dilidjan

$(GA)_{15}$, $(GT)_{15}$, (CAC)10, and (TCT)10 from KEBOLab were used to detect microsatellites. Except for the plaque hybridization procedures, library screening followed the protocol provided by Stratagene. In the first cycle of screening, duplicate lifts were performed, and the two filters for each plate were hybridized with the same probe to ensure the correct counting and isolation of positive plaques. Prehybridization of the filters was conducted in a hybridization buffer [0.5 *M* naphosphate (pH 7.2), 2.5 m*M* EDTA (pH 8.0), and 7% sodium dodecyl sulphate (SDS)] for 3 h. The oligonucleotides were labeled with [^{32}P] dCTP by the random hexamer method (Feinberg and Vogelstein 1983). The labeled oligonucleotides were hybridized with filters overnight. The incubation temperature were 45°C for the first screening and 55°C for the second screening. Membranes were washed twice for 15 min each at the hybridization temperature in 6 × SSC (0.9 *M* NaCL and 90 m*M* trisodium citrate), 0.1% SDS, then rinsed once at room temperature. Filters were exposed to X-ray film for 1 day (− 80°C).

Sequencing

Positive plaques were chosen after the second cycle of screening. The phages were then excised in vivo and recovered in Solar cells using the Exassist/Solar system (Stratagene). Plasmid DNAs were extracted according to Sambrook et al. (1989). DNA sequencing of plasmid clones was performed using the dideoxy chain termination method with the Sequenase kit version 2.0 (Amersham) with M13 (− 20) and T3 primers. In most cases, complete sequences could be obtained in a single reaction because of the small size of the cloned fragments. In some cases, sequencing from both ends was required because of difficulties encountered in sequencing through the microsatellites.

PCR primer selection and synthesis

PCR primers for unique sequence flanking microsatellite loci were designed using the computer programme OLIGO (National Biosciences). These primers were synthesized commercially (KEBOLab).

PCR amplification of the microsatellite loci

1) The PCR mixture contained 0.2 m*M* of each deoxynucleotide, 1.5–2.0 m*M* $MgCl_2$, 1 U *Taq* polymerase, 10 pmol each primer, and 20 ng template DNA in a reaction volume of 20 ml. PCR was performed in a OmniGene Temperature Cycler (Hybaid) using one of the following two PCR conditions: (1) A PCR profile consisting of 1 cycle of 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 54°C for 1 min and 72°C for 2 min, and a final elongation step of 10 min at 72°C. (2) A "touchdown" PCR consisting of 15 cycles of 94°C for a 1-min denaturation and 72°C for a 1-min extension. Annealing (30 s) temperatures were progressively decreased by 2°C every third cycle from 64°C to 54°C. The PCR continued for 30 additional cycles at 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min. The reaction ended with a 10-min extension at 72°C. The PCR products were mixed with one-tenth volume loading buffer and separated in a vertical, non-denaturing 10% polyacrylamide gels in 1 × TBE at 100 V for 14–18 h. The gels were stained with ethidium bromide.

2) PCR reactions separated on polyacrylamide were modified to contain 20 ng template DNA, 1.5–2.0 m*M* $MgCl_2$, 0.1 U *Taq* polymerase, 10 pmol each primer, and 0.2 m*M* each of dCTP, dGTP, and dTTP, 0.02 m*M* dATP, 74 TBq [S^{35}] dATP in a reaction volume of 10 ml. PCR were carried out as described previously. PCR products (4 µl per lane) were denatured for 5 min at 94°C, separated on 6% denaturing polyacrylamide gels containing 8 *M* urea at 65 W for 2.5 h, and then exposed to X-ray film for 3 days.

Results

Frequency of microsatellites

The A and B genomic libraries of *E. caninus* yielded 35,000 and 23,000 plaques, respectively. The number of plaques that hybridized to each oligonucleotide probe is given in Table 2. Thirteen (0.037%) positive clones were identified in library A and 6 (0.026%) in library B. The frequency of (GT)n positives was four times higher in library B than in library A. Within library A, the frequency of (GA)n positive clones was a little higher than that for (GT)n. Within library B, the frequency of (GA)n positive clones was about 13 times lower than that for (GT)n. The two libraries were screened with two trinucleotide probes, (CAC)n and (TCT)n. No positive plaque was detected with either of these two probes.

