COMPARATIVE INVESTIGATIONS OF MALLARD DUCK (*ANAS PLATYRHYNCHOS*) GENOMIC DNA USING CHICKEN AND DUCK SPECIFIC MICROSATELLITE PRIMERS

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Summary. Microsatellites provide an excellent opportunity for developing genetic markers of high utility because the number of repeats is highly polymorphic. In addition, the assay to score microsatellite polymorphism is quick and reliable and the procedure is based on polymerase chain reaction (PCR). In Lithuania, domestic duck is an economically important poultry species, however, developing microsatellite primers for domestic duck is technically demanding and expensive. Since such microsatellites are very common in the chicken genome, we have previously performed microsatellite – PCR analysis of genomic DNA of Mallard duck (*Anas platyrhynchos*) using chicken specific microsatellite primers. In addition, microsatellite loci have gained widespread use in genome mapping, phylogenetic and conservation genetics due to their abundance in eukaryotic organisms. Therefore, another goal of our work was to use duck specific microsatelite primers in order to detect polymophism in Mallard duck (*Anas platyrhynchos*).
Individual blood samples were collected from 61 Mallard ducks from various regions of Lithuania (Babtai, Kretuona, Bukiskis, and Vilnius).. This was followed by phenol – chloroform – isoamyl alcohol extraction of DNA from each sample. The PCR was carried out in a final volume of 25 µl consisting of 50 mM KCL, 10 mM Tris-HCL (pH 8.3), 1.5 mM $MgCl_2$, 0.1 % Triton X-100, 200µM of each deoxynucleoside triphosphate (dNTP), 1U Taq polymerase, 100nM each primer and 25 ng genomic DNA. In the polymerase chain reaction three chicken specific primers of microsatellite marker (ADL-115, ADL-209, ADL-231) and two duck specific primers of microsatellite marker (APH-23, APH-24) were used. Ten microliters of the PCR mixture were loaded onto a 5 % polyacrylamide gel, stained in 0.5 µg/ml ethidium bromide. After PCR products were visualised in a UV transilluminator, photographed and evaluated. In our study PCR products of appropriate size were resolved, suggesting that chicken specific markers may be useful for examining population structure and gene flow in a range of Anatidae. Although thegenetic markers detected may have unknown functions and perhaps do not influence trait variation, they can be used in combining information from a number of samples and provide a good characterisation of breeds. The increasing number of available markers provide an elementary and powerful tool for better understanding of the genetic variance and population architecture related to different duck species and breeds.

Keywords: duck, microsatellites, genetic markers, polymerase chain reaction, DNA, chicken.

LAUKINIŲ ANČIŲ (*ANAS PLATYRHYNCHOS*) GENOMINĖS DNR PALYGINAMIEJI TYRIMAI NAUDOJANT VIŠČIUKŲ IR ANČIŲ SPECIFINIUS MIKROSATELITINIUS PRADMENIS

Santrauka. Mikrosatelitai, susidedantys iš grupėmis besikartojančių sekų, plačiai paplitę eukariotų genomuose ir dažnai yra labai polimorfiški dėl skirtingo bepasikartojančių sekų skaičiaus. Naminė antis – ekonomiškai svarbi paukščių rūšis, tačiau jai sukurti mikrosatelitinius pradmenis techniškai sudėtinga ir brangu. Kadangi mikrosatelitai yra labai dažni viščiukų genome, savo darbe, taikydami polimerazinę grandininę reakciją, atlikome laukinių ančių (*Anas platyrhynchos*) genominės DNR analizę naudodami viščiukų specifinius mikrosatelitinius pradmenis. Mikrosatelitai taip pat plačiai taikomi identifikuojant individus, tėvystės testuose, sudarant genolapius ir kt. Todėl kitoje darbo dalyje panaudojome ančių specifinius mikrosatelitinius pradmenis. Taip siekėme įvertinti Lietuvoje gyvenančių didžiųjų ančių polimorfizmą.

