Microsatellite Variation in the Australian Dingo

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The dingo is thought to have arrived in Australia from Asia about 5,000 years ago. It is currently in danger because of interbreeding with domestic dogs. Several morphological, behavioral, and reproductive characteristics distinguish dingoes from domestic dog. Skull morphometrics are currently used to try to classify wild canids as pure dingo, dog, or hybrid. Molecular techniques based on diagnostic DNA differences between dogs and dingoes would make a much more reliable and practical test. A small number of markers (about 10) would allow detection of animals with domestic dog in their ancestry several generations back. We have typed 16 dingoes and 16 dogs of mixed breed for 14 microsatellites. The amount of variation in the Australian dingo is much less than in domestic dogs. The size distributions of microsatellites in the two groups usually overlap. The number of alleles in the dingo is much smaller in all cases. One dinucleotide repeat locus shows a size difference of 1 bp in allele classes between dog and dingo. This locus may be diagnostic for dog or dingo ancestry. The differences in distributions of alleles at other loci can also be used to classify animals using a likelihood method.

Dingoes are a type of Asian dog which is possibly derived from the Indian or Arabian wolf by domestication not more than 10,000 years (Corbett 1995). This is based on the similarity of head shape of these wolves and dingoes, which is different from domestic dogs. Analysis of mitochondrial data suggests dogs and dingoes have a common origin of domestication at a much earlier time (Vilà et al. 1997). Dingoes have been spread throughout Southeast Asia and the Pacific by man. They probably first arrived in Australia less than 5,000 years ago (Corbett 1995).

Although dingoes have many distinct physical and behavioral characteristics that differentiate them from domestic dogs, such as an annual breeding cycle and no barking, the two species interbreed and produce fertile offspring as do all wolflike species. Western influences have lead to the introduction of domestic dogs throughout the dingoes range. The proportion of pure dingoes in the wild canid population is steadily decreasing, with large proportions (>80%) known only in Thailand and Australia (Corbett 1995). Within Australia, hybridization occurs most readily in highly populated areas, with populations from the east coast, southeast, and southwest containing mostly hybrids. The more isolated central and northern areas have the largest proportions of pure dingoes, but with an increasing number of domestic dogs on properties and in aboriginal camps, the dingo is under threat even in these areas.

If the Australian dingo is to be preserved, a conservation program needs to be undertaken. Public education is probably the best way to reduce the number of dogs available for hybridization in the wild and several groups are working toward this end. Captive breeding programs are the best way to ensure the long-term future of the Australian dingo, and zoos, wildlife parks, and dingo associations are undertaking this. However, the genetic purity of the breeding stock needs to be assured. Many animals in captivity come from southeast Australia where the proportion of pure dingoes is estimated to be as little as 22% in one population with a maximum of 65% (Corbett 1995; Newsome and Corbett 1985).

Current methods of assessing dingo purity are based on skull measurements or physical appearance (Newsome and Corbett 1982, 1985; Newsome et al. 1980). According to Corbett (1995) external body characteristics are unreliable for classification even when applied by the most experienced dingo experts. Skull measurements are reliable for distinguishing pure

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Table 1. Allele frequency distributions from 16 dogs and 16 dingoes at 14 microsatellite loci showing number at smallest allele (A) and at alleles larger by *n* base pairs (A + *n*) assuming cases with a single allele are homozygotes

