HETEROCHROMATIN, THE SYNAPTONEMAL COMPLEX AND CROSSING OVER

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SUMMARY

A combined light- and electron-microscopic examination of chromosomes from two angiospermous plants, Plantago ovata and Lycopersicon esculentum, and a mammal, Mus musculus, was performed. From this investigation three observations have been made that may be relevant to the observed lack of crossing over in heterochromatin. (1) Differential staining indicates that heterochromatin represents a smaller fraction of the length of pachytene chromosomes than it represents in the length of mitotic metaphase chromosomes. Since the synaptonemal complex (SC) runs throughout the length of these pachytene chromosomes, it is under-represented in heterochromatin. Considering the evidence for a rough correlation between the length of SC and the amount of crossing over, this could result in less crossing over in heterochromatin than expected on the basis of its length in mitotic metaphase chromosomes. (2) Electron microscopy indicates that, unlike the SC in euchromatin, the SC in heterochromatin is densely ensheathed in highly compact chromatin. If crossing over occurs in the SC or even in the surrounding chromatin, the compaction of the chromatin may prevent the penetration of enzymes needed in recombination. (3) Finally, a difference in the structure of SCs in euchromatin versus heterochromatin was observed that could be associated with the lack of crossing over in heterochromatin.

INTRODUCTION

Heitz (1928) first defined heterochromatin as chromatin that remains condensed during interphase and suggested that heterochromatin is genetically inactive. Subsequently, a variety of additional properties has been attributed to heterochromatin (see Comings, 1972, for a review). Probably the best supported of these is a lack of crossing over and chiasma formation in heterochromatin compared to euchromatin (see John & Lewis, 1965, for a review). Although this property has been reported frequently, there have been relatively few attempts to explain it. For instance, Japha (1939) put forward the hypothesis that in Oenothera the contracted state of heterochromatin keeps it from synapsing. However, this generally does not appear to be the case because there have been several reports, including this one, of synaptonemal complexes (SCs) in heterochromatin. More recently, Carpenter (1975a) observed in Drosophila melanogaster structural differences in SCs in euchromatin versus heterochromatin, and Carpenter (1975b) also noted that recombination nodules (RNs), which seem to be associated with the SC at crossover sites, are absent in heterochromatin. These observations are potentially significant for the relationship of heterochromatin to crossing over, and an effort should be made to make comparable observations in other organisms.
In a further attempt to define a structural basis for the lack of crossing over in heterochromatin, I have made a light- and electron-microscopic examination of the SCs in two angiospermous plants, *Plantago ovata* and *Lycopersicon esculentum* (tomato), and a mammal, *Mus musculus* (house mouse), that have distinct pericentric masses of heterochromatin in their chromosomes. From these observations, it may be possible to explain the absence of crossing over in heterochromatin on the basis of: (1) differential representation of the SC in euchromatin compared to heterochromatin; (2) the possibility of steric hinderance of the recombination mechanism by the tightly packed heterochromatin; and, or (3) a difference in the structure of SCs in heterochromatin.

Table 1. *Giemsa staining schedules for P. ovata, L. esculentum and M. musculus chromosomes*

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed root tips and anthers treated</td>
<td>15 min in 0.2 M HCl at 30°C</td>
</tr>
<tr>
<td>Brief rinse in water</td>
<td>↓</td>
</tr>
<tr>
<td>Air-dried <em>M. musculus</em> bone marrow and testis cells on slides</td>
<td>↓</td>
</tr>
<tr>
<td>1 h at 30°C in 1% cellulase and 1% pectinase solution at pH 4.5 adjusted with 1 M HCl</td>
<td>↓</td>
</tr>
<tr>
<td>Thumb-squash root tips and anthers in 5 µl of 45% acetic acid, remove cover slip by the solid CO₂ method and air dry no more than 1 h</td>
<td>↓</td>
</tr>
<tr>
<td>10 min in a saturated solution of Ba(OH)₂ at 20°C (M. musculus) or 5 min at 30°C (P. ovata and L. esculentum)</td>
<td>↓</td>
</tr>
<tr>
<td>Three 5-min washes in water</td>
<td>↓</td>
</tr>
<tr>
<td>15 min in 0.12 M-potassium phosphate buffer (pH 6-8) at 60°C</td>
<td>↓</td>
</tr>
<tr>
<td>Brief rinse in ice-cold buffer</td>
<td>↓</td>
</tr>
<tr>
<td>Stain 20 min in 1 part Harleco 620 stock Giemsa stain and 9 parts 0.12 M-potassium phosphate buffer (pH 6-8) at 4°C</td>
<td>↓</td>
</tr>
<tr>
<td>Briefly rinse in water</td>
<td>↓</td>
</tr>
<tr>
<td>Air dry</td>
<td>↓</td>
</tr>
<tr>
<td>Mount coverglasses in Permount</td>
<td>↓</td>
</tr>
</tbody>
</table>
Squash preparations of root tips and anthers of *P. ovata* and *L. esculentum* were made essentially as described by Hyde (1953) and Ramanna & Prakken (1967), with aceto-orcein or aceto-carmine respectively, used as the stains.

