MEIOSIS IN COPRINUS LAGOPUS: A COMPARATIVE STUDY WITH LIGHT AND ELECTRON MICROSCOPY

B. C. LU*

Department of Genetics, University of Cambridge, England, and Institute of Genetics, University of Copenhagen, Denmark

SUMMARY

Meiosis within fruiting bodies of Coprinus lagopus Fr. is closely synchronized. This conveniently facilitates joint light- and electron-microscope observations. Before nuclear fusion the chromatin appears diffuse in the light microscope; after nuclear fusion individual chromosomes can be recognized. In the electron micrographs the chromatin of pre-fusion and early fusion nuclei cannot be recognized as defined structures with the fixation and staining procedures employed. At the time of synapsis the lateral components of the synaptinemal complexes can be seen in the micrographs. The pairing process of the two chromosomes of the homologous pairs is believed to involve two steps: (1) two homologous chromosomes become aligned in parallel, and (2) pairing occurs by formation of the synaptinemal complex including the central synaptic component. The term synaptic centre is coined for the central component, which is believed to be the zone where crossing-over occurs. The formation of this structure in relation to homologous pairing, and the structural organization of the synaptinemal complexes are discussed.

At meiotic metaphase, the chromosomes congregate around the central spindle microtubules. They are contracted and contain densely packed chromatin fibrils. Two types of spindle microtubules are demonstrated: (1) the chromosomal microtubules directly connecting the chromosomes to the centrosomes, and (2) the central spindle microtubules connecting the two centrosomes. The centrosomes are round, fibril-containing bodies approximately 0.3 μ in diameter. They have been observed outside the nuclear envelope at pachytene, but do not show the characteristic structure normally found in animal cells.

INTRODUCTION

The widespread use of fungi as organisms for genetic research has prompted the need for cytological information, such as chromosome behaviour, chromosome replication, pairing and disjunction, for clearly these have important genetic consequences. In recent years, several species of fungi have been investigated cytologically and the general course of their meiosis has been described (McClintock, 1945; Singleton, 1953; Carr & Olive, 1958; Knox-Davies & Dickson, 1960; Rossen & Westergaard, 1966). Electron-microscope observations have extended our knowledge of fungal nuclear cytology by showing the presence, in zygotene-pachytene nuclei, of synaptinemal complexes comparable to those seen in the cells of higher organisms (Lu, 1966; Westergaard & von Wettstein, 1966). However, problems such as the mode of chromosome pairing and the processes involved in the formation of the synaptinemal complexes remain obscure, so it is desirable to study sequential stages of meiotic division.

* Present address: Department of Botany, University of Guelph, Guelph, Ontario, Canada.
at the fine-structural level. In the present investigation, comparative observations of meiosis in *Coprinus lagopus* by means of light and electron microscopy were undertaken.

**MATERIALS AND METHODS**

The *Coprinus lagopus* used was kindly supplied by Dr D. Moore of the Department of Botany, University of Manchester. Homocaryotic cultures BC 9/55 (A^b^B^b^) and BC 9/66 (A^b^B^b^) were crossed on Brodie's agar medium (Brodie, 1948) at 37 °C and photo-induced to fruit at room temperature (25 °C).

Since meiosis takes place synchronously in the fruiting body of *Coprinus*, it is possible to correlate the meiotic stages seen with the light microscope with those observed with the electron microscope. This was done by fixing and squashing a piece of material from a given fruiting body for light microscopy and fixing another piece from the same fruiting body for electron microscopy.

For light microscopy, young developing fruiting bodies were fixed in BAC fixative (Lu, 1962) for 24 h or longer. The fixed material was hydrolysed in HCl/alcohol (1:1, v/v) at 70 °C for 2½ min or in n-HCl at 70 °C for 35 min, and then washed in Carnoy's fluid for 2 min. A small piece of a single layer of gills was removed and squashed in propionic iron haematoxylin. The preparations were examined with a Zeiss WL microscope fitted with Neofluar optics (N.A. 1.32), and photographs were taken with a 35-mm camera using Kodak Panatomic X film and developed in Kodak Microdol X developer.