Locus[a] (size of A)	Group	Numbers observed at relative allele size												N^b	Alleles	H_e^b	H_o^b
		A	A+2	A+4	A+6	A+8	A+10	A+12	A+14	A+16	A+18	A+24	A+26				
AHT103 (77 bp)	DOG	3	6	13	5	2	0	1	0					30	6	0.73	0.67
	DINGO	0	3	0	4	1	25	0	1					34	5	0.44	0.47
AHT107 (77 bp)	DOG	4	0	1	2	4	1							12	5	0.74	0.83
	DINGO	0	0	0	0	8	0							8	1	0.00	0.00
AHT109 (143 bp)	DOG	11	0	2	8	9								30	4	0.70	0.53
	DINGO	0	0	26	0	4								30	2	0.23	0.27
AHT126 (188 bp)	DOG	1	0	0	2	12	5	0	6	0				26	5	0.69	0.54
	DINGO	0	0	0	1	4	0	0	26	1				32	4	0.32	0.25
CXX101 (120 bp)	DOG	3	1	1	1	5	6	6	3	6				32	9	0.85	0.88
	DINGO	0	0	0	0	2	8	26	1	3				40	5	0.53	0.30
CXX123 (142 bp)	DOG	0	3	0	6	6	2	1						18	5	0.74	1.00
	DINGO	2	18	0	4	0	0	0						24	3	0.40	0.33
CXX127 (171 bp)	DOG	9	0	0	0	1	16	4	2					32	5	0.65	0.69
	DINGO	9	0	0	0	12	4	5	2					32	5	0.74	0.13
CXX204 (201 bp)	DOG	3	3	0	3	0	0	2	21					32	4	0.54	0.56
	DINGO	7	1	0	0	0	0	0	24					32	3	0.39	0.50
CXX263 (168 bp)	DOG	1	3	4	1	0	1	0	0	1	6	2	1	20	9	0.83	0.80
	DINGO	0	0	12	0	5	0	0	0	1	2	0	0	20	4	0.57	0.70
LEI008 (150 bp)	DOG	7	0	1	4	11	0	2	0	1				26	6	0.72	0.62
	DINGO	1	0	0	1	6	26	0	0	0				34	4	0.38	0.25
		A	A+3	A+4	A+5	A+7	A+9	A+10	A+13	A+14	A+15	A+16	A+25				
CXX30 (143 bp)	DOG	0	7	10	3	1	5	0	2	0	1	0	3	32	8	0.81	0.81
	DINGO	2	0	0	0	0	0	4	0	24	0	2	0	32	5	0.41	0.38
CXX255 (157 bp)	DOG	2	14	0	1	15								32	4	0.58	0.63
	DINGO	0	14	0	15	3								32	3	0.58	0.50
		A	A+2	A+21	A+23	A+25	A+27	A+29									
CXX377 (143 bp)	DOG	0	13	2	3	4	0	2						24	5	0.65	0.58
	DINGO	7	22	0	1	0	0	0						30	3	0.41	0.53
		A	A+2	A+4	A+6	A+7	A+9	A+11	A+13	A+15	A+17	A+19					
AHT125 (88 bp)	DOG	4	2	1	1	1	7	1	0	8	0	1		26	9	0.80	0.69
	DINGO	0	0	0	0	0	4	10	12	6	0	0		32	4	0.71	0.81

[a] CXX loci were developed by Ostrander et al.; AHT loci were developed at Animal Health Trust, UK; and LEI008 at Leicester, UK.

[b] N = total number of alleles; H_e = expected heterozygosity, $(1 - \text{sum}(p^2))$; H_o = observed proportion of heterozygotes.

dingoes from pure dogs, but it may take many years before a wild-caught animal used for breeding dies and skull measurements can be taken to assess its purity. Alternative methods of taking measurements such as X-rays or CAT scans of the skull are possible but impractical. Also, skull measurements are less useful for detecting backcross dingoes, for example, 3/4 dingoes, etc.

Alternative methods of assessing dingo purity need to devised. A number of diagnostic DNA markers can be used to assess the genetic background of a presumed dingo. The larger the number of markers, the smaller the proportion of dog ancestry that can be detected.

Development of diagnostic DNA markers will allow the maintenance of the dingo as a distinct subspecies. Attempts to use isozyme markers for this purpose were not successful (Cole et al. 1977). It is the recent availability of microsatellite and other molecular markers in dogs that make it practical now.

