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Isolation of 11 new polymorphic microsatellites from CA enriched turkey genomic libraries (Short communication)

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Abstract

Microsatellite loci from the ancient Hungarian variety of the Broad Breasted Bronze Turkey (*Meleagris gallopavo)* were isolated. CA-repeat enriched libraries were constructed from DNA of randomly collected samples. Libraries were screened for repeat-containing clones by PIMA (PCR Isolation of Microsatellite Arrays) and the DNA-sequence of 167 positive clones was determined. A total of 136 microsatellite repeat-containing sequences were found, 59 sequences were unique. Comparing these with the genomic databases, we found 7 previously annotated microsatellite sequences. The newly isolated 52 microsatellites were tested on the mapping population of the University of Minnesota, and the map position of 11 microsatellites was determined.

Keywords: turkey (*Meleagris gallopavo*), microsatellite isolation, CA-enriched, genomic library, magnetic beads, PIMA

Zusammenfassung

Isolierung von 11 neuen polymorph dinukleotid Mikrosatelliten von CA angereicherten Truthahn Genombibliotheken (Kurzmitteilung)

Bei Tieren einer alten ungarischen Variante der breitbrüstigen Bronzepute wurden Mikrosatelliten isoliert. Aus der DNA zufällig erfasster Proben wurden wiederholt CA angereicherte Genombibliotheken hergestellt. Die durch Wiederholung enthaltenen Klone der Gesamtbibliotheken wurden mit Hilfe der PIMA (PCR Isolation of Microsatellite Arrays) erfasst und die DNA-Sequenz von 167 positiven Klonen bestimmt. Gefunden wurden 136 Mikrosatelliten der angereicherten Sequenzen von denen 59 Einzelsequenzen waren. Diese wurden mit Genom Datenbanken verglichen und es konnten 7 bereits bekannte gefunden werden. Die 52 neu isolierten Mikrosatelliten wurden an der Genbank der Universität von Minnesota überprüft und die Zuordnung von 11 Mikrosatelliten wurde bestimmt.

Schlüsselwörter: Pute (*Meleagris gallopavo*), Mikrosatelliten-Isolation, CA angereicherte Genombibliothek, magnetische Perlen, PIMA

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Introduction

Development and application of genetic markers in turkey (*Meleagris gallopavo*) began in the mid 1990's (HUANG *et al.* 1999, LEVIN *et al.* 1995, SMITH *et al.* 1996b). Different methods of random genome analyses such as randomly amplified polymorphic DNA (RAPD), single primer amplification of simple sequence repeats (SPARS), and application of chicken and turkey microsatellites were used to estimate genetic diversity within the species (SMITH *et al.* 1996a). However, because of low allelic variation and heterozygosity in their test populations, LIU *et al.* (1996) concluded that chicken loci would be of little use in constructing a turkey map. At the same time avian genomes were reported to contain a lower number of microsatellites (ms), compared to mammals (PRIMMER *et al.* 1997) seemingly complicating the discovery of turkey-specific markers. Despite the slow start and the problems associated with turkey genome mapping, considerable progress was made in both marker development and construction of a comprehensive genetic map. An integrated genetic map of the turkey was prepared containing 613 loci in 41 linkage groups with a total length of 3365 cMs (REED *et al.* 2007) and the turkey genome programme is also in progress. Here we report the isolation of 52 dinucleotide sequences from the ancient Hungarian variety of the Broad Breasted Bronze Turkey (*Meleagris gallopavo*) with the characterisation and mapping of eleven new loci.

Material and methods

For microsatellite isolation and characterization DNA was isolated from blood samples of 10 individuals of the Hungarian variety of the Broad Breasted Bronze turkey with a simple salting out procedure (MILLER *et al.* 1988). These individuals were selected on the basis of genetic variability, according to previous results (SZŐKE *et al.* 2004). CA-repeat enriched libraries were constructed following the protocol of GLENN and SHABLE (2005). Three female DNA samples from the 10 were digested with restriction endonucleases: *Rsa* I. (Fermentas) and *Hae* III. (Fermentas), followed by isolation with the biotinilated oligonucleotide ($(CA)_{18}$-biotin), streptavidin-coated magnetic particles (Dynabeads) and KBB1 linkers (KBB1 forward: 5' AGG TAC CAG CCA TAT GGG CAG CAT GC 3'; KBB1 reverse: 5' CAT GCT GCC CAT ATG GCT GGT ACC TAA A 3'). Isolated fragments were cloned into a vector (pGEM-T Easy, Promega) and transformed into competent cells (XL1 Blue). Cells were grown on LB agar supplemented with ampicillin (150 µg/ml), IPTG (20 µg/ml) and X-Gal (40 µg/ml).

