

Sex without crossovers mimics clonal reproduction in *Rhynchospora tenuis*

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Meiotic recombination ensures accurate chromosome segregation and promotes genetic diversity by generating crossovers between homologous chromosomes¹. Although essential in most sexually reproducing organisms, recombination is variably regulated and can be absent in some lineages, a condition known as achiasmy². However, obligate achiasmy in both sexes of a sexual species has not been documented. Here we investigate *Rhynchospora tenuis*, a flowering plant with the lowest known chromosome number and inverted meiosis³. Combining genomics with molecular experiments, we show that *R. tenuis* undergoes obligate, genome-wide achiasmy in both male and female meiosis. Despite normal early meiotic axis formation, synapsis fails, crossovers are undetectable cytologically and genetically, and univalents persist at metaphase I. Haplotype-specific accumulation of transposable elements generates segregation distortion favouring the transmission of larger, repeat-rich chromosomes. Sexual reproduction is nevertheless retained: fertilization yields viable seeds only when translocation-compatible gametes meet, indicating strong post-meiotic selection against incompatible homozygous combinations. As a result, all surviving offspring are genetically identical, effectively maintaining heterozygosity by sexual reproduction with parental genotype restitution mimicking clonal reproduction. We propose that recombination loss, a low chromosome number, inverted meiosis and selection for compatible gamete combinations together enable faithful segregation and clonal-like inheritance despite sexual reproduction. These findings blur the boundary between sex and clonality, linking genome architecture, recombination loss and transmission bias.

Although meiotic recombination is critical for most sexually reproducing species, its frequency varies widely, often differing between sexes (heterochiasmy)^{4,5}, and can be entirely suppressed in specific chromosomes or lineages, known as achiasmy². In male *Drosophila melanogaster*, recombination and chiasmata are absent entirely, requiring alternative mechanisms such as chromatin threads or meiotic drive for correct chromosome segregation^{6–10}. The absence of recombination is expected to impair chromosome segregation, reduce genetic diversity and accelerate mutation accumulation through the Meselson

effect^{11–14}. However, obligate, genome-wide bisexual achiasmy of a sexually reproducing organism has not been described previously.

Rhynchospora tenuis Link is a small perennial beak-sedge of open, seasonally wet grasslands and savannas across tropical and subtropical America. It has the lowest known chromosome number among flowering plants ($n = 2$). *Rhynchospora tenuis* is also holocentric, with kinetochore activity distributed along the entire chromosome length, and undergoes inverted meiosis, in which sister chromatids segregate in meiosis I and homologues separate only in meiosis II^{3,15}—the reverse of

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the canonical order. Holocentric chromosomes tolerate structural rearrangements such as end-to-end translocations (hereafter, fusions)¹⁶, which may have facilitated the evolution of this extremely reduced chromosome number¹⁷. Previous cytological work showed that male meiosis in *R. tenuis* proceeds without homologous pairing or chiasmata, displaying four univalents at diakinesis and raising the possibility of achiasmy despite apparent meiotic double-stranded break formation³. Whether recombination is entirely absent in both sexes, and how accurate chromosome transmission and fertility are then maintained without crossovers, has remained unclear.

Here we combine chromosome-scale pangenomics, molecular cytogenetics and immunocytochemistry, single-gamete sequencing of pollen/seed nuclei and whole-genome sequencing of offspring from controlled crosses to characterize the recombination landscape, meiotic progression and inheritance patterns of *R. tenuis*. We show that *R. tenuis* exhibits obligate bisexual achiasmy, with failure of crossover formation, abnormal synapsis and marked segregation distortion favouring larger, transposable-element (TE)-enriched chromosomes, yet sexual reproduction repeatedly restores the parental heterozygous genotype. *Rhynchospora tenuis* therefore represents a previously undescribed mode of sexual genotype restitution, in which structurally divergent haplotypes are stably inherited across generations despite ongoing meiosis and fertilization.

Haplotype divergence and translocations

We assembled haplotype-phased chromosome-scale genomes for nine *R. tenuis* accessions (18 haplotypes) sampled across different locations in Brazil (Methods, Supplementary Table 1 and Supplementary Figs. 1–3). Despite its predominantly selfing mating system, *R. tenuis* shows very high *k*-mer-based haplotype divergence (>2% in all accessions; GenomeScope; Methods), exceeding the approximately 1% estimated for the closely related outcrossing *Rhynchospora breviuscula* (Supplementary Fig. 2). Epigenome profiling further revealed a distinctive chromatin organization of *R. tenuis* holocentromeres. Although CENH3 levels are comparable to those in *Rhynchospora pubera*¹⁷ and *R. breviuscula*¹⁸, other epigenetic features differ markedly, as *R. tenuis* shows reduced DNA methylation in CG context, together with an enrichment of the active histone mark H3K4me3 at *Tyba* satellite arrays¹⁹, in contrast to the more canonical, methylation-rich centromeric domains observed in the other species (Extended Data Fig. 1 and Supplementary Table 2). These features indicate an unconventional, unusually accessible centromeric chromatin environment that may alter holocentromere condensation and spatial organization during meiosis, potentially influencing chromosome behaviour and segregation.

Macrosynteny with closely related species reveals a stepwise fusion trajectory underlying the substantially reduced chromosome number of *R. tenuis*. The pattern of conserved collinear blocks is most parsimoniously explained by an ancestral *Rhynchospora* karyotype of $n = 5$, as found in *R. breviuscula* and most sampled *Rhynchospora* species^{17,20} and supported by the one-to-one syntenic correspondence between *R. breviuscula* and *Eleocharis vivipara*²¹. Two successive end-to-end translocations in a common ancestor appear to have produced an intermediate $n = 3$ karyotype by fusing ancestral chromosomes 2 and 5 (2 + 5), and 3 and 4 (3 + 4), an arrangement retained in the autohexaploid (6x) *Rhynchospora austrobrasiliensis*, the closest known relative of *R. tenuis*, which shares identical fusion breakpoints (Fig. 1a and Supplementary Fig. 4). A subsequent fusion of ancestral chromosome 1 with 2 + 5 yielded chromosome 1 (Chr1), while the fused 3 + 4 was retained as Chr2, forming the present $n = 2$ karyotype, conserved across all sequenced accessions (Fig. 1a and Extended Data Fig. 2).

In addition to these chromosomal fusions, pangenome-scale synteny identified marked chromosome size asymmetry between opposing haplotypes (that is, h1 and h2), varying from 8 Mb in the REC (Recife) accession to up to 32 Mb in the JGV (Jaguariaíva) and PECP

(Parque Estadual do Cerrado) accessions (Fig. 1b and Extended Data Fig. 2). Furthermore, population-specific telomeric reciprocal translocations were detected, ranging from 0.53 to 3 Mb at one end of each chromosome in *R. tenuis*, with the exchanged segments mapping consistently to different chromosomal ends in different accessions, being classified into three translocation types (Fig. 1a,b and Extended Data Fig. 2). The precise breakpoint configuration of each translocation type is described in Supplementary Note 1 (Extended Data Fig. 2b). To further validate the reciprocal translocations, we designed translocation-specific oligo probes based on the translocation type 1, found in the REC accession (Methods). Fluorescence in situ hybridization (FISH) using translocation-specific oligo probes and 45S rDNA (nucleolar-organizing regions (NORs)) probes validated the reciprocal translocations in all accessions (Fig. 1c and Extended Data Fig. 3). In REC, oligo probe 1 (magenta) and oligo probe 2 (cyan) label the ends of non-homologous chromosomes; JGV shares this arrangement on chromosome 1 but carries only a partial translocation on chromosome 2, and, as expected from the assemblies, the PECP accessions instead carry reciprocal translocations at the opposite chromosome ends, one combination of which contains the NORs (Fig. 1b,c and Extended Data Figs. 2 and 3). No reciprocal translocations were found in the close relative *R. austrobrasiliensis* ($2n = 6x = 18$) (Supplementary Fig. 5). Our results highlight the role of population-specific structural rearrangements on the unique genome organization of *R. tenuis*.

As *R. tenuis* has been reported to be achiasmatic, neither recombination nor segregation of homologous chromosomes is expected, a condition consistent with the Meselson effect. In such a system, homologues accumulate mutations independently, so divergence between haplotypes exceeds divergence among individuals or populations, generating subgenome-like differentiation in an otherwise diploid genome^{11,12}. *k*-mer similarity of all *R. tenuis* haplotypes shows a textbook signature of the Meselson effect, whereby the same haplotypes from all accessions cluster together while two haplotypes from the same individual do not cluster (Extended Data Fig. 4), mimicking an obligately asexual species¹².

To test whether the *R. tenuis* genome evolves under this regime, we have used subphaser²², a *k*-mer-based approach normally used to phase subgenomes in allopolyploid species (polyploid hybrids), on phased haplotypes to estimate divergence between haplotypes versus among-individual divergence. Notably, this analysis revealed pronounced subgenome-like differentiation between the two haplotypes across all accessions (Fig. 1d,e and Supplementary Fig. 6). This differentiation is mainly explained by haplotype-specific accumulation of TEs, which explains on average 84% of the marked chromosome size asymmetry found between haplotype 1 and 2, while other sequence types, such as *Tyba* repeats and genes, did not differ greatly between haplotypes (Fig. 1d,e and Extended Data Fig. 5). The extent and distribution of these structural variants, together with population-specific terminal reciprocal translocations, suggest long-term suppression, and effective absence, of meiotic recombination between haplotypes.

