NOTE *Anatomy*

Polymorphism of rRNA Gene Loci in the Dog

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(Received 17 June 2010/Accepted 28 October 2010/Published online in J-STAGE 11 November 2010)

ABSTRACT. We examined the number of ribosomal gene (rDNA) loci in the metaphase spreads of 54 dogs by FISH method. We found that in 16 dogs (30%) one or two loci were missing. The total number of rDNA loci was varied from 5 to 7 in males and from 4 to 6 in females. As the male dog consistently bears the rDNA on the Y chromosome, the polymorphism of the rDNA locus was ascribed to the absence of autosomal rDNA loci. Indeed, the frequency of polymorphism is almost equivalent in both sexes in the beagle. In one female beagle dog, remarkable intense fluorescence signals were observed at the four autosomal loci, indicating the *in situ* amplification of rDNA.

KEY WORDS: canine, chromosome, FISH, polymorphism, rDNA.

J. Vet. Med. Sci. 73(4): 475–477, 2011

In mammals, a cluster of tandem repeats of rRNA gene (rDNA) localizes on a single or multiple chromosomes in a species-specific manner. The polymorphisms of the rDNA loci were reported in a variety of species, especially in those with the rDNA loci located on the acrocentric chromosomes such as in the mouse [1, 9, 11, 15] and humans [7, 8]. In the dog, rDNA loci were mapped to the terminal ends of the long arms of three autosomes (#7, #11, and #27) and to the terminal end of the short arm of the Y chromosome by AgNOR method (10), or to those of chromosome #7, #9 and #22 by *in situ* hybridization autoradiography [6]. The equivalent number of rDNA loci was reported by fluorescence *in situ* hybridization (FISH) method [5, 12]. In this study, we first examined the number of rDNA loci in the metaphase spreads of the crossbreed dogs by FISH and found remarkable polymorphism in the autosomal loci or rDNA. We further investigated the rDNA loci of the inbred beagle dog that is widely used as a model experimental animal. This report represents, for the first time, the polymorphism of rDNA loci and its frequency in the dog.

The animals were 42 beagle dogs (♂ : 24, ♀ : 18) and 4 breeds (♂ :4) and 8 crossbreeds (♂ :6, ♀ :2), as detailed in Tables 1 and 2. Peripheral blood lymphocytes isolated using Ficol Hypaque PULS (GS Lifecare Sci., U.S.A.) were cultured in RPMI medium supplemented with 10% fetal bovine serum and pokeweed mitogen (2 μg/m*l*, L8777, Sigma, U.S.A.). After 48 hr culture, cells were treated with colcemid for 30 min. Subsequently, the lymphocytes were harvested by centrifugation and treated with 0.075 M KCl hypotonic solution for 15 min followed by fixation with acetic acid-methanol (1:3). Air-dried chromosome preparations were dried at 37°C for a few days in an incubator. For FISH analysis, chromosomal DNA was denatured in 70% formamide in 2× SSC at 70°C for 2 min and hybridized with the biotin labeled rDNA as described previously [9]. Human 28S-rDNA clone [pHr14E3, Human Science Research Resourse Bank (HSRRB), Japan] was labeled with biotin-dUTP by nick translation and denatured in formamide at 70°C, and then hybridized to the denatured chromosomal DNA at 37°C overnight. After washing the slide with 50% formamide in 4× SSC once, and subsequently in 4× SSC for three times, FITC-avidine was applied to detect the biotin-dUTP on the chromosomes. Fluorescence signals were captured by a CCD camera (Hamamatsu Photonics, Japan).

