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Heterochromatin and nucleolus-organizer-region behaviour at male pachytene of *Sus scrofa domestica*

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Abstract. In the domestic pig ($2n=38$) two types of constitutive heterochromatin can be differentiated by fluorescence counterstaining techniques. All 24 biarmed autosomes and the X chromosome have chromomycin A_3-positive centromeric C-bands, whereas all 12 acrocentric chromosomes exhibit DA-DAPI-positive centromeric heterochromatin. Fluorescence analysis of male pachytene nuclei revealed that the DA-DAPI-positive C-bands form one or two large chromocentres per cell, while the chromomycin A_3-bright C-material is well scattered. Hence, the bivalents formed by the acrocentric chromosome pairs are centromerically associated, whilst the submetacentric bivalents are not. – Counce-Meyer spreading techniques were used to study the structure of synaptonemal complexes (SCs) both by light and electron microscopy. In general, the SCs of the domestic pig resemble those described for other mammals. The SC formed by the X and the Y may include up to 94.5% of the Y chromosome. In silver-stained microspreads each of the bivalents (nos. 8 and 10) bearing the nucleolus-organizer-regions (NORs) is connected to a pair of nucleoli, indicating that all four NORs are active during early meiotic stages. By contrast, in the majority of mitotic metaphases of phytohaemagglutinin-stimulated lymphocytes only one pair (no. 10) exhibited Ag-NOR staining. – The significance of the chromosome disposition in the pachytene nucleus is discussed with regard to heterochromatin composition and karyotype evolution.

Introduction

Two major types of constitutive heterochromatin (C-bands) can be distinguished by fluorescence staining techniques in the porcine mitotic karyotype (Schnedl et al. 1981; Lin et al. 1982; Mayr et al. 1984). All biarmed autosomes (nos. 1–12) and the X chromosome have chromomycin A_3-positive C-band material at (or adjacent to) the centromere. All acrocentric chromosomes (nos. 13–18) have blocks of centromeric heterochromatin that stain positively by the distamycin A plus DAPI (DA-DAPI) technique. The heterochromatin of the Y chromosome is not differentially stained by either of the two fluorochromes. The autosomal heterochromatin differentiation in *Sus scrofa domestica* reflects the bimodal karyotype differentiation with submetacentric and acrocentric chromosomes.

Here we report observations on primary spermatocytes after fluorescence banding and the silver-staining method for detecting active nucleolus organizer regions (NORs). We also present results obtained by the surface microspreading technique for synaptonemal complexes (SCs) (Counce and Meyer 1973) using the light microscope (LM) and the electron microscope (EM). We show that the two kinds of C-bands in the domestic pig differ with respect to their meiotic association. An interpretation of this phenomenon is given and implications for C-band composition are discussed.

Materials and methods

Source of animals. In all experiments only animals of the commercial breed "Österreichische Landrasse" were used. The samples originated from two different breeding areas (Oberösterreich and Niederösterreich). All animals exhibited the standard karyotype (Lin et al. 1980) and were chromosomally normal.

Chromosome preparation and cytochemical staining. Mitotic chromosomes were prepared from short-term peripheral blood cultures after phytohaemagglutinin (PHA) stimulation. Meiotic chromosome preparations were obtained from seminiferous tubuli by standard air-drying procedures. For silver staining of NORs, the recipe of Howell and Black (1980) was followed. Chromomycin A_3 R-banding and DA-DAPI banding were achieved by different selective fluorescence excitation of the "tri-stained" preparations (Schweizer 1980, 1981; for conditions see Schweizer et al. 1983).

For the *relative chromosome lengths* in Table 1 and Figure 2 the mean values of six R-banded mitotic metaphases were taken. Chromosome lengths are expressed as the percentage of total karyotype length (the male diploid karyotype was arbitrarily set as 200%). Arm ratios were calculated by dividing the length of the long arm by the length of the short arm. The numbering of the chromosomes follows the standard karyotype of Lin et al. (1980).

Spreading of synaptonemal complexes. The surface-spreading technique of Counce and Meyer (1973) was used with some minor modifications. A suspension of spermatocytes

* This paper is dedicated to Prof. Hans Bauer on the occasion of his 80th birthday

in culture medium (Ham's F-10 medium containing 25% foetal calf serum) was obtained from seminiferous tubules. The cells were directly spread on a glass slide in a drop of 0.2 M sucrose containing 0.1%–0.5% Nonidet P40 (Sigma). After a few seconds the drop was distributed over half the slide and left to dry out. The slides were fixed in 4% paraformaldehyde plus 3.4% sucrose (pH 8) for 10 min and then rinsed briefly in a 0.4% Kodak Photoflo solution (pH 8). Standard air-dried preparations were made as control. For silver staining a few drops of 50% $AgNO_3$ (aqueous solution) were put on the slide and covered with a nylon mesh (diameter about 240 µm; Swiss Silk Bolting Cloth-Fabrik, Zürich) as suggested by Kodama et al. (1980). The silver-stained SCs were analysed by both LM and EM. Some slides for the EM were stained with 1% phosphotungstic acid (PTA) in 25% ethanol for 10 min and then well rinsed in ethanol. For analysis of the spread SCs in the EM, the stained slides were coated with a plastic film (0.3% Formvar in chloroform). A 1%–2% aqueous hydrofluoric acid (HF)-solution was used to float the film with the adhering cellular material (Ruzicka 1974). The film segments were picked up on single-hole EM grids, which were then examined in a Zeiss EM9.

