Karyological Characterization of Laboratory Strains of Mongolian Gerbils Using Differential Staining Techniques

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Summary We analyzed the karyotypes of laboratory strains of Mongolian gerbils showing agouti, black and albino coat colors using conventional and differential staining methods. Of these, three strains have been kept in the author’s laboratory over 30 years and one strain was obtained from a supplier. The conventionally stained karyotypes of the gerbils were fundamentally identical to those in previous studies, consisting of 2n=44 (FNa=74) with 42 autosomes: 16 pairs of bi-armed (metacentrics, submetacentrics, and subtelocentrics) and 5 pairs of uni-armed chromosomes (acrocentrics), a large-sized metacentric X chromosome and a small-sized submetacentric Y chromosome. In addition, the G- and C-banded karyotypes were identical to those in previous studies. Notably, our strains carry no intraspecific karyological variation and their karyotypes are quite stable without a polymorphic state, except for heteromorphic variations caused by size differences as a usual phenomenon. Moreover, the unique C-bands were found in Nos. 5 and 13 chromosomes but fluorescent staining results (using quinacrine mustard and chromomycin A3) at the unique C-band regions differed between Nos. 5 and 13 chromosomes. This fact indicates that such a difference has been caused by higher molecular structures containing proteins rather than nucleotide contents.

Key words C-band, G-band, Heterochromatin, Karyotype, Meriones unguiculatus.

The Mongolian gerbil (Meriones unguiculatus including some subspecies) is distributed in Asia, especially in China, Mongolia and the subcontinent of India and in Africa under natural conditions. On the other hand, these gerbils have been maintained under laboratory conditions as an experimental animal (Allanson 1970, Norris 1987). In gerbils, some coat mutants have been confirmed: non-agouti, acromelanic albino, white spot and hairless (Swanson 1980, Matsuzaki et al. 1989). The inheritance mechanisms for the expression of the coat variations have been studied, and it has been revealed that some loci are related to the expression (Matsuzaki et al. 1989).

To date, we have three strains of Mongolian gerbils (Kai et al. 1995) showing the agouti coat (agouti, here called MON/Num/a) and the non-agouti coat (black with white spots, here called MON/Num/b) (Fig. 1). In our laboratory, Mongolian gerbils (agouti) have been kept as a closed colony since 1983, when they were sourced from an original strain from Tokyo Women’s University and Tokyo University of Agriculture (Kai et al. 1995). In addition, albino gerbils were introduced from Chiba City Zoo, and their albino characteristics have been maintained as heterozygous states in the agouti strains as a closed colony. On the other hand, Mongolian gerbils with an agouti coat that can be obtained from suppliers in Japan have a different origin than our strain, according to a phylogenetic analysis (Okumura et al. 1995). Moreover, our black strain suddenly appeared from the agouti strain in our laboratory, and gerbils with such a coat color are impossible to obtain from suppliers. These coat color variations have been reported previously (Matsuzaki et al. 1989), but their genetic elements remain unclear (Okumura et al. 1995). Specifically, the chromosomal constitutions have not yet been analyzed. Particularly, if we establish a new strain with a non-agouti coat from our gerbils, their genetic backgrounds are important cues to the establishment.

In this study, we compared the karyotypes of three coat color variations, agouti, black and albino, of Mongolian gerbils using differential staining methods.

Materials and methods

Individuals of the three coat color strains in our laboratory (Fig. 1) examined in this study are listed in Table 1. As a control, the MON/JmsGbs strain (agouti, from Japan SLC, Inc.) was also analyzed.

Chromosomal preparations were obtained from fibroblast cells and bone marrow cells. The fibroblast cells were cultured from lung tissue in 10 mL of minimum
essential medium (MEM) including 15% bovine fetal serum for two weeks at 37°C, followed by colchicine treatment (final concentration 0.025 \( \mu \)g mL\(^{-1} \)) for 45 min at 37°C. The bone marrow cells were cultured in 15 mL of MEM including 15% bovine fetal serum and 0.025 \( \mu \)g/mL colchicine for 45 min at 37°C. Both cultured cells were subsequently underwent hypotonic treatment with 0.075 M KCl for 18 min at 37°C. After hypotonic treatment, the cells were fixed with Carnoy’s fixative (methanol:acetic acid=3:1) three times. The air-dried cells were conventionally Giemsa stained, C-banded (Sumner 1972) and G-banded (Sumner et al. 1971). Current numbering system for karyotyping was done according to the previous studies (Gamperl and Vistorin 1978, 1980). In addition, we applied two fluorescent staining methods using quinacrine mustard (QM) binding for AT nucleotides and chromomycin A\(_3\) binding for GC nucleotides (CMA\(_3\)) (Caspersson et al. 1971, Amemiya and Gold 1987) to their heterochromatic features.

All experimental procedures were conducted in ac-
cordance with the guidelines for animal experiments, College of Bioresource Sciences, Nihon University and permitted (Nos. AP14B023 and AP14B078).

