Hereditas 81: 211—220 (1975)

Q-, G- and C-band patterns of the mink chromosomes

NILS MANDAHL and KARL FREDGA

Institute of Genetics, University of Lund, Sweden

MANDAHL, N. and FREDGA, K. 1975. Q-, G- and C-band patterns of the mink chromosomes. — *Hereditas 81*: 211—220. Lund, Sweden. ISSN 0018-0661. Received September 23, 1975

The Q-, G- and C-band patterns of each individual chromosome of the mink (*Mustela vison*) are described. A detailed idiogram of the Q- and G-band patterns is presented, together with a nomenclature along the lines recommended for human chromosomes by the Paris Conference. The distribution, size and total amount of C-banded regions in the chromosomes of the mink and of some other carnivores are compared. The effect on chromosome banding of aging of the preparations is discussed briefly.

Karl Fredga, Institute of Genetics, University of Lund, S-22362 Lund, Sweden

The karyotype of the mink (*Mustela vison* PEALE and BEAUVOIS, Mustelidae, Carnivora, Mammalia) was described by FREDGA (1961) and by NES (1962). The 30 chromosomes were arranged by these authors into three groups with regard to chromosome size, viz. large (Nos. 1–7), medium (Nos. 8–11, X) and small chromosomes (Nos. 12–14, Y). Both authors point out that it is difficult or impossible to distinguish, by means of conventional acetic-orcein staining, chromosome pairs 3 from 4 and 9 from 10. However, recent chromosome banding techniques have given a new dimension to chromosome research and have especially improved the possibility of chromosome identification. After G-staining all the mink chromosomes show characteristic banding patterns, making feasible the identification of each autosome pair and the sex chromosomes.

It is urgent that detailed knowledge is acquired concerning the karyotypes of our laboratory and domesticated animals. The aim of the present study is to describe the Q-, G- and C-band patterns of the mink. The study is part of a comparative investigation into the karyotypes of mustelids, with particular attention to the amount and distribution of constitutive heterochromatin. Particularly the C-banding technique has opened new possibilities for identification of constitutive heterochromatin, and by combining C-banding with other banding techniques it may become feasible to subdivide the heterochromatin into different classes on the basis of staining reactions, as has actually been done in man.

Two mustelids previously studied, the pygmy weasel (*Mustela rixosa* BANGS) and the ferret (*Mustela putorius furo* L.), have 7 and 3 chromosome pairs, respectively, with block heterochromatin (FREDGA and MANDAHL 1973). One chromosome arm of these chromosomes entirely or mainly consists of constitutive heterochromatin; in both species some chromosomes lack centromeric heterochromatin (or have too little of it to be detected by the techniques applied). As will appear from the present study, the mink has quite another distribution of constitutive heterochromatin.

Material and methods

Cell cultures were initiated from biopsies of lung and heart tissues from two adult, ranch-bred minks, one female Standard type and one male Black cross. The specimens were kindly supplied by Mr. L. Åbjörnsson, Kingelstad, Tågarp.

The cell cultures were set up, grown and harvested according to our routine procedures (FREDGA 1972), and chromosome preparations were made according to the conventional air-drying technique (without heating or flaming).

For Q-bands the technique developed by CASPERSSON et al. (1968, 1971) was used with minor modifications (FREDGA 1971).

For G-bands the technique of WANG and FEDOROFF (1972) was performed as follows: Slides were treated at room temperature for 1–4 minutes in a trypsin solution (0.025% trypsin in a 1:1 mixture of Ca^{2+}- and Mg^{2+}-free balanced salt solution and versene at pH 7.0), rinsed in balanced salt solution and stained for 4–5 minutes in Giemsa solution (1 part Merck's "Giemsa Lösung" and 50 parts phosphate buffer, pH 6.8), and finally rinsed in two changes of distilled water.

For C-bands the "BSG" technique of SUMNER (1972) was used. However, the time of treatment in $Ba(OH)_2$-solution was shortened to 30–90 seconds. The time recommended by Sumner, 5–15 minutes, affected the chromosomes in these preparations far too drastically. To shorten the whole procedure somewhat, the staining time could be reduced to half an hour by using a 5% Giemsa solution instead of a 2% solution. In our materials the "BSG" technique was superior to the technique described by ARRIGHI and HSU (1971) in as far as it produced more distinct bands and more consistent results.

Results

1. Conventional staining

In the present study the chromosomes are arranged and numbered in decreasing order of size according to FREDGA (1961).

