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Genetic Analysis of Two Subspecies of Reeves' Muntjac (Cervidae: *Muntiacus reevesi*) by Karyotyping and Satellite DNA Analyses

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Pei-Yi Chiang, Chyi Chyang Lin, Shu-Ju Liao, Lie-Jiau Hsieh, Shuan-Yow Li, Ming-Chieh Chao, Yueh-Chun Li (2004) Genetic analysis of two subspecies of Reeves' muntjac (Cervidae: *Muntiacus reevesi*) by karyotyping and satellite DNA analyses. *Zoological Studies* **43**(4): 749-758. We analyzed the karyotypes of the Formosan muntjac (*Muntiacus reevesi micrurus*) including G-banding, C-banding, and NOR-staining analyses. The results showed the species has a 2n = 46 chromosome complement. The G-banding patterns as well as the localizations of rRNA gene clusters and constitutive heterochromatins were similar to those of Chinese muntjac (*M. reevesi reevesi*). In addition, satellite DNA analysis was also carried out. The restriction periodicity of FM-satI revealed a 0.75-kb register indicating that this deer species belongs to the plesiometacarpalia division. Finally, the FISH study demonstrated that the Formosan and Chinese muntjacs have similar localizations of satellite I DNA in their respective genomes. Although the Formosan and Chinese muntjacs share almost identical results of cytogenetic analyses, Southern blot and FISH studies revealed some sequence divergence of satellite I DNA between these 2 species supporting the classification of the Formosan muntjac as a subspecies of, not the same species as, the Chinese muntjac. Furthermore, the data suggest that satellite I DNA of the Formosan muntjac and that of the Chinese muntjac may have originated from different ancestral sequences or that they may have experienced different homogenization patterns in the course of evolution.
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Muntjac deer (Muntiacinae, Cervidae) are classified into 9 known species: *Muntiacus crinifrons*, *M. feae*, *M. gongshanensis*, *M. muntjak*, *M. putaoensis*, *M. reevesi*, *M. rooseveltorum*, *M. truongsonensis*, and *M. vuquangensis* (Shi and Ma 1988, Amato et al. 1991, Nowak 1991, Evans and Timmins 1994, Timmins et al. 1998, Giao et al. 1998, Wang and Lan 2000). Based on the morphological and anatomical studies, these species of the genus *Muntiacus* demonstrate quite-similar appearances, and a sterile hybrid was produced from 2 closely related species, *M. muntjak* and *M. reevesi* (Shi et al. 1980). However, these morphologically similar and closely related species have significant diversity in diploid chromosome numbers and karyotypes from 2n = 6 (female Indian muntjac; *Muntiacus muntjak vaginalis*) to 2n = 46 (Chinese muntjac, *M. reevesi reevesi*) (Fontana and Rubini 1990). Such chromosomal divergences are not uncommon within species, such as in lemurs (Dutrillaux 1979), mole rats (Nevo et al. 1994), and gibbons (2n = 38, 44, 50, and 52) (Jauch et al. 1992) or within races, such as in the house mouse (*Mus musculus domesticus*) (Nachman et al.1994). Those studies suggest that karyology might be an excellent model for investigating speciation. More recently, molecular phylogenetic studies were performed to

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identify species, which include a total DNA homology study (Schmidtke et al. 1981), restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) (Lan et al. 1993 1995, Lan and Shi 1994), RFLP analysis of highly repetitive DNA (Lima-de-Faria et al. 1984, Bogenberger 1985 1987, Grechko et al. 1997, Nijman and Lenstra 2001), and a genomic organization study of satellite DNA (Lee et al. 1997). However, actual phylogenetic distances between 2 populations (interspecific divergence) could not be determined based on the above-mentioned morphological, anatomical, karyological, and even molecular phylogenetic studies alone. It was reported that gene analysis of mtDNA can be utilized to assess evolutionary distances (Cronin 1991, Wang and Lan 2000). Moreover, sequence divergences of centromeric satellite DNA have also been useful in delineating phylogenetic relationships, thanks to its rapid evolutionary rate among species and concerted evolution within species (Hatch et al. 1976, Lin et al. 1991, Wijers et al. 1993, Kato et al. 1999, Li et al. 2000, Kato 2003). Cervid satellite I DNA is prominently localized in the cervid pericentromeric region. This satellite DNA is organized in hierarchical higher-order repeats (HORs) of 31-bp subrepeats (Bongenberger et al. 1985, Yu et al. 1986, Lee and Lin 1996). Interestingly, cervid satellite I DNA is organized primarily as 1-kb monomers in telemetacarpalia-division cervids; however, it is organized as a 0.8-kb monomer in plesiometacarpalia-division cervids (Lee at al. 1997). Comparisons of sequences of this given satellite DNA monomer showed over 95% identities within species, but lower sequence similarities between species (Lee at al. 1997). Moreover, it was reported that the interstitial distribution of satellite I DNA corresponds to the chromosomal fusion site (Lin et al. 1991, Lee et al. 1993, Yang et al. 1997, Fronicke and Scherthan 1997, Li et al. 2000). Therefore, chromosomal distribution of satellite DNA can serve as an indicator in mapping the course of karyotypic evolution.

Muntiacus reevesi (Reeves' muntjac) includes 2 subspecies: *M. reevesi reevesi* (Chinese muntjac) and *M. reevesi micruru* (Formosan muntjac) (Whitehead 1972, Wilson and Reeder 1993). While the Chinese muntjac is widely distributed throughout southeastern China, the Formosan muntjac is endemic to the island of Taiwan. The appearances of the 2 subspecies are alike except that the Formosan muntjac has a darker coat. Some aspects of the natural history of the Formosan muntjac have been reported (Chen 1992, Pei and Liu 1994), but almost no genetic information is available except for its diploid chromosome number 2n = 46 (Wang 1987). Herein, we report on detailed cytogenetic and satellite DNA analyses of the Formosan muntjac and compare results with those of the Chinese muntjac.