Length measurements of SCs were performed on photographs (9,500× and 6,200×) using a digitizer tablet (Attaker, GTCO Corporation) in conjunction with a Horizon 64K microcomputer (facilities kindly provided by the Institute of Geodetics of the Technical University of Vienna). Values given for autosomal bivalents are mean lengths of 11–14 SCs of each type; mean values for the sex bivalent are based on 11 measurements. Relative values are based on total male diploid karyotype length, arbitrarily set as 200%.

Results

Heterochromatin and karyotype architecture

Porcine metaphase chromosomes that have been stained with the GC-specific fluorescence dye, chromomycin A_3, and the AT-specific fluorescence drug, DAPI, in conjunction with the AT-specific, non-fluorescent counterstain, distamycin A (DA), are shown in Figure 1.

Selective chromomycin A_3 fluorescence excitation (maximum 436 nm) results in distinct R-banding patterns along chromosomes, which allow the identification of all autosomes and the sex chromosomes (Fig. 1a, right). The submetacentric autosomes (nos. 1–12) and the X chromosomes have chromomycin-bright centromeric heterochromatin. These C-bands comprise 15% of the karyotype length with a mean of 0.6% per biarmed chromosome. Fluorescence excitation with light predominantly at 360 nm reveals DA-DAPI-positive centromeric heterochromatin on all acrocentric chromosomes (nos. 13–18) (Fig. 1a, left). The DA-DAPI material amounts to 11% with a mean of 0.9% for each acrocentric chromosome. Figure 1b shows the distribution of chromomycin and DA-DAPI-bright heterochromatin at interphase. A schematic mitotic karyotype is presented in Figure 2. C-band polymorphisms are not indicated, although they do occur (Schnedl et al. 1981; see also Fig. 1a). The Y chromosome, which is known to have a great deal of C-band material in its long arm (Lin et al. 1982), does not exhibit bright bands either with DA-DAPI or with chromomycin A_3. Chromosomes 8 and 10 each carry a paracentromeric NOR in the short arm. The nucleolar constrictions are negative both with chromomycin A_3 and DA-DAPI. Silver staining confirmed earlier observations regarding the activity of the NORs (Lin et al. 1980; Czaker and Mayr 1980). In most cells the NORs of chromosomes 10 have distinct silver deposits and frequently exhibit association, while the NORs of chromosomes 8 are not usually labelled by the $AgNO_3$ method.

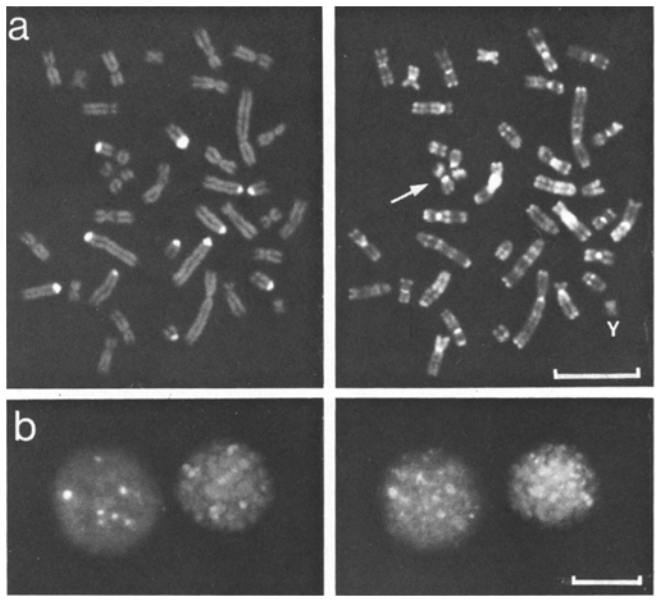

Fig. 1. Lymphocyte metaphase (**a**) and interphase (**b**) of *Sus scrofa domestica* after chromomycin/distamycin-DAPI staining and selective fluorescence excitation. The centromeric heterochromatin of the acrocentric chromosomes is DA-DAPI positive (*left*), whilst that of the biarmed chromosomes (excepting the Y) is highlighted by chromomycin fluorescence (*right*: associated NOR-chromosomes 10 are *arrowed*). Bars represent 10 µm

Chromosome length measurements. The diploid chromomycin R-banded male mitotic karyotype was 174.8 µm (SD 14.9) long. The relative chromosome length values and arm ratios given in Table 1 have been used for drawing a mitotic karyogram (Fig. 2).

