

Synaptonemal complex spreading in *Allium ursinum*: pericentric asynapsis and axial thickenings

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Summary

In *Allium ursinum* meiotic pairing of homologues is always incomplete; a proximal region on each bivalent remains regularly unsynapsed even in late pachytene. The spatial correlation of the unsynapsed region with the kinetochore suggests that the kinetochore itself exerts an inhibitory effect on synapsis in its vicinity. This can be interpreted as the cytological basis of the 'centromere effect' on recombination in this species. Moreover, the high incidence of a pericentric inversion loop in a heterozygous chromosome pair shows that proximal pairing initiation is possible and that its failure cannot be responsible for pericentric asynapsis.

The formation of the inversion loop is complicated by the need for two independent pairing initiation sites because synapsis cannot proceed across the pericentric region. It is proposed that

the meiotic bouquet polarization helps in establishing the presynaptic alignment of the homologous sites within the inverted regions and hence to achieve a high rate of inversion loop formation.

Thickenings of the axial/lateral elements are not distributed equally along the synaptonemal complex. They are underrepresented in unpaired axes but strikingly abundant at the borders with synapsed regions, suggesting their origin in the pairing forks during the process of synapsis. They are virtually always present at nucleolus-organizing regions and often they appear at corresponding sites on opposite lateral elements. Besides the thickenings several other kinds of axial deformities are present in unpaired axes.

Key words: *Allium ursinum*, synaptonemal complex, centromere effect, pericentric inversion, meiosis.

Introduction

The spreading of synaptonemal complexes (SCs) as compared to sectioning enables larger numbers of nuclei to be analysed and hence it is preferable for quantitative evaluations. During recent years SC spreading has been performed in a number of plants, such as maize (Gillies, 1981), Solanaceae (Stack, 1982), *Rhoeo spathacea* (Stack & Soulliere, 1984), *Tradescantia* (e.g. see Hasenkampf, 1984a), rye (e.g. see Gillies, 1985), *Paeonia* (Schwarzacher-Robinson, 1987), wheat (Holm, 1986; Wischmann, 1986) and several *Allium* species (e.g. see Albini & Jones, 1984; Loidl, 1986; Loidl & Jones, 1986).

Spread SCs of *Allium ursinum* have been investigated in the light microscope by Loidl (1984). The method used then was based on the spreading of the SCs by the surface tension on a drop of liquid. The results were rather unsatisfactory because of a high

rate of breakage of the SCs. It was noted, however, that the breaks were located preferentially in the median regions and it was speculated that incomplete pairing around the kinetochores was the cause.

Incomplete synapsis has been reported for several organisms, but to date in none of these has the kinetochore been shown to be causally involved in asynapsis. In this paper it will be shown that in *A. ursinum* an inhibitory effect of the kinetochore on synapsis is likely.

Axial/lateral element thickenings have been described in several plants such as *Lilium* (Moens, 1968), *Phaedranassa viridiflora* (La Cour & Wells, 1973), *Zea mays* (Gillies, 1973, 1981), *Paeonia* (Kehlhoffner & Dietrich, 1983; Schwarzacher-Robinson, 1987) and *Tradescantia* (Hasenkampf, 1984b) as well as in a fungus, *Sordaria humana* (Zickler & Sage, 1981). Axial thickenings of a different type were observed in grasshoppers (e.g. see Jones, 1973; Moens, 1973) and

a mammal (Solari, 1974), but structures comparable to the axial thickenings in plants seem to be absent from animal SCs. In the present paper the appearance and distribution of axial thickenings, splittings and other aberrations in *A. ursinum* are described and their nature is discussed.

In pachytene bivalents heterozygous for an inversion, an inversion loop may be formed or the inverted regions may remain unsynapsed or synapse heterologously (McClintock, 1931, 1933; Maguire, 1981). The larger the inverted region, the more likely it is that there is SC initiation within it, and the more frequent is loop formation. Normally, pairing is initiated midway between the inversion breakpoints (Moses *et al.* 1982). Since in *A. ursinum* this region does not pair, loop formation must be more complicated. The course of synapsis in a heterozygous pericentric inversion in *A. ursinum* will be described here in detail.

Materials and methods

Plants of *Allium ursinum* L. ($2n = 14$) were taken from natural populations in a wood near the Botanical Garden, Vienna, Austria, from near Ebensee, Upper Austria, and from near Droitwich, Worcestershire, UK. The plants from the latter two sources were used for light-microscopic studies only; in one individual from Vienna, with a chromosome pair heterozygous for a pericentric inversion, a thorough electron-microscopic investigation was performed.

Synaptonemal complexes (SCs) from pollen mother cells were prepared according to the spreading method of Albini & Jones (1984), which is based on the enzymic digestion of the pollen mother cell walls (as introduced by Stack, 1982) followed by the hypotonic swelling of the nuclei and the disintegration of the nuclear membrane with detergent. A short description of the method is also given by Loidl & Jones (1986). Enzymic treatment seems to be essential in some plants, whereas it may be omitted in others (e.g. in maize; Gillies, 1981), which probably depends on the stage of development of the callose wall of the pollen mother cell during zygotene/pachytene.

The spread SCs were contrasted with silver (see, e.g., Loidl & Jones, 1986) and analysed by light and electron microscopy (EM).

SC measurements were performed on EM micrographs (magnification $\times 1600$) with a digitizer. Smaller structures, like axial thickenings, were measured directly from EM negatives by matching them with a stage micrometer under a stereo microscope.