We next aimed to date the divergence between the two non-recombining haplotypes of *R. tenuis*. Considering that both haplotypes evolve independently without genetic exchange, we estimated their divergence time using synonymous substitution rates (K_s) calibrated with a fixed molecular clock (6.13×10^{-9} per site per year²³), and contrasted these values with insertion age estimates of haplotype-specific long terminal repeat retrotransposons (LTR-RTs) and single-nucleotide polymorphism (SNP)-based divergence estimates. The K_s -based molecular clock suggests that the two haplotypes diverged approximately 1.7 million years ago (Ma; Fig. 1f), whereas LTR insertion times indicate a more recent split, between 400,000 and 600,000 years ago (Fig. 1f). Divergence times between haplotypes calculated from SNPs divergence were estimated to range from 230,000 to 310,000 years for chromosome 1 and from 220,000 to 275,000 years for chromosome 2 (Fig. 1f). Reconciliation of these three estimates is discussed

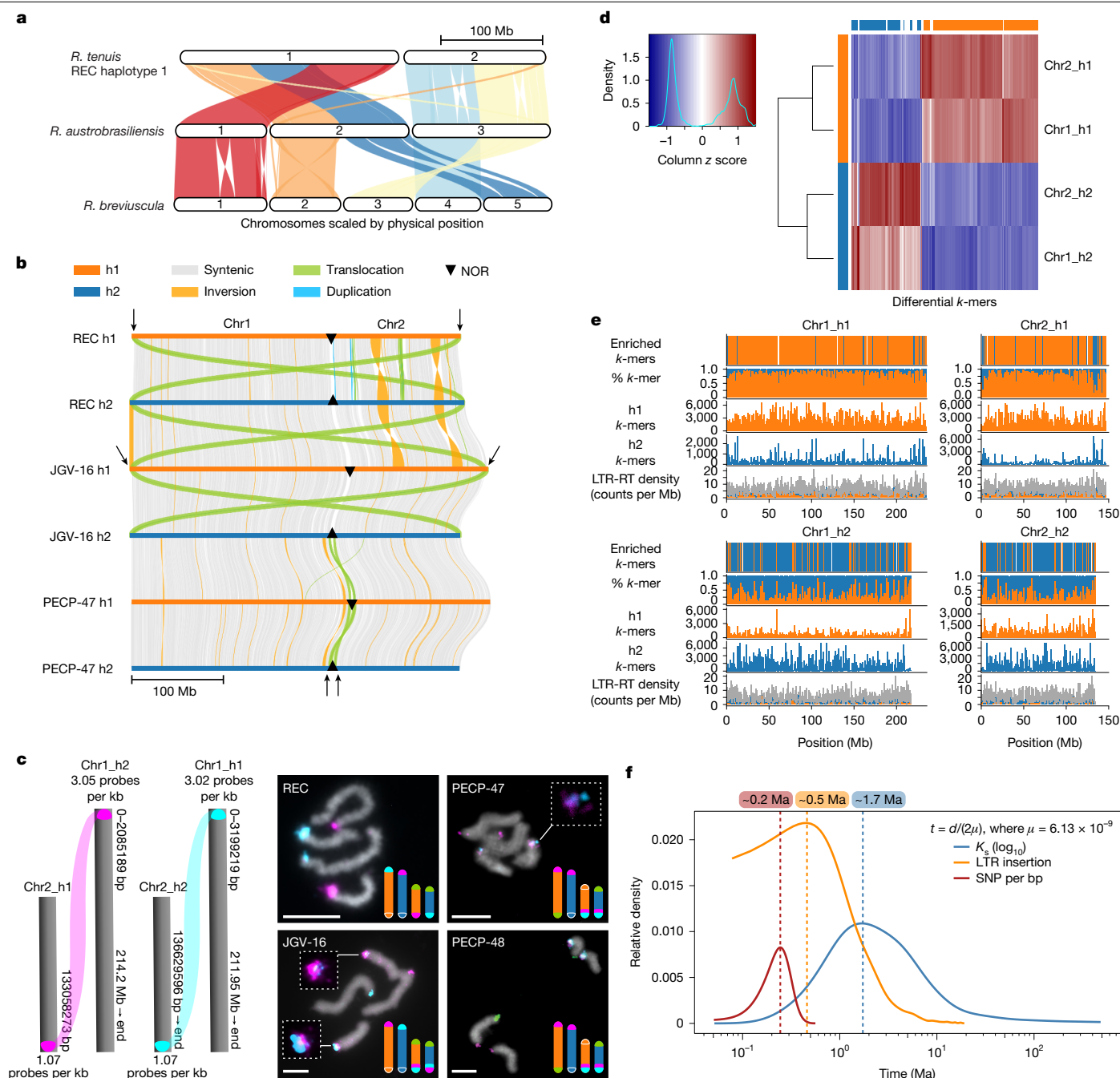


Fig. 1 | Overview of *R. tenuis* pangenome organization. **a**, Macro-synteny across *Rhynchospora* species delineates a stepwise reduction in chromosome number: conserved syntenic blocks support an ancestral karyotype of $n = 5$, fusions yielding $n = 3$ in *R. austrobrasiliensis* and a final fusion producing $n = 2$ in all *R. tenuis* accessions. **b**, Pangenome analysis identifies -1 Mb terminal reciprocal translocations at one end of each chromosome. **c**, The target regions used for haplotype-specific probe design and probe density. Oligo-FISH with translocation-specific probes (magenta, oligo probe 1; cyan, oligo probe 2) validates reciprocal exchanges in the REC and JGV accessions but not in PECP-47/PECP-48, in which 45S rDNA signals (green) lie on different homologues. Scale bars, 5 μm . $n = 10$ –34 mitotic cells per accession, examined over four independent experiments with similar results. **d**, k -mer-based subphasing reveals marked haplotype differentiation in all *R. tenuis* accessions, caused by differential accumulation of LTR retroelements; PECP-48 is shown as an

example. **d**, Unsupervised hierarchical clustering; the horizontal colour bar indicates the subgenome to which each k -mer is specific and the vertical colour bar the subgenome assigned to each chromosome. The heat map shows the z-scaled relative abundance of k -mers; a higher z indicates a greater relative abundance. **e**, Chromosomal characteristics. Tracks 1–5 show (1) significant enrichment of haplotype-specific k -mers (white, non-enriched windows); (2) normalized proportion of haplotype 1 and 2 k -mers; (3 and 4) raw counts of haplotype-1- and haplotype-2-specific k -mers; and (5) LTR-RT density (counts per Mb), coloured when significantly enriched in haplotype-specific k -mers, and grey when non-specific. Orange, enriched in haplotype 1; blue, enriched in haplotype 2. All statistics were computed in 1 Mb sliding windows. **f**, Divergence time estimates between the two non-recombining haplotypes from the density distribution of pairwise K_s ($\log_{10}$) of orthologous coding sequences, haplotype-specific LTRs and SNP density in syntenic regions.

in Supplementary Note 2. Despite this relatively recent divergence, *R. tenuis* already shows signatures that are consistent with early independent haplotype evolution, including an increase in pseudogene

abundance relative to the recombining relative *R. breviuscula* (Extended Data Fig. 5c). Pseudogenes in *R. tenuis* show a strong peak of high identity to functional genes, indicating a recent burst of duplication and

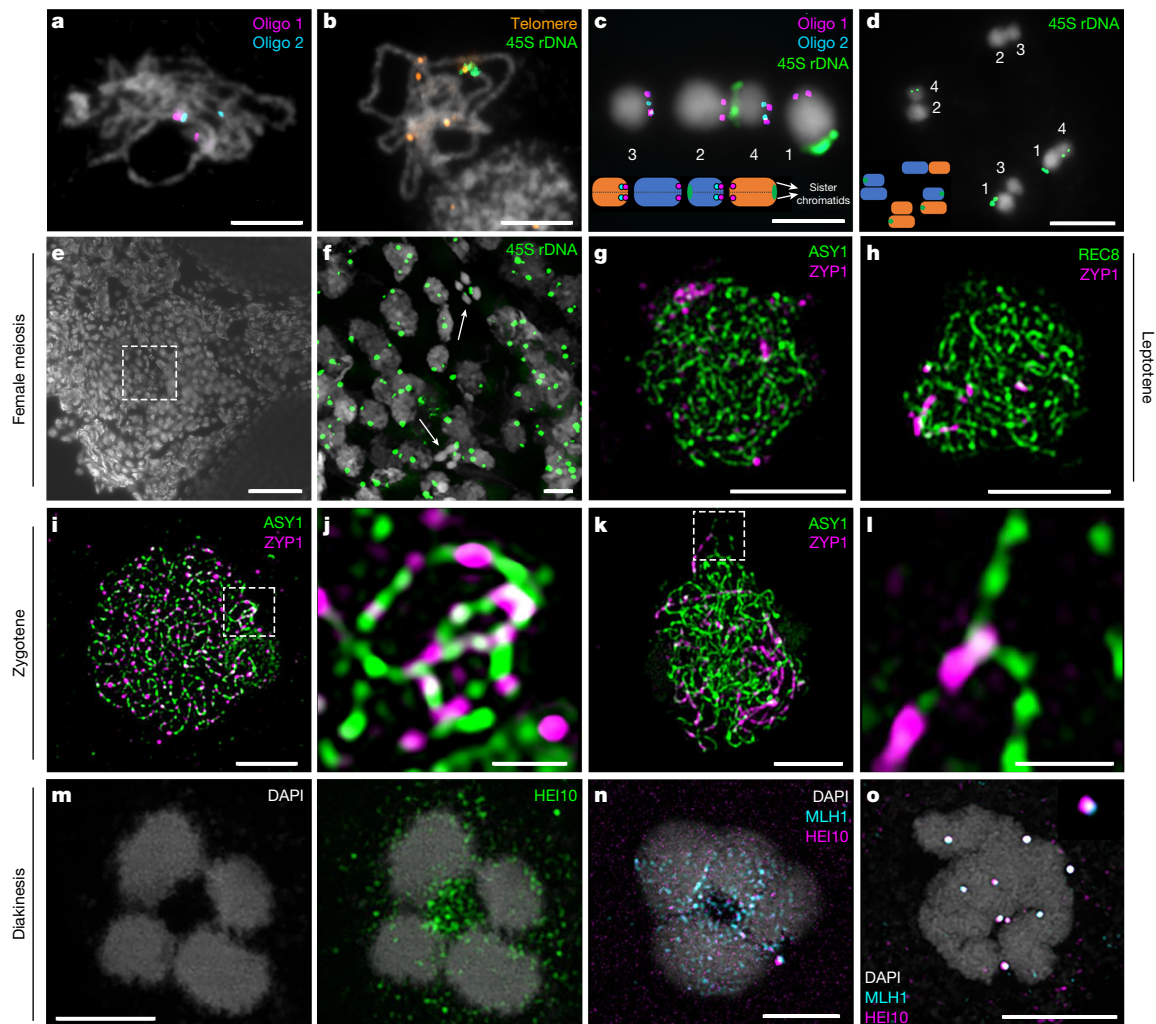


Fig. 2 | FISH and immunostaining confirm the failure of crossover formation during meiosis in *R. tenuis* (REC and PECP-48). **a, b**, FISH using translocation-specific probe sets (**a**) and 45S rDNA and telomeric probes (**b**) revealed neither pairing of translocation regions (**a**) nor bouquet formation (**b**) during early prophase I (zygotene/early pachytene-like stage ($n = 55$ cells in **a**, examined over five independent experiments). **c, d**, FISH with translocation-specific (**c**) and 45S rDNA (**d**) probes in metaphase I (**c**) and telophase II (**d**). $n = 11$ cells per stage, over two independent experiments. 1, Chr1_h1; 2, Chr1_h2; 3, Chr2_h1; 4, Chr2_h2. The schematics at the bottom illustrate the chromosome configurations corresponding to the FISH signals. **e, f**, Ovule tissue of *R. tenuis* (PECP-48) confirms analysis of female meiosis. $n = 3$ metaphase I meiocytes from two independent experiments. **f**, Magnified view of the inset in **e**,

highlighting two cells undergoing meiotic divisions, showing four univalents at metaphase I, with two 45S rDNA signals localized to one large chromosome (Chr1_h1) and one small chromosome (Chr2_h2). **g, h**, Immunostaining of ASY1 (**g**; $n = 41$) and REC8 (**h**; $n = 29$) with ZYP1 at leptotene. **i–l**, ASY1 and ZYP1 ($n = 21$) staining shows abnormal zygotene in *R. tenuis* (**i, j**), in contrast to normal axis and synapsis in *R. austrobrasiliensis* ($n = 24$) (**k, l**). Magnified views of the areas indicated by dashed boxes in **i** and **k** are shown in **j** and **l**, respectively. **m**, HEI10 immunostaining shows the lack of foci at diakinesis in *R. tenuis*. $n = 21$ meiocytes over four independent experiments. **n, o**, HEI10–MLH1 co-localization, showing no foci in *R. tenuis* ($n = 21$) (**n**), and co-localized foci in *R. austrobrasiliensis* ($n = 63$) (**o**). Scale bars, 5 μm (**a–d, f–i, k, m, n, o**), 50 μm (**e**) and 1 μm (**j, l**).

pseudogenization at around 0.2 Ma, close to the inferred haplotype divergence, whereas *R. breviscula* shows a broader, more homogeneous distribution (Supplementary Note 2). These patterns suggest that recombination suppression in *R. tenuis* may already limit the efficient removal of duplicated and non-functional sequences.