MATERIALS AND METHODS

Primary culture and establishment of a skin fibroblast cell line

Skin biopsies of male Formosan muntjacs, which were kindly provided by the Taipei Zoo, Taipei, Taiwan, were primarily grown at 37°C in Dulbecco's modified Eagle medium (DMEM) (Gibco/BRL, N.Y. U.S.A) supplemented with 15% fetal calf serum, 1% glutamine, and 1% penicillin-streptomycin-neomycin. After the 5th passage during subculture of the skin fibroblasts, skin fibroblast cells were maintained in DMEM supplemented with 10% fetal calf serum, 1% glutamine, and 1% penicillin-streptomycin-neomycin.

G-banding, C-banding, and NOR-silver staining

Chromosome preparations were obtained from an established male Formosan muntjac cell line according to standard protocols (Dracopoli et al. 2001). G-banding: A slide aged for 2 wk was treated with 0.05% trypsin/EDTA for 10~15 s at room temperature and stained with Wright's dye for 60~80 s. C-banding: The aged slide was pretreated in 0.2 N HCl at ambient temperature for 1 h, rinsed with ddH_2O, treated in an alkali solution containing 5% $Ba(OH)_2$ at 50°C for 10 min, then washed with a large amount of ddH_2O, and finally incubated in 2X SSC at 60°C for 1 h before staining with Wright's dye. Silver-NOR staining: The aged slide was treated with 3 volumes of 2% gelatin and 4 volumes of 50% silver nitrate solution at 65°C for 2~4 min. Subsequently, the slide was washed with 3% acetic acid to terminate the reaction of the silver nitrate. Finally, the slide was washed with ddH_2O, air dried, and stained with Wright's dye.

Southern blot analysis, subrepeat analysis, and copy number estimation

For Southern blot experiments, 10-μg aliquots of muntjac genomic DNA were incubated with one of 6 different restriction endonucleases. The

digested DNA samples were electrophoretically fractionated on a 0.8% agarose gel, transferred to a nylon membrane (Biodyne), and hybridized with a ^{32}P-dCTP-labeled satellite I DNA clone. The conditions used for hybridization, filter washing, and autoradiography were described previously (Lee et al. 1994). In the subrepeat analysis, a monomer of the FM-satI clone (GenBank accession no.: AY380827) (Lin et al. 2004) was subjected to single-base-shift self-comparisons based on the method of Pluciennicza k et al. (1982) to investigate the presence of internal unidirectional subrepeats. This self-comparison method is described in greater detail elsewhere (Lee and Lin 1966). Briefly, cervid satellite DNA monomer A was compared with a DNA sequence comprising 2 adjacent copies of the same monomer, AA. Monomer A was then shifted to the right in 1-base increments, with respect to AA. After each shift, the overall number of identical nucleotides detected between 2 aligned DNA sequence A's was plotted on a line graph using the CA-Cricket Graph III program (Computer Associates, CA). If monomer A contains 31-bp subrepeats, a peak in the line graph is observed every 31 base shifts due to a significantly high number of identical nucleotides between A and AA in that "in-frame" aligned position. Copy number estimation of cervid satellite I monomers in the Formosan muntjac genome was also based on an earlier described procedure (Lee et al. 1994).

Fluorescence in situ hybridization

Metaphase chromosomes were prepared from an established male Formosan muntjac cell line and a male Chinese muntjac cell line (kindly provided by Dr. F. Yang, University of Cambridge, England, UK). The FM-satI and C5 were labeled with SpectraRed-dUTP (Vysis) by nick translation. The procedures for denaturation, hybridization, post-hybridization washing, and signal detection are described in detail elsewhere (Lee et al. 1999). Fluorescent signals were captured on an Olympus (Tokyo, Japan) BX60 fluorescence microscope equipped with appropriate filter sets and a cooled charge-coupled device (CCD) camera (Photometrics KAF 1400,USA). Images were normalized and enhanced using the MacProbe v4.0 software (Perceptive Scientific Instruments, USA).

RESULTS

Karyological studies

Each of 20 chromosome spreads of a female and male Formosan muntjac was analyzed. The G-banding analysis showed 22 pairs of autosomes and 1 pair of sex chromosomes in the complement of Formosan muntjac chromosomes. A karyotype and ideogram were constructed based on the chromosome size and G-banding patterns (Fig. 1). Three pairs of larger autosomes were designated numbers 1, 2, and 3. The X chromosome has a similar size to chromosome number 4, and the Y chromosome is the smallest one in the complement. All chromosomes were shown to be telocentric/acrocentric except the Y chromosome, which was acrocentric/submetacentric. There is a secondary constriction in the middle of chromosome 1. The G-banded karyotype of the Formosan muntjac is generally the same as that of the Chinese muntjac (Fig. 1a, b). Constitutive heterochromatin banding (C-banding) was carried out to further identify the chromosomal morphology. The result showed that the constitutive heterochromatin is terminally located in every Formosan muntjac chromosome except the Y, in which the heterochromatin is located at the sub-middle region of that chromosome (Fig. 2a). Chromosomes 1, 2, and 3 appeared to have less heterochromatin, whereas the X chromosome has a larger amount of heterochromatin. The silver-NOR staining showed that there are 4 nucleolar organizer regions (NORs), two of which are located in the secondary constriction of chromosome 1 homologs at band 1q28, and the others are located in the terminal end of the q arm of chromosome 5 homologs at band 5q26 (Fig. 2b).

Satellite I DNA analysis

An *Eco*RI-digested complete monomer of FM-satI DNA (one of the Formosan muntjac's satellite I DNA clones), occupying 796 bp in length from nucleotides 202~997 of the FM-satI clone (Lin et al. 2004), was used to detect the existence of any internal subrepeats. The single-base-shift self-comparison analysis (Pluciennicza k et al. 1982) showed in-frame peaks approximately every 31 single-base shifts. This indicates the presence of internal 31-bp subrepeats in the monomer examined (Fig. 3). Therefore, these 31-bp subrepeats were organized into a higher-order repeated hierarchical structure as ~0.8-kb monomers in the Formosan muntjac genome.