The term 'bivalent' is used to denote partly or fully synapsed homologous axes in zygotene and pachytene, and has no connotation of chiasmate or other associations in later stages.

Results

Pericentric asynapsis

A. ursinum possesses 14 metacentric to submetacentric chromosomes per cell, which form seven synaptonemal complexes (SCs) in meiotic prophase (Fig. 1). EM analysis of spread SCs reveals their usual features like the tripartite structure, kinetochores and recombination nodule-like structures (Fig. 2). The nature of the latter could not be verified due to their inconsistent presence in the spreads, but they resemble those structures that were identified as recombination nodules in PTA-stained spreads of *Allium fistulosum* (Albini & Jones, 1984). Thus, although recombination nodules have not been detected in silver-stained spreads before, the nodules in *A. ursinum* are likely to be of this kind. Preservation of kinetochores was variable between different preparations but usually they could be identified as a cloudy, albeit sometimes faintly stained, material. In Fig. 3 particularly distinct kinetochores are shown. The nucleolus or its remnants could be recognized in most nuclei, associated with the telomeres of three SCs (Fig. 1).

The mean total length of SCs per nucleus as measured in 10 cells was $900 \mu\text{m}$ (s.d. 53), ranging from 810 to $972 \mu\text{m}$. This value differs considerably from the one given in an earlier paper (Loidl, 1984), which may be partly due to interindividual differences in pachytene contraction, but mainly it seems to be a consequence of the greatly improved spreading method.

A very remarkable finding in *A. ursinum* was that even in pachytene a proximal region on each bivalent remained unsynapsed (Figs 1, 3). This was observed in three widely separated populations from Vienna, from Upper Austria (some 250 km away) and from near Droitwich (UK), respectively. The observations by Loidl (1984) on a population from Bavaria (FRG) also suggest the presence of this feature. Therefore, it seems to be widely spread if not general in *A. ursinum*. The unsynapsed region always encompassed the kinetochores and its length ranged from $\approx 5 \mu\text{m}$ to over $20 \mu\text{m}$ in pachytene (where pachytene is defined as the stage of maximum homologous synapsis).

The question arises of how this asynapsis comes about. One possibility is that pairing is initiated at both bivalent ends and proceeds (in a zipper fashion) too slowly to be completed by the end of zygotene, and hence leaves a gap midway between the initiation sites. Alternatively, proximal synapsis could fail due to some sort of impairment exerted by the kinetochore. Whereas the former may be the case in most examples of partial synapsis described so far (see Discussion), the latter may be true for *A. ursinum*. Two lines of evidence point to this. One is a case of a pericentric inversion loop, indicating the existence of proximal

pairing initiation (see below), and the other is the spatial relationship of the unsynapsed region with the kinetochore in non-metacentric bivalents. It is clear that, if asynapsis were a consequence of the delayed 'zipping up' from the bivalent ends, the unpaired region should be more or less median, irrespective of the position of the kinetochore (provided that synapsis approaching from both ends proceeds equally fast).

Most chromosomes of *A. ursinum* are roughly metacentric but two of the NOR-bearing chromosomes form a distinctly submetacentric bivalent with an arm ratio near 2:1 (measured in 10 bivalents) in which the causality of the relationship between kinetochore and unpaired region could be tested. In this

bivalent the unpaired region always contained the kinetochore and it never extended to the median bivalent region. This observation reveals that the unpaired region is regularly associated with the kinetochore, which makes it likely that the kinetochore itself somehow exerts an inhibitory effect on synapsis in its vicinity.

Nevertheless, occasionally the kinetochores of the pairing partners showed a mutual attraction in pachytene (Fig. 3). This however, may be due to an unspecific association of the kinetochores (for references, see Gillies, 1984) rather than homologous attraction. Nonetheless, it indicates that *in vivo* the pericentric axial regions must (in spite of being