For differential Giemsa staining of both *P. ovata* and *L. esculentum* chromosomes inflorescences were fixed in 1:3 (v/v), acetic acid/ethanol for 1 h. Anthers and root tips were then treated as described in Table 1.

For C-banding somatic *M. musculus* (house mouse) chromosomes, 2.5 μg of vinblastine sulphate in 0.09% (w/v) saline per g body weight was injected into the abdominal cavity of a young, mature, healthy male mouse. After 1 h, the mouse was killed by cervical dislocation, and the bone marrow was used for squash preparations. Other mice that had not been treated with vinblastine were used not only for bone marrow squashes, but their testes were also used for squashes of meiotic cells. Staining was performed as described in Table 1.

Measurements of euchromatic and heterochromatic segments of chromosomes were made on either photographic prints or projected negative images.

For electron microscopy of fixed and sectioned material, anthers of *P. ovata* and *L. esculentum* were fixed for 1-2 h in 0.1 M-potassium phosphate buffer (pH 6.8) containing 3% (w/v) glutaraldehyde. The anthers were post-fixed in phosphate-buffered 2% (w/w) OsO₄ and embedded in Epon/Araldite. Sections were stained with 1% uranyl acetate in water followed by lead citrate. For serial sectioning primary microsporocytes of *L. esculentum*, the technique described by Stack (1973) was used.

As an independent means of examining SCs from *L. esculentum*, the new hypotonic bursting technique as described in detail by Stack (1982) was used. Briefly, the walls of primary microsporocytes were digested with β-glucuronidase. The protoplasts were then burst hypotonically on plastic-coated glass slides. In some preparations a dilute solution (0.1 mg/ml) of DNase I (no. 2609, Calbiochem) was used to burst the cells hypotonically. The spread SCs were subsequently fixed with formaldehyde and stained with phosphotungstic acid (PTA) or uranyl acetate and lead citrate. SCs were located by phase-contrast microscopy, and then the plastic was transferred to grids for observation and photography in the electron microscope. To measure their dimensions SCs stained with uranyl acetate and lead citrate were photographed at ×25,000, and measurements were made on negatives with a dissecting microscope using a calibrated ocular micrometer.

For electron microscopy of sectioned SCs from *M. musculus*, a mature, young, healthy mouse was killed by cervical dislocation. The testes were dissected out and placed in 0.1 M-sodium phosphate buffer (pH 7.4) containing 3% glutaraldehyde at room temperature. The testes were diced into cubes 2 mm on a side with a razor-blade. Subsequent fixation, embedding, sectioning and staining was as described for anthers.

As an independent means of examining the SCs of the mouse, the surface spreading technique described by Moses (1977) was used. The SCs were stained with phosphotungstic acid.

### RESULTS

**Aceto-orcein and differential Giemsa staining for light microscopy**

In *P. ovata* (*2n = 8*) the chromosomes are all meta- to submetacentric with heterochromatin around centromeres and nucleolus organizers (Figs 1–5). Differential Giemsa staining indicates that euchromatin undergoes relatively more contraction than heterochromatin during mitotic prophase, so that by metaphase heterochromatin comprises an average of 61.0% of the length of the chromosomes (Figs 1–3, Table 2). Since mitotic metaphase chromosomes usually stain homogeneously with aceto-orcein and the Feulgen technique, it is assumed that both euchromatin and heterochromatin are similarly condensed at this stage. Because of this and because mitotic metaphase chromosomes vary little in width, the fraction of the length of...
mitotic metaphase chromosomes that consists of heterochromatin is considered to be a close approximation of the fraction of the total chromatin that is heterochromatic, i.e. 61.0%. In contrast, differential Giemsa staining indicates that heterochromatin comprises 27.4% of the length of pachytene chromosomes (Fig. 4, Table 2). This

Figs 1–7. Squashed chromosomes of P. ovata (2n = 8). Bar, 10 μm.

Figs 1–3. Differential Giemsa staining of mid-prophase (Fig. 1), late prophase (Fig. 2) and metaphase chromosomes (Fig. 3) from root tips shows heterochromatin to be located pericentromerically in all the chromosomes and at terminal nucleolus organizers in two of the four chromosomes of the complement (arrows). Although both euchromatin and heterochromatin can be seen to contract progressively throughout prophase, euchromatin contracts relatively more than heterochromatin so that by metaphase heterochromatin represents 61.0% of the length of the chromosomes (see Table 2).