Southern blot hybridization with FM-satI DNA as a probe produced a typical type A ladder pattern with a 0.75-kb register in *Pvu*II-, *Bam*HI-,

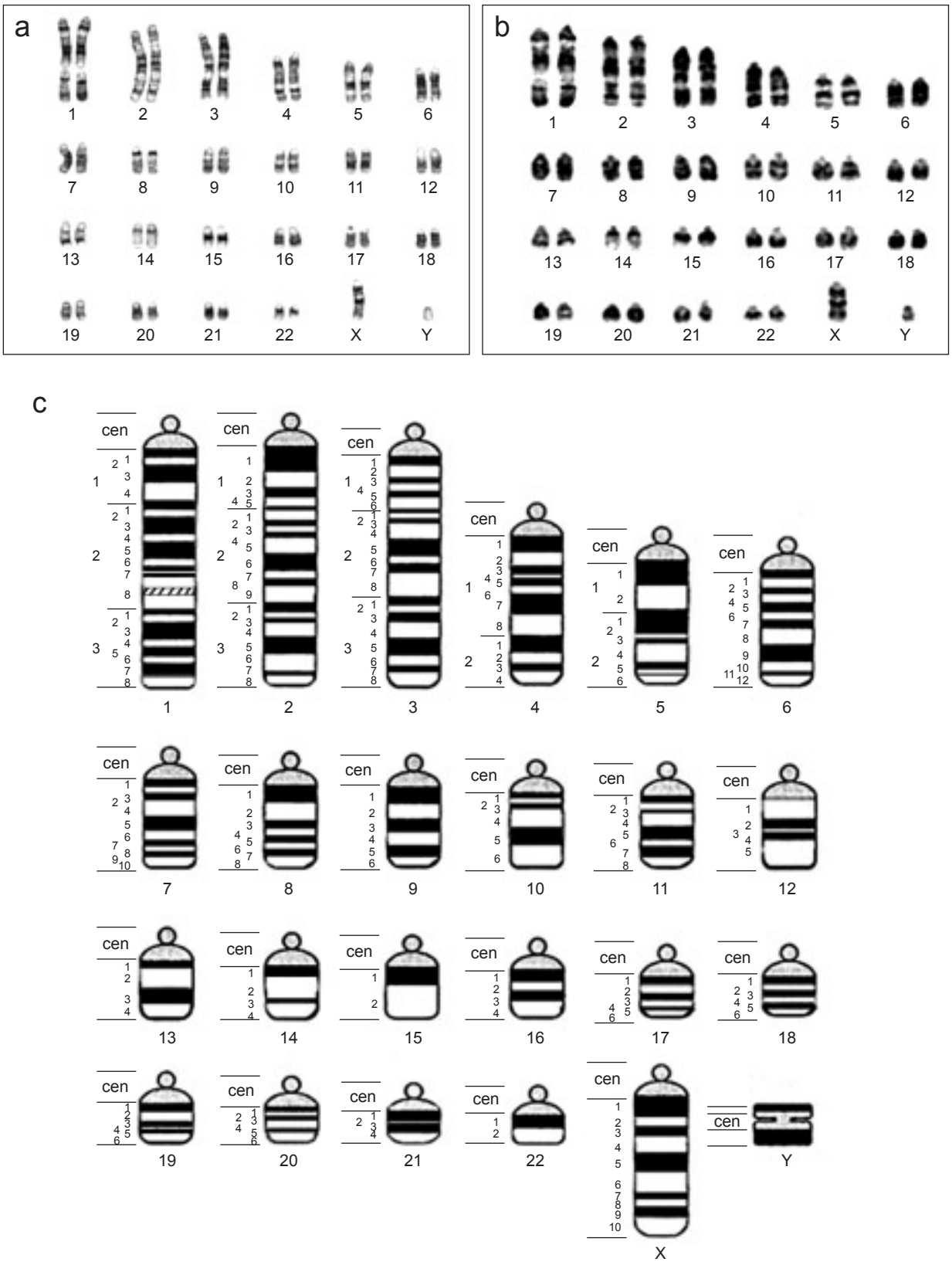

Fig. 1. G-banded chromosome analysis of 2 muntjac subspecies. (a) G-banded karyotype of the male Formosan muntjac (*Muntiacus reevesi micrurus*). (b) G-banded karyotype of the male Chinese muntjac (*Muntiacus reevesi reevesi*). (c) The ideogram was constructed based on the G-banding pattern of the male Formosan muntjac.

*Eco*RI-, and *Pst*I-digested fragments (Fig. 4a). All of these results suggest that the cervid satellite I DNA in the Formosan muntjac genome is organized primarily as 0.75-kb tandem repeats. The pattern of hybridization bands is almost the same between the Formosan and Chinese muntjacs (Fig. 4a, b) with the exception of a stronger 3.2-kb band found in *Eco*RI-digested Formosan muntjac DNA.

Chromosomal distribution of satellite I DNA

The SpectraRed-labeled satellite I DNA probe (FM-satI) was hybridized to metaphase chromosome spreads from a male Formosan muntjac. The FISH study was carried out and revealed satellite I DNA signals (which appeared as red fluorescence) at the pericentromeric region of all chromosomes, except for a large pair of autosomes (identified as chromosome 3s) and the Y chromosome (Fig. 5a). Hybridization signals were also observed at 7 specific interstitial sites on 5 different autosomes per haploid genome. Among those, 2 satellite I interstitial sites were found each on chromosomes 1 and 3, and 1 satellite I DNA interstitial site was observed on each of the other 3 autosomes (identified as chromosomes 2, 5, and 10, see Fig. 4b). In a comparison of chromosomal