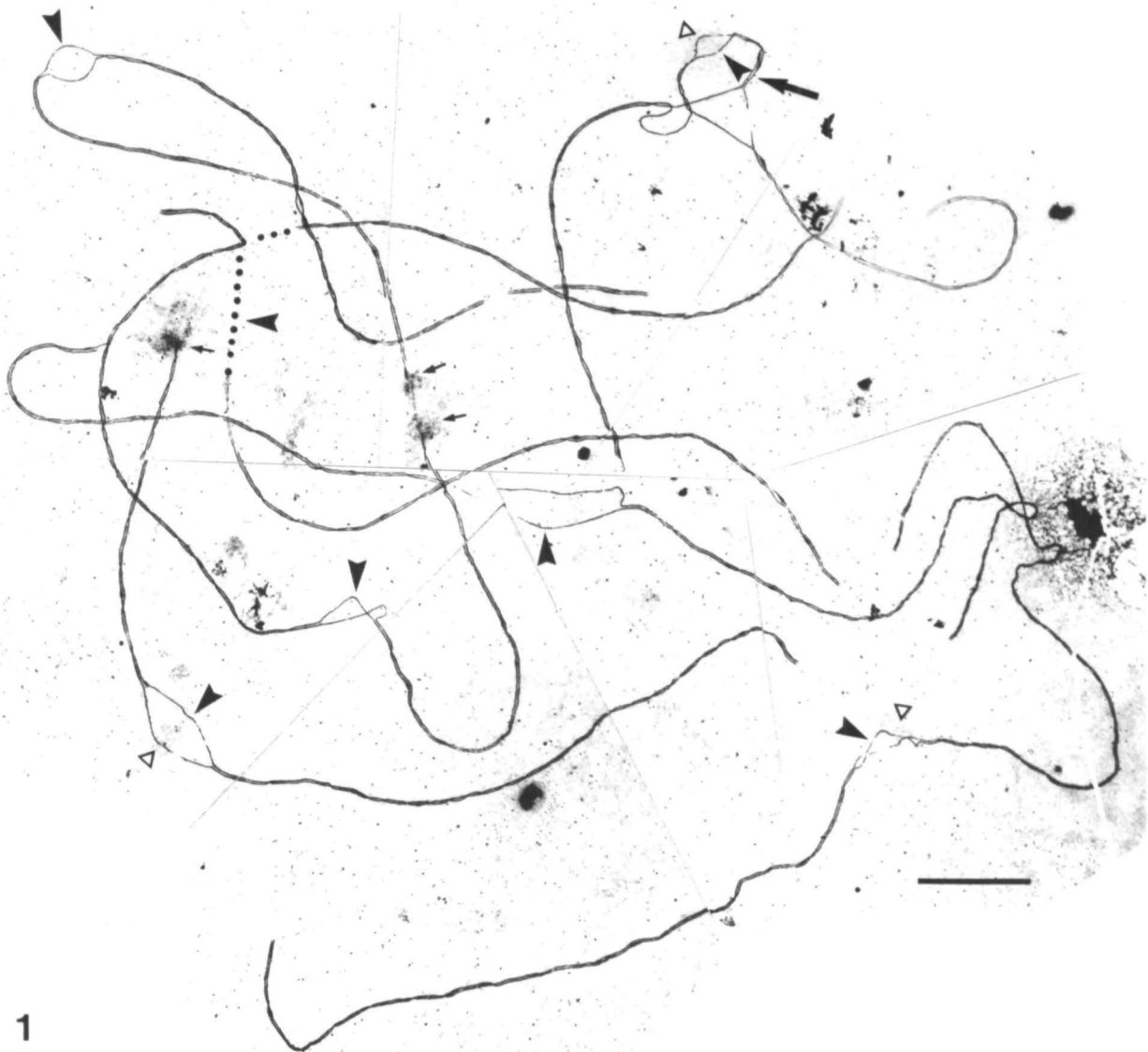


Fig. 1. Spread pachytene SC complement of *A. ursinum*. The pericentric regions are unsynapsed (filled arrowheads), one is disrupted (dotted stretch). Kinetochores can be recognized as a faintly stained material in some of them (open arrowheads; for better preserved kinetochores see Fig. 3). The small arrows denote the three terminal nucleolus-organizing regions with attached remnants of nucleoli. One SC has formed a pericentric inversion loop (large arrow). Note also the axial thickenings in the synapsed regions. Bar, 10 μ m.

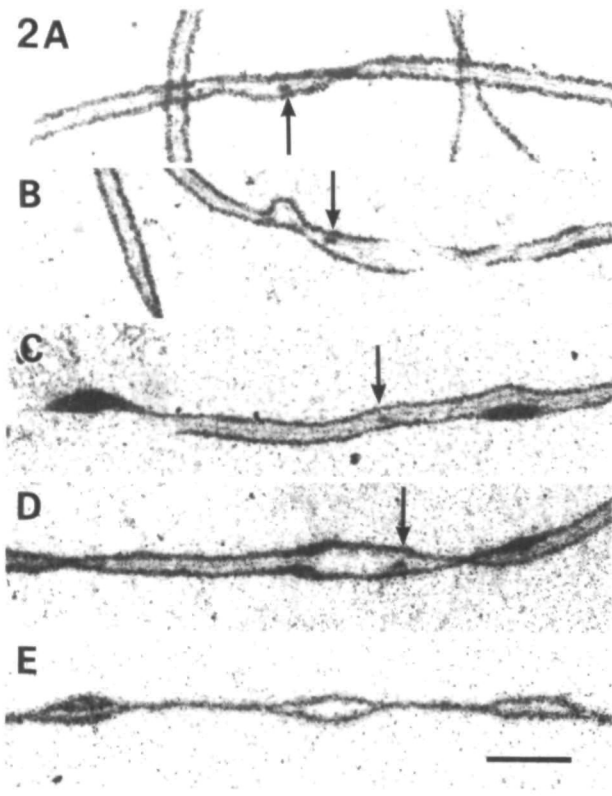


Fig. 2. Silver-stained axes at high magnification showing the fine structure of SCs and lateral elements (LEs). A–D. Examples of recombination nodule-like structures (arrows). Note the location of the nodule in B, with the LEs mismatched on both sides. C–E. Examples of axial thickenings and splittings. C. A thickening occurs at the nucleolus-organizing region (the nucleolar remnants can be seen around the thickening to the left). D. Pair of thickenings at a pairing fork of a small unsynapsed region in late zygotene. E. Axial element splittings in an unpaired axis. In one of the splittings a substrand is thickened. Bar, 1 μ m.

