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Chromosome condensation in mitosis and meiosis of rye (*Secale cereale* L.)

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Abstract. Structural investigation and morphometry of meiotic chromosomes by scanning electron microscopy (in comparison to light microscopy) of all stages of condensation of meiosis I + II show remarkable differences during chromosome condensation in mitosis and meiosis I of rye *(Secale cereale)* with respect to initiation, mode and degree of condensation. Mitotic chromosomes condense in a linear fashion, shorten in length and increase moderately in diameter. In contrast, in meiosis I, condensation of chromosomes in length and diameter is a sigmoidal process with a retardation in zygotene and pachytene and an acceleration from diplotene to diakinesis. The basic structural components of mitotic chromosomes of rye are "parallel fibers" and "chromomeres" which become highly compacted in metaphase. Although chromosome architecture in early prophase of meiosis seems similar to mitosis in principle, there is no equivalent stage during transition to metaphase I when chromosomes condense to a much higher degree and show a characteristic "smooth" surface. No indication was found for helical winding of chromosomes either in mitosis or in meiosis. Based on measurements, we propose a mechanism for chromosome dynamics in mitosis and meiosis, which involves three individual processes: (i) aggregation of chromatin subdomains into a chromosome filament, (ii) condensation in length, which involves a progressive increase in diameter and (iii) separation of chromatids.

Although chromosomes have been intensively investigated for more than 120 years, the higher order organization of chromatin and the mode of condensation are still a matter of discussion. Chromosomes are composed of approximately 1/3 DNA, 1/3 histone proteins, 1/3 non-histone proteins and about ten percent RNA molecules, and are compacted within the nucleus in a "density" of about 100 mg/ml which is comparable to a highly viscous polymer gel (Ball, 2003). In interphase chromatin forms a network arranged into three dimensional territories and attached to the nuclear matrix (Cremer et al., 2000). Chromosomes periodically undergo dramatic morphological changes when they highly condense in metaphase.

The mode of compaction of a DNA double helix by a factor of 10,000 into a mitotic metaphase chromosome is still unclear. Only a factor of 100 can be explained by binding of DNA around a histone core into a fiber of about 10 nm in diameter (= elementary fiber) and the postulated helical winding of this 10-nm fiber into a 30-nm fiber (= solenoid). The further mode of compaction to metaphase is discussed by a variety of chromosome models rather controversially, e.g. macrocoil, radial loop, helical coiling, scaffolding and dynamic matrix fibers (Du Praw, 1966; Comings and Okada, 1970; Sedat and Manuelidis, 1978; Mardsen and Laemmli, 1979; Rattner and Lin, 1985; Taniguchi and Takayama, 1986; Burkholder, 1988; Saitoh and Laemmli, 1994; Wanner and Formanek, 2000; Stack and Anderson, 2001). Meiosis is a fundamental process for sexually reproducing organisms and an evolutionary descendant from mitosis, which has acquired additional functions in recombination and reduction of the chromosomal complement (Maguire, 1992). Light microscopical (LM) investigations in meiosis I show that there are differences in chromosome arrangement into a bouquet (instead of Rabl conformation in mitosis), formation of heterochromatic knobs, higher complexity of chromosome morphology as well as formation of a unique pairing structure, the synaptonemal complex, which becomes visible as a tripartite structure only in TEM analysis (Loidl and Jones, 1986; Dawe et al., 1994; Cuvier and Hirano, 2003). In contrast, meiosis II is considered to be analogous to mitosis due to sepa-

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ration of chromatids, which implicates a similar organization and mechanism of DNA condensation.

Despite biochemical, cytogenetic and genetic approaches, which have identified proteins involved in chromosome dynamics (Oakeley et al., 1997; Prymakowska-Bosak et al., 1999; Hall et al., 2003), surprisingly little information is available about structural changes in mitosis and meiosis concerning chromosome condensation and segregation in plants. High resolution scanning electron microscopy (SEM) has proven to be an appropriate tool for investigation of chromatin ultrastructure of human, *Drosophila* and plant chromosomes (Mortin and Sedat, 1982; Allen et al., 1988; Sumner, 1991; Wanner et al., 1991). It is well documented that mitotic metaphase chromosomes are characterized mainly by 30-nm fibers, which are predominantly coiled or ordered into chromomeres (Reznik et al., 1991; Wanner and Formanek, 2000). For barley it was shown that chromatin fibers are compacted during the mitotic cycle in a polar fashion with increased condensation of pericentric regions and less condensation of distal regions (Martin et al., 1996; Wanner and Formanek, 2000). In the present communication, mitotic and meiotic chromosomes of rye are investigated – using LM and SEM – to pursue the questions, whether there is (i) a universal condensation mode for plant chromosomes during mitosis, (ii) a universal chromatin condensation during mitosis and meiosis and (iii) structural similarity in chromatin organization of mitotic and meiotic chromosomes.

Materials and methods

Root tips of germinated seedlings of rye (*Secale sereale* L.) cv. "Sorom" and anthers of florets were fixed with 3:1 ethanol:acetic acid *(v/v)*. Anthers were excised and the developmental stages of the pollen mother cells were checked by light microscopy before they were frozen for at least two days at –20 °C.

Chromosome "drop-cryo" preparation

Root tips and anthers were washed with aqua dest. and digested with 1.25 % cellulase (Onuzuka R-10) and 1.25 % pectolyase (Y-23 Kikkoman) in lyase buffer (75 mmol KCl, 7.5 mmol EDTA, pH 4.0) at 25 °C for 60–80 min. The cell suspension was treated with hypotonic solution (75 mmol KCl) and washed several times with 70 % ethanol before the last washing step followed by 3:1 ethanol:acetic acid *(v/v)*. The drop-cryo preparation was performed according to Martin et al., 1994: suspension was dropped on cold laser-marked glass slides (Laser Marking, Fischen, Germany). Shortly before the chromosome spreads dried completely, a drop of 45 % acetic acid was added, covered with a coverslip and frozen on dry ice. After removal of the coverslips the specimens were fixed in 2.5 % glutaraldehyde (buffered with 75 mM cacodylate, 2 mM $MgCl_2$, pH 7.0).

Preparation for scanning electron microscopy

The samples were washed with cacodylate buffer and aqua dest. before dehydration in an acetone series (20, 40, 60, 80 and 100 %). The specimens were critical-point dried from liquid CO_2 or dried by using HMDS (Merck), mounted on stubs and sputter-coated with approximately 3–5 nm platinum with a magnetron sputter coater (Baltec).

