SHORT COMMUNICATION

Comparative Chromosome Painting in Mammals: Human and the Indian Muntjac (*Muntiacus muntjak vaginalis*)

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We have used human chromosome-specific painting probes for *in situ* hybridization on Indian muntjac (*Muntiacus muntjak vaginalis*, 2*n* = 6, 7) metaphase chromosomes to identify the homologous chromosome regions of the entire human chromosome set. Chromosome rearrangements that have been involved in the karyotype evolution of these two species belonging to different mammalian orders were reconstructed based on hybridization patterns. Although, compared to human chromosomes, the karyotype of the Indian muntjac seems to be highly rearranged, we could identify a limited number of highly conserved homologous chromosome regions for each of the human chromosome-specific probes. We identified 48 homologous autosomal chromosome segments, which is in the range of the numbers found in other artiodactyls and carnivores recently analyzed by chromosome painting. The results demonstrate that the reshuffling of the muntjac karyotype is mostly due to fusions of huge blocks of entire chromosomes. This is in accordance with previous chromosome painting analyses between various Muntjac species and contrasts the findings for some other mammals (e.g., gibbons, mice) that show exceptional chromosome reshuffling due to multiple reciprocal translocation events.

The Indian muntjac (*Muntiacus muntjak vaginalis*) is unique among mammals with respect to its low chromosome number (2*n* = 6 in the female, 7 in male; Ref. 20). The fossil record and biomolecular data suggest that muntjacs were derived from other deer species only a few million years ago (8, 9, 16). Most extant deer species show rather high chromosome numbers, the most frequent diploid number being 2*n* = 68–70. This led to the hypothesis that the karyotype of the Indian muntjac is phylogenetically a recent derived characteristic that might have evolved by multiple chromosome fusion events (4). This idea was supported by chromosome banding studies comparing the Indian muntjac with the Chinese muntjac (*M. reevesi*, 2*n* = 46), a closely related species. Several homologous Chinese muntjac banding patterns were recognized within the large Indian muntjac chromosomes (10, 17).

Recently we established chromosome painting probes from flow sorted Indian muntjac chromosomes. Painting these probes to Chinese muntjac metaphase chromosomes supported the chromosome fusion hypothesis (21). Single Indian muntjac paints hybridized to several entire Chinese muntjac homologs. Only two chromosomes were painted by more than one probe, indicating reciprocal translocations.

Chromosome banding studies and gene mapping in various mammals show that most mammalian karyotypes have been highly conserved during evolution. Recent comparisons by cross-species chromosome painting of human and carnivores (13, 19) and human and artiodactyls (3, 14, 18) confirm these results. The 22 human autosome-specific paints hybridized to 45 segments in the pig karyotype and 49 segments in the cattle karyotype, suggesting very little chromosome reshuffling during more than 60 million years of evolution of these two mammalian orders.

According to the chromosome fusion hypothesis of the origin of Indian muntjac chromosomes and the high conservation of artiodactyl and primate karyotypes, human chromosome-specific paints should hybridize also to a very limited number of segments within Indian muntjac chromosomes. We have used human chromosome-specific painting probes for *in situ* hybridization on Indian muntjac metaphase chromosomes to identify the homologous chromosome regions of the entire human chromosome set. Hybridization signals were recorded by fluorescence *in situ* hybridization (FISH) and digital image capturing.

