Supplemental Materials

Parthenogenesis as a solution to hybrid sterility: the mechanistic basis of meiotic distortions in clonal and sterile hybrids

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Supplementary Methods

DNA flow cytometry

After manual homogenization of testes tissue we applied 0.15% trypsin treatment to separate individual cells. Blood samples and sperm suspension of sexual species were used as internal control. Testes cell suspension and blood samples were incubated with 0.1% Triton X100, 10 µ/ml DAPI and 15 mM $MgCl_2$ for 4–6 h at +4°C to realize cell nuclei which were subsequently measured by BD FACSAria™ flow cytometer. At least 10,000 events were measured. Data were further analysed by BD FACSDiva software (version 6.1.3).

Pachytene chromosomes and immunofluorescent staining

Pachytene chromosomes were obtained from male and female gonads according to Moens (2006) and Araya-Jaime et al. (2015). After manual homogenization of male gonads, 1 µl of suspension was placed into the drop (30 µl) of hypotonic solution (1/3 of 1× PBS) preliminary dropped on SuperFrost® slides (Menzel Gläser) for 20 minutes and subsequently fixed in 2% paraformaldehyde for 4 minutes. After fixation slides were washed in 1× PBS and used for immunofluorescent staining (IF). In case of female, 20 µl of manually homogenized cell suspension from ovaries was dropped on SuperFrost® slides (Menzel Gläser) together with 40 µl of 0.2 M sucrose and 40 µl of 0.2% Triron X100. Slides were incubated for 7 min and subsequently fixed in 2% PFA for 16 minutes. After washing in 1x PBS slides were stored until IF procedures.

Synaptonemal complexes (SC) of chromosomes during pachytene were visualized using immunofluorescent staining (IF) with rabbit polyclonal antibodies (ab14206, Abcam) against SYCP3 protein which is the lateral component of SC and chicken polyclonal SYCP1 (a gift from Sean M. Burgess) which is the central component of SC. Recombination foci were identified using mouse monoclonal antibodies (MLH1 (ab15093, Abcam) against MLH1 protein (mismatch repair protein). Fresh not dried slides were incubating with 1% blocking reagent (Roche) in 1x PBS and 0.01% Tween-20 for 20 min and then with primary antibody (dilutions as recommended by manufacturers) for 1h at room temperature. Slides were washed in 1x PBS at RT and incubated in combination of secondary antibodies (Cy3-conjugated goat anti-rabbit IgG (H+L) (Molecular Probes) and Alexa-488-conjugated goat anti-mouse IgG (H+L) (Molecular Probes) for 1h at RT. Slides were washed in 1x PBS with 0.05% Tween-20 and mounted in Vectashield/DAPI (1.5 mg/ml) (Vector, Burlingame, Calif., USA).

Fluorescence *in situ* hybridization and Comparative genomic hybridization

In order to precisely distinguish bi-, uni- and multivalents, fluorescence *in situ* hybridization (FISH) with telomeric probe was performed on metaphase slides and slides with pachytene chromosomes after IF staining. Telomeric FISH using a Cy3 labelled PNA probe was performed according to the manufacturer's instructions (Telomere PNA FISH Kit/Cy3, Dako, Denmark)

To distinguish whether bivalents in hybrids are formed between homologous or orthologous chromosomes, we performed comparative genome hybridization (CGH) on meiotic metaphases and

lampbrush chromosomal spreads (Majtánová *et al.* 2016). Probes for CGH experiments were prepared with whole genomic DNA (gDNA) of pure parental species *C. elongatoides* and *C. taenia*. gDNA was extracted from muscles or fins using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *C. elongatoides* gDNA was labelled with biotin-16-dUTP (Roche, Mannheim, Germany); *C. taenia* gDNA was labelled with digoxigenin-11-dUTP (Roche, Mannheim, Germany) using a Nick Translation Mix (Abbott) following the protocol supplied by the manufacturer. The best results were obtained after 2.5 – 3 hours of nick translation until labelled DNA fragments were approximately 200–500 bp long. In case of meiotic metaphase chromosomes we performed CGH on the same slides where we detected telomeric sites. Hybridization probes combined nick translated products with gDNA of both parental species and hybridization mixture (50% formamide, 2x SSC, 10% dextran sulphate, salmon sperm DNA). Hybridization was performed at 37°C for 48 hours, followed by post-hybridization washes in 50% formamide/2× SSC at 42°C for 5 min (3 times) and 2× SSC for 5 min (3 times). The biotin-dUTP and digoxigenin-dUTP labelled probes were detected using streptavidin-Cy3 (Invitrogen, San Diego, Calif., USA) and antidigoxigenin-FITC (Invitrogen, San Diego, Calif., USA) correspondingly. The chromosomes were counterstained with Vectashield/DAPI (1.5 mg/ml) (Vector, Burlingame, Calif., USA).