Fig. 4. Differentially Giemsa-stained pachytene chromosomes from a primary microsporocyte in which heterochromatin represents approximately 27.4% of the length of the chromosomes (see Table 2). The terminal heterochromatic knobs on two of the bivalents are regularly fused (arrow) and associated with the nucleolus.

Fig. 5. Nomarski differential interference microscopy of aceto-orcein-stained pachytene chromosomes from a primary microsporocyte with fused knobs (arrow) and a ratio of euchromatin to heterochromatin comparable to that illustrated in Fig. 4.

Fig. 6. Nomarski differential interference microscopy of aceto-orcein-stained metaphase I chromosomes from a primary microsporocyte with homologous chromosomes held together by single, terminal chiasmata (arrows).

Fig. 7. Differentially Giemsa-stained metaphase I chromosomes from a primary microsporocyte. Euchromatin is not visible in the two central bivalents, but in the two peripheral bivalents the chiasmata appear to lie in euchromatin (arrows).

Figs 8–12. Squashed chromosomes of L. esculentum (2n = 24). Bar, 10 μm.

Fig. 8. Differentially Giemsa stained mid-prophase chromosomes from a root tip show darkly stained pericentromeric heterochromatin in all chromosomes followed by distal euchromatin and tiny heterochromatic telomeres on many chromosomes. Two telomeres are indicated by arrows.

Fig. 9. Phase microscopy of differentially aceto-orcein-stained late prophase chromosomes from a root tip shows relatively less shortening of heterochromatin than euchromatin, so heterochromatin now represents approximately 75.3% of the length of the chromosomes (Table 2; Ramanna & Prakken, 1967). In some chromosome arms euchromatin is not visible.

Fig. 10. Nomarski differential interference microscopy of aceto-carmine-stained pachytene chromosomes. The chromosomes have pericentromeric heterochromatin (e.g. between arrowheads on the bivalent to the lower left) and relatively long distal stretches of euchromatin. Here heterochromatin comprises approximately 23.9% of the length of the chromosomes (Table 2; Ramanna & Prakken, 1967). Tiny heterochromatic telomeres are visible on some chromosomes (arrows).

Figs 11, 12. Differentially Giemsa- and aceto-carmine-stained diakinesis chromosomes, respectively. Bivalents are held together by distal chiasmata. When euchromatin is visible, the chiasmata can be seen to lie in euchromatin.

Figs 13–15. Spread C-banded M. musculus chromosomes. Heterochromatin occurs pericentromerically in all of these acrocentric chromosomes except the Y chromosome. Bar, 10 μm.

Fig. 13. In mitotic metaphase heterochromatin comprises 21.3% of the length of the chromosomes (see Table 2).

Fig. 14. In pachytene heterochromatin comprises 9.2% of the length of the chromosomes (see Table 2).

Fig. 15. In metaphase I most chiasmata are in euchromatin but a few occur in or very near heterochromatin (arrows).
Heterochromatin, SC and crossing over

Figs 1–15
Table 2. Average length and percentage of euchromatin and heterochromatin in mitotic metaphase and pachytene chromosomes

<table>
<thead>
<tr>
<th></th>
<th>P. ovata</th>
<th>L. esculentum*</th>
<th>M. musculus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotic metaphase</td>
<td>1.33 ± 0.26</td>
<td>2.23</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>Heterochromatin</td>
<td>(61.0%) (N = 100)</td>
<td>(75.3%) (N = 100)</td>
<td>(21.3%) (N = 100)</td>
</tr>
<tr>
<td>Mitotic metaphase</td>
<td>0.85 ± 0.32</td>
<td>0.73</td>
<td>3.18 ± 0.87</td>
</tr>
<tr>
<td>Euchromatin</td>
<td>(39.0%) (N = 100)</td>
<td>(24.7%) (N = 100)</td>
<td>(78.7%) (N = 100)</td>
</tr>
<tr>
<td>Pachytene</td>
<td>6.4 ± 1.3</td>
<td>9.64</td>
<td>1.06 ± 0.28</td>
</tr>
<tr>
<td>Heterochromatin</td>
<td>(27.4%) (N = 56)</td>
<td>(23.9%) (N = 56)</td>
<td>(9.2%) (N = 100)</td>
</tr>
<tr>
<td>Pachytene</td>
<td>17.0 ± 5.2</td>
<td>30.67</td>
<td>10.47 ± 2.94</td>
</tr>
<tr>
<td>Euchromatin</td>
<td>(72.6%) (N = 56)</td>
<td>(76.1%) (N = 56)</td>
<td>(90.8%) (N = 100)</td>
</tr>
</tbody>
</table>

Lengths are given in μm.
* The values for L. esculentum were calculated from Table 1 of Ramanna & Prakken (1967).

value agrees closely with the percentage of heterochromatin in pachytene chromosomes stained with aceto-carmine (Fig. 5; Hyde, 1953). Thus, heterochromatin represents 2.2 times more of the length of mitotic metaphase chromosomes than pachytene chromosomes. Assuming no interconversion between heterochromatin and euchromatin, the difference in percentage of chromosome length that is heterochromatin must be due to the relative degrees of condensation of euchromatin and heterochromatin in pachytene versus mitotic metaphase.