Tirta DNR fenoliniu–chloroforminiu metodu buvo išskirta iš didžiosios anties 61 individo kraujo pavyzdžio, paimto įvairiose Lietuvos vietovėse: Babtuose, Kretuone, Bukiškyje ir Vilniuje. Polimerazės grandininė reakcija atlikta galutiniame 25 µl tūryje, susidedančiame iš 50mM KCL, 10mM Tris-HCL, 1,5 mM $MgCl_2$, po 200µM kiekvieno dezoksiribonukleotido, 1 vieneto *Taq* polimerazės, 100nM kiekvieno pradmens ir 25 ng genominės DNR. Polimerazės grandininėje reakcijoje panaudoti trys mikrosatelitiniai specifiniai viščiukų pradmenys (ADL-115, ADL-209, ADL-231) ir du mikrosatelitiniai specifiniai ančių pradmenys (APH-23, APH-24). Po amplifikacijos produktų elektroforezės 5 % poliakrilamidiniame gelyje bei tris EDTA boratiniame buferyje DNR fragmentų skaičius ir ilgis nustatytas gelį apšvietus ultravioletiniais spinduliais. Gauti rezultatai parodė, kad viščiukų mikrosatelitinius pradmenis galima naudoti didžiųjų ančių genominės DNR analizei. Taip pat buvo nustatyta didžiosios anties vidurūšinė įvairovė naudojant APH - 23 pradmenį.

Raktažodžiai: mikrosatelitai, genetiniai markeriai, DNR

Introduction. The evaluation of genetic changes is very important in breeding research and organized breeding programs of various domestic birds and animals; it requires an objective of evaluating candidates for selection, a mating design and a way to validate the design through genetic improvement.

Domestic duck is an economically important poultry species, and in breeding research it is also very important to identify genes that control the expression of economically important traits. Most traits of this category

display a wide variation in expression and are controlled by numerous genes. In order to identify the genes that are associated with a particular trait, the predominant method uses evenly spaced deoxyribonucleic acid (DNA) markers to screen a population that exhibits phenotypic variation for the trait of interest (referred to as a resource of population). Analyzing the association of various segregating alleles at any one marker locus with the trait value allows for the identification of regions in the genome known as quantitative trait loci influencing the given trait (Cheng et al., 1995). Among the genetic markers being currently employed, microsatellites best fit the description of an ideal genetic marker. Microsatellites or simple sequence recurrences are tandem duplications with a simple motif of one to six bases as the recurrences unit. Microsatellites are so popular markers due to being highly polymorphic and randomly spreading across the genome of organism. During the past years DNR markers have contributed to the preparation of saturated genetic maps and this process is adapted to various species (Dodgson et al., 1997). However, the development of microsatellite primers for domestic ducks is technically demanding and expensive. Simple sequence recurrences that repeated over and over in a tandem are very common in the chicken genomes (Crooijmans et al., 1997; Gibbs et al., 1997). Thus in our work we have performed microsatellite – PCR analysis of genomic DNA of Mallard duck (*Anas platyrhynchos*) using chicken specific microsatellite primers corresponding to an anonymous polymorphic DNA marker. Domestic and wild ducks are classified in the order Anseriformes and subfamily Anatinae, whereas chickens in the order Galliformes. However, they are all in the same class Aves and using of chicken specific microsatellite primers in other birds are also popular. Turkey microsatellite can be amplified using chicken specific primers (Levin et al., 1994). Despite on it poly-morphic DNA was found to be only 11 % in turkey comparing to 15% in chickens (Smith et al., 1996). This notifies that the microsatellite recurrences found in chicken are similar to those in the turkey (both belong to the same family Phasianidae). Therefore one purpose of our work has been to investigate whether this genomic DNA of mallard duck can be amplified DNA pattern the same as of the chicken.

Biodiversity is the basic element of selection progress directed not only at productivity traits but also intensity of adaptive features of all organisms. Biodiversity can be described at several levels and is also applied as a marker system to estimate inter- and intraspecies genetic variation. However, isolation and breeding in closed populations in series generations also selection according to morphophysiological features and indicators can cause infringement of genes balance and minimise genetic variation in various breeds (Slavėnaitė et al., 1998). Therefore, using of frequency polymorphic loci already can control the investigation process of lines and breeds. It facilitates considerable evaluation of differentiation and similarity in lines and breeds. Such a microsatellite loci have gained widespread use in genome mapping, phylogenetic and conservation genetics due to their abundance in eukaryotic organisms. Another goal of our work – to use duck specific microsatelite primers in order to detect polymorphism in mallard duck (*Anas platyrhynchos*).

