Cloning, Characterization, and FISH Mapping of Four Satellite DNAs from Black Muntjac (*Muntiacus crinifrons*) and Fea's Muntjac (*M. feae*)

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Abstract: Recent molecular cytogenetic studies demonstrate that extensive centromere-telomere fusions are the main chromosomal rearrangements underlying the karyotypic evolution of extant muntjacs. Although the molecular mechanism of tandem fusions remains unknown, satellite DNA is believed to have facilitated chromosome fusions by non-allelic homologous recombination. Previous studies detected non-random hybridization signals of cloned satellite DNA at the postulated fusion sites on the chromosomes in Indian and Chinese muntjacs. But the genomic distribution and organization of satellite DNAs in other muntjacs have not been investigated. In this study, we have isolated four satellite DNA clones (BMC5, BM700, BM1.1k and FM700) from the black muntjac (*Muntiacus crinifrons*) and Fea's muntjac (*M. feae*), and hybridized these four clones onto chromosomes of four muntjac species (*M. reevesi*, *M. crinifrons*, *M. gongshanenisis* and *M. feae*). Besides the predominant centromeric signals, non-random interstitial hybridization signals from satellite I and II DNA clones (BMC5, BM700 and FM700) were also observed on the arms of chromosomes of these four muntjacs. Our results provide additional support for the notion that the karyotypes of *M. crinifrons*, *M. feae* and *M. gongshanensis* have evolved from a 2n = 70 ancestral karyotype by a series of chromosome fusions.

Key words: FISH mapping; Satellite DNA; *Muntiacus*; Tandem chromosome fusion

黑麂和费氏麂四种卫星 DNA 的克隆特征和染色体定位

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摘要： 近年来，分子细胞遗传学研究已基本证实了染色体的串联融合(端粒－着丝粒融合)是麂属动物核型演化的主要重排方式。尽管染色体串联融合的分子机制还不清楚，但通过染色体的非同源重组，着丝粒区域的卫星 DNA 被认为可能介导了染色体的融合。以前的研究发现在赤麂和小麂染色体的大部分假定的串联融合位点处存在着非随机分布的卫星 DNA。然而在麂属的其他物种中，这些卫星 DNA 的组成以及在基因组中的分布情况尚未被研究。本研究从黑麂和费氏麂基因组中成功地克隆了 4 种卫星 DNA（BMC5、BM700、BM1.1k 和 FM700），并分析了这些卫星克隆的特征以及在小麂、黑麂、贡山麂和费氏麂染色体上的定位情况。结果表明，卫星 I 和 II DNA (BMC5, BM700 和 FM700)的信号除了分布在这些麂属动物染色体的着丝粒区域外，也间隔地分布在这些物种的染色体臂上。其研究结果为黑麂、费氏麂和贡山麂的染色体核型也是从一个 2n＝70 的共同祖先核型通过一系列的串联融合进化而来的假说提供了直接的证据。

Muntiacus have experienced rapid and radical chromosome evolution within the last two million years (Fontana & Rubini, 1990; Yang et al, 1995; Wang & Lan, 2000). The extreme diversification in chromosomal number and structure make them an ideal model for studying karyotypic evolution. Among the five extant muntjacs that have karyotype reports, the Indian muntjac (*M. muntjak vaginalis*) has the lowest chromosome number known in mammals, 2n = 6 (♀) and 2n = 7 (♂) (Wurster & Benirschke, 1970); the Chinese muntjac (*M. reevesi*), a close relative of the Indian muntjac, has a karyotype of 2n = 46, all chromosomes are acrocentric (Wurster & Benirschke, 1967); the other species have intermediate karyotypes: 2n = 8 (♀) and 2n = 9 (♂) in black muntjac (*M. crinifrons*) (Shi, 1983) and Gongshan muntjac (*M. gongshanensis*) (Shi & Ma, 1988); 2n = 12,13,14 (♀) and 2n = 14 (♂) in Fea's muntjac (*M. feae*) (Soma et al, 1983, 1987; Tanomtong et al, 2005).

Several hypotheses were proposed to explain the great karyotypic diversity in *Muntiacus*. The most well-known hypothesis is the tandem fusion hypothesis, first proposed by Hsu et al (1975), suggesting that the 2n = 6♀/7♂ karyotype of *M. muntjak vaginalis* could have evolved from 2n = 46 *M. reevesi*-like ancestral karyotype through extensive tandem fusions and several centric fusions. Subsequent studies, including conventional comparative cytogenetics (Shi et al, 1980; Elder & Hsu, 1988; Fontana & Rubini, 1990), chromosome painting (Yang et al, 1995, 1997a; Yang, 1998; Chi et al, 2005a; Huang et al, 2006a), FISH mapping of centromeric sequences, telomeric sequences, cosmid clones and BAC clones (Scherthan,1990; Lin et al, 1991; Lee et al, 1993; Froenicke et al, 1997; Li et al, 2000a; Hartmann & Scherthan, 2004; Chi et al, 2005b; Huang et al, 2006b), and combined chromosome painting and satellite DNA mapping (Scherthan, 1995; Yang et al, 1997b,d; Froenicke & Scherthan, 1997; Huang et al, 2006c), provided direct evidence for the tandem fusion hypothesis. The chromosomal mechanism underlying the karyotype evolution in *Muntiacus* is well-established now: the karyotypes of all extant muntjacs have evolved from a common ancestor with a 2n = 70 acrocentric karyotype by extensive centromere-telomere fusions and several centric fusions.