Asynapsis in achiasmatic meiosis

We previously reported the absence of chiasmata and a reversed order of chromatid segregation (inverted meiosis) in male *R. tenuis*³, and here we tested whether both features are consistent across populations (REC, PECP and JGV). Indeed, all of the genotypes analysed always display four univalents at diakinesis and no bivalents ($n = 10$, $n = 37$, $n = 51$, respectively), followed by equational division of sister chromatids ($n = 37$, $n = 28$, $n = 12$), consistent with the concept of inverted meiosis (Fig. 2a–d and Supplementary Fig. 7). We next

tracked translocation-specific oligo and 45S rDNA probes on meiotic chromosomes. During early meiotic prophase, translocation-specific oligo probes and telomeric signals are never associated, indicating an absence of pairing and synapsis, while rDNA regions are associated at the nucleolus ($n = 55$; Fig. 2a, b and Supplementary Fig. 8a), followed by diakinesis with four univalents and no bivalents, indicating asynapsis and achiasmy (Supplementary Fig. 8b). At metaphase I, univalents were oriented with their long axes perpendicular to the equatorial plate and sister kinetochores were bioriented toward opposite poles, resulting in equational segregation of sister chromatids at anaphase I and formation of two diploid products at telophase I/interkinesis (Fig. 2c and Supplementary Fig. 8c). During meiosis II, the previously separated sisters realigned as homologous non-sisters at metaphase II and segregated at anaphase II, yielding four haploid products (Fig. 2d and Supplementary Fig. 8d). Moreover, although female meiocytes were rarely observed, analysis of ovule tissue revealed metaphase I cells

with four univalents and no detectable bivalents ($n = 3$) (Fig. 2e,f and Supplementary Video 1), indicating that achiasmy also occurs during female meiosis in *R. tenuis*. Across all of the populations, the consistent absence of chiasmata and the inversion of the canonical meiotic order demonstrate that achiasmy and inverted meiosis are conserved features of *R. tenuis* rather than accession-specific anomalies.

We extended our analysis of meiotic progression in *R. tenuis* by immunostaining male meiocytes with antibodies against the axis components ASY1 (ref. 24) and REC8 (ref. 25), the synaptonemal complex (SC) transverse filament ZYP1 (ref. 26), and the crossover markers MLH1 (ref. 27) and HEI10 (ref. 28). Early prophase I appeared to be conserved: ASY1 ($n = 41$) and REC8 ($n = 29$) localized as linear signals along chromosomal axes at leptotene in all cells analysed (Fig. 2g,h and Extended Data Fig. 6a,b). At zygotene, ZYP1 fails to elongate as a linear signal representing the SC. Instead, it forms a scattered and fragmented signal ($n = 21$; Fig. 2i,j, Extended Data Fig. 6c and Supplementary Fig. 9a). In later stages, ZYP1 can form a more-extended signal, although still fragmented. Notably, HEI10 can form discrete foci; however, these foci do not colocalize with ZYP1 ($n = 13$); Extended Data Fig. 6d). Finally, at diakinesis, meiocytes invariably displayed four univalents, and no HEI10 or MLH1 foci indicative of crossovers were observed ($n = 21$; Fig. 2m,n and Supplementary Fig. 9b–d). These features, fragmented SC signal, absence of complete synapsis and absence of crossover markers, culminated in univalents at metaphase I, consistent with achiasmy.

As a contrast, the closest relative, *R. austrobrasiliensis*²⁹, exhibited canonical chiasmatic meiosis. ASY1 localized to the axes and was progressively unloaded during synapsis ($n = 24$), the SC was assembled as continuous ZYP1 linear signals ($n = 12$; Fig. 2k,l, Extended Data Fig. 7a and Supplementary Fig. 9a), and HEI10 initially appeared as closely spaced foci on ZYP1-marked regions, later localizing as a few intense foci on bivalents, co-localizing with MLH1 ($n = 63$) and confirming normal crossover maturation (Fig. 2o, Extended Data Fig. 7b–d and Supplementary Fig. 9b–d).

To explore a genetic basis for recombination failure in *R. tenuis*, we surveyed meiotic gene content and transcription. Most core meiotic genes and their corresponding transcripts are present (Supplementary Table 3 and Supplementary Dataset 1). However, we detected notable features, such as haplotype-specific copy-number expansion of *ASY1*, *ASY4* and *ZYP1*, as well as expansion and partial pseudogenization of *MSH5*, and high structural divergence of *PTD* (Supplementary Table 3). Notably, the recombination gene *SHOC1*, which is essential for the formation of class I meiotic crossovers in plants and mammals^{30–33}, appears to be transcriptionally silenced. Two independent RNA-sequencing (RNA-seq) datasets show no detectable expression of *SHOC1* (ref. 17), and epigenomic profiling reveals strong dimethylation of Lys9 of histone H3 (H3K9me2) and DNA methylation across the gene body in the CHG and CHH contexts in both haplotypes (Extended Data Fig. 8a), consistent with epigenetic repression. By contrast, the orthologous locus in the recombining species *R. breviuscula* shows detectable transcription and lower levels of non-CG methylation (Extended Data Fig. 8b). Analysis using quantitative PCR with reverse transcription (RT–qPCR) further confirmed the substantial reduction in *SHOC1* transcripts in two *R. tenuis* accessions, in contrast to the strong expression observed in the closely related recombining species *R. austrobrasiliensis* and *R. breviuscula* (Extended Data Fig. 8c). Despite this silencing signature, the gene retains an apparently intact coding sequence, suggesting that recombination suppression in *R. tenuis* may involve recent epigenetic inactivation of key meiotic components rather than structural gene loss.

No genetic crossovers in either sex

To test genetically whether crossovers are absent in *R. tenuis*, we applied our single-gamete sequencing pipeline^{18,20} to pollen nuclei across five geographically distinct accessions, using chromosome-scale,

haplotype-phased assemblies as references. Using high-throughput single-nucleus profiling (snRNA-seq and single-nucleus assay for transposase-accessible chromatin with sequencing (snATAC-seq)), we obtained 10,997 pollen nuclei in total that yielded high-confidence, genome-wide genotypes (Fig. 3a and Supplementary Table 4). Haplotype blocks spanned entire chromosomes with no confident crossovers detected in any gamete, demonstrating that male achiasmy is pervasive across accessions. This means that, if crossovers occur at all, they must be extremely rare, consistent with a complete suppression of recombination during male meiosis.

As our single-pollen sequencing examines only male meiosis, we assessed female recombination genetically by sequencing the offspring of controlled self-crossing heterozygous mother plants. In the highly heterozygous genome of *R. tenuis*, female crossovers would be visible as haplotype switch points in F_1 individuals. Whole-genome sequencing analysis of 49 and 62 F_1 individuals coming from the controlled self-crossed seeds from heterozygous mothers of REC and PECP accessions, respectively, revealed no recombination breakpoints on either chromosome in any of the offspring (Fig. 3b). Notably, all F_1 individuals carried the same two intact parental haplotypes as the mother, with no reliable gene conversion detected (Methods), demonstrating that female meiosis, like male meiosis, is achiasmatic. Furthermore, these results also indicate that all offspring show clonal-like formation as they mirror the parental genotype.

We next examined transmission patterns among *R. tenuis* viable pollen grains, which are lower than 75% across all accessions analysed (Supplementary Fig. 10). In *R. tenuis*, as a conserved feature of the *Rhynchospora* genus, male microsporogenesis is asymmetric: only one out of the four meiotic products matures into a functional microspore (pollen), while the remaining products degenerate^{34–37} (Extended Data Fig. 9a). Such asymmetry provides a cellular context for male meiotic drive, which is not expected under symmetric microsporogenesis. Consistent with this, allelic counts at haplotype-informative markers revealed strong, accession-specific segregation distortion among viable pollen (Fig. 3c). Under inverted meiosis, sister chromatids segregate at meiosis I, yielding diploid secondary spermatocytes; if meiosis II followed Mendelian segregation and all four products matured equivalently, the four genotype classes defined by the two chromosomes should be equally frequent. Instead, in 10,997 pollen nuclei with complete genotype calls, specific chromosome combinations were significantly over-represented: the observed frequencies of the three NOR-bearing classes departed from random segregation (χ^2 goodness-of-fit test, two-sided: $\chi^2 = 9.17$, d.f. = 2, $P = 0.010$), indicating distortion favouring the larger, TE-rich NOR-bearing haplotypes. Distortion was strongest in two closely related accessions (PECP-47, PECP-48), in which the larger, TE-rich haplotype 1 was preferentially transmitted (>50%) and pollen carrying both haplotype 2 chromosomes was rare (4%; Fig. 3d). As haplotype 1 exceeds haplotype 2 by around 32 Mb, mainly through LTR-RT accumulation, these results point to male meiotic drive in which TE-associated features skew chromosomal inheritance during or immediately after meiosis II. Together with the complete absence of crossovers, these findings indicate that transmission bias in the asymmetric microsporogenesis of *R. tenuis* favours particular haplotype combinations, with chromosome size the strongest correlate.

