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Giemsa banding patterns in the chromosomes of twelve species of cats (Felidae)[1]

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Abstract

The Giemsa banding patterns in the chromosomes of 12 cat species, all previously studied with conventional techniques, have been compared. Although the basic banding pattern is largely similar among the species, several species possess distinctive banding features. Banding has permitted identification of three, rather than two, types of F-group chromosomes and has revealed that the C3 chromosome in two species is the equivalent, by banding pattern, of the F2 and F3 chromosomes in other species. The banding pattern of the X chromosome is similar among all 12 felid species and is also similar to that of the other mammalian species with "original-type" submetacentric X chromosomes which have been studied in this laboratory.

The chromosomes of 30 species of Felidae have been studied with conventional staining techniques. The results of these studies in 22 species were summarized in tabular form by WURSTER and BENIRSCHKE (1968). More recently the karyology of 8 more species has been described (WURSTER, 1969; WURSTER and BENIRSCHKE, 1969; JOTTERAND, 1971, 1972; WURSTER-HILL, 1973), bringing the total to 30. The remarkable feature of cat karyology has been the uniformity of the karyotype pattern among the species, but small differences have permitted each species to be placed in one of five distinctive karyotypic groups (WURSTER-HILL, 1973). The relatively new banding techniques (NILSSON, 1973) permit identification of individual chromosomes. The purpose of the present study was to determine if cat species could be further distinguished on the basis of their banding patterns.

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Materials and methods

Skin biopsies were taken from the following 11 species at the National Zoological Park in Washington, D.C.: *Felis caracal* (caracal lynx), *F. pajeros (colocolo)* (pampas cat), *F. geoffroyi* (Geoffroy's cat), *F. libyca* (African wildcat), *F. viverrina* (fishing cat), *F. yagouaroundi* (jaguarondi), *F. temmincki* (Asian golden cat), *F. bengalensis* (leopard cat), *F. chaus* (jungle cat), *Panthera leo* (lion), and *P. tigris* (tiger). Two specimens of *F. catus* were obtained locally. The skin explants were cultured under glass slides in large Leighton tubes and nurtured in McCoy's 5A (modified) medium fortified with 20 % calf serum. Secondary cultures were harvested at the time of maximum cell division. Mitosis was arrested with 0.04 % colchicine (0.1 ml/ml of medium) for 2 h, and the cells were freed with 0.25 % trypsin, washed with Earle's balanced salt solution, treated with 1:5 hypotonic Earle's solution, and fixed in 1:3 acetic acid-methanol fixative. The cells were dropped onto wet, cold, precleaned slides; some were air dried and some flame dried. The slides were placed in boxes with dessicant capsules and stored at 37° C for 24 to 72 h before staining. The chromosomes were banded with the use of trypsin followed by Giemsa staining, as described by SEABRIGHT (1971). Slides were placed horizontally in 0.25 % room-temperature trypsin (Gibco, lyophilized, made up in Earle's balanced salt solution) for 35 to 55 s, rinsed quickly in two changes of room-temperature isotonic saline, and stained for 5 to 8 min in Giemsa stain (Harleco original azure-blend type, No. 620). The Giemsa staining solution consisted of 1 ml of Giemsa stain, 1 ml of 0.14 M sodium phosphate (Na_2HPO_4), and 48 ml of distilled water. The *p*H was not adjusted. The staining solution was made up immediately before use. Chromosomes were photographed with a Zeiss photomicroscope on Kodak High Contrast Copy Film 5069 (HC710), which was developed in Kodak D19 developer.

Results and discussion

Banding patterns have been established from a study of 144 karyotypes. The chromosomes have been arranged in the standard pattern, based on size and centromeric position, of groups A through F as established for the cat at the San Juan Conference (JONES, 1965). The basic banding pattern of the cats, which is similar among species, with some exceptions to be discussed, is shown by a schematic representation of the haploid complement of *F. viverrina* (fig. 1). The karyotype of *F. viverrina* is shown in fig. 2. These chromosomes are extended in length, permitting the visualization of more bands than are usually seen in the typically more contracted metaphase chromosomes. The karyotypes of the other species (figs. 3–9) illustrate the more common appearance of banded cat karyotypes. Since the karyotypes of *F. libyca* and *F. catus* are identical

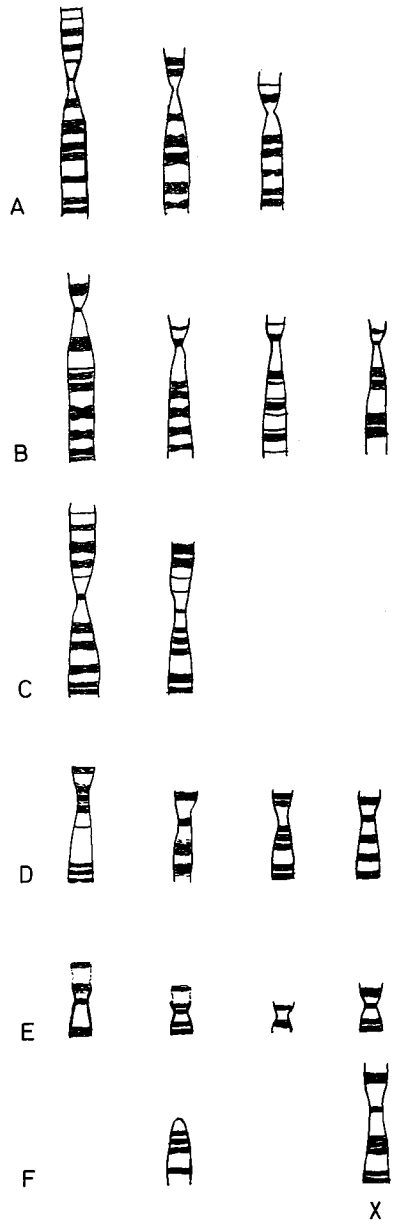

Fig. 1. Schematic representation of the haploid complement of *F. viverrina* derived from the karyotype depicted in fig. 2.