Nevertheless, the molecular mechanism that triggered such extensive tandem chromosomal fusions remains unclear. Some studies suggested that the repetitive DNA families at or near the centromeric and telomeric regions might facilitate illegitimate recombination between non-homologous chromosomes of muntjacs (Brinkley et al, 1984; Bogenberger et al, 1985, 1987; Benedum et al, 1986; Lin et al, 1991, 2004; Lee et al, 1994, 1997; Scherthan, 1995; Lee & Lin, 1996; Yang et al, 1997b; Li et al, 2000a, b, 2002; Hartmann & Scherthan, 2004). At present, four satellite DNA families are found in *Muntiacus*: satellite DNA families I, II, IV and V (Bogenberger et al, 1985; Lin et al, 1991, 2004; Li et al, 2000b, 2005). FISH mapping demonstrated that satellite I DNA of *M. muntjak vaginalis*, *M. reevesi* and *M. reevesi micrurus* and satellite II DNA of *M. muntjak vaginalis* are localized at both the centric regions and at non-random interstitial sites along the arms of the "fusion" chromosome (Lin et al, 1991, 2004; Li et al, 2000b). The findings of telomeric repetitive sequences present at several interstitial locations in *M. muntjak vaginalis* chromosomes (Lee et al, 1993; Scherthan, 1995) as well as the comparative mapping of the satellite I and II families (Li et al, 2000b) indicated that during tandem fusions the chromosomal breakpoints localized at satellite II DNA regions and subtelomeric regions of the ancestral chromosomes.

Until now, all satellite DNA families of the genus *Muntiacus* were isolated from the genomes of *M. muntjak vaginalis*, *M. reevesi* and *M. reevesi micrurus* (a subspecies of *M. reevesi,* 2n = 46), as did the FISH mapping of such satellite DNA. To date, no satellite DNA has been isolated from the genomes of *M. crinifrons*, *M. gongshanensis* and *M. feae*. Here we have cloned and characterized four satellite DNA clones from the genomes of *M. crinifrons* and *M. feae*, and studied the chromosome distribution of these four satellite DNAs in *M. crinifrons*, *M. feae*, *M. gongshanensis* and *M. reevesi*. In doing so, we hope to provide additional insights into the molecular mechanism of the tandem fusions that led to the formation of the karyotypes of *M. crinifrons*, *M. gongshanensis* and *M. feae*.

1 Materials and Methods

1.1　Cell culture and chromosome preparation

Fibroblast cell lines of a male *M. crinifrons* (KCB200004), a male *M. feae* (KCB 91006), a male *M. reevesi* (KCB91001) and a female *M. gongshanensis* (KCB 88003) were obtained from the Kunming Cell Bank (Kunming, Yunnan, PR China). Cells were cultured at 37℃ under 5% CO_2 in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Chromosome preparations were prepared as previously described (Yang et al, 1995).

1.2　Molecular cloning of repetitive DNA sequences

Genomic DNA of male *M. crinifrons* and *M. feae* were extracted from the liver tissue and cultured fibroblast cells of these two species by using a Genomic DNA Extraction kit (Bioteke, China). Genomic DNAs of *M. crinifrons* and *M. feae* were digested with five restriction endonucleases: *Eco*RI, *Bgl*II, *Bam*HI, *Hin*dIII and *Xba*I, respectively. The digestion products were subsequently fractionated on a 1.2% agarose gel. The predominant DNA fragments with a size of about 400bp were purified and cloned into pUC19 vector (Takara). PCR was carried out in a 50μL reaction volume using 100ng template DNA, Ex-Taq DNA polymerase (Takara), and 0.2μmol/L of Satellite II primers (SatII-fw[5′-GAG-CTGCCTGACAGACTCG-3′] and SatII-rv [5′-CAG-AGCCGACCTAGGATCAC-3′]) as previously described (Li et al, 2000b). PCR products were fractionated on 1.5% agarose gel and predominant DNA fragments were purified, and cloned into pMD18-T Vector (Takara). Clones containing repetitive DNA inserts were screened by PCR with the M13 forward and reverse primers.

1.3　Nucleotide sequencing and analysis

The satellite DNA clones were sequenced and deposited in NCBI GenBank. The accession numbers for BMC5, BM700, BM1.1k and FM700 are EU644506, EU644507, EU644508 and EU644509 respectively. BLASTN (http://www.ncbi.nlm.nih.gov/blast/) was performed to find similarity sequences in the Database. Tandem Repeats Finder Program (Benson, 1999) was used to reveal tandem repeats in the repetitive DNA fragment. Dot Matrix and multiple-alignment were performed by using DNAMAN software (Version 4).

