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The use of microsatellite polymorphism in genetic mapping of the ostrich (*Struthio camelus*)

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Abstract The aim of this study was to determine microsatellite polymorphism in ostriches and using it in creation the genetic map of the ostrich. The polymorphism analysis covered 30 microsatellite markers characteristic of ostrich, for the CAU (China Agricultural University) group. The material consisted of 150 ostriches (*Struthio camelus*). The 30 microsatellite *loci* was examined and a total of 343 alleles was identified. The number of alleles at a single *locus* ranged from 5 at *locus* CAU78 to 34 at *locus* CAU85. The values for the observed heterozygosity H_o ranged from 0.467 (*locus* CAU78) to 0.993 (*locus* CAU16), whereas for the expected heterozygosity H_e - from 0.510 (*locus* CAU78) to 0.953 (locus CAU85). Analyzing the individual *loci*, the highest PIC value, more than 0.7 was observed for: *loci* CAU85 (0.932), CAU64 (0.861) and CAU32, 75 (0.852), respectively. It should be noted, that the microsatellite markers used in our study were very polymorphic as evidenced by the large number of detected alleles and high rates of heterozygosity, PIC and PE as well. The analysed microsatellite markers may be used in genetic linkage mapping of ostrich, the construction of a comparative genetic map with other ratites, such as emu and rhea, and population genetics studies or phylogenetic studies of these birds.

Keywords Microsatellites · Polymorphism · Ostriches · Genetic map

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Introduction

In the recent years, the study of molecular genetics have contributed to a more profound recognition the genetic information of farm animals. This led to the creation of interdisciplinary programs of genomes mapping of important animal species. The principal aim of animal genome mapping is to determine the location and distances between genes on chromosomes as well as to search genetic markers, determining production traits (quantitative traits *loci*–QTLs). Identification of QTL provide genetic maps of high resolution i.e., containing a large number of equally distributers markers. From the breeding point of view genome mapping offers information facilitating selection for the necessary traits as it bases it on the genetic markers linked to them. Up to now, several genetic maps in agriculturally important animals have been reported such as pig [21], cattle [16], sheep [7], and chicken [8, 9].

Over the past several years, ostrich farming and breeding have been gaining popularity throughout the world as a new agricultural activity [2], since these birds provide dietetic meat, valuable skins, feathers and eggs [3, 4, 12, 22]. Recent interest in ratite farming, especially ostrich and emu, has led to an increasing demand for information about these birds [5, 13, 23], especially the genetics aspects [6, 11, 14, 17–19, 25, 26]. These studies are aimed at determining the genetic structure of these birds e.g., estimation of the genetic variability and analysis of the relationship between individuals belonging to a given populations [28].

In turn, we performed genetic analysis of the polish ostrich population using molecular methods [17]. The obtained results encourage for testing the available pool of ostrich microsatellites and identification a new microsatellite sequences. The next stage comprises the recognition of ostrich genome, which up to now has been studied very

poor. It should be emphasized that currently in available literature there is a shortage of data on genetic linkage maps for any ratites. First, a preliminary genetic map of ostrich developed Huang et al. [14], analyzing 104 polymorphic microsatellite markers using a two-generation ostrich reference family.

The main aim of this study was to determine the polymorphism of selected microsatellite markers characteristic for the ostriches, since microsatellite polymorphism is widely used in research on genome mapping. The study included also evaluation of the suitability of the analyzed *loci* for genome mapping of the ostrich.

Materials and methods

The experimental material consisted of feathers collected from 150 ostriches, collected from ostrich farm in Stypułów, which maintains the birds in conditions compliant with EU recommendations by the Committee of the European Convention for the Protection of Animals Kept for Farming Purposes (T-AP)—Draft Recommendation Concerning Ratites (Ostriches, Emus and Rheas) [24].