Highly variable genetic markers are likely to be useful because differences may have accumulated between dingoes and dogs over time. Microsatellites or simple sequence repeats are highly variable and there are a large number of microsatellite markers available for the dog (for example, Holmes et al. 1993; Ostrander et al. 1993). Dog microsatellites have been successfully used to analyze the population structure of other canids such as wolves and coyotes (Roy et al. 1994).

Materials and Methods

DNA Samples

Only captive dingoes of known origin were included in this study. Blood samples were provided by the Australian Native Dog Conservation Society from their colony of about 40 dingoes at Merigal. The Australian Dingo Conservation Association supplied 45 samples from dingoes housed with their members. Samples were obtained from animals at Northern Territory Wildlife Park (6), Taronga Zoo, Healesville Sanctuary, and Perth Zoo. Sixteen unrelated samples were selected from these for this initial screen.

Dingoes or wild dogs are still being culled for protection of stock and to control the spread of hydatids. We have a large collection of skin and tissue samples from these animals that we will analyze in the future.

DNA was extracted from blood samples collected from mixed breed domestic dogs at the Medical Research Institute, Prince of Wales Hospital.

Microsatellite Typing

Fourteen previously described canine microsatellite loci were typed (Table 1; Holmes et al. 1993, 1994; Mellersh et al. 1994; Ostrander et al. 1993). Primers were provided by the Victorian Institute of Animal Science or purchased from Genetics Research Inc. Both fluorolabeled primers and unlabeled primers were used. For reactions where both primers were unlabeled, 500 pmoles of R110-labeled dUTP was added. Ten-microliter reactions were run with 40 ng DNA, 2.5 mM $MgCl_2$, 200 μM

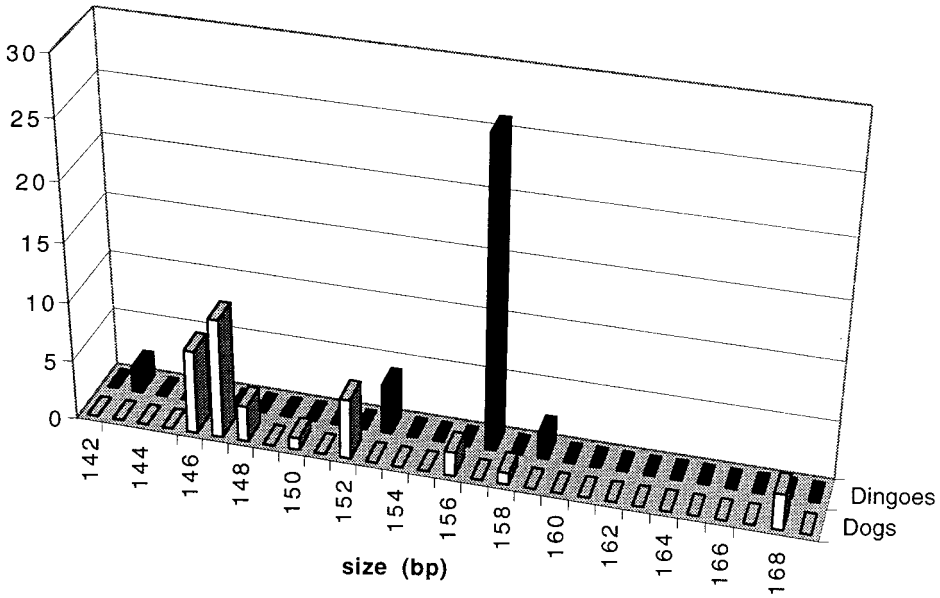

Figure 1. Distribution of allele sizes for microsatellite CXX30 for 16 dogs and 16 dingoes.

of each dNTP, 125–500 pmoles forward and reverse primers, 0.1 U AmpliTaq polymerase (Perkin-Elmer) in 10 mM Tris-HCl (pH 8.3), and 50 mM KCl. Reactions were carried out in thick-walled microfuge tubes under cycling conditions of 94°C for 3 min followed by 28 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 1 min, with a final 5 min step at 72°C. PCR products were run with GS500 size standards on ABI 377 automated DNA sequencers using GeneScan (ABI) and results processed with Genotyper software (ABI).

