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CHROMOSOME STUDIES IN HEGGEDAL AND STANDARD DARK MINK[1])

By

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The present studies of the chromosomes in mink are part of an investigation of the genetics and breeding properties of the mutant mink Heggedal and its combination types, Blue Shadow and Sapphire Shadow. This mutation which occurred in ranch-bred Standard Dark mink (Mustela Vison) has a very strong phenotypical effect. It is inherited as a dominant autosomal factor being lethal in homozygous condition. In the heterozygous state it leads to marked depigmentation of the fur and causes extensive malformations of the uterus in a great number of the females. As several congenital malformations in man have proved to be associated with numerical and/or structural abnormalities of the chromosomes (*Brögger* 1961), an investigation of the chromosomes of this mutant mink was thought justified.

At the time this investigation was started, no thorough examination of the normal morphology of the chromosomes in this animal had been published. On account of this, the investigation had a dual purpose, firstly to describe the normal chromosomes in Standard Dark mink, secondly, by comparative examination, to demonstrate possible deviations in the chromosomes of the mutant mink Heggedal.

The earlier investigations were all carried out on testicle material treated according to the classical method (*Humphrey*

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and *Spencer* 1959, *Lande* 1957, *Shackelford* and *Wipf* 1947). They were therefore limited to the male, while by the new cell culture technique the chromosomes can be studied in both sexes and with a greater degree of accuracy. *Fredga* (1961) has used the advantages of the latter method, and the same has been done in the present work.

As to the chromosome number in mink, different results are obtained. *Shackelford* and *Wipf* (1947) found 28 chromosomes while *Lande* (1957), *Humphrey* and *Spencer* (1959) and *Fredga* (1961) maintain that there are 30 chromosomes in Mustela Vison.

MATERIALS AND METHODS

The materials for the cell cultures are taken from 5 Light Heggedal (Pp S^Hs) and 2 Blue Shadow (pp S^Hs), (p = Platinum, S^H = Heggedal, p and s are the corresponding allelic factors in Standard Dark). Standard Dark mink has been used as comparative material.

In these chromosome examinations, cultures of bone marrow, skin, cornea and testicle tissue have been used. The tissues were usually taken immediately after killing the animals. A few skin samples for cultivation were taken as biopsies, and the testicle tissue was partly taken by castration.

Whilst the bone marrow cultures were explanted at once, the tissue from the cornea, skin and testicles was kept in culture medium at 4° C for a few hours — occasionally for a day or two before the primary explantation. In a few cases the material was kept at room temperature for a couple of hours during transport.

Cattle serum was used in the culture media for all types of tissue culture despite the fact that the original culture methods as a rule use human serum. This modification is general for this work and will not be declared in the special case. In comparative studies there was no noticeable difference between cultures made with sera from mink, pig, horse or man, and there was easier access to cattle serum than to the others.

Besides the primary cultures, subcultures were made by one or more transplantations, with the exception of the bone marrow cultures.

To collect mitoses the cell cultures were usually treated with colchicine during the last hours of incubation. Before preparation the cells from all cultures were pretreated with hypotonic sodium citrate solution to get the chromosomes scattered.

Below are given some details of the technique for bone marrow cultures on the one hand, and for other tissue cultures on the other.

Bone marrow tissue was taken from femur, radius or from a rib of the mink and treated according to the short-term culture method by *Ford et al.* (1958) with the exception mentioned before. The cultures were incubated in closed testtubes at 37° C for 3—18 hours — the last 1—3 hours under the influence of colchicine that was added in concentrations varying from 0.1 to 1.25 γ/ml. medium. The best results were usually achieved after an incubation time of 3—7 hours, and with a colchicine concentration of 0.1 γ/ml. medium for two hours. The cells were treated with a hypotonic citrate solution according to the method mentioned above, and the preparation was carried out according to a slightly modified method of *Tjio* and *Levan* (1956). After being fixed in 60 % acetic acid, the cells were squashed and stained in acetic orcein, a solution of 2 % natural orcein (G. T. Gurr, London) in 50 % acetic acid. The preparations were then passed through an ordinary alcohol-xylol serie and mounted in eukitt (O. Kindler, Freiburg).

Cornea, testicle and skin cells were cultered according to the method of *Fraccaro et al.* (1960a), but the trypsinisation of the fresh tissue was omitted, and the tissue was minced with a pair of sharp ophthalmological scissors. This was done in a droplet of medium on a coverslip placed in a Petri dish.

The open Petri dish coverslip cultures were incubated at 37° C in a specially made cabinet[1]) placed in an incubator. A current of carbon dioxide (5 %) and air (95 %) was continuously passing through the culture cabinet. There was water in the bottom of the cabinet to maintain the necessary moisture. The cabinet is made of transparent plastic. Inspection of the cultures is therefore possible without opening the door. This is of importance in maintaining an even carbon dioxide pressure.