Imaging by LM and SEM

Chromosomal spreads were analyzed in phase contrast (Zeiss Axioplan), and pictures of wet specimens were taken with a CCD camera (Photometrics, Tucson, USA). For analysis by SEM, specimens were examined with a Hitachi S-4100 field emission scanning electron microscope at an accelerating voltage of 8 kV. SEM images were recorded either with black and white negative film or with Digiscan™ hardware and processed with Digital Micrograph 3.4.4 software (Gatan Digital Micrograph, Inc., Pleasantdon, CA, USA). More than 700 measurements of diameters and lengths from about 100 SEM micrographs of 600 chromosome spreads – of each stage in mitosis and meiosis – were taken with an analog map ruler and averaged.

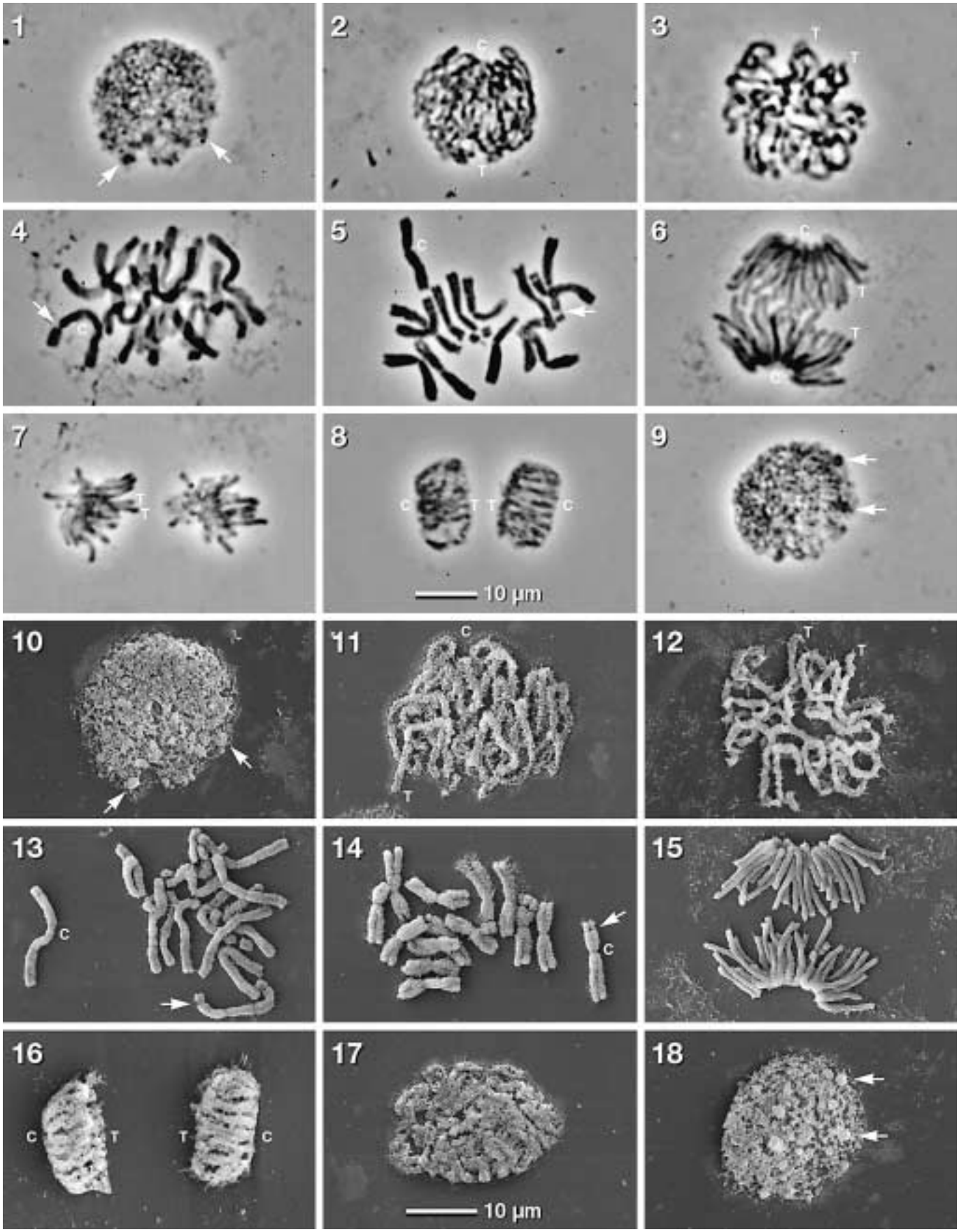
Results

The drop-cryo technique proves to be appropriate for preparation of all stages of both mitosis and meiosis I and II of rye *(Secale cereale)*. By using laser marked slides, more than 600 chromosome spreads from interphase to telophase of mitosis and meiosis I and II were prepared under the same conditions for comparison of chromosomes with light microscopy (LM) (Figs. 1–9; 19–27) and scanning electron microscopy (SEM) (Figs. 10–18; 28–42). Critical-point dried specimens for SEM show only minor shrinkage of chromosomes (compare Figs. 1 and 10; 3 and 12; 6 and 15; 9 and 18) when compared with fully hydrated chromosomes in LM. In contrast to phase contrast analysis, where halo effects prohibit a precise determination of chromosomal dimensions, SEM analysis, due to the substantially higher resolution, allows measurement of chromosomal diameters and lengths in most phases of condensation as well as discrimination of attachment sites from overlays (Figs. 3 and 12; 20 and 29; 21 and 30).

Condensation in mitosis and meiosis

Condensation patterns of mitotic and meiotic chromosomes were investigated by comparative analysis of six stages of condensation: (a) Interphase (premeiotic interphase and leptotene), when chromosomes are largely decondensed (Figs. 1 and 10; 9 and 18; 19 and 28); (b) early prophase (zygotene and pachytene), when chromosomal filaments can be discriminated for the first time (Figs. 2 and 11; 20 and 29; 21 and 30); (c) prophase (diplotene to early diakinesis), when chromosomes become individual filaments (Figs. 3 and 12; 22) (d) prometaphase (late diakinesis), when chromosomes are partly separated and constrictions begin to form in mitosis (Figs. 4 and 13; 31); (e) metaphase (metaphase I), when chromosomes are maximally condensed with prominent constrictions in mitotic stages (Figs. 5 and 14; 23 and 32); (f) anaphase (anaphase I), when chromosomes – still condensed – segregate towards the poles (Figs. 6 and 15; 24 and 33). Measurements of telophase chromosomes were not possible due to their tight association into bundles (Figs. 7, 8 and 16; 34).