Nylon membranes carrying phage were screened with the GT probe, then stripped and screened with the GA probe. About 20% of the signals obtained with the first probe showed the signals with the second probe.

Microsatellite sequences and primer selection

Sequence data were obtained for 8 of the (GA)n and 9 of the (GT) n repeats. The microsatellite sequences were classified into three types: perfect repeat sequences, imperfect repeat sequences, and compound repeat sequences. The compound repeat sequences were further divided into perfect and imperfect sequences (Table 3) according to Weber (1990). The maximum number of repeats found in the perfect category was (CT) 27 for (GA)n and (TG) 35 for (GT)n. All microsatellites that were isolated from the (GA)n positive clones exhibited $n > 10$ repeats (n equals the number of repeats), with the average number of repeats being 18.04. Over half of microsatellites isolated from the (GT)n showed $n < 10$ repeats, with the average number of repeats being 15.44.

The design of the primes flanking a microsatellite repeat allows this region to be specifically amplified by means of the PCR. Of the 17 sequenced clones, eight primer pairs were designed and synthesized. Seven primer pairs were designed for (GA) repeats, one for

Table 2 Number of plaques detected by hybridization with synthetic oligonucleotide probes

Probe	Library	Number of plaques	Positive plaques	Frequency (%)
(GA)15	A	20,000	8	0.040
	B	20,000	2	0.010
(GT)15	A	15,000	5	0.033
	B	3,000	4	0.133

Table 3 Summary of the sequenced dinucleotide microsatellites

Repeat motif	GA	GT
Number of sequenced clones	8	9
Perfect	5	3
Imperfect	0	4
Compound		
Perfect	2	1
Imperfect	1	1
Number of repeats (n)		
0–5	0	3
6–10	0	3
11–20	5	1
> 20	3	2
Mean no. of repeats	18.04	15.44
Flanking sequences too short or not suitable for designing PCR primers	1	8
Number of clones for which PCR primers were prepared	7	1
Number of successfully amplified PCR primer sets	7	1

(GT) repeats. Over half the sequenced clones were rejected (Table 3), with the most common faults being either that the microsatellite repeat was situated too close to the end of the clone for a primer to be designed or that the microsatellite repeat was considered too short to be informative (less than 10 repeat motifs).

Length polymorphism of microsatellite loci in *Elymus caninus* and other species

A total of 15 accessions of *E. caninus* were analysed using the eight primer pairs and PCR condition 1. PCR products were separated on 10% nondenaturing and 6% denaturing polyacrylamide gels. Locus *ECGA22* appeared to consist of 13 alleles on the basis of non-denaturing polyacrylamide electrophoresis. All loci displayed 1 or more alleles. The number of alleles found for the eight loci varied from 1 for *ECGA89* and *ECGT36* to 13 for *ECGA22* (Table 4). Of the eight microsatellite loci tested in the accessions of *E. caninus*, five (*ECGA22*, *ECGA114*, *ECGA125*, *ECGA126*, *ECGA210*) were polymorphic, and three (*ECGA11*, *ECGA89*, and *ECGT36*) were monomorphic. Figure 1 shows an example of the polymorphism found between

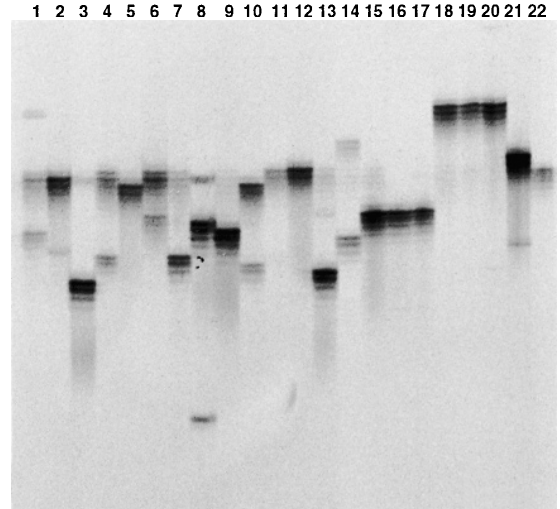

Fig. 1 Autoradiograph of a denaturing polyacrylamide gel with PCR-amplified alleles in 17 accessions of *E. caninus* using primer pair ECGA22. *Lanes* 1–14 1 individual from each accession, *lanes* 15–17 3 individuals of 1 accession, *lanes* 18–20 3 individuals of 1 accession *lanes* 21, 22 2 individuals of 1 accession

