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The Kintamani Dog: Genetic Profile of an Emerging Breed from Bali, Indonesia

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Abstract

The Kintamani dog is an evolving breed indigenous to the Kintamani region of Bali. Kintamani dogs cohabitate with feral Bali street dogs, although folklore has the breed originating 600 years ago from a Chinese Chow Chow. The physical and personality characteristics of the Kintamani dog make it a popular pet for the Balinese, and efforts are currently under way to have the dog accepted by the Federation Cynologique Internationale as a recognized breed. To study the genetic background of the Kintamani dog, 31 highly polymorphic short tandem repeat markers were analyzed in Kintamani dogs, Bali street dogs, Australian dingoes, and nine American Kennel Club (AKC) recognized breeds of Asian or European origin. The Kintamani dog was identical to the Bali street dog at all but three loci. The Bali street dog and Kintamani dog were most closely aligned with the Australian dingo and distantly related to AKC recognized breeds of Asian but not European origin. Therefore, the Kintamani dog has evolved from Balinese feral dogs with little loss of genetic diversity.

Introduction

Several hundred breeds of dogs are currently recognized around the world, and new breeds are continuously emerging. Many breeds have evolved rapidly over the past century from deliberate crosses of existing purebred dogs (Neff et al. 2004). However, many older breeds have been phenotypically modified over centuries or millennia from indigenous feral dog populations. If phenotypic selection is slow and involves large numbers of non- or distantly related males and females, the loss of genetic diversity in breed development will be small (Okumura et al. 1996). However, if phenotypic selection is rapid and involves few isolated sires and dams, genetic diversity may be low from the onset. Low genetic diversity, whether present from inception or acquired over time, has had negative disease and lifespan implications for many pure breeds of dogs (Pedersen 1999; Proschowsky et al. 2003). Therefore, the goal of breed development should be to maintain maximal genetic diversity to minimize genetic disorders, while standardizing and solidifying the desirable phenotypic traits. Such strict genetic management has not been heretofore possible; pedigrees were notoriously unreliable and genetic tests have been unavailable or too costly. However, there has been an explosion of genetic knowledge of the dog and simple, rapid, and inexpensive genetic tests have been developed. It is now possible to manage the genetic makeup of a breed.

Because many dog breeds are already inbred, the goal is to identify emerging breeds that may serve as models for genetic management of future breeds. The Kintamani dog of Bali, Indonesia, is one such breed. The objectives were to characterize the genetic evolution of the Kintamani dog from indigenous feral dogs (Bali street dogs) and to document its current genetic diversity. Such information will be useful for the maintenance of diversity as the breed is officially recognized and gains popularity.

The Kintamani dog is a common household pet in the Indonesian province of Bali. Kintamani dogs are described as intelligent, hardy, gentle, and highly loyal to the family. An attempt is now being made to establish a breed standard and a breed association. Hartaningsih and colleagues (1999) detail the desired characteristics of the Kintamani dog. Interest in studying the genetic structure of Kintamani dogs is increasing, because it is hoped to become the first Federation Cynologique Internationale–recognized dog breed from Indonesia.

The feral Bali street dogs, which live throughout the island, have undoubtedly contributed their genes to the Kintamani breed, but to what extent is unknown. The Bali street dog is more phenotypically diverse than the Kintamani dog, but all Kintamani traits are collectively present among the much larger feral population. Bali street dogs are generally short haired and of the pariah type—untamable when taken as an adult and overly jealous and territorially aggressive even when adopted as a puppy. In recent years, microsatellite analysis has been widely used to determine population structure, within and among animal populations, including dogs (Irion et al. 2003; Koskinen and Bredbacka 2000; Martines et al.

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2000; Nagamine and Higuchi 2000; Parker et al. 2004; Stahlberger et al. 2000; Saitbekova et al. 1999; Takezaki and Nei 1996; Zajc et al. 1997).

In this project, the genetic variability and relationships of the Kintamani dog was investigated by testing 40 dogs with 31 polymorphic microsatellite loci. Results were compared to previously reported findings for the Bail street dog and the Australian dingo as well as nine American Kennel Club (AKC)–recognized breeds (Irion et al. 2003). Allelic variation, breed heterozygosities, and genetic distances are presented.