1.4　Fluorescence *in situ* hybridization (FISH)

DNA fragments of the satellite DNA clones were labeled by nick translation with biotin-14-dCTP (Invitrogen) or FITC-12-dUTP (Roche). FISH was performed as described previously (Yang et al, 1997b). Biotin-labeled probes were visualized using Cy3-avidin (1:1 000, Amersham), while FITC-labeled probes were detected with a layer of rabbit-anti-FITC IgG (1:200, Invitrogen) followed by a layer of FITC conjugated goat-anti-rabbit IgG (1:250, Vector Laboratories). After detection, slides were mounted in Vectashield medium with DAPI (4'6-diamidino-2-phenylindole, Vector Laboratories) and covered with 22 mm × 32 mm cover-slips. FISH images were captured using the Genus system (Applied Imaging Corp.) as previously described (Yang et al, 2000). Hybridization signals were assigned to specific chromosomes or chromosomal regions as defined by enhanced DAPI-banding patterns (Yang et al 1995) or pre-captured G-banding patterns.

2　Results

2.1　Molecular cloning and characterization of satellite I, II, IV DNA clones from the genomes of *Muntiacus crinifrons* and *M. feae*

One clone, BMC5, was isolated from *Bam*HI digests of *M. crinifrons* genomic DNA. Three repetitive DNA clones were generated from PCR amplifications of *M. crinifrons* and *M. feae genomic* DNA using primer sequences derived from a white tailed deer satellite II DNA sequence. Among them, two clones were isolated from *M. crinifrons*, BM700 and BM1.1k, and one clone was isolated from *M. feae*, FM700. These four clones were characterized by sequencing and aligning with the known cervid satellite DNA, and shown to belong to cervid satellite DNA clones.

BMC5 had a length of 437 bp and 51.49% GC content. A *Bam*HI and *Eco*RI digested DNA fragment, with a length of 221bp from nucleotides 1–222 of BMC5 clone, had 93% homology with C5 clone (*M. reevesi* satellite I clone) with the nucleotides 561–783 (Lin et al, 1991), and nucleotides 235–437 of BMC5 clone had 89% similarity with the nucleotides 20–221 of C5 clone (Fig. 1a). When BMC5 clone was aligned with FM-sat I clone (*M. reevesi micrurus* satellite I clone) (Lin et al, 2004), 88% similarity was found between nucleotides 1–437 of BMC5 clone and nucleotides 775–1211 of FM-sat I clone, and there was also a 91% similarity between nucleotides 26–437 of BMC5 clone and nucleotides 4–416 of FM-sat I clone (Fig. 1b). These results suggest that BMC5 belongs to the satellite I DNA
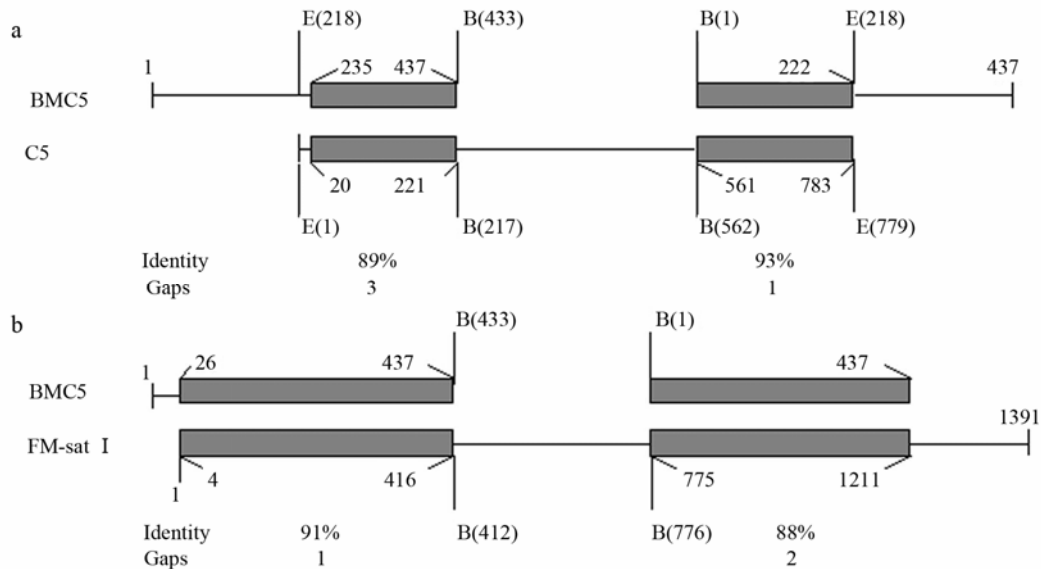
family.

Fig. 1   Comparison of satellite I DNA sequences among muntjac species

a: Schematic diagram of DNA sequences comparison between *Muntiacus crinifrons* satellite I clone (BMC5) and Chinese muntjac satellite I clone (C5) (*Eco*RI sites indicated by E and *Bam*HI sites by B); b: Schematic representation of DNA sequences comparison between *M. crinifrons* satellite I clone (BMC5) and Formosan muntjac satellite I clone (FM-sat I).