Diplotene chromosomes

Diplotene chromosomal spreads (lampbrush chromosomes) were prepared from parental and hybrid females according to the protocol initially developed for amphibian oocytes (Callan 1986) with modifications suggested by Gall et al. (1991). Vitellogenetic oocytes of 0.5–1.5 mm in diameter were taken from non-stimulated females within the OR2 saline (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$,1mM Na_2HPO_4, 5 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); pH 7.4). Nuclei were then microsurgically isolated from oocytes by jeweler forceps and needles in the isolation medium "5:1" (83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 1mM $MgCl_2$, 1 mM dithiothreitol; pH 7.0–7.2). Nuclear envelopes were manually removed in one-fourth strength "5:1" medium with the addition of 0.1% paraformaldehyde and 0.01% 1M $MgCl_2$ in a chambers attached to a slide, meaning that in each chamber we obtained chromosome spread from individual oocytes. Slide with oocyte nuclei contents were subsequently centrifuged for 20 min at +4°C, 4000 rpm, fixed for 30 min in 2% paraformaldehyde in 1x PBS, and post-fixed in 70% ethanol overnight (at 4°C). Description of bivalents morphology and lampbrush chromosome maps construction were performed according to Callan (1986) in Corel™ DRAW graphics suite X8 software.

Wide-field and fluorescence microscopy

Mitotic and meiotic chromosomes after FISH, CGH and IF were inspected using Carl Zeiss Axio Imager.Z2 and Provis AX70 Olympus microscopes equipped with standard fluorescence filter sets. Microphotographs of chromosomes were captured by CCD camera (DP30W Olympus) using Olympus Acquisition Software and CoolCube 1 using Metasystem platform for automatic search, capture and image processing. Microphotographs were finally adjusted and arranged in Adobe Photoshop, CS6 software.

Supplementary references

Araya-Jaime C., É. A. Serrano, D. M. Z. de Andrade Silva, M. Yamashita, T. Iwai, et al., 2015 Surface-spreading technique of meiotic cells and immunodetection of synaptonemal complex proteins in teleostean fishes. Mol. Cytogenet. 8: 4. https://doi.org/10.1186/s13039-015-0108-9

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Gall J. G., C. Murphy, H. G. Callan, and Z. A. Wu, 1991 Lampbrush chromosomes. Methods Cell Biol. 36: 149–166.