During metaphase I of meiosis in P. ovata the homologues of the four bivalents are usually joined by single terminal or near-terminal chiasmata (Fig. 6). Among bivalents in which euchromatin is visible after differential Giemsa staining, chiasmata are seen to be located in euchromatin, as would be expected if crossing over is inhibited in heterochromatin (Fig. 7).

In L. esculentum (2n = 24, tomato) the chromosomes are all meta- to submetacentric. Both aceto-carmine and differential Giemsa staining indicate the presence of heterochromatin around centromeres and at tiny telomeres (Figs 8, 10). During chromosome condensation in mitotic prophase, heterochromatin comprises an ever greater proportion of the length and bulk of the chromosomes (Figs 8–9) so that by mitotic metaphase the euchromatin seems to have condensed to the point that it is hardly demonstrable by light microscopy (Brown, 1949). Similarly, Schmiady & Sperling (1976) reported that in mitotic prophase the C-band in human chromosome 1 is relatively shorter than in mitotic metaphase chromosomes.

Ramanna & Prakken (1967) showed that aceto-carmine staining can differentiate between euchromatin and heterochromatin in both mitotic and meiotic chromosomes of L. esculentum. They determined the absolute length and ratio of euchromatin versus heterochromatin for each chromosome of the complement. From their data I determined that heterochromatin comprises 75.3 % of the length of what they called mitotic metaphase chromosomes and 23.9 % of the length of pachytene chromosomes.
(Fig. 10, Table 2). From their illustrations it appears they were actually measuring late prophase chromosomes rather than metaphase chromosomes (compare Fig. 9 with their fig. 3). Under any circumstance, the difference seems to be due to differential condensation of these two forms of chromatin in late prophase of mitosis compared to pachytene of meiosis, so that heterochromatin represents at least 3.2 times more of the length of late mitotic prophase chromosomes than it represents in the length of pachytene chromosomes.

During diakinesis—metaphase I in *L. esculentum* the homologues are held together by one or two distal chiasmata. As in *P. ovata*, when euchromatin and heterochromatin can be distinguished in bivalents, chiasmata are seen to be located in euchromatin (Figs 11, 12).

In *M. musculus* (*2n* = 40) the chromosomes are acrocentric with differential Giemsa staining demonstrating pericentromeric heterochromatin in all but the Y chromosome (Figs 13–15). Heterochromatin comprises an average of 21.3% of the length of C-metaphase chromosomes (induced by vinblastine, Fig. 13), 22.2% of the length of prometaphase to metaphase chromosomes that were not treated with an antimicrotubule agent, and 9.2% of the length of pachytene chromosomes (Fig. 14, Table 2). In this case heterochromatin represents 2.3 times more of the length of metaphase chromosomes than it represents in the length of pachytene chromosomes.

During diakinesis—metaphase I in *M. musculus* there are 20 bivalents held together predominantly by terminal chiasmata in euchromatin, although a few chiasmata seem to lie in or very near to terminal heterochromatin (Fig. 15).

**Electron microscopy of sectioned SCs**

Frontal and transverse sections of SCs from *P. ovata, L. esculentum* and *M. musculus* were examined in the electron microscope. Since the SCs from *P. ovata* and *M. musculus* were not serially sectioned, the terms frontal and transverse are only used in an approximate sense with reference to sections of SCs in these species. SCs are considered to lie in heterochromatin when the surrounding chromatin is condensed (Figs 16, 17, 20, 22, 25, 27, 30) or in euchromatin when the surrounding chromatin is not condensed (Figs 18, 19, 21, 23, 26, 28, 29).

In frontal and transverse sections of SCs from *P. ovata* (Figs 16–19), *L. esculentum* (Figs 20–24) and *M. musculus* (Figs 25–30) no consistent structural difference in SCs in euchromatin versus heterochromatin was observed. However, lateral elements have nearly the same electron density as heterochromatin so it was often difficult to distinguish lateral elements from heterochromatin. On the other hand, SCs can be seen relatively clearly in euchromatin and in centromeres (Fig. 24). Using transverse sections (determined from serial sections) of SCs from *L. esculentum*, two structural aspects of SCs in these two locations were measured, the centre-to-centre distance between synapsed lateral elements and the diameter of lateral elements (Table 3, Figs 23, 24). A comparison of these two measurements by Student's *t*-tests did not indicate a significant difference between SCs in euchromatin versus SCs in centromeres (Table 3).
Only a few recombination nodule-like (RN-like) structures were observed in sectioned material, and these were in euchromatin (Fig. 21).