We found remarkable polymorphism in rDNA loci of the dog. We examined the number of rDNA loci in twenty lymphocytes of an individual dog and found that each dog bears constant number for DNA loci in all cells. The loci were detected at the telomeric ends of three pairs of acrocentric autosomes and Y chromosome, although one or two autosomal loci were absent in 16 dogs (30%). Total number of the rDNA loci in the metaphase spreads of 42 beagle dogs, 4 breeds and 8 crossbreeds are summarized in Tables 1, 2. The number of the loci varied from 5 to 7 in the male (Fig. 1a–c) and from 4 to 6 in the female (Fig. 1d and 1e). As the rDNA locus on Y chromosome was consistently observed in the male (Fig. 1a–c), the difference in the number of the total rDNA loci between male and female dogs was attributed to the polymorphisms of the autosomal loci. As shown in Table 1, the incidence of locus polymorphism of rDNA was equivalent in both sexes of the beagle dog (Table 1). In the beagle, single-locus polymorphism was observed in 21–22% and double-locus polymorphism in 4–6% of the dogs examined. Such polymorphism was found in both breeds and crossbreeds of dogs as shown in Table 2. In one female beagle dog, remarkably large rDNA loci were found on the autosomes as shown in Fig. 1g.

Polymorphism found in the rDNA loci of the dog sug-

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Table 1. Number of the rDNA loci in 42 beagle dogs

Total number of rDNA loci	Number of the dogs examined	
	♂	♀
7	18 (75%)	0 (0%)
6	5 (21%)*	13 (72%)
5	1 (4%)**	4 (22%)*
4	0 (0%)	1 (6%)*
Total	24 (100%)	18 (100%)

Absence of a single rDNA ( )* or doubule loci( )**.

Table 2. Number of the rDNA loci in the dog breeds and crossbreeds

Dogs	Sex	Number of rDNA loci
Breeds		
French bulldog	♂	7
Scottish terrie	♂	6
Japanese spits	♂	7
Japanese shiba	♂	7
Crossbreeds		
1	♂	6
2	♂	6
3	♂	7
4	♂	6
5	♂	7
6	♂	7
7	♀	5
8	♀	6

gests that the rDNA loci localizing on the acrocentric chromosome are prone to translocate to other chromosomes. Indeed, the polymorphisms of the rDNA loci were found frequently in the species that bears the rDNA locus on the acrocentric chromosomes such as mouse species [1, 9, 11, 15] and human [7, 8]. As the autosomes of the dog chromosomes were all acrocentric and rDNA loci located at the telomeric end, we postulate that dog chromosomes may still expand the rDNA loci to other chromosomes by translocation over evolutionary times as has been shown for the *Mus musculus* species by FISH [9]. Such expansion of rDNA loci was reported in Indian pigmy field mice (*Mus terricolor*) in which 34 chromosomes bore rDNA in the metaphase spread [2].

The observed polymorphism of rDNA loci may be derived by the loss or gain of rDNA by translocation or an unequal crossover. Despite such polymorphisms, the loci were stably maintained on the specific chromosomes in the mouse and human [1, 8], suggesting that such loss or gain may be a rare event, and that the driving force to expand the locus by translocation might be exceeding to the loss of the corresponding loci, resulting in the stability of the rDNA loci once acquired by translocation. Indeed, in the present study, the rDNA locus on the Y chromosome was consistently maintained in all male dogs. We postulate that the absence of the rDNA loci on the corresponding dog chromosomes might reflect a genuine absence of the locus rather than the loss of rDNA by translocation.

The polymorphic nature of rDNA loci in mammals further suggests that the increase of the rDNA copy number beyond the essential necessity for cell function may have evolved together with special genetic mechanisms to control the number of active and inactive rDNA in the cell. Recent advances in molecular studies of rDNA suggest that the copy number of functional rDNA may be controlled by the epigenetic modifications of rDNA, regardless of the copy number variations of rDNA [13]. The excess copies of the rDNA on the dog Y chromosome may be balanced similary by such mechanisums.

The giant rDNA loci observed in one female beagle dog (Fig. 1g) suggest that the copy number of rDNA is unstable due to *in situ* amplification by unequal crossover of rDNA. It is supposed that the mechanism to maintain the sequence homology of rDNA copies may play a key role in such crossovers as has been proposed by concerted evolution hypothesis for rDNA [4].

ACKNOWLEDGMENTS. We wish to thank Drs. Yoshihisa Yamane and Toshiro Iwasaki of Department of Veterinary Medicine, Tokyo University of Agriculture and Technology for their supply of the dog blood samples.

