

Meiosis I and II share chromosome segregation dynamics distinct from mitosis in the firebug *Pyrrhocoris apterus*

Alexander Lindner

Technische Universität Dresden

Gunar Fabig

Gunar.Fabig@tu-dresden.de

Technische Universität Dresden

Chukwuebuka William Okafornta

Technische Universität Dresden

Silke Tulok

Technische Universität Dresden

Anja Nobst

Technische Universität Dresden

Vlastimil Smýkal

Biology Centre of the Czech Academy of Sciences

David Doležal

Biology Centre of the Czech Academy of Sciences

Leocadia V. Paliulis

Bucknell University

Thomas Müller-Reichert

Technische Universität Dresden

Research Article

Keywords: *Pyrrhocoris apterus*, meiosis, mitosis, spindle, anaphase, holocentric chromosomes, lagging chromosome

Posted Date: October 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11267621/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Meiosis I, meiosis II, and mitosis use overlapping sets of structural and regulatory molecular components to align and partition chromosomes, yet they serve different biological functions and must orchestrate chromosome segregation accordingly. Direct comparisons between division types are complicated when mitosis and meiosis are examined in different species or genetic backgrounds. Here, we used quantitative live-cell imaging to compare the dynamics of chromosome segregation and spindle elongation across all three division types within a single organism, the male germ line of the firebug *Pyrrhocoris apterus*. Tracking pole-to-pole, chromosome-to-chromosome, and pole-to-chromosome distances from metaphase through anaphase, we found that meiosis I and meiosis II are remarkably similar: the initial and final chromosome segregation distances, segregation speeds ($\approx 1.6 \mu\text{m}/\text{min}$), and the proportions of poleward chromosome movement (anaphase A) and spindle elongation (anaphase B) were nearly identical between the two meiotic divisions. Mitotic spermatogonia differed on all of these measures, segregating chromosomes over a shorter distance and at a slower speed ($\approx 1.1 \mu\text{m}/\text{min}$). Strikingly, the proportions of poleward chromosome movement and spindle elongation were inverted between division types: spindle elongation accounted for the majority of chromosome segregation in mitosis ($\approx 61\%$) but only a minority in meiosis ($\approx 26\text{--}32\%$). Furthermore, final spindle length and chromosome segregation distance correlated with cell size across the three division types but not within any single type, indicating that these dimensions are set by division identity rather than by continuous scaling with cell size. Together, our results show that the two meiotic divisions of *P. apterus* are highly similar to one another yet distinct from germ-line mitosis.

Introduction

Meiosis I, meiosis II, and mitosis all use similar sets of structural and regulatory molecular components to align and segregate chromosomes. However, each division serves a different biological function. The aim of mitosis is to distribute sister chromatids such that daughter cells are formed with sets of chromosomes that are genetically identical to one another and also to the parental cell. In contrast, meiosis aims to generate germ cells with a haploid genetic content. This is achieved by two consecutive divisions without intervening DNA replication: meiosis I separates homologous chromosomes, while meiosis II separates sister chromatids. Although the three divisions have different aims, all use bipolar spindles to segregate chromosomes. Variations in microtubule architecture and kinetochore structure and behavior allow customization of chromosome segregation by division type. What variations specifically distinguish the three division types?

Previous studies in animal systems noted that mitosis and spermatocyte meiosis are typically centrosomal (Schatten 2022), while oocyte meiosis is typically acentrosomal (Gruss 2018). Comparisons between mitosis and meiosis, however, often rely on comparisons of cells in mitosis of one species or genetic background with cells in meiosis from a different species or genetic background—or are done more general across many taxa (Fabig et al. 2020; Sato et al. 2021; Okafornta et al. 2026). A solid understanding of differences between the division types requires observations of mitosis, meiosis I, and meiosis II within the same species using identical quantitative analysis tools.

Both the kinetochore arrangement and the distribution of chromosome cohesion are built into the chromosome prior to each division, and are different in all three divisions in grasshoppers (Paliulis and Nicklas 2000, 2004, 2005). These differences are critical to achieving the aims of each division, but there remains a need for a

thorough comparison of physical characteristics (e.g., kinetochore position, chromosome behavior, cell shape, spindle size and shape, rates of chromosome movement, etc.) of cells in mitosis, meiosis I, and meiosis II. In grasshoppers, the size of cells in mitosis, meiosis I, and meiosis II appears to differ, with meiosis I cells appearing larger than meiosis II and mitotic cells (Paliulis and Nicklas 2004, 2005).

Are cell-size differences reflected in possible spindle size or chromosome segregation rate differences? Spindle scaling might explain some differences between division types. In the *Drosophila* blastoderm, for instance, the rate of anaphase chromosome movement scales directly with spindle size, with smaller spindles carrying slower anaphase chromosomes (Xu et al. 2025). In contrast, the dynamics of chromosome segregation (i.e., the speed of segregation) in *Caenorhabditis elegans* embryos remain mostly unchanged with cell size reduction during development, while spindle length elongation in this nematode system is dependent on cell size, whereas chromosome segregation is not (Okafornta et al. 2026). Whether spindle elongation (anaphase B) and segregation speed are similarly decoupled in an insect with three comparable division types was unknown.

Are differences in cell area, spindle size, and chromosome distances at the end of anaphase consistent with some form of spindle scaling? Alternatively, are differences in mitotic *versus* meiotic chromosome behavior and spindle characteristics simply division-type specific? In the nematode system, for instance, there are striking differences between the first mitotic division and the male meiotic divisions. The male meiotic divisions show a prominent shortening of the pole-to-chromosome distance (anaphase A), whereas in embryos, this is obvious in later divisions (Fabig et al. 2020; Okafornta et al. 2026).

To address these questions, we used the firebug, *Pyrhocoris apterus* (Linnaeus, 1758) as our model system. *P. apterus* is abundant in wild populations across Europe, easily collected, and amenable to culture in the laboratory. In testes of juvenile males, cells in mitosis (spermatogonia), meiosis I, and meiosis II can be found with reasonable ease, allowing a comparison of meiotic *versus* mitotic cell division, and thus a systematic study within a single system. The organism has a long history of being studied, and observations of its chromosomes were first published in 1891 by the German zoologist Hermann Henking. In cell spreads obtained from firebug testes, Henking observed a single chromosome that behaved differently from the others. Henking labeled this abnormally behaving chromosome as 'element x', which later was determined to be the largest chromosome in the firebug (Henking 1891; Wilson 1909; Paliulis et al. 2023). In 1909, E.B. Wilson confirmed Henking's observation of the accessory chromosome, which is nowadays known as the sex chromosome, namely the X chromosome, in addition to noting that the species has holocentric chromosomes in mitosis (Wilson 1909).

Here, we report on chromosome segregation and spindle characteristics in meiosis I, meiosis II, and mitosis, showing division of living cells within the same species. We note differences in spindle size and rates of anaphase movement in these different cell types. We also test hypotheses about the impact of cell size on spindle size and chromosome distances. We determined that spindle and chromosome dynamics were very similar in meiosis I and II but different from cell division in germ-cell mitosis. Spindle elongation contributed the major part of chromosome segregation in mitosis, but only a minor part in meiosis. Across the three division types, final spindle length and inter-chromosome distance correlated with cell size, but this reflected differences between division programs rather than continuous scaling within a division.

Material & Methods

Collection and maintenance of *P. apterus*

Male firebugs were either collected from natural populations in Dresden (51.0870° N, 13.6746° E; Germany) while the photoperiod (daily duration of light) exceeded 12 hours per day, or acquired as a laboratory strain (Doležal laboratory; (Pivarciova et al. 2016)) during photoperiods of less than 12 hours per day. The laboratory-acquired firebugs were kept at room temperature in glass containers lidded with a fine mesh. Linden seeds (*Tilia platyphyllos*) were added at the bottom and water was provided in glass tubes capped with pieces of gauze. A lamp positioned near the glass containers provided light for 12 hours per day.

Live-cell imaging and analysis

Sample preparation

Male firebugs were cut in half using fine scissors at a position close to the hind pair of legs. The abdomen of the animals was transferred to wells on a double-depression glass slide filled with approximately 1 mL of Clarke's Ringer (CR) solution (111 mM NaCl, 19 mM CaCl₂, 1 mM NaHCO₃, 0.1 mM Na₂HPO₄) and opened dorsally with fine tweezers. The testes and vas deferens were removed and transferred into the second well of the double-depression slide filled with CR solution. The vas deferens was discarded and residual fat tissue of the testes was removed. Testes were submerged in a culture chamber containing a thin layer of Voltalef Oil 10 S (Cat. #24627.188, VWR chemicals, USA). Testes contents were spread thinly on a coverslip under the layer of oil (Nicklas and Staehly 1967).

Imaging procedure

Utilizing a widefield microscope (Observer Z.1/7 with ApoTome, Zeiss, Germany), samples were initially imaged at low magnification using a 20×/0.45 Ph2 N-Achroplan objective (Zeiss, Germany) to check for homogeneous cell confluency and identify regions with higher density of dividing cells. In such preparations, spermatozoa were usually located around the cells of interest. Preparations were discarded when a high number of sperm surrounded the spermatocytes. After identifying regions with cells in metaphase, either meiotic (both divisions) or mitotic, a 100×/1.25 Oil Ph3 N-Achroplan objective (Zeiss, Germany) with immersion oil (Immersol 518F, Zeiss, Germany) was used. For dividing cells, a Z-stack (11 Z-planes) was recorded every 30 s. The time between Z-stack acquisitions automatically increased when imaging multiple positions sequentially. For each Z-stack, chromosomes were brought into focus, and five optical sections were acquired above and below the focal plane with a Z-spacing of 1 µm. Acquisition times ranged from 10–90 min. In total, 20 datasets were recorded for meiosis I and II and 7 for mitosis (Table 1, Table S1, Fig. S1).