Materials and Methods

Sample Collection

Bali street dogs were randomly captured and taken to the Yayasan Yudisthira Swarga clinic for treatment or sterilization, at which time buccal swabs were collected. Twice as many Bali street dogs were tested than for the comparison populations, because their relatedness was unknown. Australian dingo samples were provided by Dr. Alan Wilton of the School of Biochemistry and Molecular Genetics (University of New South Wales, Sydney, Australia). Dingoes were reported to be unrelated at the parent level.

Dogs from nine AKC breeds were sampled with buccal swabs as previously reported (Irion et al. 2003). The breeds tested were: Akita, American eskimo, Australian shepherd, Chow Chow, Jack Russell terrier, Papillon, Pomeranian, Rhodesian ridgeback, and Yorkshire terrier. These dogs were unrelated at the parent level.

Marker Selection

Thirty-one microsatellites were selected from a 100-marker multiplexed panel developed by the Veterinary Genetics Laboratory (Eggleston et al. 2002). All markers have been mapped on either the 1999 canine genetic linkage map (Neff et al. 1999) or on a radiation hybrid map (Guyon et al. 2003). Loci selected for study represented 25 of the 38 autosomes of the dog with 6 autosomes represented by two loci. Forward primers were synthesized and dye labeled with either Fam, Hex, Vic, Tamra, or Ned (Applied Biosystems [ABI], Foster City, CA). Reverse primers were synthesized by Operon (Alameda, CA).

Sample Preparation and PCR Conditions

Australian dingo samples used were supplied as extracted DNA, and remaining DNA samples were obtained from nylon bristle cytology (buccal) brushes (Medical Packaging, Camarillo, CA). DNA was extracted by heating each swab for 10 min at 95°C in 400 μl 50mM NaOH and then neutralized with 140 μl 1M Tris–HCl, pH 8.0. Two microliters were then used in each polymerase chain reaction (PCR). All markers were amplified with a PCR reagent mix of 1× PCR buffer (ABI), 2.5 mM $MgCl_2$, 0.05 μM of each dNTP (Hoffmann-La Roche, Nutley, NJ), 0.6 U AmpliTaq (ABI), and 2% dimethyl sulfoxide (DMSO). One of five thermal cycler programs was used for each primer mix, differing by the selected annealing temperature. All PCR work was done on MJ Research PTC-100 thermalcyclers (Waltham, MA). Protocols are available online at www.vgl.ucdavis.edu/research/canine/paper_data/procedures.html.

Gel Electrophoresis Conditions and DNA Fragment Analysis

One-microliter aliquots of PCR product were mixed with 2 μl Fluorescent Ladder (CXR) 60–400 bases (Promega 400) or Internal Lane Standard 600 (Promega 600, Promega, Madison, WI) fluorescent size standard, denatured on MJ Research PTC-100 thermalcyclers for 3 min at 95°C, then held at 5°C or placed on ice for at least 1 min before gel loading. Two-microliter aliquots were then loaded onto a 6% denaturing polyacrylamide gel and run on an ABI 377 Automated Sequencer using ABI 10"x 7 1/8" short plates (12 cm). Gels were run at 1.10 kV (constant) voltage, 60.0 mA current, 200 W power, 51°C, and 40.0 mW (constant) laser power for up to 2 h when using Promega 400, up to 3 h using Promega 600. DNA fragment analysis was performed with in-house designed STRand software (Hughes 1998) that replaces ABI Genotyper and Genescan software and is available online at www.vgl.ucdavis.edu/strand. This data was then transferred to an in-house database compatible with the STRand software.

Statistical Analysis

Allelic diversity and observed heterozygosities were determined by direct counting. The probability test option of the Hardy Weinberg Exact (HWE) test, pairwise F_{ST} estimates and per population F_{IS} were performed using Genepop version 3.4 (http://wbiomed.curtin.edu.au/genepop), an updated version of Genepop 1.2 (Rousset and Raymond 1995). *P*-values and F_{IS} estimates were averaged across all populations or all loci. Gene diversity (H_T) and its associate parameters H_S (average heterozygosity among subpopulations) and F_{ST} were calculated across all loci using the public domain software, DISPAN (Ota 1993; http://iubio.bio.indiana.edu/soft/molbio/ibmpc/dispan.readme). A pairwise genetic distance matrix using Nei's distance analysis (DA) was also created using DISPAN with bootstrapping.