BM700 and FM700 are 700-bp PCR products amplified from *M. crinifrons* and *M. feae genomes* respectively using satellite II primers (Li et al, 2000b) (Fig. 2a). BM700 is 667bp in length and had 62.37% GC content, and FM700 had a length of 660bp and 63.03% GC content. Nucleotides 242–634 of BM700 clone had 84% similarity with nucleotides 141–533 of FM-sat II clone (*M. reevesi micrurus* satellite II clone) (Lin et al, 2004), and nucleotides 1–195 had 74% homology with nucleotides 619–817 of FM-sat II, and nucleotides 242–543 of BM700 was of 81% similarity with nucleotides 843–1143 of FM-sat II clone (Fig. 2b). When FM700 clone was aligned with FM-sat II clone, 81% similarity was found between nucleotides 83–634 of FM700 and nucleotides 1–555 of FM-sat II clone, and nucleotides 1–520 of FM700 had 80% homology with nucleotides 621–1142 of FM-sat II (Fig. 2c). Multiple-alignment was performed among BM700, FM700 and MMV-0.7 (*M. muntjak vaginalis* satellite II clone) (Li et al 2000b), and 85.69% homology was found among these clones (Fig. 2d). These findings indicate BM700 and FM700 belong to the satellite II DNA family.

BM1.1k was a PCR product amplified from *M.*

crinifrons genome using satellite II primers (Li et al, 2002) (Fig. 2a). BM1.1k had a length of 1.1-kbp with 44.27% GC content. Multiple-alignment was performed among BM1.1k, MMV-1.0 (*M. muntjak vaginalis* satellite IV), MR-1.0 (*M. reevesi* satellite IV) (Li et al, 2002), and FM-sat IV (*M. reevesi micrurus* satellite IV) (Lin et al, 2004), 98.17% homology was found among these clones (Fig. 2e), suggesting that BM1.1k belongs to the satellite IV DNA family. No tandem repeats were found in these repetitive DNA elements using the Tandem Repeats Finder Program (Benson, 1999) and no internal sub-repeats were detected by dot matrix analysis.

2.2   Chromosome distribution of satellite I (BMC5), satellite II (BM700) and satellite IV (BM1.1k) DNAs in *M. crinifrons*

The probe from *M. crinifrons* satellite I element (BMC5) was hybridized to the metaphase chromosomes of a male *M. crinifrons* (2n = 9♂). Predominant signals were observed at the centric regions of all chromosomes except for the Y chromosome which is relatively weaker, but non-random signals were also found at some interstitial locations along the chromosomes of *M. crinifrons*, with seven interstitial signals on Chr1,
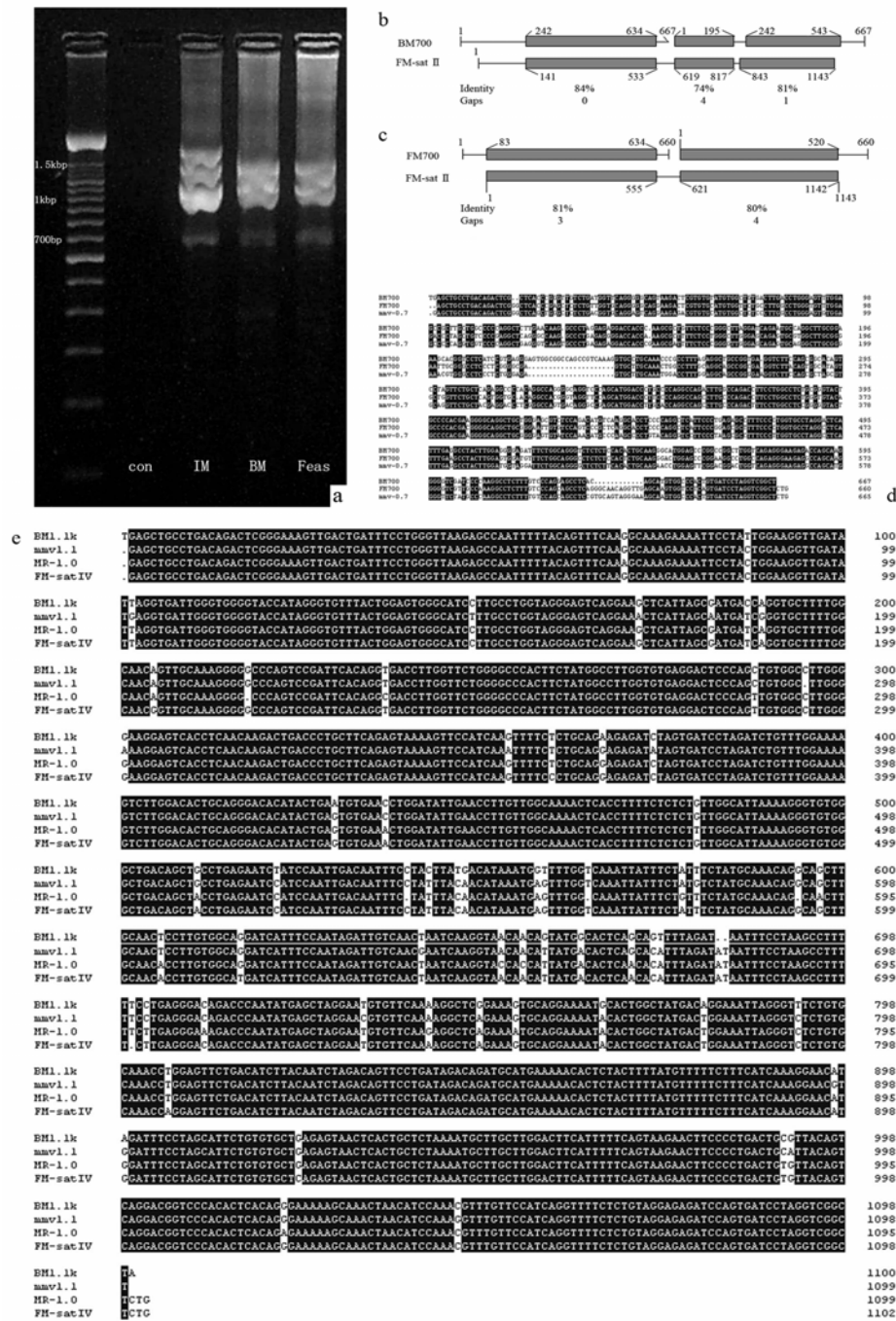
Fig. 2 Molecular cloning and characterization of sate-llite I, II, IV DNA clones from the genomes of

