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# RABBIT CHROMOSOME ANALYSIS BY IMAGE PROCESSING

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## Abstract

In this paper some aspects of automated karyotyping are discussed. We present an idiogram according to our measurements. Furthermore, suggestions for a slight revision are made pertaining to the standard published by the Committee for Standardized Karyotype of Oryctolagus cuniculus (1981). We analyzed metaphases from blood lymphozytes obtained from New Zealand White rabbits bred at our Institute. Conventional chromosomal preparations and G-banding were used prior to image processing of karyotypes. We selected 9 out of 150 metaphases of optimum quality. Objective measurements of chromosomes are achieved by using the software UNICHRO (chromosome length; centromere index, band position etc.). According to our results, and in contrast to the published standard, chromosomes 9, 10 and 11 should be arranged in group A, chromosomes 16 and 17 in group B, respectively. In addition, chromosome 12 proves to be shorter than chromosome 13 and 14. In routine karyotyping only a certain percentage of bands of the same chromosome is realized at all times, whereas other bands are visible in lower frequency. We classified our findings in three groups of banding which represent different percentages of visible bands. Karyograms analysed are compared with those reported elsewhere. Concluding we emphasize that there are promising advantages of applying image processing to cytogenetic studies with regard to the effects of rationalization and, on the other hand, adequate accurracy of karyotyping.

## Introduction

The world-wide growing significance of rabbits as a source of meat, fur and wool is well documented. On the other hand, this species is still indispensable to biomedical research (Weisbroth et al., 1974; Rudolph, 1978, Fox, 1972, 1984). Went and Stranzinger (1986) emphasized that rabbits are excellent experimental models in cytogenetic research. According to these authors karyotyping in this species is principally aimed at the following topics:

- to investigate structure of chromosomes,
- to analyse the relation between chromosomal aberrations, genetic defects and performance traits, respectively,
- to differentiate sex of embryos and newborn rabbits,
- to comparatively investigate linkage groups, gene mapping and gene expression in different species.

Thus, cytogenetic studies are not just of considerable significance for basic research but also for commercial rabbit breeding.

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In the domestic rabbit the number of diploid chromosomes equals 2n = 44 as found by Painter (1926). Melander (1956) ascertained centromers' position in rabbit chromosomes which enabled cytogeneticists to analyse the karyotype more comprehensively. An individual characterization of chromosomes, however, was made possible only after banding techniques had been introduced into karyotyping (Echard, 1973). In 1976 a standard karyotype of the laboratory rabbit was recommended by the Reading Conference (Ford et al., 1980) with special reference to chromosome morphology and G-banding as well. A slight modification pertaining to the numbering of chromosome 16 and 17 was introduced by the Committee for Standardized Karyotype of Oryctolagus cuniculus (1981). However, the discussion is carried on. Recently, Yerle et al. (1987) suggested a G-banded karyogram achieved by applying high resolution banding. On the other hand, a well-founded R-banded karyogram of the domestic rabbit was published by Poulsen et al. (1988). Popescu (1989) reviewed recent cytogenetic studies in rabbit chromosomes reproducing karyograms obtained from Gustavsson. Some brief remarks on chromosomal aberrations as well as gene mapping were made in his brief monograph.

High preparative standards are necessary to accomplish effective karyotyping in view of the increasing demand of reliable interpretations of chromosomal analyses. Routine procedures should be rationalized. Some results of partial computer-aided chromosomal analysis in rabbits were published by Stranzinger (1976) and Gustavsson (1980). Nowadays karyotyping by means of image processing proves promising.

In recent years we have applied to our studies of rabbit chromosomes the software UNICHRO originally developed for karyotypes of plants by Ahne et al. at the Institute of Breeding Research, Quedlinburg (Germany). Modified according to our recommendations to meet the requirements of analysing karyotypes of animals, this software can run on IBM-compatible hardware complemented with only a few attachments.

In this paper, karyograms analysed are compared with those reported elsewhere recently. Furthermore, some aspects of automated karyotyping are discussed. Advantages of using image processing for cytogenetic studies in rabbits are mentioned.