Fluorescence banding of spermatocytes

In our material the most frequent meiotic stages observed were early prophases up to late pachytene. Diakinesis and metaphase I stages were so rare that analyses could not be attempted. Examples of chromomycin/DA-DAPI-stained spermatogonia, pachytene nuclei, and early spermatids are shown in Figure 3. At pachytene, it is possible to distinguish between the two groups of bivalents by their centromeric fluorescence properties. The disposition of the two kinds of chromocentres can be compared in the same nucleus simply by changing the filter blocks. The chromomycin-bright centric heterochromatin of the biarmed chromosomes appears as dots or double dots (Fig. 3b–d) apparently irregularly distributed over the pachytene nucleus. The disposition of the acrocentric chromosomes as revealed by DAPI fluorescence excitation is quite different. The majority of pachytene cells have only one large DA-DAPI-positive

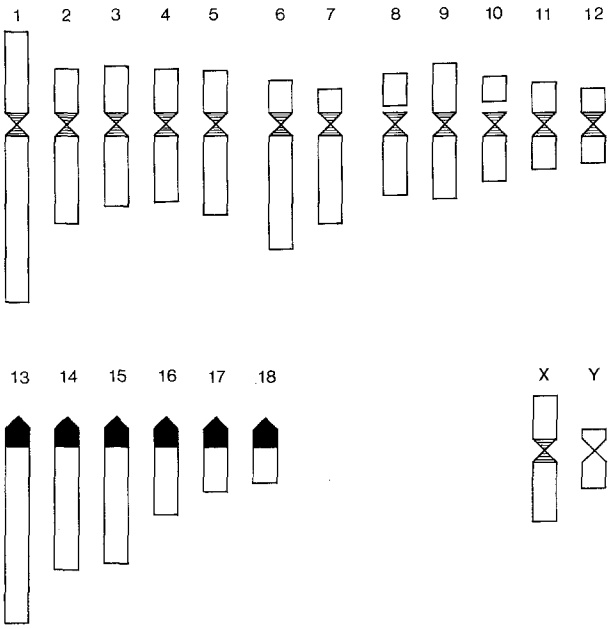

Fig. 2. Karyotype of *Sus scrofa domestica* showing the distribution of C-bands highlighted by fluorescence counterstaining. The DA-DAPI-positive centromeric heterochromatin is marked *black*, the chromomycin-positive bands are *hatched*. Each chromosome was given an equal amount of heterochromatin irrespective of the occurrence of C-band polymorphisms (compare Fig. 1). The nucleolar organizer regions are drawn as *gaps*. Relative chromosome lengths were calculated from R-banded metaphases (Table 1)

Table 1. Relative length and arm ratio of R-banded mitotic metaphase chromosomes of *Sus scrofa domestica*

Chromosome group	No.	% Relative length[a]	Arm ratio[b]
Meta-/submetacentric autosomes	1	10.8	1.9
	2	6.2	1.8
	3	5.6	1.4
	4	5.2	1.5
	5	5.8	1.6
	6	6.7	2.8
	7	5.4	2.9
	8	4.8	1.4
	9	5.4	1.2
	10	4.2	1.2
	11	3.5	1.1
	12	3.0	1.0
Acrocentric autosomes	13	8.2	
	14	6.1	
	15	5.9	
	16	3.9	
	17	3.0	
	18	2.6	
Sex chromosomes	X	5.0	1.3
	Y	2.4	1.7

[a] The length of the diploid male karyotype was arbitrarily set as 200%.
[b] Long arm/short arm

chromocenter (e.g. Fig. 3c, d). In the remaining pachytene nuclei two DA-DAPI bright spots are seen (see Fig. 3b; compare Fig. 4). This fluorescence pattern indicates that at pachytene the six bivalents formed by the acrocentric chromosomes are normally associated at their centromeric heterochromatin. Very early primary spermatocytes and spermatogonia usually exhibit a larger number of DA-DAPI-positive chromocentres (Figs. 3a, 4) indicating that there is no comparable kind of association in early stages. The haploid spermatid nuclei mostly have two to four DA-DAPI spots. A morphologically different type of interphase, which was tentatively identified as Sertoli cells, has concentric or adjacent clusters of chromomycin and DA-DAPI-bright areas suggesting that a relic Rabl orientation of the chromosomes is present.

Synaptonemal complexes

All autosomal SCs can be followed from one end to the other in well-spread pachytene cells (Figs. 5, 6). At both ends there are prominent more darkly stained knoblike structures, the attachment plates to the nuclear envelope. In some spreads parts of the nuclear envelope linking together several SCs can be seen. In many cells the silver-stained SCs look differentiated or banded along their length in the LM. However, we cannot find any characteristic arrangement of banding nor is it visible in the EM pictures. Kinetochores cannot be distinguished in silver-stained preparations by LM or EM (Figs. 5, 6). Only PTA-stained autosomal SCs show in the EM a distinct, darkly stained structure that marks the kinetochore region (Fig. 7a). The kinetochore of the acrocentric chromosomes is situated next to the endplate. Although a short-arm SC segment is not detectable, we propose the continued use of the term "acrocentric" for this type of chromosome, because this designation is established in cytogenetic literature of domestic animals.