Ostrich genomic DNA was isolated from feathers (noninvasive methods) using Dneasy Tissue KIT 250 from QUIAGEN. Each sample was examined by spectrophotometer and electrophoresis. An analysis of 30 microsatellite sequences characteristic of ostrich [25], for the CAU (China Agricultural University) group was performed. One of the primer pairs has been labeled with one of the four dyes: 6-FAM, VIC, NED, PET. The characteristic of the loci is presented in Table 1. The amplification of selected microsatellite sequences was performed using a thermal cycler PTC-200 Engine (MJ Research). The PCR was carried out in a total volume of 10 ml comprising 10 ng of template DNA, 0.5 mM of each nucleotide, 100 pmol of each primer, 1.5 mM MgCl2, 50 mM KCL, 10 mM Tris–HCl, 0.01% Tryton X-100 and 0.5 units of DNA polymerase (POLGEN). The PCR conditions were optimized for all 30 primer pairs. The PCR protocol began with a denaturing step for 5 min at 94°C, 35 cycles of 94°C for 45 s, 52.5–69.5°C for 45 s (annealing), and at 72°C for 90 s (extension), with the final 10 min elongation step at 72°C. The fluorescent PCR products were separated by electrophoresis using the four-capillary genetic analyzer Applied Biosystems 3130 and the computer software GeneScan. The results were visualized and the genotyping was completed with GeneScan 2.1. In addition, the computer program GeneMapper (Applied Biosystems) was used to automatically determine of allele size for the individual markers.

The statistical analysis of obtained results was performed using Cervus [15] program. It included: determine the frequency of identified alleles, estimate the observed and expected heterozygosity, the polymorphic information content (PIC) and the exclusion probability (PE).

The observed heterozygosity H_o was assessed for all the microsatellite *loci* examined in the population as the share of heterozygous genotypes in the overall pool of genotypes in the population. The expected heterozygosity H_e was calculated according to the formula by Ott [20] and Weir [27] but the PIC was estimated according to Botstain et al. [1]. In addition, the exclusion probability (PE) for each *locus* was valueted.

Results and discussion

We analyzed the ostrich population consisted of 150 birds. At the 30 microsatellite *loci* examined a total of 343 alleles were identified. The most polymorphic were *loci*: CAU84, CAU32, CAU7, CAU75 and CAU76, as characterized by the highest number of alleles. The number of alleles at a single *locus* ranged from 5 at *locus* CAU78 to 34 at *locus* CAU85. At each of the microsatellite *loci* studied a mean of 11.43 alleles was recorded. In the previous research Kawka et al. [17], analyzing 5 microsatellites identified 51 alleles. The number of alleles per *locus* ranged from 5 (*locus* VIAS-OS22) to 16 alleles (*locus* VIAS-OS29). The mean number of alleles per *locus* was 10.2. The similar research on isolation and characterization of 70 new microsatellite markers from ostrich conducted Tang et al. [25]. The number of alleles obtained by them ranged from 2 to 16—a mean of 5.6 per *locus*. However, in the studies of Ward et al. [26], the number of alleles per *locus* ranged from 5 to 18 and at Kimwele and Graves [19]—from 6 to 25.

Based on the frequency of individual alleles for the studied microsatellite *loci* was estimated the observed heterozygosity (H_o), which included heterozygous genotypes and the expected heterozygosity (H_e), taking into consideration the number and frequency of alleles and the polymorphic information content (PIC) as well. The values for the observed heterozygosity H_o ranged from 0.467 at *locus* CAU78 to 0.993 at *locus* CAU16 (Table 2). The mean for all *loci* value of H_o was 0.840. In turn, the values for expected heterozygosity (H_e) estimated for population analyzed, ranged from 0.510 at *locus* CAU78 to 0.953 at *locus* CAU85. The mean H_e amounted to 0.791 per *locus* (Table 2). It should be noted that both values (H_o and $H_{e)}$ in the studied ostrich population were relatively high. By comparison, Kimwele and Graves [19] indicated, that the value of mean heterozygosity He for a ostrich populations living in Nairobi National Park and ostriches kept on farms in Kenya, ranged from 0.40 to 0.79. In turn, Kawka et al. [17], examining the genetic variability within and among 3