Because the acrocentric *M. musculus* chromosomes have pericentric heterochromatin, SCs in both euchromatin and heterochromatin were expected to terminate on the nuclear envelope. This was observed (Figs 25, 29) but the ends of 18 out of 74 heterochromatic short arms terminated in a bit of euchromatin (Fig. 30). This being the case, some or all of the chiasmata observed at or near heterochromatic ends of *M. musculus* chromosomes (Fig. 15) may actually lie in these euchromatic termini.

Figs 16–24
Electron microscopy of spread SCs

Hypotonic bursting of primary microsporocytes of *L. esculentum* and surface spreading of primary spermatocytes of *M. musculus* reveal SCs laid out like ribbons in two dimensions (Figs 31–35). In both cases it is clear that SCs run through both euchromatin and heterochromatin as well as the centromere. More details of the structure of SCs are visible in hypotonically burst preparations (Figs 31–33) than in surface-spread preparations (Figs 34–35), probably because in the former case most of the protoplasm has been dispersed and the SCs are viewed more nearly free of chromatin, whereas in the latter case there is some obscuring protoplasm above and below the SCs.

In *L. esculentum* kinetochores are visible only during mid-late pachytene as amorphous spheres on the SCs with a diameter of about 1 μm (Figs 31–33). Although kinetochores are normally surrounded by pericentromeric heterochromatin, this heterochromatin as well as the more distal euchromatin is usually more or less dispersed. What appear to be recombination nodules were frequently observed on the central elements of SCs that had not been treated with DNase (Figs 31–33). Recombination nodule-like structures were more common in early pachytene SCs than in late pachytene SCs, but even so they were observed in 132 out of 139 late pachytene SCs that were examined (Figs 31–33). When RN-like structures were observed in late pachytene, they usually did not lie close to kinetochores, suggesting that they occur preferentially in euchromatin (Figs 32–33; Stack, unpublished data). A similar observation has been made on surface-spread human pachytene chromosomes (Solari, 1980).

Figs 16–19. Sections of *P. ovata* SCs from primary microsporocytes at pachytene. Bar, 0.4 μm.
Fig. 16. Approximately transverse section through a SC in heterochromatin. Lateral elements are indicated by arrows.
Fig. 17. Frontal section through a SC in heterochromatin.
Fig. 18. Approximately transverse section through a SC in euchromatin. Lateral elements are indicated by arrows.
Fig. 19. Frontal section through a SC in euchromatin.

Figs 20–24. *L. esculentum* SCs at pachytene from sectioned primary microsporocytes. Bar, 0.4 μm.
Fig. 20. Frontal section of a SC in heterochromatin.
Fig. 21. Frontal section through a SC in euchromatin. A recombination nodule-like structure is indicated by an arrow. Note that if this structure is an elipsoidal solid, it may be too large to fit on the central element of a SC in the heterochromatin of *L. esculentum* (see Fig. 22). The discontinuities in the lateral elements here and in Fig. 25 are presumably due to the angle of sectioning.
Fig. 22. Transverse section through a SC in heterochromatin. Lateral elements are indicated by arrows.
Fig. 23. Transverse section of a SC in euchromatin. Lateral elements are indicated by arrowheads.
Fig. 24. Transverse section of a SC in a centromere. Lateral elements are indicated by arrowheads.
In *L. esculentum* little difference was noted between the structure of SCs in euchromatin (far from kinetochores) and in heterochromatin (near kinetochores) after staining with phosphotungstic acid (PTA). However, adhering chromatin,

Figs 25–30. *M. musculus* SCs at pachytene from sectioned primary spermatocytes. Bar, 0.4 μm.

Fig. 25. Frontal section of a SC in heterochromatin that terminates on the nuclear envelope. Note that the heterochromatin extends to the nuclear envelope. Compare with Figs 29 and 30.

Fig. 26. Frontal section of a SC in euchromatin.

Fig. 27. Approximately transverse section of a SC in heterochromatin. Lateral elements are indicated by arrows.

Fig. 28. Near transverse section of a SC in euchromatin. Lateral elements are indicated by arrows.

Fig. 29. Frontal section of a SC in a euchromatic long arm of a bivalent at its termination at the nuclear envelope. Note the thickened lateral elements at the point of attachment.