Phylogenetic Tree Construction

Allele frequencies were used to compute a matrix of genetic distances (Saitou and Nei 1987), which were used to construct a phylogenetic tree of relationships among the 12 dog populations. Takezaki and Nei's (1996) public domain POPTREE software (http://mep.bio.psu.edu/genefreq.html) was used to create a neighbor joining tree using DA distances with 1,000 bootstrap replications. The output of POPTREE was then converted to the New Hampshire format for editing in TREEVIEW version 1.6.6 (Page 2001). The phylogram format of the tree with bootstrap values was pasted into an image editor and a representative image of each population was added.

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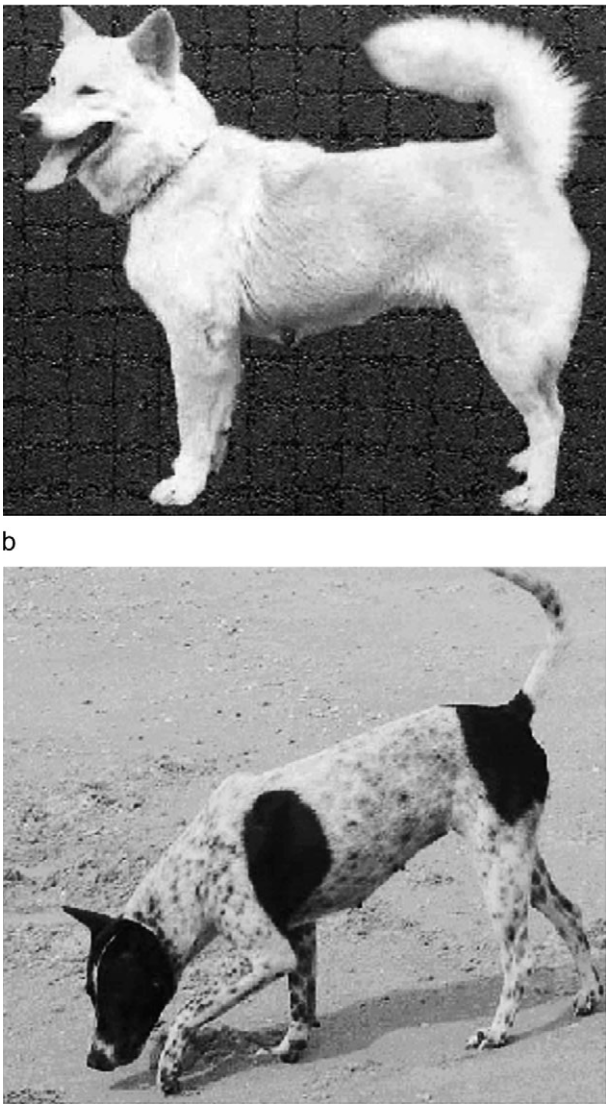

Figure 1. (**a**) Typical Kintamani and (**b**) Bali street dogs.

Results

Phenotypic Appearance of Kintamani and Bali Street Dogs

The typical physical appearances of Kintamani and Bali street dogs are shown in Figure 1. The withers height of the female Kintamani dog is 40–50 cm, 45–55 cm for the male. The stature of the Bali street dog is similar. The desired physical traits of the Kintamani dog include erect ears, forwardly curved tail held at the midline, medium to longhaired coat, almond-shaped brown eyes, and black skin pigment. The most desired coat color is white with apricot-tipped ears (Hartaningsih et al. 1999). However, other coat colors, such as black, are accepted. Bali street dogs come in many colors and coat patterns, and they are almost always shorthaired and straight to curve tailed. Both still whelp in burrows dug into the earth, a feral dog trait. However, the Bali street dog cannot be reliably tamed, even when taken as a puppy. In contrast, the Kintamani dog is gentle around people, yet retains enough assertive behavior to render it a noteworthy (but not vicious) watchdog.