SC length measurements. The autosomal SCs range from 4.5 μm to 15.5 μm long with a total length of 161.5 μm (SD 17.9). Comparisons of the total length and the length of individual SCs with the data for somatic metaphase chromosomes show that pachytene bivalents are on average about 1.9 times longer. Only 2 of the 18 autosomal bivalents were distinguishable, the 2 NOR-bearing SCs of which no. 8 is 8.7 μm (SD 1.4), and no. 10 is 7.3 μm (SD 1.0) long. In PTA-stained cells the kinetochores are detectable (Fig. 7) and therefore arm ratios can be established. However, it is only possible to distinguish between the two groups of acrocentric and submetacentric chromosomes.

Sex bivalent. The axes of the X and Y chromosomes are thicker and more darkly stained than those of the autosomes and are easy to recognize in most cells (Fig. 5). Length measurements give a mean length of 9.0 μm (SD 1.4) for the X and 3.3 μm (SD 0.6) for the Y chromosome. The paired region between the X and the Y can include up to 94.5% of the Y chromosome at early pachytene (Fig. 7b, c). During pachytene the SC region shortens progressively, and the unpaired ends of the X and Y chromosomes sometimes meet. The end of the X chromosome that is never involved in synapsis shows the greatest structural changes. Its axis is up to three times as thick as the autosomal lateral elements, and at late pachytene extreme splicing together with the formation of loops and knobs occurs.

Nucleolus-organizer-regions

The nucleoli in silver-stained surface spread pachytene cells are darkly stained and well defined at one end, and paler,

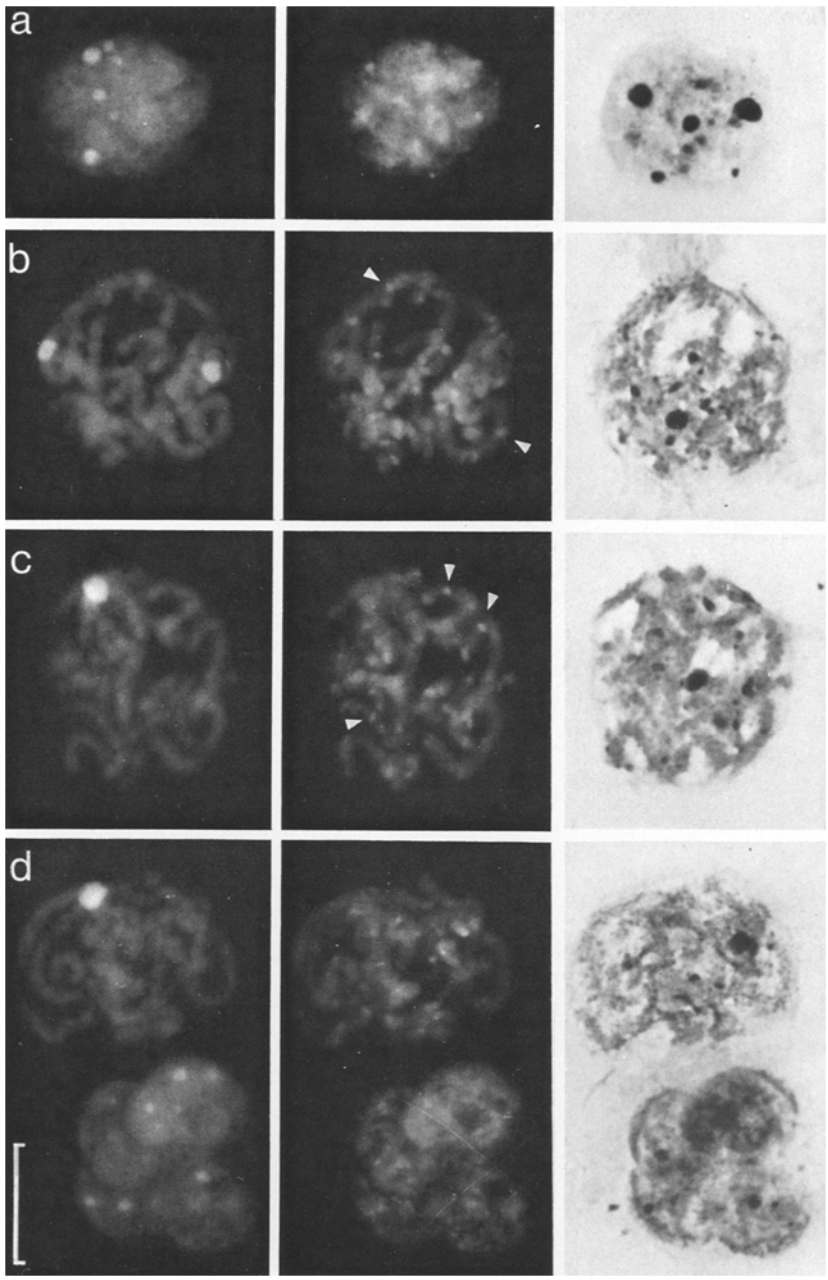

Fig. 3a–d. Cells from seminiferous tubules of the domestic pig prepared by conventional air-drying techniques and sequentially stained with chromomycin/distamycin-DAPI and $AgNO_3$. DA-DAPI fluorescence *left*; chromomycin fluorescence *middle*; and Ag-NOR staining *right*. **a** Spermatogonial cell; **b–d** primary spermatocytes and spermatids. At pachytene (**b, c** and top of **d**) the DA-DAPI-positive heterochromatin of acrocentric bivalents (nos. 13–18) is fused to form one or two chromocentres. The autosomal bivalents of the biarmed type (chromosome pairs 1–12) are not centromerically associated as indicated by the distribution of chromomycin-bright spots (some marked by *arrowheads*). Bar represents 10 μm