The cell growth was examined at intervals by means of an ordinary light microscope. Under suitable illumination the cells could be seen reasonably well. An inverted phase-contrast microscope would have been better, however, but could not be obtained.

The primary cultures from cornea and testicle tissue showed

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[1]) Designed by the author and made by Mr. *Nils Lehne.*

as a rule cell growth after about 2 days, while the skin cultures first showed growth after 4—6 days or more.

From the small tissue fragments the cells were growing in monocellular layers on the coverslips as well as in the bottom of the Petri dishes. The coverslips with the cells from such primary cultures were some times taken out and used for chromosome preparations, while the cells in the Petri dish itself were trypsinized at the same time or after a longer growth period to be used in the preparation of subcultures. These subcultures and subcultures after two or more transplantations were, however, nearly always preferred in making permanent chromosome preparations in this work. In so doing, tissue fragments in the preparations could be avoided to a greater degree.

Where the cell density in the subculture was great, the greatest number of mitoses was achieved if harvested 8—10 hours after the trypsinisation. In other cases 18—20 hours passed before there were sufficient cells for preparation. In the last part of the incubation period the cell cultures were treated with colchicine. Ten different concentrations varying from 0.050—1.25 γ/ml. medium were tried as well as variation in time. A colchicine treatment of 2—3 hours with a concentration of 0.1 γ/ml. usually gave a good result. A concentration of 0.075 γ/ml. was, however, sufficient to accumulate mitoses in these cultures, and the number of mitotic cells was not increased by increasing the concentration. The mitotic activity was just estimated.

The cells were treated with hypotonic citrate solution in the same way as the bone marrow cells and the preparation was carried out according to the air-drying technique for flattening chromosomes by *Rothfels* and *Siminovitch* (1958). The chromosomes were stained in acetic orcein, but instead of making the preparations permanent by means of the dry-ice method of *Conger* and *Fairchild* (1953) they were dehydrated and mounted in the same way as the preparations of bone marrow cells.

EXAMINATION OF THE CHROMOSOMES

The number and structure of the chromosomes was examined directly by phase-contrast microscope (400—1600 ×) and indirectly by means of photomicrographs.

A Zeiss photomicroscope was employed and the preparations photographed with phase-contrast, using the magnifications

400 × or 512 × (film Adox KB 14). After being cut out of photographic enlargements (4000 ×) the chromosomes were matched and serially ordered according to size and centromere position (see Figs. 1, 2 and 3).

The chromosome counts were made in cells in metaphase and only cells with reasonable distinct chromosomes were used. Cells in which the chromosomes were lying so closely that they could not be clearly distinguished from each other, and cells mingling their chromosomes with those of adjacent cells, were not used for counting the chromosomes.

The chromosomes were counted at least three times. If there was any doubt about the chromosome number, or if this differed from the normal, further counts were undertaken by one of my colleagues. If there were differing results, the chromosomes were regarded as uncountable and excluded from the material.

Chromosome measurements were performed on karyograms by means of an Amsler's curve-meter (Alfred J. Amsler & Co., Schaffhausen, Switzerland). This gives the length in mm. with an accuracy of 0.02 mm.

By moving the curve-meter along the median line of the chromosome it can be measured regardless of whether straight or coiled. The measurement was started at the distal end of the shorter chromatid arm and the instrument was moved proximally along the median line to the centromere. At this point of the chromatid the length of the shorter arm was read off the instrument which was subsequently moved further along the longer chromatid arm to its distal end where the length of the longer arm was registered. In so doing an inaccuracy in the fixation of the centromere position did not influence the measurement of the total length of the chromatid. It did, however, effect the proportion between its two arms i. e. the arm ratio.

The length of the chromosome was taken to be equal to the average length of the corresponding sister chromatids. At the magnification used (4000 ×) the chromosome lengths ranged between 3 and 30 mm. The relative length of each chromosome is stated as millesimals of the total length of a normal X-containing haploid chromosome set (see Table II).

Abbreviations and details concerning special terms

a. r. — arm ratio, expressed as the length of the longer chromosome arm relative to the shorter one.

c. i. — centromeric index, expressed as the ratio of the length of the shorter arm to the whole length of the chromosome.

r. l. — relative length of a chromosome.

D. S. — Denver System or Denver Scheme, the proposed standard of nomenclature of human mitotic chromosomes (A Human Chromosomes Study Group 1960).

Karyogram — here used instead of karyotype when talking of the serially ordered chromosomes of a single cell without underlining or concluding this being the karyotype of the individual or the species.