In ideal non-banded metaphases all chromosome pairs except Nos. 3–4 can be identified. In less favourable cells, difficulties exist in distinguishing Nos. 2, 3 and 4 from each other and, in addition, 9 from 10, and 11 from X. Pair No. 2 may be distinguished from Nos. 3–4 by the presence of a secondary constriction in its long arm close to the centromere. Contrary to NES (1962) we regard the secondary constriction as located in the long arm of No. 2. In the present study this secondary constriction was quite prominent in one or both homologues of the female individual studied (Fig. 5a) but was rarely seen in the male, and when seen, appeared narrow and indistinct. The other secondary constriction of the karyotype, in the short arm of No. 8, was always distinct in both individuals.

In chromosomes 1–4 with their low arm ratio (1.1–1.2 according to NES 1962) it was often difficult to distinguish between the short (p) and the long arm (q). No systematic measurements were undertaken in the present study, but by comparison of a reasonable number of complete karyotypes (9 unbanded, 4 Q-banded, 13 G-banded and 16 C-banded) it was possible to identify p and q in the chromosomes 1–4. It was decided, in agreement with previous studies, that in No. 8 the arm with the secondary constriction was the short arm.

2. Q-bands

In accordance with studies on other materials, it was found that in the mink negative Q-bands corresponded to light-staining G-bands, and positive Q-bands to dark-staining G-bands. In contrast to findings in Homo, no exception to this rule was found in the two specimens of mink studied. For this reason we have chosen just to present the Q-banded karyotype of Fig. 1 and to reserve any details to the description of the G-banded chromosomes.

The most intensely fluorescent portion of the karyotype is the major part of the long arm of No. 8.

3. G-bands

The G-band patterns permit exact identification of every chromosome pair in the mink, as is apparent from the 3 cells karyotyped in Fig. 2. Fig. 3 is a diagrammatic representation of the Q- and G-bands of the mink. The short arms and the long arms of the chromosomes are divided, whenever convenient, into 2 or 3 regions, separated by landmark bands. These and all other bands have been given numbers in accordance with the

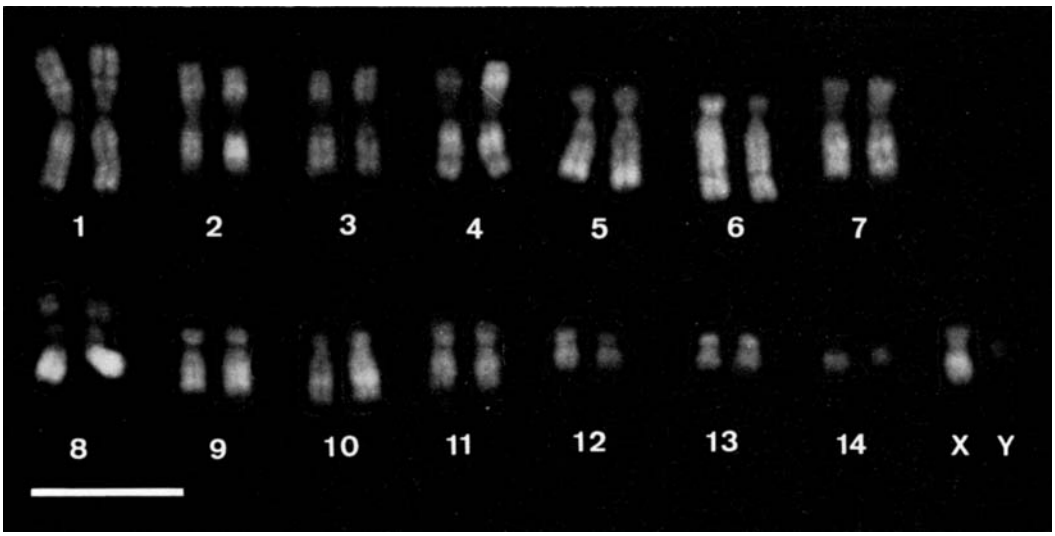

Fig. 1. Q-banded male karyotype of the mink. Note the bright fluorescence of the long arm of chromosome No. 8. Scale indicates 10 μ.

nomenclature recommended for the human karyotype by the Paris Conference (1971). In the subsequent list of the 16 chromosome types of the mink, the most characteristic and striking bands are recorded. In the present paper we have designated the intensity of staining as light, weak and dark.

Chromosome No. 1. – A large number of more or less distinct bands are distributed rather evenly along the chromosome. A typical feature of the short arm of this chromosome is the distinct dark band (p21), followed by the fairly extended light band (p22). The long arm is characterized by a heavy distal band (q33).

