NOTE *Anatomy*

Identification of rRNA Gene Loci in the Wild Mouse (*Mus musculus molossinus*) Captured at Hachioji, Tokyo

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(Received 7 June 2007/Accepted 2 August 2007)

ABSTRACT. *Mus musculus* (*M. m.*) *molossinus* has been considered an independent subspecies of *Mus musculus*. To elucidate the evolutional origin of this subspecies, we carried out double-color FISH using 18s-28s ribosomal DNA and mouse chromosome paint probes. Among eleven rDNA loci detected, five loci on chromosomes 12, 15, 16, 18 and 19 were common to both *Mus musculus* (*M. m.*) musculus and *M. m. molossinus* and the other six loci, on chromosomes 1, 5, 10, 11, 13 and 17, were characteristic in *M. m. molossinus*. As *M. m. molossinus* is thought to originate from a hybrid between ancestral colonies of *M. m. musculus* and *Mus musculus castaneus*, we supposed that these six rDNA loci might have evolved after geographical isolation of the ancestral hybrid animals from *M. m. musculus* and *M. m. castaneus*.

KEY WORDS: FISH, *Mus musculus molossinus*, rDNA.

J. Vet. Med. Sci. 69(12): 1277–1279, 2007

The ribosomal RNA genes (rDNA) are reiterated within the genome and the increase in the number of rRNA genes is roughly correlated with the evolutionary increment in the DNA content per haploid genome [8]. However, large variations in rDNA copy numbers exist not only between closely related species, but also between different strains of the same species. In laboratory strains of the mouse, rDNA loci have been mapped by *in situ* hybridization autoradiography, and the gene clusters were mapped on chromosomes 12, 15, 16, 18, and 19, although a large difference in the number of loci among these mouse strains has been reported [1–3, 5]. Furthermore, genetic variation among mouse strains were extensively studied based on the polymorphism of microsatellite DNA loci, demonstrating the wide-spread polymorphism of microsatellite loci in the mouse strains [7].

M. m. molossinus has long been thought to be an independent subspecies of *Mus musculus* [4]. Molecular analyses of mitochondrial DNA have suggested that this subspecies evolved from an ancestral hybrid between *M. m. musculus* and *M. m. castaneus* [10]. Suzuki *et al.* analysed the distribution of nucleolar organizer region (Ag-NORs) in *M. m. molossinus* and found the variation in the number of loci of NOR from 3 to 8 in 21 mice [9]. *M. m. molossinus* is characterized by multiple rDNA loci on eleven pairs of autosomes, as shown by the direct R-banding FISH method [6]. In the present study, we reexamined the chromosomal rDNA loci of this subspecies by double-color fluorescence *in situ* hybridization (FISH) using mouse chromosome paint probes.

Lymphocytes were isolated from the spleen of a male *M. m. molossinus* captured at Asakawa, Hachiouji. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 3 μg/m*l* conacavalin A (Sigma), 10 μg/m*l* lipopolysaccharide (Sigma) and 5×10^{-5} M mercaptoethanol. The cells were cultured for 48 hr and then colcemid (0.02 μg/m*l*) was added 60 min before harvesting. The cell suspension was centrifuged and the collected cells were treated with 0.075 M KCl for 15 min and fixed with 3:1 methanol: acetic acid. After fixation at 4°C o/n, the cells were resuspended in fresh fixative and air-dried chromosome slides were prepared following the standard protocol. The slides were dried at 37°C for a few days prior to FISH.