Mitotic interphase nuclei of rye typically show few dark and polar-orientated heterochromatin "domains" in phase contrast (Figs. 1 and 9), which can be correlated with more or less compact chromatin regions in SEM (Figs. 10 and 18). Rabl configuration becomes clearly visible in early prophase (Figs. 2 and 11). Individual chromosomes, which can be discriminated in SEM show thickenings at their telomeric regions (Fig. 11). Chromosomes in early prophase exhibit a "rough" surface, due to emanating fibers, which disappears during further condensation to metaphase. Fibrillar "threads" are typically observed in prophase, connecting neighboring chromosomes and seeming to "force" them into an S-like conformation (Fig. 12). Contrary to findings in barley, where individual chromatids are less condensed and are separated in early prophase (Martin et al.,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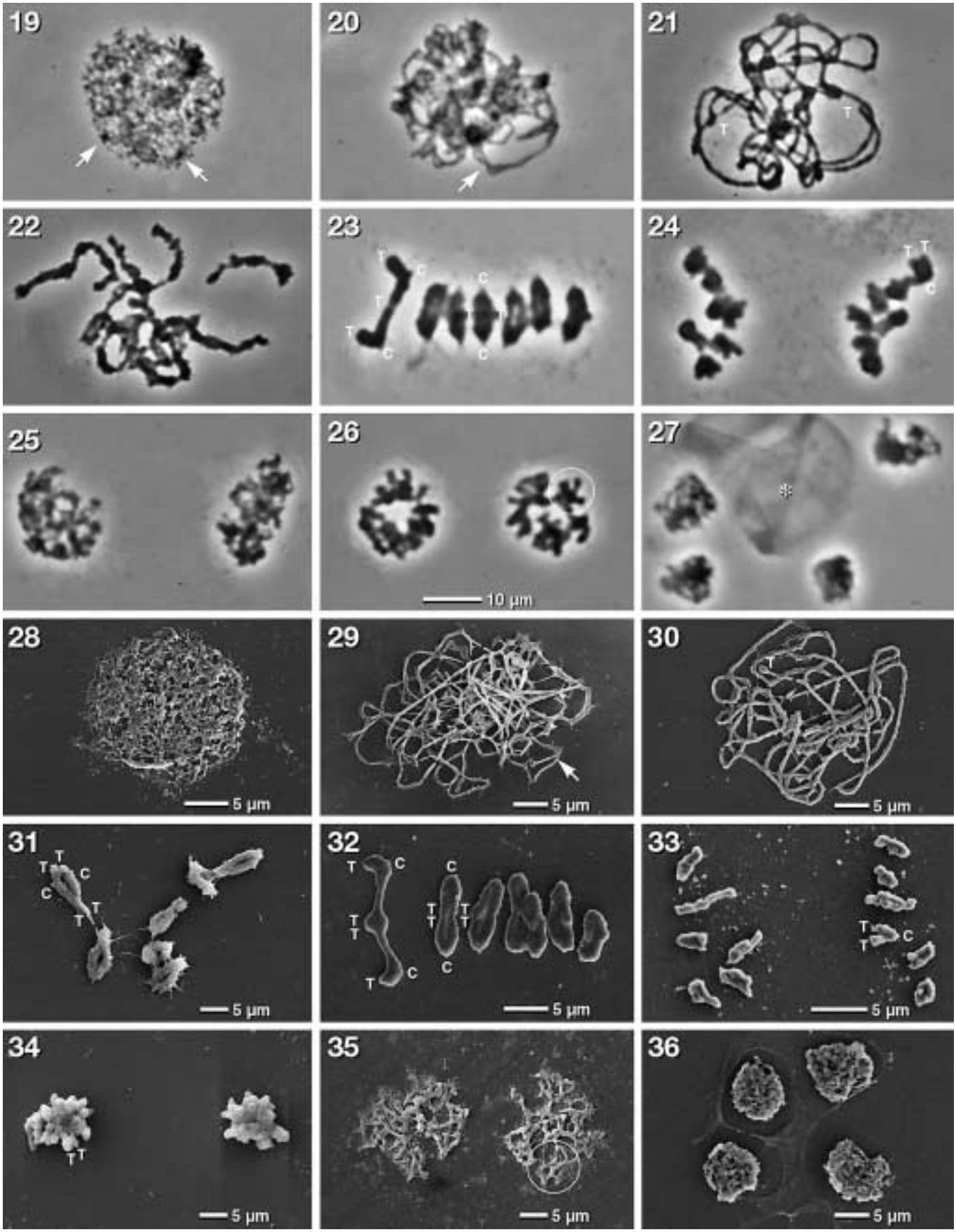
1996), chromatids of rye cohere from initial condensation stages and cannot be discriminated in prophase with LM or SEM. Primary and secondary constrictions (centromere and NOR) are not detectable (neither with LM nor SEM) before chromosomes enter prometaphase (Figs. 4 and 13). The progression from prophase to prometaphase is accompanied by the loss of interconnecting threads (visible only with SEM). Separation of sister chromatids is initiated equally along the chromosome arms in metaphase, but they still cohere at the pericentric region until anaphase (Fig. 14). During anaphase chromatids/chromosomes lengthen and segregate in twin bundles to the poles (Fig. 15). Secondary, but not primary, constrictions are still visible. In telophase decondensation starts more or less evenly over the chromosome arms (Fig. 16), in contrast to barley where decondensation is initiated at the telomeric regions (Martin et al., 1996). Chromosome surface becomes rough again (Figs. 16 and 17). Multiple interconnecting threads between chromatids are visible at higher magnifications (data not shown) before chromosomes decondense to interphase (Fig. 18).

In interphase of meiosis I heterochromatic regions are less pronounced and not oriented to the poles. In leptotene chromosomes become visible as diffuse filaments in LM (Fig. 19). SEM investigation shows a chromatin network of short filaments, which prevents discrimination of single chromosomes over a longer distance (Fig. 28). In zygotene and pachytene chromosomes become visible in LM as thin strands which are more or less regular in thickness (Figs. 20 and 21); in SEM they exhibit a rather smooth surface (Figs. 29 and 30). In contrast to mitosis, chromosomes show only few interconnecting fibrillar threads (Figs. 29 and 30). Chromosomes have no apparent orientation (Figs. 20 and 21; 29 and 30), and bouquet arrangement was not observed after spreading. Typically, 1 to 4 club-shaped telomeres are prominent (Figs. 21 and 30). From zygotene to early diplotene chromosome arms condense uniformly. Beginning in late diplotene, chromosomes become significantly shorter with extreme changes in diameter, leading to a highly irregular appearance (Fig. 22). In diakinesis bivalents separate longitudinally, and chiasmata become visible at terminal regions (Fig. 31). In metaphase I, when centromeres are opposed, no constriction sites are visible in LM or SEM (Figs. 23 and 32). In contrast to anaphase in mitosis, where chromosomes segregate as chromatid bundles, in anaphase I of meiosis chromosomes segregate individually (Figs. 24 and 33; compare with Figs. 6 and 15). From late anaphase I to telophase I separation of individual chromatids becomes barely visible at the telomeres (Figs. 24; 33 and 34). Meiosis II is initiated by a slight decondensation in interkinesis (Fig. 25). In prophase II and metaphase II chromatids are widely separated with exception of the pericentric region (Figs. 26 and 35). Multiple connections between non-homologous chromatids cause a "kinky" appearance of the chromosome arms (Fig. 35). In telophase of meiois II, chromosomes are largely decondensed (Figs. 27 and 36).