## Methods and Procedure

We analysed metaphases from blood lymphozytes of male New Zealand White rabbits. The animals belong to two strains developed at our Institute.

Conventional chromosomal preparations and G-banding, according to the method of Seabright (1971), were used prior to image processing of karyotypes. For image processing we selected 9 out of 150 metaphases of optimum and equal quality. Image analysis was carried out with the system K 7076 (IMTRONIC) using the software UNICHRO. After image preprocessing and measuring of chromosome features all the chromosomes of equal type (e.g.1a) were displayed on the screen and then viewed together. The position of the bands were compared directly,

thus enabling us to characterize equal bands in the same manner. Bands that fuse during condensation were given contiguous numbers. Data were transmitted to a d-base-file and then analyzed with a special program written by the author. Graphic description was achieved by windows-paintbrush.

## Results

In selecting the metaphases attention was paid to a relatively equal and medium degree of chromosome condensation. We considered only metaphases realizing a close connection between chromatids to avoid inaccurate measurements possibly occuring by spreaded chromatids. Results of measuring length of chromosomes are listed in table 1. Total length of haploid chromosome set (without sex chromosomes) amounted to $\bar{x}$=122.43 ±13.036 µm ($\bar{x}$=253.54±27.19 µm for 2n, including X and Y chromosome). In view of optimum comparability we refer to the arm ratio AR=q/p and, on the other hand, to the centromere index CI=p/p+q. Noteworthy is the mean centromere index (and large AR) of chromosome 9b and 10b as well as 16c and 17c.

Length of chromosomes varies from 9,915 µm (1a) to 2,705 µm (Y chromosome). The coefficient of variation ranges from 11 to 15 %. The small length of chromosome 12c in comparison to 13c and 14c is worthy of emphasis. The same is true with regard to chromosome 17d vs. 18d, and 20d vs. 21d. The Y chromosome plainly proved to be the smallest one. It belongs to the submetacentric group of chromosomes. The X chromosome is equally submetacentric. As to the length it is in proper order between chromosome 9b and 10b. Variation between homologous chromosomes is presented in fig. 2. We ascertained the maximum (max) and mean difference in length, respectively. The graph reflects a certain tendency to larger differences in length if chromosome length increases. The maximum difference between homologous chromosomes of the same metaphase amounted to 1.74 µm (chromosome 3a). There is a correlation (r = 0.562) between variation and length of the chromosomes (fig. 2).

Banding patterns are shown in fig. 1. In view of different frequencies of bands visible on chromosomes of the same type (e.g. 1a) we chose a figuration which presents each chromosome in three variants of banding patterns. At the left the idiogram stands for banding pattern realized at 100 % (black bands). In the center bands are represented which occurred by $\geq$ 50 %, at the right bands are shown that would be measured at least once. Bands in this paper are characterized as such if they are darker than contiguous areas. We measured up to 141 bands of the karyotypes. This corresponds to 600-650 bands defined in the nomenclature issued by the Reading Conference (Ford et al., 1980).

## Discussion

This paper presents results achieved in measuring rabbit chromosomes by means of interactive image analysis. Numbering of chromosomes and arrangement in the karyogram are based on the

proposals made by the Reading Conference (Ford et al., 1980) and the recommendations published by the Committee for Standardized Karyotype of Oryctolagus cuniculus (1981). The latter are quite similar to those published by Hsu and Benirschke (1967) who arranged chromosomes, according to the position of their centromere, in groups of meta-, submeta-, subtelo- and acrocentric ones. Most of cytogeneticists were in accordance with these proposals (Ducayen et al., 1974; Chan et al. 1977; Fox, 1975; Hageltorn and Gustavsson, 1979; Yerle, 1987). However, Echard (1977) suggested six groups of chromosomes (A-F) in the light of their length and centromere index.

Taking into consideration the arrangement of chromosomes recommended by Levan et al. (1964), i.e.