Results

In all the individuals of all the coat color strains, including the MON/JmsGbsSlc strain, the chromosomal constitutions consisted of $2n=44$ (FNa=74) with 42 autosomes: 16 pairs of bi-armed (metacentrics, submetacentrics, and subtelocentrics) and 5 pairs of uni-armed chromosomes (acrocentrics), a large-sized metacentric X chromosome and a small-sized submetacentric Y chromosome (Fig. 2). Slight size variations as heteromorphic states within a homologue were sometimes observed, especially in larger homologues (Fig. 2). In addition, current G-band patterns of all the individuals examined were fundamentally identical without intraspecific variations (Fig. 3).

In the present C-banding analysis, all the centromeric regions, the proximal regions of the long arm of the No. 5 homologue, the terminal half of the long arm of the No. 13 homologue and the entire Y chromosome were positively stained in all the individuals of all the coat

![Fig. 2. Typical examples of conventionally stained karyotypes of the four coat color strains: agouti (specimen No. SLC#5, obtained from Japan SLC, Inc. as MON/JmsGbsSlc), agouti (specimen No. #2424 as MON/Num), black (specimen No. #2367 as MON/Num/b) and albino (specimen No. #2223 as MON/Num/a).](image-url)
color strains (Fig. 4). In particular, Nos. 5 and 13 homologues are distinctive with the unique C-band patterns from the other chromosomes carrying only centromeric C-bands and are characterized for Mongolian gerbil as marker chromosomes (Fig. 4).

In the C-band patterns of current gerbils, Nos. 5 and 13 chromosomes carried the unique C-bands (Fig. 4). To characterize the heterochromatic features of these unique C-bands, we compared the band patterns of two fluorescent stainings. In No. 5 chromosomes, the C-band positive region at the proximal region of long arm was dull fluorescence by both QM-banding and CMA$_3$-banding, and the QM bands were fundamentally reversible to the CMA$_3$ bands (Fig. 5). On the other hand, in No. 13 chromosomes, entire segments were bright fluorescence by both QM-banding and CMA$_3$-banding, and obvious differences between both banding patterns were not recognized (Fig. 5).

Discussion

Current chromosomal constitutions (Fig. 2) were fundamentally identical to those of previous karyological studies of Mongolian gerbils (Pakes 1969, Lenoard and Deknudt 1970, Gamperl and Vistorin 1978, 1980, Qumsiyeh 1986). Slight size variations within a homo-
logue were confirmed but these heteromorphic states have been constantly observed as intercellular variations in previous and current analyses (Gamperl and Vistorin 1978, 1980). Thus, it is estimated that such size variations are a usual phenomenon in Mongolian gerbils. In addition, the G-band patterns of all of the individuals were fundamentally identical to those in the previous studies (Fig. 3) (Gamperl and Vistorin 1978, 1980). Moreover, in the present C-banding analysis, all the C-band patterns were identical to those in the previous studies (Fig. 4) (Gamperl and Vistorin 1978, 1980). Accordingly, considering the chromosomal features of the agouti, black and albino strains by current analysis, we conclude that there are no differences in chromosomal

Fig. 4. Typical examples of C-banded karyotypes of the four coat color strains: agouti (specimen No. SLC#5, obtained from Japan SLC, Inc. as MON/JmsGbsSlc), agouti (specimen No. #2424 as MON/Num), black (specimen No. #2367 as MON/Num/b) and albino (specimen No. #2223 as MON/Num/a).

Fig. 5. Comparisons of staining patterns in Nos. 5 and 13 chromosomes (Conv., conventionally Giemsa stained; C, C-banded; G, G-banded; QM, QM-banded; CMA3, CMA3-banded).
constitutions among Mongolian gerbil strains (Figs. 2, 3 and 4).

In the fluorescent analyses for the C-band regions of Nos. 5 and 13 chromosomes, staining results differed between Nos. 5 and 13 chromosomes (Fig. 5). The C-band region of No. 5 chromosomes was dull fluorescence but that of No. 13 chromosomes was bright fluorescence, by both QM-banding and CMA3,-banding. On the basis of these results, the heterochromatic feature of No. 5 chromosomes is considered to be different from that of No. 13 chromosomes, at least. In mammals, there are heterochromatic features caused by differences of nucleotide contents, such as AT-rich and GC-rich (Obara et al. 1997, Sumner 2003). However, it is suggested that the current difference of the heterochromatic features would be caused by higher molecular structures containing proteins rather than nucleotide contents because of there was no difference between the fluorescence stainings (Sumner 1990, 2003).

In laboratory animals, for example in mice (Mus musculus), chromosomal aberrations sometimes occur as intra- and inter-chromosomal rearrangements and aneuploidy (Lutz et al. 2012). Such chromosomal aberrations affect gene order constitutions and expressions as position effects (White 1973, King 1993) and sometimes influence viability. On the other hand, in this study, Mongolian gerbils did not have chromosomal variations. Notably, the chromosomal constitutions of Mongolian gerbils have been quite stable genetically throughout inbreeding maintenances over 30 years in the author’s laboratory as a closed colony. Furthermore, we estimate that any gene expression abnormalities caused by chromosomal aberrations would be absent in Mongolian gerbils. Thus, we concluded that the cytogenetic background of Mongolian gerbils is not varied irrespective of the coat color variations and the differences between strains.

References