Materials and methods. Blood from 61 samples of Mallard ducks habituating in various regions of Lithuania (Babtai, Kretuona, Bukiskis, and Vilnius) has been individually withdrawn via the wing vein directly to microtube overlaid with EDTA as an anticoagulant. The erythrocytes were lysed by adding lysis buffer (20 mM tris-HCl, pH 8,0; 100 mM Nacl; 10mM EDTA, pH 8,0 and 0,5 % SDS) and then proteinase K was added. The mixture was incubated overnight at 37.6 °C followed by phenol – chloroform – isoamyl alcohol extraction and ethanol precipitation. The polymerase chain reaction was carried out in a final volume of 25 µl consisting of 50mM KCL, 10 mM Tris-HCL (pH 8.3), 1,5 mM $MgCl_2$, 0,1 % Triton X-100, 200 µM of each deoxynucleoside triphosphate (dNTP), 1U Taq polymerase, 100 nM each primer and 25 ng genomic DNA. In the polymerase chain reaction three chicken specific primers of microsatellite marker (ADL 115 - GGA TGA GAA GAA GAA AGG CA (forward primer) and CAA TGG TGG TTC AGG TAA TC (reverse primer); ADL 209 - GGT TAG CTC CCT CCT TCC AG (forward primer) and TCA CTC CAG CTT GAG ACA GG (reverse primer); ADL 231 - ACT ATT AGC CTG GGG AGA GC (forward primer) and AAG GAA ACA AAG AGA AAT CC (reverse primer)) also two specific duck primers of microsatellite marker (APH23 - GGA TGA GAA GAA GAA AGG CA (forward primer) CAA TGG TGG TTC AGG TAA TC (reverse primer), APH24 - GGA TGA GAA GAA GAA AGG CA (forward primer) and CAA TGG TGG TTC AGG TAA TC (reverse primer)). The data of primer identification are shown in Table 1. Reactions were initially denatured for 3min at 94 C, then 30 cycles of 94 C for 1 min, 43 to 57 C for 1 min and 72 C for 1 min with a final 5 min elongation step at 72 C in Thermal Cycler. The PCR products were mixed with 5 µl bromophenol blue. Ten microliters of the PCR mixture were loaded onto a 5 % polyacrylamide gel, stained in 0.5 µg/ml ethidium bromide. Electrophoresis was performed with constant voltages at 200V in a Tris - borate EDTA buffer. After that PCR products were visualised in a UV transilluminator also photographed and evaluated.

Results and discussion. In our data, appropriate size of PCR products as resolved, by using chicken and duck specific microsatellite primers. The highest number of DNA fragments in Mallard duck (*Anas platyrhynchos*) as gained with primer ADL 209 (ranged 6 to 7 bands) whereas the lowest – with ADL 231 (ranged 4 bands). Primers ADL 115 and APH23 ranged within the same number of bands (3 to 5) (Table 2) By using primers ADL 209 fragments of 190-70 bp length of Mallard duck were amplified. The polymorphism (two alleles) has been detected in the range of 70 to 80 bp. Also clearly polymorphic fragments of DNA were detected by using APH23 primers in the largest 180 to 190 bp range. In this range two allies were also detected. By using other three pairs of primers the size of monomorphic fragments fluctuated from 60 bp (APH-24) to 840 (ADL-115).

In our data we detected genetic variation within species of Mallard duck and the increasing number of available markers provided an elementary and especially powerful tool for better understanding of population architecture related to species and breeds of ducks. Both between and within breed genetic variations are important

for the future viability of livestock. Continued genetic improvement of livestock is dependent on the fact that substantial genetic variation exists within individual breeds allowing them to respond to selection for different traits. Within species genetic variation is, however just as important as between species diversity (Caballero, Toro, 2002) because there has been much concern in recent years over the loss of biodiversity. Human activities worldwide are devastating the whole ecosystem and resulting in the extinction of a large number of species. Breeds or populations that lack genetic variation may have reduced fertility and fitness due to inbreeding depression. The value of genetic markers was also recognized by the Food and Agriculture Organization (FAO) in the guidelines they produced for measurement of domestic animal diversity.