We used standard chromosome preparations derived from two different established cell lines of the Indian muntjak. Line MMV1 was spontaneously transformed from a diploid male fibroblast line provided by the Kunming Cell Bank of the Chinese Academy of Sciences. Most of the metaphases recorded were normal diploid. Banding analysis suggested no further chromosome rearrangements. Line MMV2 was a gift from K. Sperling (Berlin, Germany) and was close to a triploid

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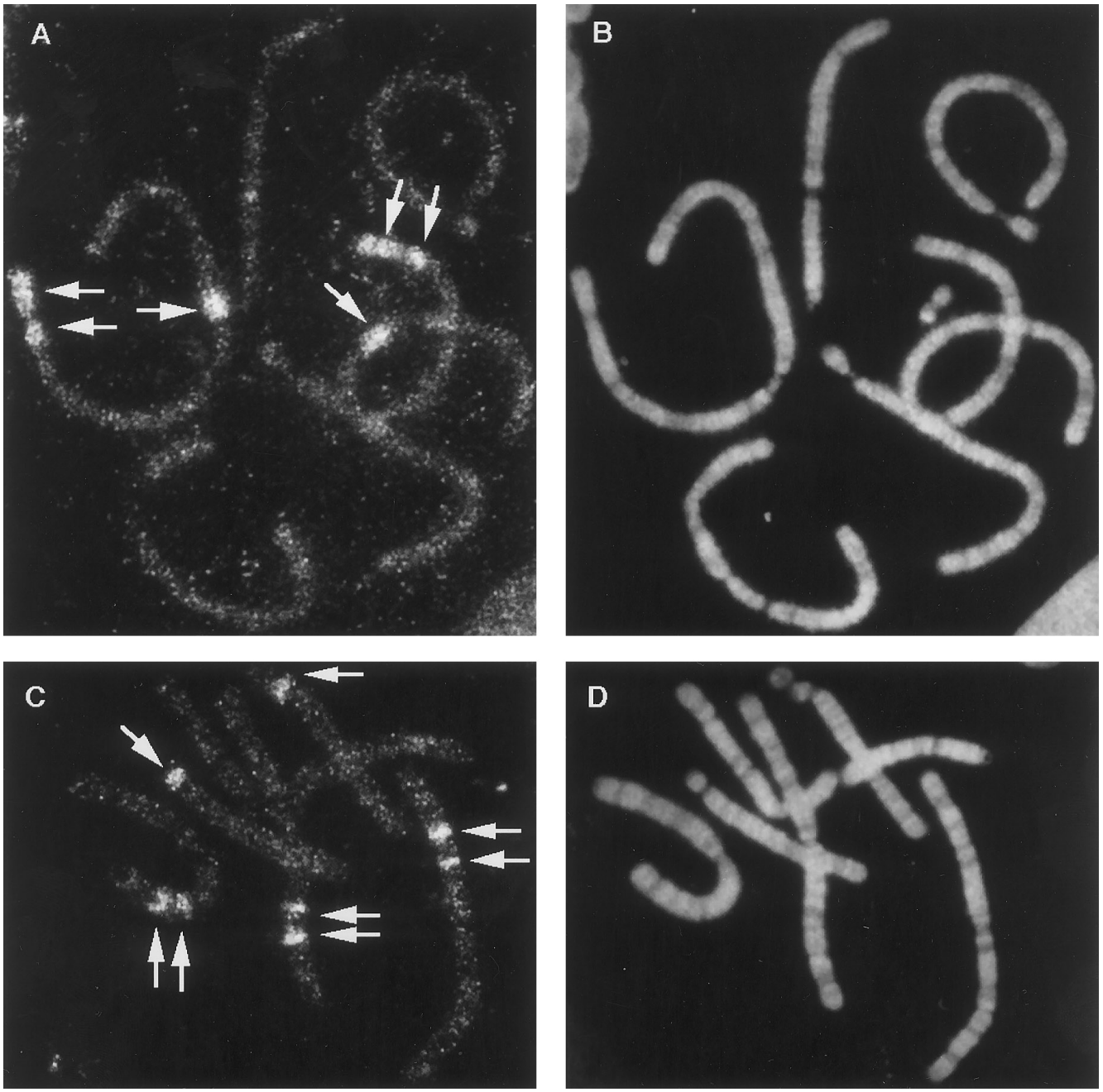