For electron microscopy, a piece of hymenium was carefully cut into small cubes in a drop of 3.4% glutaraldehyde in 0.067 M phosphate buffer, pH 6.5 or 7.0, and fixed for 2½–3 h at 4 °C. In a few samples, 0.7% NaCl was added to increase tonicity; the membranes appeared to be better preserved, but the nuclear fine structure was not altered. The material was washed in 3 changes of phosphate buffer overnight and then post-fixed in 1% osmium tetroxide in phosphate buffer for 2 h; these procedures were carried out at 4 °C. The material was further washed in 3 changes of phosphate buffer at room temperature, dehydrated in ascending series of ethanol, and embedded in a new resin mixture developed by Dr A. Spurr (private communication). Thin sections were cut with a Porter–Blum ultramicrotome using a glass knife and were put on Formvar/carbon-coated copper grids. The preparations were examined with a Zeiss EM 9 electron microscope using an accelerating voltage of 60 kV. Photographs were taken with an automatic camera.

**OBSERVATIONS**

*Nuclear synchrony and meiotic prophase*

All basidia in a developing fruiting body are in remarkably close synchrony with respect to the meiotic process, though this is not to say that there is absolutely no variation within a fruiting body. In general, the basidia at the bottom of the gills are slightly more advanced in their development than are those from the apex. Variations
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have also been observed among neighbouring basidia, but on the whole, about three-quarters of the basidia are at the same stage. For example, in one fruiting body (CL 026) as seen in Fig. 1, the majority of the basidia (about 70–80%) were at the pre-fusion stage, containing two nuclei of compatible mating types. Nuclear fusion was observed only in a small percentage of the cells, and in none of them had meiosis proceeded beyond the stage shown in Figs. 2 and 3. When thin sections were prepared from this fruiting body and examined with the electron microscope, they were seen to include many more cells at the pre-fusion stage (Fig. 10) than at the fusion stage (Fig. 11), as expected from the light-microscope observations.

The nucleus, at both the pre-fusion and post-fusion stages, is enclosed by an annulate nuclear envelope (Figs. 10–12, 14). Inside the nucleus is a prominent electron-dense, ovoid body, the nucleolus, which includes numerous granular particles resembling ribosomes (Figs. 10, 11, 13). In the pre-fusion and early post-fusion nuclei, the chromatin material is not well defined, the nucleoplasm appearing evenly fibrillar throughout the nuclear sphere, although there are faint patches of higher electron density which may represent the chromocentres. The fine structure of the pre-fusion nucleus (Fig. 10) is very similar to that of the post-fusion (diploid) nucleus (Fig. 11), and this resemblance suggests that the organization of the chromosomes changes little during and immediately after nuclear fusion.

Another fruiting body (CL 027) was at a slightly more advanced stage of development. In the light microscope it could be established that about 20% of the basidia were at a prefusion–fusion stage comparable to that described for CL 026 above, while the majority (about 60%) were at the synaptic stage (Fig. 4). The slightly later stage, early pachytene, was also observed in a few basidia (Fig. 6). Electron microscopy revealed, in addition to the pre-fusion and early post-fusion nuclei shown in Figs. 10 and 11, two new types of nuclei, differing in their fine structure. In type I, many structures resembling chromocentres are seen in a homogeneous nucleoplasm (Figs. 12, 13). They apparently represent the lateral components of the synaptinemal complexes in longitudinal or transverse section. They are undivided structures and are frequently distributed in pairs (Fig. 13). By comparative light and electron microscopy, this can be explained most simply as the beginning of the pairing process at zygotene when two homologous chromosomes become associated. In the other nuclear type II, synaptinemal complexes including the central synaptic component are present. It was established that type I precedes type II, which represents the completion of chromosome synapsis. By comparing this observation with those made with the light microscope, one can say that the formation of the lateral components of the synaptinemal complexes precedes the formation of the central synaptic component.