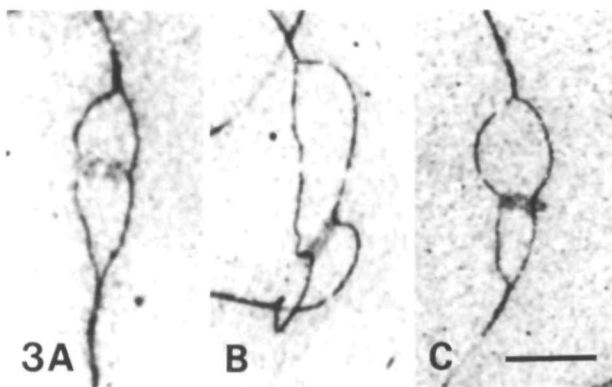


Fig. 3. Light micrographs of intensely stained kinetochores located within unsynapsed regions. Note that opposite kinetochores are associated. Bar, 5 μ m.

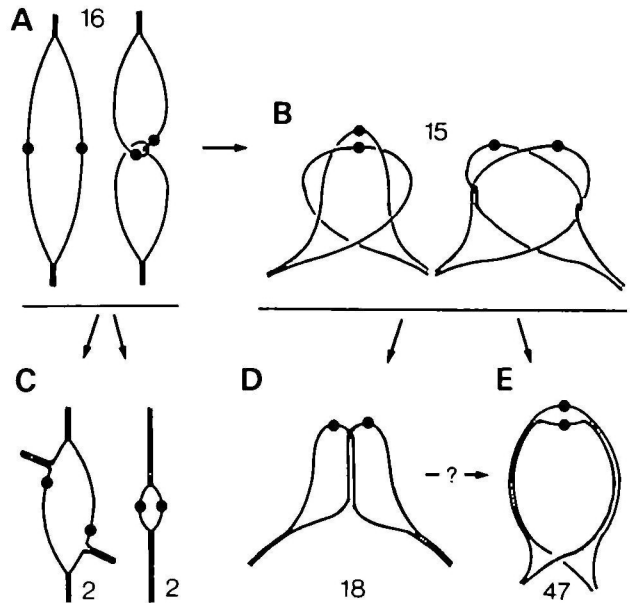


Fig. 4. Frequencies of pairing configurations in 100 heterozygous inversion bivalents in late zygotene to pachytene and the presumed sequence of formation (arrows). A. In 16 bivalents the inverted region was totally unsynapsed and appeared as a large, sometimes twisted, gap (see Fig. 5A). B. In 15 bivalents the inverted regions of the axes were aligned homologously and were often interlocked or had already initiated synapsis in places (see Fig. 5B–D). In four configurations the inverted regions were paired non-homologously (C), in two of them both axial elements showed foldback pairing, in the other two synapsis has proceeded from outside into the inverted region. In 18 bivalents, only one pairing initiation had taken place within the inversion, whilst the region on the other side of the kinetochore was totally unbound, making it doubtful if this configuration had the potential to become a complete inversion loop (D) (an example is shown in Fig. 5E). In 47 bivalents complete inversion loops, with various extents of synapsed regions, were formed (F) (examples in Fig. 5F–H).

unsynapsed) be closely aligned in order to allow the homologous kinetochores to fuse.

Synapsis in a heterozygous pericentric inversion

One individual from Vienna had a bivalent heterozygous for a pericentric inversion. It formed an inversion loop in a high proportion of pachytene nuclei (Figs 1, 4, 5). This finding proves that proximal regions are also capable of initiating synapsis. Since synapsis cannot proceed across the kinetochore region, at least two independent pairing initiations are required, one on each side of the kinetochore, within the borders of the inverted region.

The breaks of the inversion must have occurred symmetrically with respect to the kinetochore, since the kinetochores are located opposite one another in the heterozygous bivalent. The length of the inverted

region can be estimated as $35.6 \pm 2.3 \mu\text{m}$. It was measured as the length of the asynaptic region in 10 presumed inversion bivalents in which no inversion loop had yet formed. Therefore, they are likely to be in late zygotene, when heterosynapsis is not likely to occur. This estimation may be subject to error since the location of the pairing fork does not necessarily reflect the inversion breakpoint (see Ashley *et al.* 1981).

Pairing within the inverted regions is much delayed compared to all other regions except the pericentric parts, and the course of synapsis in the inversion loop could be studied in late zygotene/pachytene nuclei.

Pairing of the inverted regions is probably facilitated by the bouquet polarization where the chromosomes are looped back around the centromeres. This ensures close association of the homologous stretches within the inversion (Figs 4, 5, 6). At this stage, those regions within the loops that are about to initiate pairing are often interlocked with each other

(Fig. 5B–D). This seems to be a consequence of the relational twisting of the homologous axes.

Finally, on one or both sides of the kinetochores an SC is initiated within the inverted region. If synapsis is achieved on both sides, an inversion loop is the result. This was the case in 47 out of 100 bivalents scored (Figs 4E, 5F–H). In 18 bivalents synapsis had taken place on only one side of the kinetochores but it could not be decided if these incomplete inversion loops represented the final stage or had the potential to become complete loops. In four bivalents the formation of an inversion loop had totally failed and heterosynapsis had taken place, which resulted in hairpin loops in two of them. The other two were indistinguishable from normal bivalents and could be identified only in complete and easily analysable nuclei. However, their absence from less suitable nuclei is established, since in these the presence of other types of the inversion bivalent was always easily ascertained by their characteristic appearance.