Chromosome No. 2. – There are faintly stained areas on both sides of the centromere. The extension of the area into the long arm (q11) is dependent on the size of the secondary constriction, which is sometimes visible as a pale gap on the borderline to q12. A dark band in the middle of the short arm (p21) is a prominent landmark. The long arm is characterized by three dark bands, the middle one (q21) being the most prominent. Sometimes these bands fuse into a broad dark region in the middle of the arm.

Chromosome No. 3. – Similar to No. 2, this chromosome has faintly stained areas in both arms adjacent to the centromere; these areas are, however, somewhat smaller than in No. 2. The short arm has three dark bands of similar size evenly spaced. There are four dark bands in the long arm, the two central ones sometimes fusing into one.

Chromosome No. 4. – The proximal half of the short arm consists of a large, light-staining region. The distal half contains two dark bands, the proximal (p21) being the most conspicuous one. In the long arm there are three dark bands. The light band, q22, is often striking.

Chromosome No. 5. – The short arm has two prominent dark bands in the middle of it, p21 and p23, which often fuse. There are two distinct dark bands in the long arm, one near the centromere (q12) and one, broader, close to the telomere (q18). In addition, 2 dark bands (q14 and 16) separated by a weak band (q15) are found in the middle of the long arm. One of these bands would have been a natural landmark for subdividing this long chromosome arm into two regions. Since, however, they were irregularly appearing in most