Genetic Diversity

The Bali street dog had the highest level of diversity of the populations sampled, exhibiting 59.2% (239 of 404) of the alleles observed in all populations and an average of 7.7 observed alleles per locus versus the 5.4 average for all AKC populations (Table 1). The Kintamani dog had the second highest total number of observed alleles (217), averaging 7.0 alleles per locus. The observed (H_O) heterozygosity was only slightly lower for the Kintamani dog versus the Bali street dog (0.681 versus 0.692), and the expected heterozygosity was 0.700 for the Kintamani dog versus 0.746 for the Bali street dog. The Bali street dog and the Kintamani dog barely differed in overall *P*-values (.354 versus .359) and in F_{IS} values (0.097 versus 0.089). Although there was allele loss in the Kintamani dog (217 versus 239 observed alleles), heterozygosities were also not significantly different.

Genetic Comparison by Loci

The diversity of each locus was further explored for the Kintamani and Bali street dog populations with representative AKC populations in Table 2. The number of observed alleles ranged from 3 to 11 in the Kintamani versus from 3 to 14 in Bali street dog. The Bali street and Kintamani dogs were similar to each other at each locus but with some exceptions. At AHT111 and CPH02, the H_O of the Kintamani dog is near one-half that of the Bali street dog. At AHT139, both populations are out of HWE, although the pairwise F_{ST} for that locus is the highest (0.1383). Allele frequencies for loci AHT139, C20.446, and CPH02 show the greatest difference between the Bali and Kintamani dogs (see supplement). The Kintamani dog shows a shift in frequencies from the 149 allele to the 153 allele at AHT139. At C20.446 the 197 allele had a 43.8% frequency in the Kintamani dog, whereas it was at 5% or lower for all the other populations. The Kintamani dog did not display the 106 allele at CPH02. This allele is fairly common in the Bali street dog and several of the AKC populations. In contrast, the 96 allele of CPHO2 had a frequency of 88.8% in the Kintamani dog, similar to the dingo and Akita populations.

Genetic Relatedness

The Kintamani dog is most closely related to the Bali street dog by genetic distance analysis (0.083), with the next most related population being the Australian dingo at 0.263 (Table 3). Bali street dog pairs had lower genetic distance values than the Kintamani dog pairs, indicating that the Kintamani dog was not a hybrid of the Chow Chow and Bali street dog. The same result held true for the pairwise F_{ST} values. The Kintamani/Bali street dog comparison value was 0.029, and the next closest population to the Kintamani dog was the Chow Chow. All pairwise F_{ST} values were higher for the Kintamani dog pairs than the Bali Street dog pairs.

An unrooted neighbor joining dendogram, showing the genetic relationships among 12 dog populations and using Nei's DA genetic distance, was constructed (Figure 2). Asian

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Table 1. Breed code, sample size, mean and total number of observed alleles, observed (H_O) heterozygosity, expected (H_E) heterozygosity, mean *P*-values, and per population F_{IS} across all 31 loci

Population	Code	Sample size	Mean no. observed alleles	Total no. observed alleles	H_O	H_E	Mean *P* value	F_{IS}
Bali dog	Bali	40	7.7	239	0.692	0.746	.354	0.097
Kintamani	Kinta	40	7.0	217	0.681	0.700	.359	0.089
Dingo	Dingo	20	4.6	144	0.426	0.524	.282	0.194
Chow Chow	Chow	20	5.3	165	0.640	0.649	.516	0.117
Akita	Akita	20	4.8	148	0.582	0.629	.513	0.145
American eskimo	AES	20	5.4	166	0.642	0.674	.472	0.123
Australian shepherd	AS	20	5.5	172	0.616	0.659	.424	0.139
Jack Russell terrier	JRT	20	6.3	194	0.723	0.731	.543	0.098
Pomeranian	PM	20	5.7	177	0.671	0.703	.463	0.119
Papillon	PN	20	5.5	172	0.671	0.705	.448	0.127
Rhodesian ridgeback	RR	20	4.7	146	0.611	0.626	.595	0.104
Yorkshire terrier	YT	20	5.6	175	0.671	0.700	.438	0.118
All			5.7	404	0.635	0.671	.451	0.122

breeds (Akita, Chow Chow), Australian dingo, Bali street dog, and Kintamani dog clustered together in 90% of the trees, separate from European breeds. The Chow Chow and Akita appeared to have branched from the common Asian limb, before the branching of SE Asian dogs. The long branch length of the Australian dingo indicates that dingoes diverged from indigenous SE Asian dogs in the distant past, but also after SE Asian dogs separated from their more northerly Asian ancestors. The Bali street dog and the Kintamani dog clustered together in 80% of the trees with