Muntiacus crinifrons and *M. feae*

a: Electrophoretic analysis of PCR products. PCR products were amplified with a pair of Satellite II primers (Li et al, 2000b, 2002) from three muntjac species (IM, *Muntiacus muntjak vaginalis*; BM, *M. crinifrons*; Feas, *M. feae*) and 'con' stands for negative control without template. The products were fractionated on a 1.5% agarose gel. Three bands of 0.7, 1.1 and 1.4kb are detected in these species; b: Schematic illustration of sequence comparison between *M. crinifrons* satellite II DNA clone (BM700) and Formosan muntjac satellite II clone (FM-satII); c: Schematic diagram DNA sequences comparison between *M. feae* satellite DNA II clone (FM700) and Formosan muntjac satellite II clone (FM-satII); d: Multiple-alignment of *M. crinifrons* satellite II clone (BM700), *M. feae* satellite II clone (FM700) and *M. muntjak vaginalis* satellite II clone (MMV-0.7) shows 85.69% homology (identical nucleotide sequences from these clones are shown in black boxes); e: Multiple-alignment of *M. crinifrons* satellite IV clone (BM1.1k), MMV-1.0 (*M. muntjak vaginalis* satellite IV) (Li et al, 2002), MR-1.0 (*M. reevesi satellite* IV) (Li et al, 2002), and FM-sat IV (*M. reevesi micrurus* satellite IV) (Lin et al, 2004) shows 98.17% similarity.
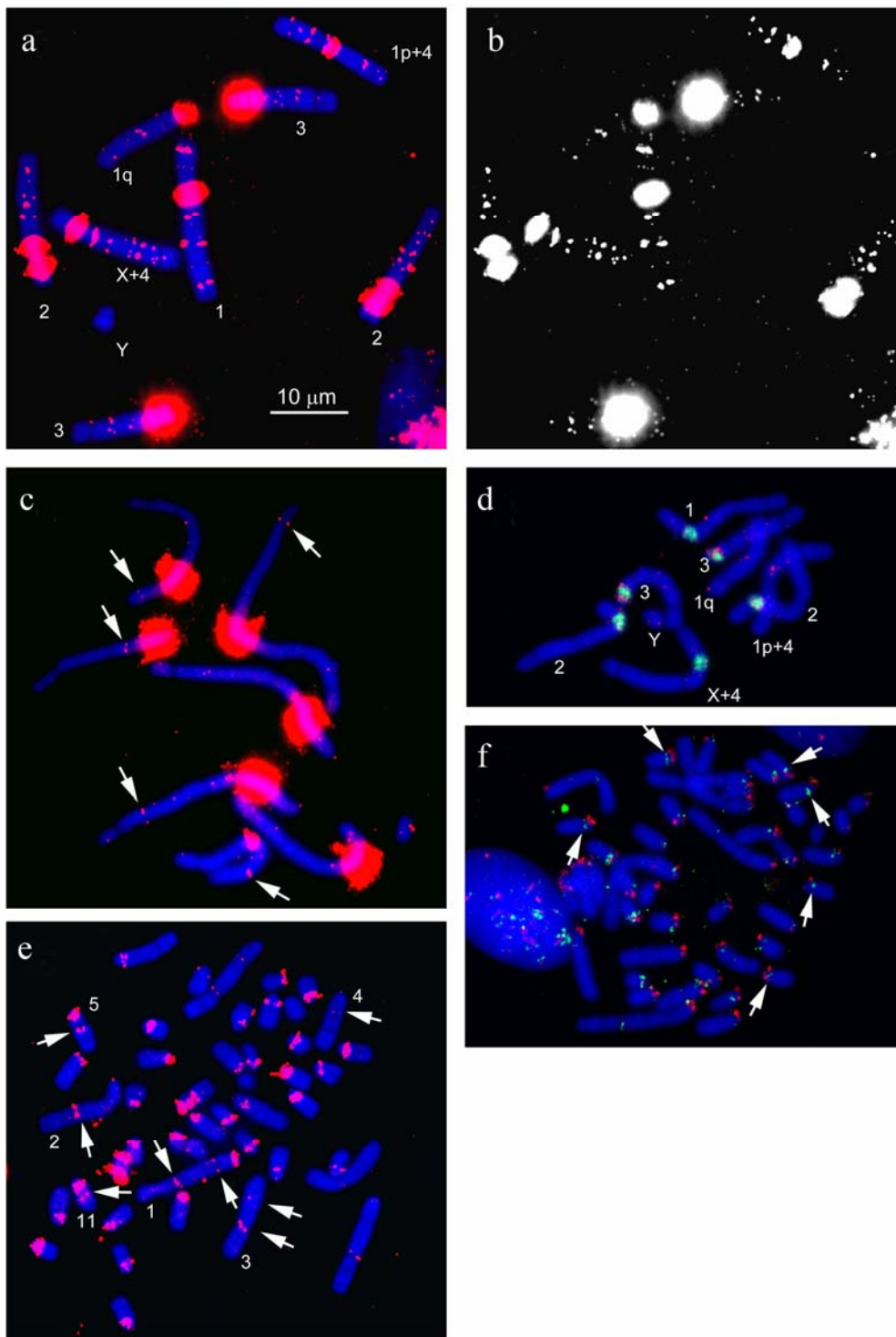
Fig. 3   FISH mapping examples