Since the nuclear synchrony is so regular, a fruiting body at pachytene, a relatively long stage, would be expected to show the same stage throughout. This is indeed the case as found in fruiting bodies CL 019 and CL 041 (Figs. 5, 7). When thin sections were prepared from these fruiting bodies and examined with the electron microscope, it was evident that the chromosomes within these nuclei, whether in longitudinal section (Figs. 14–16) or in transverse section (Fig. 17), exhibit the tripartite synaptinemal complexes which are known to be a feature of paired bivalents. The lateral com-
ponents are considered to be axes of two homologous chromosomes which first appear aligned in pairs, and then become physically synapsed with the formation of the central synaptic component. Since this component is a direct consequence of synapsis and is believed to be the area where pairing of homologous fibrils occurs, the functional term synaptic centre is proposed. It should be pointed out that the chromosome axis is only the condensed part of the chromosome from which chromatin fibrils extend laterally. The lateral chromatin fibrils do not show sufficient contrast to be clearly visible in the electron microscope with the fixation and staining procedure used.

A fine-structural analysis of the synaptinemal complexes is beyond the scope of the present paper, and will be considered separately. However, a few points of interest may be raised here for they are relevant to chromosome pairing.

The ends of the lateral components of a synaptinemal complex, possibly telomeres, are frequently observed to be associated with the inner nuclear membrane. Such an association is evident in nuclei with unpaired chromosomes (Fig. 12) as well as in those with synaptinemal complexes (Figs. 14, 16). It should be pointed out, however, that while the two lateral components of a complex are associated with the nuclear membrane, the synaptic centre does not extend right up to the nuclear membrane. A gap of about 2500 Å is always seen between the end of the synaptic centre and the nuclear membrane (Figs. 14, 16). It is likely that the pairing does not extend to the telomere regions.

Since a synaptinemal complex is a bivalent, it is to be expected that each of the two chromosomes of the homologous pairs should consist of two chromatids. This is by no means readily resolvable by the electron microscope, however. In transverse sections, the synaptinemal complex is identified as having a synaptic centre flanked by two electron-dense lateral components, the chromosome axes, which are either round or oval in shape, approximately 450 × 650 Å in diameter (Fig. 17). Thus one must conclude that the two chromatids are so closely associated that their identity cannot be resolved. In some favourable sections, however, the lateral component does appear to show some structural doubleness (Fig. 15) and it is possible that this may be correlated with the presence of functional chromatids.

**Meiotic metaphase through telophase**

The progress from metaphase I to telophase II is very rapid, and consequently all these stages may be found within a single fruiting body (for example CL 037). However, synchrony is still quite evident (Fig. 9).

At metaphase I, all 10 pairs of chromosomes of *C. lagopus* are clumped into an area about 1–1½ μ in diameter at the apex of the basidium (Fig. 9). At this time, the two centrosomes are not readily discernible by the light microscope. Electron microscopy (Fig. 19) revealed that they are very closely associated with the chromosome mass (Fig. 19). In later stages, they pull further away from the chromosome mass (Fig. 8).

With electron microscopy, the metaphase chromosomes are seen to be highly electron-dense, consisting of closely packed chromatin fibrils (Figs. 19–23). The space between the chromosomes is filled with ribosomes like those in the cytoplasmic ground substance (Figs. 19–22). In early metaphase, as shown in Fig. 19, a trace of the nuclear
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membrane is still present, but it is not seen at later stages (Figs. 21–23). The nuclear membrane therefore disappears at metaphase I and division is not intranuclear.

All of the spindle microtubules converge towards the centrosomes (Figs. 21–23). They may be classified into two categories: chromosomal microtubules, which directly connect the chromosomes to the centrosomes, and central spindle microtubules, which extend from pole to pole. The first type is shown in Fig. 22, where 2 tubules, having one end inserted into a chromosome, are seen to connect the chromosome to the centrosome. The second type is better seen in cross-section (Fig. 20); a bundle consisting of more than two dozen microtubules forms the central core around which the chromosomes are congregated.