- metacentric: AR = 1.0-1.7
- submetacentric AR > 1.7-3.0
- subtelocentric AR > 3.0-7.0
- telocentric AR > 7.0,

which is widely accepted, our results (table 1) indicate that chromosomes 9 - 11 have to be arranged in group A, whereas chromosomes 16 and 17 should be placed to the submetacentric group B.

Furthermore, as observed in previous analyses, chromosome 12 seems to be smaller than chromosome 13 and 14. The same is true with regard to chromosome 18 and 19. Our detailed measurements confirm these findings. Therefore, in view of the conclusions drawn at the Reading Conference to group chromosomes according to their length and centromere position, our measurements carried out by image analysis could be a suggestion to reconsider idiograms which have been hitherto recommended.

It follows from the measured variability of homologous chromosomes of one and the same metaphase that it is next to impossible to identify single chromosomes only by their length and position of their centromere. Issa et al. (1968) measuring the length of chromosomes in homogeneous stained metaphases found standard deviations from 5 to 10 %. Patau (1960) pointed out that there is often a greater difference between the homologous than between the non-homologous chromosomes. This leads, according to our findings, to the conclusion that differences of homologous chromosomes cannot be attributed to the G-banding procedure.

The correlation between the absolute length of chromosomes and the difference of homologous ones is smaller than expected (r=0.562). The condensation of chromosomes which, as a rule, does not come off precisely synchronous may affect large chromosomes more than smaller ones. Detailed studies are needed analysing condensation of chromosomes during definite points of mitosis.

Results of analysing the banding pattern are shown in fig. 1. The data are based on 18 chromosomes per type (except X and Y). Thus some overlapping occurs which in fact is non-existent. Differences in hatching do not stand for staining intensity of bands but for the frequency of their occurrence.

As mentioned above we figured three variants of chromosomes characterized by different degrees of visible bands. We are of opinion that our findings reproduced in the center of fig. 1 will be most practicable as to the routine identification of a single chromosome.

In conclusion, our findings resemble more the recommendations of the Reading Conference (Ford et al., 1980) than those published by the Committee of Standardized Karyotyping (1981). As to the band position of the Y chromosome we concur with Echard (1973) and Stranzinger (1979) who observed only one large band on the q-arm reaching the p-arm but not covering the whole q-arm (fig. 3). A comparison pertaining to chromosome 7b (fig. 4) shows a striking similarity with the findings of Yerle (1987). Obviously, some areas of the chromosome seem to be more liable to condensation than others. The figure suggests that bands from 7.p.1.2.1.1. to 7.p.1.2.3.3. (Yerle) tend to fuse into band 2 (see our findings), whereas bands from 7.q.1.3.1. to 7.q.5.3. tend to fuse into band 5, and so on. It would be possible to show similar effects for other bands and chromosomes.