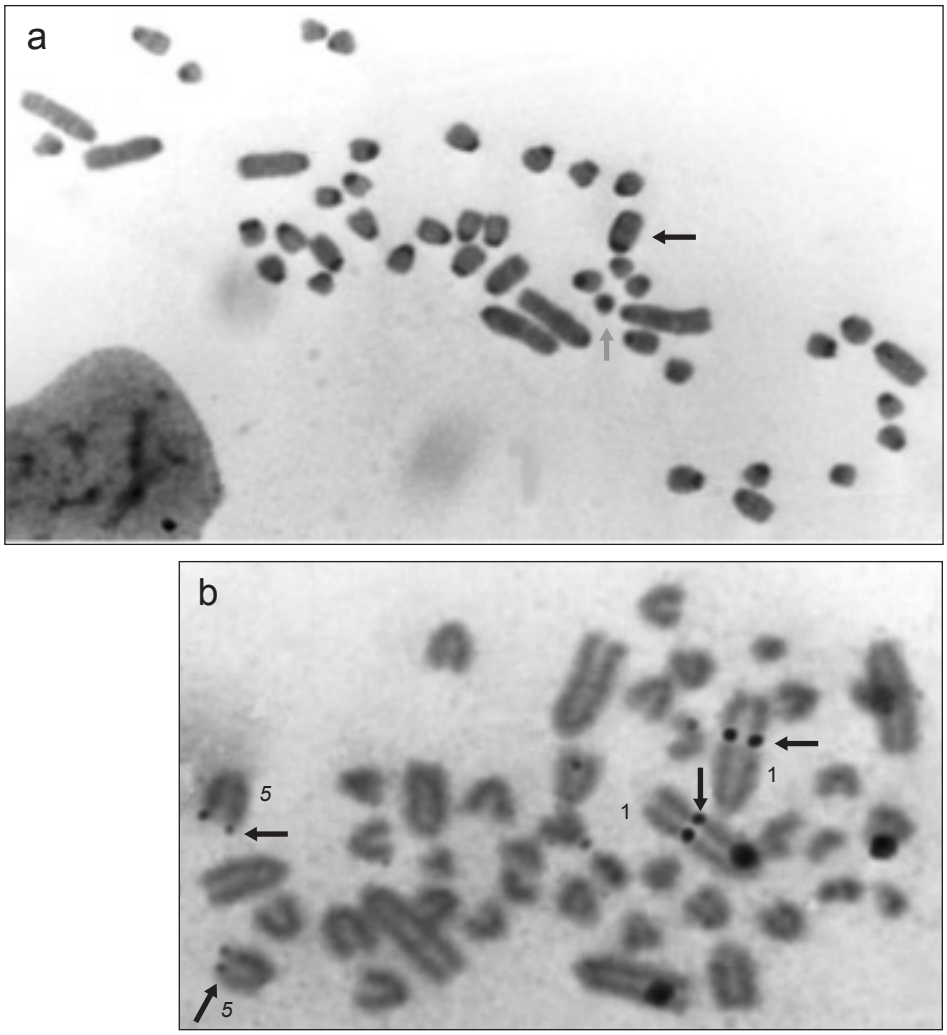

Fig. 2. C-banding and NOR-banding analyses of the Formosan muntjac chromosomes. (a) C-banded metaphase spread of the male Formosan muntjac. Positive C-bands are located on every terminal pericentric heterochromatin region with the exception of the Y chromosome which is mainly heterochromatic (the red arrow indicates the Y chromosome and the black arrow indicates the X chromosome). (b) Localization of NOR sites on male Formosan muntjac chromosomes. There are 4 NOR sites, two of which are located at the secondary constriction region of chromosome 1s and the other two at the terminal end of the q arm of chromosome 5s (as indicated by arrows).

localization of Chinese muntjac satellite I DNA (C5) (Fig. 5c, d), there was a stronger satellite I DNA signal present at the pericentromeric region of Formosan muntjac chromosome 4s; in addition, only 1 interstitial signal was detected in each chromosome 2 of the Formosan muntjac, whereas 2 interstitial signals were observed in Chinese muntjac chromosome 2. No interstitial signal was found in chromosome 4 of the Formosan muntjac, whereas, an interstitial signal was observed in the counterpart of the Chinese muntjac chromosome.

DISCUSSION

In the present karyological study, we observed that the Formosan and Chinese muntjacs share similar G-banded karyotypes. The location of the NOR and the C-banding pattern of the Formosan muntjac are also the same as those of the Chinese muntjac (Shi et al. 1980). The G-banded ideogram of the Formosan muntjac showed some minor differences with the enhanced DAPI-banded ideogram of the Chinese muntjac as reported by Yang et al. (1995). We exchanged chromosome 11 of the Chinese muntjac, identified by DAPI-enhanced banding (Yang et al. 1995), with chromosome 10, based on the high resolution of G-banding and the chromosome size. Even so, the karyotypes of these 2 subspecies of *M. reevesi* are highly conserved. This differs from a subspecies of the house mouse (*Mus musculus domesticus*) which shows a wide range of variations of karyotypes (Nachman et al. 1994). Furthermore, it was reported that the greater the similarity of a given satellite DNA family among species, the closer the phylogenetic distances are among those species, by analyses of RFLP patterns, monomer size, sequence divergence, and chromosomal localization of satellite DNA among species (Lin et al. 1991, Wichman et al. 1991, Lee et al. 1997, Kato et al. 1999, Li et al. 2000, Slamovits et al. 2001, Kato 2003). In the present study, we found an almost identical restriction periodic pattern of satellite I DNA arrays between the Formosan and Chinese muntjacs with the exception of a stronger 3.2-kb band in *Eco*RI-digested Formosan muntjac genomic DNA. The genomic organization of this satellite I DNA in the Formosan muntjac was characterized by a ~0.8-kb higher-order repeat (HOR) monomer which in turn is comprised of degenerate 31-bp subrepeats. Such a