Chromosome ultrastructure

SEM analysis confirmed that the dominant structural element of chromosomes in mitosis and meiosis are 30-nm fibers, visible from interphase to prophase (Figs. 37 and 38; 40 and 41; Table 1); 10-nm fibers are observed less frequently. In prophase of mitosis 30-nm fibers are predominantly arranged into coiled structures, which are characteristically organized into larger globular elements (= chromomeres) interspersed by regions of longitudinal fibers (Figs. 37 and 38). In prometaphase interspersed segments of longitudinal fibers are less frequent, while chromomeres of variable sizes are formed. In metaphase the chromosomal surface is characterized by increasingly compacted chromomeres (Fig. 39). Longitudinal fibers are no longer visible with exception of primary and secondary constriction sites. Similar to mitosis, chromosomes in meiosis are predominantly organized by coiled and longitudinally arranged 30-nm fibers. In meiosis however, the alternating distribution of these elements is only visible in early prophases I (Figs. 40 and 41) and II (data not shown). In zygotene and early diplotene longitudinal fibers alternate with chromomeres, which are less prominent and more elongated in contrast to mitosis (Fig. 41; compare with Fig. 38). During condensation in meiosis I, chromosome surface changes structurally in several respects: In prophase I (diplotene to diakinesis) chromosomes vary widely in diameter. The chromosome surface, which appears "smoother" at moderate magnifications and exhibits only few protuberances, is typically organized by 30-nm fibers

Figs. 1–18. Mitotic cycle of rye *(Secale cereale)* in LM (**1–9**) and SEM (**10–18**) (C = centromere; T = telomere; NOR = nucleolus organizer region). In LM, interphase chromatin appears decondensed, with dark polar-orientated heterochromatic regions (**1**, arrows). In prophase, chromosome strands become visible lying in Rabl configuration; chromosome arms are parallely ordered (**2**). In late prophase chromosomes shorten; some telomeres become visible (**3**). In prometaphase constrictions of NORs (**4**, arrow) become visible for the first time, while primary constrictions (C) are hardly detectable. In metaphase chromosomes spread well and show characteristic constrictions at the centromere (**5**) and NOR (**5**, arrow). In anaphase chromatids are bundled at the centromeric regions but free at telomeres (**6**). In telophase chromosome bundles are so compact that only telomeric regions are discernible (**7**). Decondensation in late telophase is characteristically accompanied by elongation of chromosome arms which become diffuse (**8**). During decondensation chromosomes adopt an interphase stage in which individual chromosomes can no longer be distinguished and heterochromatic "spots" can be observed (**9**, arrows). In SEM, chromatin in interphase shows a fibrillar network in which some compact regions can be correlated with dark heterochromatic regions in LM (**10**, arrows; same interphase spread as **1**). In prophase chromosomes exhibit a rough surface and are connected by chromatin threads (**11**); in contrast to LM analysis, individual chromosomes can be clearly recognized. In late prophase chromosomes condense to a higher degree, appear smoother and are still connected by chromatin threads, forcing chromosomes into an S-like conformation (**12**, same late prophase spread as in **3**). In prometaphase chromosome surface becomes even smoother (**13**); constrictions of NOR (**13**, arrow) and centromere are still hardly visible. In late metaphase chromosomes are highly condensed, and separation of chromatids becomes apparent (**14**); primary and secondary constrictions are clearly expressed only at this stage (**14**, arrow). In anaphase chromosomes appear smooth and are bundled at the centromeres (**15**, same anaphase spread as in **6**). In late telophase chromosome arms start to decondense while chromosome surfaces become rough again (**16**). During transition to interphase individual chromosomes are no longer discernible, while interconnecting chromatin threads become visible again (**17**). In interphase several compact regions can be again correlated with dark heterochromatic "spots" (**18**, same nucleus as in **9**).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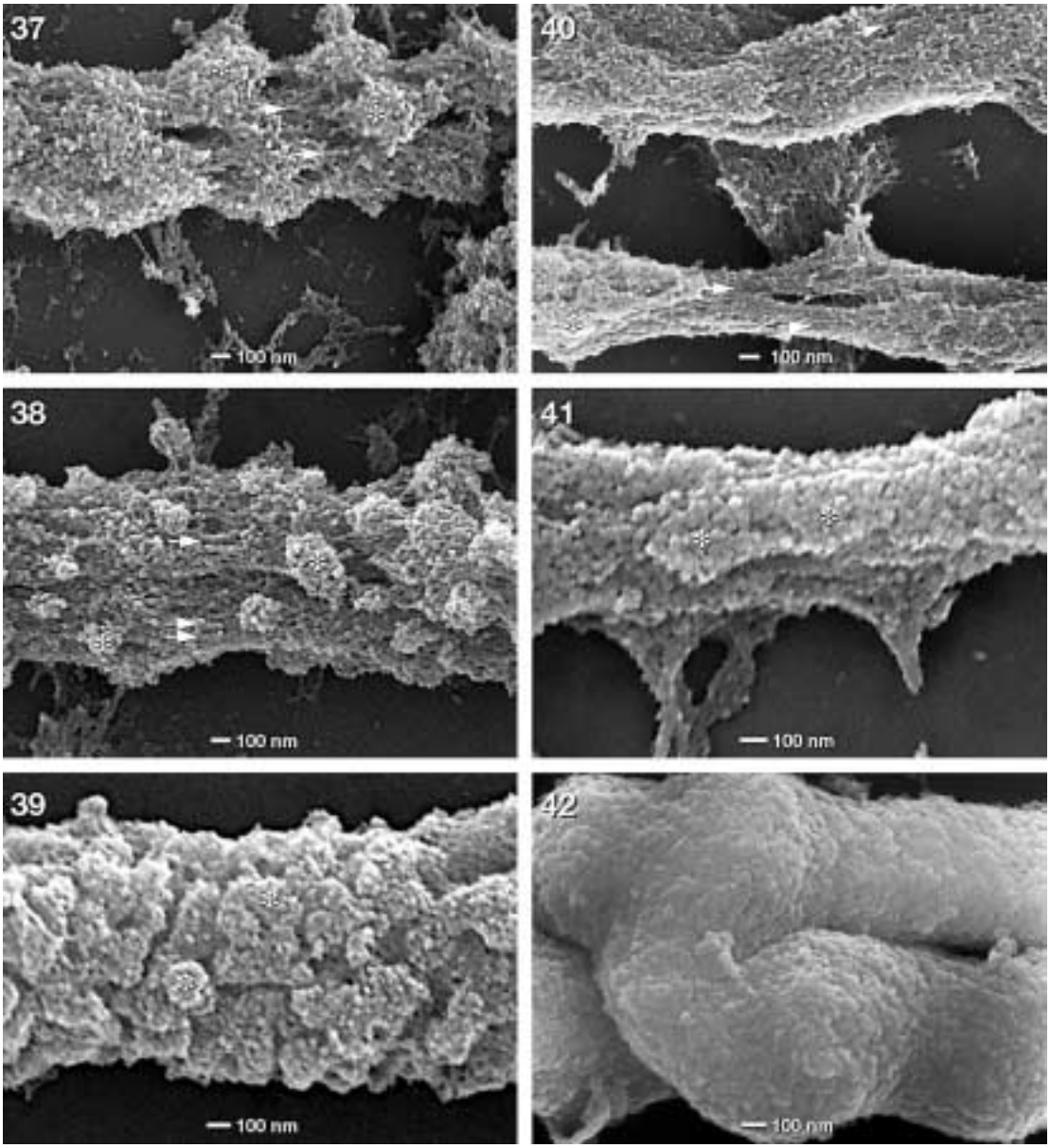