Table 1 Characteristics of 30 ostrich microsatellite loci used in the study

Microsatellite	Sequence of microsatellite	Repeat motif	Number of alleles	Length of alleles (bp)
CAU1	TTACAAGCAAGGTAGAACCCA GCAAGCAACCCAATCCCTG	$(AC)_8AT(GC)_3(AC)_7$	10	86–104
CAU3	AACTAAGTATAGCCCTGTTACA TGCGAGTCTTTCTAGTTCTAC	$(CA)_9$	6	115–125
CAU7	CACTCCTGTCCCCTACTTG CTGTAGTGTATTTAGAGACTGA	$(AC)_{18}$	12	185–211
CAU11	CCTTGACAGTCTTCCCATATGAC AACACAGAGGGCTTAGTCCTACA	$(CA)_{12}$	7	98–114
CAU14	ATTTAACTTCTCTAAGGCACTC GAGGAGCAATTCAGACAGAC	$(CA)_{16}$	14	142–178
CAU16	TGTCCCTGCAGTCTCAGTTTT GCCAGGTATGTGCATGTGTC	$(CA)_{27}$	7	188–204
CAU17	CGTAAACCCAGATAATCACAA AGTGGCATTGTAGCTCTTCA	$(CA)_{22}$	11	160–180
CAU22	TGACTGTTAAATAAGCGAATGT CATATATTAAGCCACTCTAAAAT	$(AC)_{11}$	7	140–154
CAU23	AGGAACCGTGGAACACATTT GAGCTGTGAACGTCTTCATCC	$(CA)_{10}$	7	165–193
CAU25	ATGGGGCAGCATAAGAGTGT CCAGGTGAATTTGCCACATA	$(CA)_5CT(CA)_8$	6	197–207
CAU30	AGGGGAGCGTTCTCACTCA GCCACAAAGCAAAAGACCAC	$(CA)_{19}$	9	117–137
CAU32	ATACTGGTTTTGATTTGTGTGAT CATGGGAAGGGCAATAGATTT	$(CA)_{10}$	7	177–205
CAU34	ATTTGATAGCCAGAGCAGTTC TCTTACAAGATTTCACTATATACA	$(CA)_{12}$	7	194–208
CAU40	ACGGGGAGACTCAAGGATG GCTTGCGTGTGCATGAGTAT	$(CA)_9$	9	138–156
CAU42	AGTCCAGCCCGCATACAC CCTCTGTGGAGAGAACTGTGTG	$(CA)_{10}$	7	182–198
CAU43	ACTGAGTGCCCAGGTTTGAG TGCTGTTTCTTCTTCTTTTAGGG	$(CA)_{17}$	6	211–221
CAU44	GCAAAGCAGTGTCCTTAGTCAA AGCGTGTATCTGCCACATGA	$(CA)_{12}$	5	227–237
CAU57	AAGAGGCAACAGGAATAGGTA CAAAAATCTGGCTTGTCACTTA	$(CA)_7(TA)_5(CA)_5$	6	201–221
CAU64	AGCACCTCATCCCTCAAAC AGATTTGGAGCATGGACTATT	$(CATA)_7(CA)_6(TA)_4$ $(CA)_{15}$	9	161–183
CAU65	TGAGAGTCTCCCAGAAATGC CAGAGAAATATATGCCTGTAAAT	$(TA)_{12}(CA)_9$	6	181–191
CAU68	TCTAAGCACTACCATCACGG GCTCCTTTTCATCTTTTAGGC	$(TA)_7(CA)_8\ldots(CA)_{15}$	6	265–275
CAU69	TGAGTAAGGCATGCTGCTTC CCTAAATGCAACCCTTCTGTTT	$(GA)_{19}$	6	100–112
CAU75	ACAGACCAGGGAGTCCAGCA ACCCTGCACCTTGACAACAT	$(GC)_7(AC)_{18}$	7	186–210
CAU76	GCACCAATCTTGATGTCCTG ACCTACCCAGAATGGCTTGA	$(CA)_{11}CG(CA)_5$	10	220–254

Table 1 Characteristics of 30 ostrich microsatellite loci used in the study

Microsatellite	Sequence of microsatellite	Repeat motif	Number of alleles	Length of alleles (bp)
CAU78	CAGGTGGAAAGTGGGTATGC GCTTTGTAAGTGTGGGTGTGG	$(AC)_8C_5$	5	113–121
CAU83	AAACAAGCCGCTAGTGAGGA TGCAGACTCAGACCAGCATC	$(AC)_{16}$	8	198–218
CAU84	TATCAGTGCCATTATCGTCTC TGTCCCTTCTGTTTCTAATACT	$(CA)_{12}$	7	202–214
CAU85	GAGGTGCCTGTCTTGTTTAC AAAAGCACCTTCCCACATTG	$(AC)_{26}$	16	204–276
CAU97	TGCACGCACTAACTCCTGTC AGTTCCCCTTCCAAATGCTT	$(CA)_{10}$	5	152–166
CAU98	CACTCCACCGAATGCCTTTA TTTGTTCAGGTGCAGAATGC	$(CA)_{12}$	8	134–178

ostrich breeds reported a mean observed and expected heterozygosity ranking from 0.463 to 0.663 and from 0.481 to 0.679, respectively. In a preliminary study of genetic diversity of emu populations (based on 5 microsatellite loci) kept on farms in Australia and Thailand and in the wild emu [10] obtained a wide range of value of He. In turn, for emus kept on a farm in Australia, this ratio ranged from 0.44 to 1, whereas in Thailand from 0.28 to 0.89.