REFERENCES

1. Akeson, E. C. and Dasisson, M. T. 1996. Nucleolus organizer regions. pp. 1509–1511. *In*: Genetic Variants and Strains of the Laboratory Mouse (Lyon, M. F., Rastan, S. and Browns, S. D. M. eds.), Oxford University Press, Oxford.
2. Bardhan, A. and Sharma, T. 2000. Dynamic of nucleolus organizer regions and karyotype evolution in Indian pygmy field mice. *Cytogenet. Cell Genet*. **91**: 47–51.
3. Breen, M. 2008. Canine cytogenetics—from band to basepair. *Cytogenet. Cell Genet*. **120**: 50–60.
4. Eickbush, T. H. and Eickbush, D. G. 2007. Finely orchestrated movements: Evolution of the ribosomal RNA genes. *Genetics* **175**: 477–485.
5. Fisher, P. E., Holmes, N. G., Dickens, H. F., Thomas, R., Binns, M. M. and Nacheva, E. P. 1996. The application of FISH techniques for physical mapping in the dog (*Canis familiaris*). *Mamm. Genome* **7**: 37–41.
6. Fujinaga, T., Yamashit, M., Yoshida, M. C., Mizuno, S., Tajima, M., Okamoto, Y. and Otomo, K. 1989. The banding patterns of normal canine chromosomes. *Jpn. J. Vet. Sci.* **51**: 294–299.
7. Garkavtsev, I. V., Tsvetkova, T. G., Yegolina, N. A. and Gudkov, A. V. 1988. Variability of human rRNA genes: inheritance and nonrandom chromosomal distribution of structural variants of nontranscribed spacer sequences. *Hum. Genet*. **81**: 31–37.
8. Hirai, H., Taguchi, T. and Godwin, K. 1999. Genomic differentiation of 18S ribosomal DNA and β-satellite DNA in the hominoid and its evolutionary aspects. *Chromosome Res*. **7**: 531–540.
9. Ito, T., Tsuchiya, K., Osawa, S., Shibata, H. and Kanda, N. 2008. Mapping of rRNA gene loci in the mice, *Mus musculus molossinus* (Japan) and *Mus musculus musculus* (Russia) by double color FISH. *J. Vet. Med. Sci*. **70**: 997–1000.