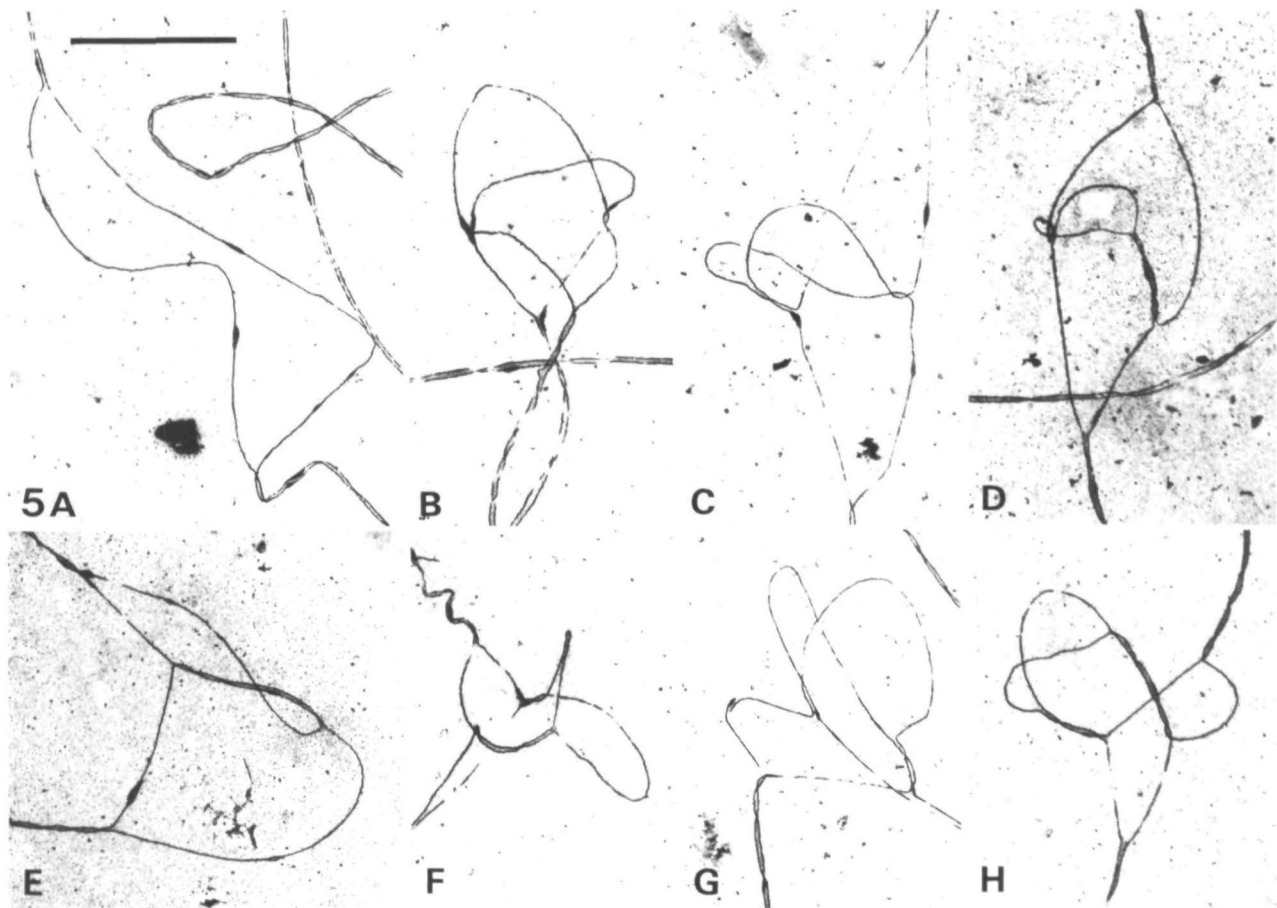


Fig. 5. Various aspects of pericentric inverted regions. A. Complete asynapsis of the inverted region. In B–D the inversion loop is about to be formed, in E only one SC initiation had taken place, hence the inversion loop is incomplete. F–H. Complete inversion loops with various degrees of synapsis. Note thickenings at pairing forks in B, E, F and G, and at sites of homologous association or pairing initiation in B and C. Thickenings (in most of which the axes can be seen to be split at higher magnifications) and splittings are present in those regions of the unpaired axes that potentially do synapse (A, C, D, E, G) but not in the regions close to the kinetochores, which remain unsynapsed in any case (see also Fig. 1). Bar, $10 \mu\text{m}$.