a: Satellite I DNA (BMC5) signals (red) are detected at centric regions of all *Muntiacus crinifrons* chromosomes except Y chromosome as well as at non-random interstitial fusion sites; b: Cy3 image of the same metaphase; c: Hybridization of *M. crinifrons* satellite II DNA clone probe (BM700) on a male *M. crinifrons* metaphase. Arrows indicate the satellite II DNA signals; d: Co-hybridization of satellite II (BM700, green) and satellite IV (BM1.1k, red) probes on a male *M. crinifrons* metaphase; e: Localization of *M. crinifrons* satellite I clone (BMC5) probe on male *M. reevesi* metaphase; f: Localization of *M. crinifrons* satellite I (BMC5, green) and satellite II (BM700, red) DNA probes on male *M. reevesi* metaphase. Satellite II DNA is located distal to satellite I DNA (indicated by arrows).

ChrX+4 and Chr1P+4, six interstitial signals on Chr1q and Chr2, five interstitial signals on Chr3, respectively (Fig. 3a, b). The hybridization patterns were summarized on a *M. crinifrons* idiogram (Fig. 4a). In total, 49 site-specific autosomal interstitial signals were detected by BMC5 probe on the nine *M. crinifrons* chromosomes.

The probes from satellite II element (BM700) and satellite IV element (BM1.1k) of *M. crinifrons* were also hybridized onto the metaphase chromosomes of *M. crinifrons*. Besides the predominant pericentromeric signals, several non-random interstitial signals were found along the chromosomes of *M. crinifrons* by BM700 probe (Fig. 3c). The probe of BM1.1k gave signals exclusively at the centromeres of all chromosomes in *M. crinifrons*. Two-color FISH, simultaneously hybridizing satellite II DNA (BM700) probe and satellite IV DNA (BM1.1k) probe onto the metaphases of male *M. crinifrons*, demonstrated that the hybridization signals of satellite IV (red) co-localized with the satellite II signals (green) at the centric regions of all *M. crinifrons* chromosomes (Fig. 3d).

2.3　Chromosome distribution of satellite I (BMC5) and satellite II (BM700 and FM700) DNA in *M. reevesi*, *M. feae* and *M. gongshanensis*

The probe from *M. crinifrons* satellite I element (BMC5) was also hybridized to the metaphase chromosomes of *M. reevesi* (2n = 46), *M. feae* (2n = 14) and *M. gongshanensis* (2n = 8). Besides the centromeres, BMC5 probe also gave signals at specific interstitial locations along chromosome 1–5, 11 of *M. reevesi* (Fig. 3e). The hybridization patterns of BMC5 on the chromosomes of *M. feae* and *M. gongshanensis* were very similar to that of BMC5 on the chromosomes of *M. crinifrons*. Some non-random interstitial signals were observed along the chromosomes of *M. feae* and *M. gongshanensis*. Altogether 28 and 46 site-specific autosomal interstitial signals were detected in the genomes of male *M. feae* and female *M. gongshanensis* (Fig. 4b, c).

To confirm the location of satellite I DNA and satellite II DNA on chromosomes of *M. reevesi*, two-color FISH was performed by simultaneously hybridizing the satellite I (BMC5) probe and satellite II (BM700 or FM700) probe onto chromosomes of *M. reevesi*. The results indicate satellite II element (BM700, red) is distal to satellite I (BMC5, green), and interstitial signals (BMC5, green) were also observed on chromosomes 1–5 and 11 of *M. reevesi* (Fig. 3f). Similar chromosome distribution patterns were also detected on the chromosomes of *M. reevesi* using *M. crinifrons* satellite I DNA (BMC5) and *M. feae* satellite II DNA (FM700) probes (data not shown).

3　Discussion

We have successfully cloned and characterized four centromeric satellite DNA clones, which belong to three different satellite DNA families: I (BMC5), II (BM700 and FM700), and IV (BM1.1k), from *M. crinifrons* and *M. feae*. Mapping these four satellite DNA clones onto the chromosomes of *M. reevesi*, *M. crinifrons*, *M. feae* and *M. gongshanensis* allowed us to further investigate the distribution and organization of satellite DNAs in other muntjac species and the nature of the chromosome fusions that lead to the origin of diverse karyotypes in different muntjac species.