Fig. 30. Frontal section of a SC in a short arm of a bivalent at its termination at the nuclear envelope. Note that the pericentric heterochromatin does not extend to the nuclear envelope, but there is a distal segment of euchromatin.
Table 3. Dimensions of SCs in euchromatin and heterochromatin measured on transverse sections of SCs and spread SCs from L. esculentum primary microsporocytes

<table>
<thead>
<tr>
<th></th>
<th>Sectioned SCs in euchromatin</th>
<th>Sectioned SCs in centromeres</th>
<th>Spread SCs in euchromatin</th>
<th>Spread SCs in heterochromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre-to-centre distance between synapsed lateral elements (nm)</td>
<td>I 166·2 ± 14·4 N = 25</td>
<td>II 169·5 ± 8·0 N = 15</td>
<td>III 149·5 ± 11·4 N = 55</td>
<td>IV 150·9 ± 11·5 N = 29</td>
</tr>
<tr>
<td>Diameter of lateral elements (nm)</td>
<td>V 44·1 ± 5·1 N = 12</td>
<td>VI 43·9 ± 3·7 N = 13</td>
<td>VII 47·0 ± 5·3 N = 110</td>
<td>VIII 38·8 ± 5·8 N = 58</td>
</tr>
</tbody>
</table>

SCs were stained with uranyl acetate and lead citrate.

Student’s t-tests were performed to compare the centre-to-centre distance between synapsed lateral elements to determine the probability that they represent populations with different means.

- For I and II, \( t = 0.814 \) (38 d.f., \( P > 0.5 \)).
- For I and III, \( t = 5.62 \) (78 d.f., \( P > 0.999 \)).
- For I and IV, \( t = 4.35 \) (52 d.f., \( P > 0.999 \)).
- For II and III, \( t = 6.39 \) (68 d.f., \( P > 0.999 \)).
- For II and IV, \( t = 5.59 \) (42 d.f., \( P > 0.999 \)).
- For III and IV, \( t = 0.611 \) (82 d.f., \( P > 0.4 \)).

Student’s t-tests were also performed to compare the diameter of lateral elements to determine the probabilities that they represent populations with different means.

- For V and VI, \( t = 0.113 \) (23 d.f., \( P < 0.1 \)).
- For V and VII, \( t = 1.80 \) (120 d.f., \( P > 0.9 \)).
- For V and VIII, \( t = 7.69 \) (68 d.f., \( P > 0.999 \)).
- For VI and VII, \( t = 2.05 \) (121 d.f., \( P > 0.98 \)).
- For VI and VIII, \( t = 3.03 \) (69 d.f., \( P > 0.99 \)).
- For VII and VIII, \( t = 9.22 \) (166 d.f., \( P > 0.999 \)).

particularly euchromatin, often obscures the SCs to some degree (Fig. 32). This chromatin can be largely eliminated by bursting the cells in a dilute solution of DNase I, which also eliminates most RN-like structures (not illustrated). In addition, we have recently found that compared to PTA, uranyl acetate and lead citrate give finer and more specific staining of the SC (compare Figs 31 and 32 with Fig. 33). Because of this, a combination of digestion with DNase I and staining with uranyl acetate and lead citrate was used for a comparison of the structure of SCs in euchromatin versus heterochromatin. In this comparison the same two aspects of the structure of SCs that were measured in sectioned SCs were measured in spread SCs, i.e. the centre-to-centre distance between synapsed lateral elements and the diameter of lateral elements (Table 3). When these measurements are compared using Student’s t-tests, there is no significant difference in the centre-to-centre distance between synapsed lateral elements in euchromatin versus heterochromatin, but there is a highly significant difference in the diameter of lateral elements (Table 3). In the latter case, the diameter of lateral elements in heterochromatin is less than in euchromatin (Fig. 33). In other comparisons, the centre-to-centre distances between synapsed lateral elements in sectioned SCs in euchromatin are significantly greater than in spread SCs in euchromatin (Table 3). Possibly, this is due to some shrinkage of spread SCs during air drying. On the other hand, the diameters of lateral elements in euchromatin from sectioned and spread SCs are not significantly different (Table 3).
And finally, in both euchromatin and heterochromatin lateral elements in and adjacent to twists in the SC have the same width (Figs 31–33). Since lateral elements should be seen in side-view in a twist, lateral elements must be roughly isodiametric. This is also their appearance in sectioned SCs.

In *M. musculus* kinetochores occur near one end of the SCs surrounded by pericentric heterochromatin (Figs 34–35). As in *L. esculentum* the chromatin is usually dispersed, but in *M. musculus* pericentric heterochromatin sometimes remains associated with the SC (Fig. 34). Usually a structure that can be identified as a kinetochore cannot be observed, but the terminal segments of the SCs that were associated with kinetochores and pericentric heterochromatin have a characteristic structure. Here in the terminal 1 μm of the SCs, the lateral elements are thicker and more darkly stained than the distal lateral elements that were associated with euchromatin. Since twists were never observed in segments of SCs in heterochromatin (possibly indicating rigidity), the height of the lateral elements in relation to their width in heterochromatin is uncertain. On the other hand, twists in euchromatic segments of the SCs indicate that lateral elements here are isodiametric.