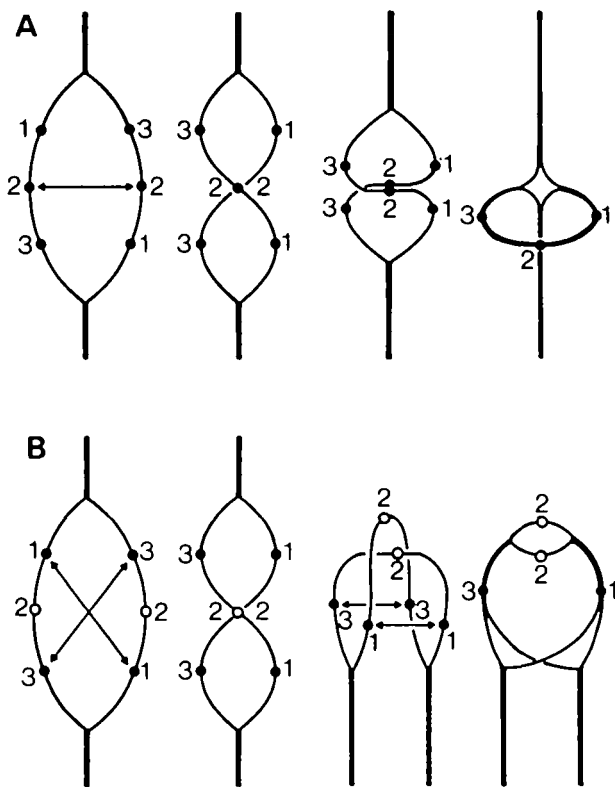


Fig. 6. Comparison of the modes of SC initiation in a normal inversion loop (A) and in a pericentric inversion loop with an unpaired median region as encountered in *A. ursinum* (B). In A the median homologous regions interact over a short distance to achieve alignment and pairing. In B the homologous regions that have to interact are separated by the median region. The suggested mechanism for this case is that the bivalent folds back in the centric region (which corresponds with 2 in the drawing), thereby bringing the homologous regions into proximity and allowing SC initiation at them. The fold-back in the median region could be a consequence of the bouquet polarization.

Axial thickenings

Thickenings of the axial/lateral elements (LEs) were a regular feature in the individuals from Vienna (Figs 1, 2, 5). They were lens-shaped, up to $1.6 \mu\text{m}$ in length (mostly around $1 \mu\text{m}$) and up to $0.5 \mu\text{m}$ wide. On the other hand, others were only slightly thicker than the LE that measured $50\text{--}75 \text{ nm}$. The highest number of thickenings in a pachytene nucleus was 66, the lowest 25. The mean number, as derived from 15 nuclei is 46 ± 11 . Altogether, 1371 thickenings were counted in 207 late zygotene/pachytene bivalents (including those from incomplete nuclei), the mean number per bivalent being 6.6. The highest number of thickenings per bivalent was 16 (found once); two bivalents had 0.

No complete early to mid-zygotene nuclei could be analysed; from several observed fragments it seems, however, that the thickenings are much less abundant than in pachytene.

The thickenings were of such a size and density, that they could be recognized even in the light microscope. They have already been noted in light-microscopic preparations of plants from a population in Bavaria (Loidl, 1984). They were observed in light-microscopic preparations from Droitwich and from Upper Austria, too, although they seemed to be less frequent in the latter.

The thickenings often appear associated with a gap in the LE (see also Gillies, 1981). This can be interpreted to indicate that the thickenings are structures of the subunits of the LEs, which are pushed apart by the thickening.

The thickenings are not distributed equally along the bivalents. They are virtually always present at the nucleolus-organizing regions (NORs) in either one or both of the lateral elements that are mostly mismatched (Figs 1, 2C). In the 15 completely analysed nuclei all three NORs were associated with at least one thickening. In 44 additional NOR bivalents only three SCs showed no thickening at the NOR. In sum, the 89 NORs examined had 105 thickenings. A similarly high incidence of thickenings at NORs was reported for maize SCs (Gillies, 1983). The NOR-associated thickenings will be excluded from the following considerations about the intrachromosomal distribution of thickenings because their location makes them a distinct category.

In *A. ursinum* the thickenings are underrepresented in the unpaired pericentric regions but strikingly abundant at the junctions between the paired and the unpaired regions (pairing forks) (Figs 2D, 5). The 207 bivalents scored for thickenings had a total of 486 pairing forks (comprising those at the pericentric unsynapsed regions and several other intercalary and terminal pairing gaps) and 1266 thickenings (excluding those at the NORs), of which 91 were located directly at a fork. If for the sake of calculation, one assigns to a pairing fork a dimension of $1 \mu\text{m}$ within which the thickening is located, then on average each $5.3 \mu\text{m}$ of the border region harbours one thickening. The sum of all synapsed regions in these 207 bivalents is $\approx 23950 \mu\text{m}$ (the mean length of the synapsed parts of a bivalent having been calculated as $115.7 \mu\text{m}$) and they harbour 1155 thickenings, i.e. one thickening per $20.7 \mu\text{m}$. Hence, in pairing forks, thickenings are about four times more frequent than in synapsed regions.

On the other hand, the unpaired pericentric region accounts on average for 9.6% of the bivalent length (S.D. 2.4, measured in 70 bivalents), but only six thickenings (more than $1 \mu\text{m}$ away from the pairing

fork) were found within this region in the 207 bivalents. (If the distribution were random one would have expected 9.6% of the total of 1266, namely 122 thickenings.) This means that they are much less abundant than in the paired regions.

The above findings suggest that the thickenings originate preferentially at the pairing forks where at the same time synapsis is delayed.

It was reported by some authors, that thickenings on opposite LEs were more often than by chance opposite each other or considerably overlapping (Kehlhoffner & Dietrich, 1983; Hasenkampf, 1984b). Therefore, this aspect was examined as well. In the 207 bivalents 68 pairs of thickenings were counted, which were situated opposite each other on the LEs. A calculation to determine if pairs of thickenings are more frequent than expected from independent distribution, was done as follows: the accumulated lengths of synapsed regions in the 207 bivalents give a total of 23 950 μm (see above). They harbour a total of 1246 thickenings (including those at the pairing forks). It may be assumed that the thickenings are distributed equally to the LEs, i.e. each total of LEs has 623 thickenings. Knowing that an average thickening measures 1 μm , the proportion of thickened regions per LE is one in 38. If the distributions of thickenings in the two LEs were independent of each other, then one out of 38 thickenings in one LE would be expected to be situated opposite (or shifted by half of its length) a thickening in the other LE. As there are 623 thickenings per LE this event is expected to take place 16 times. Hence, the 68 opposite thickenings found are more than four times the expected number.