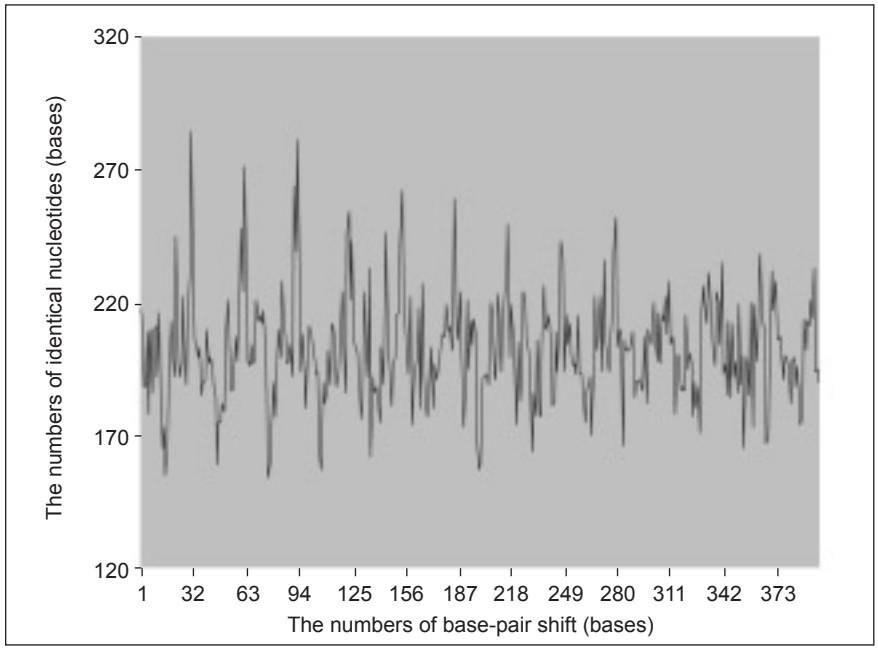

Fig. 3. Presence of 31-bp subrepeats in the FM-satI clone of the Formosan muntjac. The line graph shows increased DNA sequence similarities ("in-frame" peaks) in a 31-bp shift periodicity when monomer A (nucleotides 202~997) of the FM-satI clone is compared with 2 adjacent copies of the identical monomer AA itself and that which is shifted to the right at 1-base intervals. The vertical axis indicates the total number of identical nucleotides between the 2 aligned DNA sequences. The horizontal axis represents the number of base-pair shifts during sequence alignment. As the complete graph is a symmetrical image defined by the vertical axis of symmetry halfway across the graph, only the results of the 1st 398 shifts are presented.

hierarchical pattern of HORs of satellite I DNA further implies that the Formosan muntjac should also be classified as a plesiometacarpalia deer (Lee et al. 1997). In comparison to an earlier FISH study of satellite I DNA (C5) distribution in the Chinese muntjac (Li et al. 2000), the Formosan muntjac has the same chromosomal localization of satellite I as the Chinese muntjac with the exception of 2 interstitial satellite I DNA signals that were undetectable in the haploid genome of the Formosan muntjac compared to its Chinese muntjac counterparts. The 2 interstitial signals being undetectable may have been due to lesser amounts of satellite I DNA or to degradation of that particular satellite DNA in the course of tandem fusion. Previously, by a comparative G-banding study (Fontana and Rubini 1990) as well as FISH with chromosome-specific painting probes and centromeric satellite DNA probes (Yang et al. 1995 1997), it was suggested that the karyotype of the Chinese muntjac had evolved from a 2n = 70 ancestor by 12 sequential repeated-tandem fusions without involvement of Robertsonian translocation. In this study, the G-banding karyotype analysis and FISH results obtained together support the notion that the Formosan muntjac is a subspecies of *M. reevesi*, and that its karyotype was also derived from a 2n = 70 ancestor. Moreover, fossil records indicate that the Formosan muntjac may have existed in the early Pleistocene as did the Chinese muntjac (Ma et al. 1986, Dong 1993). Furthermore, the sequence divergence of satellite I DNA also draws into questions whether the Formosan muntjac is only a different race of the Chinese muntjac. Indeed, satellite I DNA sequence comparisons show that satellite I of the Formosan muntjac is more similar to that of the Indian muntjac (86% homology) than to