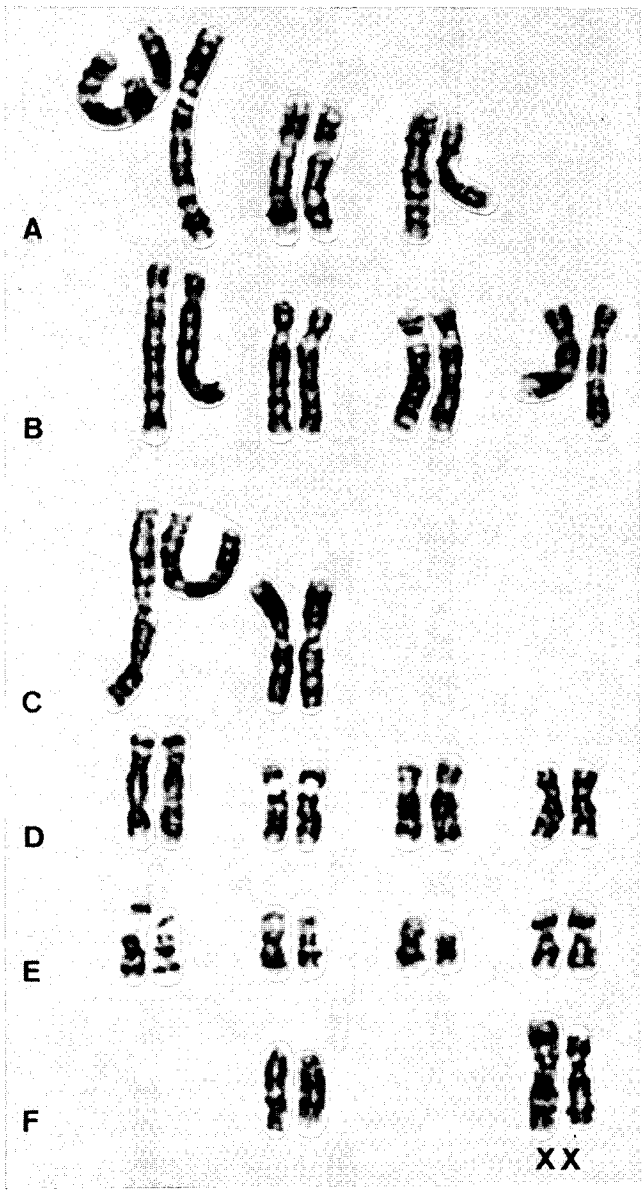

Fig. 2. Banded karyotype of female *F. viverrina*. Note the presence of an E4 chromosome. The F group has only the F2 chromosomes in this species. The X chromosomes are in the lower-right corner. Reproduced at 1800 ×.

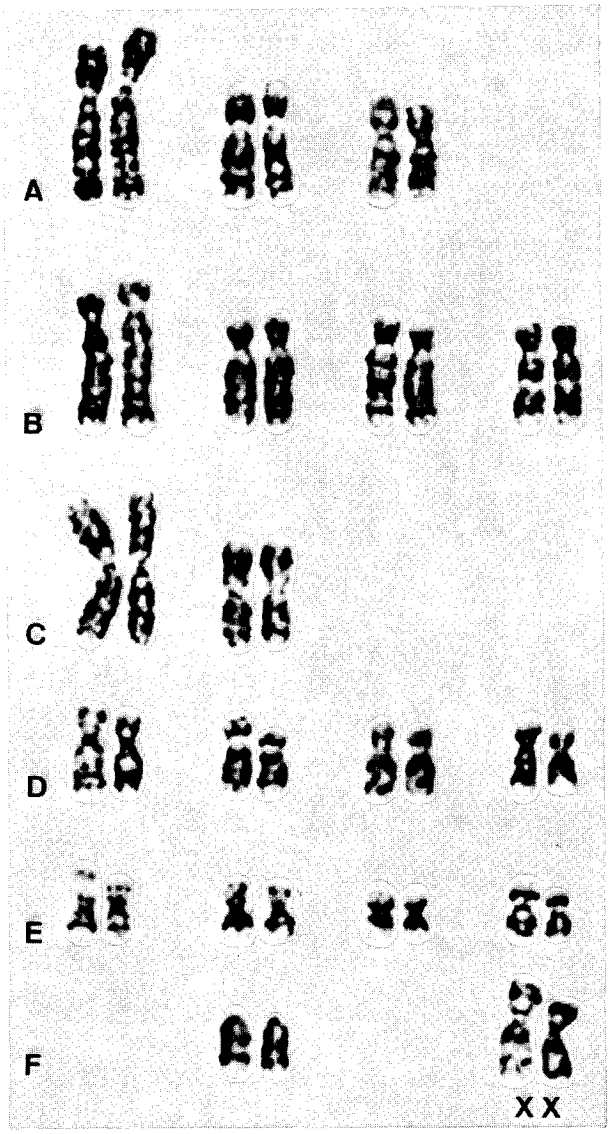

Fig. 3. Banded karyotype of female *F. bengalensis.* The X chromosomes are in the lower-right corner. The karyotype is similar to *F. viverrina* but shows the more typical appearance of banding in cat chromosomes. Reproduced at 1800 ×.