more diffuse and tail-like at the other (Fig. 6). Of the 42 NOR-bearing bivalents analysed in 21 cells in the EM 34 are each associated with a *pair* of nucleoli. None of the cells has more than four nucleoli, and only 6 have less than four. Similar results were obtained from SC spreads that were studied in the LM in which 73% of the analysable pachytene cells have two pairs of nucleoli. However, in many cases it is unclear to what extent the silver-positive material can be correlated with ribosomal gene activity. A number of unidentified cellular components and debris from the nuclear membrane may also stain darkly with silver nitrate. In conventional air-dried spreads of hypotonically treated and methanol/acetic acid-fixed spermatocytes the NORs and nucleoli are very weakly stained and are difficult to distinguish in pachytene from other structures, so that a comparison with what is seen in the surface-spread preparations is impossible (Figs. 3, 6).

In spermatogonia and early primary spermatocytes (leptotene and zygotene) four nucleoli are found in most cells, indicating activity of all four NORs in the complement. Spermatids cannot be identified after surface spreading but after conventional air drying 60% of the cells have one nucleolus and 25% have two nucleoli. It is not clear whether the single nucleolus represents a fusion product of two active NORs or indicates the activity of only one NOR. The size correlation and the observation made on different material by Schwarzacher and Wachtler (1983) that cells beginning rRNA synthesis normally do not show nucleolar fusion suggests that the latter possibility is more likely.

Discussion

The present observations demonstrate that *Sus scrofa* provides favourable material for studying heterochromatin and

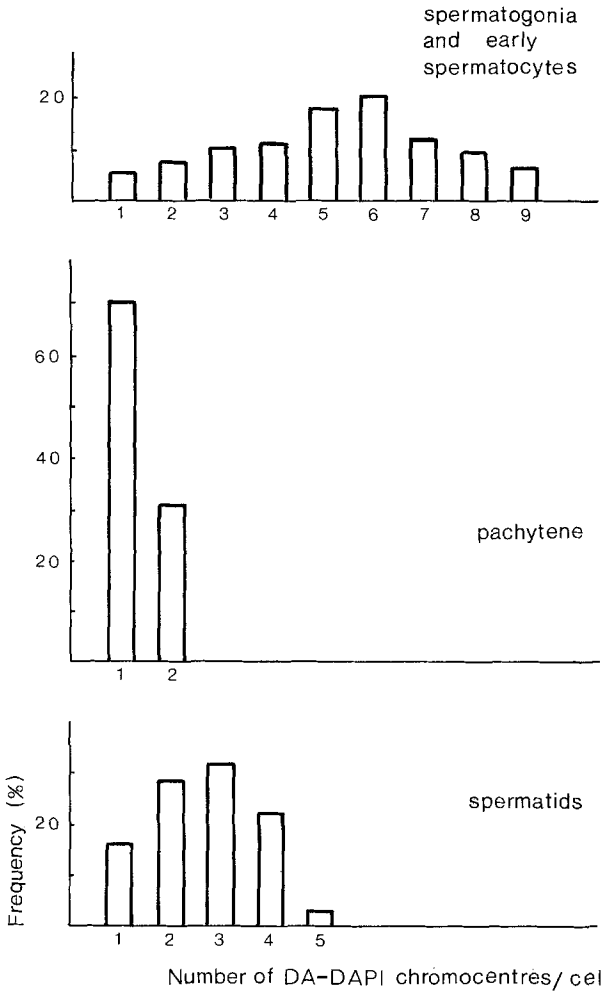

Fig. 4. Frequencies of cells with different numbers of DA-DAPI chromocentres at early and middle stages of spermatogenesis in the domestic pig. Preferential association of DA-DAPI heterochromatin is seen at pachytene

the behaviour and disposition of chromosomes at those stages of division when these aspects are normally not amenable to analysis. The low number of NORs per cell also makes the system suitable for the investigation of rRNA gene activity in different cell types and division stages by cytochemical techniques. The present study centres on heterochromatin and NOR behaviour at male pachytene. Observations made after Ag-NOR staining, Counce-Meyer surface spreading of synaptonemal complexes, and fluorescence counterstaining are discussed in turn.