Table 2. Observed number of alleles, observed (H_O) heterozygosity, and pairwise F_{ST} for 31 loci for the Kintamani dog, Bali dog, Chow Chow, and Jack Russell terrier populations

		No. of observed alleles				Observed heterozygosity				Bali pairwise F_{ST} by locus		
	CFA	Kintamani	Bali dog	Chow	JRT	Kintamani	Bali dog	Chow	JRT	Bali dog	Chow	JRT
AHT111	CFA02	6	7	5	6	0.325	0.725	0.950	0.750	*0.041*	*0.086*	*0.252*
AHT121	CFA13	9	14	5	9	0.825	0.900	0.700	0.800	*0.025*	*0.031*	*0.004*
AHT130	CFA18	9	8	5	7	0.750	0.775	0.550	0.900	*−0.002*	*0.134*	*0.064*
AHT137	CFA11	11	9	6	7	0.825	0.850	0.700	0.750	*0.030*	*0.181*	*0.095*
AHT139	CFA15	3	4	5	3	0.300	0.275	0.750	0.400	*0.138*	*0.195*	*0.001*
C01.424	CFA01	4	5	5	5	0.525	0.450	0.550	0.550	*0.023*	*0.045*	*0.390*
C03.877	CFA03	5	10	4	6	0.850	0.875	0.500	0.750	*0.009*	*0.116*	*0.025*
C06.636	CFA06	8	7	3	5	0.675	0.600	0.450	0.700	*−0.003*	*0.004*	*0.302*
C08.618	CFA08	6	7	3	6	0.500	0.700	0.100	0.550	*0.016*	*0.114*	*0.332*
C09.250	CFA09	7	8	5	6	0.875	0.750	0.700	0.500	*0.026*	*0.080*	*0.151*
C10.404	CFA10	10	12	5	7	0.800	0.725	0.700	0.600	*0.021*	*0.127*	*0.193*
C14.866	CFA14	5	8	7	8	0.675	0.750	0.700	0.800	*0.043*	*0.249*	*0.213*
C20.446	CFA20	7	8	5	6	0.825	0.725	0.700	0.600	*0.106*	*0.232*	*0.173*
C22.279	CFA22	8	7	7	6	0.825	0.775	0.850	0.800	*0.012*	*0.118*	*0.066*
C23.123	CFA23	6	7	5	5	0.800	0.700	0.800	0.750	*0.016*	*0.037*	*0.041*
C28.176	CFA28	4	7	3	4	0.250	0.325	0.150	0.700	*0.007*	*0.012*	*0.277*
C31.646	CFA31	10	10	6	9	0.725	0.750	0.800	0.950	*0.030*	*0.025*	*0.018*
CPH02	CFA32	4	5	3	5	0.225	0.550	0.450	0.750	*0.128*	*0.374*	*0.235*
CPH03	CFA06	10	10	7	6	0.800	0.850	0.700	0.900	*0.020*	*0.171*	*0.033*
CPH08	CFA19	6	7	7	6	0.675	0.825	0.750	0.600	*0.010*	*0.098*	*0.227*
CPH16	CFA20	8	7	7	7	0.900	0.675	0.900	0.850	*0.032*	*0.116*	*0.054*
FH2001	CFA23	6	6	5	7	0.725	0.775	0.750	0.750	*0.049*	*0.172*	*0.151*
FH2004	CFA11	9	11	6	7	0.675	0.800	0.650	0.650	*0.015*	*0.077*	*0.056*
FH2054	CFA12	9	8	6	9	0.775	0.775	0.650	0.950	*0.012*	*0.045*	*0.030*
FH2140	CFA05	9	12	10	10	0.825	0.775	0.900	1.000	*0.022*	*0.105*	*0.059*
LEI002	CFA27	5	3	3	4	0.700	0.575	0.300	0.600	*0.038*	*0.288*	*0.017*
LEI004	CFA37	5	5	4	4	0.550	0.525	0.300	0.650	*0.007*	*0.054*	*0.040*
PEZ02	CFA17	9	9	6	5	0.800	0.775	0.900	0.650	*0.046*	*0.102*	*0.204*
PEZ08	CFA17	7	6	7	9	0.625	0.625	0.700	0.700	*−0.001*	*0.103*	*0.127*
RVC1	CFA15	6	4	5	5	0.650	0.500	0.700	0.700	*0.016*	*0.180*	*0.153*
VIASD10	CFA07	6	8	5	5	0.825	0.775	0.550	0.800	*0.006*	*0.036*	*0.050*
All		217	239	165	194	0.681	0.692	0.640	0.723	*0.029*	*0.123*	*0.131*