3.1　Satellite I clone-BMC5

The hybridization pattern of BMC5 to the chromosomes of *M. reevesi* closely resembles that of the C5 clone probe in the metaphase of *M. reevesi* (Yang et al, 1997d; Li et al, 2000b), indicating that BMC5 satellite I DNA, like the C5 centromeric satellite DNA, was probably inherited from the ancestral acrocentric chromosomes, and represents the remnant of centromeric heterochromatin of ancestral chromosomes after tandem chromosomal fusion.

Recent chromosome painting and BAC mapping studies demonstrated that 27 and 28 centromere-telomere tandem fusions are needed to "reconstruct" the haploid karyotypes of *M. feae* (2n = 14), *M. crinifrons* and *M. gongshanensis* (both 2n = 8,9) respectively from a 2n = 70 ancestral karyotype (Yang et al, 1997c; Huang et al, 2006c). BMC5 clone probe revealed 49, 28 and 46 interstitial hybridization signals in the diploid cells of *M. crinifrons*, *M. feae* and *M. gongshanensis*, respectively (Fig. 4). Moreover, these interstitial hybridization signals, apparently mapped to the putative tandem fusion sites along the chromosomes of *M. crinifrons*, *M. feae* and *M. gongshanensis* defined previously by chromosome painting and comparative BAC mapping. Although 28 interstitial hybridization signals detected by BMC5 clone probe in *M. feae* was only half of the number of putative fusion sites (54), 49 and 46 interstitial hybridization
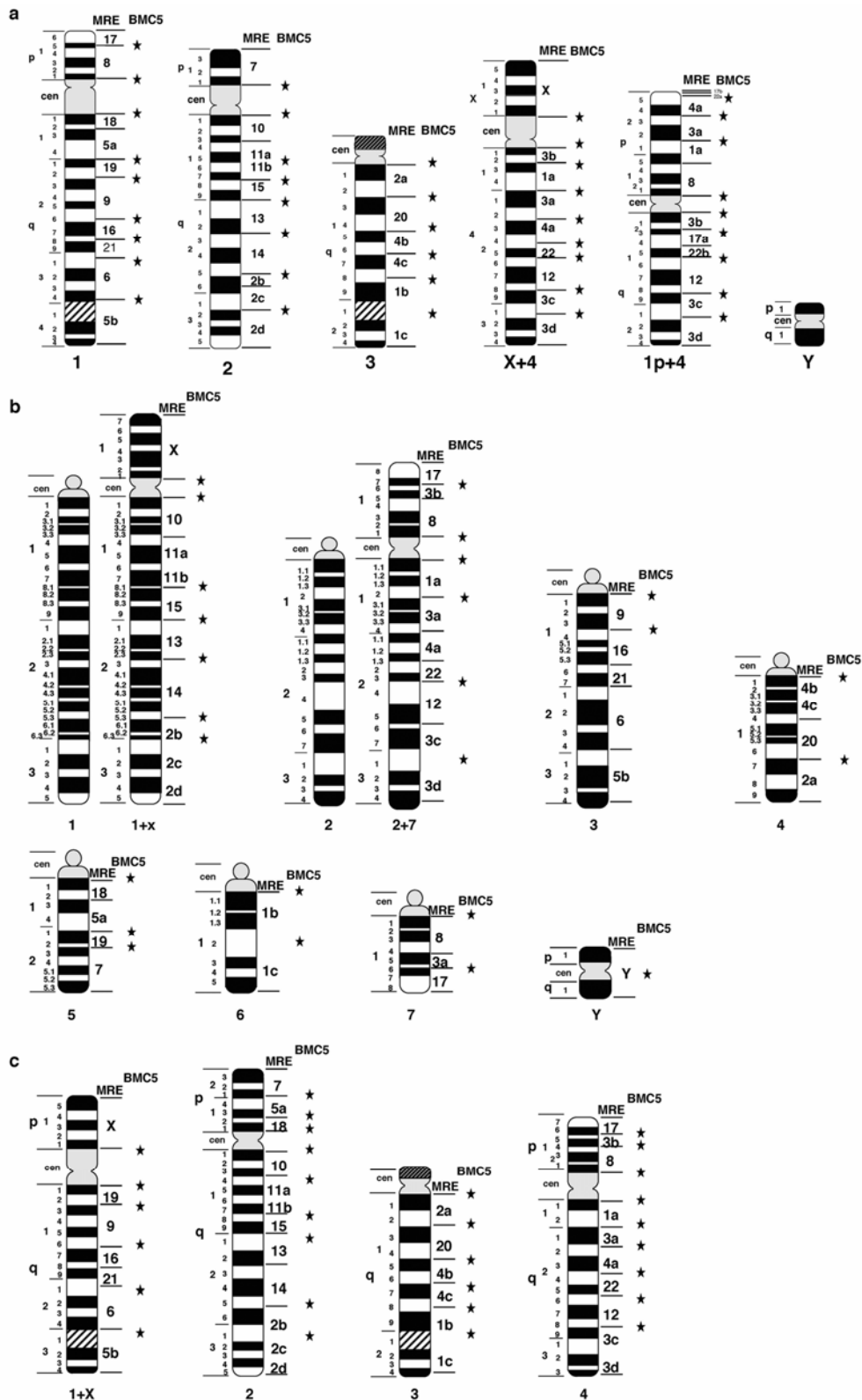
Fig. 4   Summary of the non-random interstitial signals of *Muntiacus crinifrons* satellite I clone (BMC5) on a high-
resolution G-banded idiogram of *M. crinifrons* (a), *M. feae* (b) and *M. gongshanensis* (c)