Table 1

Spindle and chromosome dynamics in meiosis I, meiosis II, and mitosis of *P. apterus*. For each division type, the pole-to-pole (P-P, spindle length), chromosome-to-chromosome (C-C, inter-chromosomal distance) and derived pole-to-chromosome (P-C) distances are given as the initial (metaphase) distance, the final (anaphase) distance, and the segregation speed. Initial and final distances are the fitted logistic evaluated at anaphase onset, $d(0)$, and at the last imaged frame, $d(t_{\text{max}})$, respectively; speed is the slope of a linear regression over the movement window (from anaphase onset to the point at which the logistic reaches 95% of its total change, or the last frame; see Methods). Distances are in μm and speeds in $\mu\text{m}/\text{min}$; negative P-C speeds denote the decreasing P-C distance as chromosomes approach the poles. For each measure and division, n = number of imaged cells, Average = mean, and SD = standard deviation. P-C distances were derived per cell as $(\text{P-P} - \text{C-C})/2$ and are therefore available only for cells with both a pole and a chromosome track ($n = 19$ per meiotic division), whereas C-C was measured in all cells ($n = 20$ per meiotic division; $n = 7$ for mitosis in all measures).

Division		P-P initial [μm]	P-P final [μm]	P-P speed [$\mu\text{m}/\text{min}$]	C-C initial [μm]	C-C final [μm]	C-C speed [$\mu\text{m}/\text{min}$]	P-C initial [μm]	P-C final [μm]	P-C speed [$\mu\text{m}/\text{min}$]
Meiosis I	n	19	19	19	20	20	20	19	19	19
	Average	15.4	19.6	0.5	1.8	14.6	1.6	6.8	2.3	-0.5
	SD	± 2.0	± 2.0	± 0.2	± 0.5	± 1.5	± 0.3	± 1.0	± 1.0	± 0.1
Meiosis II	n	19	19	19	20	20	20	19	19	19
	Average	13.4	16.3	0.5	1.4	12.7	1.6	6.0	1.7	-0.6
	SD	± 1.4	± 1.3	± 0.3	± 0.3	± 0.9	± 0.3	± 0.7	± 0.3	± 0.1
Mitosis	n	7	7	7	7	7	7	7	7	7
	Average	7.2	10.9	0.6	1.2	7.2	1.1	3.2	1.8	-0.4
	SD	± 1.2	± 0.9	± 0.5	± 0.4	± 1.4	± 0.4	± 0.4	± 0.3	± 0.2

Data pre-processing

Anaphase onset (AO) was defined by the frame prior to the first frame when chromosome segregation was visible. Then, the Z-stacks across the time-series were cropped to the Z-planes in which the chromosomes were clearly visible. The feature 'Extended Depth of Focus (EDF)' in the application ZEN blue edition (version 3.3.89.0008, Zeiss, Germany) was then used to merge the Z-stack time-series to a single Z-plane time-series for subsequent analysis (Method Parameters: Variance; Z-Stack Alignment: No alignment). The EDF always selected the sharpest edges of an object across all Z-planes of the Z-stack. This resulted in a composite image where all objects appear in the best focus, even if they span multiple focal planes. The time-series were then contrast inverted.

Cell size determination

Using FIJI (version v1.54p; (Schindelin et al. 2012)), the cross-sectional area A of each cell was measured at AO. Assuming a circular cross-section, the cell diameter d was calculated as

$$d = 2\sqrt{\frac{A}{\pi}}$$

Distance tracking

Spindle poles were manually tracked in each frame using the 'Manual Tracking' plugin in FIJI. Spindle poles were identified as the center of a brighter, phase-clear region delimited by aligned mitochondria. The Python script 'Kymograph generation for 3D image' (Okafornta 2025), was used to generate a kymograph from each Z-stack time-series by computing the mean intensity of a 10-pixel-wide line between the two tracked poles in every frame; the profile was exported as a color-coded intensity image and a spreadsheet. In the contrast-inverted phase-contrast images, chromosomes appeared as the brightest objects, i.e. the highest pixel-intensity values. For each frame (row), the positions of the intensity maxima in the left and right halves were taken as the two chromosome masses; their separation provides the chromosome-to-chromosome (C-C) distance, and the separation of the left and right profile boundaries denotes the pole-to-pole (P-P) distance, both in pixels. Tracking errors producing spurious jumps in C-C distance were corrected manually. Distances were converted to micrometers using the pixel size, 0.0454 $\mu\text{m}/\text{pixel}$. The pole-to-chromosome (P-C) distance was computed at each matched time point as $P-C = \frac{PP-CC}{2}$, since the two chromosome masses lie symmetrically between the poles. In total, 20 cells were recorded in meiosis I, 20 in meiosis II, and 7 in mitosis (Table 1, Table S1, Fig. S1).

Quantification of spindle and chromosome dynamics

All subsequent analyses were performed in Python (version 3.12; Python Software Foundation, USA) using NumPy, pandas, and SciPy libraries, and is provided as a single reproducible script (https://github.com/gunarf/firebug_spindle_analysis). For every track (C-C, P-P, and the derived P-C), the same procedure was applied so that all measures are directly comparable. First, a four-parameter logistic function $d(t) = a + \frac{b}{1 + e^{-\frac{t-t_0}{k}}}$ was fitted to the distance over time data (a , lower plateau; $a + b$, upper plateau; t_0 , inflection time; k , steepness). The fit was bounded to biologically meaningful values: t_0 was constrained to the observation window, and for C-C tracks the lower plateau a was restricted to the measured congressed range (0–1.5 μm), preventing degenerate fits to shallow, non-saturating traces. The logistic was used only to define the movement window; it does not itself provide the reported speed. The movement window was defined as the interval from AO ($t = 0$ s, or the first imaged frame if imaging began after AO) to the earlier of (i) the time at which the fit reached 95% of its total change or (ii) the last imaged frame, the latter accommodating P-P traces that had not plateaued by the end of imaging. The initial (metaphase) and final (anaphase) pole and chromosome distances were read from the fitted curve at the window endpoints, $d(0)$ and $d(t_{\text{max}})$, respectively (Suppl. Fig. S2). The segregation speed was defined as the slope of an ordinary linear regression fitted to the measured points within the movement window (Suppl. Fig. S3). Group values (Table 1) are mean $\pm$ SD of these per-cell quantities.

Anaphase A and B components of chromosome segregation

For each cell with both a P-P and a C-C fit, the fractional component of spindle elongation (anaphase B) to total chromosome segregation was calculated from the paired changes in distance as anaphase B = $100 \times \Delta(\text{P-P}) / \Delta(\text{C-C})$, where Δ denotes the difference between final and initial values; the anaphase A component (poleward chromosome movement) was defined as 100 – anaphase B percentage points (Table 2).

Table 2

Anaphase A and B components and cell size in meiosis I, meiosis II, and mitosis of *P. apterus*. For each division type, the proportion of poleward chromosome movement (anaphase A, from the decrease in pole-to-chromosome distance) and of spindle elongation (anaphase B, from the increase in pole-to-pole distance) driving total chromosome separation are provided as percentages, together with the cell area at anaphase onset and the corresponding cell diameter. Anaphase A and B were computed per cell from paired changes in distance (anaphase B = $100 \times \Delta\text{P-P} / \Delta\text{C-C}$; anaphase A = 100 – anaphase B) and therefore sum to 100%; because B = 100 – A, the two contributions share the same SD. Cell diameter was derived from the measured cell area assuming a circular cross-section, $d = 2\sqrt{A/\pi}$. For each division, n = number of cells, Average = mean, and SD = standard deviation. Anaphase components require both a pole and a chromosome track and are thus available for the paired cells only (n = 19 per meiotic division), whereas cell area and diameter were measured in all cells (n = 20 per meiotic division; n = 7 for mitosis).

Division		Anaphase A component (P-C) [%]	Anaphase B component (P-P) [%]	Cell area [μm^2]	Cell diameter [μm]
Meiosis I	n	19	19	20	20
	Average	68.1%	31.9%	585.4	27.2
	SD	$\pm 10.2\%$	$\pm 10.2\%$	± 104.8	± 2.4
Meiosis II	n	19	19	20	20
	Average	74.4%	25.6%	353.3	21.2
	SD	$\pm 10.1\%$	$\pm 10.1\%$	± 46.0	± 1.4
Mitosis	n	7	7	7	7
	Average	39.1%	60.9%	84.4	10.3
	SD	$\pm 14.4\%$	$\pm 14.4\%$	± 10.5	± 0.6

Scaling analysis

To test whether final spindle length (P-P) and final chromosome separation (C-C) scale with cell size, linear regressions of the final distance against cell area were computed both pooled across all three division types and separately within each division (Table S2). Cell-area records were paired to tracked cells by division and AL number; where one AL number contained several sub-cells within the same division, area records sorted by descending cell area were assigned to the remaining unmatched candidate track with the largest fitted final chromosome-to-chromosome distance, and no track was assigned twice. All 47 pairings are listed in Table S2.

Statistics

Group differences in C-C segregation speed and in cell area were assessed with Welch's unequal-variance *t*-test and confirmed with the non-parametric Mann–Whitney *U* test, given the small mitotic sample (n = 7). Three pre-planned pairwise contrasts (meiosis I vs meiosis II, meiosis I vs mitosis, meiosis II vs mitosis) were tested without correction for multiple comparisons. Statistical significance is indicated as: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

0.001, **** $p < 10^{-4}$; ns, not significant. To test whether the cell-area dependence of final spindle length differed between division types, an ordinary least-squares model including an area $\times$ division interaction was fitted (statsmodels 0.14; Python 3.12).