The centrosomes are frequently contained in an invagination of the nuclear membrane at prophase stages (Fig. 18) but their fine structure is obscure. They are fibril-containing bodies about 0.3 μ in diameter approaching the size of a condensed chromosome (Figs. 19, 21, 23), in agreement with light-microscope observations (Fig. 8). In many sections, whether fixed in osmium tetroxide alone or in osmium tetroxide with glutaraldehyde, there is no structural pattern apparent which conforms to that of the centrosomes with centrioles of animals. The centrosome seen in Fig. 25 appears to have a central dense core surrounded by a more diffuse periphery. The fibrils of the central core appear to be morphologically similar to the chromatin fibrils of the metaphase chromosomes. Since the centrosomes are self-replicating organelles, it is possible that some of these fibrils present in the central core represent DNA material, but this requires further investigation.

At anaphase, homologous chromosomes are separated and move to opposite poles, often asynchronously (Fig. 23). Then they remain clumped at the pole, near the centrosome, while a new nuclear membrane is being formed (Fig. 24). Later the chromosomes become uncoiled and the nucleus has almost the same fine structure as the pre-fusion nucleus (Fig. 25).

DISCUSSION

With thin-section electron microscopy, difficulties often arise when attempting to identify stages of meiosis. Moses (1958), by cutting thick and thin sections for light- and electron-microscope observations respectively, was able to correlate the synaptinemal complexes he observed in crayfish with the pachytene chromosomes. This method, though quite adequate, may be tedious technically. In a recent paper, Roth (1966) has found that meiosis in the mosquito ovary is synchronous, and by virtue of this synchrony, he was able to follow the fate of the synaptinemal complexes with success.

The fruiting bodies of all Coprinus species examined (Lu, unpublished) were found to undergo meiosis in close synchrony, and they therefore offer excellent material for comparative light- and electron-microscope studies. Although there are slight variations within a fruiting body the variations have not hampered the present investigation. In fact, they facilitated the assignment of the chromosome figures observed to their respective sequence by virtue of relative percentages of the overlapping stages found in different fruiting bodies at different stages of development. From the present
investigation the following conclusions may be drawn with respect to chromosome pairing in *C. lagopus*. Before synopsis, the chromosomes are organized in such a way that the chromosome axes (the lateral components) become visible in the electron microscope. The pairing process and hence the formation of the synaptinemal complex appears to be accomplished in two steps: (1) two homologous chromosomes become closely aligned, and (2) pairing occurs with the formation of the synaptic centre. This conclusion is in agreement with the observations made on rat oocytes by Franchi & Mandl (1963).

The synaptic centre was first suggested by Fawcett (1956) to result from the deposition of chromatin material from two homologous chromosomes. As pointed out earlier, the synaptic centre is only slightly shorter than the length of a given pachytene chromosome. This is to say that the synaptic centre may be envisaged as a plane on which synopsis of homologous genetic materials can occur. The actual process of forming this centre is not understood, but one might speculate on this from what one knows of chromosome organization. If a chromosome is organized with lateral loops extending from the chromosome axis (see Moses & Coleman, 1964), the ends of these loops could constitute a pairing interface when two homologous chromosomes become paired along their length. Since chromosome pairing at pachytene provides a precise means for genetic exchanges between the homologues, the synopsis must be precise with respect to the genetic loci or the nucleotide segments. In other words, each loop needs to be paired with its non-sister homologue. It is conjectured that some 'fittings' may be necessary before such precise pairing can be accomplished, and only then will the synaptic centre become apparent.

It may be further suggested that, during chromosome pairing, the protein moiety of the nucleoprotein or some newly synthesized protein molecules may be deposited among DNA fibrils and serve to lock or stabilize the homologous pairing and to facilitate crossing over. These proteins may disintegrate or be detached as casts at a later stage. This idea is compatible with the suggestion that the protein moiety of the synaptinemal complexes is released from the chromosomes and may form the multiple complexes seen in some animal cells subsequent to meiosis (Schin, 1965; Sotelo & Wettstein, 1964; Roth, 1966; Wolstenholme & Meyer, 1966).