Nucleolus-organizer-regions

Analysis of mitotic metaphases from PHA-stimulated lymphocytes by silver nitrate staining confirmed that only the NOR of chromosome 10 is normally active (in both homologues), whereas the ribosomal site of chromosome 8 is very seldom active and then shows only weak transcriptional activity (Lin et al. 1980). This contrasts with our findings in silver-stained synaptonemal complexes at pachytene (Figs. 5, 6). In more than 70% of the cells both NOR-bearing bivalents are associated with nucleoli, and hence all four rDNA clusters are active. This enhanced activity

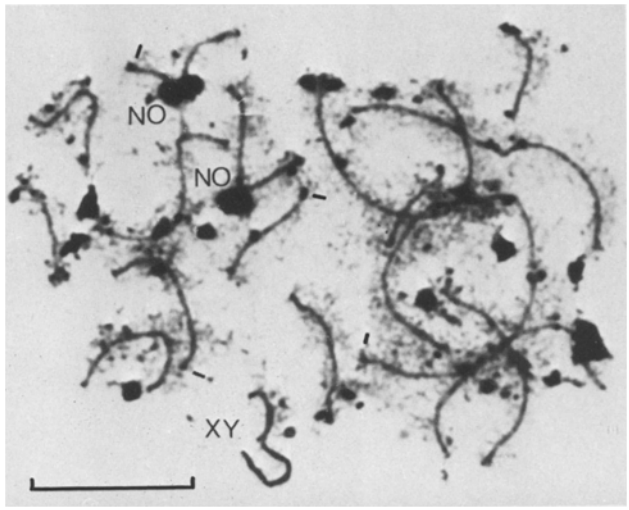

Fig. 5. Silver-stained Counce-Meyer spread of synaptonemal complexes (SCs) in the light microscope. The SCs appear as single threads ending in more densely stained endplates (*dashed*). The X and Y chromosomes are distinguishable by their relative sizes and their thicker and more densely stained axes. In this cell the X and the Y are presumably attached only at their ends. Two bivalents are associated with pairs of nucleoli (*NO*). Bar represents 10 μm

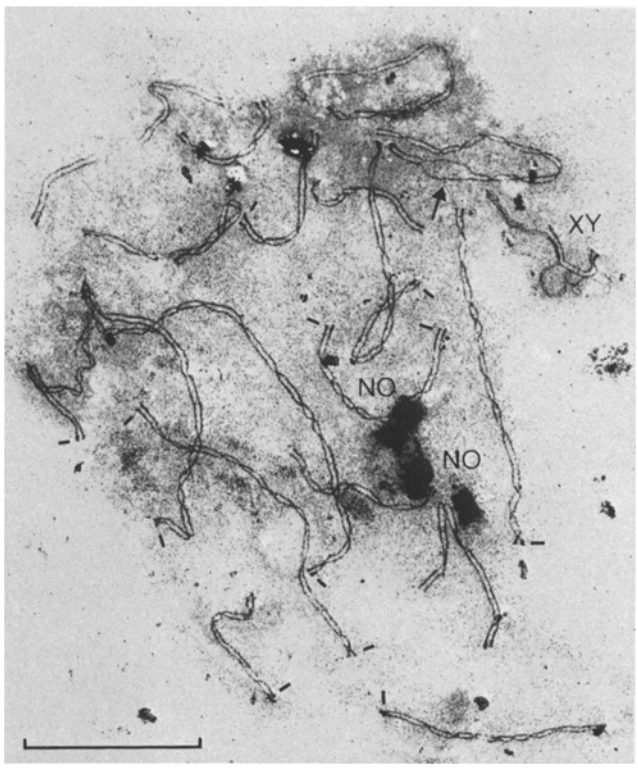

Fig. 6. Silver-stained late zygotene to early pachytene spermatocyte of the domestic pig as seen in the electron microscope. The lateral elements and endplates (*dashed*) are heavily stained. The central element and the kinetochores however are not differentiated. The two pairs of nucleoli (*NO*) of bivalents 8 and 10 are partly associated, which is rather atypical. One bivalent is not yet fully paired (*arrow*); the discontinuity (*top left*) is probably an artefact. The synapsed region of the sex bivalent involves nearly the entire Y chromosome. Bar represents 5 μm