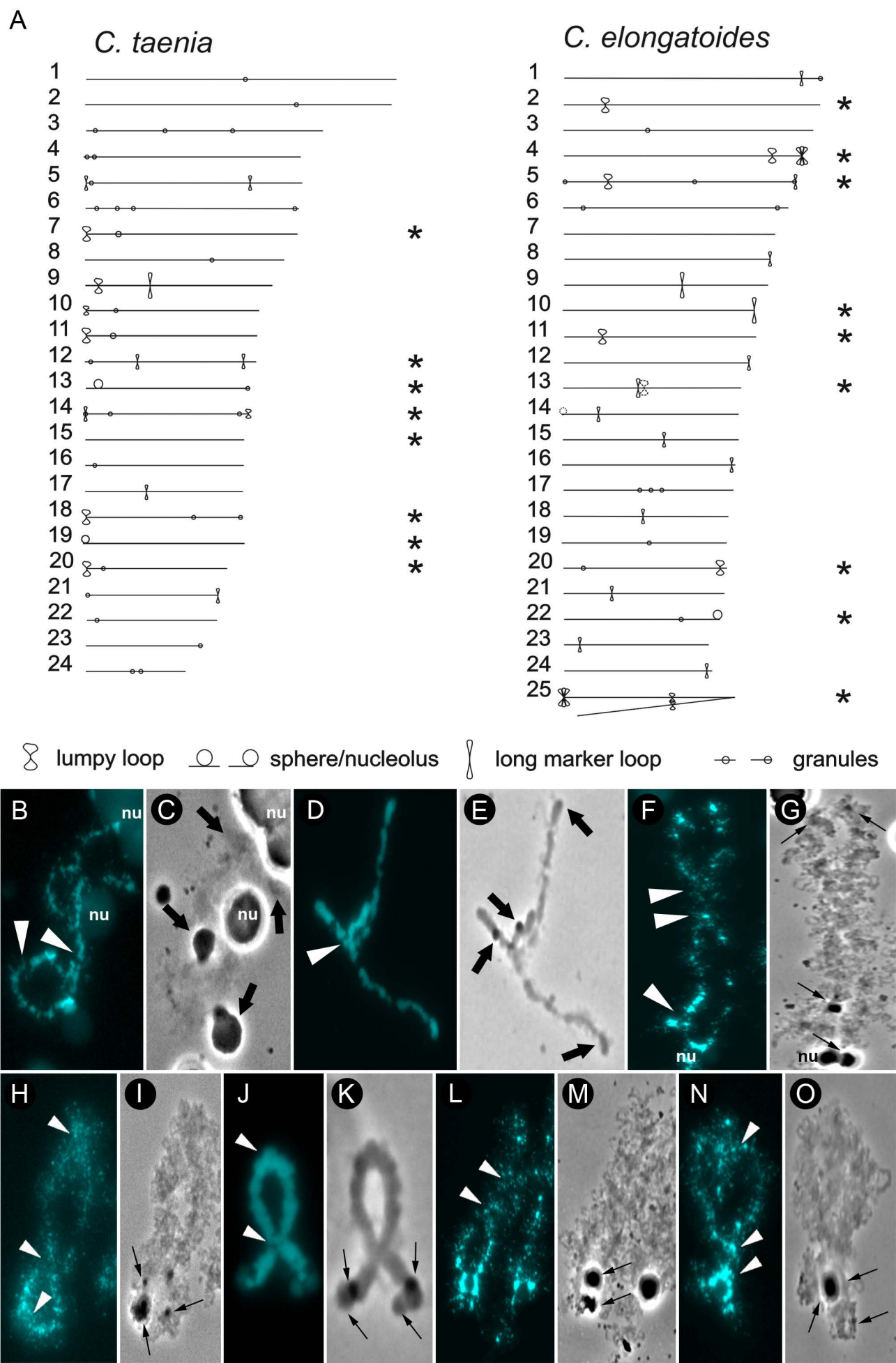
Majtánová Z., L. Choleva, R. Symonová, P. Ráb, J. Kotusz, et al., 2016 Asexual Reproduction Does not apparently increase the rate of chromosomal evolution: karyotype stability in diploid and triploid clonal hybrid fish (*Cobitis*, Cypriniformes, Teleostei). PLOS ONE 11: e0146872. https://doi.org/10.1371/journal.pone.0146872

Moens P. B., 2006 Zebrafish: chiasmata and interference. Genome 49: 205–208. https://doi.org/10.1139/g06-021