Figs. 19–36. Meiotic cycle of rye *(Secale cereale)* in LM (**19–27**) and SEM (**28–36**); (C = centromere; T = telomere). In LM, early leptotene chromatin appears granular with minor dark chromodomains (**19**, arrows). In zygotene chromosomes are densely coiled; at few sites paired and unpaired homologs are apparent (**20**, arrow). In pachytene homologs are completely paired and lying disordered (**21**); only few telomeres can be discriminated. In late diplotene paired homologous chromosomes are irregularly shaped (**22**). In metaphase I bivalents are still connected at terminal chiasmata, whereas centromeres are opposed to each other; constriction sites are not visible (**23**, note: left bivalent is lying in an open ring formation). Homologs segregate individually in anaphase I where separation of chromatids becomes visible at the telomeres (**24**). From interkinesis to early prophase II chromosomes lie disordered (**25**). In metaphase II chromatids are largely separated but still cohere at the centromeres, leading to X-shaped chromosomes (**26**, circle). In telophase II chromatin of tetrads is characteristically decondensed (**27**, asterisk designates wall of pollen mother cell). SEM micrographs show that in leptotene chromatin is arranged as in interphase; chromosomes are hardly detectable (**28**). In zygotene "pairing forks" at sites of paired and unpaired homologs can be discriminated (**29**, arrow). In pachytene homologs are completely paired; only few club-shaped telomeres become visible (**30**). In diakinesis chromosomes are highly condensed, still paired at their chiasmata at the telomeric regions (**31**). In metaphase I chromosome surface is "smooth"; neither primary nor secondary constrictions are detectable (**32**). The telomeric regions are characteristically fused. In anaphase I homologs separate individually (**33**). In telophase I forked telomeres become evident (**34**, each T represents the telomere of one chromatid). In prophase II chromatids are widely separated; the "kinky" chromosomes can hardly be discerned as individuals (**35**, circle). In telophase II of early tetrad stage chromosomes are decondensed again with a granular (interphase-like) appearance (**36**).

Figs. 37–42. High resolution SEM micrographs of mitotic (**37–39**) and meiotic chromosomes (**40–42**) of rye *(Secale cereale)*. In mitosis the dominant substructures of early prophase chromosomes are "chromomeres" (**37**, asterisks) interspersed by longitudinally arranged fibers (**37**, arrows). In prophase the number of chromomeres increases; longitudinal fibers (**38**, arrow) are prominent at regions with lower density of chromomeres (**38**, asterisks). In metaphase, surface of mitotic chromosomes is composed of densely packed chromomeres of different sizes (**39**, asterisks). In meiosis I chromosomes of early prophase (pachytene) are organized by 30-nm fibers forming knobby substructures (**40**, asterisk), which are interspersed by longitudinally arranged fibers (**40**, arrows). In prophase (diplotene) surface appears more granular; single elongated chromomeres become obvious (**41**, asterisks); longitudinal fibers are hardly detectable. In metaphase I chromosome surface is characteristically smooth: neither chromomeres nor parallel fibers are visible (**42**).