A structural basis for this distortion emerges from the position of the nucleolar organizer region (NOR, 45S rDNA). In REC and JGV, the NOR is not interchanged between non-homologous chromosomes, whereas in PECP-47 and PECP-48, the NOR-bearing segments are reciprocally translocated between Chr1 and Chr2. Consequently, one genotype class, Chr1_h2 + Chr2_h1, lacks any NOR, predicting compromised ribosome biogenesis and gamete inviability. Notably, single-nucleus genotypes retrieved pollen grains in PECP-47/PECP-48 showing the following frequencies: Chr1_h1 + Chr2_h1 (50%, bearing one NOR), Chr1_h1 + Chr2_h2 (28%, bearing two NORs), Chr1_h2 + Chr2_h1

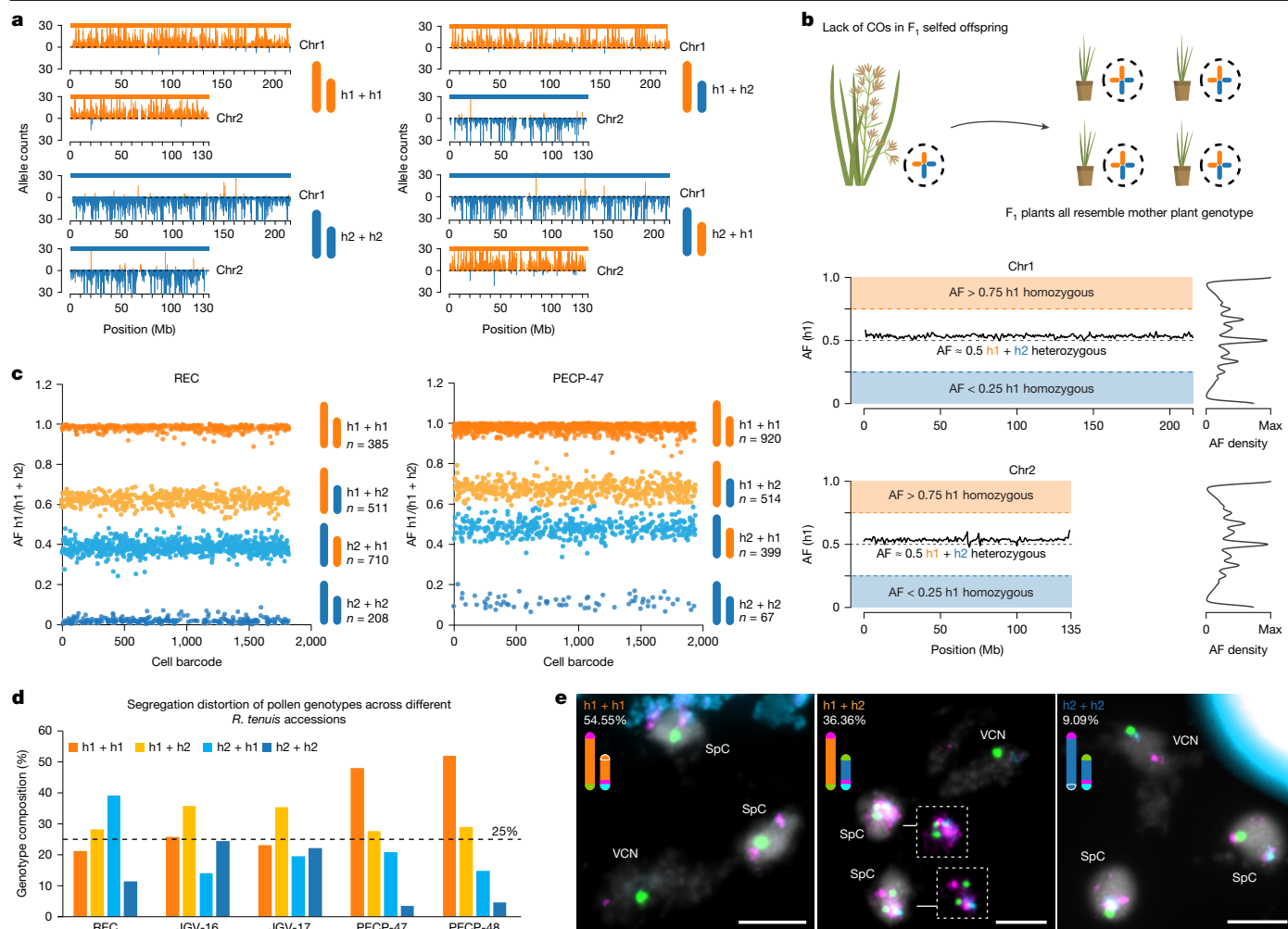


Fig. 3 | Single-gamete sequencing reveals complete crossover suppression and male meiotic drive in *R. tenuis*. **a**, Four pollen genotype combinations show chromosome-scale haplotype blocks without recombinants. The vertical lines show the allele counts of haplotype-specific SNP markers (orange, haplotype 1; blue, haplotype 2). No confident crossovers were detected in 10,997 pollen nuclei from five accessions. **b**, F_1 offspring (sample A03) of self-crossed mothers show chromosome-wide 1:1 allele ratios (haplotype 1 allele frequency (AF) ≈ 0.5 , in 1 Mb windows, 500 kb steps), indicating an absence of crossovers (COs) and mirroring of the maternal genotype; the same pattern occurred in all F_1 individuals ($n = 49$ REC, $n = 62$ PECP biologically independent plants). Orange and blue shading indicates regions homozygous for haplotype 1 (AF > 0.75) or haplotype 2 (AF < 0.25), respectively. The marginal density plots show the marker AF distribution. Window-based genotyping is described in the Methods. **c**, The AF (haplotype 1 markers/total markers) per pollen nucleus

reveals four genotype groups; each dot represents one nucleus ($n = 10,997$ nuclei, five accessions). Chromosomes with over 75% of markers from one haplotype were assigned to it; none was ambiguous. **d**, Genotype class frequencies, showing segregation distortion, which is strongest in PECP-47/PECP-48 and favours the larger, TE-rich haplotype 1. Two-sided χ^2 goodness-of-fit versus Mendelian segregation (25% per class, d.f. = 3): REC, $\chi^2 = 295.61$, $P < 2.2 \times 10^{-16}$; JGV-16, $\chi^2 = 39.27$, $P = 1.52 \times 10^{-8}$; JGV-17, $\chi^2 = 47.52$, $P = 2.69 \times 10^{-10}$; PECP-47, $\chi^2 = 782.71$, $P < 2.2 \times 10^{-16}$; PECP-48, $\chi^2 = 349.32$, $P < 2.2 \times 10^{-16}$. **e**, Haplotype-specific barcoding of mature pollen grains shows intact, non-recombinant transmission; in PECP-47/PECP-48, reciprocal NOR translocations eliminate NOR-null gametes post-meiotically. Oligo probe 1, magenta; oligo probe 2, cyan; 45S rDNA, green. SpC, sperm cell; VCN, vegetative cell nucleus. Scale bars, 5 μ m. $n = 33$ pollen grains, 2 independent experiments.

(18%, NOR-null) and Chr1_h2 + Chr2_h2 (4%, bearing one NOR). FISH on PECP-48 mature pollen grains ($n = 33$) with oligo probes and 45S rDNA recovered similar abundances, but only for genotype combinations retaining at least one NOR; the NOR-null class (Chr1_h2 + Chr2_h1) was never observed in mature pollen or in sperm cells from germinated pollen tubes (Fig. 3d,e and Extended Data Fig. 9b). Notably, in 90.5% ($n = 21$) of the germinated pollen tubes, we found only one single NOR signal in both tube nucleus and sperm cells, which is consistent with chromosomes from the same haplotype being inherited together (Extended Data Fig. 9b). In earlier pollen stages the NOR-null genotype was found only in the degenerating, non-selected cells (Extended Data Fig. 9c,d), indicating post-meiotic selection of haploid products during early pollen development. Together with a consistent bias favouring the larger, TE-rich haplotype 1 in PECP-47/PECP-48, these results

reveal asymmetric male meiotic drive in *R. tenuis*, in which structural features, including NOR placement and TE-associated genome expansion, skew chromosomal transmission in the complete absence of recombination.

Selection yields clonal-like progeny

To exclude that the obtained seeds from our F_1 offspring are derived from apomixis, a form of asexual seed formation in which embryos develop without fertilization or meiosis³⁸, we performed crossing experiments to confirm that seed formation requires fertilization (Fig. 4a). Notably, viable seeds were obtained only when both parents carried the same reciprocal translocation type (for example, PECP-47 $\times$ PECP-48; $n = 3$), whereas all other combinations failed to set

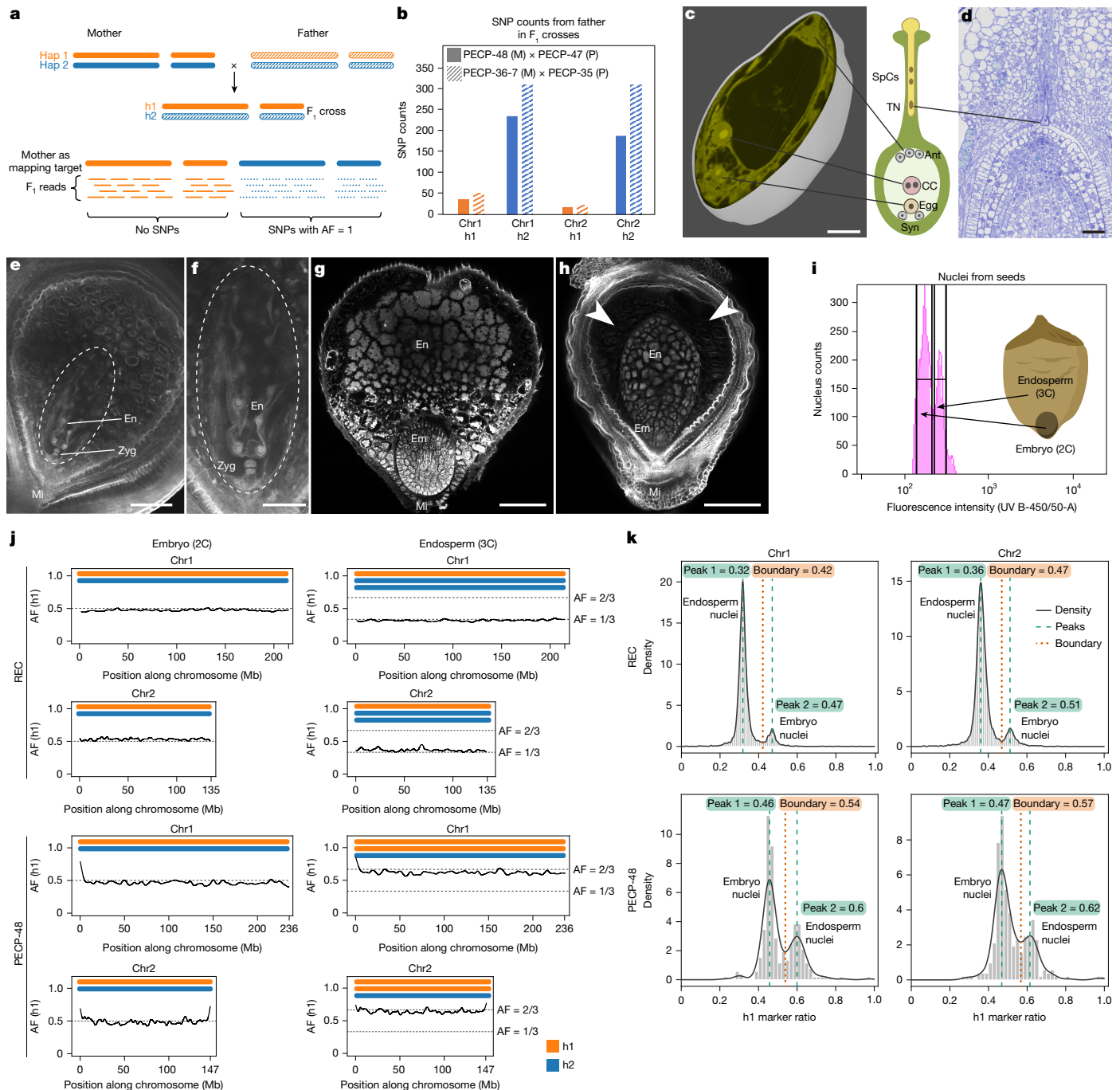


Fig. 4 | Female achiasmy and post-meiotic selection produce clonal-like progeny despite sexual reproduction. **a**, The strategy for inferring parental haplotype transmission: reads were mapped to the maternal phased assembly, whereby unchanged maternal haplotypes produce no SNPs and paternal haplotypes give SNPs with AF = 1. Orange and blue, maternal haplotypes 1 and 2; hatched, paternal homologues. **b**, SNP counts from two F_1 crosses between PECP accessions. Progeny reads aligned to the maternal genomes (PECP-48, PECP-36-7) show paternal-specific SNPs mostly on both haplotype 2 chromosomes (blue bars), confirming biparental fertilization. $n = 3$. M, maternal; P, paternal. **c–h**, Histology of gametophyte and embryo development in PECP-48; $n = 5$ independent ovules or seeds, from 2 independent experiments. **c**, 3D reconstruction of serial sections: egg cell (egg), synergids (syn), central cell (CC) with fused polar nuclei and antipodals (ant). $n = 15$. **d**, Pollen tube growth towards the ovule. $n = 15$. **e**, Early post-fertilization stage: zygote (zyg)