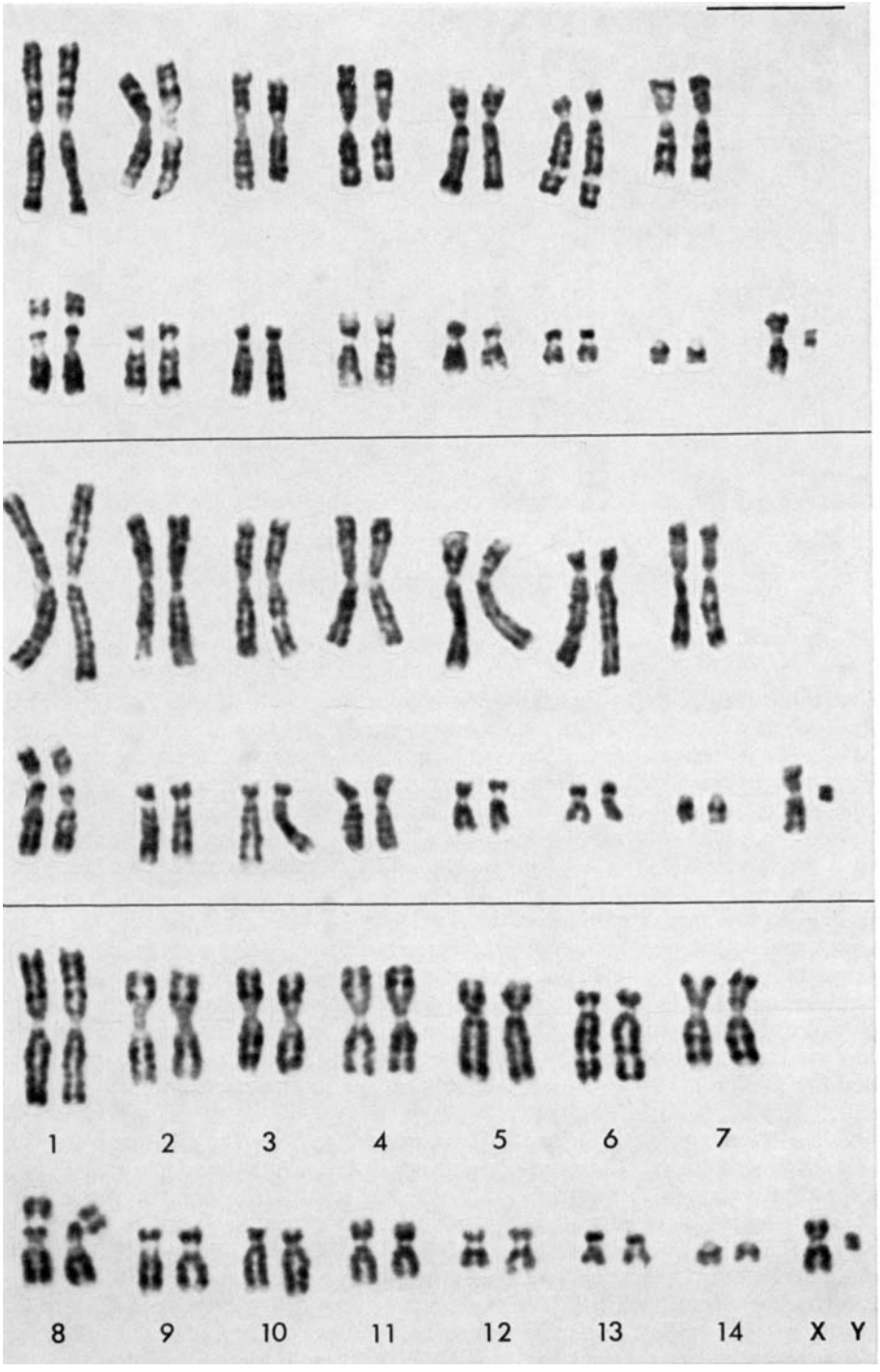

Fig. 2. Three G-banded male karyotypes of the mink. Note the characteristic appearance of the centromeric region of chromosome No. 9. Scale indicates 10 μ.

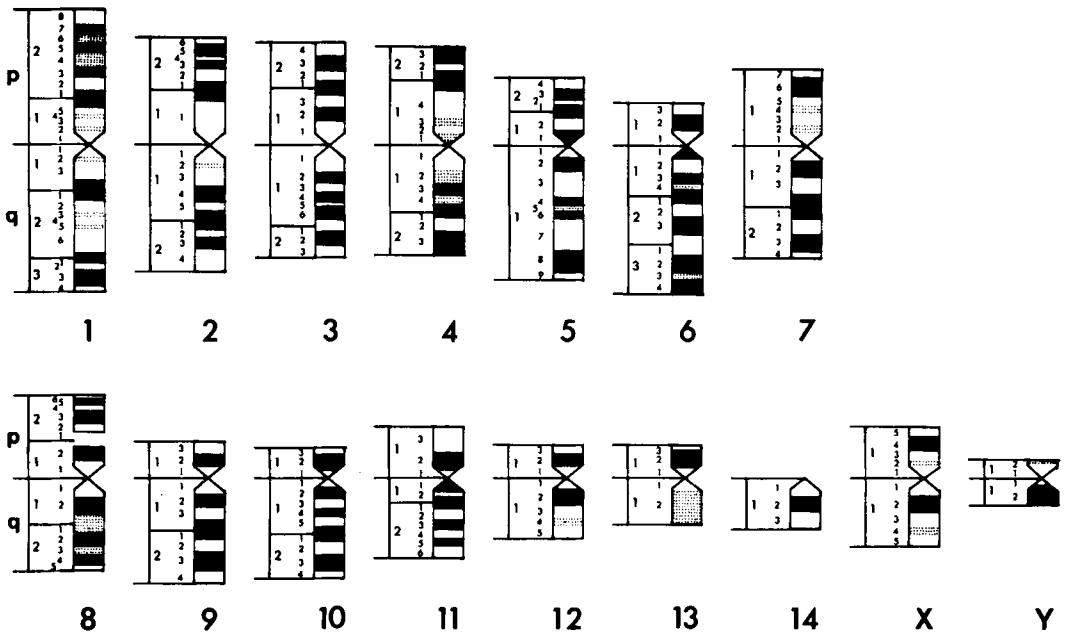

Fig. 3. Diagram of G-band patterns of mink chromosomes. In the text the white, dotted and black bands are referred to as light, weak and dark bands, respectively.

cells, we preferred to let the entire arm be one region.

Chromosome No. 6. – A dark band (p12) is located in the center of the short arm, which is clearly smaller than the short arm of No. 5. Six dark bands are present in the long arm, the most distinct ones being q23 and q32, which have a striking light band, q31, inbetween.

Chromosome No. 7. – Most of the short arm is faintly stained, except the distal part where a heavy dark band (p16) is found. The long arm has three dark bands rather evenly spaced.

Chromosome No. 8. – The characteristic secondary constriction in the short arm is visible as an unstained gap (p21). Proximal to the constriction there is a distinct dark band (p12). The distal region has two dark bands (p23 and 25). The long arm contains three dark bands (q12, 22 and 24) interspaced by weak bands. The arm gives a generally dark impression, except in the proximal part.

Chromosome No. 9. – This chromosome has a relatively broad, unstained centromeric region, which looks like a hole in the chromosome. This gives the chromosome a characteristic appearance, immediately distinguishing it from No. 10. The short arm has one dark band (p12), the long arm three (q12, 21 and 23). The long arm ends in a relatively broad, light band.