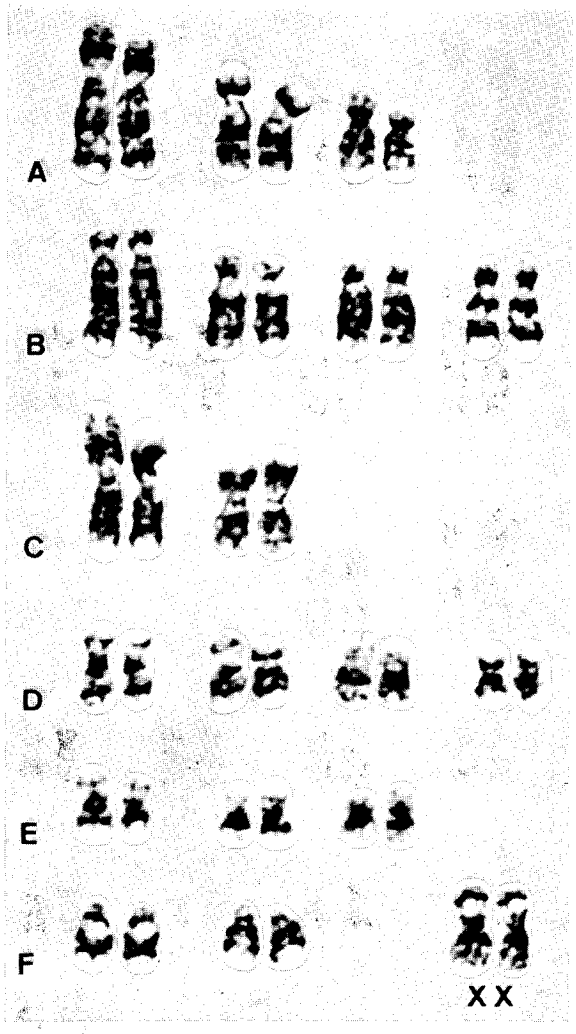

Fig. 4. Banded karyotype of female *F. caracal.* The X chromosomes are in the lower-right corner. The F group contains F1 and F2 chromosomes. Note the negatively stained area in the short arms of the X. Reproduced at 1800 ×.

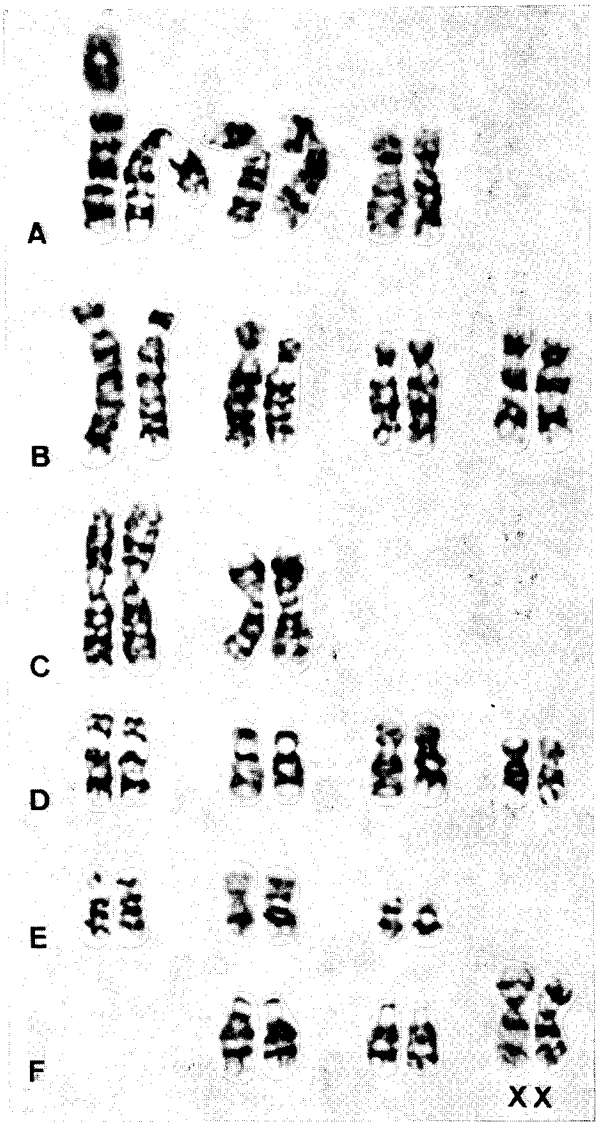

Fig. 5. Banded karyotype of female *F. temmincki.* The X chromosomes are in the lower-right corner. The F group contains F2 and F3 chromosomes. Note the negatively stained areas in the short arms of the A1 and D1 chromosomes. Reproduced at 1800 ×.

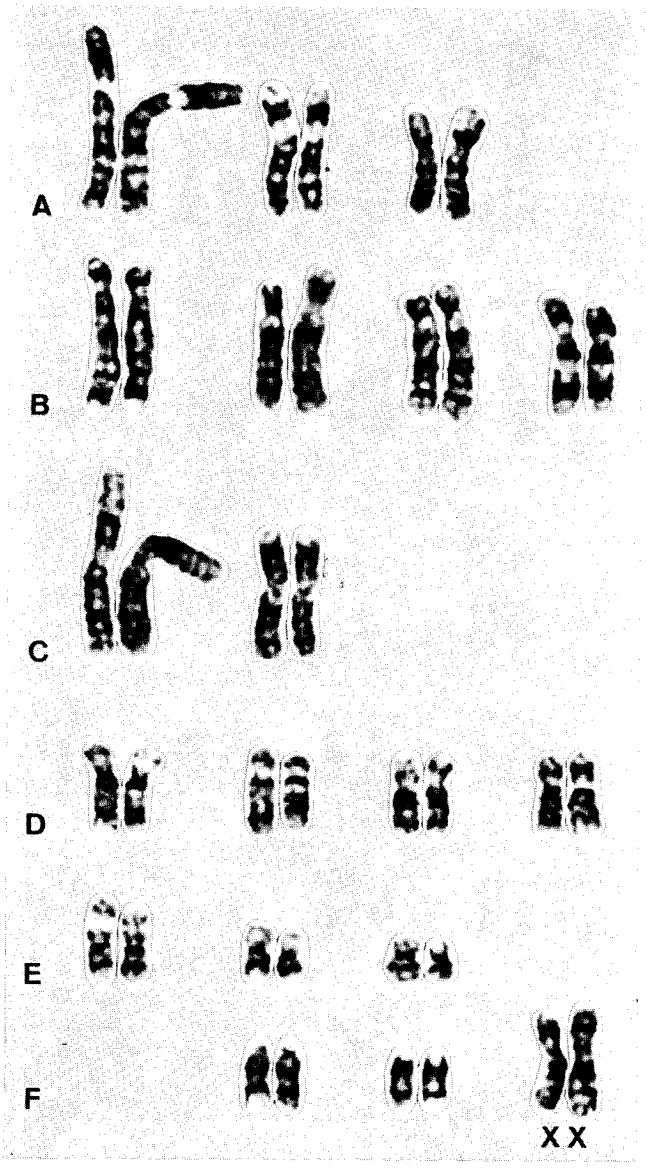

Fig. 6. Banded karyotype of female *P. leo*. The X chromosomes are in the lower-right corner. The F group contains F2 and F3 chromosomes. The A1 chromosome has a small, negatively stained area in the short arms adjacent to the centromere. Reproduced at 1800 ×.