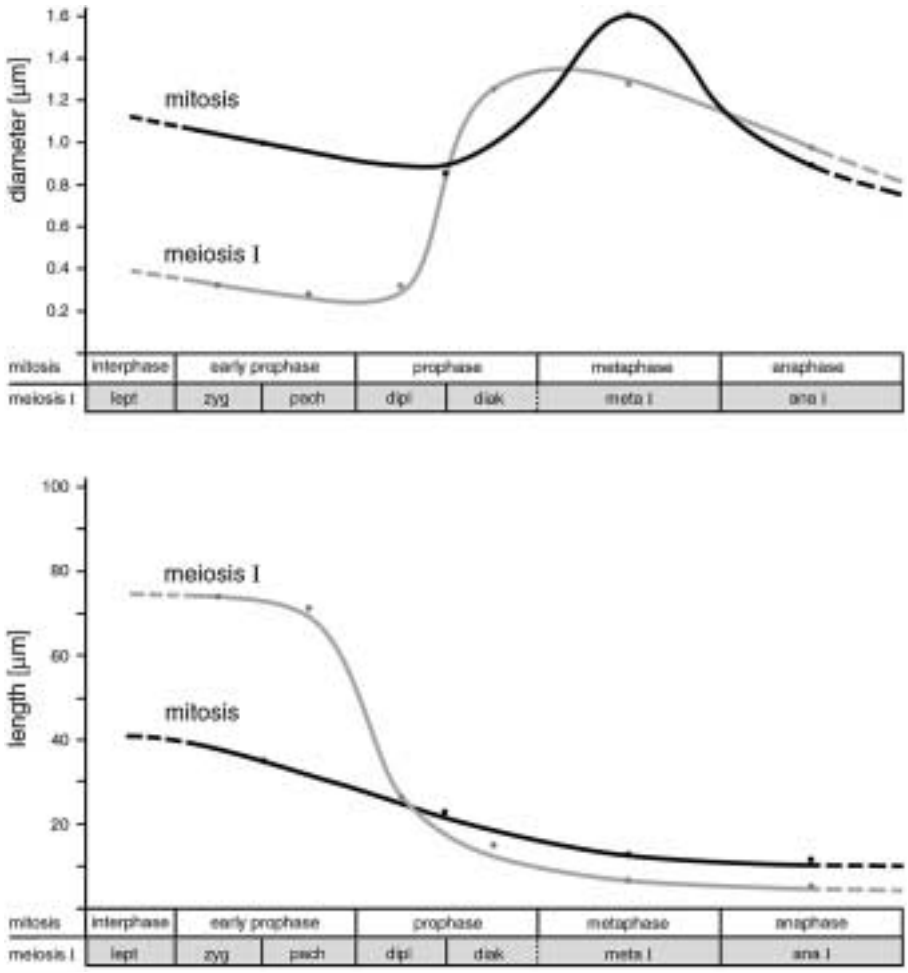

Fig. 43. Schematic representation of condensation mode in mitosis and meiosis I of rye *(Secale cereale)* deduced from measurements of chromosomal diameters and lengths. Individual stages of mitosis are correlated with meiosis I as follows: interphase of mitosis (premeiotic interphase to leptotene = lept), early prophase (zygotene = zyg, pachytene = pach), prophase (diplotene = dipl, diakinesis = diak), metaphase (metaphase I = meta I), anaphase (anaphase I = ana I). In meiosis the decrease in diameter and shortening in length create a sigmoidal curve, in contrast to mitosis where changes in dimension are more linear.

Table 1. Chromosome and chromatin (30-nm fiber) characteristics in meiosis (I and II) compared to that in mitosis

	centromere/NOR[b]				telomeres[b]				chromomeres[b]				fibers and surface[c]			
cell cycle[a]	pro	met	ana	telo	pro	met	ana	telo	pro	met	ana	telo	pro	met	ana	telo
mitosis	–	+	±	–	+	+	+	+	+	+	–	–	p/c	p/c	c	s
meiosis I	–	–	–	–	±	±	+	+	±	–	–	–	p/c	c/s	s	s
meiosis II	–	–	–	–	+	+	+	+	–	–	–	–	p/c	c	c	p/c

[a] Not synchronized and not arrested. pro = prophase; met = metaphase; ana = anaphase; telo = telophase.
[b] + = visible; ± = rarely visible; – = not observed.
[c] p = parallel fibers; c = coiled fibers; s = smooth surface.

and small knobs of about 30 nm in diameter, leading to a granular appearance (Fig. 41). Longitudinally arranged fibers and chromomeres are no longer visible when condensation progresses to the maximum. Chromosomes in metaphase I exhibit the highest degree of compaction and a strikingly smooth surface at moderate magnifications (Fig. 42). Constriction sites are not formed in metaphase of meiosis I and II (Table 1).

Chromosomal dimensions

For quantitative comparison, lengths and diameters of chromosomes were measured from SEM micrographs taken at various stages in mitosis and meiosis. In contrast to mitosis of barley, both chromosome arms of rye condense without preference of centromeric regions. From prophase to metaphase in mitosis, the chromosome diameter increases from 0.86 to 1.6 µm with a continuous reduction of the length from 35.6 to 12 µm. However, during early prophase a minor but significant reduction of the diameter of about 13% becomes obvious (Fig. 43). In general, in early prophases the chromosomal diameter is greater in regions with chromomeres, compared to those exhibiting longitudinal fibers (Fig. 37). Over a short period, telomeric regions become more strongly condensed. In metaphase the

diameter is characteristically decreased by 10% compared to twice the diameter of a single chromatid in anaphase, indicating an intimate association of sister chromatids in metaphase (Fig. 43). After separation in anaphase and telophase, chromosomes start to decondense by elongation and expansion in width.

Measurements establish that condensation in mitosis and meiosis of rye proceeds more or less uniformly along the chromosome arms, but also accentuate differences in time, degree and mode of compaction: In early prophase I (zygotene and pachytene) chromosomes are characterized by a remarkably reduced diameter (380 nm) and significantly increased length (74 μm) compared to mitosis (1 and 35.6 μm respectively). From zygotene to pachytene, chromosomal diameter and length remain rather constant, indicating that condensation pauses, with exception of few telomeres which appear club-shaped in pachytene (Fig. 30). Measurements of aligned and not aligned chromosomes show that the diameter decreases when homologs pair in pachytene, indicating the intimate association of sister chromatids and/or synapsis of homologs (Fig. 43). During diplotene the chromosomes condense in length from about 45 μm in early diplotene to 17.2 μm in late diplotene, while the diameter increases from about 400 nm to 1.0 μm. Condensation seems to be uneven from diplotene to diakinesis, as deduced from variations in diameter (380 nm to 1.27 μm). Chromosomes in metaphase are compacted by a factor of 1.7 in length in meiosis I (7.1 μm) compared to mitosis (12.4 μm) and by a factor of 1.3 comparing diameter of meiosis I (1.25 μm) with mitosis (1.6 μm). By extrapolation of diameter and length (and the presumption of a cylindrical chromosome shape) it becomes obvious that the calculated chromosome volume of 8.7 μm^3 is remarkably low – only 35% – in meiosis I compared to mitotic metaphase with a volume of 24.9 μm^3. Meiosis II is initiated by an incomplete decondensation of the chromosomes. In metaphase II, diameters of individual chromatids (0.91 μm) are more or less comparable to diameters of chromatids in mitosis, in contrast to chromosomal lengths (6.7 μm) which are strikingly decreased by a factor of 1.8 compared to mitosis.

Discussion

Differences in condensation in mitosis of barley and rye

Chromosome compaction in mitosis has been described by Sakar et al. (2002) for animals as gradual thickening of chromosomes, which is in accordance with our observations in mitosis of rye *(Secale cereale)*, where chromosomes condense more or less uniformly along the arms. Only few telomeres show a higher degree of compaction in prophase. This finding differs from observations in barley, where chromosomes condense and decondense in a polar fashion: telomeric regions are less condensed and do not cohere in prophase, while proximal regions are highly condensed (Martin et al., 1996; Wanner and Formanek, 2000). These differences in condensation of two closely related cereals, with genome and chromosomes of similar size, indicate that compaction can vary significantly. Obviously condensation may be influenced by subdomain-specific characteristics, e.g. position of highly repetitive sequences within C- and N-bands, which are located proximally in barley and distally in rye (Gill and Kimber, 1974; Schlegel and Gill, 1984; Pedersen et al., 1996; Zoller et al., 2001).