Chromosome No. 10. – The major part of the short arm is dark. The long arm has four dark bands, the two distal ones being broader than the other two.

Chromosome No. 11. – There is a heavy dark band in the short arm close to the centromere (p12), while the distal part of the short arm, in contrast to this part of the X chromosome, is rather light. Four dark bands are present in the long arm, q21 being the most distinct of them.

Chromosome No. 12. – A large part of the short arm consists of one dark band. The long arm has two positive bands, the proximal one (q12) being the most distinct.

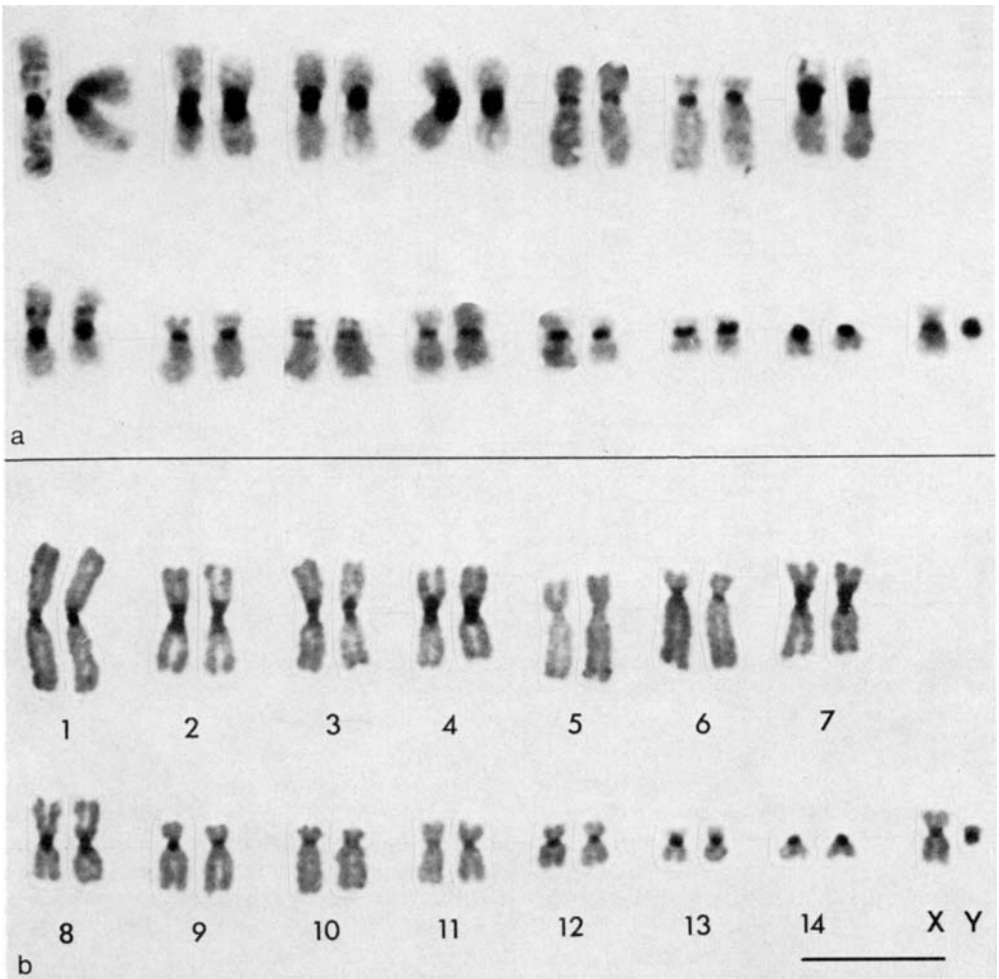

Fig. 4a and b. Two C-banded male karyotypes of the mink; a: chromosomes from a fresh preparation; b: from a two-months-old preparation, in the latter some C-bands have been lost, e.g. in chromosomes No. 5 and No. 8. Scale indicates 10 μ.

Chromosome No. 13. – Most of the short arm is heavily stained while the long arm is stained more weakly and has no distinct banding.

Chromosome No. 14. – This is a telocentric chromosome with only one dark band slightly closer to the centromere than to the distal end.

The X chromosome. — The short arm contains two positive bands, one proximal, narrow and weak (p12), and one distal, broad and distinct (p14). In the long arm there are also two positive bands, the proximal one (q12) being the most conspicuous of them.