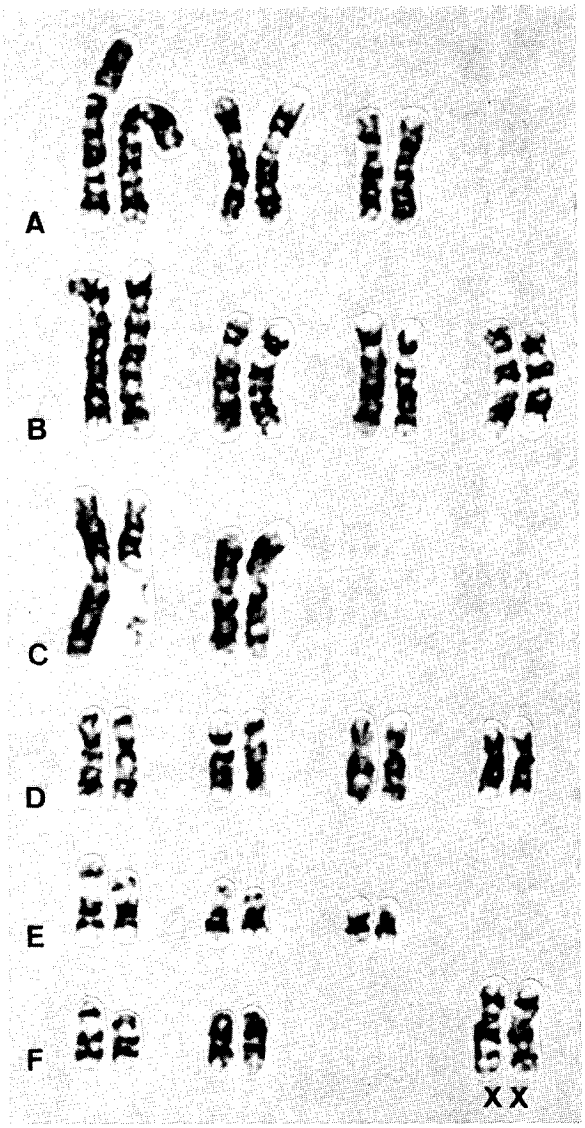

Fig. 7. Banded karyotype of female *F. chaus*. The X chromosomes are in the lower-right corner. The C1 chromosome is distorted by artifact. The F group contains F1 and F2 chromosomes. Reproduced at 1800 ×.

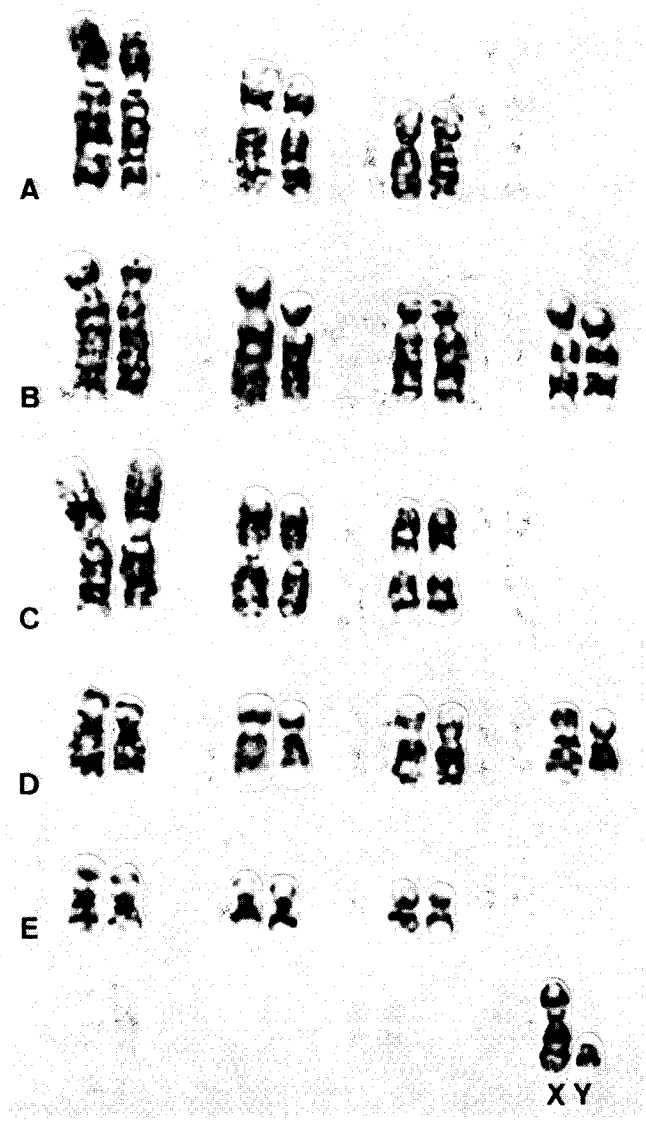

Fig. 8. Banded karyotype of male *F. pajeros*. The sex chromosomes are in the lower-right corner. There are no F-group chromosomes. Note presence of C3 chromosome. Reproduced at 1800 ×.