Table 4 *Elymus caninus* microsatellites

Locus	Repeats	Primers (5′ → 3′)	Number of alleles[a]	Tm (°C)	Expected[b] size (bp)[b]
*ECGA*22	(CT)27	GAAGGTGACTAGGTCCAAC ATAGTCTCGGTCAGGCTC	13	54	166
*ECGA*210	(GA)22	CGACAACTAGTGGATCAAA GAAGTACTCTCGAGAAGCTT	6	54	196
*ECGA*114	(TC)15	CTTATATCTTGTGGGTTATCAT GATCTGATACGTGACATCTCA	8	54	129
*ECGA*125	(AG)23	TGCTTCCAACTTGCTCA TGCATCTGTGTGTCCACA	10	54	204
*ECGA*11	(GT)11(GA)13(GGGA) 2	TGAACACTAAATCAACATTCA TACTTCTTGGTTCAGGTGAC	6	54	125
*ECGA*89	(GA)17	TTAGCTCTTTACTTATTCAAAC TCCTATGATCAAGCACAAG	1	54	232
*ECGA*126	(GA)15	GTCACTAGTGGATCGTGCC GATTTGGTGTCGTTCTGATC	9	54	186
*ECGT*36	(GT)18	TCACAGAGTGATTACATGCG ACATGTAACTGGAGTCGAGC	1	54	195

[a] Number of alleles was calculated on non-denaturing polyacrylamide gel electrophoresis
[b] The expected size range was obtained by counting the number of base pairs between the starting point of the primer pairs

accessions of *E. caninus* using the primer pair *ECGA22* and denaturing polyacrylamide gel electrophoresis.

All the primers were also tested using "touchdown" PCR on 6 accessions of *E. caninus*, in which the final annealing temperature was 54°C. One additional primer pair, *ECGA11*, also generated polymorphic PCR products under these conditions.

The microsatellite primers described above produced clear amplification products with DNA isolated from 3 other *Elymus* species. Seven of the primers (except for *ECGA89*) function in *E. alaskanus* and *E. mutabilis*, which both have the same genome constitution as *E. caninus*. In *E. caucasicus*, which has a different genome constitution, four primer pairs (*ECGA22*, *ECGA125*, *ECGA210*, and *ECGT35*) could amplify the DNA.

Discussions

Elymus caninus microsatellites were obtained by screening genomic DNA libraries (A and B) for clones containing (GA)n and (GT)n. A total of 35,000 plaques from library A and 23,000 plaques from library B were screened. Assuming an average insert size of 300 bp and 600 bp for library A and B, respectively, the libraries contained 10,500 and 13,800 kilobase pairs (kbp) of genomic DNA. The former thus gives an estimate of 1 microsatellites motif per 807 kbp, and the latter 1 per 2,300 kbp. These estimates are roughly similar to those obtained from maize (Taramino and Tingey 1996), but much lower than those reported in genomes of *Triticum aestivum* (Röder et al. 1995), *Brassica napus* L. (Kresovich et al. 1995), *Paspalum vaginatum* (Liu et al. 1995), and *Hordeum vulgare* L. (Liu et al. 1996). Previous studies on plant genome microsatellites showed that for all plants, (GA)n sites are more frequent than (GT)n sites (Lagercrantz et al. 1993; Thomas and Scott 1993; Röder et al. 1995; Liu et al. 1996). It is noteworthy that this conclusion resulted from the screening of the approximately 200- to 600-bp size library. In our study, the GA- and GT- microsatellites were estimated to be present every 750 kbp and 900 kbp of the DNA in library A (200–500 bp). These results are consistent with the conclusion that (GA)n repeats are more abundant than (GT)n repeats in plant genomes. However, in library B (500–900 bp), the GA- and GT- microsatellites were estimated to be present every 6,000 kbp and 450 kbp of DNA, respectively. These results seem to indicate a nonrandom distribution of the two classes of microsatellites, with (GT)n clustered to a greater degree in the *Elymus caninus* genome. A similar finding was observed in the wheat genome by Ma et al. (1996), who observed a non-random distribution of the (AC)n and (AG)n microsatellites, with the (AC)n repeat clustered to a greater degree in the wheat genome.

