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Identification of the X chromosome of the domestic pig *(Sus scrofa domestica)*

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Summary

As the banding patterns of the X chromosome and chromosome No. 8 of the domestic pig are very similar, this identification problem has been studied. Depending on the metaphase stage, the banding pattern of the two chromosomes differ very much. Revised landmarks for the X chromosome and for chromosome No. 8 are presented, but it is not possible to use the landmarks on long chromosomes from the early metaphase stage.

Identification of the X chromosome of the domestic pig has been difficult from the early investigations of the karyotype. Different authors have described the X chromosome as meta-, submeta-, or telocentric, HULOT (1969). After introduction of the banding methods, GUSTAVSSON *et al.* (1972) and HANSEN (1972) described different banding patterns of the X chromosome by the Q-band technique; BERGER (1972), ECHARD (1973), HANSEN-MELANDER *et al.* (1974), PACE *et al.* (1975), SYSA (1975), MICHELMANN *et al.* (1977), and MIYAKE *et al.* (1978) have described different banding patterns by the G-band technique. Some authors found one distinct band on the middle of the short arm (*p*), other found two and three bands on p. From one to four bands have been described on the long arm (*q*) with one or two of them, but not in all cases the same, having a higher intensity. A diagrammatic representation of the Q-, G-, and R-band patterns of the pig chromosomes was published by HANSEN (1977), but still some identification problems have persisted.

The only chromosome in the pig karyotype, which is difficult to distinguish from the X chromosome by banding methods, is chromosome No 8. In attempting to solve this problem, the G-band pattern of the X chromosome and chromosome No 8 were studied in different metaphase stages of leucocytes.

The G-band technique was chosen because this technique gives more distinct bands than the Q-band technique, HANSEN (1975).

Materials and methods

Blood cultures from pigs of different strains of Danish Landrace were prepared according to the method described previously, HANSEN (1972, 1977). It must be stated that the culture time was 48 hours, because it seems that the number of prometaphase stages at 48 hours is a little bit higher than for 72 hours. The G-band staining was carried out according to the method described by WANG and FEDOROFF (1972), slightly modified.

The chromosomes were subdivided in groups according to the system by LEVAN *et al.* (1964), and arranged in karyotype according to the *Reading Conference* (1976), which does not follow the Levan system precisely. Landmarks are indicated by L, short arms by *p* and long arms by *q*. The numbering of bands is according to the *Paris Conference* (1971), and *Supplement* (1975). The terms distal and proximal refer to the position of bands or parts of arms in respect to the centromere.

Results

Depending on the length or the degree of contraction of the chromosomes, the banding pattern becomes more or less distinct. For that reason the early metaphase stages are very useful for banding purpose. Figure 1 shows pair No. 1, No. 8 and the sex chromosomes from four male pig leucocytes in different metaphase stages. Chromosome pair No. 1 demonstrates the metaphase stage by means of the length. Figure 2 shows the diagrammatic representation of the bands and the landmarks on chromosome No. 8 and the X chromosome. Figure 3 shows a karyotype based on cell No. 175-7, representing an early metaphase stage with long chromosomes.

The description of the banding patterns of the X chromosome and chromosome No. 8 is given for *a*) contracted, *b*) medium contracted, and *c*) long chromosomes.

Pair No. 19. *The X chromosome.* L : on contracted and on medium contracted chromosomes two bands on each side of the middle of *q*. On long chromosomes each of these bands are subdivided into two bands. (*p*) : on contracted chromosomes two bands are visible; a proximal pale band, and on the middle or just distal to it a band of medium intensity. On medium contracted chromosomes, three bands : a pale proximal and a pale distal band, and on the middle, a medium band. On long chromosomes the proximal and the distal band are subdivided into two very pale bands, Fig. 1 cell 175-7. (*q*) : on contracted and medium contracted chromosomes four bands are visible. A distal and a proximal pale band, and two medium to intense bands on each side of the middle, X*q*21 and X*q*31. Band X*q*21 is the most intensely stained band. On long chromosomes each of these intense bands are subdivided into two bands. The distal pale, X*q*33, is very often visible as a small distinct dot on each of the chromatids.