FIG. 1. Hybridization patterns of human biotin-labeled painting probes specific for chromosomes 2 (**A**) and 12 (**C**) on chromosome preparations of the Indian muntjac. (**B**) and (**D**) show the respective metaphase stained with DAPI. The metaphase in (**C**) and (**D**) shows three chromosomes for muntjac chromosome 1. Arrows point to the hybridization signals obtained in all metaphases analyzed with the respective probe. The images are not further processed and were obtained with a CCD camera as 8-bit black and white images.

male fibroblast cell line, with the small Y chromosome missing. Chromosome banding and painting with probes derived by flow sorting from normal Indian muntjac diploid fibroblasts indicated no further rearrangements of the karyotype except for a small translocation of X chromosome material to one of the three chromosomes 1 and a small deletion on the 3 + X chromosome. The triploid status of this cell line simplified signal detection since each hybridization pattern was present on all three homologs within one cell.

Cy3 and DAPI signals were captured separately as 8-bit black and white images through a quadruple bandpass filter (Chroma Technology, 84000 504) with a cooled CCD camera (Photometrics KAF-1400) (Fig. 1). Banding was enhanced by processing the DAPI image with a 5×5 high-pass spatial filter (Digital Scientific, Cambridge, UK). Assignments of hybridization signals were achieved by merging the enhanced DAPI-banded image with the unprocessed Cy3 image (Fig. 2). All image processing was performed on a Macintosh Quadra 950 using IPLab Spectrum software.