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Table 3. Nei's DA distance (lower triangle) and mean F_{ST} estimates (upper triangle) between each pair of 12 dog populations (see Table 1 for breed codes)

	Bali dog	Kintamani	Dingo	Chow	Akita	AES	AS	JRT	PM	PPN	RR	YT
Bali Dog		*0.029*	*0.125*	*0.092*	*0.126*	*0.141*	*0.136*	*0.107*	*0.110*	*0.127*	*0.146*	*0.125*
Kintamani	0.083		*0.146*	*0.123*	*0.149*	*0.173*	*0.178*	*0.131*	*0.138*	*0.151*	*0.189*	*0.159*
Dingo	0.242	0.263		*0.232*	*0.257*	*0.265*	*0.273*	*0.253*	*0.246*	*0.271*	*0.288*	*0.252*
Chow	0.242	0.274	0.397		*0.190*	*0.221*	*0.209*	*0.165*	*0.171*	*0.196*	*0.223*	*0.173*
Akita	0.286	0.303	0.414	0.363		*0.192*	*0.189*	*0.166*	*0.166*	*0.199*	*0.200*	*0.194*
AES	0.285	0.340	0.426	0.431	0.369		*0.098*	*0.101*	*0.099*	*0.117*	*0.129*	*0.128*
AS	0.266	0.311	0.423	0.377	0.358	0.198		*0.101*	*0.104*	*0.129*	*0.134*	*0.118*
JRT	0.251	0.276	0.432	0.324	0.345	0.214	0.201		*0.081*	*0.105*	*0.133*	*0.075*
PM	0.264	0.298	0.457	0.390	0.380	0.231	0.251	0.215		*0.108*	*0.127*	*0.108*
PPN	0.256	0.287	0.422	0.370	0.345	0.220	0.212	0.201	0.241		*0.157*	*0.127*
RR	0.278	0.330	0.435	0.396	0.369	0.246	0.217	0.259	0.276	0.243		*0.158*
YT	0.298	0.331	0.445	0.372	0.411	0.287	0.223	0.185	0.256	0.249	0.287	

relatively short branch lengths, though the branch length of the Kintamani is longer than that of the Bali street dog. Therefore, the Kintamani dog is derived from the indigenous feral dog population and is not a mixture of Chow Chow and native Bali dogs. The lack of significant bootstrap values between the Akita and Chow Chow or between these two breeds and the Bali street dog/Kintamani dog/Australian dingo cluster further refutes the local folklore that Kintamani dogs originated from Asian dogs.

Discussion

Origin of the Kintamani Dog

It is said that a wealthy Chinese man moved to Bali in the 1400s and brought with him his Chow Chow dog (Hartaningsih et al. 1999). He settled in the mountainous region of Kintamani and married into the Balinese family of King Jaya Pangus. The Chow Chow interbred with the local dogs and created a unique type—the Kintamani dog. This study confirms that the Kintamani dog was derived from the local feral Bali street dogs and not the Chow Chow. The even closer relationship of the Kintamani and Bali street dogs to Australian dingoes was significant and thus supports the theory that Australian dingoes originated from dogs of East Asia that followed human Austronesian expansion into the islands of SE Asia (Savolainen et al. 2004). The Australian dingo has been isolated from its parent population for ~5,000 years (Savolainen et al. 2004), yet both dingo and Bali feral dog populations remain genetically close. This suggests that the Bali street dog population has also been relatively isolated for millennia. The Kintamani and Bali street dogs showed different relatedness to AKC breeds, depending on whether the breed was of European or Asian origin. Asian breeds were more closely related to SE Asian dogs than European breeds, supporting the concept that the various dog breeds have evolved as human races have evolved.