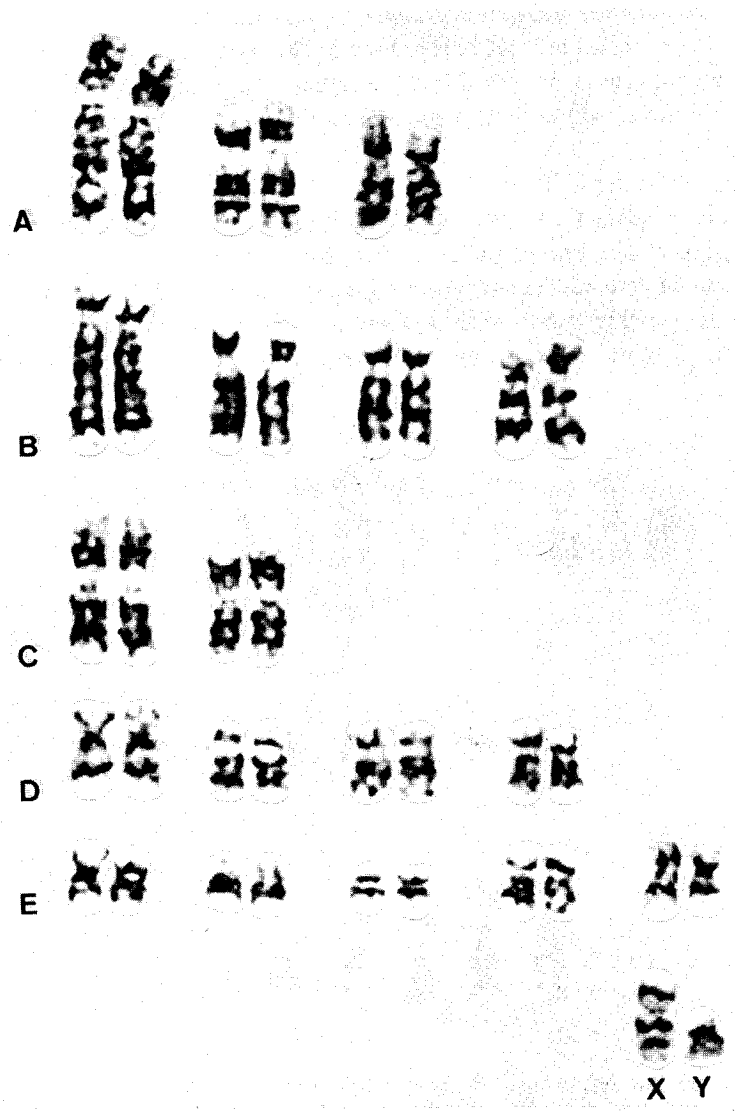

Fig. 9. Banded karyotype of male *F. yagouaroundi*. The sex chromosomes are in the lower-right corner. There are no F-group chromosomes. Note the presence of E4 and E5 chromosomes. Reproduced at 1800 ×.

to that of *F. chaus* and that of *F. geoffroyi* is identical to that of *F. pajeros*, these three species are not represented in the figures. A description of the banding patterns is not considered necessary, since the karyotypes present the data more accurately and the schematic drawing presents the authors' interpretation.

The distinction of cat species according to their karyotypes is summarized in table I. As described previously, the conventionally stained karyotype of any one of the 30 investigated species of cats can be placed into one of five distinctive groups (WURSTER-HILL, 1973). Banding has permitted further subdivision of these groups. These 12 species formerly fell into 4 of the 5 groups and now, restudied with banding, are divisible

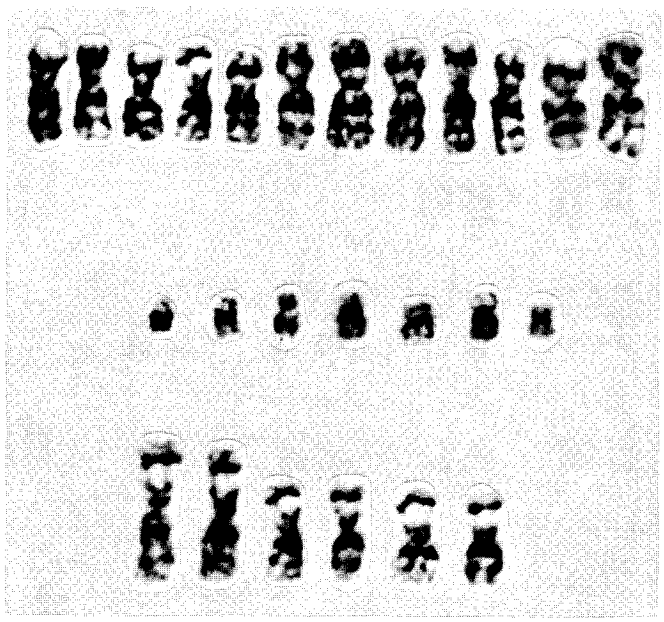

Fig. 10. Top row, left to right: X chromosome from *F. catus, F. geoffroyi, F. libyca, F. caracal, F. bengalensis, F. viverrina, F. pajeros, F. temmincki, P. leo, F. chaus, F. yagouaroundi,* and *P. tigris.* Note similarity of all except for pronounced negatively stained area in short arms of *F. caracal.* Middle row, left to right: Y chromosome from *F. geoffroyi, F. libyca, F. caracal, F. bengalensis, F. pajeros, F. yagouaroundi,* and *P. tigris.* Note terminal banding on long arms of *F. caracal*'s Y. With better banding, species might be distinguishable by the patterns on the Y chromosome. Bottom row: X chromosome from several cells of *F. caracal* demonstrating consistent appearance of negatively stained area in short arms.

Table I. Summary of most distinctive features of each species studied.

Species	No. of species ♂	♀	2n	Chromosomes[1] A1	B4	C3	D1	E4	E5	F1	F2	F3	X	Y	G-banding	Remarks
F. libyca	2	0	38			0		0	0	+	+	0			identical	karyotypes indistinguishable before banding
F. chaus	0	2	38			0		0	0	+	+	0				
F. catus	0	2	38			0		0	0	+	+	0				
F. caracal	1	1	38			0		0	0	+	+	0	+	+	unique	
F. temmincki	0	2	38	+		0	+	0	0	0	+	+			unique	
P. leo	0	2	38	+	+	0		0	0	0	+	+			similar	
P. tigris	1	1	38		+	0		0	0	0	+	+				
F. viverrina	0	1	38			0		*	0	0	+	0			identical	banding has not enabled any further distinction of these species
F. bengalensis	1	1	38			0		*	0	0	+	0				
F. geoffroyi	1	0	36			*		0	0	0	0	0			identical	
F. pajeros	1	0	36			*		0	0	0	0	0				
F. yagouaroundi	1	0	38			0		*	*	0	0	0			unique	