In addition to the (GA) and (GT) repeat sequences, we screened the library for (CAC) and (TCT) repeats. However, we failed to identify any positive plaques after the second cycle of hybridization of the library with these two probes, even though the experimental conditions were carefully controlled to ensure low-stringency hybridization and filter washes. It appeared that trimeric repeats occurred at much lower frequencies in our library than the dimeric repeats (GA)n and (GT)n. Our results correspond with the result reported by Liu et al. (1995), who also failed to identify trimeric repeats in the *Paspalum vaginatum* library. A relatively lower abundance of trimeric repeats in comparison to dimeric repeats has been reported previously (Morgante and Olivieri 1993; Wang et al. 1994).

The clustering of repeat regions is also suggested by the observation that 20% of the phage inserts that carried a GT site also carried a GA site. Clustering of two-base repeat regions was further implicated by the sequence data. Condit and Hubbell (1991) made similar observations about AC repeats in tropical tree genomes, noting that 10–20% of the phage inserts that carried a AC repeat also carried an GA repeat.

The majority of the repeat sequences were perfect for the (GA) repeat, about 90% (including the perfect repeats from compound repeats), which corresponds with the 81% perfect repeat sequences for GA repeats reported by Liu et al. (1995). In this study, all of the (GA) microsatellites isolated had more than 20 bp of the repeat sequences. The relatively simple (GA)n microsatellite structures resemble those reported for the *Arabidopsis* genome (Bell and Ecker 1994), *P. vaginatum* genome (Liu et al. 1995), and wheat genome (Ma et al. 1996). Results from a database search also indicated that the sequences of (GA)n microsatellites are simple (Morgante and Olivier 1993). Though GA-microsatellite markers are less abundant than AT-microsatellites in plants (Condit and Hubbell 1991; Lagercrantz et al. 1993; Morgante and Olivieri 1993; Wang et al. 1994), it is possible that the former may serve as the dinucleotide repeat candidates for use because of their relative ease of isolation and characterization when compared to AT-microsatellites.

DNA sequencing of clones containing microsatellites revealed the prevalence of imperfect repeats within the (GT)n class of dinucleotide repeats. More than 50% of the (GT)n clones that were sequenced fell into this category. Over half of the (GT)n repeats contained short repeats (less than 10 repeats), all of which were found to be imperfect. This finding concurs with the results in maize reported by Taramino and Tingey (1996).

The eight microsatellite loci were tested on *Elymus caninus*. The highest number of alleles was detected with the longest dinucleotide locus *ECGA22* [(CT) 27]. This corresponds with the results from Weber (1990) and Thomas and Scott (1993) who found that longer dinucleotide loci are genetically more variable and detect more alleles than those with a short repeat length. The higher level of polymorphism associated with

microsatellites was demonstrated in the present study in *E. caninus*. Among the eight microsatellites assayed for polymorphism, six microsatellite loci detected polymorphism in a sample of 15 accessions of *E. caninus*. The *ECGA22* locus exhibited a higher amount of polymorphism than the others.

Polymorphic microsatellite loci can be detected across species boundaries. The seven microsatellite primers yielded a product (or products) on the *E. alaskanus* and *E. mutabilis*, and four primers yielded a product (products) on *E. caucasicus*. This evidence indicates the existence of a greater divergence between *E. caninus* and *E. caucasicus*. From a perspective of the utility of microsatellite primers, our results are encouraging. Cross-species amplification has been found in mammals (Hearne et al. 1992) and plants (Lagercrantz et al. 1993; Thomas and Scott 1993). We also successfully amplified the DNA from 19 *Elymus* species using microsatellite primers developed for wheat, *Triticum aestivum* (Sun et al. 1997). Sharing of primer pairs between researchers working with plant families may be highly desirable and fruitful.

The current report demonstrates that microsatellite markers can be found in *E. caninus*. The GA-microsatellite structure is relatively simple, and relatively easy to isolate and characterize in the *E. caninus* genome. Microsatellite markers represent a DNA-marker type of which there is an extremely large number of potential markers available in the *E. caninus* genome, the individual markers being highly polymorphic and informative. Based on the results presented here, microsatellites appear to be promising markers in detecting variation in *E. caninus*.