Human rDNA clone (pHr14E3, Human Science Research Resource Bank (HSRRB).) was labeled with digoxigenin-11-dUTP by nick translation and ethanol-precipitated with salmon sperm DNA and tRNA. This probe DNA was dissolved in 8 μ*l* of 100% formamide and denatured at 75°C for 10 min. The mouse paintings (Cambio) were denatured for 10 min following the manufacturer's protocol. The labeled probes, 8 μ*l* of rDNA and 3 μ*l* of chromosome paints, were mixed with 11 μ*l* of hybridization buffer (10 × SSC, BSA and 50% dextran sulfate). Prior to hybridization, the slides were treated with RNase (100 μg/m*l* in 2 × SSC) for 1 hr to remove the rRNA in the cells. The slides were washed three times in 2 × SSC for 3 min each, dehydrated in 70, 90 and 100% ethanol series for 3 min each, denatured at 70°C for 2 min in 70% formamide in 2 × SSC and dehydrated in the ethanol series at 4°C. The probe mixture was placed on slides and covered with parafilm for 30–60 min. Hybridization was carried out at 37°C in a moist chamber o/n. The slides were washed with 50% formamide at 37°C for 5 min followed by three washes with 4 × SSC at 37°C. Probe DNAs were detected by avidin-FITC and anti-dig-rhodamine and fluorescence signals were captured by a CCD camera (Hamamatsu Photonics).

Eleven rDNA loci were mapped at the distal ends of the autosomes in *M. m. molossinus* (Table 1, Fig. 1). A rDNA

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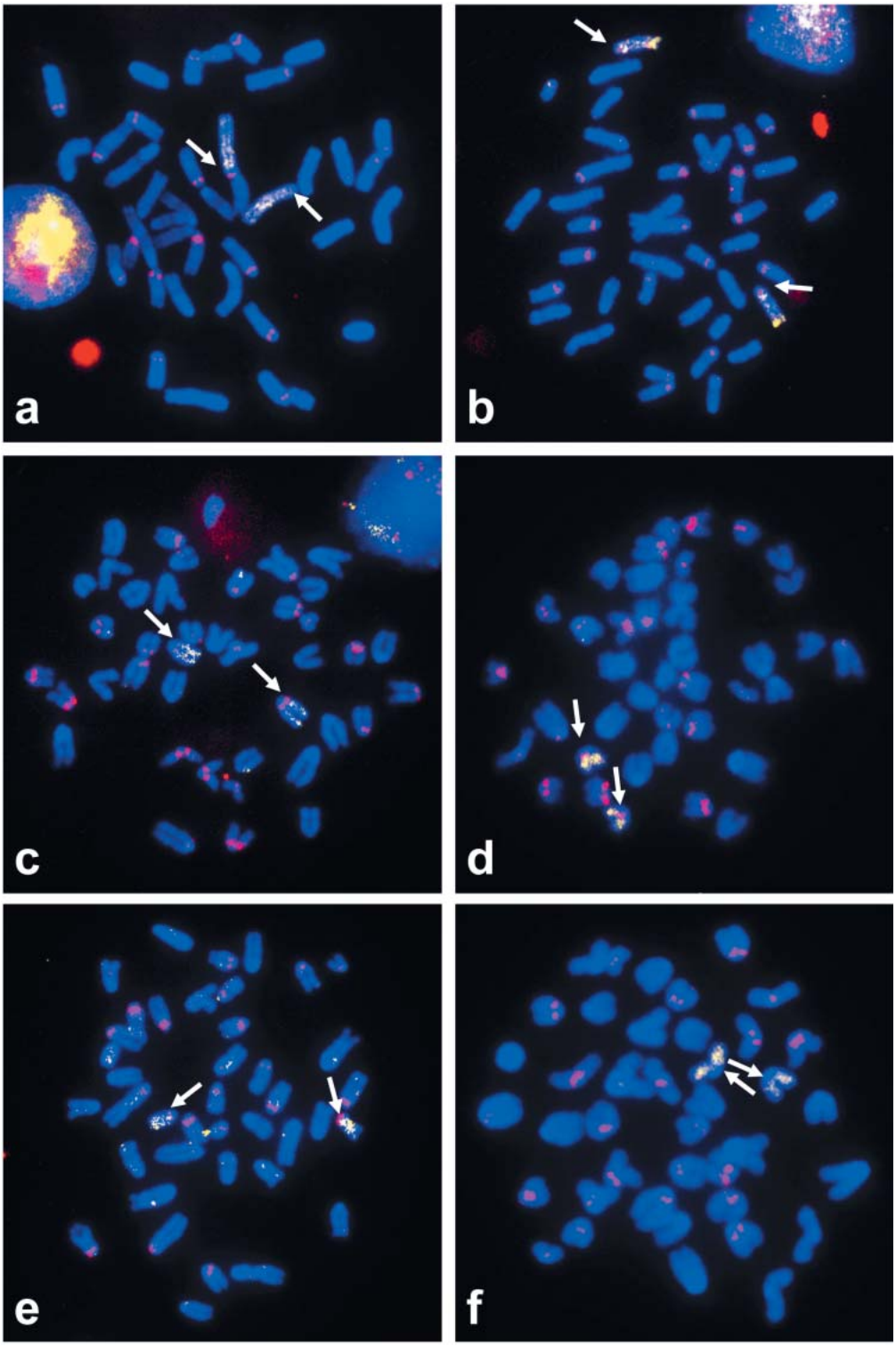