[1] An asterisk signifies the presence of a chromosome that distinguishes that species by placing it in one of the five karyotypic patterns previously described (WURSTER-HILL, 1973). A plus sign signifies a chromosome that distinguishes, or partly distinguishes, that species by its banding pattern. A zero indicates absence of that chromosome from the karyotype. An unmarked space signifies the presence of that chromosome with a banding pattern similar to its homolog in the other species, i.e., a nondistinctive chromosome.

into seven groups or individual categories (table I). *Felis caracal* (fig. 4) has a unique X chromosome, with a large, negatively stained area in the short arms and, consequently, a band in the short arms that is much more distal than the equivalent band in the X of all the other species (fig. 10). The Y of *F. caracal* is also unique (fig. 10) in having a dark centromere and a pronounced terminal band on the long arms. Possibly some species could be distinguished by the banding pattern of the Y chromosome, but males of every species were not available for study. Good banding on the tiny Y chromosome is also difficult to obtain. *Felis temmincki* has unique A1 and D1 chromosomes, with a large, negatively stained area in the short arms adjacent to the centromere (figs. 5, 11, and 12). It also has distinguishing F-group chromosomes. *Felis temmincki, P. leo,* and *P. tigris* are the only species having F2 and F3 chromosomes (fig. 13), but those of *F. temmincki* have prominent satellites, whereas

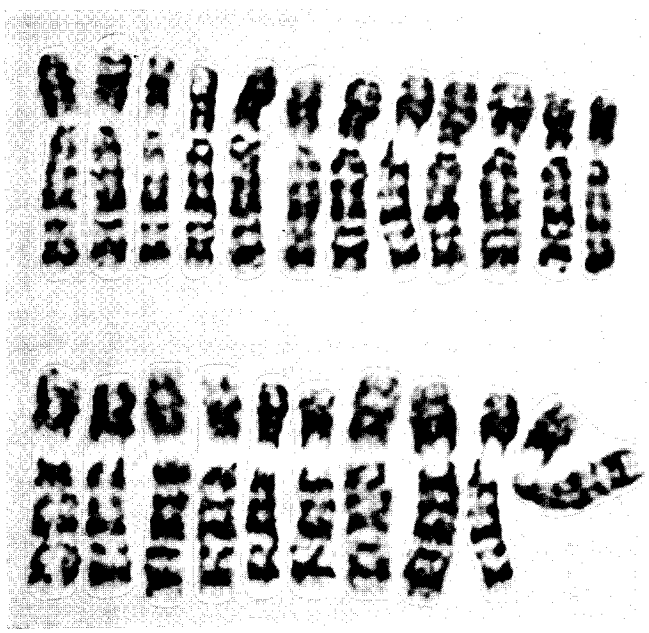

Fig. 11. Top row, left to right: A1 chromosome from *F. pajeros, P. leo, F. geoffroyi, F. viverrina, F. yagouaroundi, F. libyca, F. bengalensis, F. temmincki, F. catus, P. tigris, F. chaus,* and *F. caracal.* Note pronounced negatively stained area in short arms of *F. temmincki* and, to a lesser degree, in the short arms of *P. leo.* Bottom row: A1 chromosome from several cells of *F. temmincki* showing consistent appearance of the large, negatively stained area in the short arms.

those of the other two species do not. These features may be peculiar to the individual rather than the species, however, and this could not be determined since so few specimens were studied. *P. leo* has a slightly pronounced negatively stained area in the paracentric region of the short arms of the A1 and D2 chromosomes, but it does not have this feature in the D1 chromosome (figs. 6, 11, and 12). *P. tigris* lacks the negatively stained area in all these chromosomes. It might thus be distinguishable from *P. leo,* but more specimens would have to be studied to confirm this difference. *P. tigris* and *P. leo* have in common a distinctive B4 chromosome which is different from the B4 in all the other species (fig. 14). There is a heavy band or set of bands in the long arm immediately adjacent to the centromere and a negatively stained paracentric area in the short arm. The derivation of this chromosome is easily envisioned as a pericentric inversion of the B4 chromosome that occurs in all the other species.

The banding patterns in these 12 species show that these karyotypes are, as assumed from earlier studies, largely similar to one another. Species with two pairs of acrocentrics in the F group were previously assumed to have homologous pairs. Banding has shown that this is not

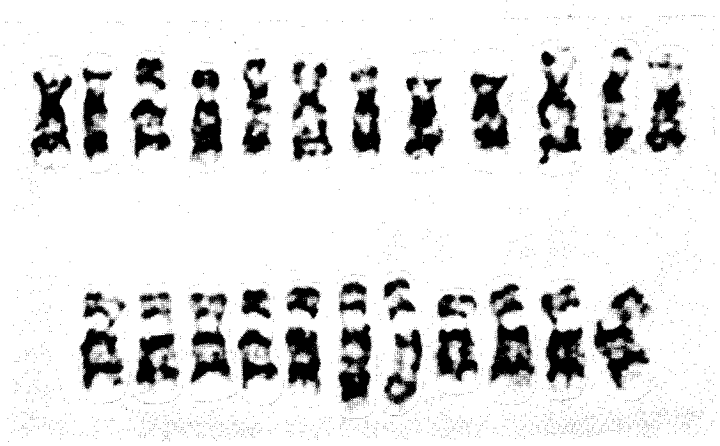

Fig. 12. Top row, left to right: D1 chromosome from *F. catus, F. libyca, F. temmincki, P. leo, F. chaus, F. bengalensis, F. viverrina, F. caracal, F. yagouaroundi, F. pajeros, F. geoffroyi,* and *P. tigris.* Note pronounced negatively stained area in short arms of *F. temmincki.* Bottom row: D1 chromosome from several cells of *F. temmincki* showing consistent appearance of the large, negatively stained area in the short arms.