and coenocytic endosperm (en). $n = 11$. **f**, Enlargement of the region circled in **e**. **g**, A mature seed with a late-stage embryo surrounded by endosperm; embryos consistently developed from the micropyle (mi), indicating sexual origin. $n = 59$. **h**, A late aborted seed with arrested embryo and endosperm. $n = 9$. The arrowheads indicate collapsed embryo sac. **i**, Flow cytometry analysis of seed nuclei ($n = 150$ seeds) reveals diploid embryos and triploid endosperms. The gating strategy is shown in Supplementary Fig. 16. **j**, Single-nucleus sequencing of embryos ($n = 327$ REC, 76 PECP-48 nuclei) and endosperms ($n = 3,365$ REC, 185 PECP-48 nuclei) shows biparental contributions: embryos heterozygous, endosperms with the expected 2:1 maternal:paternal dosage. **k**, Allelic ratio distributions confirm this dosage bias and the post-meiotic selection restoring heterozygosity, with maternal transmission biased towards the larger, TE-rich haplotype (2 in REC, 1 in PECP-48). Scale bars, 10 μm (**c**), 20 μm (**d**) 50 μm (**f**) and 100 μm (**g** and **h**).

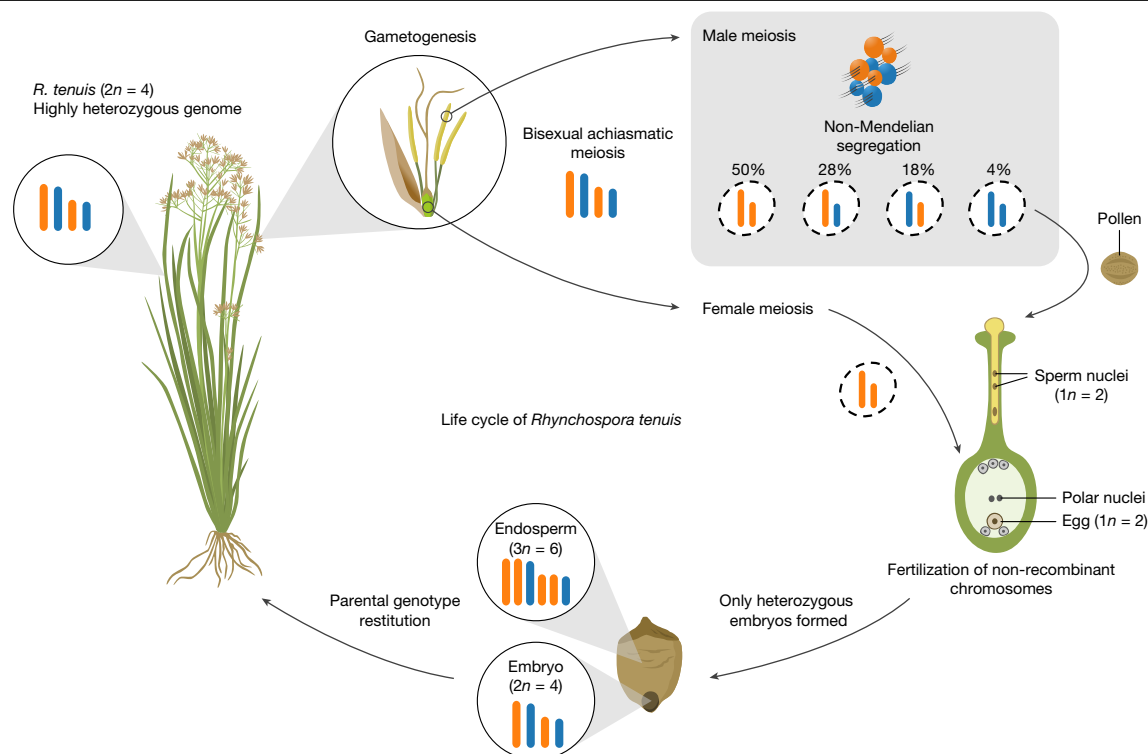


Fig. 5 | Life cycle of *R. tenuis* (PECP-48 genotype) under achiasmatic meiosis. Schematic representation of the *R. tenuis* life cycle ($2n = 4$). Both male and female meiosis proceed without crossovers (achiasmatic inverted meiosis). Male gametogenesis is asymmetric, yielding a single functional sperm nucleus

and non-Mendelian segregation. Fertilization restores the diploid state with intact haplotypes, but only heterozygous seeds develop into viable embryos, ensuring faithful restoration of the highly heterozygous parental genome each generation.

seeds ($n = 21$; Supplementary Fig. 11). Sequencing of offspring from these crosses confirmed that all plants were heterozygous, consistent with fertilization but indicating that heterozygosity is obligatory (Fig. 4b; Supplementary Dataset 2).

We next examined female gametophyte and early seed development histologically in early and mature flowers. Female gametophytes showed normal development, with well-differentiated embryo sacs and initiation of seed development consistent with fertilization (Fig. 4c). At early stages of megagametogenesis, multiple embryo sacs were frequently observed, of which only one continues to mature in 98% of the cases ($n = 59$; Supplementary Fig. 12). Pollen tube growth towards the ovule was consistently observed (Fig. 4d, Extended Data Fig. 10a,b and Supplementary Figs. 13 and 14). Although healthy embryos consistently developed from the micropyle, indicating sexual origin (Fig. 4e,f), many ovules failed to form seeds (Fig. 4h and Extended Data Fig. 10c–f). Undeveloped ovules contained intact, apparently unfertilized gametophytes, suggesting failed fertilization, possibly due to male gametophytic defects, whereas others had collapsed shortly after fertilization (Fig. 4h and Extended Data Fig. 10d–f). Furthermore, a high frequency (87%) of aborted seeds was observed ($n = 167$, 145 aborted and 22 fertile for PECP-48; Extended Data Fig. 10d–f), indicating strong post-meiotic and post-fertilization selection.

Flow cytometry analysis of full mature seeds ($n = 150$) confirmed diploid embryos and triploid endosperms (Fig. 4i), consistent with meiosis followed by double fertilization. snATAC-seq analysis of flow-sorted embryo ($n = 327$ (REC) and $n = 76$ (PECP-48)) and endosperm ($n = 3,365$ (REC) and $n = 185$ (PECP-48)) nuclei verified biparental contributions: embryos were uniformly heterozygous, while endosperms showed the expected 2:1 maternal:paternal dosage (Fig. 4j), enabling direct inference of female gamete haplotype composition. Notably, in both REC and PECP-48 accessions, a single maternal haplotype was always transmitted (haplotype 2 in REC and haplotype 1 in PECP-48; Fig. 4k).

In both cases, the driving haplotype corresponded to the physically larger, TE-enriched chromosomes.

Importantly, none of the imaged ovules or seeds showed evidence of apomictic development ($n = 11$). In all cases, embryos originated exclusively from the micropyle, never from sporophytic tissues, and only a single embryo was observed per seed. Flow cytometry analysis of mature seeds confirmed the expected 2:3 embryo:endosperm ratio, consistent with double fertilization. These results exclude diplospory, apospory and adventitious embryony as mechanisms of seed formation, supporting sexual reproduction as the sole reproductive mode in *R. tenuis*.

Our findings indicate that seed production in *R. tenuis* is strictly sexual, but its success depends on translocation compatibility and the maintenance of heterozygosity, which appears to be essential for embryo viability. Together with the single-gamete sequencing data, these findings establish obligate, genome-wide achiasmy in both sexes. The apparent paradox of clonal-like offspring arising through sexual reproduction can be explained by extreme segregation distortion, reinforced by post-meiotic selection, which ensures that only heterozygous zygotes with specific haplotype combinations survive. As a result, viable progeny repeatedly reconstitutes the parental heterozygous genotype despite originating from biparental fertilization (Fig. 5).

Discussion

Previous cytological work established inverted meiosis and suggested achiasmy in *R. tenuis*³. Here we show, at the genomic, molecular and population levels, that recombination is undetectable in both sexes despite normal initiation of meiosis, and that meiotic drive with post-meiotic selection sustains sexual genotype restitution despite the loss of crossovers. Thousands of sequenced gametes and multiple progenies reveal intact parental haplotypes and offspring genetically

identical to their heterozygous mothers. This clonal-like inheritance arises through extreme segregation distortion, haplotype-biased transmission and strong post-meiotic selection that restricts viable zygotes to specific heterozygous combinations.

Although achiasmy occurs in limited contexts, such as *Drosophila* males⁷, *Bombyx mori* females¹⁰ or non-recombining sex chromosomes², *R. tenuis* appears to represent a case of complete, genome-wide achiasmy in both sexes of an otherwise sexually reproducing multicellular eukaryote. A partial parallel exists in the budding yeast *Saccharomyces ludwigii*, which suppresses crossovers while retaining SPO11-dependent double-stranded break formation and high heterozygosity through intratetrad mating³⁹; however, in contrast to *R. tenuis*, this unicellular, automictic system lacks the developmental, cytological and selective constraints of multicellular reproduction, gametophyte competition and post-zygotic viability. The unusual karyotype of *R. tenuis* provides a permissive framework: holocentric chromosomes stabilize segregation in the presence of rearrangements¹⁷, inverted meiosis reduces reliance on chiasmata³ and an extremely reduced chromosome number minimizes missegregation, together creating a context in which recombination can be lost without compromising segregation or reproductive success.

The system both parallels and diverges from other non-Mendelian inheritance mechanisms. In *Ooceraea biroi* ants and *Mesorhabditis belari* nematodes, heterozygosity is retained through co-segregation of recombinant chromatids even when recombination is active^{40,41}. A related plant analogue occurs in *Oenothera*, where balanced lethal and extensive reciprocal translocations enforce permanent translocation heterozygosity and restrict recombination to subterminal regions, thereby maintaining heterozygous haplotypes across generations^{42–45}. By contrast, *R. tenuis* achieves the same functional outcome through a combination of bisexual achiasmy and gametic selection.

