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Characterization of Duck Microsatellite Repeat Sequences

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Abstract

We cloned duck microsatellite repeat sequences from genomic DNA of an Indonesian native duck to evaluate the genetic relationships among native breeds of duck. A $(CA)_n$-enriched library was constructed using the method of Takahashi et al. (1996) with modifications. In the library, 8.3% of the clones were CA-positive. The average number of uninterrupted $(CA)_n$ repeats among 22 duck microsatellites was 11.4. Fifteen clones which could be developed as PCR primer pairs to detect $(CA)_n$ repeat length polymorphisms were identified. They should provide a valuable tool for studies related to biodiversity and population structure in duck.

Discipline: Genetic resources
Additional key words: DNA, Indonesia

Introduction

Duck farming in Indonesia is important and aimed mainly at egg production. Currently, the duck population in Indonesia amounts to about 30 million and most of them are raised in Java and Sumatra islands. The duck eggs contribute to about 25% of the national egg production, which amounts to about 140,000 t per year. Recently, duck production for eggs has expanded and an increasing number of farmers raise a larger population of ducks under intensive system as their main farming activities. Therefore, the demand for improved breeds is also increasing. The Research Institute for Animal Production of Indonesia has initiated a breeding program for improving the efficiency and consistency of egg production through selection and crossbreeding among native ducks. Major 3 breeds, Tegal, Alabio and Mojosari, have been used as breeding resources at the Institute. Tegal in Java and Alabio in Sumatra are used for egg production. Mojosari in Java is used for egg and meat production. However, genetic relationships among Indonesian native ducks have not been elucidated. For the evaluation of genetic relationships among duck breeds and effective use, it is necessary to develop an efficient method for analysis. Microsatellite repeat sequences, for example $(CA)_n$ repeats, are well dispersed in the genome, highly polymorphic and have been shown to be powerful tools for population genetics[4]. Although Maak et al.[1] reported 7 duck microsatellites, many more microsatellites are needed. As a first step in duck genetic research, we cloned and characterized microsatellite repeat sequences in duck.

Materials and methods

A $(CA)_n$-enriched library was constructed using the method of Takahashi et al.[3] with modifications. In the chicken $(CA)_n$-enriched library, about half of the clones are over-represented[3]. Takahashi et al.[3] suggested that these results were due to a severe bias of genomic DNA fragments which were used for ligation into a plasmid

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vector. To alleviate this shortcoming, sonicated fragments were used for constructing a duck $(CA)_n$-enriched library, as described below.

The genomic DNA of the library was derived from a female individual of Tegal. Genomic DNA was extracted from blood using a DNA isolation kit (SepaGene, Sanko Junyaku, Japan) at the Research Institute for Animal Production of Indonesia. To obtain non-biased DNA fragments, genomic DNA was fragmented by sonication. Sonicated fragments were blunted by mung bean nuclease (Takara, Japan) according to the instructions of the supplier. The fragments were electrophoresed in a 1.2% agarose gel and fragments ranging from 300 to 500 bp were recovered. The fragments were repaired by T4 DNA polymerase (Toyobo, Japan) and Klenow Fragment (Toyobo, Japan) according to the supplier's manual, and then ligated into the *Srf* I site of the pCR-Script Amp SK(+) vector (Stratagene, USA). Recombinant plasmid vector was transformed into TG1 competent cells (#200123, Stratagene, USA), and single-stranded DNA was prepared according to the method of Takahashi et al.[3].

Selective double-stranded DNA synthesis was employed using $(CA)_{12}$ oligonucleotide and cloned *Takara EX Taq*™ DNA polymerase (Takara, Japan), according to the method of Takahashi et al.[3]. Single-stranded DNA remaining in the reaction mixture was digested with mung bean nuclease. The resultant double-stranded DNA was transformed into TG1 competent cells again and these transformants were referred to as "$(CA)_n$-enriched library". From the library, colonies were randomly chosen and inoculated with 50 µL of LB medium containing ampicillin in separate wells of 384-well plates (#242757, Nalge Nunc International, USA). The libraries were duplicated on the surface of nylon membranes (#1209299, Boehringer Mannheim GmbH, Germany) using 384-pin replicators (X5050, GENETIX, UK). The membranes were incubated on 2 × YT agar plates at 37ºC for 16 h. Then, the membranes were subjected to hybridization screening as previously described[3]. The sequences of the CA-positive clones were determined using the BigDye terminator sequencing kit (PE-Applied Biosystems, USA) with a DNA sequencer (model 310, PE-Applied Biosystems, USA).