Another parameter characterizing the genetic variability of the *locus* and used to determine the value of markers in analyzing the linkage with other *loci* is the polymorphism information content (PIC). Analyzing the individual *loci*, the highest value for this parameter more than 0.7 was observed, among others: for *loci* CAU85 (0.932), CAU64 (0.861) and CAU32, 75 (0.852) (Table 3). These microsatellites are the most polymorphic and most useful in the linkage analysis for ostrich. The lowest values of the PIC (0.462) was recorded for *locus* CAU78. In previous study conducted on ostriches by Kawka et al. [17], the PIC value ranged from 0.117 to 0.786. One must emphasise that almost all the microsatellite markers selected for our analysis were characterized by a high polymorphism of heterozygosity or by high values of the polymorphism information content. Among the least polymorphic microsatellite markers one may count *locus* CAU78.

It was estimated also the probability of exclusion (PE) for each *locus* when data of both parents are available, taking into consideration the frequency of the n-th co-

Table 2 Observed heterozygosity (H_o) and expected heterozygosity (H_e) within the ostrich analysed

Locus	H_o	H_e	Locus	H_o	H_e
CAU1	0.880	0.867	CAU43	0.913	0.836
CAU3	0.953	0.737	CAU44	0.860	0.688
CAU7	0.807	0.701	CAU57	0.713	0.832
CAU11	0.960	0.839	CAU64	0.893	0.876
CAU14	0.960	0.821	CAU65	0.700	0.820
CAU16	0.993	0.862	CAU68	0.780	0.850
CAU17	0.840	0.859	CAU69	0.967	0.840
CAU22	0.913	0.814	CAU75	0.867	0.869
CAU23	0.780	0.724	CAU76	0.873	0.853
CAU25	0.820	0.738	CAU78	0.467	0.510
CAU30	0.967	0.809	CAU83	0.833	0.781
CAU32	0.687	0.868	CAU84	0.833	0.841
CAU34	0.780	0.685	CAU85	0.953	0.939
CAU40	0.953	0.757	CAU97	0.853	0.754
CAU42	0.513	0.664	CAU98	0.900	0.722
Mean	0.840	0.791			
SE	0.023	0.018			

Table 3 Polymorphism information content (PIC) and probability of exclusion (PE) for the microsatellite *loci* examined within the ostrich analyzed

Locus	PIC	PE	Locus	PIC	PE
CAU1	0.850	0.888	CAU43	0.812	0.841
CAU3	0.692	0.689	CAU44	0.642	0.637
CAU7	0.677	0.730	CAU57	0.812	0.857
CAU11	0.819	0.860	CAU64	0.861	0.905
CAU14	0.794	0.820	CAU65	0.792	0.815
CAU16	0.845	0.889	CAU68	0.828	0.859
CAU17	0.840	0.877	CAU69	0.818	0.852
CAU22	0.785	0.808	CAU75	0.852	0.895
CAU23	0.704	0.763	CAU76	0.834	0.876
CAU25	0.690	0.677	CAU78	0.462	0.446
CAU30	0.781	0.808	CAU83	0.756	0.797
CAU32	0.852	0.897	CAU84	0.818	0.846
CAU34	0.643	0.656	CAU85	0.932	0.972
CAU40	0.718	0.731	CAU97	0.719	0.743
CAU42	0.646	0.710	CAU98	0.689	0.720

dominant allele. The values of the probability of exclusion is presented in Table 3. The PE value ranged from 0.446 at *locus* CAU78 to 0.972 at *locus* CAU85. Kawka et al. [17] analyzed five microsatellite *loci* obtained a very high probability of exclusion from 0.77 to 0.98. Analysis of 30 microsatellite *loci* presented gives a very high probability of exclusion incorrect parent from 0.77 to 0.98. The presented results show that the analysis of these 30 microsatellite *loci* may be successfully applied in identification the origin of ostriches kept in Poland.

In summary, the microsatellite markers used in our study were very polymorphic as evidenced by the large number of detected alleles and high rates of heterozygosity, PIC and PE as well. The microsatellite markers we have analyzed may contribute to genetic linkage mapping of ostrich, the construction of a comparative genetic map with other ratites such as emu and rhea. Further research aimed at creation of two-generation ostrich reference family and evaluation of the distances between markers is recommended.

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