Rhynchospora tenuis exhibits a form of sexual genotype restitution in which structurally divergent haplotypes are transmitted intact across generations, producing clonal-like inheritance despite ongoing meiosis and fertilization. This resembles the Meselson effect, which predicts progressive haplotype divergence in non-recombining lineages through independent mutation accumulation^{11,12}; however, in contrast to the parthenogenetic booklouse *Liposcelis bostrychophila*⁴⁶, *R. tenuis* retains meiosis and fertilization while eliminating homozygous combinations by post-meiotic selection. Moreover, meiotic drive consistently favours larger, TE-rich haplotypes, potentially constraining haplotype turnover during gametogenesis. Consistent with a relatively recent onset of recombination suppression, *R. tenuis* already shows early signatures of independent haplotype evolution, including recent pseudogene accumulation and asymmetric sequence divergence, but without evidence of extensive long-term genomic degeneration.

Stepwise structural rearrangements can also progressively suppress recombination before the evolution of asexuality, as in the planarian *Schmidtea mediterranea*⁴⁷, and altered meiosis underlies stepwise transitions between hybridogenesis, parthenogenesis and triploidy in *Bacillus* stick insects⁴⁸. However, in *R. tenuis*, recombination disappears through obligate achiasmy while meiosis and fertilization persist, and clonal-like inheritance arises from meiotic drive and post-meiotic selection rather than from a transition to asexuality—possibly an intermediate state on a trajectory towards obligate asexuality. These comparisons are developed in Supplementary Note 3.

The genetic basis of recombination failure in *R. tenuis* remains unclear. Despite copy-number differences (such as *ASY1*, *ASY4*, *ZYP1* and *MSH5*) and divergent variants (for example, *PTD*), the recombination machinery is intact at the sequence level and early meiotic processes appear to initiate normally before pairing and synapsis fail. However, our data suggest that recombination failure may involve epigenetic inactivation of the key crossover component *SHOC1* (refs. 30–33), which shows no detectable transcription in *R. tenuis* and exhibits strong CHG and CHH DNA methylation across the gene body in both

haplotypes, consistent with transcriptional silencing. As *SHOC1* is normally expressed in recombining *Rhynchospora* species, including *R. austrobrasiliensis* and *R. breviuscula*, this pattern suggests that heritable epigenetic silencing of an essential crossover gene may have contributed to the transition to obligate achiasmy in *R. tenuis*. The meiotic phenotype of *R. tenuis* resembles that of *SHOC1* mutants in *Arabidopsis* and rice^{31–33}, in which axis formation and early recombination proceed normally but crossovers fail to mature; in contrast to these mutants, *R. tenuis* tolerates the resulting segregation defects through its extremely low chromosome number and inverted meiosis, which allow stable segregation of univalents.

Alternatively, structural divergence between haplotypes, including reciprocal telomeric translocations and asymmetric TE accumulation, may disrupt homologous recognition and promote univalent formation. We consistently observe female drive favouring the larger, TE-rich haplotype, in independent accessions. As kinetochore activity extends along the entire chromosome in holocentric chromosomes during *Rhynchospora* meiosis^{3,18,37}, TE accumulation could enlarge the functional kinetochore and bias segregation consistent with centromere and holokinetic drive models^{49–51}. Over evolutionary time, such transmission bias, together with recombination suppression and structural rearrangements, could reinforce the maintenance of divergent haplotypes, although the contribution of genetic drift cannot currently be excluded.

Haplotype-specific TE expansion may reflect germline mobilization during gametophyte development^{52,53}, gradually enriching the haplotype preferentially transmitted through the ovule, or may derive from ancient hybridization followed by recombination suppression. Notably, the opposite direction of female drive observed in different accessions (haplotype 1 in PECP and haplotype 2 in REC) suggests that haplotype bias is dynamic rather than fixed, potentially reflecting local selection or stochastic shifts in drive polarity.

In conclusion, obligate bisexual achiasmy in *R. tenuis* expands the known limits of meiotic flexibility and blurs the distinction between sexual and clonal reproduction. Despite the apparent absence of crossovers, meiosis and fertilization remain functional through the combined effects of holocentricity, inverted meiosis, meiotic drive and strong post-meiotic selection, which together stabilize clonal-like inheritance without apomixis. Our findings establish *R. tenuis* as a unique system for investigating the evolutionary consequences of recombination loss and the emergence of alternative meiotic strategies.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-11057-7>.

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Methods

Plant material

Plants of *R. tenuis* and *R. austrobrasiliensis* were collected from natural populations in Brazil under appropriate permits and cultivated under controlled greenhouse conditions. Nine geographically distinct accessions of *R. tenuis* were used for genome sequencing and cytological analyses. Individual flowers and pollen were staged for meiotic analyses. All experimental material was propagated clonally from field-collected individuals to ensure genetic identity across assays.

Sequencing

High-molecular-mass DNA was extracted from young leaf tissue using a modified cetyltrimethylammonium bromide protocol. PacBio HiFi libraries were prepared and sequenced on the Sequel IIe platform. Illumina short-read sequencing was used for genome polishing and transcriptome profiling. Hi-C libraries were constructed using Arima Hi-C kits and sequenced on the Illumina NovaSeq 6000 platform. RNA was extracted from young inflorescences for expression analyses.

DNA isolation

High-molecular-mass DNA from *R. tenuis* and *R. austrobrasiliensis* was isolated from 1.5 g of material using the NucleoBond HMW DNA kit (Macherey Nagel). Quality was assessed using a FEMTO-pulse device (Agilent), and the quantity was measured using the Quantus Fluorometer (Promega).

PacBio

HiFi libraries were prepared according to the 'Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0' manual, with an initial DNA fragmentation by Megaruptor-3 (Diagenode) and final size-selection by BluePippin (Sage Science). Size distribution was again controlled by FEMTO-pulse (Agilent). Size-selected libraries were then sequenced on a Revo device using the Revo polymerase kit and Revo chemistry for 30 h (Pacific Biosciences).

Arima Hi-C

Plant tissues were cross-linked with 1% formaldehyde for 30 min at room temperature, and the reaction was quenched with 125 mM glycine for 10 min. Subsequently, the tissues were ground using a TissueLyser at a frequency of 30 Hz for 3 min. Nucleus extraction was performed using the CellLytic PN Plant Nuclei Isolation/Extraction Kit (Sigma-Aldrich) according to the manufacturer's protocol. Hi-C libraries were prepared using the Arima High Coverage Hi-C Kit (Arima Genomics, A410110) according to the manufacturer's instructions, and were then sequenced (paired-end, 2 × 150 bp) on the NextSeq 2000 instrument (Illumina).

Methyl-seq analysis

To investigate the methylome space in *R. tenuis* (REC accession), the relatively non-destructive NEBNext Enzymatic Methyl-seq Kit was used to prepare an Illumina-compatible library, and paired-end sequencing (2 × 150 bp) was performed on the NextSeq 2000 (Illumina) instrument. For each library, 10 Gb of reads was generated.

RNA-seq analysis

Total RNA was isolated from flower buds (REC accession). Poly(A) RNA was enriched from 1 µg total RNA using the NEBNext Poly(A) mRNA Magnetic Isolation Module. RNA-seq libraries were prepared as described in the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs). A total of 11 cycles was applied to enrich the library concentration. Sequencing was performed at BGI Genomics using the BGISEQ-500 system on the DNBseq platform in paired-end mode with a read length of 2 × 150 bp.

Illumina libraries (TPase and DNA FS)

Genomic DNA from 49 and 59 controlled self-crossed *R. tenuis* REC and PECP35-7 accessions, respectively, were deep-sequenced on the Illumina HiSeq 3000 system or, alternatively, using DNBseq short-read sequencing (BGI Genomics) in 150 bp paired-end mode.

k-Mer-based haplotype divergence estimation

Heterozygosity levels of *R. breviscula*, *R. austrobrasiliensis* and all *R. tenuis* accessions were estimated by GenomeScope2.0 (ref. 54). *k*-Mer counting was performed using FASTK (v.1.1; <https://github.com/thegenemyers/FASTK>) with PacBio HiFi reads, which were used for genome assembly, as the input. The *k*-mer size was 31 (Fastk -k31). The resulting binary output (.hist) from FASTK was then converted to a text histogram file by Histex (part of FASTK): Histex -G hifi_31mer.hist > hifi_31mer.histo. The histogram was then analysed with GenomeScope2 using *k* = 31 and ploidy = 2, except for *R. austrobrasiliensis*, for which ploidy = 6.

To avoid ambiguity, we interpret the GenomeScope estimate as a reference-free measure of haplotype divergence rather than as strict SNP heterozygosity. GenomeScope infers this value from the *k*-mer frequency spectrum by modelling the relative contribution of haplotype-specific and shared *k*-mers. Thus, the estimate captures sequence differences that generate haplotype-specific *k*-mers, including SNPs, indels and unique haplotype-specific regions, but it may treat repetitive divergent sequence differently from unique sequence. We therefore use this value as a complementary measure of genome-wide haplotype divergence, particularly useful in *R. tenuis*, where highly divergent or structurally variable regions may be excluded from alignment-based estimates.

Genome assemblies

HiFi reads were assembled using Hifiasm (v.0.25.0) with the default settings, combining both PacBio HiFi reads and Hi-C reads to generate haplotype-resolved contigs. The assembly quality was assessed using BUSCO (v.5.2.2)⁵⁵ and QUAST (v.5.3.0)⁵⁶. Duplicated haplotypes were retained for downstream analysis.

Scaffolding

Chromosome-scale scaffolding was performed using the Juicer and 3D-DNA pipelines^{57,58}. Contigs from each haplotype generated from Hifiasm were taken as Hi-C alignment targets. Hi-C contact maps were visualized using Juicebox Assembly Tools to manually curate misjoins and validate the final pseudomolecules. Haplotypes were scaffolded independently and phased using *k*-mer-based alignment strategies.

k-Mer completeness and assembly artefact evaluation

To evaluate whether haplotype size discrepancies reflected true biological variation rather than assembly artefacts or missing sequence, we performed *k*-mer completeness and copy-number spectrum analyses using Merqury (v.1.3). For each accession, 19-mers were derived from raw PacBio HiFi reads using Meryl (canu v.2.1). We evaluated *k*-mer multiplicity distributions across individual haplotype assemblies (h1 and h2) and the combined diploid assembly. Assembly completeness and structural integrity were confirmed by inspecting the presence of unrepresented read *k*-mers (read-only *k*-mers) relative to expected heterozygous (1×) and homozygous (2×) coverage peaks. A 2× multiplicity peak in the isolated h1 spectrum representing shared homozygous *k*-mers was omitted from h2. The absence of read-only *k*-mer peaks at heterozygous and homozygous coverage positions in the diploid spectrum confirms high assembly completeness across both haplotypes, demonstrating that observed haplotype size differences represent genuine structural variation rather than differential assembly collapse or gapped repetitive regions.