Insert-containing colonies chosen on the basis of blue-white selection and were screened with *P*CR *I*solation of *M*icrosatellite *A*rrays (PIMA) consisting of two reactions: first using transforming vector-specific primers M13 Forward and Reverse primers only (reaction contained: 200nM each primer, 2mM MgCl2; 320nM dNTP; 1U Taq polymerase, Roche) and a second reaction containing an additional primer (CA primer: 5' ACA CAC ACA CAC ACA CDN 3') specific for the repeat (200nM each primer, 2mM MgCl2; 320nM dNTP; 1U Taq polymerase, Roche), supplemented with 4 % DMSO (LUNT *et al.* 1999). In case of repeat containing clones the second reaction gives two bands. The transformed colonies were used as templates. Amplifications were performed in an Applied Biosystems 9700 thermal cycler and the reaction profile was: 3 cycles of 95 °C for 2 min, 45 °C for 1 min, 72 °C for 1 min 15 s, followed by 41 cycles of 95 °C for 30 s, 45 °C for 30 s, 72 °C for 1 min 15 s, then 72 °C 5 min. PCR products were visualized on 2 % agarose gels stained with ethidium bromide. If a

fragment smaller than the insert was also detected in the second reaction, the sequence of the insert was determined. Sequencing was performed in an ABI Prism 310 Genetic Analyser using ABI Prism Big Dye Terminator 3.1 kit. Sequences were compared with each other and the NCBI database and primers were designed for unique sequences with the OligoExplorer programme.

Microsatellite primers were tested on the 10 Broad Breasted Bronze turkeys and mapping was done on the F1 and F2 generation of the mapping population of the University of Minnesota (UMN/NTBF, REED *et al.* 2003). PCR reactions included 25 ng genomic DNA, 2 mM dNTP (Roche), 720 nM forward and reverse primers, 2000 ng BSA and 0,2 U Taq polymerase (Roche). Amplifications were performed with the following reaction conditions: 94 °C 6 min; 10 cycles of 94 °C 30 s, 63-56 °C or 60-53 °C 30 s, 72 °C 45 s; 30 cycles of 94 °C 30 s, 56 °C or 53 °C 30 s, 72 °C 45 s and 72 °C 5 min. Products were run on denaturing acrylamide gels (50 cm x 38 cm x 0.4 mm) using a Sequi-Gen GT Sequencing Cell (BIORAD) and fragments were visualized by silver staining (VARGA *et al.* 1997). Genotypes of loci genetically informative in the mapping families were determined by PAGE and detected with ^{32}P-labeled nucleotides and autoradiography. The map position of the microsatellite loci were determined using Locusmap software (GARBE and DA 2003).

Results and discussion

We constructed a CA-enriched turkey genomic library. After screening, the DNA-sequence of 167 positive clones was determined, all of them contained CA-repeats, 136 were typical microsatellites sequences.

Comparing these sequences with each other, 59 unique sequences were found, 7 of them were previously annotated in GenBank (NCBI). Primers were designed for the newly isolated 52 microsatellies and tested on the 10 Broad Breasted Bronze turkey samples and on the UMN/NTFB mapping families (University of Minnesota).

A total of eleven markers were found to be genetically informative in the mapping families. LOD scores ranged from 3.54 to 40.08 (Table 1). Two markers (MGP40 and MGP46) on turkey chromosome 1 (MGA1) were linked at a distance of 14.95 cM (LOD=7.51) as were two of the markers (MGP18 and MGP 35) on MGA 2 (distance=26.84 cM, LOD=9.16). The addition of MGP47 and its linkage with the chicken microsatellite ADL184 allowed for identification of the new linkage group MGA35 that is homologous to GGA18 (REED *et al.* 2007). The turkey genome project (DALLOUL *et al.* 2010) revealed the chromosomal position of the markers too (Table 1).

