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SHORT REPORT

A search for sequence similarity between chicken (*Gallus domesticus*) and ostrich (*Struthio camelus*) microsatellite markers*

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Although the ostrich (*Struthio camelus*) has been farmed for many years for skins, eggs, meat and feathers, very little is known about the genetic structure of this species. The suitability of 29 chicken microsatellite markers was evaluated as potential genetic linkage markers in the ostrich. No sequence homology was stated (0.00% similarity) between any of the 29 chicken microsatellites and the genome of the ostrich. This leads to the conclusion that the former are not suitable for genome mapping of the latter. In light of this, more work should especially be done to widen our knowledge of ostrich specific markers.

KEY WORDS: chicken / genome / mapping / markers / microsatellite / ostrich

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Although ostrich farming as a viable novel branch of agriculture became a reality in South Africa around 1863, it was only since the 1990's that the ostrich has successfully been acclimatized in Australia, Asia, South and North America as well as in most of Europe as a producer of skins, eggs, meat and feathers [Horbańczuk 2002].

Compared to chicken and some other avian species, our knowledge about the ostrich microsatellites that can be used for e.g. linkage mapping or parentage control, as well as in population genetics, molecular evolution, ecological genetics, or phylogenetic studies, is almost totally unexploited [Ward *et al*. 1994, 1998, Kimwele *et al*. 1998, Kimwele and Graves 2003, Kawka *et al*. 2003, 2007, Tang *et al.* 2003]. So far, over 800 microsatellite markers of chicken have been described and several comprehensive genetic maps constructed [Groenen *et al*. 2000]. Although it has been demonstrated that in chicken genome many DNA sequences occurring at high repetition frequency have a much lower repetition frequency than in the DNA of the ostrich [Eden *et al*. 1978], the problem of generating the amplification products in the ostrich genomic DNA by chicken-specific microsatellites needs detailed investigation. The present work aimed at recognizing the suitability of chicken microsatellites as genetic linkage markers in the ostrich.

Material and methods

Twenty-nine specific chicken microsatellite *loci* (Tab. 1) were selected from Microsatellite Chicken Wageningen, The Netherlands [Crooijmans *et al*. 1996, Groenen *et al*. 1997] and University of Leicester, Leicester, UK [Gibbs *et al*. 1997] as described in the Roslin Institute Database (http://www.thearkdb.org).

The material was collected from two well-established ostrich farms of northern Poland managed according to European Union regulations [Horbańczuk 2002]. The investigated group of birds was composed of 66 individuals by 66 sires and out of 66 dams maintained in reproduction pairs, unrelated back to second generation.

DNA from blood samples was isolated by incubating with proteinase K, and purified using standard methods with phenol-chloroform extractions [Sambrook *et al*. 1989]. PCR was carried out in a volume of 7.5 µl containing about 100 ng of template DNA, 2.5 pmol of each primer, 100 mM of each dNTP, 0.5 units of DNA Taq polymerase, 10 mM tris- HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100. Primers for each microsatellite marker were tested with a Gradient Thermal Cycler to determine accurate annealing temperatures for the ostrich DNA. The PCR amplification of microsatellite markers was performed with a PTC-200 Programmable Thermal Controller (MJ RESEARCH, USA). The PCR reaction was started with an initial denaturation for 5 min at 94°C followed by 25-37 thermal cycles composed of 45 s denaturation at 94°C, annealing at 50-68°C (temperature suitable for each marker established in twelve gradient ranges) and an extension at 72°C for 60 s. The fluorescent PCR products were separated on 6% denaturing polyacrylamide gel, using an Automated Laser Fluorescent (ALFexpress) DNA Sequencer (PHARMACIA