RESULTS

The chromosome number

As Table I shows, the chromosome have been counted in 308 cells, 158 bone marrow cells and althogether 150 cells from testicle, skin and cornea tissue. In the major part (294 cells) the chromosome number was found to be $2n = 30$, i. e. 14 pairs of autosomes + 2 sex chromosomes. 14 cells showed variations from 27 to 32 in chromosome number. The bone marrow cells showed the most marked and greatest number of variations. 9 out of the 158 bone marrow cells gave differing values of chromosome number, i. e. in 5.7 % of the cells, while the correspond-

Table I.

The result of chromosome counts in mitotic cells from different tissue cultures.

Colour type and number	Sex	Tissue	Chromosome number						Cells counted
			27	28	29	30	31	32	
B. S. 547 S	♀	Bone marrow	1	—	—	20	—	—	21
B. S. 277 A	♂	Bone marrow	—	—	—	21	—	—	21
L. H. 393 T	♂	Bone marrow	—	—	2	38	1	—	41
L. H. 244 P	♀	Bone marrow	1	—	1	45	1	1	49
L. H. 529 B	♂	Bone marrow	—	—	—	25	—	1	26
L. H. 529 B	♂	Testicle	—	—	—	92	1	2	95
L. H. 226 B	♀	Skin	—	—	1	32	—	—	33
L. H. 492 B	♀	Cornea	—	—	—	21	1	—	22
Total for bone marrow			2	—	3	149	2	2	158
Total for the other tissues			—	—	1	145	2	2	150
Total for all tissues investigated			2	—	4	294	4	4	308

B. S. = Blue Shadow. L. H. = Light Heggedal.

ing figures for the other tissues were 5 of 150 or 3.3 % with differing numbers.

A few tetraploid cells were observed in all cultures. The frequency seemed, however, to be a little higher in the bone marrow cultures than in the others. There was also found an octoploid bone marrow cell in which 117 chromosomes, were counted. These polyploid cells are presumably formed by endomitosis.

The morphology and identification of the chromosomes

Chromosome measurements were performed in 4 female and 4 male bone marrow cells and in one testicle cell. With the exception of the latter one, all of them had been pretreated with colchicine. As the measurements did not show any certain difference between corresponding chromosomes in male and female cells, the r. l. and a. r. of the different chromosomes are given as the means of those in the nine chromosome set measured, Table II, column 2 and 4, respectively. In column 3 and 5 the corresponding standard deviations are recorded.

Table II.

Average relative length and arm ratio of the chromosomes of 9 mitotic cells from mink, 4 from females and 5 from male. 8A the satellite is excluded and 8B the satellite is included in computing the relative length and arm ratio of chromosome No. 8. s = standard deviation.

Chrom. No.	Rel. length	s	Arm ratio	s
1	114	6.8	1.1	0.11
2	94	3.7	1.2	0.05
3	87	2.5	1.1	0.02
4	83	2.5	1.1	0.07
5	82	3.9	1.8	0.15
6	80	3.5	2.7	0.15
7	76	3.5	1.5	0.15
8A	(51)	3.2	2.3	0.18
8B	72	3.8	1.1	0.05
9	60	2.7	2.2	0.30
10	55	1.3	2.3	0.24
11	54	1.7	1.5	0.09
12	38	2.0	1.7	0.14
13	32	3.0	1.4	0.13
14	24	2.2	—	—
x	48	3.0	1.3	0.05
y	17	2.5	—	—

To get an idea of the error of the measurements the coefficient of variation has been calculated for ten measurements of three chromosomes of different size. A large one, No. 1, a medium sized one, No. 11 and a small one, No. 13. (The left chromosome over the respective numbers in Fig. 2). The results are given in Table III. As will be seen from column 3 in this table, the coefficients of variation of the length measurements though increasing with decreasing size of the chromosomes, are small for all three chromosomes examined. The coefficients of variation for the a. r. were a little higher than those for the length measurements, and were increasing with increasing values of the a. r., but are still acceptable.

Table III.

The coefficients of variation for the measurements of three different chromosomes measured on an enlarged photomicrograph (4000 ×).

Chromosome No.	Mean length mm.	Coeff. of var.	Arm ratio	Coeff. of var.
1	23.11	0.68 ± 0.15	1.02	1.66 ± 0.35
11	10.87	1.13 ± 0.25	1.47	3.81 ± 0.85
13	7.66	1.46 ± 0.33	1.43	4.04 ± 0.90

According to the D. S. the autosomes are serially numbered, 1—14, as nearly as possible in descending order of length. When the chromosomes are equal in size the one with the most median centromere is ranged at first. The X and Y chromosomes are here placed at the end of this array (see the karyotypes of mink, Figs. 1, 2 and 3). This is however, not in agreement with the D. S. after which the sex chromosomes are placed near to, but separated from the autosomes they resemble.

As it will appear from Table II and from Figs. 1, 2 and 3, the chromosomes in mink may be divided into three main groups according to size, the large, medium and small chromosomes. The first one comprises of seven pairs of autosomes, the second one of four pairs of autosomes + the X chromosome and the third one of three autosomes + the Y chromosome.