For chromosome painting we used chromosome-spe-

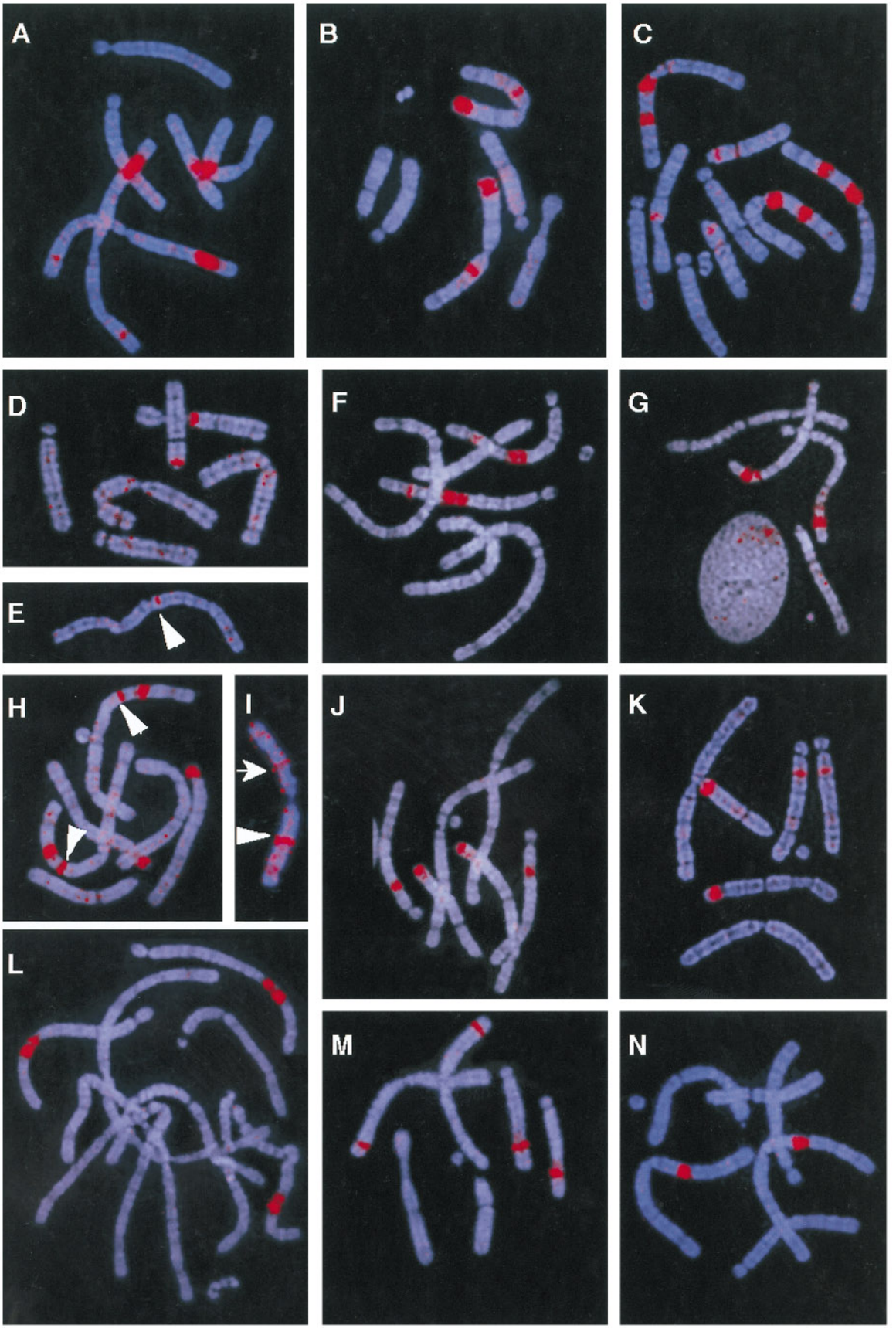

FIG. 2. Chromosome assignment of the hybridization signals obtained with human painting probes on chromosome preparations of the Indian muntjac. Images with the hybridization signal (red) were merged with those of the respective counterstained metaphase chromosomes (blue). (**A**) Human chromosome 1 probe paints two segments on muntjac chromosome 1 and one segment on chromosomes 3 and X + 3; (**B**)

cific painting libraries ("pBS libraries"; Ref. 1) kindly provided by J. Gray (San Francisco, CA) and "chromosome paints" obtained from Cambio (Cambridge, UK). Hybridization conditions and detection of the signals were as reported before (15, 21). Briefly, compared to the standard use of chromosome paints on human chromosomes, the concentration of the biotin-labeled probe was increased threefold to give a final concentration of 10μg/μl paint and 400 ng/μl human cot-1 in 50% deionized formamide, 10% dextran sulfate, 2× SSC. The probe was denatured at 65°C for 10 min and preannealed by incubation at 37°C for 1 to 3 h. Slides were denatured by incubation in 70% formamide/2× SSC solution at 65°C for 2 min. The preannealed paints were applied to slides and allowed to hybridize for 3 days at 42°C. Posthybridization washes were two 5-min incubations in 50% formamide, 50% 2× SSC at 42°C, followed by two 5-min incubations in 2× SSC at 42°C. Biotin-labeled probes were visualized by one layer of Cy3-avidin (Amersham, 1:500). After detection, slides were counterstained in 0.08 μg/ml DAPI solution.

For both cell lines and each painting probe, at least five metaphases were recorded. With the pBS libraries significant results were obtained with probes specific for human chromosomes 1, 2, 4, 5, 7, 13, 14, 16, 20, and 21. Except for the human Y chromosome probe the "Cambio paints" identified homologous segments from all human chromosomes within the Indian muntjac karyotype in both cell lines analyzed. Hybridization patterns were consistent both between the two sources of painting probes and between the two cell lines. The Cambio paints, however, provided better resolution and thus allowed additional details to be resoved. For example, the small signals obtained with paints 1, 7, and 8 on muntjac chromosomes 1p and 2q, respectively, were not identified with the pBS libraries. The same is true when comparing our results obtained with paints with previous work (15) using pBS libraries for chromosomes 1, 16, and X probes for FISH on muntjac chromosomes. Although the result for the human chromosome X probe was the same in both experiments, paints from chromosomes 1 and 16 each provided more detail than the respective pBS library.