Acknowledgements The authors are grateful to M. Lenman, M. Lee and C. Lanner for frequent assistance and helpful advice, and to M. Röder, at Institute fur Pflanzengenetik und Kulturpfanzenforschung, Germany, for kind advice and suggestions. The research was supported by Swedish Council for Forestry and Agricultural Research. Genlou Sun was supported by a postdoctoral fellowship from the Swedish Biodiversity Center.

References

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

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

Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo J, Beattie CW (1994) A genetic linkage map for cattle. Genetics 136:619–639

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez-Candelas F, Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench). Theor Appl Genet 93:190–198

Condit R, Hubbell SP (1991) Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome 34:66–71

Cornall RJ, Airman TJ, Hearne CM, Todd JA (1991) The generation of a library of PCR-analysed microsatellite variants for genetic mapping of the mouse genome. Genomics 10:874–881

Díaz O, Salomon B, Bothmer von R (1996) Genetic variation in Nordic *Elymus* species (Poaceae). In: Geirsson RT, Ingvarsson S, Jónsdóttir S (eds) Fifth Workshop Nordic Genome Initiative. University Hospital of Iceland, Reykjavik, p 56

Díaz O, Salomon B, Bothmer von R (1998) Isozyme characterization of genetic diversity in species of the genus *Elymus*. In: Jaradat AA (ed) Third Triticeae Symp. Aleppo: ICARDA, Aleppo

Edwards A, Civitello H, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Hearne CM, Ghosh S, Todd JA (1992) Microsatellites for linkage analysis of genetic traits. Trends Genet 8:288–294

Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES (1991) Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. Cell 67:213–224

Junghans H, Metzlaff M (1990) A simple and rapid method for the preparation of total plant DNA. Biotechniques 8:176

Katzir N, Danin-Poleg Y, Tzuri G, Karchi Z, Lavi U, Cregan PB (1996) Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor Appl Genet 93:1282–1290

Kostina EV, Agafonov AV, Salomon B (1998) Electrophoretic properties and variability of endosperm proteins in *Elymus caninus* (L.) L. In: Jaradat AA (ed) Third Triticeae Symp ICARDA, Aleppo (in press)

Kresovich S, Szewc-McFadden AK, Bliek SM, McFerson JR (1995) Abundance and characterization of simple sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (Rapessed). Theor Appl Genet 91:206–211

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

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

Liu Z-W, Jarret RL, Kresovich S, Duncan RR (1995) Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (*Paspalum vaginatum* Swartz). Theor Appl Genet 91:47–52

Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Love JM, Knight AM, McAleer MA, Todd JA (1990) Towards construction of a high resolution map of the mouse genome using PCR-analysed microsatellites. Nucleic Acids Res 18:4123–4130

Ma ZQ, Röder M, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123–130

Moore SS, Sargeant LL, King TJ, Mattick JS, Georges M, Hetzel DJS (1991) The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10:654–660

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

Provan J, Powell W, Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). Theor Appl Genet 92:1078–1084

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

Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW (1994) A microsatellite linkage map of the porcine genome. Genetics 136:231–245

Salomon B (1995) Biodiversity in arctic and alpine Eurasian *Elymus* L. (*Poaceae*). In: Borgen L et al. (eds) Variation Evolution Arctic Alpine Plants: IV Int Symp IOPB. University of Tromsø, Tromsø, p 23

Salomon B, Díaz O, Bothmer von R (1996) Species diversity and genetic diversity in Eurasian *Elymus* L. (Poaceae). In: Geirsson RT, Ingvarsson S, Jónsdóttir S (eds) Fifth Workshop Nordic Genome Initiative. University Hospital of Iceland, Reykjavik, p 64

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

Sun GL, Salomon B, Bothmer von R (1997) Analysis of tetraploid *Elymus* species using wheat microsatellite markers and RAPD markers. Genome 40 : 806–814

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

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor Appl Genet 86 : 985–990

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

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

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millacceau P, Vaysseix G, Lathrop M (1992) A second-generation linkage map of the human genome. Nature 359 : 794–801

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

Zheng L, Collins FK, Kumar V, Kafatos FC (1993) A detailed genetic map for the X chromosome of the malaria vector, *Anopheles gambiae*. Science 261 : 605–608