Chromosome 1 is metacentric and the largest chromosome in mink. This is usually easily distinguishable in size from the other chromosomes, but in preparations where the chromosomes are unevenly contracted, mistakes can be made (see Fig. 1).

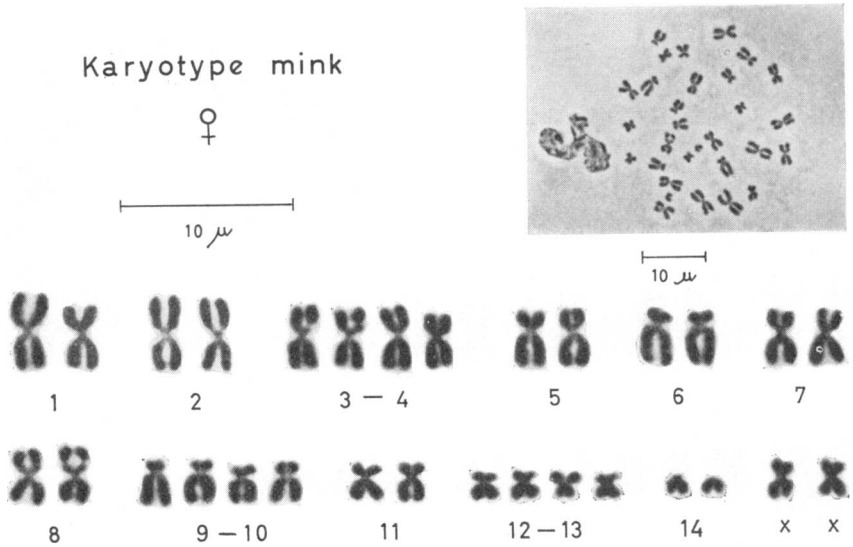


Fig. 1. Karyotype of a mink female. The photomicrograph in the upper right corner shows the chromosomes at metaphase in a bone marrow cell grown in vitro and treated with colchicine (0.1 γ/ml. medium, 1¾ hrs.), and with hypotonic citrate (10 min.). Acetic orcein squash preparation. Below, the chromosomes are arranged according to size and centromere position. Original magnification 512 ×.

Chromosome 2 is nearly metacentric and is about the same size as Nos. 3 and 4, both of which are also metacentric, or nearly so. It can, however, usually be distinguished from those by its secondary constriction. This lies very near the centromere or primary constriction, see Fig. 3. If the chromosomes are greatly contracted as shown in Figs. 1 and 2, this secondary constriction can hardly be seen.

The chromosomes Nos. 3 and 4 are of about the same size, and as mentioned above, they are both metacentric or nearly so. As no clear difference has been found with the technique used, these two pairs of chromosomes are placed in one group (3—4) in the present karyograms (Figs. 1, 2 and 3).

The chromosomes Nos. 5 and 6 are of about the same size as Nos. 3 and 4. They are, however, usually easy to identify because of the centromere position. No. 5 is a submetacentric chromosome and No. 6 is an acrocentric one. Chromosome 7 is slightly smaller than the former. Like No. 5, No. 7 must be said to be submetacentric, but in the latter the centromere is slightly nearer the centre of the chromosome, i. e. it has a little smaller a. r. Because

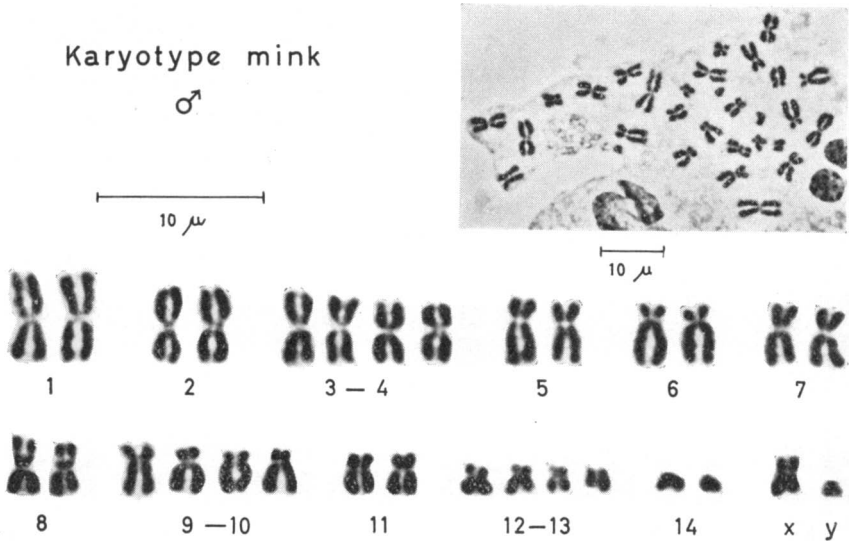


Fig. 2. Karyotype of a mink male. The photomicrograph in the upper right corner shows the chromosomes at metaphase in a bone marrow cell grown in vitro and treated with colchicine (0.3 γ/ml. medium, 2 hrs.), and with hypotonic citrate (10 min.). Acetic orcein squash preparation. Below, the chromosomes are arranged according to size and centromere position. Original magnification 512 ×.

of this and the difference in size, it is usually possible to distinguish these chromosomes from each other.