Supplementary Figures

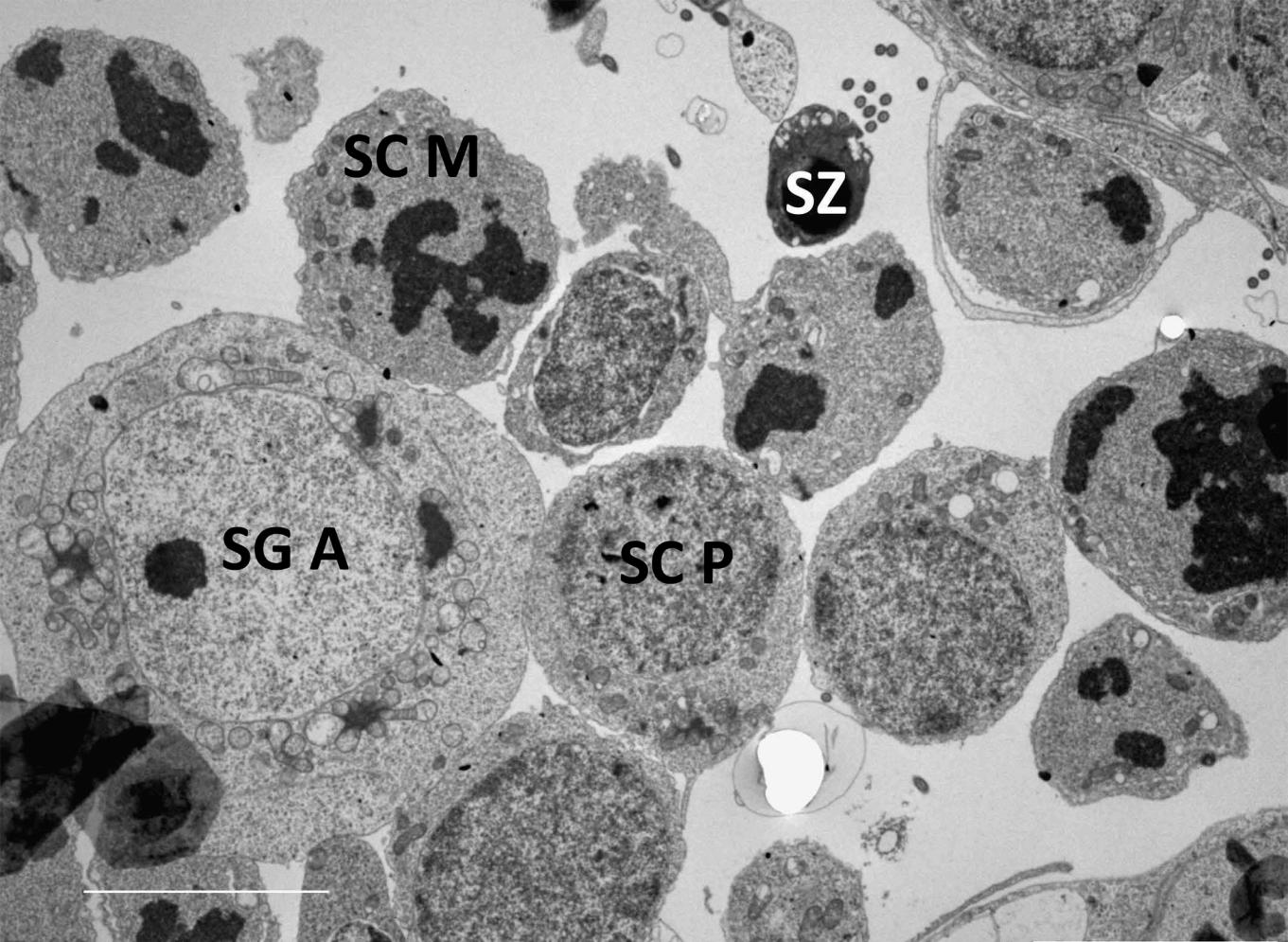
Supplementary Figure 1

A: Cytological maps of lampbrush chromosomes from *C. taenia* and *C. elongatoides* with indication of relative position of marker loops, associated spheres or nucleoli and prominent granules. Asterisks denote diagnostic lampbrush chromosomes with remarkable markers which are reliable for the identification of individual chromosomes in hybrids. Panels B-G demonstrate the morphology of one *C. elongatoides* diagnostic chromosome (chromosome 5) as appearing in spreads of *C. elongatoides* (B, C), diploid (D,E) and triploid (F,G) hybrid females, respectively Panels H-O demonstrate the morphology of one *C. taenia* diagnostic chromosome (chromosome 7) as appearing in spreads of *C. taenia* (H,I), diploid (J,K) and triploid (L-O) hybrid females, respectively (note that ETT female has two copies of such chromosome). Both DAPI and phase-contrast image are shown. Arrows indicate the most prominent marker structures. Arrowheads indicate chiasmata. Scale bar = 10μm


A
C. taenia
C. elongatoides
lumpy loop
sphere/nucleolus
long marker loop
granules
B
C
D
E
F
G
H
I
J
K
L
M
N
O
nu