Immunofluorescence

Immunostaining

The testes of *P. apterus* were removed from the abdomen and its contents were spread under a thin layer of Voltalef Oil 10 S to produce a single-cell layer of living spermatocytes. Fixation was performed as described (Nicklas et al. 1979). Immunostaining was carried out using the primary antibodies, anti- β -tubulin (Clone Tu27/Tubulin; 903401; 1:25; BioLegend, USA) and anti-mitotic proteins (Clone MPM2; ab14581; 1:25; Abcam, UK) followed by the secondary antibody anti-mouse conjugated to Cy3 (M30010; 1:25; Thermo Fisher Scientific, USA) as previously described (King and Nicklas 2000). The cells were then mounted on glass slides using Vectashield Antifade Mounting Medium containing DAPI (Vector Laboratories, USA) (Felt et al. 2017).

Imaging and data processing

Immuno-labeled samples were imaged using a confocal laser scanning microscope (Stellaris 8, Leica Microsystems, Germany) controlled by the LAS X software (version 4.8, Leica Microsystems), equipped with an HC PL APO 63 $\times$ /1.40 oil immersion objective (Leica Microsystems, Germany), a diode laser (405 nm for DAPI) and a pulsed white light laser (tuned to 552 nm for Cy3). For signal detection, the following detectors were used: for DAPI a hybrid detector (HyD) X (range 430–543 nm), and for Cy3 a HyD S detector (range 557–818 nm). Image stacks with a z-spacing of 0.3 μ m were acquired at 400 Hz. Maximum intensity projections (MIPs) from the Z-planes displaying the clearest tubulin, kinetochore, and chromosome signals were generated in FIJI (Schindelin et al. 2012).

Results

Spindle elongation in meiosis

To characterize meiosis I in *P. apterus*, we imaged spermatocytes from metaphase through late anaphase (Fig. 1A; Suppl. Movies S1–S3). Cell shape in meiosis I was elliptical, and the region containing chromosomes between the spindle poles appeared free of any phase-dense particles. Using the vertices of the cleared area containing the spindle as the spindle pole positions and by detecting the positions of the chromosomes, we created kymographs from our time-lapse images (Fig. 1B). We then plotted the pole-to-pole (P-P, the spindle length), the chromosome-to-chromosome (C-C, the inter-chromosomal distance), and the pole-to-chromosome distances (P-C) over time (Fig. 1C, Table 1). Our analysis revealed that the average pole-to-pole distance increased during meiosis I, starting from 15.4 ± 2.0 μ m (mean $\pm$ SD, $n = 19$) in metaphase to a final spindle length of 19.6 ± 2.0 μ m (mean $\pm$ SD) in late anaphase, with a net increase of about 4.2 μ m. From the data we also calculated the speed of spindle elongation which was 0.5 ± 0.2 μ m/min.

In the second meiotic division, cells appeared less elongated and more circular in shape (Fig. 1D, Suppl. Movies S4–S6). As with meiosis I spermatocytes, we created kymographs to analyze spindle elongation (Fig. 1E) and determined the same properties for meiosis II over time (Fig. 1F). The average spindle length in our sample set increased from 13.4 ± 1.4 μ m (mean $\pm$ SD, $n = 19$) in metaphase II to a final average length of 16.3 ± 1.3 μ m

(mean $\pm$ SD) in late anaphase II. Thus, spindle length in meiosis II increased by about 2.9 μm . We also calculated the speed of spindle elongation which was also $0.5 \pm 0.3 \mu\text{m}/\text{min}$. From these measurements we concluded that spindle elongation proceeded at approximately the same speed in anaphase I and anaphase II. In addition, our observations confirmed the presence of a lagging chromosome in anaphase II (the X chromosome), thus verifying the initial observations of Henking from 1891 (Fig. 1D, yellow arrowhead).

Immunofluorescence images of meiosis I (Fig. 2A) and meiosis II (Fig. 2B) spermatocytes with stained microtubules confirmed our phase-contrast observations that each spindle has well-defined spindle poles. We observed a prominent array of mid-spindle microtubules in anaphase I and in anaphase II. Staining of tubulin and chromosomes confirmed lagging of the X chromosome in anaphase II. (Fig. 2B, yellow arrowhead). In addition, MPM2 antibody staining, if specific to kinetochores in this species as seen in other insect species (Logarinho and Sunkel 1998; Nicklas et al. 1998; Warren et al. 2000), supports the holocentric nature of the chromosomes from metaphase to late anaphase in meiosis I (Fig. 2C-D) and in meiosis II (Fig. 2E-F). Further we observed a bipolar attachment of the X chromatid through microtubules (yellow arrowhead in Fig. 2B) and also a holocentric kinetochore around this lagging chromosome (Fig. 2F) in *P. apterus*.

Chromosome dynamics in meiosis

We also analyzed the C-C distances over time. At metaphase I, homologous chromosomes were tethered together, but both homologs could be clearly distinguished from one another, resulting in a distance of $1.8 \pm 0.5 \mu\text{m}$ (mean $\pm$ SD; $n = 20$; Fig. 1B). This distance then increased to a final length of $14.6 \pm 1.5 \mu\text{m}$ (mean $\pm$ SD, Table 1) in late anaphase I within about 9.7 min (Suppl. Fig. S4, Table 1). The speed of segregation was $1.6 \pm 0.3 \mu\text{m}/\text{min}$. In meiosis II, the C-C distance increased from $1.4 \pm 0.3 \mu\text{m}$ to $12.7 \pm 0.9 \mu\text{m}$ (mean $\pm$ SD) in late anaphase II, and autosomes were segregated at a speed of $1.6 \pm 0.3 \mu\text{m}/\text{min}$. Chromosomes in meiosis II stopped separating after approximately 7.8 min (Suppl. Fig. S4, Table 1).

Using our measurements of spindle length and inter-chromosomal distance, we calculated the decrease in pole-to-chromosome (P-C) distance over time. Our analysis revealed that the P-C distance decreased from $6.8 \pm 1.0 \mu\text{m}$ to $2.3 \pm 1.0 \mu\text{m}$ in meiosis I ($n = 19$), and from $6.0 \pm 0.7 \mu\text{m}$ to $1.7 \pm 0.3 \mu\text{m}$ in meiosis II ($n = 19$). From these measurements of chromosome-to-chromosome distance, pole-to-chromosome distance, and rates of chromosome movement in anaphase, we conclude that both meiotic cell divisions are very similar in *P. apterus*. Interestingly, the presence of the lagging X did not appear to have an effect on autosomal chromosome dynamics in meiosis II, as rates of chromosome separation and spindle pole separation were nearly identical in both division types.

Comparison of spindle and chromosome dynamics in mitosis versus meiosis

We then compared the dynamics of the meiotic divisions to those observed during mitosis. Using our live-cell approach, we analyzed chromosome dynamics and spindle elongation in the round and relatively small spermatogonia (Fig. 3A; Suppl. Movies S7–S9). Analysis of the kymograph (Fig. 3B) revealed a pole-to-pole distance of $7.2 \pm 1.2 \mu\text{m}$ in metaphase and $10.9 \pm 0.9 \mu\text{m}$ in late anaphase (Fig. 3C). The spindle elongation rate was $0.6 \pm 0.5 \mu\text{m}/\text{min}$. As a result, spindle elongation rates were almost identical in meiotic and mitotic divisions in *P. apterus*.

We also analyzed chromosome dynamics. In mitosis, chromosomes showed an initial separation distance of $1.2 \pm 0.4 \mu\text{m}$ and a final distance of $7.2 \pm 1.4 \mu\text{m}$, approximately half the distance observed in cells during meiosis I and meiosis II. Pole-to-chromosome distance decreased from $3.2 \pm 0.4 \mu\text{m}$ at metaphase to $1.8 \pm 0.3 \mu\text{m}$ in late anaphase. Immunofluorescence images revealed that mitotic cells in metaphase appear to have bundled microtubules, similar to k-fibers in mammalian metaphase spindles (Kiewisz et al. 2022). Anaphase cells showed a prominent array of mid-spindle microtubules with relatively few astral microtubules visible (Fig. 3D). Although chromosome segregation speed was significantly slower in mitosis compared to both meiotic divisions (Fig. 4A; $1.1 \pm 0.4 \mu\text{m}/\text{min}$; Welch's *t*-test, $p < 0.05$), spindle elongation speed was comparable across all three divisions (Fig. 4A; $0.5\text{--}0.6 \mu\text{m}/\text{min}$; Table 1), and the directed-movement window was shorter in mitosis than in meiosis (Fig. S4).

We then determined the proportion of spindle elongation (anaphase B) to total chromosome segregation for both types of division. Our analysis revealed that the increase in the P-P distance accounted for about 61% of chromosome segregation in mitosis but only 32% in meiosis I and 26% in meiosis II (Table 2, Fig. 4B). Conversely, poleward chromosome movement (anaphase A) accounted for 39% in mitosis *versus* 68% in meiosis I and 74% in meiosis II. For each cell, the anaphase A component was significantly higher in both meiotic divisions than in mitosis but did not differ between meiosis I and II (Fig. 4C; Welch's *t*-test, $** p < 0.01$, $*** p < 0.001$, ns). Thus, the proportional involvement of poleward movement and spindle elongation in total chromosome segregation are inverted between mitosis and meiosis in *P. apterus*.

Comparative analysis of spindle scaling

To analyze the impact of cell size on spindle size and chromosome separation, we measured and correlated the area of the cells with our dynamic measurements. Cells in anaphase I averaged $585.4 \pm 104.8 \mu\text{m}^2$ ($n = 20$); anaphase II cells were significantly smaller at $353.3 \pm 46.0 \mu\text{m}^2$ ($n = 20$, $\sim 60\%$ of meiosis I); mitotic cells were smallest at $84.4 \pm 10.5 \mu\text{m}^2$ ($n = 7$). Cell size thus decreased significantly from meiosis I to meiosis II to mitosis (Fig. 4D; Welch's *t*-test, $**** p < 10^{-4}$).