Chromosome 8 is the largest one of the medium sized chromosomes in mink. It is nearly metacentric and has a secondary constriction located at a distance from the centromere approx. ⅓ of the length of the chromosome arm concerned. The secondary constriction is usually distinct, and serves as a fairly certain method of identification.

The chromosomes Nos. 9 and 10 are a little smaller than No. 8, but there is not much difference between the two. As they are both acrocentric, there is no certain way of differentiating between them, and they are therefore placed together in one group (9—10) in the karyograms.

Chromosome 11 is of about the same size as Nos. 9—10, but is in contrast to those submetacentric and therefore easy to identify.

The chromosomes Nos. 12 and 13 belong to the small chromosomes in mink and are considerably smaller and therefore easily distinguished from those described previously. But the

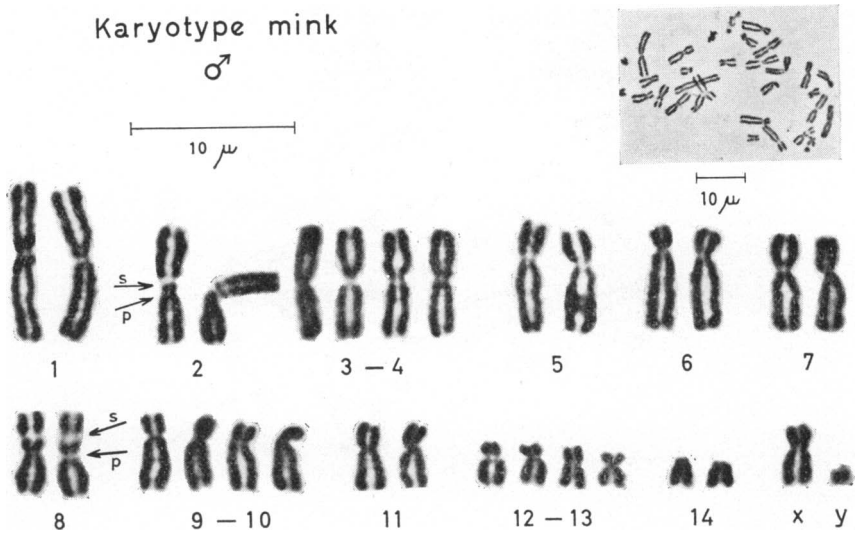


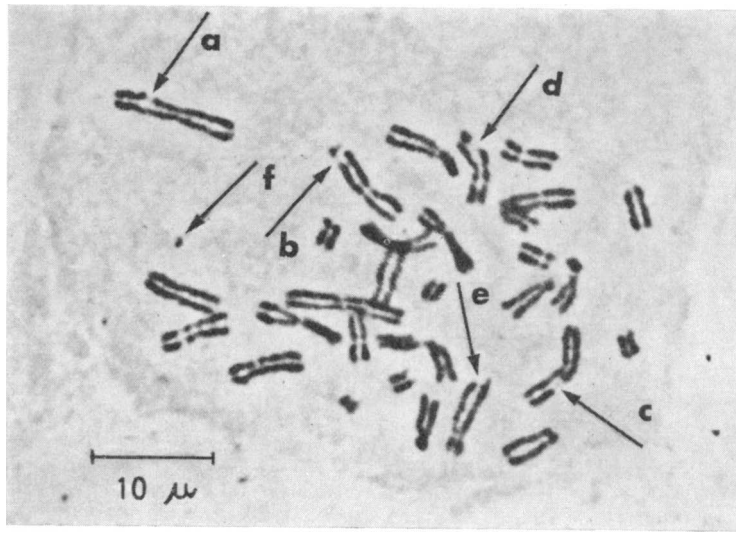
Fig. 3. Karyotype of a mink male. The photomicrograph in the upper right corner shows the chromosomes at metaphase in a testicle cell grown in vitro. No colchicine treatment, but hypotonic treatment (10 min.). Air-drying acetic orcein preparation. Original magnification 400 ×. The chromosomes are less contracted than the colchicine treated chromosomes shown in Figs. 1 and 2. Note the secondary constrictions in the chromosomes Nos. 2 and 8. s — secondary constriction, p — primary constriction (centromere).

difference in size between the two pairs is negligible, and as both are submetacentric, a differentiation between the two is uncertain. These two pairs of chromosomes have therefore been placed together in one group (12—13) in the karyograms.

