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Genetic Characterstics of the Kintamani Dogs Using *Microsatellite* Markers

Karakterisasi Genetik Anjing Kintamani Menggunakan Petanda *Mikrosatelit*

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Abstrak

Karakterisasi molekuler anjing Kintamani berperan penting dalam penentuan status trah dan mencegah penurunan kemurnian akibat perkawinan silang dengan trah anjing lain. Anjing Kintamani sangat popular di Bali. Anjing Kintamani dipercaya berasal dari Kintamani, Bangli, tetapi asal usulnya sampai kini belum jelas. Mikrosatelit merupakan runutan nukleotida terulang yang didistribusikan secara acak dalam gen vertebrata. Lokus mikrosatelit ini telah diketahui sangat polimorfik, karena keragaman jumlah nukleotida yang terulang. Mikrosatelit merupakan petanda allel utama yang dapat digunakan untuk pemetaan gen, genetika populasi dan identifikasi individu serta alat untuk mengungkap karakter genetik hewan. Pada penelitian ini diungkap karakteristik genetik anjing Kintamani menggunakan mikrosatelit. Jumlah dan ukuran allel pada 425 anjing Kintamani dianalisis menggunakan 116 macam primer mikrosatelit. DNA diisolasi dari sel hasil swab pipi. Amplifikasi 116 lokus mikrosatelit menggunakan PCR dalam 12 multiplek. Produk PCR dipisahkan dengan gel bis-akrilamid 6% dalam *automated DNA sequencer*. Flurosesnsi yang dihasilkan dideteksi dengan program *Genescan 3.1*, dan program *Strand* versi 2.2.39 digunakan untuk menghitung jumlah allel. Hasil penelitian menunjukkan bahwa jumlah allel yang didapat adalah 1128. Jumlah allel perlokus berkisar antara 3 (AHT136) sampai 41 (FH2138). Rataan PIC adalah 0,68 dan semua lokus bersifat polimorfik.

Kata kunci : anjing kintamani, mikrosatelit, polymorphic information content (PIC)

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Introduction

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. In recent years microsatellite analysis has been widely used to determine population structure within and among population (Koskinen and Bredbacka, 2000; Martines *et al*., 2000; Stahlberger-Saitbekova *et al*., 2001).

Microsatellites are repetitive DNA sequence that are randomly distributed throughout vertebrate genomes. Microsatellite repeat sequences for example $(CA)_n$ repeats are well dispersed in the genome, highly polymorphic and have been shown to be powerful tools in genome mapping of dog (Mellersh *et al*., 2000). They are based on short 1-5 base repeats and commonly referred to as simple sequence repeats. The repeat motif is most widely distributed in eucaryotic genome is $(dC\text{-}dA)_n$ - $(dG\text{-}dT)_n$ (Stalling *et al*., 1991). These microsatellites loci have been shown to be highly polymorphic in the population because of variations in the number of repeat unit (Zajc *et al*., 1997), with mutation rate

often exceeding 10^{-4} per generation (Weber and Wong, 1993). Their variability stems from different numbers of basic repeated units. As polymorphic genetics markers, they have been used to improve the accuracy in classification of domestics animals (Nagamine and Higuchi, 2000) and are increasingly becoming the markers of choice for population genetics studies (Bruford and Wayne, 1993). Microsatellites are the best available molecular tools for characterization domestics animal, breed assignment (Canon *et al*., 2000) and parental assignment (Koskinen and Bredbacka, 1999, Schnabel *et al*., 2000, Villanueva *et al*., 2002). They have been used for parentage testing in many species, but the application of microsatellite to parentage testing in dogs is relatively recent (Ichikawa *et al*., 2001). Microsatellite have been used as one of the most useful tools for genetic identification and to studies of canine pedigrees (Oishi *et al*., 2005).

Sukawana, a village in a distric of Kintamani, Bangli, province Bali, is an area just 5 km from Kintamani and home to more than 5200 people. Approximately 2100 dogs also live in this area. The Kintamani dogs are highly popular household pets in Bali. The Kintamani dogs have a good appearance and the capacity for circus performances (Puja, 2001). Although Kintamani dogs have a wide range of coat colors, most of the dogs 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 is 51.21 cm. Kintamani dogs are bold and not aggressive (Puja, 2000). A preliminary studies have been initiated to characterize of Kintamani dogs using craniometry analysis (Puja 2001). This result suggest that the Kintamani dogs are considerably pure. However, the origin of Kintamani dogs remains unknown and this breed is believed to be native to the region of Kintamani, Bali, Indonesia.

The ease of isolation and the utility of microsatellites have made it possible to use them for investigation 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, the present study was carried out to characterize of Kintamani dogs using microsatellites markers. Hundreds sixteen canine microsatellite loci, which are known to be polymorphic 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. The samples were taken randomly from individuals from known areas, without consideration of relationship between animals.

DNA Extraction

Genome DNA was extracted from buccal swab cell by heating a single swab for 10 min at 95^0C in 400 µl 50mM NaOH and then neutralized with 140 µl 1 M Tris-HCL pH 8.0.

Microsatellite Genotyping

Hundreds sixteen microsatellites were amplified in 12 multiplexes reaction. The multiplexing strategy involves amplifying combinations of markers so that no two markers with the same dye and product size overlap (Cargill *et al*., 2002). Polymerase chain reaction (PCR) amplification was performed on PCT 100 (MJ Research, Inc, Watertown, Mass, USA) using 30 cycles: denaturation at 95^0C (10 min), annealing at 56^0C (30 min) and extension at 72^0C (1min). All PCR work was done in polycarbonate 96-well v-bottom microtiters plates. 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 was determined by direct counting and polymorphism information content (PIC) was determined for all markers in each animal of the population.

Result and Discussion

The goal of the present study was to characterize the Kintamani dogs. The microsatellite loci analyzed in the present investigation were selected from a total of 116 sequenced loci. Genetics variabilities within Kintamani dogs population were quantified with microsatellites allel size and polymorphic information content (PIC).

One hundred sixteen 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). Frequency distribution and allele size in microsatellite loci in Kintamani dogs are almost the same. The PIC values in Kintamani dogs were 0.68. This is higher than 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. A considerable reduction in intrabreed population variation was observed in Kintamani dogs population. This result almost certainly reflects high inbreeding coefficient, which varies from breed to breed in domestic dogs. Although the Kintamani dogs were supposedly unrelated, they are probably more inbreed within population.

Population data collected on microsatellites polymorphism in Kintamani dogs can be applied in breed assignment, linkage analysis, studying a segregation of microsatellites and loci associated with quantitative traits or numerous genetics diseases that affect the Kintamani dogs. Screening the canine genome for linkage of markers with various hereditary diseases facilitates identification of affected and carrier individuals, thereby providing researchers and clinicians with an additional diagnostic tool.

This study contributes to the knowledg of genetic structure and molecula characterization of the dog population. It als show how microsatellites can be used t establish the genetic relationship betwee population providing reasonable statistica power for breed assignment, regardless o whether they are closely related or not allowing their future management to be base on greater knowledge of genetics structurin and relationship between population.

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