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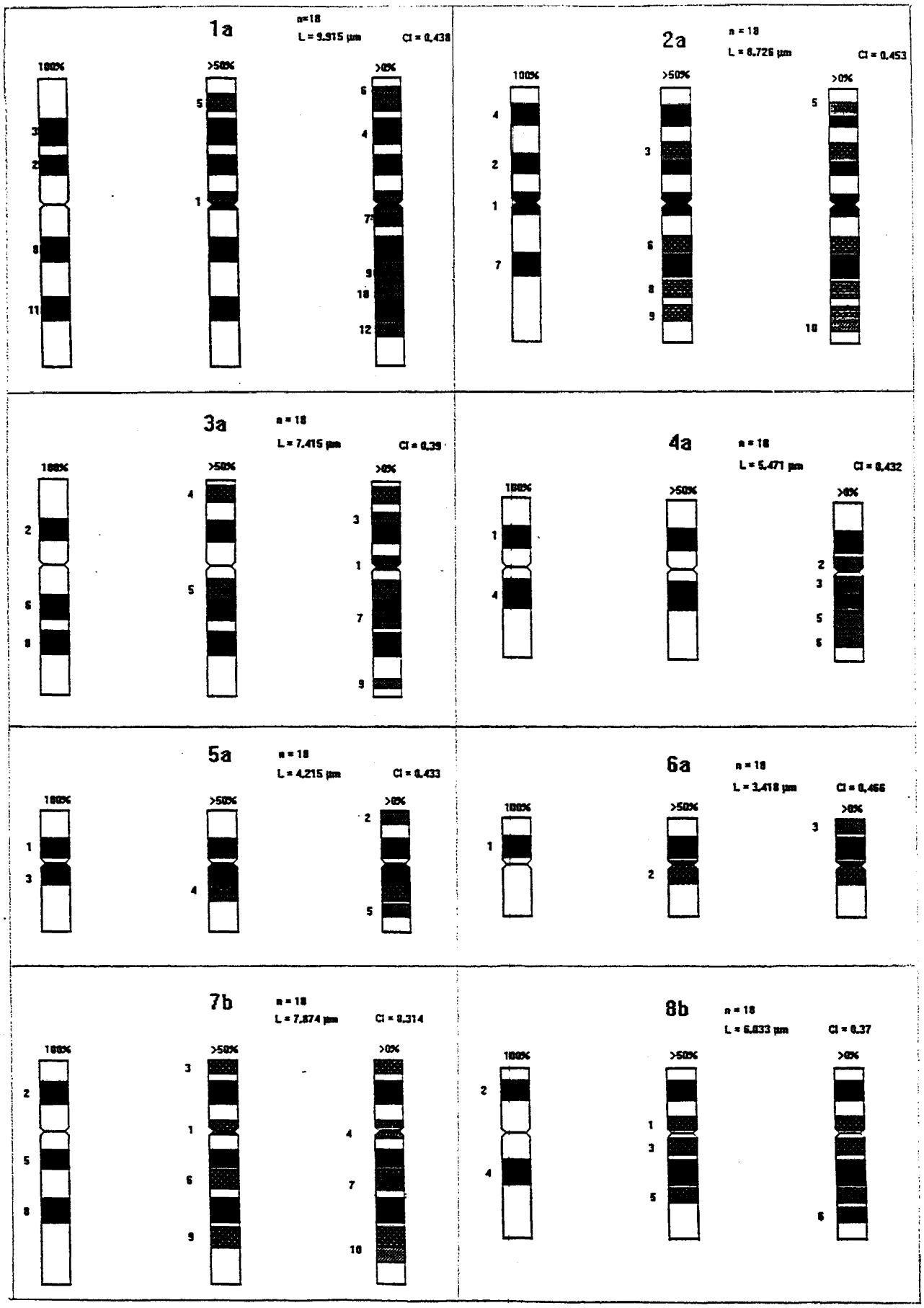
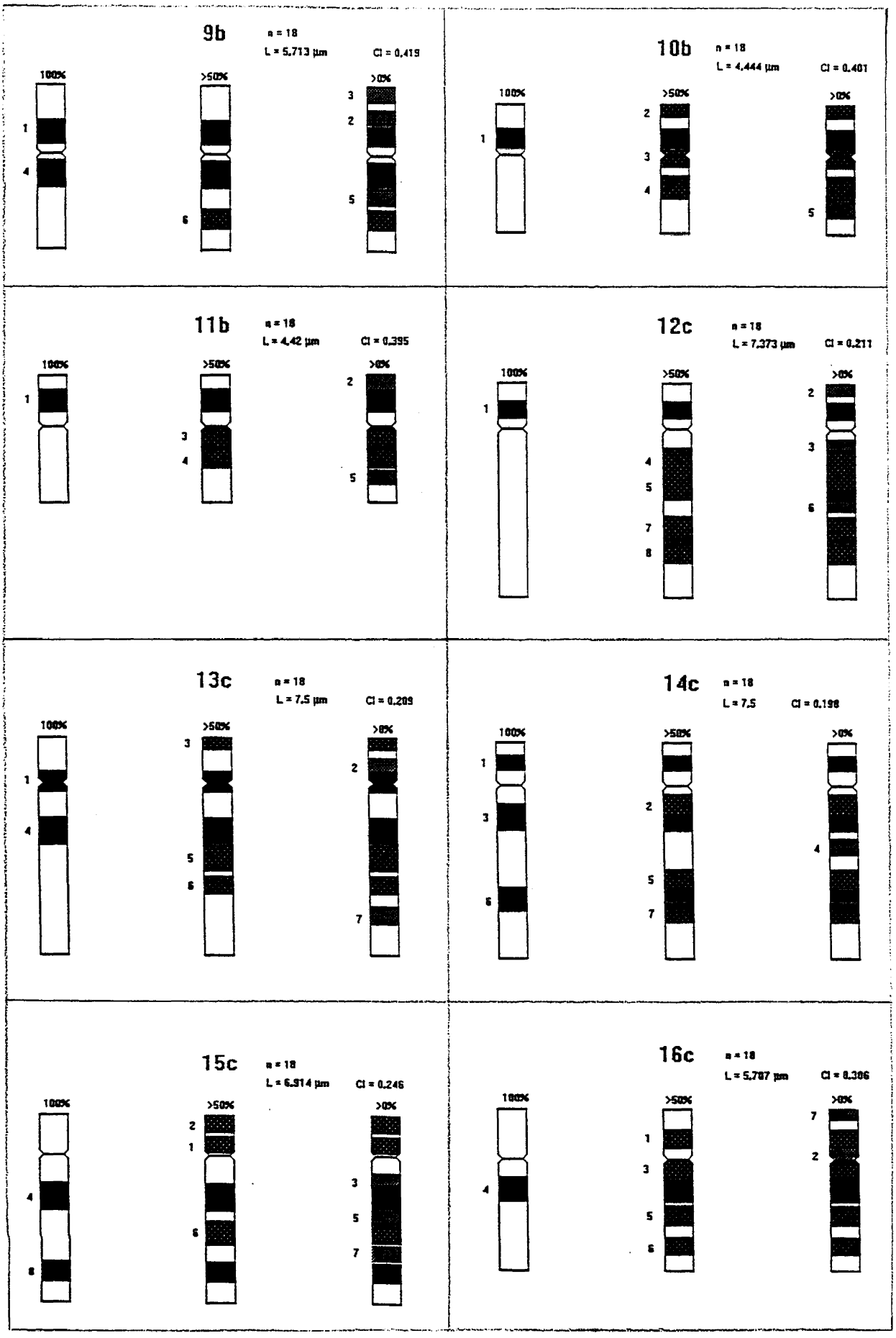
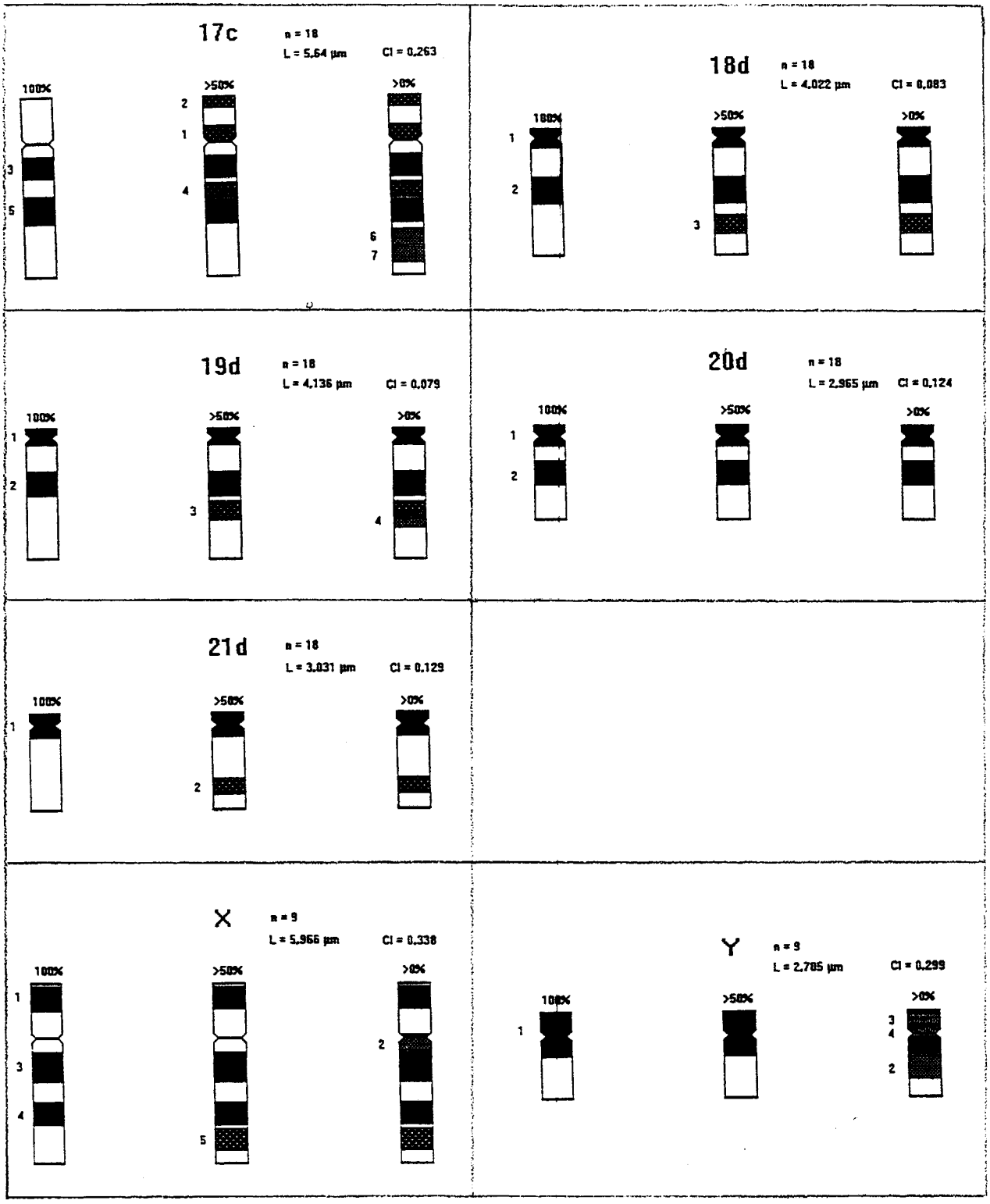
| Nb. | l µm | SD | SD % | AR q/p | CI p/(p+q) | Diff. of homol. (µm) max. | mean | p µm | SD | SD % | q µm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 9,915 | 1,25 | 12,6 | 1,282 | 0,438 | 0,87 | 0,51 | 4,348 | 0,56 | 12,9 | 5,568 |
| 2a | 8,726 | 1,19 | 13,6 | 1,206 | 0,453 | 1,74 | 0,74 | 3,955 | 0,56 | 14,1 | 4,771 |
| 3a | 7,415 | 1,04 | 14,0 | 1,537 | 0,394 | 1,74 | 0,74 | 2,923 | 0,40 | 13,7 | 4,493 |
