Variation of Short Tandem Repeats in Kintamani Dogs

Variasi Runutan Pendek Terulang pada Anjing Kintamani

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Abstract

Microsatellites are repetitive DNA sequences that are randomly distributed throughout vertebrate genomes. These microsatellite loci have been shown to be highly polymorphic, because of variation in the number of repeats units. They can be amplified faithfully with polymerase chain reaction (PCR), enabling precise allele designation both in pedigree and population surveys. This makes them applicable both in gene mapping, population genetics, and for individual identi fication. In this project, I have investigated the genetics variability in Kintamani dogs. Fr equency distribution and allele size in 116 canine microsatellite loci were analyzed in 425 Kintamani dogs. Genome DNA was isolated from swab cell. Hundreds sixteen microsatellites were amplified by PCR in 12 multiplexes reaction. PCR products were run on 6% bis-Acrylamide gel in automated DNA sequencer. Fluorescent signals from the dye-labeled microsatellites were detected using Genescan 3.1 software and Strand Version 2.2.39 program was employed in calculation of the allele number. The result showed that a total of 1128 alleles were found. The number of alleles per locus ranged from 3 (AHT136) and 41 (FH2138). Mean PIC value is 0.68. All of the microsatellite loci were polymorphic.

Keywords: Kintamani dogs, microsatellite, PIC.

Introduction

The Kintamani dog is a highly popular household pet in Bali. Although Kintamani dogs have wide range of coat color, most of dog have similar morphological characteristic. The Kintamani dogs are small to medium in size. The wither heights of the female dog is 44.65 cm while the male one is 51.21 cm. Kintamani dogs are bold and not aggressive (Puja, 2000). However, the origin of Kintamani dogs remains unknown and this breed is believed to be native to the region of Kintamani, Bali, Indonesia.

The discovery of double helical of DNA brought biology into chemistry and gave new direction to the fields of molecular biology and molecular evolution. Population structure of living organism which were originally constructed based on morphological similarities, have increasingly been assessed from comparisons of DNA (Lowenstein, 1985). In recent years microsatellite analysis has been widely used to determined population structure within and among population (Koskinen and Bredbacka, 2000; Martines et al., 2000; Stahlberger-Saitbekova et al., 2001). Microsatellites are repetitive DNA sequences that are randomly distributed throughout vertebrate genomes (Fredholm and Wintero, 1995). They are based on short 1-5 base repeats and can be highly polymorphic in the population (Zajc et al., 1997). Their variability stems from different numbers of basis repeats unit. As polymorphic genetics markers, they have been used to improve the accuracy in classification of domestic animals (Nagamine and Higuchi, 2001) and used extensively in application as diverse as mapping studies, linkage analysis (Bruford and Wayne, 1993). Microsatellites are the best available molecular tools for characterization domestics animal (Metta et al., 2004), breed assignment (Canon et al., 2000), and parental assignment (Koskinen and Bredbacka, 1999; Schnabel et al., 2000; Villanueva et al., 2002)

The ease of isolation and utility of microsatellite have made it possible to investigate and apply them to a wide range of different species in which extensive basic genetic analysis has not previously been feasible. As the knowledge about the frequency distribution of microsatellite alleles within canine species currently is sparse, I have investigated the genetics variability in Kintamani dogs population. Hundreds sixteen canine microsatellite loci, which are known to be polymerphic were chosen from a bank of canine microsatellites.

Materials and Methods

Sample Collection

Buccal swab samples were collected from 425 Kintamani dogs living in their habitat in Kintamani Bali. If the dogs has been eating or drinking, wait 10 to 15 minutes before taking samples. The samples were taken randomly from individuals from known areas, without consideration of relationship between animals.

DNA Extraction

Genome DNA was isolated from buccal swab cell by 50mM NaOH treatment and then resuspended in 1 M Tris pH 8.0

Microsatellite Genotyping

Hundreds sixteen microsatellites were amplified in 12 multiplexes reaction. Polymerase chain reaction (PCR) amplification was performed on PCT 100 (MJ Research, Inc, Watertown, Mass, USA) using 30 cycles with denaturation at 95 C (10 min), annealing at 56 C (30 min) and extension at 72 C (1min). PCR products were run on 6% bis-Acrylamide gel in automated DNA sequencer (ABI Prism 377 DNA Sequencer-PE Biosystem, Foster City, CA, USA). Fluorescent signals from the dye-labeled microsatellites were detected using Genescan 3.1 software (PE Biosystem and Strand Version 2.2.39 program was employed in calculation of the allele number.

Computation

Allele frequency were determined by direct counting and polymorphism information content (PIC) were determined for all markers in each animal of the population.

Result and Discussion

Genetics variabilities within Kintamani dogs population were quantified with microsatellites allel size and polymorphic information content (PIC). In total, 116 primer pairs designed for analyzing canine microsatellites were used for amplification of homologous sequences in Kintamani dogs. A total of 1128 alleles were found in Kintamani dogs. The microsatellite loci revealed that all loci were polymorphic with the number of allele varying from 3 (AHT136) to 41 (FH2138). Size range of alleles and the frequency distribution detected for each animal is almost similar. The PIC values in Kintamani dogs is 0.68 was higher to the value of 0.52, calculated by Ostrander and coworkers (1993), and 0.5 by Zajc et al.1997. PIC might provide a better estimate of the degree of variability, because it depends on the number as well as the frequency of alleles. The mean PIC value mean that a considerable reduction in intrabreed variation was observed in Kintamani dogs. This result almost certainly reflects high inbreeding coefficient, which in domestic dog vary from breed to breed. Although the Kintamani dogs were supposedly unrelated, a breed is probably more inbreed within population.

This study shows that microsatellite markers are useful in characterize the kintamani dogs. This study contributes to the knowledge of the genetic structure and molecular characterization of Kintamani dogs population. It also shows how microsatellites can be used to establish the genetics relationship of Kintamani dogs. Population data collected on microsatellites polymorphism in Kintamani dogs can be applied in studying breed assignment and segregation of microsatellites and loci associated quantitative traits or numerous genetics diseases that affect the Kintamani dogs.

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