Pair No. 8. L : on contracted and medium contracted chromosomes, a proximal band on *q*. On long chromosomes this band is subdivided into two bands. (*p*) : on contracted chromosomes two bands; a proximal pale band, and a « distal » medium to intensely stained band. On medium contracted and on long

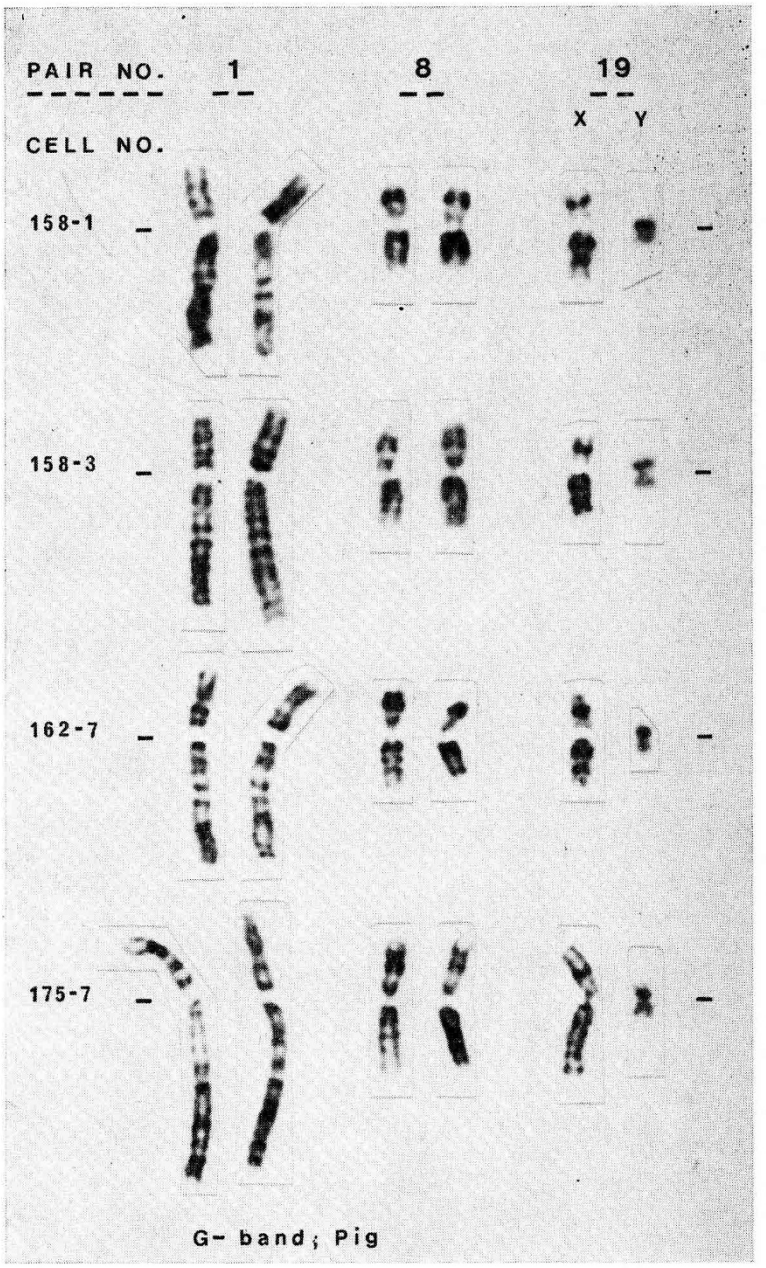

FIG. 1. — *Chromosome pair Nos. 1, 8, and 19 (the sex chromosomes) from four pig leucocytes in three different metaphase stages. Cell 158-1 shows contracted, cells 158-3 and 162-7 medium contracted, and cell 175-7 long chromosomes.*

Paires chromosomiques n° 1, 8 et 19 (chromosomes sexuels) extraites de 4 leucocytes de porc possédant 3 stades métaphasiques différents. Les chromosomes de la cellule 158-1 sont contractés, ceux des cellules 158-3 et 162-7 ont un degré de contraction moyen et ceux de la cellule 175-7 sont très despiralisés.

chromosomes the « distal » band is in fact a median band, which is subdivided into two bands, 8*p*14 and 8*p*16. Band 8*p*14 is the most heavily stained one. The very long and pale telomere region on 8*p* usually disappear on contracted chromosomes, Fig. 1 cell 158-1. On medium contracted and long chromosomes the telomere region is sometimes difficult to identify, too, because the region entangles or doubles up with the distal band. Band 8*p*14 is placed on the middle of *p*, and 8 *p*16 just distal to the middle, when the telomere region is identifiable, Fig. 1 cells 158-3 and 175-7. If the telomere is not visible, it seems that band 8*p*16 is placed at the end, Fig. 1 cell 162-7. (*q*) : on contracted chromosomes the intensity of the staining decreases from the proximal to the distal end. On medium contracted chromosomes four bands are visible. The breadth of the

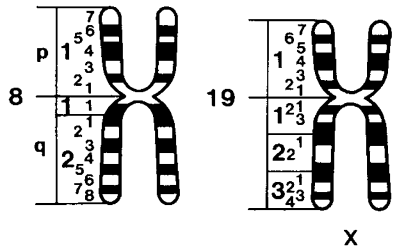

FIG. 2. — *Diagrammatic representation of the bands and landmarks on chromosome No. 8 and the X chromosome of the domestic pig*
Représentation schématique des bandes et des landmarks du chromosome X et du chromosome 8 du Porc domestique

bands decreases along the arm. On long chromosomes the proximal band is subdivided into two bands as shown in Fig. 1, cell 175-7.