The ideograms were modified from Yang et al, 1998. The homologous chromosomes or segments of the Chinese muntjac (MRE)
indicated on the right of *M. crinifrons*, *M. feae* and *M. gongshanensis* chromosomes. The interstitial sites are indicated by *.

signals in *M. crinifrons* and *M. gongshanensis* detected by BMC5 clone probe respectively were very close to the number of putative fusion sites (56). The absence of interstitial hybridization signals in some putative fusion sites of *M. feae* is most likely due to variations in copy number of satellite I DNA (if the copy number is too low, the hybridization signals will be too weak to be detected by FISH). These data, together with the interstitial signals detected on *M. reevesi* chromosomes 1–5 and 11 by BMC5 clone probe, provide direct molecular evidence for the origin of *M. crinifrons*, *M. feae* and *M. gongshanensis* karyotypes from an ancestral karyotype (2n = 70).

3.2　Satellite II clones - BM700 and FM700

The FISH mapping results of *M. crinifrons* (BM700) and *M. feae* (FM 700) demonstrated that satellite II DNA was mainly found in the centromeric regions of the chromosomes of *M. crinifrons* (Fig. 3c) and *M. feae* (data not shown). Several interstitial hybridization signals were also detected on the arms of chromosomes of *M. crinifrons* (Fig. 3c) and *M. feae* (data not shown) by these two satellite II DNA clone probes. But the number of interstitial hybridization signals detected by satellite II DNA clone probes was far less than that by satellite I DNA clone probes. In contrast, Li et al (2000b) showed that the *M. muntjak vaginalis* satellite II clone (MMV-0.7) gave signals on most sites of tandem fusions along *M. muntjak vaginalis* chromosomes. This discrepancy could be due to either the variation in the amounts of target satellite II DNA between species, or most likely, the variation in FISH protocols as the MMV-0.7 probes made by us using the same PCR primer reported by Li et al (2000b) only gave several interstitial hybridization signals in the Indian muntjac genome (data not shown). The two-color FISH results of satellite I (BMC5) and II (BM700 and FM700) DNA probes further indicate that satellite II DNA localized distal to satellite I DNA at centromeric regions in *M. reevesi* (Fig. 3f). This finding

is in agreement with previous reports on the distribution of other satellite I and II DNA in *M. reevesi* and *M. reevesi micrurus* (Li et al, 2000b; Lin et al, 2004). The distribution pattern of satellite I and II DNA suggests that in addition to ancestral satellite I DNA at least some ancestral satellite II DNA may have been retained at the chromosome fusion sites during the process of tandem chromosome fusion in the karyotype evolution of *M. crinifrons* and *M. feae*, even though satellite II DNA seems to have a higher tendency to be eliminated than satellite I DNA.

3.3　Satellite IV clone-BM1.1k

It has been demonstrated that satellite IV DNA isolated from different deer species are highly conserved in DNA sequence and co-locate exclusively at the centromeric regions with satellite II DNA (Li et al, 2002; Lin et al, 2004). BM1.1k, a type of satellite IV DNA isolated from *M. crinifrons* in this study, also showed similar characteristics with other satellite IV DNA: co-localizing with satellite II DNA at centromeric regions of all the chromosomes of *M. crinifrons* (Fig. 3d). Since these satellite IV DNAs were isolated from different deer species using the same primer for satellite II DNA, it can be considered a newly evolved family derived from the satellite II DNA family and may have a functional centromeric role (Li et al, 2002).

Furthermore, the findings of the satellite V DNA family in genus *Muntiacus* and satellite III DNA in Hydropotes inermis (a close relative of the muntjac ancestor) but not in *Muntiacus* suggests complex rearrangements among satellite DNA might underly tandem chromosome fusions (Buntjer et al, 1998; Li et al, 2005; Lin et al, 2006). Further investigation of the organization and distribution of various centromeric satellite DNA families in muntjacs and other deer is needed to better understand the process of karyotypic evolution of muntjacs and the role of satellite DNA in tandem chromosomal fusions.

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《云南两栖爬行动物》出版

由中国科学院昆明动物研究所杨大同研究员任主编、饶定齐副研究员任副主编的《云南两栖爬行动物》一书，近日由云南出版集团公司和云南科技出版社正式出版发行。

本书记述了云南两栖动物 115 种，分别隶属于 11 科 3 目；爬行动物 162 种，分别隶属于 16 科 2 目。其中，两栖动物是在原有《云南两栖类志》（1991）102 种的基础上修改和增加了 7 个新种。为力求做到准确鉴定、描述清楚、信息正确，书中还配置了 240 幅彩色或黑白照片，可达到"一目了然"识别物种的目的。

本书包含物种的分类文献、分类地位、分类系统、生活习性、栖居环境、地理分布等内容，还对部分分类地位存在"争议"的属、种，以标本和原始文献为依据，科学地、实事求是地进行了论证，并作了必要的更正。是一本记述云南两栖爬行动物物种多样性的专著。

此书可供国内外从事动物分类学、分支生物地理学、生物进化学、动物生态学、两栖爬行动物行为、保护生物学、环境科学、系统发育、分子进化等研究的人员参考。