Plotting final pole-to-pole distance against cell area (Fig. 4E), spindle length correlated strongly with cell size across all three divisions (pooled $y = 0.016x + 10.43$, $R^2 = 0.80$, $n = 45$). Within individual divisions, cell area varied over a much narrower range (SD = 105, 46 and $11 \mu\text{m}^2$ for meiosis I, meiosis II and mitosis) and explained little of the residual variation in spindle length ($R^2 \leq 0.27$), although the within-division slopes ($0.008\text{--}0.033 \mu\text{m} \mu\text{m}^{-2}$) were of the same order as the pooled slope and did not differ significantly between divisions (area $\times$ division interaction, $p = 0.48$ and $p = 0.67$ for meiosis II and mitosis relative to meiosis I). Final chromosome-to-chromosome distance behaved similarly (Fig. 4F; pooled $y = 0.011x + 8.03$, $R^2 = 0.62$, $n = 47$; within-division $R^2 \leq 0.22$). The large between-division differences therefore dominate the apparent scaling relationship, which is set primarily by division identity rather than resolvable as a continuous dependence on cell size within a division.

Discussion

We applied quantitative live-cell imaging to systematically compare spindle and chromosome dynamics in meiosis I, meiosis II, and mitosis in *P. apterus*. We found that meiosis I and meiosis II were quantitatively nearly identical to each other yet differed consistently from germ-line mitosis in chromosome segregation distance, speed, and anaphase A and B components.

Spindle elongation

We observed that final spindle length scales with cell size across cell types in *P. apterus*, being longer in the large meiosis I cells and shorter in the small mitotic cells. Within each division type, the sampled cells spanned only a narrow range of cell sizes, so a continuous dependence of final spindle length on cell size could not be resolved, although the within-division slopes were of the same order as the pooled slope. However, scaling across division types largely depends on the vast differences in cell sizes. This might indicate that final pole-to-pole and chromosome-to-chromosome distances depend more on division programs rather than a continuously-tuned response to cytoplasmic volume. Notably, this differs from the continuous within-system scaling reported in many embryonic and cellular systems, where spindle length tracks cell size across a developmental size range, e.g. in *C. elegans*, *Paracentrotus lividus*, *Danio rerio* and *Xenopus laevis* (Brown et al. 2007; Greenan et al. 2010; Good et al. 2013; Farhadifar et al. 2015; Lacroix et al. 2018; Rieckhoff et al. 2020) and mammalian cells (Kletter et al. 2022). In contrast to fission yeast, where elongation velocity scales with cell size (Krüger et al. 2019), we found comparable elongation rates across our three divisions despite their large size range (see below).

Mechanistically, spindle scaling has been attributed to the cytoplasmic volume available for spindle assembly (Good et al. 2013; Hazel et al. 2013), with a limiting pool of spindle components setting the achievable spindle length (Greenan et al. 2010; Nannas et al. 2014); consistent with this, microtubule dynamics themselves scale with cell size to set spindle length (Lacroix et al. 2018). Importantly, the relationship is often biphasic rather than strictly linear: in very large cells, spindle length reaches an upper limit that is uncoupled from cell size, and a strong correlation emerges only below a threshold cell size (Good et al. 2013; Novakova et al. 2016; Rieckhoff et al. 2020). In the literature, several models have been proposed for mitotic spindle elongation, e.g., through motor proteins at the spindle midzone creating outward sliding forces that push the poles apart (McIntosh and Landis 1971; McDonald et al. 1977; Winey et al. 1995; Krüger et al. 2019) or through microtubule polymerization that sets the final spindle length (Masuda and Cande 1987; Lera-Ramirez et al. 2022). The mechanism for this aspect of spindle scaling in *P. apterus*, however, remains to be determined. Recently, we have proposed a biophysical model for spindle length scaling in the early embryo in *C. elegans*. In this model, mitotic spindle elongation is driven by cortically anchored motor proteins and astral microtubules, rendering it sensitive to cell size (Okafornta et al. 2026). Incorporating experimental results into an established stoichiometric model (Farhadifar et al. 2020), we observed that allowing only cell size and microtubule catastrophe rates to vary, reproduces elongation dynamics across development (Okafornta et al. 2026). It is an interesting question whether similar rules might apply in an insect system.

Force generation in spindles

A striking difference between the division types was the proportions of spindle elongation (anaphase B) and poleward chromosome movement (anaphase A) driving chromosome segregation. In germ-line mitosis, spindle elongation accounted for the majority of chromosome separation ($\approx 61\%$), whereas in both meiotic divisions it constituted only a minority ($\approx 26\text{--}32\%$), with poleward movement dominating instead. This inversion is particularly notable because it occurred despite comparable spindle-elongation rates across all three divisions ($0.5\text{--}0.6\text{ }\mu\text{m}/\text{min}$; Table 1): the divisions differ not in the rate of spindle elongation, but in the extent to which elongation drives total chromosome segregation. Given that meiotic chromosomes segregate across roughly twice the distance than mitotic chromosomes ($\text{C-C} \approx 12.7\text{--}14.6\text{ }\mu\text{m}$ versus $\approx 7.2\text{ }\mu\text{m}$), a similar amount of spindle elongation represents a much smaller fraction of the meiotic total. This division-specific balance is

reminiscent of the situation in the nematode *C. elegans*, where the male meiotic divisions show a prominent anaphase A that is largely absent in early embryonic mitoses (Fabig et al. 2020; Okafornta et al. 2026), and it contrasts with the *Drosophila* blastoderm, in which anaphase chromosome speed scales directly with spindle length (Xu et al. 2025). We did not observe such a coupling here, as the short mitotic spindles segregated chromosomes at a P-P elongation rate comparable to the much longer meiotic spindles. Importantly, because we measured distances (i.e., P-P and C-C, and calculated P-C) in the *P. apterus* spindles (Table 1) but did not perturb chromosome movements, the mechanistic basis of chromosome segregation remains to be examined in this insect system. Whether the measured distances reflect division-specific force generation by cortical pulling forces or midzone-microtubule pushing will require further work, including micromanipulation and/or laser-ablation. Such additional experiments, however, have to be subject to a follow-up publication. Notably, an inverted balance of anaphase A and B does not necessarily imply distinct force-generating machineries; the same molecular components may simply be deployed to different extents in each division type.

Along this line, we observed a prominent array of mid-spindle microtubules in *P. apterus* mitosis (Fig. 3D), reminiscent of the inter-chromosomal microtubules that mediate chromosome segregation in mitosis in the model system *C. elegans*. Using laser ablation and serial-section electron tomography in combination with manipulation of cortical pulling forces, we reported that inter-chromosomal microtubules mediate chromosome segregation in *C. elegans*. This is achieved by plus-end directed motors exerting pushing forces on antiparallel microtubules (Yu et al. 2019). As mentioned, this subpopulation of microtubules remains uncoupled from cell size up to the 64-cell stage of embryonic development in *C. elegans* (Okafornta et al. 2026). Whether such a size invariance of the final C-C distance can be observed for early mitotic divisions in *P. apterus* remains a fascinating question.

Using the X chromosome as a model for single-chromatid segregation

Lagging X chromosomes are observed in a number of animal systems including nematodes, insects, and spiders (reviewed in (Fabig et al. 2015)). Such systems differ in the number of single sex chromosomes that have to be segregated and in the occurrence of univalent X chromosomes in either meiosis I or II. In some XX|X0 sex-determination systems, single X chromosomes are visible in meiosis I, when the X is segregated to one of the spindle poles after the homologous autosomes have been partitioned. This scenario has been studied in depth in the nematode, *C. elegans*, and in multiple Hemipteran insects (Felt et al. 2017; Fabig et al. 2020; Felt et al. 2022; Borseth et al. 2023). In an alternate scenario, a univalent X chromosome is visible in meiosis II. In this 'inverted meiosis', the X chromatids are segregated in meiosis I without any lagging, while appearing in meiosis II as a lagging chromosome (Melters et al. 2012; Fabig et al. 2015). This behavior has rather recently been observed in the nematode, *Auanema rhodense* (Shakes et al. 2011; Winter et al. 2017); it was observed, however, also in hemipteran insects (Schrader 1935; John and Claridge 1974) and plant species (Nokkala et al. 2004; Li et al. 2021; Liu et al. 2022). The presence or absence of a lagging X chromosome does not discernibly affect either spindle or chromosome dynamics in *P. apterus* meiosis, as both meiotic divisions show very similar dynamic patterns. The mechanism of X chromosome segregation in general is poorly understood. It is not fully clear what triggers the initiation and direction of the X segregation. In *C. elegans*, also a species with a holocentric kinetochore, it was proposed that an imbalance of microtubules attached to the holocentric kinetochore causes the X to segregate to the pole with more attached kinetochore microtubules (Fabig et al. 2020). Whether such a 'tug-of-war' mechanism operates in *P. apterus* remains to be determined. *P. apterus*, however, would be an

excellent system to study single chromosome missegregation by using microneedle manipulations (Nicklas 1967; Nicklas and Koch 1969; Suresh et al. 2022; Rux et al. 2026).

Conclusions

By comparing all three division types within a single organism and with identical analysis, we found that the two meiotic divisions of *P. apterus* are quantitatively almost indistinguishable from each other yet consistently distinct from germ-line mitosis, most strikingly in the inverted proportions of poleward movement and spindle elongation driving chromosome segregation. Spindle length and chromosome-segregation distances were set by division identity rather than by continuous scaling with cell size. Together with its holocentric chromosomes, lagging X, and amenability to micromanipulation, *P. apterus* emerges as a tractable system for dissecting how a shared molecular toolkit is tuned to the distinct demands of mitosis and meiosis.

Declarations

Acknowledgements

The authors acknowledge the use of the Core Facility Cellular Imaging (CFCI, MF-TUD). We also thank Aayush Poddar for critical reading of the manuscript.