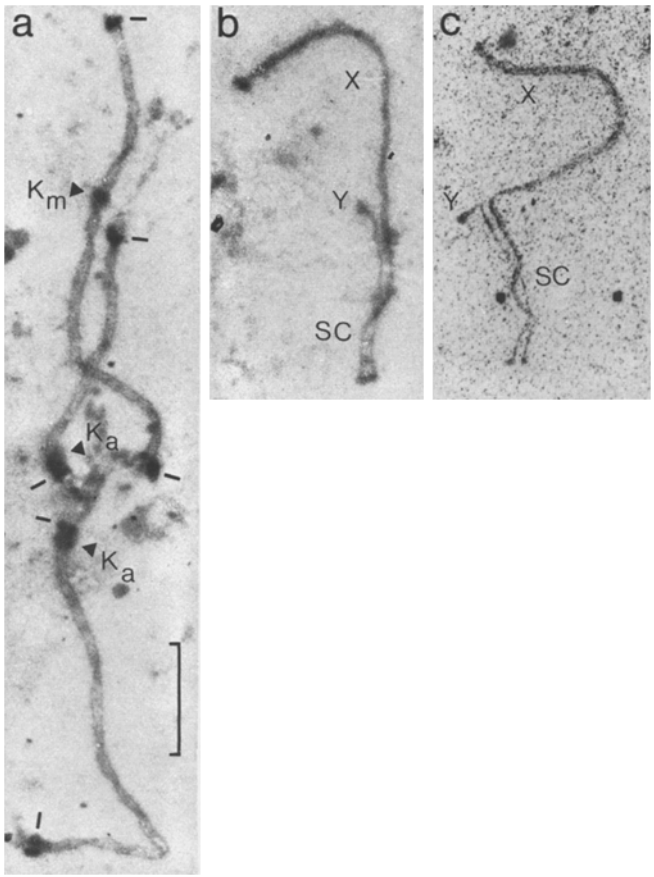

Fig. 7a–c. PTA- and silver-stained SCs in the EM. **a** PTA-stained autosomal SCs showing that the kinetochore in the acrocentric chromosomes (K_a) is situated next to the end plate (*dashes*; K_m kinetochore region of a submetacentric bivalent). **b, c** Examples of sex bivalents stained with PTA (**b**) and silver nitrate (**c**) from early pachytene nuclei. The paired region comprises over 90% of the Y chromosome. Bar represents 2 μm

of rRNA genes is presumably initiated at premeiotic interphase and persists through leptotene, zygotene to early pachytene.

Close association and fusion of nucleolar material was rare in pachytene nuclei in surface spreads. In addition to the activity of NORs and their number per cell, the degree of nucleolar fusion probably depends on their spatial disposition (compare Solari 1980; see Schwarzacher and Wachtler 1983). In the porcine karyotype, the rDNA is located next to the centromere in the submetacentric chromosomes 8 and 10 (Fig. 2), which differ in relative length (4.8% and 4.2%) and arm ratio (1.4 and 1.2). The Rabl orientation favours NOR association during mitosis, but the bouquet arrangement hinders their association during meiotic prophase.

Synaptonemal complexes

The most conspicuous structures of zygotene/pachytene nuclei prepared after the surface-microspreading technique (Counce and Meyer 1973) are the nucleolus-forming bivalents 8 and 10 (as discussed above) and the X–Y bivalent. Nearly the whole length of Y chromosome is involved in synapsis (Fig. 7b, c). In other species, e.g. Chinese hamster and man, only about 50% of the Y is paired with the X (Moses 1977b; Solari 1980). Presumably, the amount of constitutive heterochromatin in the Y chromosome of these species accounts for these differences.

The structure of the autosomal synaptonemal complexes in surface-spread preparations of porcine spermatocytes is similar to that seen in other mammals (e.g. Moses 1977a; Dresser and Moses 1980; Tres 1977). All bivalents have distinct attachment plates at their ends detectable both with silver and PTA staining (Figs. 5–7). The kinetochores are visible only in PTA-stained autosomal SCs, whereas the centromere position of both X and Y remained elusive with both techniques. Although there are thickenings along the sex chromosome axes, their occurrence and position are not constant, which makes it unlikely that they are connected with the kinetochores. It was, therefore, impossible to decide which part of the X chromosome undergoes synapsis.

It appeared reasonable to investigate the occurrence of proximal associations in SC spreads similar to those seen between acrocentric chromosomes in air-dried pachytene nuclei. In our limited number of EM photographs taken from PTA-stained SCs, we could not find significant centromeric associations of the acrocentric bivalents. This may be explained by the disruption of the collective chromocentre by the spreading forces.

The mitotic karyotype

The measurements made on chromomycin R-banded metaphase chromosomes (Figs. 1a, right, 2; Table 1) are generally in good agreement with the values for G- and Q-banded chromosomes of Lin et al. (1980). Major differences, however, are found for chromosomes 5 and 8 that can be explained by the cytochemical properties of the fluorescence dyes used. With the exceptions of the long arms of chromosomes 4 and 8 and the short arm of chromosome 10, all other chromosomes have R-band-positive material at both telomeres (see Fig. 1a, right). In the karyotype of Lin et al. (1980) the G- and Q-negative band (=R-positive band) at the end of chromosome 5 was omitted giving rise to their lower length value of 4.5%. In our previous paper (Schnedl et al. 1981), in which the chromosomes are ranked according to their lengths, chromosomes 4 and 5 are in reversed order (see Fig. 1 in Schnedl et al. 1981). The positive chromomycin band which Lin et al. (1980) postulated for chromosome 8 could not be found in our preparations, and hence their length of 5.4% for this chromosome seems to be overestimated. Our value of 4.8% is slightly lower than the value we find in silver-stained synaptonemal complex spreads in which chromosome 8 can be identified by the associated nucleoli. Differences in material can be excluded because Lin et al. (1982) present a chromomycin-stained porcine karyotype with banding patterns for chromosomes 5 and 8 identical to those observed in this study.