Chromosome rearrangements that have been involved in the karyotype evolution of these two species belonging to different mammalian orders could be reconstructed from the hybridization patterns. Although, compared to human chromosomes, the karyotype of the Indian muntjac seems to be highly rearranged, we could identify a limited number of highly conserved homologous chromosome regions for each of the human chromosome-specific probes. Examples of the hybridizations obtained are given in Fig. 1 and 2. A summary of the hybridization results is given in the idiogram in Fig. 3. Chromosome painting identified 48 homologous autosomal chromosome segments, which is in the range of the numbers found in other artiodactyls and in carnivores (3, 13, 14, 18, 19).

Some hybridization patterns attract special attention because they show a remarkable conservation in mammalian evolution (3, 13, 14, 18, 19): (i) Human chromosome 1 and 2 painting probes hybridized adjacent to one another on muntjac chromosome 1p. In all reports on nonprimate species analyzed with human

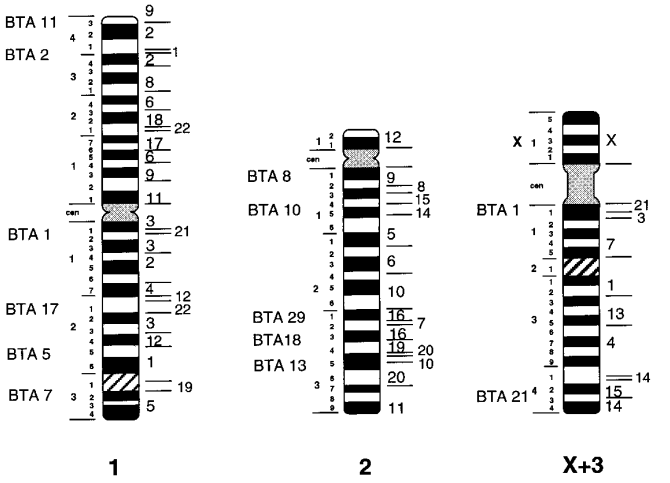

FIG. 3. Summary of the results hybridizing human chromosome-specific painting probes to chromosomes of the Indian muntjac in an idiogram. Chromosome numbers of the Indian muntjac are given below the chromosomes; the human homologous chromosome segments are indicated on the right. Some hybridizations resemble patterns found with chromosome painting of bovine chromosomes, indicating ancestral chromosome forms of artiodactyls (see text). The assumed homologous bovine (BTA) chromosome numbers are shown to the left of each muntjac chromosome idiogram.

chromosome 2 paints give two signals on muntjac chromosome 1; (**C, D,** and **E**) human chromosomes 3 and 21 paints, respectively. (**C**) A partial triploid metaphase with two signals from human paint 3 on muntjac chromosome 1 and one signal on chromosomes 3 and X + 3. The signal on muntjac chromosome 1 is colocalizing with the paint for human chromosome 21 (**E,** arrowhead). The second signal from paint 21 is found adjacent to paint 3 on muntjac chromosomes 3 and X + 3 (**D**). (**F** and **G**) The hybridization pattern of paints 10 and 20, respectively. The signal of paint 20 on muntjac chromosome 2 (**G**) is split by a segment homologous to human chromosome 10 (**F**). A second large signal from chromosome 10 is found on the same chromosome (**F**). (**H** and **I**) The hybridization pattern of human paints 12 and 22. Paint 22 gives two signals on muntjac chromosome 1 (**I,** arrow and arrowhead); the larger signal (arrow) is found adjacent to one of the two signals from the chromosome 12 paint (**H,** arrowheads). A third signal is evident on muntjac chromosome 2. (**J** and **K**) Hybridization pattern of human chromosome 14 and 15 paints, respectively. Both probes show two adjacent signals on muntjac chromosomes 2 and X + 3. (**L**) The pattern of paint 16. The signal of human paint 16 is split into two segments on muntjac chromosome 2 (**L,** partial triploid cell). The probe for human chromosome 7 hybridizes between these two segments. A second signal from this probe is found on muntjac chromosome 1. (**M**) Human chromosome 19 probe delineates two regions—one region on Indian muntjac chromosome 1 and the other on chromosome 2. (**N**) Human chromosome 17 paint gives one distinct signal on muntjac chromosome 1.