Table 1. **The data of specific chicken and duck primer identification**

Locus name	Recurrence	Forward Primer	Reverse Primer	Gene Bank ID	Length
ADL 115	(TA)8(GTT)9	GGA TGA GAA GAA GAA AGG CA	CAA TGG TGG TTC AGG TAA TC	G0 1556	109 bp
ADL 209	(AC)21	GGT TAG CTC CCT CCT TCC AG	AAG GAA ACA AAG AGA AAT CC	G0 1629	163 bp
ADL 231	(GT)125	ACT ATT AGC CTG GGG AGA GC	TCA CTC CAG CTT GAG ACA GG	G0 1651	136 bp
APH23	(CA)4 TA(CA)12	GGA TGA GAA GAA GAA AGG CA	AAG GAA ACA AAG AGA AAT CC	AJ515898	205bp
APH24	(CA)2TA (CA)9	GGA TGA GAA GAA GAA AGG CA	CAA TGG TGG TTC AGG TAA TC	AJ515899	147bp

Table 2. **Number and length of DNA fragments amplified with various microsatellite primers**

Genomic markers	Taxon	Number of DNA fragments	Length of DNA fragments
ADL - 115	*Anas platyrhynchos*	5 - 3	120 - 840
ADL - 209	*Anas platyrhynchos*	6 - 7	80 - 260
ADL - 231	*Anas platyrhynchos*	4	110 - 760
APH - 23	*Anas platyrhynchos*	5 - 4	50 - 190
APH - 24	*Anas platyrhynchos*	5 - 3	60 - 230

In our data we detected that the chicken specific microsatellite primers can be used to amplify the polymorphic DNA fragments in Mallard duck. It is important that majority of traits would remain stable with minor differences observed from generation to generation, which could be caused by environmental variation. Therefore, the chicken is an ideal domestic animal for identifying quantitative trait loci for a variety of reasons. Some of these reasons are that the chicken has a short generation time, large families can be rapidly generated, selected lines are available for many traits and controlled environments limit the influence of environmental variations.

The ideal measure of diversity within and between breeds is based on the genes that control variation in all relevant quality, disease, resistance, fitness and other traits and it is currently not possible to derive such measure because most of the relevant genes have yet to be identified. Our detected genetic markers have unknown function and perhaps they do not influence trait variation but they are valuable for various reasons. Firstly, many DNA based genetic markers can be used in combining information from a number of samples and provide a good characterisation of the breed. Also birds can be typed for a large number of markers using DNA from a small amount of tissue (e.g. feathers or a blood sample) and taken from any bird at any stage of its development (alive or dead) DNA; samples can be safely stored for along periods of time and used in future analysis, and the markers are relatively robust and unaffected by environmental variation.

Most common features that are accounted in the studies of biodiversity of wild and domestic avians (including Anseriformes) are morphophysiological and productivity traits. In some cases a breed may be superior for most traits of economic importance but lack a desirable characteristic of another breed. The ideal outcome is to transfer the advantageous genes without losing superior characteristic of the original (recipient) breed. In many cases this is achieved by forming an initial cross between breeds followed by a number of generations of back - crossing to replace the genome of the donor breed that is inferior for most traits of economic importance. Finally there is a generation of inter - crossing to make the individuals homozygous for the desired alleles. Microsatellites as ideal genetic markers could be used in this case in two ways. Firstly markers could be used to select at each back - cross which is heterozygous for the desired allele. Secondly, markers can be used against the remainder of the donor genome. The latter is not strictly necessary but observations have shown that it can speed up the genome recovery in the recipient breed by one to two generations (Kospital et al., 1992; Vissher et al., 1996).

Microsatellites provide an excellent opportunity for developing genetic markers of high utility because the number of recurrences is highly polymorphic and the assay to score microsatellite polymorphism is quick and

reliable because the procedure is based on the polymerase chain reaction. Also, polymerase chain reaction primers are easily shipped or synthesized from the DNA sequence; thus, microsatellite markers are readily exchanged (Beckmann, Soller, 1990). Subsequently and assisted by the development of appropriate software packages for data handling and linkage analysis we expected that in ducks many associations between markers and traits will be reported in the near future.

Conclusions.

1. In our data, appropriate size of PCR products was resolved, suggesting that chicken specific markers may be useful for examining population structure and gene flow in a range of Anatidae.

2. Despite the fact that our detected genetic markers have unknown function and perhaps they do not influence trait variation, they can be used in combining information from a number of samples and provide a good characterisation of the breed.

3. Increasing number of available markers provide an elementary and especially powerful tool for better understanding of the genetic variance and population architecture related to species and breeds of ducks.

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