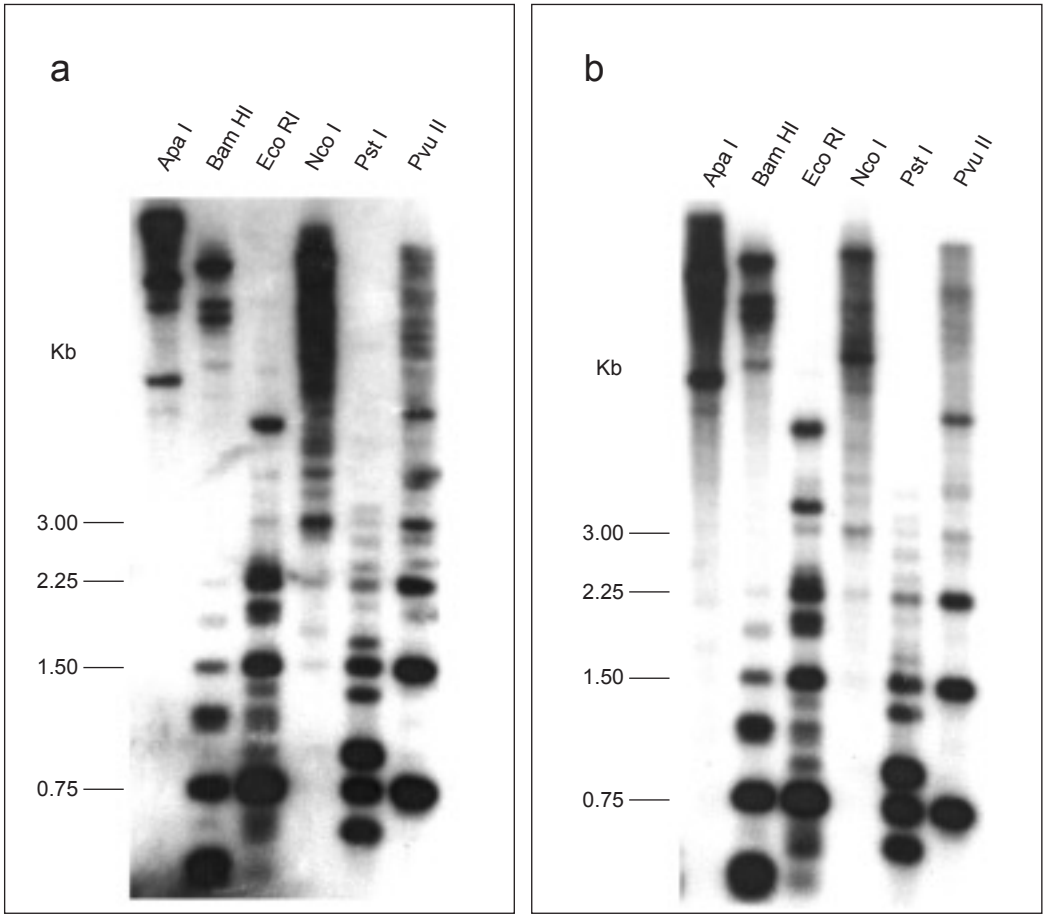

Fig. 4. Restriction periodicity of cervid satellite I DNA in the genomes of Formosan and Chinese muntjacs. (a) Southern blot of Formosan muntjac genomic DNA hybridized to the ^{32}P-labeled FM-satI DNA clone. (b) Southern blot of Chinese muntjac genomic DNA hybridized to ^{32}P-labeled C5 DNA. Fragment sizes are indicated on the left hand side showing a 0.75-kb register for 5 restriction enzymes, *Bam*HI-, *Eco*RI-, *Nco*I-, *Pst*I-, and *Pvu*II-digested genomic DNA, but not for *Apa*I digests.

the Chinese muntjac (82% homology) (Lin et al. 2004). Based on satellite DNA sequence comparison data alone, one could argue that the Formosan and Indian muntjac ancestors shared very high sequence homology of satellite I DNA. On the other hand, if satellite I DNA of the Formosan and Chinese muntjacs indeed originated from the same ancestral sequence, they might have separately experienced different homogenization patterns in the course of evolution (Nijman