Author contributions

Conceptualization: G.F., L.V.P., T.M.-R. Investigation: A.L., G.F., S.T., A.N., L.V.P. Data curation: A.L., G.F. Formal analysis: A.L., G.F., C.W.O. Methodology: A.L., G.F., C.W.O., V.S., D.D., L.V.P. Resources: V.S., D.D., L.V.P. Software: G.F., C.W.O. Validation: G.F., C.W.O. Supervision: G.F., L.V.P., T.M.-R. Visualization: A.L., G.F. Writing – original draft: A.L., L.V.P., T.M.-R. Writing – review & editing: all authors. Funding acquisition: T.M.-R.

Funding

Research in the Müller-Reichert laboratory is supported by grants from the Deutsche Forschungsgemeinschaft (DFG 258577783 and 282354882) to T.M.R. The Müller-Reichert laboratory also acknowledges support from the CCBx program (SF 1157392) of the Center for Computational Biology of the Flatiron Institute (NY, USA).

Data availability

All microscopic images used in this study and all derived images (kymographs) are publicly available and accessible: <https://omero.med.tu-dresden.de/webclient/?show=project-2201>

All software code used for image analysis can be found on GitHub and Zenodo: <https://doi.org/10.5281/zenodo.15010690> (Okafornta 2025) and <https://doi.org/10.5281/zenodo.23153660> (Fabig et al. 2026).

Competing interests

The authors declare no competing interests.

Ethics approval

Not applicable.

References

1. Borseth AB, Felt KD, Stowe EL, Paliulis LV (2023) Segregation of the Univalent X Chromosome in the Two-Striped Planthopper *Acanalonia bivittata*. *cytologia* 88:137–142. <https://doi.org/10.1508/cytologia.88.137>
2. Brown KS, Blower MD, Maresca TJ, Grammer TC, Harland RM, Heald R (2007) *Xenopus tropicalis* egg extracts provide insight into scaling of the mitotic spindle. *J Cell Biol* 176:765–770. <https://doi.org/10.1083/jcb.200610043>
3. Fabig G, Müller-Reichert T, Paliulis L (2015) Back to the roots: segregation of univalent sex chromosomes in meiosis. *Chromosoma* 125:277–286. <https://doi.org/10.1007/s00412-015-0550-9>
4. Fabig G, Kiewisz R, Lindow N, Powers JA, Cota V, Quintanilla LJ, Brugués J, Prohaska S, Chu DS, Müller-Reichert T (2020) Male meiotic spindle features that efficiently segregate paired and lagging chromosomes. *Elife* 9. <https://doi.org/10.7554/eLife.50988>
5. Fabig G, Lindner A, Okafornta CW, Müller-Reichert T (2026) Analysis code: Quantitative spindle and chromosome dynamics in meiosis I, meiosis II and mitosis of *Pyrrhocoris apterus* (Version v1.0.1) [Software]. Zenodo. <https://doi.org/10.5281/zenodo.23153660>
6. Farhadifar R, Baer CF, Valfort A-C, Andersen EC, Müller-Reichert T, Delattre M, Needleman DJ (2015) Scaling, selection, and evolutionary dynamics of the mitotic spindle. *Curr Biol* 25:732–740. <https://doi.org/10.1016/j.cub.2014.12.060>
7. Farhadifar R, Yu C-H, Fabig G, Wu H-Y, Stein DB, Rockman M, Müller-Reichert T, Shelley MJ, Needleman DJ (2020) Stoichiometric interactions explain spindle dynamics and scaling across 100 million years of nematode evolution. *Elife* 9. <https://doi.org/10.7554/eLife.55877>
8. Felt KD, Lagerman MB, Ravida NA, Qian L, Powers SR, Paliulis LV (2017) Segregation of the amphitelically attached univalent X chromosome in the spittlebug *Philaenus spumarius*. *Protoplasma* 254:2263–2271. <https://doi.org/10.1007/s00709-017-1117-9>
9. Felt KD, Lagerman MB, Maurer S, Qian L, Oluwafemi O, Pedraza-Aguado N, Stowe EL, Paliulis LV (2022) Segregation of the univalent X chromosome in the wide-footed treehopper *Enchenopa latipes* (Say 1824). *Caryologia* 75:165–171. <https://doi.org/10.36253/caryologia-1411>
10. Good MC, Vahey MD, Skandarajah A, Fletcher DA, Heald R (2013) Cytoplasmic volume modulates spindle size during embryogenesis. *Science* 342:856–860. <https://doi.org/10.1126/science.1243147>
11. Greenan G, Brangwynne CP, Jaensch S, Gharakhani J, Jülicher F, Hyman AA (2010) Centrosome size sets mitotic spindle length in *Caenorhabditis elegans* embryos. *Curr Biol* 20:353–358. <https://doi.org/10.1016/j.cub.2009.12.050>
12. Gruss OJ (2018) Animal Female Meiosis: The Challenges of Eliminating Centrosomes. *Cells* 7. <https://doi.org/10.3390/cells7070073>
13. Hazel J, Krutkramelis K, Mooney P, Tomschik M, Gerow K, Oakey J, Gatlin JC (2013) Changes in cytoplasmic volume are sufficient to drive spindle scaling. *Science* 342:853–856. <https://doi.org/10.1126/science.1243110>

14. Henking H (1891) Über Spermatogenese und deren Beziehung zur Entwicklung bei *Pyrrhocoris apterus* L. Z. wiss. Zool. 51:685–736
15. John B, Claridge MF (1974) Chromosome variation in British populations on *Oncopsis* (Hemiptera: Cicadellidae). *Chromosoma* 46:77–89. <https://doi.org/10.1007/BF00332340>
16. Kiewisz R, Fabig G, Conway W, Baum D, Needleman D, Müller-Reichert T (2022) Three-dimensional structure of kinetochore-fibers in human mitotic spindles. *Elife* 11. <https://doi.org/10.7554/eLife.75459>
17. King JM, Nicklas RB (2000) Tension on chromosomes increases the number of kinetochore microtubules but only within limits. *J Cell Sci* 113 Pt 21:3815–3823. <https://doi.org/10.1242/jcs.113.21.3815>
18. Kletter T, Reusch S, Cavazza T, Dempewolf N, Tischer C, Reber S (2022) Volumetric morphometry reveals spindle width as the best predictor of mammalian spindle scaling. *J Cell Biol* 221. <https://doi.org/10.1083/jcb.202106170>
19. Krüger LK, Sanchez J-L, Paoletti A, Tran PT (2019) Kinesin-6 regulates cell-size-dependent spindle elongation velocity to keep mitosis duration constant in fission yeast. *Elife* 8. <https://doi.org/10.7554/eLife.42182>
20. Lacroix B, Letort G, Pitayu L, Sallé J, Stefanutti M, Maton G, Ladouceur A-M, Canman JC, Maddox PS, Maddox AS, Minc N, Nédélec F, Dumont J (2018) Microtubule Dynamics Scale with Cell Size to Set Spindle Length and Assembly Timing. *Dev Cell* 45:496–511.e6. <https://doi.org/10.1016/j.devcel.2018.04.022>
21. Lera-Ramirez M, Nédélec FJ, Tran PT (2022) Microtubule rescue at midzone edges promotes overlap stability and prevents spindle collapse during anaphase B. *Elife* 11. <https://doi.org/10.7554/eLife.72630>
22. Li X, Huang F, Chai J, Wang Q, Yu F, Huang Y, Wu J, Wang Q, Xu L, Zhang M, Deng Z (2021) Chromosome behavior during meiosis in pollen mother cells from *Saccharum officinarum* × *Erianthus arundinaceus* F1 hybrids. *BMC Plant Biol* 21:139. <https://doi.org/10.1186/s12870-021-02911-z>
23. Liu B, Tao X, Dou Q (2022) Meiotic Chromosomal Abnormality Detected in a Heterozygote of *Elymus nutans*. *Front Plant Sci* 13:895437. <https://doi.org/10.3389/fpls.2022.895437>
24. Logarinho E, Sunkel CE (1998) The *Drosophila* POLO kinase localises to multiple compartments of the mitotic apparatus and is required for the phosphorylation of MPM2 reactive epitopes. *J Cell Sci* 111 (Pt 19):2897–2909. <https://doi.org/10.1242/jcs.111.19.2897>
25. Masuda H, Cande WZ (1987) The role of tubulin polymerization during spindle elongation in vitro. *Cell* 49:193–202. [https://doi.org/10.1016/0092-8674\(87\)90560-5](https://doi.org/10.1016/0092-8674(87)90560-5)
26. McDonald K, Pickett-Heaps JD, McIntosh JR, Tippit DH (1977) On the mechanism of anaphase spindle elongation in *Diatoma vulgare*. *J Cell Biol* 74:377–388. <https://doi.org/10.1083/jcb.74.2.377>
27. McIntosh JR, Landis SC (1971) The distribution of spindle microtubules during mitosis in cultured human cells. *J Cell Biol* 49:468–497. <https://doi.org/10.1083/jcb.49.2.468>
28. Melters DP, Paliulis LV, Korf IF, Chan SWL (2012) Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. *Chromosome Res* 20:579–593. <https://doi.org/10.1007/s10577-012-9292-1>
29. Nannas NJ, O'Toole ET, Winey M, Murray AW (2014) Chromosomal attachments set length and microtubule number in the *Saccharomyces cerevisiae* mitotic spindle. *Mol Biol Cell* 25:4034–4048. <https://doi.org/10.1091/mbc.E14-01-0016>