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**Figs 31–33.** SCs from hypotonically burst primary microsporocytes of *L. esculentum*. The chromatin has been largely dispersed to leave the SCs intact. Except for twists, SCs are seen in frontal view. While kinetochores are not visible on early pachytene SCs (Fig. 31), kinetochores appear as prominent dark spheres about 1 μm in diameter on late pachytene SCs (Figs 32, 33). The ends of the SCs are usually capped by dark structures where the SCs were attached to the nuclear envelope before hypotonic bursting disrupted the nuclear envelope. RN-like structures are marked by arrowheads. Because there is often an accumulation of stain at twists, densities at twists are probably not RN-like structures. However, in some cases including those where RN-like structures were not observed, RN-like structures could be obscured in twists. Bar, 2 μm.

Fig. 31. PTA-stained early pachytene SC. Typically, several RN-like structures are visible (arrowheads).

Fig. 32. PTA-stained late pachytene SC with two RN-like structures (arrowheads).

Fig. 33. Uranyl acetate/lead citrate-stained late pachytene SC with two RN-like structures (arrowheads). Note that lateral elements have a smaller diameter near kinetochores (in heterochromatin) than distal to kinetochores (in euchromatin). This is not so easily observed in PTA-stained SCs (compare with Fig. 32).

Figs 34, 35. Surface spread SCs from *M. musculus* primary spermatocytes that have been stained with PTA. All of the autosomes are acrocentric with pericentromeric heterochromatin. The chromatin has been largely dispersed to leave SCs intact. Except for twists, SCs are seen in frontal view. In each case the terminal micrometer of the lateral elements in the heterochromatic short arms are thickened and stain more darkly than the lateral elements that run through euchromatin. The ends of these thickened termini were attached to the nuclear envelope that was dispersed during spreading (compare with Figs 25, 30). At the opposite end each SC has shorter thickenings of the lateral elements that are part of the attachment plaques where the SCs in the euchromatic long arms terminate on the nuclear envelope (compare with Fig. 29). RN-like structures were not observed on these SCs. Bar, 2 μm.

Fig. 34. SC with its centromeric end embedded in and partly obscured by incompletely dispersed heterochromatin on the right.

Fig. 35. A SC similar to that in Fig. 34, but here the pericentric heterochromatin has been dispersed to reveal the thickened lateral elements in the centromeric region on the right.
Fig. 36. Model for the relation of synaptomeres to the structure of the SC in which ———— represents a chromonema with synaptomeres (●), ———— represents the SC, and \( \equiv \) represent homologous chromonemas associated with the SC at their synaptomeres in euchromatin and heterochromatin, respectively. Above and below are homologous mitotic metaphase chromosomes based on a unineme model in which, for convenience, the chromonemas of only one chromatid per chromosome are represented. In this hypothetical case synaptomeres are three times more concentrated in euchromatin (EU) than in heterochromatin (HET). As a result of the distribution of synaptomeres, after synapsis (centre) heterochromatin is twice as long in pachytene as it is in metaphase, and euchromatin is six times as long in pachytene as it is in metaphase. Thus, while heterochromatin represents one third of the length of these metaphase chromosomes, at pachytene heterochromatin represents one seventh of the length of the bivalents. Different distribution of synaptomeres would result in different ratios of euchromatin to heterochromatin in pachytene chromosomes. Although synaptomeres are illustrated as evenly spaced in both euchromatin and heterochromatin, the model should not be taken to indicate regularity in the spacing of synaptomeres but a relatively greater concentration of synaptomeres in euchromatin compared to heterochromatin.

**DISCUSSION**

*Under-representation of the SC in heterochromatin*

During pachytene in certain species of the genera *Oenothera* and *Tribolium* and in the three species studied here, heterochromatin ranges from about the same length to five times longer than it is at mitotic metaphase, and euchromatin ranges from 3–30 times longer than it is in mitotic metaphase (Japha, 1939; Smith, 1952; Table 2). As a result heterochromatin is on average two to five times under-represented in the length of pachytene chromosomes compared to its representation in mitotic metaphase chromosomes. It has been noted often that recombination maps are poorly correlated with the physical dimensions of chromosomes (Mather, 1938, and subsequently many other reports). A part of the explanation for this is undoubtedly the presence of heterochromatin that contributes to the physical length of chromosomes, while it is
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hardly if at all represented in recombination maps. Considering that the SC occurs between synapsed chromosomes during pachytene in both euchromatin and heterochromatin, the SC must be two to five times under-represented in heterochromatin compared to euchromatin. The potential significance of this under-representation is suggested by observations that the SC seems to be a necessary, although not sufficient, prerequisite for the high levels of recombination associated with typical meiosis (see Moses, 1968, for a review) and that recombination seems to occur during pachytene (see Henderson, 1970, for a review). These observations coupled with a positive correlation between length of metaphase chromosomes and the number of chiasmata (Mather, 1938; John & Lewis, 1965) and a positive correlation between the relative lengths of metaphase chromosomes and their relative lengths at pachytene (Moses, 1968), suggest that the length of the SC may be more closely related to the number of recombination events than chromosome size per se (Mogensen, 1977). Observations on Zea mays, Stethophyrum grossum and humans support this hypothesis (Gillies, 1973; Mogensen, 1977; Holm & Rasmussen, 1977; Fletcher, 1977). Following this line of thought, there should be two to fivefold less recombination in heterochromatin than expected on the basis of its physical length in mitotic metaphase chromosomes and (2) recombination maps should be better related to the structure of pachytene chromosomes than mitotic metaphase chromosomes (but see below).