It is generally accepted that the synaptinemal complexes are involved in chromosome pairing at zygotene and pachytene (Moses & Coleman, 1964; Franchi & Mandl, 1963; Roth, 1966). But the question of whether these complexes contain DNA is still unanswered. The enzyme digestion studies of Nebel & Coulon (1962b) and of Coleman & Moses (1964) were inconclusive. Two possibilities may be entertained at present. First, if the two structures involved in the formation of the synaptinemal complex are considered to be chromosome axes, as intended in the present paper, one must conclude that the complex represents specially organized bivalent chromosomes and must therefore be nucleoprotein in nature. An alternative possibility is to assume that the lateral components are not parts of the chromatin material but are some kind of proteinaceous backbone to each of which a chromosome is attached. This idea has been favoured by Nebel & Coulon (1962a) and Roth (1966).

It has recently been established decisively that crossing over and chiasma formation
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take place at zygotene or pachytene (Henderson, 1966). In addition, there is evidence that the synaptinemal complexes are associated with chiasma formation (Meyer, 1960, 1964). Although the fine-structural organization of synaptinemal complexes is still far from clear, the organization must accommodate homologous pairing and crossing over.

The association of the telomeres with the nuclear membrane has been found in many plant and animal species. It is interesting to note that no synaptic centre is formed between the telomeres in any organism so far examined (Nebel & Coulon, 1962a; Moses, 1958; Moses & Coleman, 1964; Lu, 1966; Woollam & Ford, 1964). Whether this has anything to do with the structure of the telomeres remains to be seen.

It is remarkable that the chromosomes contract from about 1 μ wide and 7–10 μ long at pachytene to 0.3 μ in diameter at metaphase I. Since the chromosomes at metaphase are seen to contain only chromatin fibrils, condensation must clearly be accomplished by the packing of these fibrils, though the mode of fibril packing is still far from clear.

The presence of a spindle mechanism in fungi has been known for some time from light microscopy (Dodge, 1927; Lu, 1964). On the basis of light-microscope observations, Lu (1964) suggested that there were two types of spindle fibres in the basidiomycete Cyathus stercoreus: (1) chromosomal fibres that connect chromosomes to the pole, and (2) central spindle fibres that connect between two poles. The present electron microscope observations confirm this suggestion and show chromosomal and central microtubules comparable to those found in higher organisms (Harris & Mazia, 1962).

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REFERENCES


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<td>c</td>
<td>centrosome</td>
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<tr>
<td>ch</td>
<td>chromosome</td>
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<td>cm</td>
<td>cell membrane</td>
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<td>cw</td>
<td>cell wall</td>
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<td>lc</td>
<td>lateral component of the synaptinemal complex</td>
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<td>m</td>
<td>mitochondrion</td>
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<td>mt</td>
<td>spindle microtubules</td>
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<td>n</td>
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Figs. 1–3. Basidia from fruiting body CL 026 of *C. lagopus* at pre-fusion and post-fusion stage; nuclear behaviour is closely synchronized.

Fig. 1. All basidia except one (arrowed) contain two nuclei of compatible mating types at pre-fusion state. × 2000.

Figs. 2, 3. Post-fusion stage before synopsis begins. × 3000.

Fig. 4. Basidia from fruiting body CL 027 of *C. lagopus* showing stages at zygotene. In some basidia (for example, arrow a) homologous chromosomes are associated in pairs, in others (for example, arrow b) chromosome pairing has been completed (see also Fig. 6). × 2000.
Fig. 5. Basidia from fruiting body CL 019 of *C. lagopus*, showing good synchrony of nuclei at pachytene. (A squashed nucleus at the same stage is shown in Fig. 7.) \( \times 2000 \).

Fig. 6. A basidium at early pachytene from fruiting body CL 027 of *C. lagopus*. The chromosomes are synapsed. \( \times 3000 \).

Fig. 7. Late pachytene (see Fig. 5). \( \times 3000 \).