Results

Of the 384 clones screened from a duck $(CA)_n$-enriched library, 32 clones (8.3%) gave positive signals with the $(CA)_{12}$-oligonucleotide probe. We subjected them to sequencing analysis and in 31 clones the sequences could be read. Thirty clones were unique and one clone duplicated. Of the unique clones, 29 clones showed runs of CA repeats. Three clones were discarded because runs of CA repeats were directly ligated into the *Srf* I site of the pCR-Script™ Amp SK(+) vector. Four clones were also discarded because the inserts were too long (>500 bp), presumably due to the production of fragments in the ligating step. The length of the $(CA)_n$ repeat units of the other 22 clones ranged from 6 to 24 units with a mean value of 11.4 (Table 1). Fifteen clones could be developed as PCR primer pairs to detect $(CA)_n$ repeat length polymorphisms. Seven clones were discarded because sequence information was insufficient to develop primers.

Table 1. Characterization of 22 unique clones from the duck $(CA)_n$-enriched library

Clone	Length of repeat	Primer design
1	$T(CA)_9GA_5$	Yes
2	$T(CA)_{11}GAA$	Yes
3	$A(CA)_{10}C$	Yes
4	$A_2(CA)_{18}A$	Yes
5	$A(CA)_{12}G$	Yes
6	$A(CA)_{10}A_{14}$	Yes
7	$A(CA)_4GA(CA)_6$	Yes
8	$C(CA)_{11}C_6$	Yes
9	$A(CA)_8A_7$	Yes
10	$A(CA)_{10}T$	Yes
11	$A(CA)_{18}C$	Yes
12	$T(CA)_{12}C$	Yes
13	$G(CA)_8A_2$	Yes
14	$A(CA)_{11}T$	Yes
15	$G(CA)_8G$	Yes
16	$A(CA)_{18}G$	No
17	$A(CA)_{11}T$	No
18	$A_3(CA)_{11}G$	No
19	$A_2(CA)_{24}C$	No
20	$G(CA)_8A_2$	No
21	$A_2(CA)_8A_4$	No
22	$G(CA)_9A_2$	No
	Mean continuous repeat length 11.4	

Discussion

The frequency of avian microsatellites is almost tenfold lower than that in mammals[2]. Therefore, an efficient method should be developed for cloning new duck microsatellites. Takahashi et al.[3] reported that the average number of uninterrupted $(CA)_n$ repeats among the 32 chicken microsatellites is 13.1 unit. Since this study and the previous report used the same procedures for the

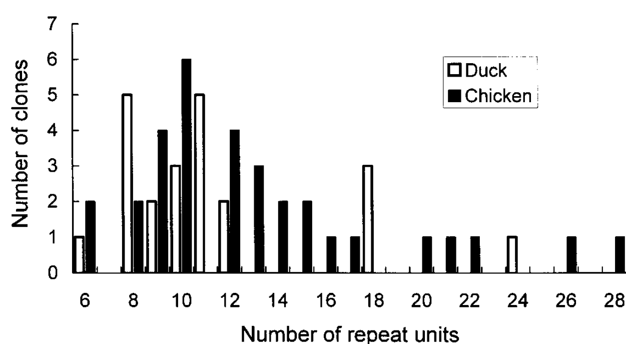

Fig. 1. Distribution of duck and chicken microsatellites classified by the length of CA repeat units

selection of $(CA)_n$ microsatellites *in vitro*, the data of duck and chicken microsatellites are considered to be comparable. We classified 22 duck and 32 chicken microsatellites by the length of CA repeat units (Fig. 1). Primmer et al.[2] estimated that the number of $(CA)_{\geqq 10}$ would be around 7,000–9,000 and the number of $(CA)_{\geqq 14}$ about 3,000 per haploid genome in chicken. The distribution of the 32 chicken $(CA)_n$ microsatellites ($(CA)_{\geqq 10}$: $(CA)_{\geqq 14}$ = 25 : 11) is in agreement with this estimation. In contrast to chicken, in 50% (11/22) of the duck clones, $(CA)_{\leqq 10}$ was observed. Since the length of the CA repeat units did not seem to distribute normally (Fig. 1), we compared both data using the Mann-Whitney's rank test based on unpaired measurements. As a result, the length of the duck $(CA)_n$ repeat units was significantly ($P<0.01$) shorter than that of chicken $(CA)_n$ repeat units. This report provides additional evidence on the characteristics of microsatellites in avian species.

Recently, Takahashi has constructed a new chicken $(CA)_n$-enriched library without the bias of genomic DNA fragments which were used for ligation into the vector, by applying the same method as that described in this study. The library contains 37.5% CA-positive clones (unpublished data, Takahashi), although only 8.3% of the clones were CA-positive in the duck library. The discrepancy can be ascribed to the difference in the length of the $(CA)_n$ repeat units between duck and chicken. If so, the efficiency of cloning duck microsatellites could be improved by lowering the temperature of the selective second-stranded DNA synthesis *in vitro*.

In conclusion, we isolated 15 microsatellite repeat sequences that could be developed as PCR primer pairs to detect $(CA)_n$ repeat length polymorphisms. They should provide a valuable tool for studies related to biodiversity and population structure in duck.

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