As shown in Fig. 1, one of the chromosomes of pair No. 8 are sometimes shortened and more heavily stained compared to the homologue. This heavy staining of one of the chromosomes usually causes indistinct banding patterns, and for that reason the identification of the chromosome is sometimes difficult.

Because the frequency of cells with long chromosomes is not very high in cultures, the diagrammatic representation of the banding patterns in Fig. 2 are based on medium contracted chromosomes as a previous paper, HANSEN (1977).

Table I indicates the bands which on medium contracted chromosomes serve as landmarks on the X chromosome and on chromosome No. 8.

TABLE I

Bands serving as landmarks which divide the chromosomes into cytologically defined regions
Bandes utilisées comme landmarks qui divisent les chromosomes en regions cytologiquement définies

Chromosome No.	Arm	Number of regions	Landmarks as shown in the diagram
8	*q*	2	Proximal band of medium intensity(21)
19 X	*q*	3	Two median bands of medium intensity (21, 31)

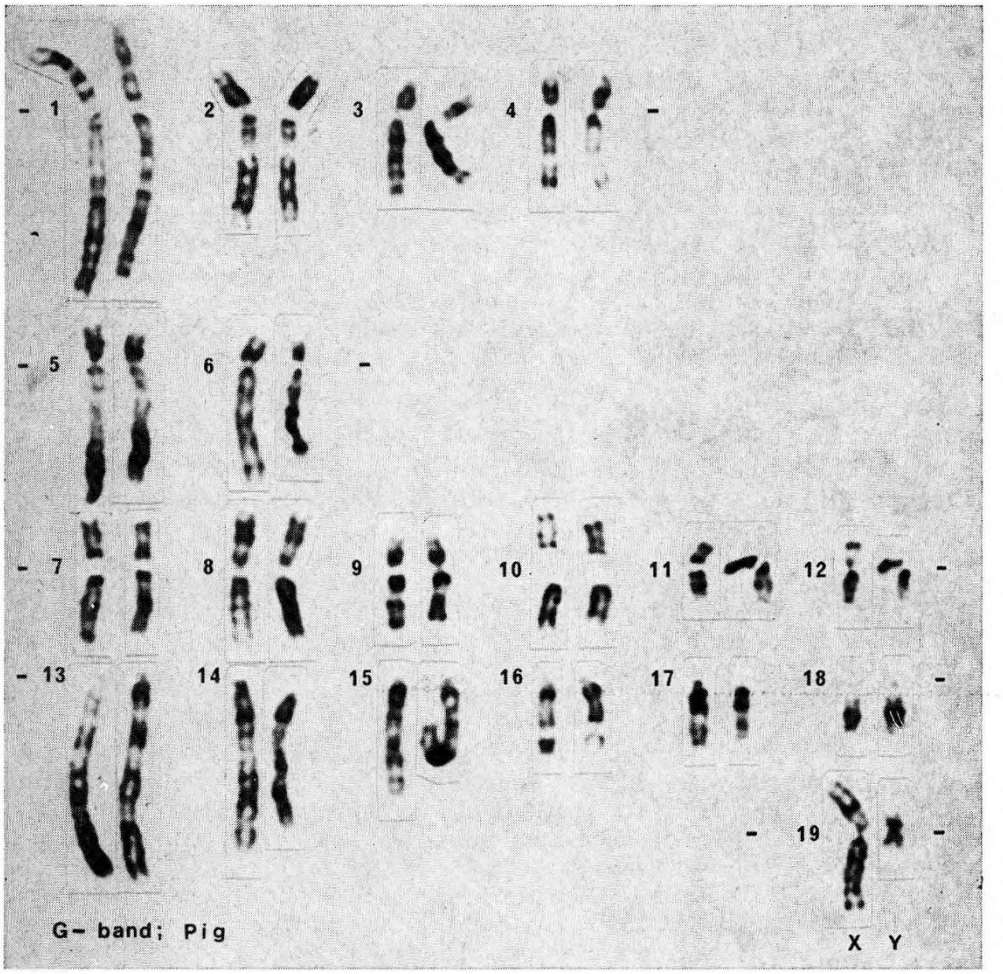

FIG. 3. — *G-band karyotype of the domestic pig*, (cell No. 175-7).
Early metaphase stage with long chromosomes.
Caryotype en bandes G du Porc domestique (cellule n° 175-7).
Prémétaphase avec des chromosomes despiralisés

Discussion and conclusion

In conventional *Giemsa* stained metaphase plates it was impossible to identify with certainty the X chromosome of the pig, because it belongs to the metacentric group, HULOT (1969). Because of the great similarity of the banding pattern of the X chromosome and pair No. 8, these can be difficult to destinguish by the banding methods, too.