Gene-based synteny analysis

Genes used for synteny analysis were annotated by a deep-learning-based tool Helixer (v.0.3.4)^{59,60} with the land plant mode. Gene synteny was analysed for *R. breviscula* haplotype 1, *R. austrobrasiliensis* haplotype 1, and all haplotypes of nine *R. tenuis* accessions using GENESPACE (v.1.3.1)⁶¹ running in R (v.4.2.0) along with the dependent tools OrthoFinder (v.2.5.5)⁶² and MCScanX (v.1.0.0)⁶³. The R script for running GENESPACE (run_genespace.R) can be found in our project GitHub page (https://github.com/Raina-M/Rhynchospora_tenuis_project).

DNA sequence-based synteny analysis

Collinearity of all *R. tenuis* haplotypes was analysed by SyRI (v.1.5.3)⁶⁴. The alignment between haplotypes was conducted using minimap2 (v.2.28)^{65,66} with the following parameter settings: -ax asm5 --eqx. The plot was generated using plotsr (v.0.5.3) with a minimal 50 kb syntenic block size. NOR regions labelled on the haplotypes were searched by BLAST (v.2.12.0) with the *Arabidopsis* rDNA sequences as the template.

Pangenome analysis

Pangenomes were generated using minigraph (v.0.17) with phased chromosome-scale assemblies from nine accessions. Variants were extracted using vg toolkit v.1.40.0. Structural variants and sequence divergence were quantified across homologous haplotypes. Repeat annotation was performed with DANTE (v.0.2.10) and DANTE_LTR (v.0.4.0.5)⁶⁷ and EDTA (v.2.0)⁶⁸ using the plant TE library. Pairwise divergence and TE composition were visualized using custom R scripts.

PanKmer analysis and haplotype clustering

To evaluate haplotype conservation and relationships across all 18 *R. tenuis* haplotypes, pangenome *k*-mer profiling was conducted using PanKmer (v.0.20.4). An index of canonical 31-mers was constructed from the assembled fasta files for all accessions using 'pankmer index'. Pairwise sequence overlaps were subsequently quantified by generating an adjacency matrix with 'pankmer adj-matrix'. To visualize haplotype relationships, hierarchical clustering was performed on the matrix through 'pankmer clustermap' using the Jaccard similarity index as the distance metric, yielding an adjacency clustermap in which the colour intensity reflects the average nucleotide similarity across haplotypes (Extended Data Fig. 4a).

Pseudogene analysis

RNA sequences were mapped to diploid *R. tenuis* REC and *R. breviscula* by STAR (v.2.7.11b). RNA data for *R. breviscula* was from our previous publication¹⁷. The gene annotations from Helixer with at least one RNA coverage were taken as functional genes. All functional genes, repeats and TEs were hard-masked. We used miniprot (v.0.18) to map the functional gene sequences to the hard-masked genome then parse the output with our custom script. Only alignments matching a $\geq 40\%$ sequence identity and $\geq 50\%$ query coverage threshold were considered. Alignments displaying at least one inactivating open reading frame (ORF) disruption (frameshifts or premature stops) were classified using structural criteria: (1) processed pseudogenes required an intronless, compact footprint (≤ 2 CDS blocks and a genomic span $\leq 1.20 \times$ the expected mRNA length); (2) non-processed pseudogenes were identified by a retained multi-exon structure or non-compact genomic span. Scripts are available in our GitHub repository (folder: search_pseudogenes; https://github.com/Raina-M/Rhynchospora_tenuis_project).

ChIP-seq

Chromatin immunoprecipitation (ChIP) was performed as described previously⁶⁹ with minor modifications. Young leaves and flower buds were collected from greenhouse-grown plants and immediately frozen

in liquid nitrogen before storage at -80°C . Tissue samples were cross-linked with 4% formaldehyde under a vacuum on ice for 1 h, and the reaction was quenched by adding 1 M glycine. Nuclei were isolated using NIB buffer (50 mM HEPES pH 7.4, 5 mM MgCl_2 , 25 mM NaCl, 5% sucrose, 30% glycerol, 0.25% Triton X-100, 0.1% β -mercaptoethanol and 0.1% protease inhibitor), and chromatin was extracted in TE-SDS buffer. Chromatin was sheared by sonication for 30 s on/30 s off cycles for a total of 25 cycles. For immunoprecipitation, the sonicated chromatin was incubated overnight at 4°C with 2 ng of the following antibodies: anti-CENH3⁷⁰, anti-H3K4me3 (Abcam, ab8580), anti-H3K9me2 (Abcam, ab1220) and anti-H3K27me3 (Merck, 07-449). A control without antibodies was performed simultaneously. The antibody-chromatin complexes were captured using rProtein A Sepharose Fast Flow (Sigma-Aldrich, GE17-1279-01) or Protein G Sepharose 4 Fast Flow (Sigma-Aldrich, GE17-0618-01). Bound chromatin was eluted, and de-cross-linked by treatment with proteinase K. DNA was purified by ethanol/sodium acetate precipitation and resuspended in nuclease-free water. The purified DNA was sent for library preparation and sequencing.

Sequencing reads were aligned to the reference genome using Bowtie2⁷¹ using the --very-sensitive-local option. ChIP and control alignments were compared using bamCompare⁷² with the parameters --binSize 50 --normalizeUsing RPKM --operation log2. The resulting $\log_2$ ratio tracks were visualized using pyGenomeTracks⁷³. Metaplots were generated using computeMatrix⁷² (scale-regions mode) with the parameters --regionBodyLength 20000 --beforeRegionStartLength 10000 --afterRegionStartLength 10000 --binSize 50, and plotted using plotHeatmap. The mean of each column of the matrix over *Tyba* arrays was extracted, which is the actual value plotted on the metaplots. As all epigenetic signals (CENH3, H3K4me3, H3K9me2, CG, CHG and CHH methylation) are not normally distributed (Shapiro-Wilk normality test $P < 0.05$), we performed spearman correlation analysis between every signal in *R. pubera*, *R. breviscula* and *R. tenuis* to test whether these signals present the same pattern across different species.

Probe design and oligo-FISH

After phased genome assembly and synteny analysis, a haplotype-specific translocation in *R. tenuis* reference plant was identified at the chromosome ends. To validate these data, we designed oligo probes specific to each translocated region and hybridized in the reference plant, in different accessions, plus *R. austrobrasiliensis*. Probes were designed for the following regions to each chromosome/haplotype: for chromosome 1 (Chr1_h1, 0–3100219 bp; Chr1_h2, 0–2085189 bp) at a density of 3 probes per kb (Fig. 1c), and for chromosome 2 (Chr2_h1, 133059273 bp to end; Chr2_h2, 136628596 bp to end) at a density of 1 probe per kb. After designing and setting the regions corresponding to each haplotype, these data were submitted to Daicel Arbor Biosciences for synthesis and labelling of the probes. Probes for haplotype 1 were labelled with Atto633 (far red), while probes for haplotype two were labelled with Alexa Fluor 488 (green). For the slide preparation, young roots (mitosis) and flower buds (meiosis and microgametogenesis), from plants maintained in pots at greenhouse facilities of Max-Planck Institute for plant Breeding Research, were collected and fixed in Carnoy solution (methanol:acetic acid, 3:1, v/v) for 2–24 h at room temperature and then immediately used or kept at -20°C until the moment of use. After fixation, the material was digested in enzymatic solution containing 2% cellulase Onozuka (Serva), 2% pectolyase Y-23 (Duchefa Biochemie), 2% cyto-helicase (Sigma-Aldrich) and 10% pectinase (Sigma-Aldrich) in citrate-phosphate buffer (pH 4.5), washed in water and macerated in 60% acetic acid, post-fixed with ice-cold fresh Carnoy solution and air dried. For removal of cytoplasm, the slides were washed in 60% acetic acid for at least 30 min, then air dried. For the pollen tube germination experiment, 30 mature flower buds were collected into 3 ml of sterile distilled H_2O and vortexed to release pollen grains. From the bottom of the tube, 300 μl of the pollen suspension

was collected and added to 3 ml of liquid medium for trinucleate pollen as described previously⁷⁴. Pollen grains were incubated at 28 °C for 24 h. After incubation, the medium containing germinating pollen grains was transferred into a conical tube and centrifuged at 5,000 rpm for 3 min. After removing the supernatant, pollen nuclei were suspended in 60% acetoacetate, transferred onto a slide, post-fixed and air-dried. For oligo-FISH, the procedures were similar to the ones described previously⁷⁵; in brief, the hybridization mix was composed of 50% formamide (Sigma-Aldrich), 2× SSC (saline sodium citrate) solution (pH 7.0), 10% dextran sulfate, 350 ng of probe labelled in Alexa Fluor 488 and 200 ng of the probe labelled in Atto633 (a total of 15 µl per slide). After denaturation and hybridization, the slides were passed through stringency washes in 2× and 0.1× SSC at 42 °C, which corresponds to a final stringency of about 76%. The slides were counterstained with 2 µg ml⁻¹ DAPI in Vectashield H-100 antifade mounting medium (Vector Laboratories) and photographed on the Zeiss Microscope coupled with a Zeiss Axio Imager 2 camera system and software ZEN Blue (v.3.1). Images were treated in brightness and contrast using Adobe Photoshop software.

Immunocytochemistry

Immunocytochemistry was performed as described previously¹⁸, with some variations. In brief, young flowers of *R. tenuis* and *R. austrobrasiliensis* were sampled and fixed in ice-cold 4% (w/v) paraformaldehyde (PFA) in PBS solution (pH 7.5, 1.3 M NaCl, 70 mM Na₂HPO₄, 30 mM NaH₂PO₄) and 0.1% (v/v) Triton X-100 for 30 min in a vacuum. Flowers were dissected to select anthers of the appropriate size to obtain meiocytes in prophase I. Anthers were opened from the tip in a drop of PBS with 0.1% (v/v) Triton X-100 and squeezed to release the meiocytes from the locules. The suspension of meiocytes was stirred to separate individual nuclei and remove excess debris. A coverslip was pressed onto the suspension to squash the meiocytes and later removed with liquid nitrogen. Slides were mounted with Vectashield containing 0.2 µg ml⁻¹ DAPI and checked for prophase I stages of interest. Selected slides were incubated with blocking buffer (3% (w/v) BSA in PBS + 0.1% (v/v) Triton X-100) for 1 h at 37 °C to block and permeabilize the cells. The following antibodies were used: anti-AtASY1 raised in rabbit (PAK006)²⁴, anti-AtMLH1 raised in rabbit (PAK017)²⁷ and anti-AtZYP1-cter raised in chicken (PAK053). Another anti-RpZYP1 was raised in rat against the peptide KLTAERLVKDQASVKNDLEC (gene ID: RP1G00482580, RP4G01479980, RP2G00810370, RP5G01738420) and affinity-purified (Lifeprotein). The anti-RpREC8 was a combination of two antibodies raised in rabbit against the peptides CEEPYG EIQISKGPNN and CYNPDDSVEMRDDPG (gene ID: RP1G00316120/ RP2G00915110, RP4G01319620, RP5G01638170) and affinity-purified (Eurogentec)¹⁸. The anti-RpHEI10 was a combination of four antibodies raised in rabbit and rat against the peptides CNRPNQSRRATNMFLQ, CPVRQRNNKSMVSGGP, CIDIMDSRDLRQGKREREIWI and CDTDS AVNMGPPSGDTSNRR (gene ID: RP3G01271190, RP3G01008630, RP1G00269340, RP2G00699130) and affinity-purified (Eurogentec and Lifeprotein)¹⁸. Primary antibodies were diluted in the blocking buffer to a final volume ratio of 1:200. Slides were incubated with primary antibodies overnight at 4 °C. The next day, the slides were washed three times for 5 min each with PBS + 0.1% (v/v) Triton X-100. The samples were incubated with secondary antibodies for 2 h at room temperature or 1 h at 37 °C. Secondary antibodies were conjugated with STAR ORANGE (Abberior, STORANGE-1007) or Alexa Fluor 488 (Thermo Fisher Scientific, goat anti-rabbit IgG (H+L), Superclonal Recombinant Secondary Antibody, A27034) diluted 1:250 in blocking buffer. The slides were washed again three times for 5 min with PBS + 0.1% (v/v) Triton X-100 and allowed to dry. The samples were then prepared with 10 µl of mounting solution (Vectashield + 0.2 µg ml⁻¹ DAPI). Specimens were covered with a coverslip and sealed with nail polish for storage. Images were taken with a Zeiss Axio Imager Z2 with Apotome system for optical sectioning. Images were deconvolved and processed with ZEN Blue (v.3.1) software and Adobe Photoshop (v.27.0.0).