Rabl and bouquet stages

In mitosis centromeres and telomeres of plants like barley, rye, sorghum and wheat are characteristically oriented into Rabl configuration in interphase and prophase (Dong and Jiang, 1998; Cowan et al., 2001). In early prophase of meiosis I, chromosomes of most fungi, plants and animals are ordered into the bouquet, with only telomeres attached to distinct regions of the nuclear membrane (Loidl, 1990; Scherthan et al., 1996; Bass et al., 1997; Cowan et al., 2001; Carlton and Cande, 2002). For rye it was recently reported that bouquet arrangement of chromosomes is variable to some extent (Noguchi, 2002). With SEM it is shown that chromosome spreads in mitosis are integrated in a network of interconnecting threads when lying in Rabl orientation, which might stabilize single chromosomes into a polar orientation after degradation of the nuclear envelope (see Figs. 11 and 12). These connections possibly force mitotic prophase chromosomes into an S-like conformation which might represent "macrocoils" described by several authors (Paulsen and Laemmli, 1977; Rattner and Lin, 1985; Hadlaczky et al., 1986). In meiosis I chromosomes apparently lack such interconnecting threads which may stabilize the chromosomal arrangement in a bouquet after removal of the envelope, causing them to spread onto the glass slides apparently disordered.

Ultrastructural changes in chromatin arrangement in mitosis and meiosis

As shown by SEM, chromatin in leptotene of meiosis I is organized – comparable to interphase – into a network of coiled regions and interspersed longitudinal fibers which can be correlated with the "striped pattern" and "patches" of chromatin density (Sakar et al., 2002) – characteristics, which are attributed to heterochromatic and euchromatic regions in LM. Chromosome threads, which become clearly visible in early prophase of mitosis, are hardly detectable in leptotene. These observations might reflect a loose grouping of chromosomal domains in early meiosis I, as proposed for first search of homology, before homologs synapse intimately from zygotene to pachytene (Kleckner, 1996; Bhatt et al., 2001). As speculated for years, chromatin in meiosis I adopts a specific conformation and undergoes a general structural rearrangement, elongation of heterochromatic knobs and reduction of heterochromatin when starting recognition, alignment and synapsis of homologs (Stack, 1984; Dawe et al., 1994; Kleckner, 1996; Zickler and Kleckner, 1998).

Although the basic "equipment" of mitotic and meiotic chromosomes is similar (30-nm fibers; coiled and parallel), there is a significant difference especially in the formation of chromomeres (Martin et al., 1994, 1996; Iwano et al., 1997; Wanner and Formanek, 2000), which increase in size and number from prophase to metaphase in mitosis. Chromosomes in meiosis, however, exhibit elongated chromomere-like structures in early prophase. They completely disappear during con-

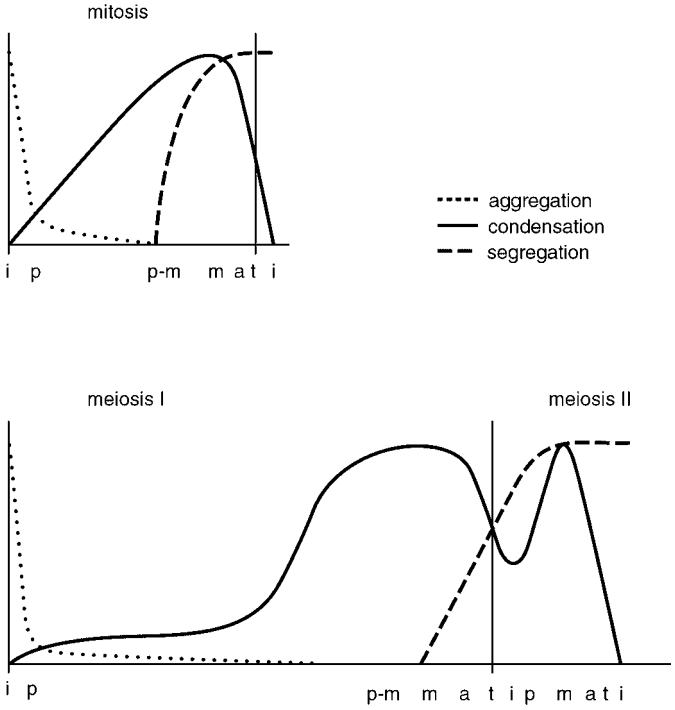

Fig. 44. Schematic comparison of chromosome condensation in mitosis and meiosis: Three mechanisms (aggregation, condensation, segregation) are proposed for chromosome condensation in width and length which are triggered in a mitotic and meiotic specific mode. Correlating stages are labeled as follows in mitosis/meiosis II and meiosis I respectively: interphase (premeiotic interphase to leptotene) = i; prophase (zygotene to diplotene) = p; prometaphase (diakinesis) = p-m; metaphase (metaphase I) = m; anaphase (anaphase I) = a; telophase (telophase I) = t.

densation to metaphase I and II, which may be explained by the extreme degree of compaction of meiotic chromosomes (approximately 2/3 more than in metaphase of mitosis) that obscures these structures. Chromosome surface in meiosis I becomes rather smooth (see Figs. 40–42) which is in accordance with SEM investigations on cryo-fractured pollen mother cells of *Tradescantia reflexa* (Inaga et al., 2000).

Mode of chromosome condensation in mitosis and meiosis

Mitosis and meiosis of rye differ in time and mode of condensation: chromosomes in mitosis condense within 11.5 h, in meiosis I within 48 h, and in meiosis II within 5.6 h (Scoles and Kaltsikes, 1977). During compaction, chromosomes in mitosis and meiosis become dynamically reorganized by a non-autonomous behavior – which involves physical interactions between chromosomes and segregation of chromatids by the spindle fiber apparatus – and an autonomous behavior – which results in a global reorganization of chromosomes (Cowan et al., 2001). Comparison of correlating stages of condensation shows that the autonomous behavior is apparently affected by specific mechanisms both in mitosis and meiosis. Differences pertain to (i) initiation of condensation, (ii) mode of aggregation, (iii) degree of compaction and (iv) sister chromatid segregation (Fig. 44).