The Y chromosome. – Due to its small size it is very difficult to establish any banding pattern. It is a biarmed chromosome and in some good metaphases the long arm was darker than the short one.

4. C-bands

The C-bands of the mink chromosomes are mainly restricted to the centromeric regions (Fig.

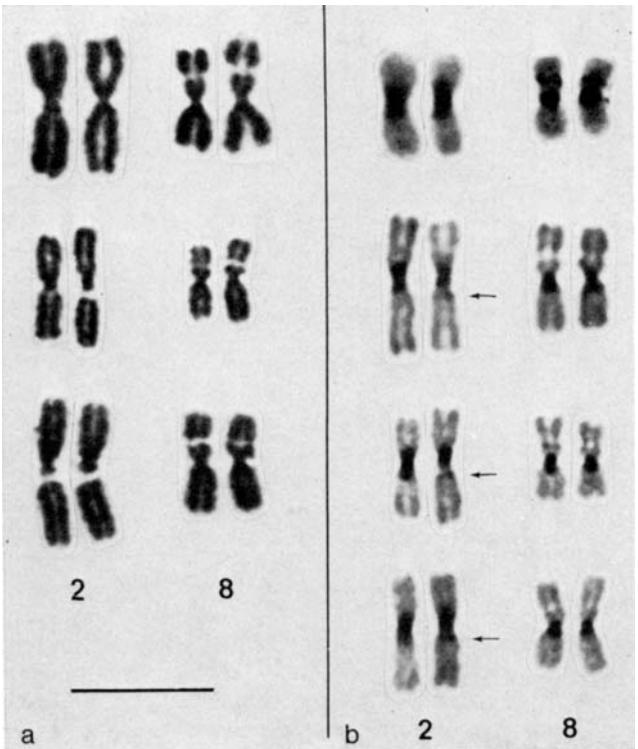

Fig. 5a and b. Chromosomes No. 2 and No. 8 of the mink selected from different cells. — a: Giemsa stained unbanded chromosomes, top row from the male; middle and bottom rows from the female. Note the difference between the two sexes in the expression of the secondary constriction of chromosome No. 2. — b: C-banded chromosomes from the male, top row from a fresh preparation; rows two to four from a two-months-old one. Note the variation in the appearance of the secondary constriction of chromosome No. 8. In some No. 2 chromosomes the C-band in the region of the secondary constriction (arrows) can be seen well separated from the centromeric band. — Scale indicates 10 μ.

4a–b). The secondary constrictions of Nos. 2 and 8 and the distal part of the short arm of No. 10, however, show rather faint C-bands (see below). The variable extension of the bands in the different chromosomes is summarized in Table 1. Due to more or less uncontrollable technical variations of the preparations the size and intensity of a specific region may vary in different cells, but the relative length and intensity of the bands within each cell are the same from cell to cell. The bands can be roughly grouped according to size.

Large bands, extending from the centromere into both arms, are present in chromosomes Nos. 2, 3, 4 and 7. The centromeric regions of all these chromosomes are faintly stained after G-banding. It is difficult to distinguish these pairs on the basis of their C-bands, but No. 3 is the pair with the smallest C-band region.

The C-band region of No. 2 often overlaps into the secondary constriction in the long arm. In quite a few cells, however, the secondary constriction is visible as a narrow dark band separate from the centromeric heterochromatin (Fig. 5b, arrows).

The secondary constriction (p21) of No. 8 exhibits variable staining properties. It was noticed that in some preparations it was darkly

Table 1. The size of C-bands in mink chromosomes

Unless otherwise indicated, each C-band corresponds in position to the centromeric region

No. 1	Medium—large
Nos. 2 - 4	Large, extends from centromere into p and q. Smallest in No. 3. Includes usually secondary constriction of No. 2
No. 5	Small
No. 6	Medium
No. 7	Large, extends from centromere into p and q
No. 8	Medium—large. Often C-bands in secondary constriction.
No. 9	Medium
No. 10	Medium—small. Faint C-bands in p 13
No. 11	Medium—small
Nos. 12—14	Medium
X	Medium
Y	Medium—large, extends from centromere into proximal part of p and all q

stained (most frequently in fairly fresh preparations, i.e. less than 2 weeks old); in others it appeared as an unstained gap only containing two thin threads (seen only in preparations more than 2 months old); in still other metaphases it did not diverge from the rest of the chromosome arm, although the constriction was evident in all metaphases of preparations not exposed to the C-staining procedure.