Chromosome 14 is the smallest of the autosomes and is easily identified on the basis of length. It is an acrocentric chromosome, but in most preparations it is impossible to discern the separate arms.

The X chromosome belongs to the medium sized chromosomes in mink, and is of about the same size as No. 11. It is submetacentric, but the centromere has a slightly more median position than is the case with No. 11 and it is usually possible to distinguish between these two.

The Y chromosome is the smallest chromosome found in mink. It is submetacentric, but because of its small size, it is usually impossible to discern the separate arms.



F i g. 4. Mitotic metaphase in a testicle cell of mink. The cell is grown in vitro, treated with colchicine (0.15 γ/ml. medium, 3½ hrs.), and with hypotonic citrate (10 min.). Air-drying acetic orcein preparation. Original magnification 400 ×. The arrows show "secondary constrictions" or chromosome breakage. The chromosomes concerned are probably a No. 1, b and c Nos. 3—4, d No. 8 and e No. 6 (see Fig. 3), f is probably a fragment of No. 6.

In addition to the secondary constrictions on the chromosomes Nos. 2 and 8, others have been observed in various chromosomes. In this paper they are described as irregularly occurring secondary constrictions. These constrictions are of varying lengths. Some times such a constriction displays itself as a rather long unstained gap on the chromosome. Up to now it has been observed in only one of the two sister chromatids of a chromosome, but it has a tendency to occur more frequently in certain chromosomes than in others. Thus in chromosome 1 a subterminal secondary constriction has been observed several times, and also in a few cases submedian constriction in one of the chromatids. The same phenomenon has been observed in the chromosomes Nos. 3—4. This can be seen in Fig. 1 where there is a submedian secondary constriction on one of the chromatids of two chromosomes belonging to this group, presumably No. 3. Chromosome breakages are often seen in connection with these secondary constrictions (see Fig. 4).

DISCUSSION AND CONCLUSION

Cell cultures as well as pretreatments, fixing and staining etc. are carried out according to methods developed for human cells and human chromosomes. At the present investigation in mink, these methods have been used without, or with only slight, modifications.

The chromosome measurements were made with an Amsler's Curve-meter on enlarged photomicrographs because this method was found to be more exact than a method of *Fraccaro* and *Lindsten* (1960). The latter method was found less accurate especially as the centromere and the end points of the chromatid could not be fixed as exactly as with the first method. Through a lens on Amsler's Curve-meter these points will usually be seen fairly well. On photomicrographs the centromere and the boundaries of the chromatids are indeed more or less indistinct and the determination of the centromere and end points will to a certain degree be a matter of estimation. Besides the exactness of the instrument used, the accuracy will further depend on how exactly the median line of the chromatid is followed, which in turn is dependent on whether the chromatid is straight or coiled. It should be noted that the three chromosomes for which the coefficients of variations of ten measurements are calculated, Table III, were just moderately coiled and the photomicrographs were reasonably distinct. If the chromosomes are more coiled and the photomicrographs less distinct, the error of measurements will be greater than these figures indicate. In spite of this the method seems to be well fitted for use as far as the exactness of the measurements is concerned.

As appears from Table II and from the karyograms, the value of the chromosome measurements, however, is not beyond dispute.

As for the relative chromosome lengths given in Table II, they are the means for pairs of chromosomes whose components are matched on considering their r. l., a. r. and secondary constrictions. These matchings seem to be fairly certain for all chromosomes except Nos. 3—4, 9—10, and 12—13. The separate matching within each of these three groups leaves some doubt and may have caused mistakes in one chromosome set or more. If so, the corresponding standard deviations are greater in reality than calculated in Table II, the values of which consequently

will have to be considered minima. However, even these low values of standard deviation are so high that these measurements seem to be of little use for the identification of the chromosomes of mink.

The variation in r. l. and a. r. within pairs is not shown in the table. It is, however, seen in the karyograms that chromosomes of same pair and even sister chromatids differ considerably in length, probably because of an unequal squashing or a different degree of contraction, depending upon the intensity of the pretreatment. Different contraction can to a certain degree be judged by inspection and taken into account at the identifications. Measurements alone, however, might in such cases lead to mistakes. This finding also limits the importance of chromosome measurements.

Beside the a. r. the c. i. is not likely to make any difference as to the confidence in the identification of the chromosomes. Therefore it is omitted in the present paper.

In man, different characters are shown to be due to chromosome abnormality. Klinefelter's syndrome is found in connection with two X chromosomes and a Y chromosome (*Jacobs* and *Strong* 1959, *Mowe et al.* 1961) and Turner's syndrome with only one sex chromosome, a single X (*Ford et al.* 1959, *Fraccaro et al.* 1960 a). Mongoloids are found with an additional autosome making up a total of 47 chromosomes (*Jacobs et al.* 1959, *Lejeune et al.* 1959) or with a normal chromosome number but with translocations (*Fraccaro et al.* 1960 b, *Polani et al.* 1960). Translocations are also found in polydysspondylism with 45 chromosomes (*Turpine et al.* 1959) and in a congenital auricular septal defect of the heart, with 46 chromosomes (*Böök et al.* 1961).