However, crossing over is almost, if not completely, suppressed in heterochromatin. For the preceding line of reasoning to explain this observation, there should be little or no SCs in heterochromatin. Since appreciable lengths of SC do occur in heterochromatin another factor(s) must contribute to the inhibition of recombination.

As a further consideration, several current models for the structure of the SC require interspersed DNA sequences along the length of the DNA double helix of each chromatin. These hypothetical sequences, called ‘synaptomeres’ by King (1970), are supposed to be recognized and bound by proteins of the SC. The relatively large amount of SC associated with euchromatin could be due to abundant synaptomeres in euchromatin compared to heterochromatin (Fig. 36), or, alternatively, many of the synaptomeres in heterochromatin could be unable to be involved in SC formation because of the condensed state of the heterochromatin. An effectively low number of synaptomeres in heterochromatin could minimize the synaptic inequalities that would seem to be inherent in the widespread occurrence of intraspecies polymorphism for heterochromatin.

Structural variation of the SC in euchromatin versus heterochromatin

Carpenter (1975a) observed that in D. melanogaster the SCs in heterochromatin are less thick, less distinct and appear less rigid than the SCs in euchromatin. The lateral elements of L. esculentum SCs in heterochromatin have a significantly smaller diameter than in euchromatin, while in M. musculus lateral elements in heterochromatin have a larger diameter than lateral elements in euchromatin (Table 3). Thus, on the basis of this limited sample, there may be structural differences between SCs in euchromatin versus heterochromatin. Because of this and as Carpenter
(1975a) suggested, structural differences in SCs are a potential explanation for the lack of crossing over in heterochromatin. However, it may be important that in *L. esculentum* the SCs in euchromatin and centromeric chromatin were indistinguishable (Table 3) and yet crossing over does not seem to occur in the centromere.

**Steric hinderance of recombination in heterochromatin**

In another approach to the inhibition of crossing over in heterochromatin, one may observe in *P. ovata, L. esculentum* and *M. musculus* that a particular segment of sectioned SC is surrounded by diffuse chromatin (euchromatin), compact chromatin (heterochromatin) or centromeric chromatin. Carpenter (1975a) made similar observations on SCs in euchromatin and heterochromatin in *D. melanogaster*. Whether recombination takes place in the SC or in the surrounding chromatin, the needed enzymes may have difficulty penetrating heterochromatin or centromeric chromatin to ensure that a sufficient number of the complex and seemingly haphazard events that appear to be necessary for recombination take place (Holliday, 1977). This suggestion is supported by the report of Hotta & Stern (1978) that little repair synthesis occurs in mouse satellite DNA during pachytene. Since mouse satellite DNA is concentrated in heterochromatin and repair synthesis is thought to be one of the steps in recombination, their observation is probably related to the lack of recombination in heterochromatin and could be explained on the basis of recombination enzymes not being able to penetrate heterochromatin. The potential penetration problem might be greatly increased if recombination nodules are necessarily involved in recombination (Carpenter, 1979). These 100 nm spheroids are found exclusively on the central element of the SC in euchromatin (Carpenter, 1975b), possibly because there is no way for them to penetrate heterochromatin nor room for them even to be assembled on the central element in heterochromatin. The actual positioning of recombination nodules in euchromatin (Carpenter, 1979) presumably would be influenced by factors other than steric hinderance (see the next section).

**Other influences on recombination**

If recombination occurs only in euchromatin, the length of euchromatic chromosome arms in pachytene, i.e. the length of SC in euchromatin during pachytene, may be correlated better than any other chromosomal dimension with the number of recombination events. However, other factors may distort this generalization. These factors include genetic influences (Catcheside, 1977), positive chiasma interference, the location of the centromere (Mather, 1938), and the total amount and location of heterochromatin (Yamamoto, 1979).

**Practical consideration**

Aside from any potential value of the foregoing hypotheses in explaining the lack of crossing over in heterochromatin, the results on the under-representation of heterochromatin in the length of pachytene chromosomes should be taken into consideration in any attempt to identify pachytene chromosomes on the basis of their
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relative lengths and arm ratios compared to the relative lengths and arm ratios of mitotic metaphase chromosomes. Solari (1980) has taken this into consideration in identifying human pachytene chromosomes.

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