Results

We have tested 14 canine microsatellites in the dingo and all amplify fragments of similar size to the dog and all are polymorphic in a sample of 16 crossbred dogs and 16 dingoes (Table 1). Most microsatellites show different distributions of alleles in the two groups with an overlap in allele sizes in 13 of 14 loci. For one locus, CXX30, there is no allele common to both groups (Figure 1). For this dinucleotide repeat, dingoes have allele sizes which differ by 1 bp from some dog alleles. If this difference holds up with a larger sample size, the locus would make a good diagnostic marker for introgression of dog genes.

The level of variation in the dingo is noticeably lower than in domestic dogs at all loci, as shown by the heterogeneity estimates in Table 1. Locus CXX127 shows a large deficit of observed heterozygotes compared to that expected in the dingo. The allele sizes reported here for CXX109, CXX123, CXX204, and CXX377 are approximately 40 bp larger than those reported for domestic dogs in a study on Mexican wolves (García-Moreno et al. 1996). Genescan gels and sequencing gels with ^{32}P-labeled products and *Msp*I cut pBR322 as size standards give similar size estimates for these loci.

Discussion

The method of incorporating fluorolabeled dUTPs during PCR labels both DNA strands of the product. The two strands often migrate at different rates on denaturing sequencing gels and are both detected. Allele calling is possible by recognizing patterns of peaks that represent a single allele, but is not as simple as when fluorolabeled primers are used.

We cannot be absolutely certain of the purity of the dingoes used in this study. The animals have been classed as dingoes on the basis of external physical characteristics and, for many that were born in captivity, their status has been supported by the classification of their parents as pure dingoes based on skull measurements. The difference between the allele distributions for dogs and dingoes suggests that there is not a large amount of contamination of the dingo stock. The lack of sharing of any alleles at locus CXX30 suggests that none of the dingoes used are hybrids. To determine the genotypes at the test loci in the dingo before the introduction of dogs, typing of DNA from old preserved specimens is recommended.

There is much lower heterozygosity in dingoes than dogs at all loci and fewer alleles at each locus. This could represent a small founding population for dingoes in Australia or may be characteristic of dingoes in general. Locus CXX127 shows a large excess of apparent homozygotes (Figure 2). There are two alleles with relatively high frequencies but very few het-

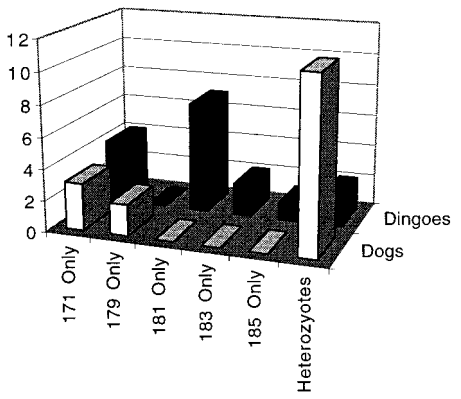

Figure 2. Genotypes at locus CXX127 for 16 dogs and 16 dingoes.