A distinction has to be made between the unpaired regions associated with a kinetochore, and those that are due to the formation of an incomplete inversion loop or to delayed synapsis. Whereas in the former complications of the axes are rare, in the latter they are more frequent. These are thickenings, splittings, ramifications and interruptions of the axes with overlapping ends (Figs 2E, 5). In spite of the different aspects of these structures, their common basis seems to be the bipartite nature of the axial elements, which in paired axes becomes apparent occasionally near the site of a thickening.

Very often splittings or thickenings were found at the sites where the axes in the inversion loop are interlocked prior to SC initiation (Fig. 5). This phenomenon could be related to the occurrence of thickenings in pairing forks. Another possible explanation is that the interlocked axes can slip along each other until the movement of the interlocking is hampered where the axes are thickened or split.

Discussion

Incomplete synapsis of bivalents has been described as a regular feature in several organisms, mainly grasshoppers, and a dependence of crossing-over localization from the extent of synapsis has been established (e.g. see Henderson, 1969; Fletcher, 1977; Wallace & Jones, 1978; Oakley & Jones, 1982; Moens & Short, 1983). In all the cases investigated so far, synapsis seems to start at one or both bivalent ends, but ceases before completion. There exists no obvious responsibility of the kinetochore for the partial asynapsis. In other organisms it was found that synapsis is initiated less frequently or late and hence delayed in proximal regions (Hasenkampf, 1984a; Gillies, 1985; Holm, 1986; Loidl & Jones, 1986). In these cases it is uncertain whether this is due to the membrane attachment of the telomeres, which promotes distal SC initiation, or to a negative interference of the kinetochore with pairing.

In *A. ursinum*, however, the unsynapsed region is consistently associated with the kinetochore, even when it is located in a submedian position and the pericentric region remains unpaired although proximal SC initiation does occur.

As in many organisms, the frequency of chiasmata decreases towards the centromere in *A. ursinum* (Loidl, unpublished results). This phenomenon of reduced crossing-over rate in the vicinity of the centromere has been termed the 'centromere effect' or 'centromere interference'. Beadle (1932) attributed the centromere effect to the inhibitory influence of the spindle fibres on crossing over. Other authors thought it was due to the presence of centromeric heterochromatin, which is devoid of chiasmata (see, e.g., John & Lewis, 1965). This is certainly not a general explanation, since in *Allium sipyleum* there is a clear centromere effect in the absence of pericentric heterochromatin (Loidl, 1982). Also in *A. ursinum* no trace of centromeric heterochromatin (as defined by C-banding) was found (Loidl, unpublished results). Therefore, asynapsis in *A. ursinum* represents a true case of centromere interference with pairing and is obviously the cytological basis of centromeric interference with recombination in this species.

It has to remain open whether the inhibition of synapsis is due to a merely steric hindrance by the kinetochore material or to the lack of homology in the pericentric regions, or if it has another cause. It could also be speculated that it is a genetically fixed precaution against crossing over within the kinetochore region, for (as discussed by Charlesworth *et al.* 1986) unequal crossing over could lead to a variation in the number of microtubule binding sites, causing non-disjunction.

If homologous synapsis is achieved in a heterozygous inverted region, it often lags behind synapsis in other regions, due to the sterical difficulties that occur with the formation of an inversion loop. Sometimes homologous synapsis fails completely. This seems to be the more frequent the smaller the inverted region (McClintock, 1933; Maguire, 1966) and the smaller the size of the involved bivalent (Ashley *et al.* 1981). The inversion found in *A. ursinum* is relatively large, but the formation of an inversion loop is complicated by the need for two independent SC initiation sites within the inversion. Normally, for the formation of a loop one initiation will suffice, which takes place midway in the inversion (see Moses *et al.* 1982; Moses & Poorman, 1984) by the interaction of directly opposite homologous regions (Fig. 6A). (Strictly speaking, at least two recognition sites have to be involved in order to permit alignment of the axes in correct orientation.) This is not possible in *A. ursinum*, where the median region of the inversion, containing the kinetochore, does not synapse. Therefore, widely separated homologous regions have to be brought into contact. This is probably made possible by the bouquet polarization of the bivalent (Fig. 6B). Thus, in spite of the complicated mode of alignment and pairing, a reasonably high proportion of fully synapsed inversion loops is achieved (Fig. 4).

The absence of synaptic adjustment from the pairing configurations in *A. ursinum* is inferred from the absence of inversion loops of different sizes, which would suggest a desynapsis and heterologous resynapsis of the inverted regions as described by Moses *et al.* (1982). It seems as if in *A. ursinum* there are two synaptic phases, with only homologous synapsis being possible in the first, whereas in the second stage heterosynapsis also occurs (see Moses *et al.* 1982). This is inferred from the appearance of the unsynapsed regions (Figs 4A, 5A), which are all roughly equal in size, and which probably approximately represents the size of the inverted region. The absence of intermediary sizes between the unsynapsed and the fully heterosynapsed inverted regions (Fig. 4) suggests that synapsis is arrested for a certain time span until heterosynapsis can take place. A similar phenomenon was observed in triploid *Allium sphaerocephalon* by Loidl & Jones (1986). There, of three homologous axes, two form an SC, whilst the third remains closely attached. Only in late pachytene are the formerly unpaired axes released from homologous alignment to engage in heterosynapsis, possibly to satisfy an inherent urge to pair.