Heterochromatin behaviour at pachytene

During male meiotic prophase the bivalents of acrocentric chromosomes are joined together at their DA-DAPI-stained centromeric regions while the autosomal bivalents 1–12, which have chromomycin A_3-positive centromeric heterochromatin, remain unassociated (Fig. 3). It seems that this differential behaviour of the C-bands in the two autosomal chromosome groups is a result of both their position and composition. It is clear that proximity is a

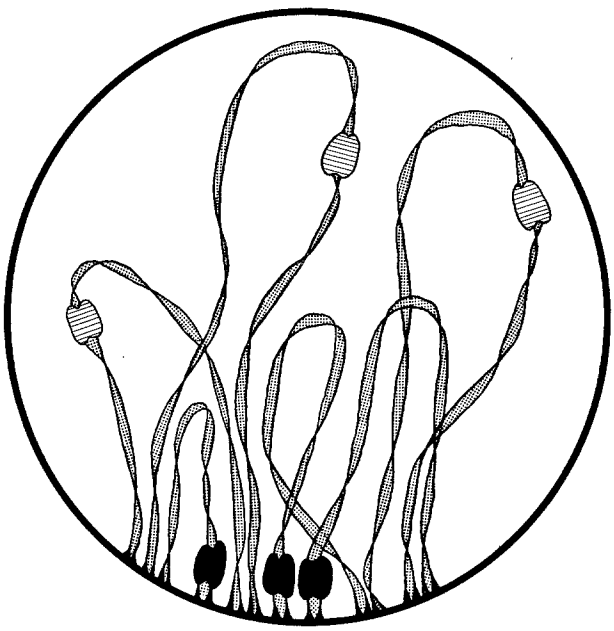

Fig. 8. Schematic drawing of the bouquet stage at meiotic prophase in the spermatocytes of *Sus scrofa domestica* showing the likely disposition of the centromeric heterochromatin in the acrocentric bivalents (*black*) and in submetacentric chromosome pairs (*hatched*). For the sake of clarity only 6 bivalents are drawn.

necessary but not a sufficient requirement for heterochromatin fusion (compare the heteromorphic regions of bivalent 1 in *Triturus cristatus*; Sims et al. 1984). The observed association of acrocentric bivalents in *Sus scrofa* is not a specific property of DA-DAPI bands. Centromeric association at pachytene has also been reported for the acrocentric chromosomes of cattle, which have chromomycin-positive centric C-bands (Mayr et al. 1979) and for the mouse (Hsu et al. 1971), in which the centric C-bands are DA-DAPI and chromomycin-negative but DAPI/AMD positive (see Schweizer 1981). In each case, the proximity of similar C-blocks (DNA sequences) during the meiotic bouquet stage may favour "ectopic pairing" and the formation of collective chromocentres (Fig. 8). Thomas and Kaltsikes (1976) provided a comparable explanation for the association of the telomeres of rye chromosomes in leptotene.

Heterochromatin and karyotype evolution

Differential "ectopic pairing" of C-bands in meiotic prophase may have implications for heterochromatin composition and karyotype evolution. The formation of centric associations during the meiotic process may facilitate those molecular events that give rise to C-band polymorphisms (see Driscoll et al. 1979). Moreover, the proximity of heterochromatic DNA during zygotene/pachytene, i.e., at stages when the enzymatic endowment for recombination (both reciprocal and non-reciprocal) is available, may present a cytological basis for molecular processes responsible for maintenance of satellite sequence homogeneity between non-homologous chromosomes as discussed by Dover (1982). If this view is correct, then in *Sus scrofa domestica* the intrachromosomal rate of homogenisation of chromomycin-positive centric heterochromatin DNA in biarmed chromosomes should be greater than the interchromosomal rate of homogenisation, because meiotic association is rare, and this would not be the case for the DA-DAPI type centric heterochromatin DNA of the acrocentric chromosomes (Fig. 8). This hypothesis seems amenable to experimental analysis and therefore an investigation of highly repeated DNA in *S. s. domestica* has been started in this laboratory.

The proximity of the centric regions of acrocentric chromosomes 13–18 of the domestic pig may aid mechanisms that result in Robertsonian fusion and the formation of biarmed chromosomes. Fusion of acrocentrics has taken place in the European wild pig (chromosomes 15; 17) and in the Asian wild pig (chromosomes 16; 17) (see Mayr et al. 1984). A spontaneously occurring Robertsonian translocation (13; 17) has been observed twice in the domestic pig (see Alonso and Cantu 1982). Situations comparable to that described for the acrocentric chromosomes of the domestic pig are known to occur in other domestic animals including cattle, sheep, and goats (Kurnit et al. 1978). The latter authors have shown that in the sheep the three largest chromosomes, which are stable Robertsonian centric fusion translocations, contain sheep DNA satellite II but little or no satellite I sequences, whilst the centromeric C-bands of the remaining acrocentric autosomes contain sequences homologous to both satellites I and II. As the authors point out "we cannot tell whether such losses accompanied and were part of the fusion process, or whether they developed subsequently." A rationale for the apparent conservation of such compositional differences of centromeric sequences in metacentric and acrocentric autosomes is presented in the following.

Comparative analysis of the wild and domestic pig has led us to suggest that a hypothetical ancestral karyotype in the Suidae was unimodal consisting of similar chromosomes with only one type of autosomal heterochromatin situated in "equilocal" centromeric positions (Mayr et al. 1984). As a result of Robertsonian changes, the spatial distribution of centric heterochromatin at zygotene/pachytene has been altered and, consequently, this may have allowed the heterochromatic DNA in the different chromosome groups to diverge (compare Figs. 2, 8). This model suggests that in *S. s. domestica* and probably in other stable Robertsonian systems such as the sheep, heterochromatin differentiation has been driven by karyotypic differentiation.

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