30. Nicklas RB (1967) Chromosome micromanipulation. II. Induced reorientation and the experimental control of segregation in meiosis. *Chromosoma* 21:17–50. <https://doi.org/10.1007/BF00330545>
31. Nicklas RB, Koch CA (1969) Chromosome micromanipulation. 3. Spindle fiber tension and the reorientation of mal-oriented chromosomes. *J Cell Biol* 43:40–50. <https://doi.org/10.1083/jcb.43.1.40>
32. Nicklas RB, Staehly CA (1967) Chromosome micromanipulation. I. The mechanics of chromosome attachment to the spindle. *Chromosoma* 21:1–16. <https://doi.org/10.1007/BF00330544>
33. Nicklas RB, Brinkley BR, Pepper DA, Kubai DF, Rickards GK (1979) Electron microscopy of spermatocytes previously studied in life: methods and some observations on micromanipulated chromosomes. *J Cell Sci* 35:87–104. <https://doi.org/10.1242/jcs.35.1.87>
34. Nicklas RB, Campbell MS, Ward SC, Gorbsky GJ (1998) Tension-sensitive kinetochore phosphorylation in vitro. *J Cell Sci* 111 (Pt 21):3189–3196. <https://doi.org/10.1242/jcs.111.21.3189>
35. Nokkala S, Kuznetsova VG, Maryanska-Nadachowska A, Nokkala C (2004) Holocentric chromosomes in meiosis. I. Restriction of the number of chiasmata in bivalents. *Chromosome Res* 12:733–739. <https://doi.org/10.1023/B:CHRO.0000045797.74375.70>
36. Novakova L, Kovacovicova K, Dang-Nguyen TQ, Sodek M, Skultety M, Anger M (2016) A Balance between Nuclear and Cytoplasmic Volumes Controls Spindle Length. *PLoS One* 11:e0149535. <https://doi.org/10.1371/journal.pone.0149535>
37. Okafornta CW (2025) Kymograph generation for 3D image (Version v0.0.1) [Software]. Zenodo. <https://doi.org/10.5281/zenodo.15010690>
38. Okafornta CW, Farhadifar R, Fabig G, Wu H-Y, Köckert M, Vogel M, Baum D, Haase R, Shelley MJ, Needleman DJ, Müller-Reichert T (2026) Cell size reduction distinctly scales spindle elongation and chromosome segregation in *C. elegans*. *Nat Commun* 17. <https://doi.org/10.1038/s41467-026-76360-3>
39. Paliulis L, Fabig G, Müller-Reichert T (2023) The X chromosome still has a lot to reveal - revisiting Hermann Henking's work on firebugs. *J Cell Sci* 136 4. <https://doi.org/10.1242/jcs.260998>
40. Paliulis LV, Nicklas RB (2000) The reduction of chromosome number in meiosis is determined by properties built into the chromosomes. *J Cell Biol* 150:1223–1232. <https://doi.org/10.1083/jcb.150.6.1223>
41. Paliulis LV, Nicklas RB (2004) Micromanipulation of chromosomes reveals that cohesion release during cell division is gradual and does not require tension. *Curr Biol* 14:2124–2129. <https://doi.org/10.1016/j.cub.2004.11.052>
42. Paliulis LV, Nicklas RB (2005) Kinetochore rearrangement in meiosis II requires attachment to the spindle. *Chromosoma* 113:440–446. <https://doi.org/10.1007/s00412-005-0330-z>
43. Pivarciova L, Vaneckova H, Provaznik J, Wu BC-H, Pivarci M, Peckova O, Bazalova O, Cada S, Kment P, Kotwica-Rolinska J, Dolezel D (2016) Unexpected Geographic Variability of the Free Running Period in the Linden Bug *Pyrrhocoris apterus*. *J Biol Rhythms* 31:568–576. <https://doi.org/10.1177/0748730416671213>
44. Rieckhoff EM, Berndt F, Elsner M, Golfier S, Decker F, Ishihara K, Brugués J (2020) Spindle Scaling Is Governed by Cell Boundary Regulation of Microtubule Nucleation. *Curr Biol* 30:4973–4983.e10. <https://doi.org/10.1016/j.cub.2020.10.093>
45. Rux CJ, Chong MK, Myers V, Cho NH, Dumont S (2026) Mechanical force locally damages, remodels, and stabilizes the lattice of spindle microtubules. *Curr Biol* 36:760–772.e4. <https://doi.org/10.1016/j.cub.2025.12.047>

46. Sato M, Kakui Y, Toya M (2021) Tell the Difference Between Mitosis and Meiosis: Interplay Between Chromosomes, Cytoskeleton, and Cell Cycle Regulation. *Front Cell Dev Biol* 9:660322. <https://doi.org/10.3389/fcell.2021.660322>
47. Schatten H (2022) Centrosomes in Reproduction. *Adv Anat Embryol Cell Biol* 235:55–73. https://doi.org/10.1007/978-3-031-20848-5_6
48. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682. <https://doi.org/10.1038/nmeth.2019>
49. Schrader F (1935) Notes an the Mitotic Behavior of Long Chromosomes. *Cytologia* 6:422–430. <https://doi.org/10.1508/cytologia.6.422>
50. Shakes DC, Neva BJ, Huynh H, Chaudhuri J, Pires-Dasilva A (2011) Asymmetric spermatocyte division as a mechanism for controlling sex ratios. *Nat Commun* 2:157. <https://doi.org/10.1038/ncomms1160>
51. Suresh P, Galstyan V, Phillips R, Dumont S (2022) Modeling and mechanical perturbations reveal how spatially regulated anchorage gives rise to spatially distinct mechanics across the mammalian spindle. *Elife* 11. <https://doi.org/10.7554/eLife.79558>
52. Warren WD, Steffensen S, Lin E, Coelho P, Loupart M, Cobbe N, Lee JY, McKay MJ, Orr-Weaver T, Heck MM, Sunkel CE (2000) The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis. *Curr Biol* 10:1463–1466. [https://doi.org/10.1016/S0960-9822\(00\)00806-X](https://doi.org/10.1016/S0960-9822(00)00806-X)
53. Wilson EB (1909) Studies on chromosomes. IV. The “accessory” chromosome in *Syromastes* and *Pyrrochoris* with a comparative review of the types of sexual differences of the chromosome groups. *J. Exp. Zool.* 6:69–99. <https://doi.org/10.1002/jez.1400060103>
54. Winey M, Mamay CL, O'Toole ET, Mastronarde DN, Giddings TH, McDonald KL, McIntosh JR (1995) Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J Cell Biol* 129:1601–1615. <https://doi.org/10.1083/jcb.129.6.1601>
55. Winter ES, Schwarz A, Fabig G, Feldman JL, Pires-daSilva A, Müller-Reichert T, Sadler PL, Shakes DC (2017) Cytoskeletal variations in an asymmetric cell division support diversity in nematode sperm size and sex ratios. *Development* 144:3253–3263. <https://doi.org/10.1242/dev.153841>
56. Xu Y, Chao A, Rinaldin M, Kickuth A, Brugués J, Di Talia S (2025) The cell cycle oscillator and spindle length set the speed of chromosome separation in *Drosophila* embryos. *Curr Biol* 35:655–664.e3. <https://doi.org/10.1016/j.cub.2024.11.046>
57. Yu C-H, Redemann S, Wu H-Y, Kiewisz R, Yoo TY, Conway W, Farhadifar R, Müller-Reichert T, Needleman D (2019) Central-spindle microtubules are strongly coupled to chromosomes during both anaphase A and anaphase B. *Mol Biol Cell* 30:2503–2514. <https://doi.org/10.1091/mbc.E19-01-0074>