Medium to large bands are present in Nos. 1, 8 and Y. In the Y chromosome the entire long arm and the proximal part of the short arm stain darkly. In spite of the small size of the Y the C-band region may be classified as medium to large.

Medium bands are present in Nos. 6, 9, 12, 13, 14 and X.

Medium to small bands are present in Nos. 10 and 11; in some preparations the heterochromatin is observed as two small dark dots. Faintly stained telomeric knobs are sometimes visible in the short arm of No. 10 (cf. Fig. 4a).

Small bands. The chromosome with the smallest amount of heterochromatin is No. 5. Usually a pair of discrete dots are seen in the centromere, but sometimes no dark staining at all is visible (cf. Fig. 4a and b).

Discussion

Although the mink karyotype is not really "difficult" as far as chromosome identification is concerned, some chromosomes are easily confused with one another with conventional aceto-orcein staining. The G-band patterns, however, permit an unambiguous identification of each individual chromosome pair. The only observable difference between the autosomes of the two specimens studied was the secondary constriction of chromosome No. 2, which was quite prominent in most female metaphases but was rarely seen in the male cells. However, this constriction is much less distinct than the one in No. 8, which is seen regularly in both homologues of the two sexes. As far as No. 8 is concerned, we have adopted the nomenclature of NES (1962) and designate it as a metacentric chromosome with a secondary constriction in the short arm, and not as a subterminal chromosome with large satellites attached to the short arm (FREDGA 1961).

As pointed out before, the four big m chromosome pairs 2, 3, 4 and 7 may be difficult or impossible to identify without chromosome banding. In C-banded cells only chromosome No. 3 may possibly be picked out on account of its C-bands being slightly smaller. In G-stained preparations chromosome No. 3 may be distinguished from No. 2, especially by its smaller faintly stained area in the short arm. The three dark bands in the short arm of No. 3 are of similar size and evenly spaced. Chromosome No. 4 is characterised by the two heavy dark bands in the distal part of the short arm and the prominent terminal dark band in the long arm. Contrary to Nos. 2, 3 and 4, chromosome No. 7 has only one dark band in the short arm, distally located.

In our experience there is an almost undetectable difference in size between Nos. 9 and 10. However, there is a difference in arm ratio, and we have chosen, in agreement with Nes, to designate the pair with the lower arm ratio as No. 9 and that with the higher as No. 10. Unlike Nes, we had no difficulty in distinguishing No. 12 from No. 13 even in unbanded karyotypes. No. 12 is clearly larger, mainly due to a larger long arm, and consequently it has also a higher arm ratio than No. 13. Nes stresses that the centromere of No. 14 is subterminal rather than terminal, as held by Fredga. On the basis of the present material we still regard this chromosome as one-armed, having

a terminal centromere. In G-banding, No. 14 has a faintly stained, rather broad band close to the centromere, which stains heavily in C-banding. We have not been able to detect any short arm, even in weakly contracted chromosomes, even though a pair of centromeric knobs may give this impression. Accordingly we regard No. 14 of the mink as a telocentric chromosome, comparable, for instance, to mouse chromosomes. Since, however, variations in the amount of centromeric heterochromatin have been described in "acrocentric" chromosomes of other species (PATHAK et al. 1973), this may be the case in the mink, too, and may explain the differences in opinion concerning the presence or absence of a short arm in No. 14.

The X chromosome is somewhat smaller than No. 11 and the location of its centromere is more median. In G-banded preparations the best criterium for distinction between X and 11 is that the main dark band of the short arm is located in the distal part in the X but in the proximal part in No. 11. The X chromosome of the mink is very similar in morphology and banding pattern to that of other mustelids. It also conforms with the X of felids, for instance the domestic cat, and of viverrids such as various species of mongooses (FREDGA and MANDAHL, unpubl.). In other words, the X chromosome of the mink exhibits the pattern expected in biarmed X chromosomes of the "standard" type (PATHAK and STOCK 1974). The very small Y chromosome of the mink is clearly biarmed, its long arm being C-band positive in its entire length.

The distribution of constitutive heterochromatin in the mink chromosomes, as revealed by the C-staining method, differs strikingly from that of some other mustelids, e.g. the pygmy weasel and the ferret. In the latter two species the heterochromatin is mainly concentrated to some large blocks (in 7 and 3 autosome pairs, respectively), constituting practically entire chromosome arms (FREDGA and MANDAHL 1973). Contrary to this, the C-bands of the mink are mainly situated in the centromeric regions, just as in the majority of other mammals. The large C-bands in chromosome Nos. 2, 3, 4 and 7, extending into the proximal parts of both arms, however, are larger than ordinary centromeric C-bands and are in fact quite comparable in size with the heterochromatic blocks of the pygmy weasel and the ferret.