| 4a | 5,471 | 0,68 | 12,4 | 1,317 | 0,432 | 0,76 | 0,41 | 2,361 | 0,34 | 14,4 | 3,110 |
| 5a | 4,215 | 0,50 | 11,8 | 1,311 | 0,433 | 0,65 | 0,29 | 1,824 | 0,21 | 11,5 | 2,391 |
| 6a | 3,418 | 0,45 | 13,2 | 1,144 | 0,466 | 0,65 | 0,31 | 1,597 | 0,19 | 11,9 | 1,824 |
| 7b | 7,874 | 0,97 | 12,3 | 2,180 | 0,314 | 0,98 | 0,31 | 2,476 | 0,34 | 13,7 | 5,399 |
| 8b | 6,033 | 0,69 | 11,4 | 1,700 | 0,370 | 1,30 | 0,45 | 2,234 | 0,31 | 13,9 | 3,798 |
| 9b | 5,713 | 0,70 | 12,2 | 1,389 | 0,419 | 0,54 | 0,22 | 2,391 | 0,35 | 14,6 | 3,321 |
| 10b | 4,444 | 0,59 | 13,3 | 1,495 | 0,401 | 0,98 | 0,50 | 1,781 | 0,36 | 20,2 | 2,663 |
| 11b | 4,420 | 0,64 | 14,5 | 1,533 | 0,395 | 0,54 | 0,22 | 1,754 | 0,26 | 14,8 | 2,675 |
| 12c | 7,373 | 0,97 | 13,1 | 3,732 | 0,211 | 0,87 | 0,43 | 1,558 | 0,24 | 15,4 | 5,815 |
| 13c | 7,500 | 0,83 | 11,0 | 3,777 | 0,209 | 0,54 | 0,30 | 1,570 | 0,23 | 14,6 | 5,930 |
| 14c | 7,500 | 0,82 | 10,9 | 4,047 | 0,198 | 0,98 | 0,31 | 1,468 | 0,22 | 15,0 | 6,014 |
| 15c | 6,914 | 0,78 | 11,3 | 3,060 | 0,246 | 1,52 | 0,52 | 1,703 | 0,25 | 14,7 | 5,211 |
| 16c | 5,707 | 0,69 | 12,1 | 2,270 | 0,306 | 0,54 | 0,25 | 1,745 | 0,28 | 16,0 | 3,961 |
| 17c | 5,640 | 0,62 | 11,0 | 2,796 | 0,263 | 0,54 | 0,27 | 1,486 | 0,25 | 16,3 | 4,155 |
| 18d | 4,022 | 0,47 | 11,7 | – | – | 0,43 | 0,16 | – | – | – | 4,022 |
| 19d | 4,136 | 0,55 | 13,3 | – | – | 0,43 | 0,17 | – | – | – | 4,136 |
| 20d | 2,965 | 0,40 | 13,5 | – | – | 0,76 | 0,28 | – | – | – | 2,965 |
| 21d | 3,031 | 0,39 | 12,9 | – | – | 0,43 | 0,12 | – | – | – | 3,031 |
| x | 5,966 | 0,89 | 14,9 | 1,956 | 0,338 | – | – | 2,017 | 0,29 | 14,4 | 3,949 |
| y | 2,705 | 0,35 | 12,9 | 2,344 | 0,299 | – | – | 0,809 | 0,22 | 35,8 | 1,896 |
| 1n without gonosomes: | | | | | | | | | | | |
| 122,433 | | 13,04 | 10,6 | | | | | | | | |
| 2n including X and Y: | | | | | | | | | | | |
| 253,54 | | 27,19 | 10,72 | | | | | | | | |

