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

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
Materials and methods

The experimental material consisted of feathers collected from 150 ostriches, collected from ostrich farm in Stypulów, 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).

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 (Hₒ), which included heterozygous genotypes and the expected heterozygosity (Hₑ), taking into consideration the number and frequency of alleles and the polymorphic information content (PIC) as well. The values for the observed heterozygosity Hₒ ranged from 0.467 at locus CAU78 to 0.993 at locus CAU16 (Table 2). The mean for all loci value of Hₒ was 0.840. In turn, the values for expected heterozygosity (Hₑ) estimated for population analyzed, ranged from 0.510 at locus CAU78 to 0.953 at locus CAU85. The mean Hₑ amounted to 0.791 per locus (Table 2). It should be noted that both values (Hₒ and Hₑ) 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           | TTACAAGCAAGTGAGAAGCCA      | (AC)$_3$(AT)(GC)$_3$(AC)$_3$ | 10              | 86–104                |
|                | GCAAGCAAGCAATCCCTCTG       |              |                  |                        |
| CAU3           | AACAGGTATAGCCCTGTTACA      | (CA)$_9$     | 6                | 115–125               |
|                | TGCGAGTCTTTCTAGTCTAC       |              |                  |                        |
| CAU7           | CACCTCTGCTCCCTACTTTG       | (AC)$_{18}$  | 12               | 185–211               |
|                | CTGTAAGTTAAGAGACTGGA       |              |                  |                        |
| CAU11          | CTTTGAGCAGTCTGCTCATTGCAC  | (CA)$_{12}$  | 7                | 98–114                |
|                | AACCAGAGGGCTAGTCTTCTCACA   |              |                  |                        |
| CAU14          | ATTTAACCTCTCTAAAGGACCTC    | (CA)$_{16}$  | 14               | 142–178               |
|                | GAGGAAGATTCAGACGACAGC      |              |                  |                        |
| CAU16          | TGCCCTGCGTTGACTTACTTTT     | (CA)$_{27}$  | 7                | 188–204               |
|                | GCCAGGTAATGTCAGTGTC        |              |                  |                        |
| CAU17          | CTGAAAGCCAGAATACACACAA     | (CA)$_{22}$  | 11               | 160–180               |
|                | AGTGGGCATTGTGACGCTCTCA      |              |                  |                        |
| CAU22          | TGACTGTAAATAAGCAGAATG      | (AC)$_{11}$  | 7                | 140–154               |
|                | CTTATATGAAAGCACCCTCTAAAT   |              |                  |                        |
| CAU23          | AGGAAAGTGGAAACACTATTT      | (CA)$_{10}$  | 7                | 165–193               |
|                | GAGGTAGAAAGCTCTTATCACCC    |              |                  |                        |
| CAU25          | ATGGGGAAGCAGATAGAGTGT      | (CA)$_{3}$CT(CA)$_{8}$ | 6          | 197–207               |
|                | CCAGGTGAAATGCGCCACATA      |              |                  |                        |
| CAU30          | AGGGGACGCTCTCCTACCA        | (CA)$_{19}$  | 9                | 117–137               |
|                | GCCAAGAGAAGACAGCAGC        |              |                  |                        |
| CAU32          | ATACTGGTTTGTATTGTGTGAT     | (CA)$_{10}$  | 7                | 177–205               |
|                | CATGGGAAGGGCAATAGATTT      |              |                  |                        |
| CAU34          | ATTTGATAGCAGAGCAGTTC       | (CA)$_{12}$  | 7                | 194–208               |
|                | TCTTACAAGATTTTCACATATAC    |              |                  |                        |
| CAU40          | ACGGGGAAGCTCAAGGATG        | (CA)$_{9}$   | 9                | 138–156               |
|                | GCCTTGGAGTGACGATGAT        |              |                  |                        |
| CAU42          | AGTCCAGCCCCGCATACAC        | (CA)$_{10}$  | 7                | 182–198               |
|                | CCTCTGTGAGAGAACTGTTG       |              |                  |                        |
| CAU43          | ACTGAGTGCCCGAGGTGAGG       | (CA)$_{17}$  | 6                | 211–221               |
|                | TGCTGTATTTTGTCACTCTTTCTTTT |              |                  |                        |
| CAU44          | GCAAGAGCAGTGCTCTAGTCCAA    | (CA)$_{12}$  | 5                | 227–237               |
|                | AGCGTGTACTCTGACACATGA      |              |                  |                        |
| CAU57          | AAGAGCCAAGCAGGAATAGGTCA    | (CA)$_{7}$(TA)$_{3}$(CA)$_{3}$ | 6          | 201–221               |
|                | CAAAATCTGGCTTTGCTAATCA     |              |                  |                        |
| CAU64          | AGCACCCTCATCTCCCCAAC       | (CATA)$_{3}$(CA)$_{6}$(TA)$_{4}$ | 9          | 161–183               |
|                | AGATTTGGAGCATGAGCATATT     | (CA)$_{15}$  |                  |                        |
| CAU65          | TGAGAGCCTCCAGAAATGCGC      | (TA)$_{12}$(CA)$_{9}$ | 6          | 181–191               |
|                | CAGAGAATAATATGCGCTGAAATAT |              |                  |                        |
| CAU68          | TCTAAGCAGATCCATCACG        | (TA)$_{9}$(CA)$_{8}$...(CA)$_{15}$ | 6          | 265–275               |
|                | GCCTCCCTTCTACATCTCTAGGCG   |              |                  |                        |
| CAU69          | TGAGTAAGGGACTGCTTC         | (GA)$_{19}$  | 6                | 100–112               |
|                | CCTAAAGGCAACCCCTCTGTCTT    |              |                  |                        |
| CAU75          | ACAGACCAGGGAGTCCACGA       | (GC)$_{2}$(AC)$_{18}$ | 7          | 186–210               |
|                | ACCCTCGACGCTTGAACACAT      |              |                  |                        |
| CAU76          | GCACCAATCTTGATGCTTGC       | (CA)$_{11}$CG(CA)$_{3}$ | 10         | 220–254               |
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 $H_e$. 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 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       | (AC)$_8$(C)$_5$ | 5                | 113–121                |
|               | GTCTTGTAGTGGTGGTGG         |              |                  |                        |
| CAU83         | AAAACAAGCGCTAGTGAGGA       | (AC)$_{16}$  | 8                | 198–218                |
|               | TGCAGACTCAGACCGACATC       |              |                  |                        |
| CAU84         | TATCAGTGCCATTATCGCTCTC     | (CA)$_{12}$  | 7                | 202–214                |
|               | TGTCCCTTCTTCCAATACCTCTC   |              |                  |                        |
| CAU85         | GAGGTGCCTGCTCCTTTTAC      | (AC)$_{26}$  | 16               | 204–276                |
|               | AAAAGCACCTCCACACATGG       |              |                  |                        |
| CAU97         | TGCACGCAATTACCTCTGTGTGCAAG | (CA)$_{10}$  | 5                | 152–166                |
|               | AGTTCCCTTCTCAATGCTT        |              |                  |                        |
| CAU98         | CACTCCACGAATGTGCTTTTA     | (CA)$_{12}$  | 8                | 134–178                |
|               | TTTGTCAGGTGCAGAATGC        |              |                  |                        |

### 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 |       | 0.023 | 0.018 |
| SE    | 0.023 | 0.018 |       | 0.023 | 0.018 |
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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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   |
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