paints (cat, pig, and cattle) a chromosome homologous to parts of human chromosomes 1 and 2 is found. The hybridization pattern found on muntjac chromosome 1 suggests that this chromosome has been conserved in deer species. (ii) Gene mapping data suggest that in most mammals and even in marsupials the human chromosome 21 homolog is associated with part of the chromosome homologous to human chromosome 3 (12). This association can also be found by painting pig, cow, and cat chromosomes (3, 13, 14, 18, 19) and is also present on muntjac chromosomes 1 and 3. (iii) In all mammals analyzed except for hominoid apes (5) chromosome painting indicates an association of human chromosome 14 and 15 homologs. This association is also found on muntjac chromosomes 2 and 3 + X. (iv) The association of signals obtained with human chromosome 16 and 19 probes on muntjac chromosome 2q is conserved in carnivores and artiodactyls (3, 13, 14, 18, 19). (v) Two chromosomes each showing homologous segments to human chromosomes 12 and 22 are found in all nonprimate species thus far analyzed by chromosome painting. This association is also found in the muntjac (chromosome 1q).

Various adjacent hybridization signals on muntjac chromosomes find their counterparts in single bovine chromosomes. In the cow 14 of 29 chromosomes are painted with more than one human chromosome-specific probe (3, 18). Twelve of these 14 chromosomes (*Bos taurus* chromosomes 1, 2, 5, 7, 8, 10, 11, 13, 17, 18, 21, and 29), including 29 hybridization signals, can be found conserved as individual signals in the muntjac karyotype, suggesting that they have been conserved by evolution from common ancestral chromosome forms. This allows us to predict cow versus muntjac chromosome homologies for these chromosomes (Fig. 3). The remaining 19 hybridization signals on muntjac chromosomes may be derived from ancestral deer chromosomes painted by just one human probe. Two human chromosome-specific paints (chromosomes 13 and 17) hybridized to a single bovine chromosome (3, 18), indicating that these chromosomes have been conserved. Since these chromosomes give only one signal each on the muntjac karyotype, it is likely that they have also been conserved within the large muntjac chromosomes.

These results indicate that the reshuffling of the muntjac karyotype is due to fusions of huge blocks of entire chromosomes rather than due to various reciprocal translocations. This is in accordance with previous analyses of hybridizations between different muntjac species (21) and contrasts with the findings for some other mammals (e.g., gibbons, mouse) that show exceptional chromosome reshuffling due to multiple reciprocal translocation events (2, 5, 6, 7, 11). Cross-species *in situ* hybridization with chromosome painting probes derived from cattle or from deer species that show the proposed ancestral high chromosome number karyotype will allow a more detailed analysis of the chromosome reshuffling in muntjac species. This work is in progress.