The capacity of axes that are prevented from homologous pairing to eventually synapse non-homologously, was described by Ashley *et al.* (1981), in an inversion heterozygote and by Sharp (1986) in a C-band heterozygote, as synaptic adjustment. However,

this term seems questionable for these cases of heterosynapsis, since synaptic adjustment in the strict sense implies the replacement of homologous synapsis by heterologous synapsis (see also Maguire, 1981).

It is a remarkable fact that thickenings of almost identical appearance are formed by the axial/lateral elements of distantly related dicotyledoneous and monocotyledoneous plants (Moens, 1968; Gillies, 1973, 1981, 1984; La Cour & Wells, 1973; Kehlhoffner & Dietrich, 1983; Hasenkampf, 1984b; Schwarzacher-Robinson, 1987) and even a fungus (Zickler & Sage, 1981), whereas they are not consistent within one genus. For instance, thickenings were not observed in *Allium fistulosum* (Albini & Jones, 1984), *A. cepa* (Albini, personal communication) or *A. sphaerocephalon* (Loidl & Jones, 1986). In *A. vineale* very occasionally a few per nucleus were found (Loidl, 1986), the situation in *A. senescens* being similar (Loidl, unpublished results). Moens (1968) observed thickenings in the allotriploid *Lilium tigrinum*, but not in the autotetraploid *L. longiflorum* (Moens, 1970). Similarly, Schwarzacher-Robinson (1987) found thickenings in the tetraploid *Paeonia officinalis* but not in the diploid *P. tenuifolia*, although in the latter thickenings were reported by Kehlhoffner & Dietrich (1983).

The explanation for this variability may be that the underlying structures are present (latently) but their expression as thickenings depends on environmental conditions (see La Cour & Wells, 1973) or the individual genetical background. The variable incidence of the thickenings is also documented in maize, where Gillies (1981) found pachytene nuclei with as many as 40 and others with none; the variation within individuals being almost as high.

Axial thickenings have been found in animals, also (Jones, 1973; Moens, 1973; Solari, 1974). However, they differ considerably with regard to size, location and number per nucleus, and hence it is doubtful if they belong to the same class. The thickenings in the ascomycete *Sordaria*, on the other hand, share many features with those in higher plants (Zickler & Sage, 1981).

The morphology of thickenings in plants is fairly uniform. Their maximal length ranges from about 500 nm in maize (Gillies, 1984) to 2000 nm in *Lilium tigrinum* (Moens, 1968). In most other plants the values are around 1000 nm. The width of thickenings is about 120 nm in *Tradescantia* (Hasenkampf, 1984b), about 300 nm in maize (Gillies, 1984) and up to 500 nm in *A. ursinum*. EM sections have revealed that the thickenings are hollow tubes (Moens, 1968; Gillies, 1973; La Cour & Wells, 1973; Zickler & Sage, 1981). The observations presented in this paper (see Fig. 2E) suggest that a thickening is formed by one

substrand of the axes, which are bipartite (for review, see Moses, 1968; Gillies, 1984).

There are also similarities with respect to the location of the thickenings within the axes between different plants. Thickenings are very frequent at the NOR sites of the axes in maize (Gillies, 1983) and *A. ursinum*. In *Tradescantia* (Hasenkampf, 1984b), *P. officinalis* (Schwarzacher, 1984) and *A. ursinum* they are underrepresented, in *L. tigrinum* (Moens, 1968) and *Phaedranassa viridiflora* (La Cour & Wells, 1973) they are even totally missing in unsynapsed regions. In *Tradescantia*, *P. officinalis* and *A. ursinum* they are more often located opposite one another in the LEs than expected from a random distribution; in *Paeonia tenuifolia* this is even the rule. Finally, thickenings are abundant at pairing forks (boundaries between synapsed and unsynapsed regions) in *Tradescantia* and *A. ursinum*.

The fact that thickenings are more frequent in paired than in unpaired regions permits the conclusion that most of them originate during or after synapsis (see Moens, 1968). The additional observation that they are especially frequent in the pairing forks (and perhaps also in SC initiation sites) (see Figs 2D, 5) suggests that they originate there whilst synapsis is retarded or temporarily halted. It may be speculated that the thickenings are a transitory and somewhat inconsistent concomitant of some rectifying process that brings the axial elements into phase with each other. This could be necessary if the sites for homologous recognition were differentially spaced in the two axes (e.g. due to slight heterozygosities or differential condensation between the homologues) and the intervening axial segments had to be adjusted in order to form a regular SC (compare with Moens, 1970). The high incidence of pairs of opposite thickenings fits this hypothesis, since a correction would be most efficient if carried out in both axes simultaneously.

As shown by Hasenkampf (1984b), the number of thickenings decreases during late zygotene, when synapsis approaches completion. This suggests also that the thickenings are concomitant with the event of synapsis and are eliminated from the paired axes after a while. Thickened axes in wheat, on the contrary, occurred exclusively in late diplotene (Holm, 1986) and probably represent a different class of structural abnormalities of the axes.

The same can be said of the structures in the unsynapsed axes of *A. ursinum*. They are of a less-regular appearance than the thickenings at the borders of and within the paired axes and seem to be heterogeneous. Most of them seem to represent various aspects of axial splitting, which in general is more conspicuous in unpaired than in paired axes. Some may be accumulations of central element material (see

Loidl & Jones, 1986), others may simply reflect degeneration of the axes.

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