Initiation of condensation

Earliest condensation of chromatin in meiosis I is accompanied by a dramatic decrease of the chromosomal diameter (compared to mitosis) indicating substantial differences to mitosis. Dark heterochromatic regions (= chromodomains) which are best visible in mitotic interphase can be correlated with highly compact chromatin in SEM (see Figs. 1 and 10; 9 and 18). They colocalize with prominent FISH signals of the subtelomeric repeat pSc 119.2 (data not shown). These heterochromatic regions are less pronounced (both in LM and SEM) in meiosis, indicating that chromatin of this stage is less condensed. Observations of multicolor banding analysis on human chromosome 18 proves that the "chromosomal axis" in mitotic interphase has a comparable length to that of prophase at a 600-band resolution (Lemke et al., 2002). If this observation is also valid for meiosis, it would imply that condensation in meiosis starts earlier in interphase, when chromosomes are more elongated – in contrast to mitosis – probably due to more decondensed heterochromatic domains. Aggregation from less dense and more "relaxed" chromatin status in premeiotic S-phase could subsequently affect chromosomal diameter and length as well as the further mode of chromatin compaction. LM data of early meiosis I of mouse and man show that the association of chromosome territories and grouping is determined prior to the association of homologs (Scherthan et al., 1996). This specialized conformation, which occurs in rye when chromosomes are still decondensed, may facilitate homolog recognition (Dawe et al., 1994; Noguchi, 2002).

Mode and degree of condensation

In contrast to chromosome models postulating a helical winding of individual chromatids/chromosomes during condensation (Stack and Anderson, 2001), no structural evidence was found for helical winding in either mitosis or meiosis. Mitotic chromosomes of rye condense in a linear fashion from prophase to metaphase, with a linear decrease of the transcription rate up to metaphase (Cho et al., 1998). In meiosis I the condensation curve is sigmoidal (see Figs. 43 and 44), retarded in early prophase I (leptotene to pachytene) and accelerated from prophase to metaphase I (diplotene to diakinesis). Only very little information is available about transcription rates during meiosis (Morohashi et al., 2003), but a chromatin state, open for transcription as well as open for late replication events as described for zyg-DNA in *Lilium*, has been discussed to be important for initiating of homolog recognition and recombination (Hotta et al., 1985). Elongated and less condensed chromosomes would present more potential sites of homology which might facilitate recognition and synapsis of homologs. Retardation of condensation in meiosis may also facilitate crossing-over and Holiday junction which probably would be disturbed by simultaneous condensation. Perturbations of meiocytes may have severe effects on synapsis and crossing-over resulting in aneuploidy and miscarriage. Indications for coupling of condensation and recombination are supported by observations with ditelosome rye-wheat lines, which are deficient in synapsis, leading to an increased condensation and incorrect segregation in meiosis I (Maestra et al., 2002).

Both chromosome and chromatid segregation in anaphase of meiosis I and II are generally regarded to be more critical than in mitosis. The extreme condensation in metaphase – which possibly prevents formation of constriction sites – may be important for a proper segregation of meiosis I and II, as there is a greater necessity for structural compactness and genome stability, to prevent loss of genetic material and to maintain the genetic continuity of germlines. Little is known about physical forces onto centromere regions when the spindle fiber apparatus separates paired bivalents, which are still connected at their chiasmata. It has been discussed for *Oenothera* that during ring formation in meiosis, the physical forces at the chromosomes are twice of those operating during mitosis (Hejnowicz and Feldman, 2000).

Sister chromatid aggregation and segregation

Sister chromatid cohesion in mitosis differs in rye and barley: in rye chromatids are completely paired in early prophase, in contrast to barley where chromatids are dissociated at distal regions up to late prophase. For meiosis I sister chromatids are sketched in most textbooks as separated along the chromosome arms. Noguchi (2002) describes that four chromatids are co-aligned in parallel but remain separated up to diakinesis with telomeric regions that were fixed by chiasmata. SEM analysis proves that this is not true for rye, where cohesion of sister chromatids forms one single "structural" chromosome which remains intact (with exception of "pairing forks") from zygotene up to early anaphase I (Zoller et al., 2004). The slight decrease of about 10 % of the chromosome diameter from early prophase to prophase in mitosis and meiosis I (Fig. 43) may be explained by a transversal condensation process, but could also be due to a tight cohesion, or even, in the case of meiosis, linked to homologous pairing and formation of synaptonemal complex.

Sister chromatid dissociation is reported to be initiated in late meiosis I to ensure a correct segregation of chromosomes (Miyazaki and Orr-Weaver, 1994; Cohen-Fix, 2000; Zoller et al., 2004) and subsequently proceeds to metaphase II. These observations indicate that association of sister chromatids is not analogous (in terms of time and mode) to that of mitosis. These structural data further suggest that chromosome condensation is essential for a proper segregation of chromatids, but association of chromatids does not seem to be a prerequisite for condensation in meiosis II. This would imply that condensation and segregation are two processes which are coupled and may be regulated by cell-specific and chromosome region-specific factors.

A dynamic model for chromosome condensation in mitosis and meiosis

Chromosome condensation, both mitotic and meiotic, can be regarded as a dynamic and autonomous multi-step process concerning segregation of replicated DNA. Based on ultrastructural data, we propose that there are three main processes important for correct chromosome dynamics: aggregation, condensation and segregation (see Fig. 44):

I. Aggregation of interphase chromatin into distinct chromosomal filaments involves a transversal condensation process, manifested by a decrease in diameter in early prophases.

II. Condensation progression to metaphase is a uniform process along the chromosome arms in rye, leading to a reduction in length and increase in diameter.

III. Segregation of chromatids, which is initiated at maximum of condensation in metaphase and (as shown in meiosis II) can be maintained once initiated.

The proper balance between these three processes seems to be important for chromosome dynamics in mitosis and meiosis. Each of these three processes may be modulated in a cell cycle-specific manner (Bomar et al., 2002), which would explain structural differences in mitosis and meiosis observed in this investigation.

Structural changes documented in this study conform with the "dynamic matrix" model for chromosome condensation (Wanner und Formanek, 2000), which proposes that solenoids bind to "matrix fibers" and aggregate to higher substructures (= chromomeres). As condensation progresses in mitosis, chromomeres accumulate, causing successive shortening and thickening of chromosomes as more chromomeres are formed. To what extent the dynamic matrix model can be applied for chromosome condensation during meiosis is speculative. As chromomeres and parallel fibers are observed in principle, and condensation proceeds in a linear fashion without formation of any helical structures, we would expect that the mode of chromatin compaction in meiosis is similar to mitosis. Further experiments, e.g. controlled loosening after fixation and DNA specific staining with platinum blue (Wanner and Formanek, 2000), could provide more information on chromosome substructure and the mechanisms underlying meiosis.

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