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REFERENCES

1. Collins, C., Kuo, W. L., Segraves, R., Pinkel, D., Fuscoe, J., and Gray, J. W. (1991). Construction and characterisation of plasmid libraries enriched in sequences from single human chromosomes. *Genomics* **11:** 997–1006.
2. Copeland, N. G., Jenkins, N. A., Gilbert, D. J., Eppig, J. T., Maltais, L. J., Miller, J. C., Dietrich, W. F., Weaver, A., Lincoln, S. E., Steen, R. G., Stein, L. D., Nadeau, J. H., and Lander, E. S. (1993). A genetic linkage map of the mouse: Current application and future prospects. *Science* **262:** 57–66.
3. Hayes, H. (1995). Chromosome painting with human chromosome-specific DNA library reveals the extent and distribution of conserved segments in bovine chromosomes. *Cytogenet. Cell Genet.* **71:** 168–174.
4. Hsu, T. C., Pathak, S., and Chen, T. R. (1975). The possibility of latent centromeres and a proposed nomenclature system for total chromosome and whole arm translocations. *Cytogenet. Cell Genet.* **15:** 41–49.
5. Jauch, A., Wienberg, J., Stanyon, R., Arnold, N., Tofanelli, S., Ishida, T., and Cremer, T. (1992). Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting. *Proc. Natl. Acad. Sci. USA* **89:** 8611–8615.
6. Koehler, U., Arnold, N., Wienberg, J., Tofanelli, S., and Stanyon, R. (1995). Genomic reorganization and disrupted chromosomal synteny in the siamang (*Hylobates syndactylus*) revealed by fluorescence *in situ* hybridization. *Am. J. Phys. Anthropol.* **97:** 37–47.
7. Koehler, U., Bigoni, F., Wienberg, J., and Stanyon, R. (1995). Genomic reorganization in the concolor gibbon revealed by chromosome painting. *Genomics* **30:** 287–292.
8. Lan, H., and Shi, L. (1994). Restriction endonuclease analysis of mitochondrial DNA of muntjacs and related deer. *Science China Ser. B* **37:** 294–302.
9. Ma, S., Wang, Y., and Xu, L. (1986). Taxonomic and phylogenetic studies on the genus *Muntiacus*. *Acta Theriol. Sinica* **6:** 191–208.
10. Neitzel, H. (1987). Chromosome evolution of Cervidae: Karyotypic and molecular aspects. *In* "Cytogenetics—Basic and Applied Aspects" (G. Obe and A. Basler, Eds.), pp. 91–112, Springer-Verlag, Heidelberg/New York.
11. O'Brien, S. J., Seuánez, H. N., and Womack, J. E. (1988). Mammalian genome organization: An evolutionary view. *Annu. Rev. Genet.* **22:** 323–335.
12. O'Brien, S. J. (1993). "Genetic Maps: Locus Maps of Complex Genomes," 6th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
13. Rettenberger, G., Klett, C., Zecher, U., Bruch, J., Just, W., Vogel, W., and Hameister, H. (1995). Zoo-FISH: Cat and human karyotypes closely resemble the putative ancestral mammalian karyotype. *Chromosome Res.* **3:** 479–486.
14. Rettenberger, G., Klett, C., Zecher, U., Kunz, J., Vogel, W., and Hameister, H. (1995). Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. *Genomics* **26:** 372–378.
15. Scherthan, H., Cremer, T., Arnason, U., Weier, H.-U., Lima-de-Faria, A., and Fronicke, L. (1994). Comparative chromosome painting discloses homologous segments in distantly related mammals. *Nature Genet.* **6:** 342–347.
16. Schmidtke, J., Brennecke, H., Schmid, M., Neitzel, H., and Sper-

ling, K. (1981). Evolution of muntjac DNA. *Chromosoma* **84:** 187–193.

17. Shi, L., Ye, Y., and Duan, X. (1980). Comparative cytogenetic studies on the red muntjac, Chinese muntjac and their F_1 hybrids. *Cytogenet. Cell Genet.* **26:** 22–27.
18. Solinas-Toldo, S., Lengauer, C., and Fries, R. (1995). Comparative genome map of human and cattle. *Genomics* **27:** 489–496.
19. Wienberg, J., Stanyon, R., Nash, W. G., O'Brien, P., Yang, F., O'Brien, S. J., and Ferguson-Smith, M. A. Conservation of human vs. feline genome organization revealed by reciprocal chromosome painting. Submitted for publication.
20. Wurster, D. H., and Benirschke, K. (1970). Indian muntjac, *Muntiacus muntjak:* A deer with a low diploid chromosome number. *Science* **168:** 1363–1366.
21. Yang, F., Carter, N. P., Shi, L., and Ferguson-Smith, M. A. (1995). A comparative study of karyotype of the muntjacs by chromosome painting. *Chromosoma* **103:** 642–652.