Fig. 8. Metaphase I. The chromosomes are at the equatorial plate; a pair of centrosomes and the spindle are clearly seen. \( \times 4500 \).

Fig. 9. Basidia from fruiting body CL 037 of *C. lagopus* at metaphase I. The chromosomes are clumped at the apex of the basidium. The centrosomes are not visible in most nuclei, because they are closely associated with the chromosome mass (arrowed). \( \times 2000 \).
Figs. 10–11. Basidia from fruiting body CL 026 of *C. lagopus*.

Fig. 10. Two nuclei of compatible mating types before nuclear fusion are shown. \(x\ 40000\).

Fig. 11. The post-fusion diploid nucleus before synapsis begins. In both cases the chromosomes are not recognizable though there are patches of slightly higher electron density. The nucleolus contains many ribosomes. \(x\ 40000\).
Figs. 12–13. Nuclei from fruiting body CL 027 of *C. lagopus* during synapsis. The lateral components (lc) of the synaptinernal complexes are visible. These structures are frequently distributed in pairs (Fig. 13), and they are sometimes associated with the nuclear membrane (Fig. 12, arrow). × 30000.
Fig. 14. Basidium from fruiting body CL 019 of *C. lagopus* at pachytene showing two synaptinemal complexes (*syn*) in the nucleus. One complex is associated with the nuclear membrane (arrow); the synaptic centre is absent in this region. \(\times 28000\).

Fig. 15. Nucleus at pachytene from fruiting body CL 041 of *C. lagopus*, showing a synaptinemal complex which is made up of a synaptic centre (*sc*) flanked by two lateral components (*lc*). Some part of the lateral components appears as a double structure (arrowed) which may correspond to the two chromatids of the chromosome. \(\times 100000\).
Fig. 16. Nucleus at pachytene from fruiting body of *Coprinus curtis*, showing two synaptinemal complexes (syn) in longitudinal section, one of which (arrowed) is associated with the nuclear membrane (nm). × 35000.

Fig. 17. A nucleus at pachytene from fruiting body CL 041 of *C. lagopus*, showing four synaptinemal complexes (syn) in transverse section, each consisting of a synaptic centre (sc) flanked by two lateral components (lc). × 28000.

Fig. 18. Nucleus at pachytene of *C. lagopus*, showing a centrosome outside the nuclear membrane; fixation in osmium tetroxide at pH 6.0 in Kellenberger’s buffer. × 60000.
Figs. 19–20. Basidium from fruiting body CL 037 of *C. lagopus* at metaphase I.

Fig. 19. The chromosomes (ch) are highly contracted and clumped at the apex of the basidium. Two centrosomes (c) are closely associated with the chromosome mass (compare with Fig. 9); a trace of the nuclear membrane is still present. × 50,000.

Fig. 20. Basidium, showing cross-section of the central spindle; the chromosomes (ch) are arranged around the central spindle microtubules (mt) which connect two poles. The nuclear membrane has disappeared. × 50,000.
Figs. 21–23. Basidia from fruiting body CL 037 of *C. lagopus*.

Fig. 21. Metaphase I, showing chromosomes (ch), spindle microtubules (mt) and a centrosome (c) at the pole. × 50,000.

Fig. 22. Metaphase I, two chromosomal microtubules (arrowed), whose ends are inserted into a chromosome, directly connect between the chromosome and the centrosome. × 35,000.

Fig. 23. Early anaphase I, in which chromosome separation is beginning. It should be noted that these chromosomes are highly contracted and contain densely packed chromatin fibrils. × 50,000.
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Figs. 24–25. Basidium from fruiting body CL 037 of *C. lagopus*.

Fig. 24. Early telophase I. The chromosomes are clumped at the pole near the centrosome and the nuclear membrane is being formed.  x 50000.

Fig. 25. Late telophase I. The chromosomes have uncoiled and the nuclear membrane has been formed. The centrosome includes a central electron-dense region surrounded by a less electron-dense zone (indicated by opposing arrows). The central region appears to contain fibrils comparable to the chromatin fibrils.  x 50000.