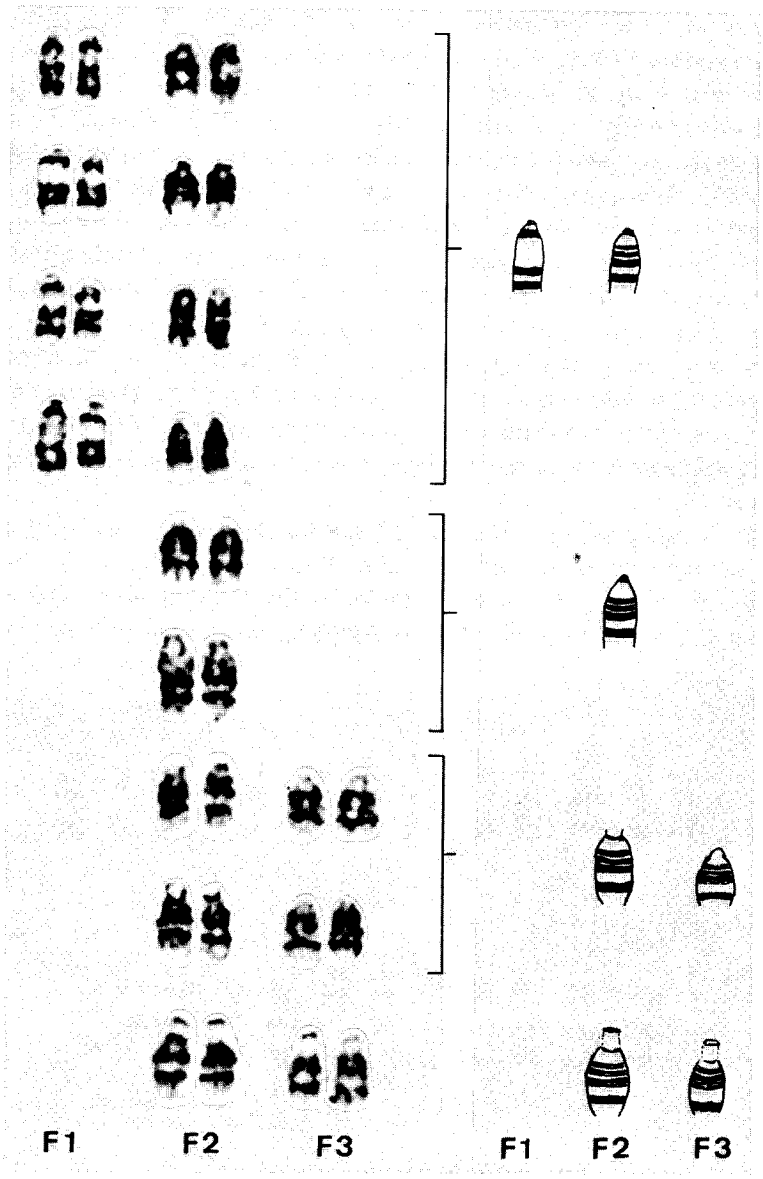

Fig. 13. Photos, top to bottom: F-group chromosomes from *F. catus, F. libyca, F. chaus, F. caracal, F. viverrina, F. bengalensis, P. tigris, P. leo,* and *F. temmincki.* Freehand drawings illustrate schematic interpretation of F-group banding patterns derived from study of many karyotypes.

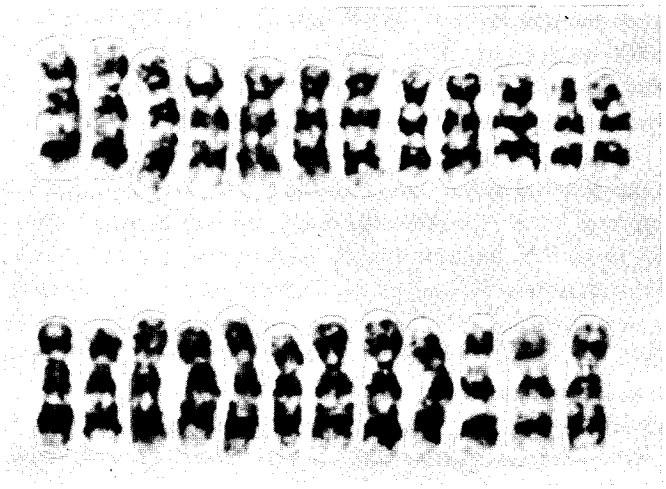

Fig. 14. Top row, left to right: B4 chromosome from *P. tigris, P. leo, F. chaus, F. pajeros, F. catus, F. yagouaroundi, F. temmincki, F. libyca, F. viverrina, F. bengalensis, F. geoffroyi,* and *F. caracal.* Note relatively longer short arms and shorter long arms of *P. tigris* and *P. leo* owing to pericentric inversion. Bottom row: B4 chromosome from several cells of *P. leo* and *P. tigris* demonstrating the consistent appearance of this chromosome within and between these two species (first eight chromosomes from *P. leo,* last four from *P. tigris*).

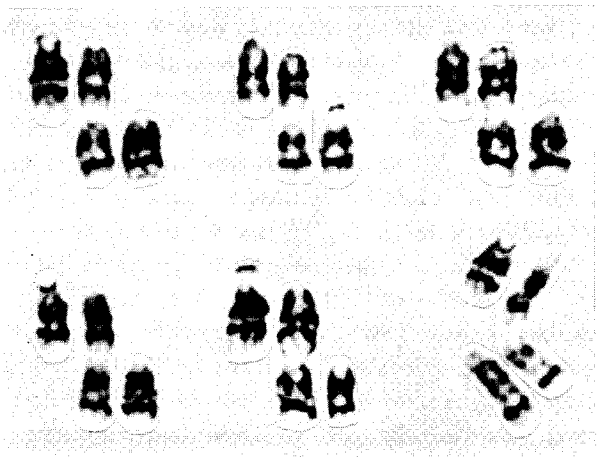

Fig. 15. C3 chromosome from several cells of *F. pajeros* and *F. geoffroyi* paired with F2 chromosomes (top half) and F3 chromosomes (bottom half) from several species. Note similarity of banding in the F2 and top half of C3 and in the F3 and bottom half of C3, presumably representing sections of homologous material.

always true and that three different types of F-group chromosomes exist (fig. 13). Although *F. libyca, F. chaus, F. caracal, F. catus, F. temmincki, P. leo,* and *P. tigris* each have two pairs in the F group, the first four species each have F1 and F2, whereas the last three species have F2 and F3.