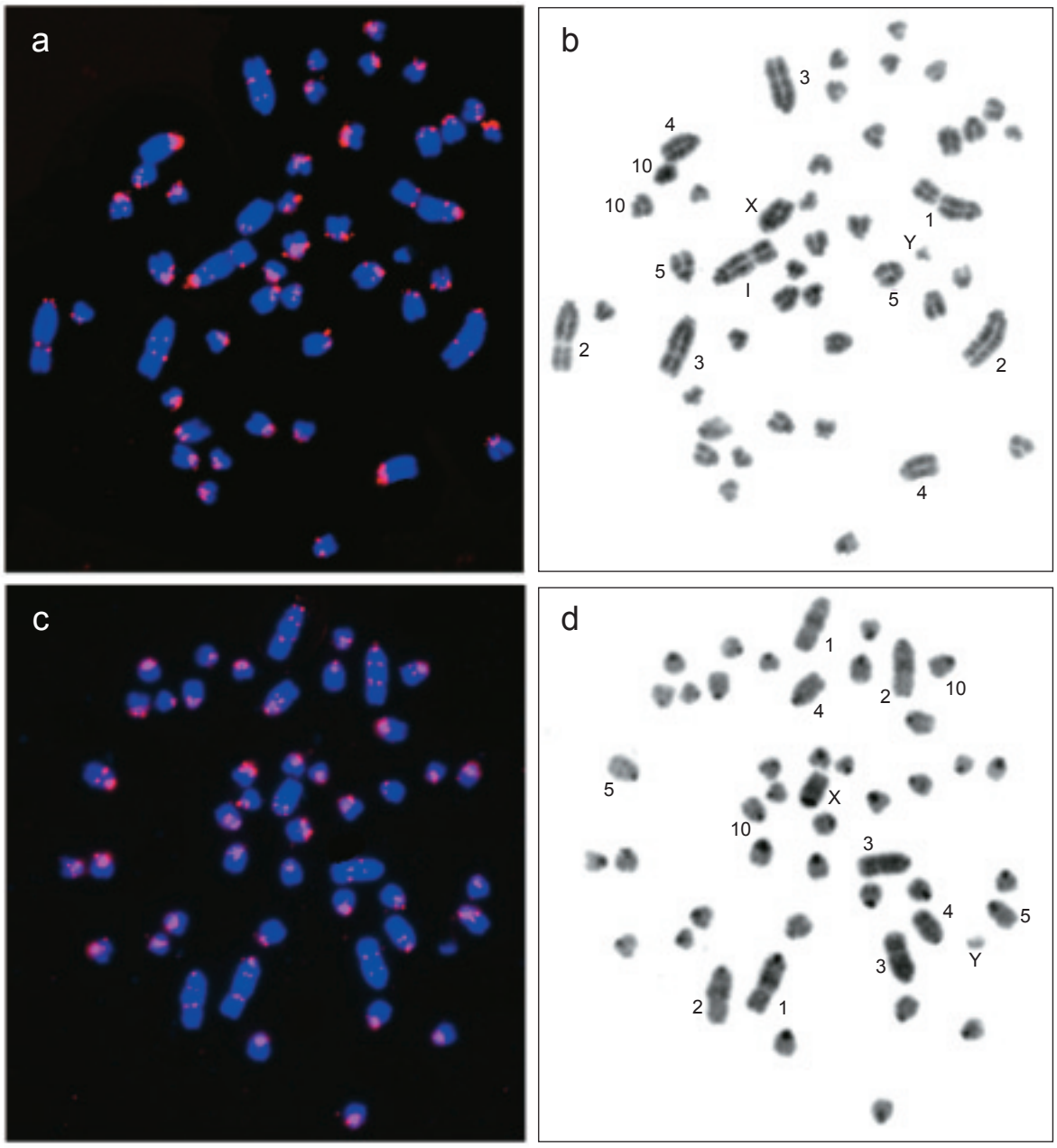

Fig. 5. Chromosomal distribution of cervid satellite I DNA in Formosan and Chinese muntjacs. (a) SpectraRed-labeled FM-satI DNA probe hybridized to the metaphase spread of the Formosan muntjac and hybridization signals (appearing as red fluorescence) localized at all pericentromeric regions except for chromosome 3s and the Y chromosome. Identification of chromosomes by inverse DAPI-banding on the same metaphase in (a) is shown in (b). There were 7 interstitial hybridization signals observed in 5 autosomes (1, 2, 3, 5, and 10), as indicated in panel (b), in a haploid set. (c) Hybridization signals of the SpectraRed-labeled C5 probe observed at all pericentromeric regions of the Chinese muntjac with the exception of chromosome 3s and the Y chromosome. (d) Inverse DAPI-banding of the same metaphase as (c) with the identified chromosomes indicated. There were 9 interstitial signals of C5 observed in 6 autosomes (1, 2, 3, 4, 5, and 10) as indicated in panel (d), in a haploid set.

and Lenstra 2001).

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REFERENCES

Amato G, MG Egan, A Rabinowitz. 1991. A new species of muntjac, *Muntiacus putaoensis* (Artiodactyla: Cervidae) from northern Myanmar. Anim. Conserv. **2:** 1-7.

Bogenberger JM, H Neitzel, F Fittler. 1987. A highly repetitive DNA component common to all Cervidae: its organization and chromosomal distribution during evolution. Chromosoma **95:** 154-161.

Bongenberger JM, PS Neumaier, F Fittler. 1985. The Muntjak satellite IA sequence is composed of 31-bp-pair internal repeats that are highly homologous to the 31-base pair subrepeats of the bovine satellite 1.715. Eur. J. Biochem. **148:** 55-59.

Chen YC. 1992. The ecological study of Formosan muntjac (*Muntiacus reevesi micrurus*). Master's thesis, National Taiwan Univ., Taipei, Taiwan. (in Chinese)

Cronin MA. 1991. Mitochondrial DNA phylogeny of deer (Cervidae). J. Mammal. **72:** 533-566.

Dod B, E Mottez, E Desmarais, F Bonhomme, G Roizes. 1989. Concerted evolution of light satellite DNA in genus *Mus* implies amplification and homogenization of larger blocks of repeat. Mol. Biol. Evol. **6:** 478-491.

Dong W. 1993. The fossil records of deer in China. *In* N Ohtaishi, H Sheng, eds. Deer of China, biology and management. Amsterdam: Elsevier, pp. 95-102.

Dracopoli NC, JL Haines, B Korf, DT Moir, CC Morton, CE Seidman, JG Seidman, DR Smith. 2001. Chromosome banding techniques: centromeric heterochromatin staining (C-banding). *In* Current protocols in human genetics. Vol. 1, Unit 4.2. New York: J Willey, pp. 25-28.

Dutrillaux B. 1979. Chromosomal evolution in primates: tentative phylogeny from *Microcebus murinus* (Prosimian) to man. Hum. Genet. **48:** 251-314.

Fontana F, M Rubini. 1990. Chromosomal evolution in Cervidae. Biosystems **24:** 157-174.

Fronicke L, H Scherthan. 1997. Zoo-fluorescence in situ hybridization analysis of human and Indian muntjac karyotypes (*Muntiacus muntjak vaginalis*) reveals satellite DNA clusters at the margins of conserved syntenic segments. Chromosome Res. **5:** 254-261.

Giao PM, D Touc, VV Dung, ED Wikramanayake, G Amato, P Arctander, JR MacKinnon. 1998. Description of *Muntiacus truongsonensis*, a new species of muntjac (Artiodactyla: Muntiacidae) from central Vietnam, and implications for conservation. Anim. Conserv. **1:** 61-68.

Grechko VV, LV Fedorova, AN Fedorov, SY Slobodyanyuk, DM Ryabinin, MN Melnikova, et al. 1997. Restriction endonuclease analysis of highly repetitive DNA as a phylogenetic tool. J. Mol. Evol. **45:** 332-336.

Hatch FT, AJ Bodner, JA Mazrimas, DH Moore. 1976. Satellite DNA and cytogenetic evolution. DNA quantity, satellite DNA and karyotypic variations in kangaroo rats (genus Dipodomys). Chromosoma **58:** 155-168.

Kato M. 2003. Evaluation of intra- and interspecific divergence of satellite DNA sequences by nucleotide frequency calculation and pairwise sequence comparison. Biol. Proceed. Online **5:** 63-68.

Kato M, A Kato, N Shimizu. 1999. A method for evaluating phylogenetic relationship of a-satellite DNA suprachromosomal family by nucleotide frequency calculation. Mol. Phylogenet. Evol. **13:** 329-335.

Lan H, L Shi. 1994. Restriction endonuclease analysis of mitochondrial DNA of muntjacs and related deer. Sci. China. (Ser. **B**) **37:** 294-302.

Lan H, LM Shi, H Suzuki. 1993. Restriction site polymorphism in ribosomal DNA of muntjacs. Chin. Sci. Bull. **38:** 1659-1664.

Lan H, W Wang, LM Shi. 1995. Phylogeny of *Muntiacus* (Cervidae) based on mitochondrial DNA RFLPs. Biochem. Genet **33:** 377-388.

Lee C, DR Court, C Cho, J Haslett, CC Lin. 1997. High-order organization of subrepeats and the evolution of cervid satellite I DNA. J. Mol. Evol. **44:** 327-335.

Lee C, CC Lin. 1996. Conservation of a 31-bp bovine subrepeat in centromeric satellite DNA monomers of *Cervus elaphus* and other cervid species. Chromosome. Res **4:** 427-435.