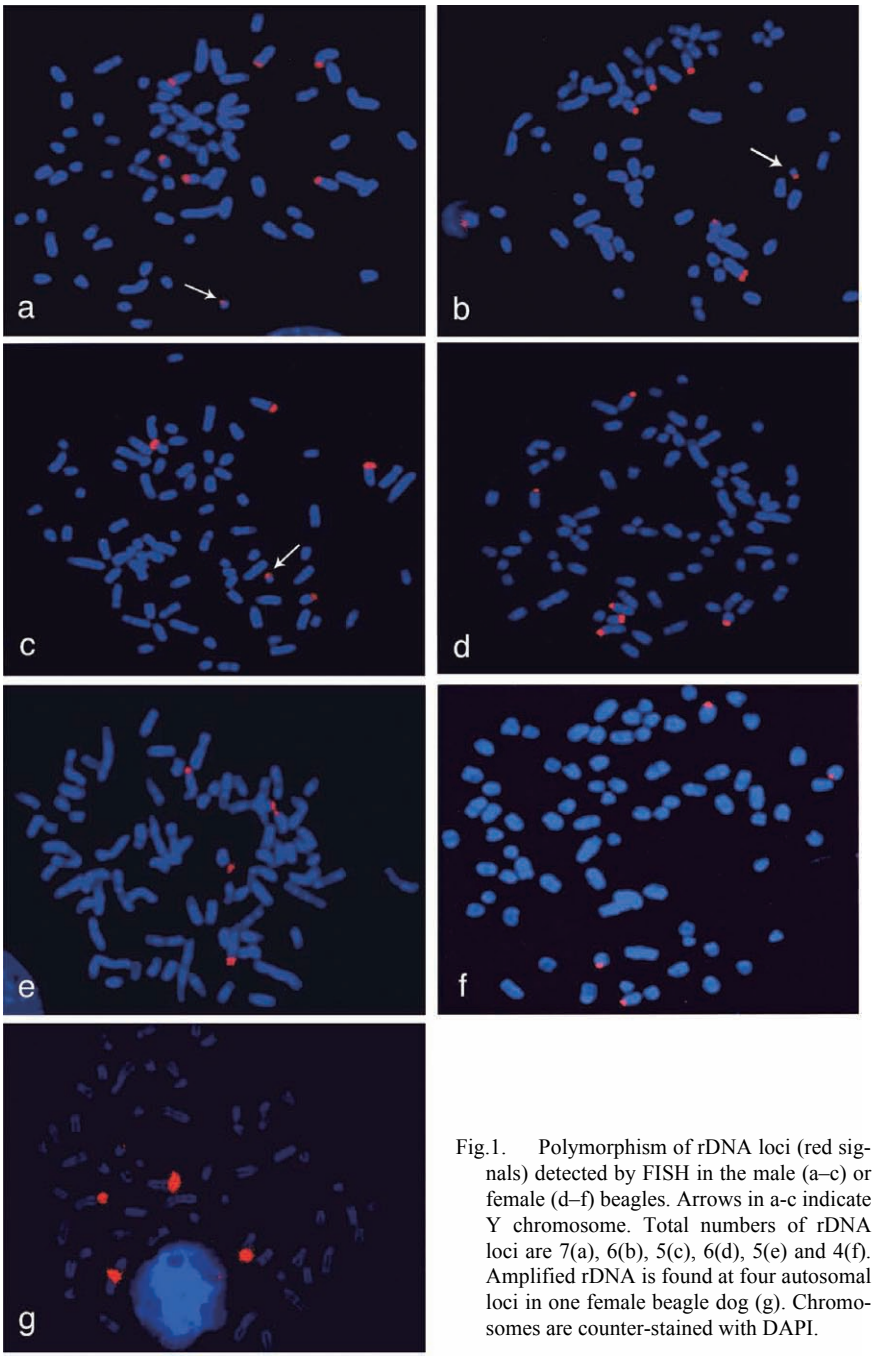


Fig.1. Polymorphism of rDNA loci (red signals) detected by FISH in the male (a–c) or female (d–f) beagles. Arrows in a-c indicate Y chromosome. Total numbers of rDNA loci are 7(a), 6(b), 5(c), 6(d), 5(e) and 4(f). Amplified rDNA is found at four autosomal loci in one female beagle dog (g). Chromosomes are counter-stained with DAPI.

10. Kopp, E., Mayr, B. and Schieger, W. 1982. Nucleolus organizer regions on chromosomes of the demestic dog. *J. Heredity* **73**: 230.
11. Kurihara, Y., Suh, D. S., Suzuki, H. and Moriwaki, K. 1994. Chromosomal locations of Ag-NORs and clusters of ribosomal DNA in laboratory strains of mice. *Mamm. Genome* **5**: 225–228.
12. Makinen, A., Zijlstra, C., de Haan, N. A., Mellink, C. H. M. and Bosma, A. A. 1997. Localization of 18S+28S and 5S ribosomal RNA genes in the dog by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet*. **78**: 231–235.
13. McStay, B. and Grummt, I. 2008. The epigenetics of rRNA genes: From molecular to chromosome biology. *Annu. Rev. Cell Dev. Biol*. **24**: 131–157.
14. Pathak, S., Van Tuinen, P. and Merry, D. E. 1982. Heterochromatin, synaptonemal complex, and NOR activity in the somatic and germ cells of a male domestic dogs, *Canis familiaris* (mammalia, Canidae). *Cytogenet. Cell Genet*. **34**: 112–118.
15. Suzuki, H., Kurihara, Y., Kanehisa, T. and Moriwaki, K. 1990. Variation in the distribution of silver-staining nuceleolar organizer regions on the chromosomes of the wild mouse, *Mus musculus*. *Mol. Biol. Evol*. **7**: 271–282.