From previous studies it was considered a likely possibility that the C3 chromosome in those species with a C3 and no F-group chromosomes (*F. geoffroyi* and *F. pajeros,* fig. 8) represented a centric fusion of the F-group chromosomes (WURSTER and BENIRSCHKE, 1968; WURSTER, 1969). It can be seen from fig. 15 that C3 can be considered to be the product of a tandem fusion, an F3 fused at the centromere to the telomeric end of an acentric F2. Tandem translocation involving an autosome and an X chromosome has been described in several mammalian species, for example, *Potorus tridactylus* (SHAW and KROOTH, 1964), *Carollia perspicillata* (HSU et al., 1968), *Muntiacus muntjak* (WURSTER and BENIRSCHKE, 1970), and *Gazella dorcas* (WURSTER, 1972). This type of translocation involving two autosomes has been reported recently (STOCK and HSU, 1973) to have occurred in several chromosomes of *Cercopithecus aethiops.* Thus, with the help of banding techniques, tandem translocations may be discovered to be a more common mechanism in karyotype evolution than thought previously.

Assuming similar banding patterns can be considered indicative of homology between two chromosomes, *F. pajeros* and *F. geoffroyi,* with C3 chromosomes, and *F. temmincki, P. leo,* and *P. tigris,* with F2 and F3 chromosomes, all have mainly similar chromosomal material (although *F. temmincki* has distinctive negatively stained areas in the A1 and D1 chromosomes, and *P. leo* and *P. tigris* have apparent inversions in the B4 chromosome) and might be considered more closely related to one another than to the species having F1 and F2 chromosomes. *Felis bengalensis* and *F. viverrina* each have only an F2 pair, but they also have an E4 pair which the species with two F pairs lack (figs. 2 and 3). The E4, which is similar in these two species, is similar in size to an F-group chromosome. *Felis yagouaroundi* (fig. 9) has no F-group chromosomes but has instead an E4 similar to that of *F. bengalensis* and *F. viverrina* and an E5. Both of these are the size of F-group chromosomes (fig. 16). On the basis of previous studies (WURSTER, 1969), it was suggested that E4 and E5 resulted from a pericentric inversion of the F-group chromosomes. The banding patterns of E4 and E5 do not support this interpretation, however, unless the authors are just not successful in envi-

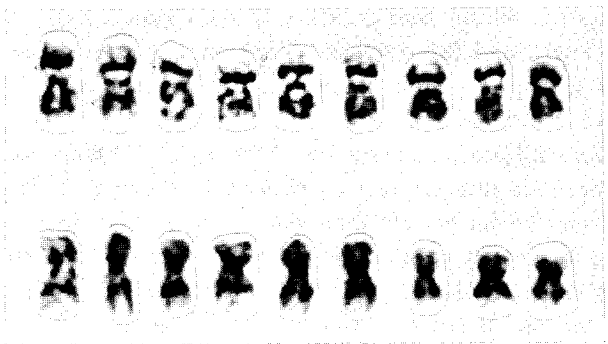

Fig. 16. Top row: E4 chromosome from several cells of *F. viverrina, F. bengalensis,* and *F. yagouaroundi.* The banding is similar in all three species. Bottom row: E5 chromosome from several cells of *F. yagouaroundi.* This chromosome seems resistant to banding, usually appearing fuzzy, with dull bands.

sioning the transformation. Hybridization studies, if successful, could provide definite answers to this problem.

Fig. 10 shows the similarity of the banding pattern in the X chromosome of all 12 species. *Felis caracal,* as noted above, is distinguished by the large, negatively stained area in the short arms. It is of interest, however, that the felid X is banded similarly to the X of the human and to the X of a number of other mammalian species with submetacentric Xs that have been studied in this laboratory. These include *Arctictis binturong, Fossa fossa,* and *Atelocynus microtis* of the Carnivora, *Cacajo calvus* and *Macaca tonkeana* of the Primates, *Sus scrofa domestica* of the Artiodactyla, and *Lariscus insignis* of the Rodentia. *Muntiacus reevesi* and *Cervus timorensis* of the Artiodactyla have acrocentric X chromosomes with a banding pattern, similar in the two, easily derived by a pericentric inversion of the submetacentric X possessed by the species mentioned above. The uniformity of banding pattern in the "original type" X (OHNO et al., 1964) is not unexpected and offers strong support for the suggestion of OHNO et al. (1964), based on comparative X-chromosome sizes, "that the X still retains much of the genetic material originally comprising the primitive X of a common ancestor." This same banding pattern should be recognizable within the unusually large Xs and the complicated autosomal-gonosomal translocations found in some species.

The banding patterns have lent some support to those who would further subdivide the large genus *Felis* as discussed previously (WURSTER

and BENIRSCHKE, 1968). And contrary to the opinion based on previous studies (WURSTER and BENIRSCHKE, 1968), there is now some karyotypic support for placing *F. temmincki* into the genus *Profelis,* as suggested by THENIUS and HOFER (1960).

While the uniformity among cat karyotypes remains striking even with banding, it is not as great as previously thought. Relationships of the species may be further clarified when more species become available for banding studies. It is interesting that the B-group chromosomes are identical in most of these species. Chromosomes identical in banding pattern to the cat B-group chromosomes in the majority of these 12 species have been found in *Arctictis binturong* and *Fossa fossa* of the Viverridae (WURSTER-HILL, unpublished material), indicating that these chromosomes were probably present in the common ancestral stock of the Felidae and Viverridae prior to the Upper Pliocene. This would also imply that the inversion of the B4 chromosome in *P. leo* and *P. tigris* was a more recent event.

Very small differences that have not been detected in these preparations may exist among these cat species. Such minute differences have been reported in the banding of several species of Eurasian mice (WURSTER-HILL et al., 1973). Hopefully, banded preparations can be refined in the future and more mammalian species re-examined for meaningful elucidation of interspecific relationships.

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