Fig. 1. Mapping of rDNA loci distinctive to *M. m. molossinus* by double-color FISH. Red signal: rDNA, White signal: mouse chromosome paint probes. (a) Chromosome 1, (b) Chromosome 5, (c) Chromosome 10. (d) Chromosome 13, (e) Chromosome 17, (f) Chromosome 9.

Table 1. Comparison of the rDNA loci in *M. m. molossinus*, *M. m. musculus* and *M. m. castaneus*

Species	Chromosome												
	1	4	5	9	10	11	12	13	15	16	17	18	19
M. m. molossinus[a)]	+	–	+	–	+	+	+	+	+	+	+	+	+
M. m. molossinus[b)]	+	–	+	+	–	+	+	+	+	+	+	+	+
M. m. musculus[c)]	–	–	–	–	–	–	+	–	+	+	–	+	+
M. m. castaneus[b)]	–	+	–	–	–	+	+	–	+	–	–	+	+

a) Double-color FISH (present study).
b) Direct R-banding FISH (6).
c) In situ hybridization autoradiography (1–3).

locus was detected at the distal ends of chromosome 10 (Fig. 1-c) and, this observation is not in agreement with a previous report by Matsuda *et al.* [6]. They found the rDNA locus on chromosome 9 instead of chromosome 10 using the R-banding FISH method, although we did not detect the rDNA locus on chromosome 9. This discordance can be explained by polymorphism of rDNA loci as has been reported in strains of *M. m. musculus* [1]. Indeed, Suzuki *et al.* reported rDNA loci on both chromosome 9 and 10 in defferent individuals of *M. m. molossinus* by Ag-NORs method [9]. These observations suggest the possibility of locus polymorphism of rDNA between these two chromosomes.

Among the eleven rDNA loci, five on chromosomes 12, 15, 16, 18, and 19 were common to the rDNA loci of both *M. m. musculus* and *M. m. molossinus*, although the six loci on chromosomes 1, 5, 10, 11, 13, and 17 were only found in *M. m. molossinus* (Table 1, Fig. 1). Furthermore, *M. m. molossinus* is classified as an independent subspecies which evolved from the ancient hybrid between *M. m. musculus* and *M. m. castaneus* based on a sequence comparison of the mitochondrial genome [10]. Our data indicated that the six rDNA loci distinctive to *M. m. molossinus* might have evolved by chromosomal translocation of a rDNA cluster after geographical isolation of this subspecies from *M. m. musculus* and *M. m. castaneus*. As the karyotypes of these subspecies are cytogenetically homologous, even after the evolution of *M. m. molossinus* as an independent subspecies, the translocation of the rDNA cluster in the genome of *M. m. molossinus* occurred more frequently than that in *M. m. musculus*, without any morphological changes in the chromosomes. Indeed, even in *M. m. castaneus*, the number of rDNA loci was fewer by five than that of *M. m. molossinus*.

The DNA sequence of rDNA is highly conserved beyond species, although the copy number and the number of loci vary remarkably among species. Comparative cytogenetic studies in mammals will provide us further insight into the evolution of mammalian rDNA in the genome.

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