Table 1: Results of length measurements from 9 rabbit metaphases

fig. 1: (next pages) Graphic presentation of rabbit chromosomes (idiogram). For details see text of our contribution


1a
n=18
L = 9,515 µm
CI = 0,438
2a
n = 18
L = 8,726 µm
CI = 0,453
3a
n = 18
L = 7,415 µm
CI = 0,39
4a
n = 18
L = 5,471 µm
CI = 0,432
5a
n = 18
L = 4,215 µm
CI = 0,433
6a
n = 18
L = 3,418 µm
CI = 0,466
7b
n = 18
L = 7,874 µm
CI = 0,314
8b
n = 18
L = 6,833 µm
CI = 0,37


9b
n = 18
L = 5,713 µm
CI = 0,419
100%
>50%
>0%
10b
n = 18
L = 4,444 µm
CI = 0,401
11b
n = 18
L = 4,42 µm
CI = 0,395
12c
n = 18
L = 7,373 µm
CI = 0,211
13c
n = 18
L = 7,5 µm
CI = 0,209
14c
n = 18
L = 7,5
CI = 0,198
15c
n = 18
L = 6,914 µm
CI = 0,246
16c
n = 18
L = 5,787 µm
CI = 0,306


17c
n = 18
L = 5,64 µm
CI = 0,263
100%
>50%
>0%
18d
n = 18
L = 4,022 µm
CI = 0,083
19d
n = 18
L = 4,136 µm
CI = 0,079
20d
n = 18
L = 2,965 µm
CI = 0,124
21d
n = 18
L = 3,031 µm
CI = 0,129
X
n = 9
L = 5,966 µm
CI = 0,338
Y
n = 9
L = 2,785 µm
CI = 0,299

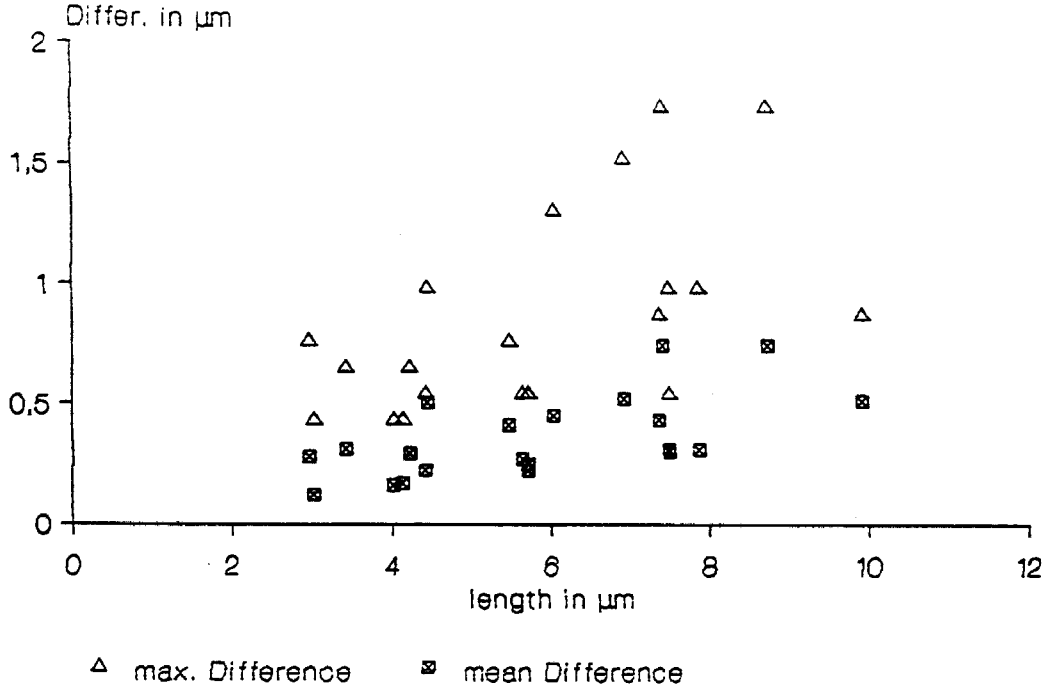



fig. 2: Length differences of homologous chromosomes

fig. 3: Comparison of diagrammatic representations of the Y chromosome as made by ECHARD (1973), STRANZINGER (1979), CHAN (1977), Committee (1981), our findings (2 figures) and Yerle (1987) (from left to right)

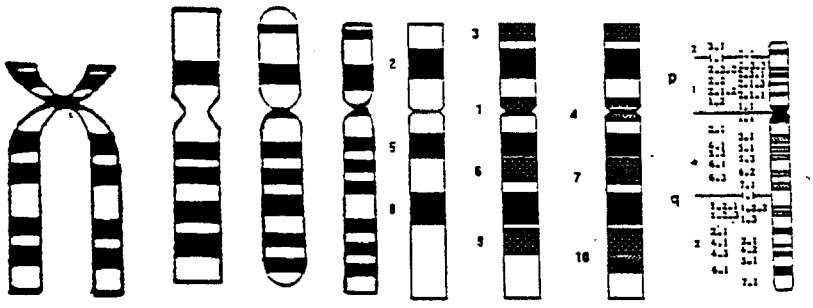


fig. 4: Comparison of diagrammatic representations of chromosome 7 as made by ECHARD (1973), STRANZINGER (1979), CHAN (1977), Committee (1981), our findings (3 figures) and Yerle (1987) (from left to right)