Table 1. List of 29 selected chicken microsatellite sequences

Microsatellite	Annealing temperature (°C)	Sequence of microsatellite
MCW0018	55	TCCCTAGGCAAACCTGCTTAC AAGACCCCACAACTTGACTTG
MCW0029	68	CATGCAATTCAGGACCGTGCA GTGGACACCCATTTGTACCCTATG
MCW0032	55	AAGTTCCTTGTACAATTGTTA CATTACTAGTACAATCAAGATGG
MCW0036	55	CCTCATGTGAAGCATCTTTTCATA TGTCTTCAGTAGGACTGTGATAC
MCW0040	50	ACCGAAATTGAGCAGAAGTTA ACTCAAAAATGTGGTAGAATATAG
MCW0041	60	CCCAATGTGCTTGAATAACTTGGG CCAGATTCTCAATAACAATGGCAG
MCW0047	60	GGATTACGGCCGTTTGTGCACAAA AATGGAACGCCGAACTCGCGTGCA
MCW0051	60	GGAACAAGCTCTTTCTTCTTCCCG CATGGAGGTGCTGGTACAAAGAC
MCW0056	55	TGGTAACCTCTAACCTTGACG AGTGAAGGAGACTCCACAGCCTCT
MCW0063	55	GAAAACCAGTAAAGCTTCTTAC GGCTCCAAAAGCTTGTTCTTAGCT
MCW0068	55	CCTCACTGTGTAGTGTGGTAGTCA GAGAAGCTTGAACCTACCAGTCTT
MCW0081	55	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC
MCW0082	65	GATCTTTAAGGGGAAAGATAT CTTTTCATGCCTCTCCATTTC
MCW0096	55	ATCTAATAGTTTTGCTACCATC AGAACATTAGGTACTACAGTTC
MCW0114	60	AGCAAACTGCTCAGTGCTGTG GCGTTGAAAGTAGTGCTTCCG
MCW0115	55	ATACCAACATCTGCCTCTGAC GCAGTGTGTCTGACTAGCTCT
MCW0126	65	ACAGAGGAAGCCTGAATGAGT GGTGTACAGCACAGGCAACA
MCW0129	55	CATGCAATTCAGGACCGTGCA GTGGACACCCATTTGTACCCTATG
MCW0131	55	GTTGCTGATTCTAAGGCAGGC TTGCAGTTGTAAAGGTGTAGC
MCW0139	55	TCTGCCACACTTCATTTATA AAGTAGTTGCTACTGTACTTG
MCW0145	55	ACTTTATTCTCCAAATTTGGCT AAACACAATGGCAACGGAAAC
MCW0167	55	GATCCCAAAACAAATGCACAC CTTACATGAGTGCTATCTGCT

Table 1. Continued.

Microsatellite	Annealing temperature (°C)	Sequence of microsatellite
MCW0170	55	TTGTGAAACTCACAGCAGCTG TTATAGCAGGCTGGCCTGAAG
MCW0200	60	GAGACATTGCAAATACTCAGC TAGTCAGGGAGTTCAGGAAGG
MCW0261	68	GTAGTAGCAGCTACACCAGAG GAGCAGTTCATATGAAGTGCAG
MCW0264	55	AGACTGAGTCACACTCGTAAG CTTACTTTTCACGACAGAAGC
MCW0283	60	GATCCTAAATATTTTAATTAACAC TTTCTGTGAATGCTGACTGAG
MCW0297	65	TGCCAAACATGACCTCCAGTC ACTTCACTGCAGGGTGGTGAG
LEI0113	62	ATGGGATGCTGGAAAGGGGT TTCTGCAAACCTATGTTGGGC

BIOTECH, Uppsala). The PCR products were electrophoretically analysed after 5 min denaturation in a 50% formamide solution containing blue dextran (2,5 mg/ml). The results were visualized and the genotyping completed with Allele Links 1.01 programme (PHARMACIA BIOTECH, Uppsala). After automated allele calling and binding within the Allele Links 1.01 software, individual genotypes were inspected manually before transferring the genotype database to Excel.

Results and discussion

None (0.00%) out of the 29 chicken microsatellite primers evaluated and described in this report showed specific "amplificons" for any of the 66 ostriches tested. Furthermore, the occurrence of stutter pictures was observed.

Similar studies investigating the similarity between chicken and turkey microsatellites were carried out by Levin *et al*. [1995] and Liu *et al*. [1996]. In the latter study, 51% of chicken markers gave a PCR product suitable for turkey what implied that chicken markers can amplify turkey DNA *loci*. However, a few years later, Reed *et al.* [2000] comparing microsatellite sequences of chicken with those of turkey, showed that only 20% of total microsatellite markers in the chicken were homologous with turkey markers and could be used for the construction of comparative maps for these species.

The present result is in accordance with the conclusion of Inoue-Murayama *et al.* [2001] based on studies with chickens and Japanese quail, that the use of heterologous primers in avian species is ineffective. This is in contrast to mammals, where the feasibility of microsatellite marker exchange has been reported between closely related species such as cattle, sheep and goats [Crawford *et al*. 1995, de Gortari *et al*.

[1997]. Although the present report indicates no sequence similarity between chicken and ostrich microsatellite markers, the result could be affected by the relatively low number of primers compared and, therefore, be treated with criticism. The search for homology with a wider range of chicken-specific and ostrich-specific primers, and especially the recognition of ostrich-specific markers, is necessary in further investigations.

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Poszukiwanie podobieństw sekwencji microsatelitarnych między kurą (*Gallus domesticus*) a strusiem (*Struthio camelus*)

S t r e s z c z e n i e

Zbadano przydatność 29 markerów mikrosatelitarnych kury jako potencjalnych markerów genetycznych strusia. Homologii między starterami dla markerów mikrosatelitarnych kury i strusia nie stwierdzono. Wnioskuje się, że markery mikrosatelitarne kury nie są przydatne do mapowania genomu strusia.