The reasons for that are several : *a*) similar morphology; *b*) similar banding pattern of *p*; *c*) next to the centromere a heavily stained band on *q*; *d*) overfluorescence /overstaining.

In the first description of the Q-band pattern of the pig karyotype by GUSTAVSSON *et al.* (1972), the X chromosome was described with a bright band on the middle of the short arm, and two bright bands on the long arm. At the same time HANSEN (1972) described the X chromosome as follows : (*p*) distal half distinct, proximal half pale. (*q*) three bands. In a later paper HANSEN (1977) found on *q* two main bands on the middle, and a very narrow pale band next to the centromere. BERGER (1972) and SYSA (1973) described a slightly brighter fluorescence on the middle of *p*, and HANSEN-MELANDER *et al.* (1974) found one band on the middle of *p*, and two bands on *q*. In a later paper SYSA (1975) described one distinct narrow band on the middle of the short arm. In certain plates he found this band accompanied by narrow bands on both sites. On *q* he found four bands, the second one most intensely stained. The present results confirm the median band on the short arm as described by GUSTAVSSON *et al.* (1972), BERGER (1972), SYSA (1973), HANSEN-MELANDER *et al.* (1974), SYSA (1975), MICHELMANN *et al.* (1977), MIYAKE *et al.* (1978), and LIN *et al.* (1980). However, very distinct, but pale bands are present proximally as well as distally to the median band on *p*, and both of the pale bands are subdivided into two bands on long chromosomes. Especially, the distal very small band, X*p*16, is lost on contracted chromosomes, and for that reason only two bands are visible, see Fig. 1 cell 158-1, and LIN *et al.* (1980). On long chromosomes band X*p*16 can be twisted, and give a heavy staining at the end of *p*. Possibility for these reasons ECHARD (1973) and PACE *et al.* (1975) described two bands on *p*. The long arm shows two very distinct median bands as described by GUSTAVSSON *et al.* (1972), ECHARD (1973), HAGELTORN *et al.* (1973), HANSEN-MELANDER *et al.* (1974) and HANSEN (1977). But the present results show a distinct narrow band proximally as well as distally to the two median bands, i.e. on medium contracted chromosomes a total of four bands on *q*. This is in accordance with SYSA (1975), with Fig. 3 in the paper by HANSEN-MELANDER *et al.* (1974), and in part with HANSEN (1972) and LIN *et al.* (1980), because the last two authors only describe three bands. As demonstrated in Fig. 1 cell 175-7, each of the two broad bands on q, X*q*21 and X*q*31, are subdivided into two bands on long chromosomes.

On chromosome No. 8 the very long telomere region of *p* is often twisted or doubled up with band 8*p*16, see e.g. Fig. 1 cell 162-7. Possibly for that reason band 8*p*16 is usually described on the distal part of *p*, GUSTAVSSON *et al.* (1972). When the telomere is visible, band 8*p*14, which has the highest intensity of staining, is placed exactly on the middle, and 8*p*16 just distally to the middle of *p*, see Fig. 1 cell 175-7. The banding pattern of the long arm is very characteristic, because of the decreasing intensity of the bands from 8*q*21 to 8*q*27, which is in contrast to X*q*. The banding pattern of 8*q* has also been described in different ways in the literature.

It seems that bands which serve as landmarks only can be used as landmarks on contracted and medium contracted chromosomes. On long chromosomes these landmarks are very often subdivided, and the intensity of the subdivided bands are usually equal. Furthermore, bands which are negative on contracted or medium contracted chromosomes often show one or two very pale, but distinct bands, on long chromosomes.

The karyotype in Fig. 3 is in accordance with the agreement of the *Reading Conference* (1976), because it was decided to put chromosome No. 5 in the temporarily used pig karyotype into the right position, i.e. into the group of metacentric chromosomes, if further measurements confirm the results by HANSEN (1976, 1977). Recently LIN *et al.* (1980) have measured the relative length of the pig

chromosomes, and the results agree very well with the previously and the revised results by HANSEN (1980).