As mentioned before, one of the purpose of this investigation was to find out if the present mutation in mink was associated with similar abnormality of the chromosomes. However, no peculiarity in the karyotype of this mutant mink has been found compared with that of the Standard Dark mink, though some morphological characteristics not described before are observed.

The chromosome number found in Heggedal is $2n = 30$, which is the same number as found in other colour types of Mustela Vison by *Lande* (1957), *Humphrey* and *Spencer* (1959), and *Fredga* (1961). Deviations from this number is observed. It will be noticed, however, that they are nearly twice as frequent and to a certain degree greater for bone marrow cultures than for

the other tissues cultivated. This is what could be expected if the deviations were due to technical errors. With the squash technique used for bone marrow cells there is a great chance that a chromosome will go astray and be lost for counting, or be counted together with chromosomes of other cells. By the air-drying technique used for the other cells there is a smaller chance of this happening.

The results have not been specified for the different subcultures used in this work. On the other hand, no morphological or numerical differences between the primary cultures and the subcultures have been found. This is in accordance with the observation of *Fraccaro et al.* (1960 a).

As to the morphology of the sex chromosomes the present investigation supports *Fredga*'s results (1961). This is also the case with the autosomes as far as their serial order in the karyogram is concerned. In this work, however, the chromosomes Nos. 3 and 4, have been grouped together and in the same way Nos. 9 and 10 and Nos. 12 and 13. This is done because within each of these groups no reliable method of identification could be found.

In addition to the morphological criteria, described by *Fredga* (1961), it is found that chromosome 2 has a secondary constriction. It is also found that chromosome 14 has a subterminal instead of a terminal centromere. If strongly contracted, especially in colchicine treated preparations, the chromosome 14 will indeed look like a telocentric chromosome, (see Figs. 1 and 2), but if less contracted it will present itself as an acrocentric chromosome (see Fig. 3).

The secondary constriction on chomosome 2 may some times escape notice because of its position near the primary one, and in preparation treated with colchicine it has a tendency to disappear in the chromatin mass (see Figs. 1 and 2). In most preparations, however, it is easily discovered. Therefore it is of great importance for the identification of the chromosome. Without this criterion, chromosome 2 can easily be mistaken for the chromosomes Nos. 3—4.

Chromosome 8 is by *Fredga* (1961) characterized as a satellited chromosome with subterminal centromere. By "satellite" is usually meant a small body set off by the secondary constriction when occurring near the distal end of a chromosome. In this case the secondary constriction delimits about ⅔ the

chromosome arm concerned. The terms satellite, satellited chromosomes and SAT-chromosomes are very convenient, but when used on chromosome 8 in mink, it should be remembered that this is an extension of their usual meaning.

As to his indication of the centromere position it is in accordance with the D. S. which expresses the rule as follows: "... in view of the apparent morphological variation of satellites they and their connecting strands are excluded in computing indices." In spite of the secondary constriction the variation of the length of chromosome 8 is rather small. As shown in Table II one will get but a little increase of the standard deviation of relative length by including this "satellite" in the measurement. This is one of the reasons why this chromosome is characterized as a metacentric chromosome in this paper instead of an acrocentric one. Another reason is that in preparations where this secondary constriction cannot be discerned, this characterization is more adequate than the above mentioned one.

As mentioned before, irregulary occurring secondary constrictions are found in various chromosomes. The irregularity of the appearance of these constrictions exclude the possibility of their being used as a mean of identification. These secondary constrictions are neither restricted to special types of cells nor to cells from certain animals or colour types. They are, however, traced only in part of the cell cultures. But if they do appear, they are often found in more cells and also repeatedly in the same cell (see Fig. 4). They have occurred irrespective of method of preparation.

Concerning the manifestation there is a marked difference between these secondary constrictions and those found in the chromosomes Nos. 2 and 8. They are therefore likely to have a different causation. Whilst the latter ones seem to be of the same nature as those found to be associated with the nucleolar formation (*Heitz* 1931; *Gates* 1942), the former ones are more suggestive of those induced by low temperature. *Darlington* and *La Cour* (1938, 1940) who first demonstrated this phenomenon in Trillium, postulated that these regions could not synthesize nucleic acid as efficiently as could adjacent euchromatin. The different segments consequently would appear negatively heteropycnotic, i. e. lightly staining regions and create gaps that appear as constrictions (*Swanson* 1958). It does not seem unlikely that the irregular occurring secondary constrictions described have

Table IV.
Conspectus of mitotic chromosomes in mink.