Figures

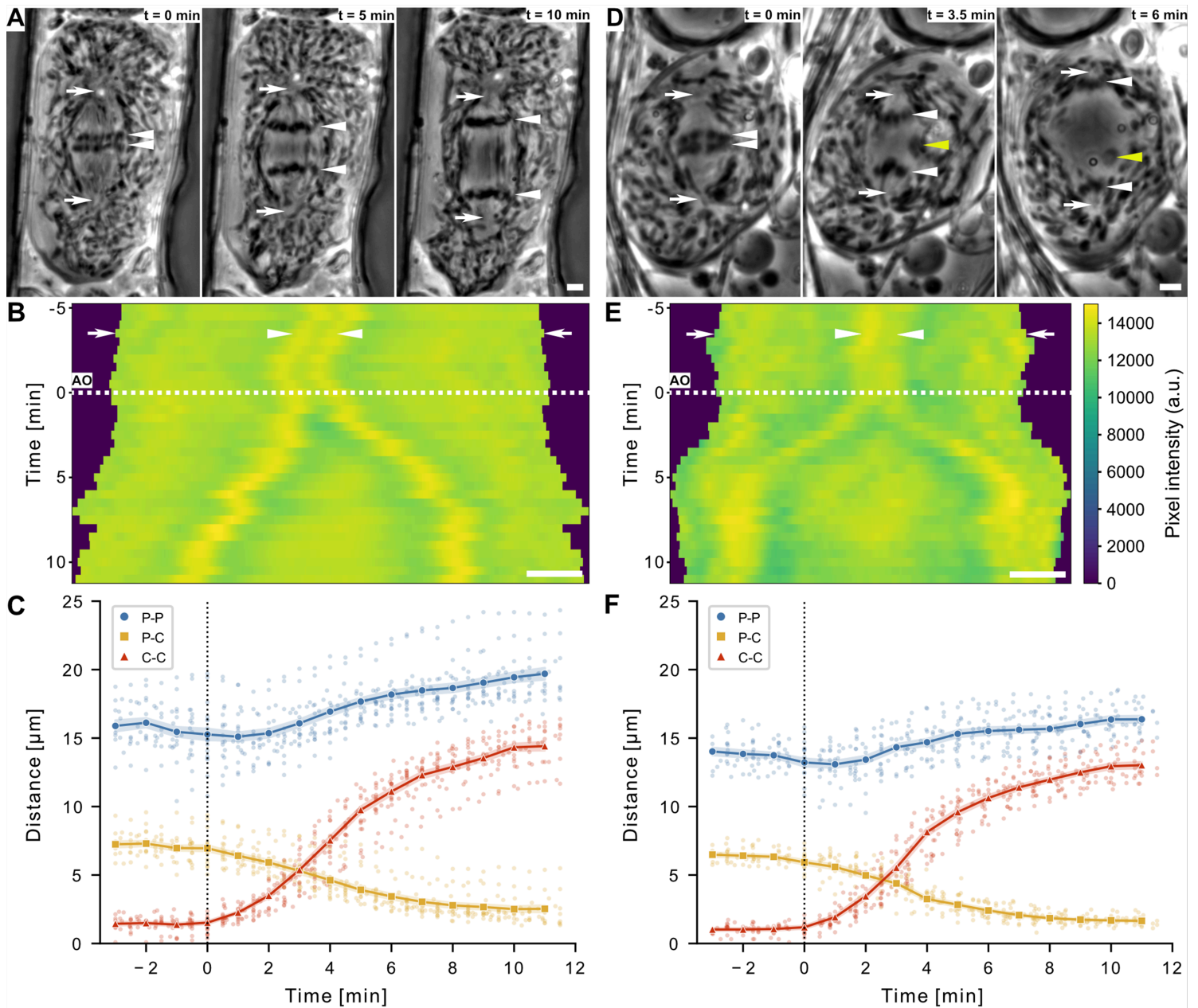


Figure 1

Comparative analysis of spindle and chromosome dynamics in meiosis I and meiosis II. (A) Phase-contrast extended-depth-of-focus (EDF) projections from live-cell imaging of a representative spermatocyte during meiosis I, from anaphase onset (AO, $t = 0$ min) to $t = 10$ min. Spindle poles (white arrows) and chromosomes (white arrowheads) are indicated. Scale bar, 2.5 μm . (B) Kymograph of the cell in (A), showing the positions of the spindle poles and the chromosomes over time; AO is marked by a dashed line. The color bar indicates pixel intensity (a.u.). Scale bar, 2.5 μm (horizontal); time axis as indicated. (C) Pole-to-pole (P-P, blue), pole-to-chromosome (P-C, yellow) and chromosome-to-chromosome (C-C, red) distances in meiosis I as a function of time (min). Points show onset-centered binned means of all cells ($n = 19$ for P-P and P-C; $n = 20$ for C-C); shaded bands, $\pm$ SEM; the dotted vertical line marks AO. (D) EDF projection of a representative meiosis II spermatocyte, from anaphase onset (AO, $t = 0$ min) to $t = 6$ min. The lagging univalent X chromosome is marked (yellow arrowhead). Scale bar, 2.5 μm . (E) Kymograph of spindle and chromosome dynamics in meiosis II, displayed as in (B). Scale bar, 2.5 μm . (F) P-P, P-C and C-C distances in meiosis II over time, displayed as in (C) ($n = 19$ for P-P

and P-C; n = 20 for C-C). Distances were obtained by tracking as described in Methods; for each measure, the plotted curve summarizes all cells. See Table 1 for numerical values.

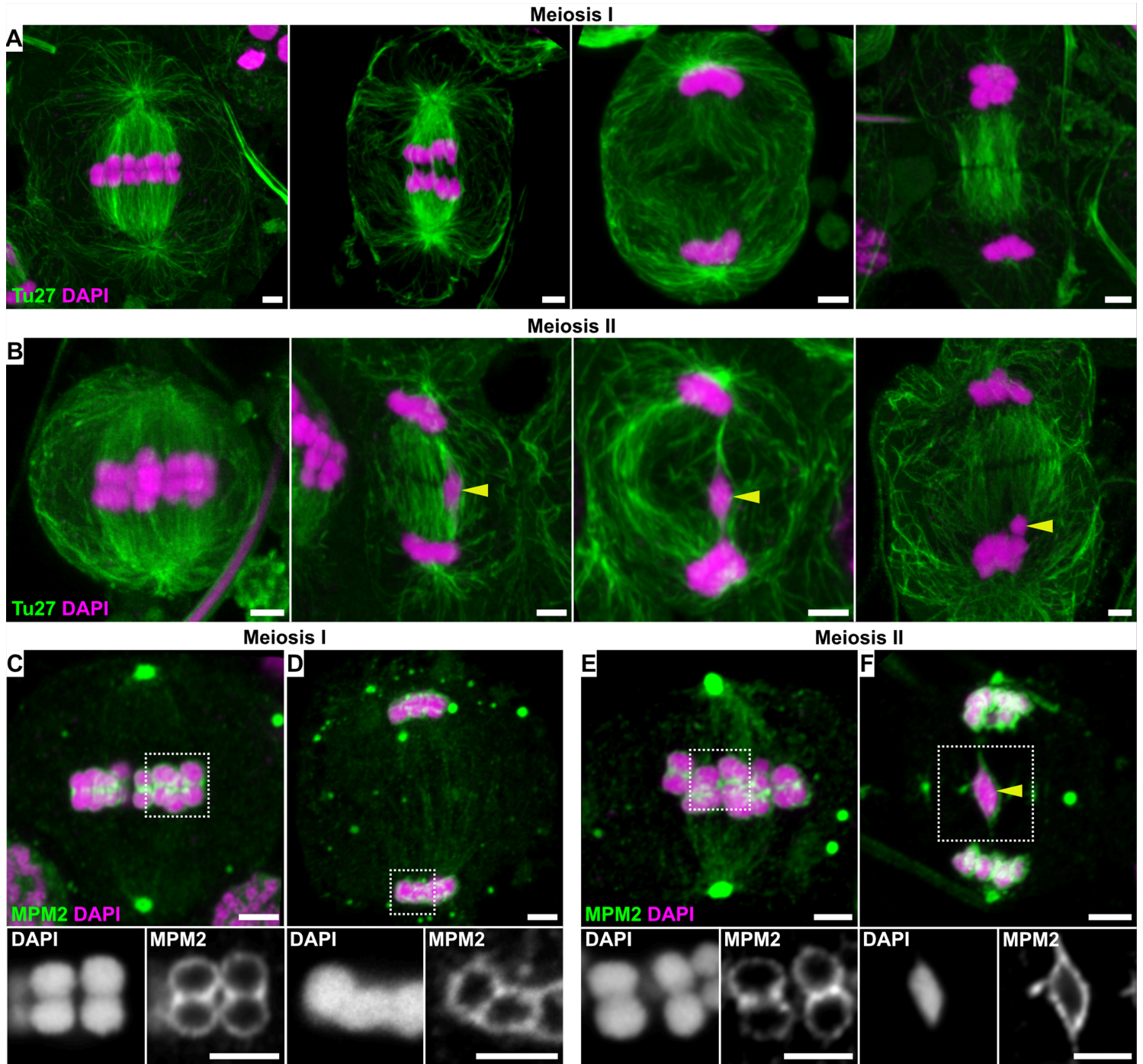


Figure 2

Immunofluorescence imaging of spermatocytes during meiosis I and II in *P. apterus*. (A) Spindle architecture during meiosis I from metaphase (left panel) to mid- (middle panels) and late anaphase (right panel). Spindle microtubules are stained with anti- β -tubulin (clone Tu27, green) and chromosomes with DAPI (magenta). (B) Spindle and chromosome organization in meiosis II; the lagging univalent X chromatid is visible during anaphase II (yellow arrowhead) and remains off-centered in late anaphase II. (C, D) Cells in meiosis I stained for mitotic phosphoproteins (MPM2, green), a marker of kinetochores in other insects, and for chromosomes (DAPI,

magenta), in (C) metaphase I and (D) anaphase I. (E, F) Cells in meiosis II stained as in (C, D), in (E) metaphase II and (F) anaphase II. Dashed boxes indicate the higher-magnification views (lower rows) that highlight the holocentric kinetochores throughout meiosis I and II; the lagging X chromatid is indicated by a yellow arrowhead. All images are maximum-intensity projections. Scale bars, 2.5 μm .