The efficiency of microsatellite enrichment was very high (100 %), however it led to redundancy. The observed number of alleles was nearly similar in the Broad Breasted Bronze turkeys and in the mapping population. Compared to other studies on turkey microsatellites, the number of detected alleles was comparable, but the number of monomorphic markers was higher (REED *et al.* 2003, BURT *et al.* 2003), whereas the microsatellites were tested only on Broad Breasted Bronze turkey individuals from an inbred stock and on the UMN/NTBF mapping population. Domestic turkeys generally show significantly reduced numbers of alleles per locus when compared to wild turkeys (LATCH *et al.* 2002). Position of monomorphic microsatellites can further be determined on the basis of the turkey genome sequence (DALLOUL *et al.* 2010) and can be polymorphic and usable in other populations.

Table 1
Summary information for the polymorphic turkey microsatellites.
Daten der polymorphen Puten-Mikrosatelliten

Marker (GenBank Accession)	Primers (5′ – 3′)	Alleles in test samples	Alleles in the mapping population	Turkey linkage group	Nearest linked locus	LOD score	Chicken homologue	Turkey genome seq. (2.01) Turkey chromosome	Start	% ID	Length
MGP010 (DQ526388)	AGT AAA AAT AGG TTT GCC CGT A TTA CGA GGC ACC AGG ACC	2	2	MGA3	MNT LEI070	11,7	GGA2q	Chr_3	88707519	98,68	304
MGP012 (DQ497633)	AGG TAG CAC AGC CTT ATT TCA ACC CAA GCC TCA TTA CAA CA	4	2	MGA11	MNT 149	3,54	GGA4p	Chr_13	14830633	99,73	364
MGP018 (DQ497634)	GAG CAG GCT TTG AGC AGT C CAG AGA TGT CCA GGT GTG TTG	6	3	MGA2	RHT 031	27,6	GGA3	Chr_2	23122419	100	169
MGP022 (DQ526381)	GGA GGT CAG TGA ACA CAG GAA ACA CGG GTG AGA AGG CAG T	3	4	MGA8	RHT 078	40,1	GGA6	Chr_8	27930796	98,73	314
MGP031 (DQ526391)	TCA GAG CGA GCA GGC AC GAG CTG GAG GGG AGG ATC	2	2	MGA2	LEI 043	4,21	GGA3	Chr_15	7986027	97,35	453
MGP035 (DQ526382)	CAG GGG AGA TTT CTG GAG TT GTG CCA ATC ACT AGG ACT GTC T	3	2	MGA2	MNT 388	14,8	GGA3	Chr_2	11984877	99,71	341
MGP040 (DQ526383)	GGC TGC TCC TCA CCA TCT G GAC CGC CTC ACT TGG ATT C	1	2	MGA1	RHT 138	33,7	GGA1	Chr_1	34603484	96,86	318
MGP043 (DQ526392)	AGC AGG TGG AAT AAA GTA GCA GCT GTG GAG GGG AAA GG	3	2	MGA19	RHT 044	22,2	GGA13	Chr_15	15321719	99,26	269
MGP046 (DQ526385)	AGG CTT CAA GTG CTA CCT GG GCA AAC AGA ACA TTA GTT TGT AAC A	1	2	MGA1	MNT 174	12,3	GGA1	Chr_1	41985095	99,33	150
MGP047 (DQ526393)	GAT GAA CAG GAT CTT TGG AGG CAC AGG CTT TGG GAA GGA	1	2	MGA35	ADL 184	13,9	GGA18	Chr_20	5418244	99,07	321
MGP049 (DQ526386)	TGG GCA TCA CTC ATT ACA CAA GAG AAA AGG GAA AAT AAT GAT GAC	2	2	MGA2	MNT 217	20,7	GGA3	Chr_2	81397330	95,45	107

Included for each marker are the Genbank accession number, primer sequence, number of alleles detected in the 10 Broad Breasted Bronze turkey individuals and in the UMN/NTBF mapping population, turkey linkage group, nearest linked locus, LOD score, corresponding chicken chromosome homologue, the position of each sequence (based on BLAT search) in the turkey whole genome sequence, identity % and length.

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