Chromosome No.	Characteristics and remarks
1	Large, metacentric. The largest one.
2	Large, nearly metacentric. Secondary constriction.
3 and 4	Large, metacentric. Usually impossible to distinguish from each other with certainty.
5	Large, submetacentric.
6	Large, acrocentric.
7	Large, submetacentric. The smallest of the "Large chromosome" in mink.
8	Medium, metacentric, secondary constriction.
9 and 10	Medium, acrocentric. Difficult to distinguish from each other.
11	Medium, submetacentric.
12 and 13	Small, submetacentric. Often difficult to distinguish from each other with certainty.
14	Small, acrocentric.
X	Medium, submetacentric. Sometimes difficult to distinguish from No. 11.
Y	Small, submetacentric. The smallest of all chromosomes in mink.

a similar interpretation. Their real nature and causation, however, have not been elucidated and an investigation in that direction is outside the scope of this work.

Shackelford and *Wipf* (1947) described two nonhomologous satellited chromosomes which they considered to be the sex chromosomes. The present investigation, however, has failed to support this result.

The normal morphology of the chromosomes in Heggedal and Standard Dark mink is found to be the same. A survey of the morphologic criteria of the mitotic chromosomes is given in Table IV.

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SUMMARY

Chromosome Studies in Heggedal and Standard Dark Mink.

The number and morphology of the chromosomes of the mutant mink Heggedal have been studied by means of the cell culture technique. The chromosomes are measured and the method described. The morphology of the chromosomes of this mutant has been compared with that of the Standard Dark mink by comparative studies, while the chromosome number is compared with those found by other investigators in Standard Dark and other colour types of Mustela Vison.

The chromosome number is found to be 2n = 30, which is in accordance with the number stated by *Lande* (1957), *Humphrey* and *Spencer* (1959) and *Fredga* (1961). As to the morphology of the chromosomes the author has found a secondary constriction on chromosome 2. He has also found that chromosome 14 is acrocentric instead of telocentric as maintained by *Fredga*. These findings, not described before, are demonstrated in all colour types investigated. Compared with Standard Dark mink neither morphological nor numerical peculiarities are found in the chromosomes of the mutant mink Heggedal.

ZUSAMMENFASSUNG

Chromosomstudien an Heggedal und Standard Nertz.

Mit Hilfe der Zellenkulturtechnik wurden Anzahl und Morphologie der Chromosome des norwegischen Nertzmutanten Heggedal studiert. Die Chromosome wurden gemessen und das Verfahren beschrieben. Die Morphologie der Chromosome bei diesem Mutanten wurde mit der Morphologie der Chromosome des Standardnertzes durch vergleichende Studien verglichen, während die Chromosomzahl mit derjenigen verglichen wurde, die andere Forscher bei Standardnertz und anderen Farbtypen des Mustela Vison gefunden haben.

Es wurde eine Chromosomzahl von 2n = 30 ermittelt. Dies stimmt mit den Ergebnissen von *Lande* (1957), *Humphrey und Spencer* (1959) und *Fredga* (1961) überein. Bezüglich der Morphologie der Chromosome fand der Verfasser eine sekundäre Einschnürung im Chromosom 2. Er fand desweiteren, dass Chromosom 14 acrozentrisch ist statt

telozentrisch, wie dies von Fredga behauptet wurde. Diese Funde, die bisher noch nicht beschrieben sind, wurden bei allen untersuchten Farbtypen nachgewiesen. Im Vergleich mit dem Standardnertz wurden bei den Chromosomen des Nertzmutanten Heggedal weder morphologische noch numerische Abweichungen festgestellt.

SAMMENDRAG

Kromosomstudier hos Heggedal og standard mink.

Ved hjelp av cellekulturteknikk har en studert kromosomenes antall og morfologi hos den norske minkmutant heggedal. Kromosomene er målt og metoden beskrevet. Morfologien av kromosomene hos denne mutant er sammenliknet med morfologien av standardminkens kromosomer ved sammenliknende studier, mens kromosomtallet er sammenliknet med de som andre forskere har funnet hos standardmink og andre fargetyper av Mustela Vison.

Kromosomtallet er funnet å være 2n = 30. Dette er i overensstemmelse med det som er funnet av *Lande* (1957), *Humphrey og Spencer* (1959) og *Fredga* (1961). Når det gjelder kromosomenes morfologi har forfatteren funnet en sekundær innsnøring på kromosom 2. Han har også funnet at kromosom 14 er acrocentrisk i steden for telocentrisk som hevdet av Fredga. Disse funn som ikke er beskrevet tidligere, er påvist hos alle de undersøkte fargetypene. Sammenliknet med standardmink er det funnet hverken morfologiske eller nummeriske avvikelser hos kromosomene til minkmutanten heggedal.

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