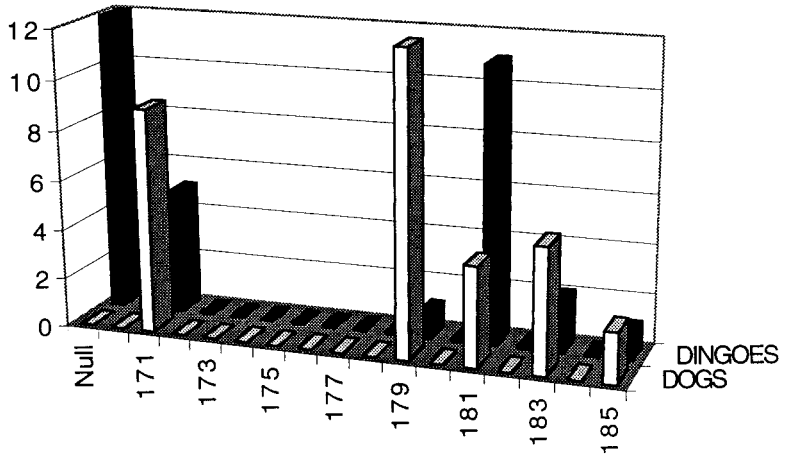

Figure 3. Distribution of alleles in 16 dogs and 16 dingoes at locus CXX127 including estimated null allele numbers.

erozygotes. Even the two rare alleles appear alone. This suggests that a null allele is present in dingoes and many apparent homozygotes are heterozygotes for the null. The estimated frequency of the null is shown in Figure 3.

Since only 14 loci have been tested and one looks promising as a diagnostic marker, it suggests that other microsatellite loci will be useful as genetic markers for detecting dog ancestry in Australian wild dogs. Microsatellites that have different allele size distributions in the two groups but still have alleles in common can also be used to determine an animal's origins based on likelihood estimates. The fewer alleles shared the more useful the marker. One marker has been identified as possibly diagnostic for dog or dingo origins. This will be useful for detecting offspring from dingo-dog crosses, but one locus is not sufficient to reliably detect backcross progeny. More diagnostic markers are needed. Microsatellite loci will also be useful in examining the possible population subdivision of the Australian dingo into tropical, desert, and alpine races (Corbett 1995). If there are marked genetic differences between these groups which are not related to the level of introgression of dog genes, then several different breeding programs may be needed to preserve each race separately.

References

Cole SR, Baverstock PR, and Green B, 1977. Lack of genetic differentiation between domestic dogs and dingoes at a further 16 loci. Aust J Exp Biol Med Sci 22: 229–232.

Corbett, LK 1995. The dingo in Australia and Asia. Sydney: University of New South Wales Press.

García-Moreno J, Matocq MD, Roy MS, Geffen E, and Wayne RK, 1996. Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. Conserv Biol 10:376–389.

Holmes NG, Mellersh CS, Humphreys SJ, Binns MM, Holliman A, Curtis R, and Sampson J, 1993. Isolation and characterization of microsatellites from canine genome. Anim Genet 24:289–292.

Holmes NG, Strange NJ, Mellersh CS, Humphreys SJ, and Sampson J, 1994. Three polymorphic canine microsatellites. Anim Genet 25:200.

Mellersh CS, Holmes NG, Binns MM, and Sampson J, 1994. Dinucleotide repeat polymorphisms at four canine loci (LEI003, LEI007, LEI008 and LEI015). Anim Genet 25:125.

Newsome AE and Corbett LK, 1982. The identity of the dingo. II. Hybridisation with domestic dogs in captivity and in the wild. Aust J Zool 30:365–374.

Newsome AE and Corbett LK, 1985. The identity of the dingo. III. The incidence of hybrids and their coat colours in remote and settled regions of Australia. Aust J Zool 33:363–375.

Newsome AE, Corbett LK, and Carpenter SM, 1980. The identity of the dingo. I. Morphological discrimination of the dingo and dog skulls. Aust J Zool 28:615–625.

Ostrander EA, Sprague GF, and Rine J, 1993. Identification and characterization of dinucleotide repeats (CA)n markers for genetic mapping in dog. Genomics 16: 207–213.

Roy MS, Geffen E, Smith D, Ostrander EA, and Wayne RK, 1994. Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Mol Biol Evol 11:553–570.

Vilà C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, and Wayne RK, 1997. Multiple and ancient origins of the domestic dog. Science 276:1687–1689.

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