From the present results it is obvious that the criteria for identification of chromosome No. 8 and the X chromosome are very different in cells from the early to the late metaphase. If these are observed it is easy in well spread metaphase plates to identify with certainty the X chromosome of the domestic pig. The landmark system, however, is only usable on contracted and medium contracted chromosomes.

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Résumé

Identification du chromosome X du Porc domestique (Sus scrofa domestica)

La similitude de marquage du chromosome X et du chromosome 8 du Porc domestique conduit à étudier ce problème d'identification. Le marquage de ces deux chromosomes varie selon le stade métaphasique. Les landmarks (interbandes) ont été revues mais il n'a pas été possible de les utiliser sur les chromosomes très despiralisés provenant de prométaphases.

References

BERGER R., 1972. Étude du caryotype du porc avec une nouvelle technique. *Exptl. Cell Res.*, **75**, 298-300.

ECHARD G., 1973. Étude des bandes chromosomiques du porc et de trois différentes souches de rein de porc en culture (PK 15, F et RP). *Ann. Génét. Sél. Anim.*, **5**, 1-21.

GUSTAVSSON I., HAGELTORN M., JOHANSSON C., ZECH L., 1972. Identification of the pig chromosomes by the quinacrine mustard fluorescence technique. *Exptl. Cell Res.*, **70**, 471-474.

HAGELTORN M., GUSTAVSSON I., 1973. *Giemsa* staining patterns for identification of the pig mitotic chromosomes. *Hereditas*, **75**, 144-146.

HANSEN K. M., 1972. The karyotype of the pig (*Sus scrofa domestica*), identified by quinacrine mustard staining and fluorescence microscopy. *Cytogenetics*, **11**, 286-294.

HANSEN K. M., 1975. Animal chromosomes and banding methods. Proceeding of: 2. *Europäisches Kolloquium über Zytogenetik* (Chromosomenpathologie) in Veterinärmedizin, Tierzucht und Säugetierkunde. Giessen, 29 und 30 September 1975, 33-43.

HANSEN K. M., 1976. The karyotype of the pig. Contribution to the Reading Karyotype Conference, 2nd-6th August 1976. Unpublished results.

HANSEN K. M.,.1977. Identification of the chromosomes of the domestic pig (*Sus scrofa domestica*). An identification key and a landmark system. *Ann. Génét. Sél. Anim.*, **9**, 517-526.

HANSEN K. M., 1980. The relative length of pig chromosomes, and a suggestion for a karyotype system. In press.

HANSEN-MELANDER E., MELANDER Y., 1974. The karyotype of the pig. *Hereditas*, **77**, 149-158.

HULOT F., 1969. Les chromosomes des Suiformes. *Ann. Génét. Sél. Anim.*, **1**, 315-336.

LEVAN A., FREDGA K., SANDBERG A. A., 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, **52**, 201-220.

LIN C. C., BIEDERMAN B. M., JAMRO H. K., HAWTHORNE A. B., CHURCH R. B., 1980. Porcine (*Sus scrofa domestica*) chromosome identification and suggested nomenclature. *Canad. J. Genet. Cytol.*, **22**, 103-116.

MICHELMANN H. W., EL NAHASS E. M., PAUFLER S., 1977. Vergleichende Chromosomenuntersuchung bei Zucht- und Mastschweinen mit Hilfe der Giemsa-Färbung und der Bänderungstechnik. *Züchtungskunde*, **49**, 294-300.

MIYAKE Y. I., ISHIKAWA T., 1978. The possibility of chromosome Classification as identified by trypsin-Giemsa banding patterns. *Zuchthyg.*, **13**, 33-37.

PACE J. W., SRIVASTAVA P. K., LASLEY J. F., 1975. G-band patterns of swine chromosomes. *J. Hered.*, **66**, 344-348.

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

Paris Conference (1971), *Supplement* (1975) : *Standardization in Human Cytogenetics*. Birth Defects: Original Article Series, XI, **9**, 1975. The National Foundation, New York.

Reading Conference (1976) : Standardization of banded karyotypes of domestic animals. *Hereditas*, **92**, 145-162.

SYSA P., 1973. Fluorescence characteristics of the karyotype of the pig. *In Vitro*, 2 /2*A*, 56-67.

SYSA P., 1975. Studies on the karyotype of domestic pig (*Sus scrofa dom.*) by means of banding patterns techniques and autoradiography. Proceeding of: 2. *Europäisches Kolloquium über Zytogenetik* (Chromosomenpathologie) in Veterinärmedizin, Tierzucht und Säugetierkunde. Giessen, 29 und 30 September 1975, 83-91.

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