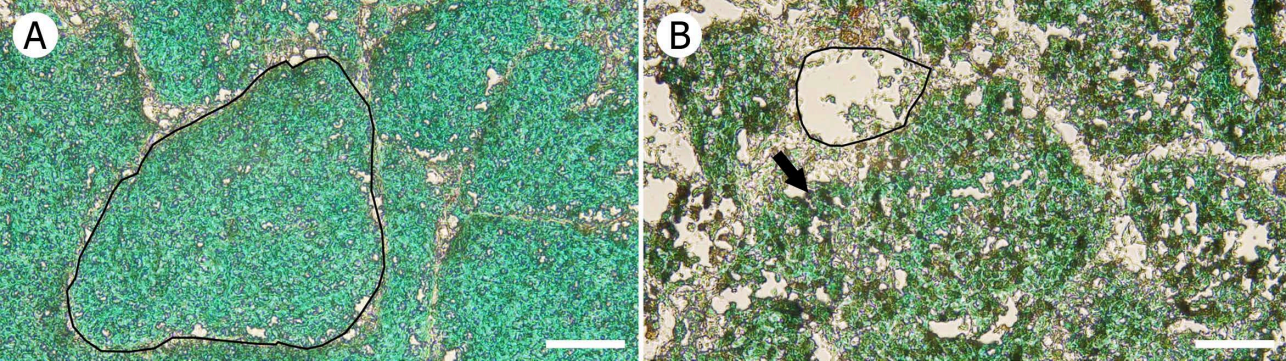
Supplementary Figure 2

A detailed view of the testicular cyst of ETT male with asynchronous development of germ cells containing spermatogonia A (SG), spermatocytes in prophase I (SC P), spermatocytes in metaphase I (SC M) and spermatozoa (SZ). Scale bars = 5 μm.


SC M
SZ
SG A
SC P

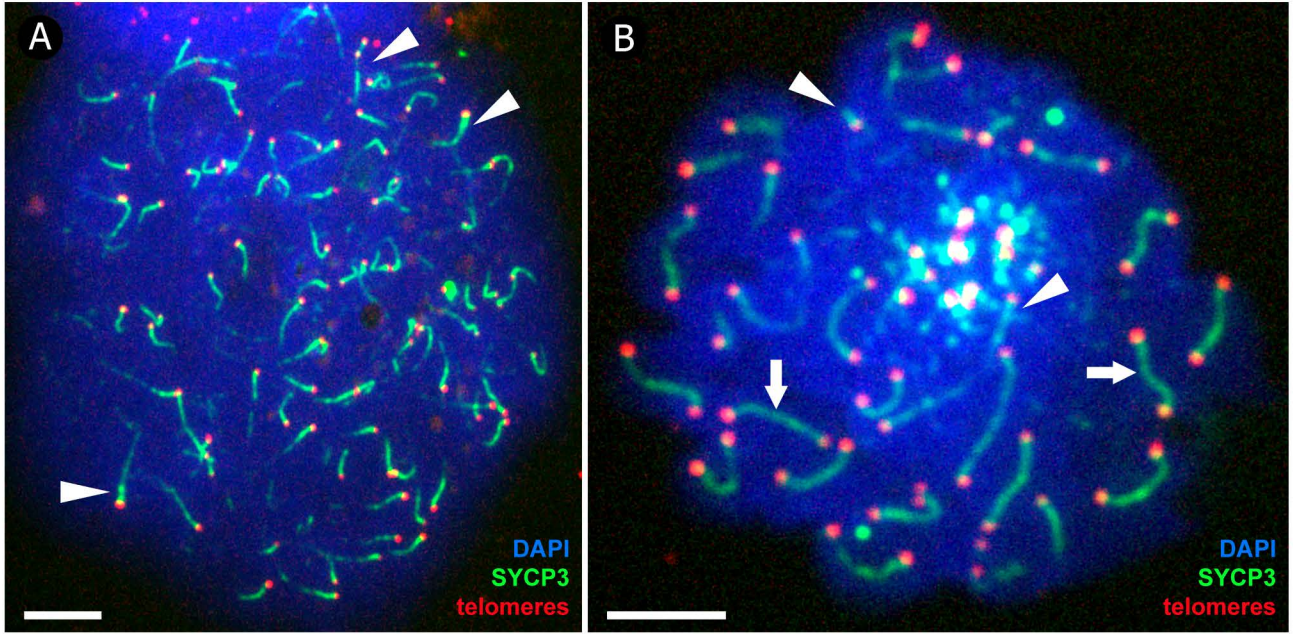
Supplementary Figure 3

Evidence for apoptosis from paraffin sections of testes from *C. elongatoides* (EE, left panel) and triploid hybrid (ETT, right panel) using terminal deoxynucleotidyl transferase detected using streptavidin-horseradish peroxidase (dark stain) with methyl green counterstain (green). The blackline encircles sperm cyst with spermatozoa; arrow shows apoptotic spermatocyte. Magnification 400x.


A
B

Supplementary Figure 4

Spread pachytene spermatocytes of diploid ET (A) and triploid ETT (B) hybrid males stained with DAPI (blue) showing SCs visualized by immunolabelling with antibodies against SYCP3 protein (green). FISH with telomeric PNA probes (red) indicated chromosomal ends. Note that hybrids usually show a partial formation of SCs at the subtelomeric regions only (indicated by arrowheads). Sometimes, the normal formation of SCs was also observed (indicated by arrows). Scale bar = 10µm.


A
DAPI
SYCP3
telomeres
B
DAPI
SYCP3
telomeres