Lee C, DBC Ritchie, CC Lin. 1994. A tandemly repetitive, centromeric DNA sequence from the Canadian woodland caribou (*Rangifer tarandus caribou*): its conservation and evolution in several deer species. Chromosome. Res. **2:** 293-306.

Lee C, R Sasi, CC Lin. 1993. Interstitial localization of telomeric DNA sequences in the Indian muntjac chromosomes: further evidence for tandem chromosome fusions in the karyotypic evolution of the Asian muntjacs. Cytogenet. Cell Genet. **63:** 156-159.

Lee C, R Stanyon, CC Lin, MA Ferguson-Smith. 1999. Conservation of human gamma-X centromeric DNA among primates with an autosomal location in certain Old World monkeys. Chromosome. Res. **7:** 43-47.

Li YC, C Lee, D Sanoudou, TH Hseu, SY Li, CC Lin. 2000. Interstitial colocalization of two cervid satellites DNAs involved in the genesis of the Indian muntjac karyotype. Chromosome. Res. **8:** 363-373.

Lima-de-Faria A, V Arnason, B Widegreen, J Essen-Moller, N Isakson, E Olsson, J Haworska. 1984. Conservation of repetitive DNA sequences in deer species studied by Southern blot transfer. J. Mol. Evol. **20:** 17-24.

Lin CC, PY Chiang, LJ Hsieh, MC Chao, YC Li. 2004. Cloning, characterization and physical mapping of three cervid satellite DNA families in the genome of Formosan muntjac (*Muntiacus reevesi micrurus*). Cytogenet. Genome Res. **105:** 100-106.

Lin CC, R Sasi, YS Fan, ZQ Chen. 1991. New evidence for tandem chromosome fusions in the karyotypic evolution of Asian muntjacs. Chromosoma **101:** 19-24.

Ma SL , YX Wang, LH Xu. 1986. Taxonomic and phylogenetic studies on the genus *Muntiacus*. Acta Theriol. Sin. **6:** 191-207.

Nachman MW, SN Boyer, JB Searle, CF Aquadro. 1994. Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. Genetics **136:** 1105-1120.

Nevo E, MG Filippucci, C Redi, A Korol, A Beiles. 1994. Chromosomal speciation and adaptive radiation of mole rats in Asia Minor correlated with increased ecological stress. Proc. Natl. Acad. Sci. USA **91:** 8160-8164.

Nijman IJ, JA Lenstra. 2001. Mutation and recombination in cattle satellite DNA: a feedback model for the evolution of satellite DNA repeats. J. Mol. Evol. **52:** 361-371.

Nowak RM. 1991. Order artiodactyla. *In* Walker's mammals of the world. 5th ed. Vol. 2. Baltimore, MD and London: The Johns Hopkins Univ. Press, pp. 1334-1400.

Pei K, HW Liu. 1994. The reproductive biology of male Formosan Reeves' muntjac (*Muntiacus reevesi micrurus*). J. Zool. (Lond.) **233:** 293-306.

Pluciennicza A, J Skowronski, J Jaworski. 1982. Nucleotide sequence of bovine 1.715 satellite DNA and its relation to other bovine satellite sequences. J. Mol. Biol. **158:** 293-304.

Schmidtke J, H Brennecke, M Schmid, H Neitzel, K Sperling. 1981. Evolution of muntjac DNA. Chromosoma **84:** 187-193.

Shi LM, CX Ma. 1988. A new karyotype of muntjac (*M.* sp.) from Gongshan County in China. Zool. Res. **9:** 343-347.

Shi LM, YY Ye, XS Duan. 1980. Comparative cytogenetic studies on the red muntjac, Chinese muntjac and their F1 hybrids. Cytogenet. Cell Genet. **26:** 22-27.

Slamovits CH, JA Cook, EP Lessa, MS Rossi. 2001. Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*, Rodentia: Octodontida): a phylogenetic approach. Mol. Biol. Evol. **18:** 1708-1719.

Timmins RJ, TD Evans, K Khounboline, C Sisomphone. 1998. Status and conservation of the giant muntjac, *Megamuntiacus vuquangensis*, and notes on other muntjac species in Laos. Oryx **32:** 59-67.

Wang ST. 1987. Studies on karyotype and blood types of Taiwan native and exotic deer. Master's thesis, National Taiwan Univ. Taipei, Taiwan. (in Chinese)

Wang W, H Lan. 2000. Rapid and parallel chromosomal number reductions in muntjac deer inferred from mitochondrial DNA phylogeny. Mol. Biol. Evol. **17:** 1326-1333.

Whitehead GK. 1972. Deer of the world. London: Constable and Company, 194 pp.

Wichman HA, CT Payne, OA Ryder, MJ Hamilton, M Maltbie, RJ Baker. 1991. Genomic distribution of heterochromatic sequences in equids: implication to rapid chromosomal evolution. J. Hered. **82:** 369-377.

Wijers ER, C Zijlstra, JA Lenstra. 1993. Rapid evolution of horse satellite DNA. Genomics **18:** 113-117.

Wilson DE, DM Reeder. 1993. Mammal species of the world: a taxonomic and geographic reference (2nd ed.). Washington DC: Smithsonian Books.

Yang F, NP Carter, L Shi, MA Ferguson-Smith. 1995. A comparative study of karyotypes of muntjacs by chromosome painting. Chromosoma **103:** 642-652.

Yang F, PCM O'Brien, J Wienberg, H Neitzel, CC Lin, MA Ferguson-Smith. 1997. Chromosomal evolution of Chinese muntjacs (*Muntiacus reevesi*). Chromosoma **106:** 37-43.

Yu LC, D Lowensteiner, EFK Wong, I Sawada, J Mazrimas, C Schmid. 1986. Localization and characterization of recombinant DNA clones derived from the highly repetitive DNA sequences in the Indian muntjac cells: their presence in the Chinese muntjac. Chromosoma **93:** 521-528.