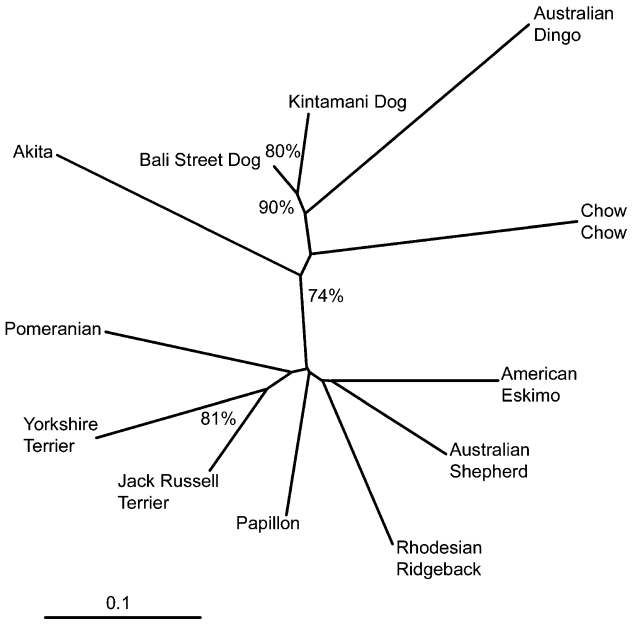

Figure 2. Unrooted neighbor joining dendogram showing the genetic relationships among 12 dog populations using Nei's DA genetic distance. Bootstrap values above 70% are shown.

Selection for the Kintamani Dog Phenotype

The Kintamani dog phenotype is associated with the husbandry practices of the farmers of the Kintamani region over hundreds or thousands of years. Kintamani bitches, presumably those that were more tractable and of the "desirable type," have been kept by regional farmers and their puppies sold as pets. Bitches are allowed to randomly mate with males roaming the area; therefore, phenotypic selection involves mainly females. In contrast, comparatively few founder animals have been used in many modern Western breeds (Neff et al. 2004) and the influence of a few males has been inordinately great (Bannasch et al. 2005). The formation of the Kintamani dog parallels that of older and more regional breeds. Vila and colleagues (1999) concluded that dog breeds originate from a larger number of founder animals in the indigenous dog population. The fact that significant phenotypic changes can be made in a breed without losing genetic diversity has also been demonstrated for eight Japanese breeds (Okumura et al. 1996).

Even though Kintamani dogs and Bali street dogs were virtually identical at the loci tested, there was nonetheless

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evidence for genetic selection. The Kintamani dog tended to have the 153, rather than 149, allele at AHT139. The 197 allele of C20.446 had a 43.8% frequency in the Kintamani dog, but 5% or lower for all the other dog populations. Kintamani dogs did not display the 106 allele at CPH02, an allele that is fairly common in the Bali street dog and several of the AKC breeds. In contrast, the frequency of allele 96 of CPH02 was 88.8%, similar to the dingo and Akita but not other populations.

Implications for Future Breed Development

The Kintamani dog is currently the second (to the Bali street dog) most genetically diverse dog population studied at this laboratory. As the Kintamani dog gains local and international recognition, it will undoubtedly be selected for formal breed status. Such recognition usually involves the creation of a specific phenotypic standard and ultimately the closing of the founder population. Whether the Kintamani dog retains its great genetic diversity and robust health will be determined by the stringency of the breed standard and the breeding practices employed to meet that standard. The Kintamani dog has been established to date with virtually no loss of genetic diversity yet remains in the early stages of breed development. It could be possible to maintain that diversity with careful genetic testing and selective breeding. Emerging breeds such as the Kintamani dog should serve as a paradigm for how a breed can be developed, standardized, and maintained (Meyers-Wallen 2003).

Supplementary Data

Supplementary tables are available at *Journal of Heredity* online (www.jhered.oxfordjournals.org).

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