So far, only few publications on C-staining in carnivores exist but preliminary results are available also from the domestic cat and from six species of the genus *Herpestes* (FREDGA and MANDAHL, unpubl.). The mongooses in general have very small to medium-sized heterochromatic zones at the centromeric regions. The most striking exception was found in *Herpestes edwardsi* which had two pairs of autosomes with relatively large distal blocks of heterochromatin (FREDGA and MANDAHL 1973).

The total length of the C-banded regions in the ten species of carnivores studied in our laboratory was calculated and expressed as percentage of the female diploid set. The extremes were represented by the pygmy weasel and the cat: in the pygmy weasel the C-banded regions constituted approximately 22–25% of the chromatin, and in the cat some 4%. In this series, the mink would come next to the pygmy weasel with some 18–19% C-banded regions. These figures underline the great interspecific differences in the amount of constitutive heterochromatin existing in the carnivores. It must be kept in mind, however, in all these considerations that intraspecific variation, too, is not uncommon.

The C-staining of the secondary constriction of chromosome No. 8 was variable and, as mentioned, it could appear either as dark-, weak- or unstained (Fig. 5b). The last-mentioned staining reaction was found only in preparations older than 2 months, while dark staining was found both in old and fresh preparations, although it was much more frequent in the fresh ones. Except for this behaviour of the secondary constriction the staining properties of the remaining heterochromatic segments were relatively consistent. The C-bands in the centromere of chromosome No. 5 and in the distal end of the short arm of chromosome No. 10, however, appeared less distinct or were completely absent in old preparations. In spite of the variable staining the secondary constriction of No. 8 is considered to be C-band positive, the variability being referred to changes in the chromosomes during storage which should first affect the delicate C-bands.

Acknowledgments. — We are much indebted to Mr. Leif Åbjörnsson who kindly supplied the minks used in this study. We also wish to thank Professor Albert Levan for critically reading the manuscript. This work was supported by grants from the Carl Trygger Foundation and the Swedish Natural Science Research Council.

Literature cited

ARRIGHI, F. E. and HSU, T. C. 1971. Localization of heterochromatin in human chromosomes. — *Cytogenetics 10*: 81—86.

CASPERSSON, T., FARBER, S., FOLEY, G. E., KUDYNOWSKI, J., MODEST, E. J., SIMONSSON, E., WAGH, U. and ZECH, L. 1968. Chemical differentiation along metaphase chromosomes. — *Exp. Cell Res. 49*: 219—222.

CASPERSSON, T., LOMAKKA, G. and ZECH, L. 1971. The 24 fluorescence patterns of the human metaphase chromosomes — distinguishing characters and variability. — *Hereditas 67*: 89—102.

FREDGA, K. 1961. The chromosomes of the mink. — *J. Hered. 52*: 90—94.

—— 1971. Idiogram and fluorescence pattern of the chromosomes of the Indian muntjac. — *Hereditas 68*: 332—337.

—— 1972. Comparative chromosome studies in mongooses (Carnivora, Viverridae) I. Idiograms of 12 species and karyotype evolution in Herpestinae. — *Ibid. 71*: 1—74.

FREDGA, K. and MANDAHL, N. 1973. Autosomal heterochromatin in some carnivores. — *Nobel Symp. 23*: 104—117.

NES, N. 1962. Chromosome studies in Heggedal and standard dark mink. — *Acta Vet. Scand. 3*: 275—294.

Paris Conference 1971. Standardization in Human Cytogenetics. — *Birth Defects: Original Article Series, VIII*: 7, *1972. The National Foundation, New York.*

PATHAK, S., HSU, T. C. and ARRIGHI, F. E. 1973. Chromosomes of *Peromyscus* (Rodentia, Cricetidae) IV. The role of heterochromatin in karyotypic evolution. — *Cytogenet. Cell Genet. 12*: 315—326.

PATHAK, S. and STOCK, A. D. 1974. The X chromosomes of mammals: karyological homology as revealed by banding techniques. — *Genetics 78*: 703—714.

SUMNER, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. — *Exp. Cell Res. 75*: 304—306.

WANG, H. C. and FEDOROFF, S. 1972. Banding in human chromosomes treated with trypsin. — *Nature New Biol. 235*: 52—53.