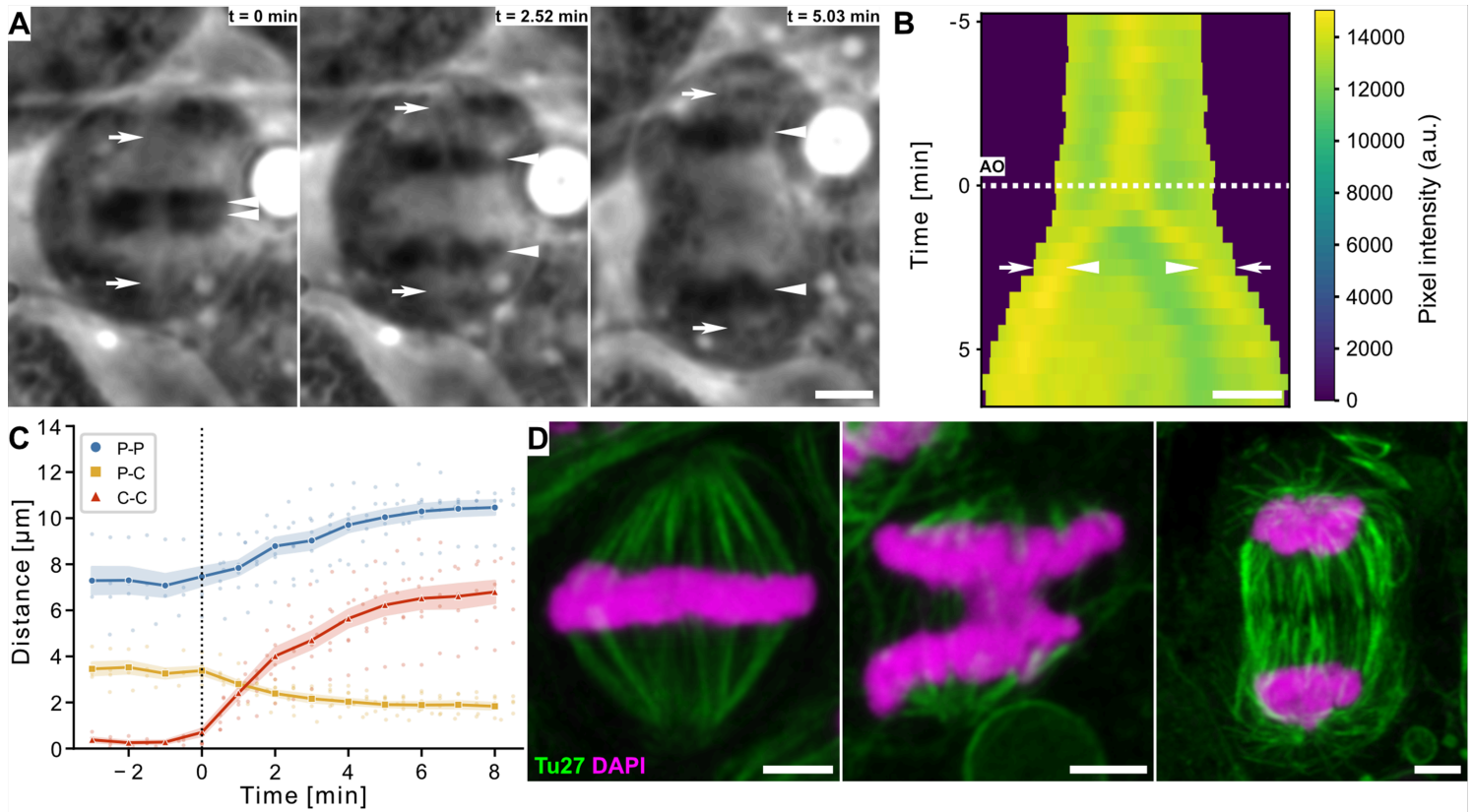


Figure 3

Analysis of mitotic spindle and chromosome dynamics. (A) Phase-contrast extended-depth-of-focus (EDF) projections from live-cell imaging of a representative mitotic spermatogonium, shown from anaphase onset (AO, $t = 0$ min) to $t = 5.03$ min. Spindle poles (white arrows) and chromosomes (white arrowheads) are indicated. Scale bar, 2.5 μm . (B) Kymograph of the cell in (A), showing the positions of the spindle poles and the chromosomes over time (min); AO is marked by a dashed line. The color bar indicates pixel intensity (a.u.). Horizontal scale bar, 2.5 μm . (C) Pole-to-pole (P-P, blue), pole-to-chromosome (P-C, yellow) and chromosome-to-chromosome (C-C, red) distances in mitosis as a function of time (min). Points and lines show onset-centered binned means across all cells ($n = 7$); shaded bands, $\pm$ SEM; the dotted vertical line marks AO. (D) Immunofluorescence of representative mitotic spindles in metaphase (left panel), early anaphase (middle panel) and late anaphase (right panel). Spindle microtubules are stained with anti- β -tubulin (clone Tu27, green) and chromosomes with DAPI (magenta). All images are maximum-intensity projections. Scale bars, 2.5 μm .

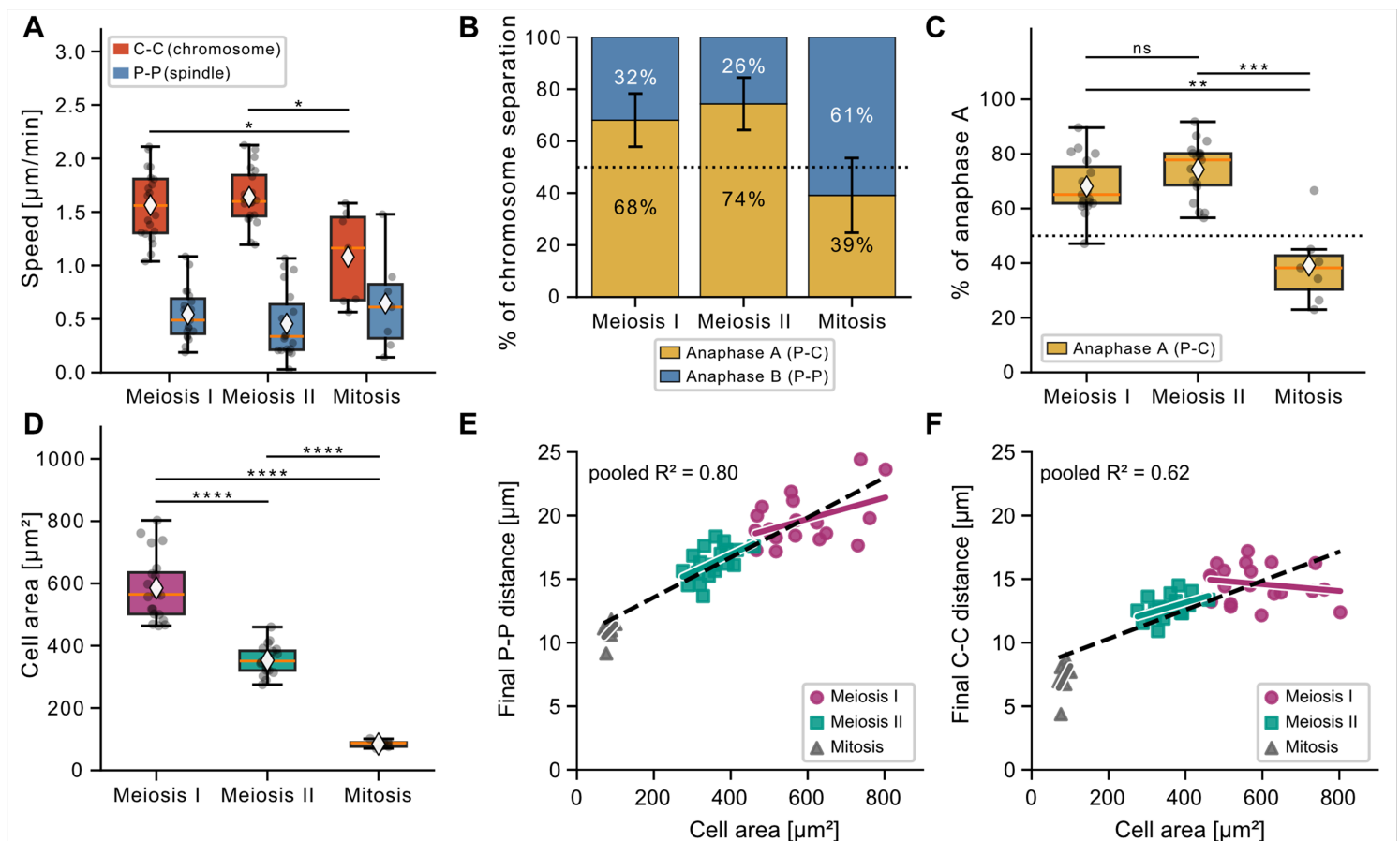


Figure 4

Comparative quantitative analysis of cell size, anaphase mechanism, and segregation speed across division types in *P. apterus*. (A) Chromosome (C-C, red) and spindle (P-P, blue) segregation speeds per division. Box plots show the median and interquartile range; group means are indicated (white diamonds). C-C speed was significantly slower in mitosis than in either meiotic division (Welch's *t*-test; * *p* < 0.05); C-C (n = 20, 20, 7) and P-P (n = 19, 19, 7). (B) Mean proportions of poleward chromosome movement (anaphase A, derived from the decrease in pole-to-chromosome distance; yellow) and spindle elongation (anaphase B, derived from the increase in pole-to-pole distance; blue) in driving total chromosome segregation, per division. Values represent percentages of total chromosome segregation; error bars denote standard deviation (SD) for the anaphase A component (n = 19, 19, 7). (C) For each cell, anaphase A component per division. Box plots show the median and interquartile range; individual cells are overlaid (grey dots) and group means are indicated (white diamonds). The anaphase A component was significantly higher in both meiotic divisions than in mitosis but did not differ between meiosis I and II (Welch's *t*-test; ** *p* < 0.01, *** *p* < 0.001, ns, not significant). Dotted horizontal lines in (B, C) mark 50%. (D) Cell area at anaphase onset for meiosis I (purple), meiosis II (green) and mitosis (grey; box, individual cells, and means as in C). Cell area decreased significantly from meiosis I to meiosis II to mitosis (n = 20, 20, 7; Welch's *t*-test, **** *p* < 10⁻⁴). (E) Final pole-to-pole (P-P) distance *versus* cell area. Points are individual cells colored by division; colored lines are within-division linear fits, and the black dashed line is the pooled fit (pooled slope, 0.016 µm per µm²). (F) Final chromosome-to-chromosome (C-C) distance *versus* cell area, displayed as in (E) (pooled slope, 0.011 µm per µm²). In both (E) and (F), the dimensions correlate with cell area across the three division types but not within any single division.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table2contributionssize.xlsx](#)
- [Table1dynamics.xlsx](#)
- [FigS4Movementtime.pdf](#)
- [TableS2scalingmatch.xlsx](#)
- [TableS1percell.xlsx](#)
- [FigS2Logisticfits.pdf](#)
- [FigS3Linearfits.pdf](#)
- [FigS1Traces.pdf](#)
- [TableS3datasetproperties.xlsx](#)
- [MovieS1MeiosisIAL01.avi](#)
- [MovieS2MeiosisIAL42.avi](#)
- [MovieS5MeiosisIIAL27.avi](#)
- [MovieS3MeiosisIAL47.avi](#)
- [MovieS6MeiosisIIAL35a.avi](#)
- [MovieS4MeiosisIIAL17.avi](#)
- [MovieS9MitosisAL126.avi](#)
- [MovieS8MitosisAL64b.avi](#)
- [MovieS7MitosisAL23.avi](#)