Image processing and analysis

To measure the degree of fragmentation of the SC of *R. tenuis* compared with *R. austrobrasiliensis*, deconvolved nuclei at zygotene for both species were selected. The ZYP1 signal was isolated and converted to 8-bit in Fiji. The signal threshold was adjusted based on the maximum entropy and the signal was skeletonized. The skeleton number representing the fragment number and branch lengths representing SC elongation were extrapolated. For each cell, the number of skeletons was divided by the total length of the SC. We named the resulting value the SC fragmentation coefficient, representing the degree of fragmentation of the SC signal per unit of SC length (Supplementary Fig. 9a). To measure the ability of recombination proteins to form foci, deconvolved nuclei at diakinesis were selected. ZEN Blue software (v.3.1) was used to analyse the histograms of MLH1 and HEI10 signals. The skewedness of the distribution of pixel intensities was used as a measurement of tendency to form foci, as high-intensity foci skew the distribution of pixels towards higher intensities (Supplementary Fig. 9b,c). To measure the ability of HEI10 and MLH1 signals to colocalize, deconvolved nuclei at diakinesis were analysed using the colocalization tool in ZEN. Quadrants were adjusted based on Costes test for statistical significance, and the Pearson's colocalization coefficient was extrapolated (Supplementary Fig. 9d). R scripts used for the plots are available at Zenodo (<https://doi.org/10.5281/zenodo.20847664>).

Meiotic gene analysis

Meiotic genes with a role specifically in meiotic recombination were selected from the literature based on plants and other eukaryotes. Sequences were selected from *Arabidopsis* or closer relatives in the case of poorly conserved genes (for example, rice, maize, barley). Protein sequences were used as a query for TBLASTN with our in-house genomes, annotations and expression datasets. The number of hits on the same query sequence was used as a starting measure of copy number. The results were then manually curated for validation (Supplementary Fig. 15). Expression evidence for meiotic genes was based on the analysis of the inflorescence transcriptome obtained by RNA-seq data.

To further evaluate *SHOC1* transcriptional activity, flower buds of *R. tenuis* (REC and PECP-47 accessions), *R. breviscula* and *R. austrobrasiliensis* were used for total RNA isolation using the Sigma-Aldrich Spectrum Plant Total RNA-Kit (STRN50-1KT) together with the On-Column DNase I Digestion Set (DNASE70-1SET). Isolated RNA (1 µg) was used for cDNA synthesis using the Thermo Scientific RevertAid First Strand cDNA-Synthesis-Kit (K1622). The obtained cDNA was used for qPCR analysis using the NEB Luna Universal qPCR Master Mix (M3003S) on the BIO-RAD CFX96 Real-Time PCR Detection System. List of oligos is provided in Supplementary Table S5.

Epigenomic profiles at the *SHOC1* locus were examined using newly generated chromatin and DNA methylation datasets. For *R. tenuis*, tracks corresponding to histone modifications (H3K4me3, H3K9me2 and H3K27me3), RNA-seq coverage and DNA methylation levels (CG, CHG and CHH contexts) were visualized for both haplotypes using pyGenomeTrack⁷³ views centred on the annotated *SHOC1* gene. DNA methylation profiles were derived from whole-genome enzymatic methylation sequencing datasets, and RNA-seq from flower buds data were used to assess transcriptional activity at the locus. For comparison, epigenomic data for the orthologous *SHOC1* locus in *R. breviscula* were obtained from published datasets¹⁸. All tracks were visualized and compared using the same genomic window to assess transcriptional activity and methylation patterns across species.

Pollen nucleus isolation and sequencing

The protocol was adapted from a previous study¹⁸. For collecting the pollen, mature flowers of *R. tenuis* were collected into 5 ml tubes containing woody pollen buffer (scWPB: 200 mM Tris-HCl, 4 mM MgCl₂, 2 mM EDTA, 86 mM NaCl, 10 mM Na₂S₂O₅, 250 mM sucrose, 0.5 mM

spermine, 0.5 mM spermidine; 1% PVP-10), and vortexed for 30 s at maximum speed. The obtained solution was filtered through a 50 µm CellTrics cell strainer into a 5 ml tube. The samples were centrifuged at 5,000g for 5 min, the supernatant was removed and the pollen pellet was frozen in liquid nitrogen. Pollen viability was assessed by Alexander staining (Morphisto, 13441-00250). Pollen samples were stored at -70 °C until further use.

For extracting the pollen nuclei, frozen pollen samples were thawed on ice for 5 min and resuspended in scWPB. The solution was filtered through a 5 µm CellTrics cell strainer, retaining the pollen. Pollen grains inside the cell strainer were crushed with a plastic rod. Pollen nuclei were filtered from the macerated pollen by adding scWPB with some additives to preserve RNA (scWPC; 5 mM DTT, 1% BSA, 0.2 U µl⁻¹ Protector RNase Inhibitor). Pollen nucleus solution was stained with DAPI (1 µg µl⁻¹) and used for fluorescence-activated cell sorting (FACS) to enrich the nucleus population. A BD FACSAriaIII Fusion Flow Cytometer with a 70 µm nozzle and 483 kPa sheath pressure, operated with BD FACSDiva software (v.8.0.3), was used for this purpose; the sequential gating strategy is shown in Supplementary Fig. 16. Nuclei were dispensed into a 96-well plate containing collection buffer (1× PBS, 1% BSA and 0.2 U µl⁻¹ Protector RNase Inhibitor). The quality and number of nuclei were evaluated with the LUNA-FX7 Automated Cell Counter. Nucleus solutions were used for library preparation using Chromium Next GEM Single Cell ATAC or Chromium Next GEM Single Cell 5' Kits according to the manufacturer's instructions. Libraries were sequenced at BGI Genomics according to the Chromium 10x Kit recommendations.

Single-cell analysis

Single-cell RNA data were analysed with a similar pipeline reported in the previous study in *R. breviscula*¹⁸. The genotyping markers were defined by haplotype-specific polymorphisms by remapping the PacBio HiFi reads that were used for genome assembly to the assembled haplotype 1 genome then calling SNPs. Remapping was implemented by minimap2 (v.2.28) with argument options: -ax map-hifi. SNPs were called by bcftools (v.1.9): (1) bcftools mpileup -Oz -min-MQ1; then (2) bcftools call -Avm -Oz. The output VCF file was converted by 'SHOREmap convert' (v.3.6) to extract three key pieces information on alternative alleles: mapping quality, read coverage and AF. Only the alternate alleles with mapping quality over 100, read coverage and AF close to the main peaks were used as markers for genotyping. All snRNA-seq data were first analysed using cellranger (v.9.0.1), and snATAC data were analysed using cellranger-atac (v.2.1.0). The output alignment file (.bam) from cellranger was then demultiplexed to single-cell alignment files based on barcodes. We next called variants in each cell and compared them with genotyping markers, based on which we can determine the genotype along the chromosomes. Crossover was then called when a genotyping switch occurred. A full description of the pipeline and parameters is provided at GitHub (https://github.com/Raina-M/detectCO_by_scRNAseq).

Statistical analysis of segregation distortion

Differences between observed and expected gamete genotype frequencies were assessed using χ^2 goodness-of-fit tests, considering only the three detectable NOR-bearing genotype classes. The expected frequencies were calculated assuming equal transmission among these classes under random segregation in inverted achiasmatic meiosis. The NOR-null class was excluded because it was not observed among viable pollen grains or sperm cells. Statistical analyses were performed in R.

Subgenome-aware phasing of *R. tenuis* haplotypes

We used SubPhaser²² (default parameters) to phase and partition the haplotypes of *R. tenuis* by assigning chromosomes to subgenomes based on differential repetitive *k*-mers. These were assumed to have expanded during the period of independent evolution after the loss of recombination. A subgenome is considered to be well phased when it

displays distinct patterns of both differential *k*-mers and homoeologous chromosomes, confirming the presence of subgenome-specific features.

Estimating divergence times between haplotypes

Divergence times between haplotypes were estimated using the method outlined previously⁷⁶. In brief, all SNPs, derived from SyRI (v.1.5.3)⁶⁴ analysis explained above, between two haplotypes were used to estimate divergence times according to the formula $g = d/2\mu$, where g is the number of generations, μ is the assumed mutation rate of 6.13×10^{-9} (ref. 23), and d is the number of SNPs per bp (d = total number of SNPs between two haplotypes/chromosome size). We assumed a generation time of one per year.

LTR insertion times were calculated by Subphaser as follows: LTR-RTs were de novo detected using LTRharvest (v.1.6.1)⁷⁷ and LTRfinder (v.1.07)⁷⁸. To reduce false positives, TESorter (v.1.3.0)⁷⁹ was used to reconstruct the classification of LTR-RTs and further refine this classification. The subgenome-specific *k*-mer sequences were mapped to the LTR-RT sequences using a substring match procedure to identify the subgenome-specific LTR-RTs using Fisher's exact test. Two LTRs of each subgenome-specific LTR-RT were retrieved and the nucleotide divergence was estimated using the Jukes-Cantor 1969 model. The insertion time (T) was calculated using the equation $T = K/2r$, where $r = 6.13 \times 10^{-9}$ substitutions per year, and K represents the divergence of the LTRs from the LTR-RT.

The K_s between two haplotypes was computed based on the CDS sequences annotated by Helixer by CoGe (<https://ghibli.bti.cornell.edu/coge/>). Genomes and gene annotations were uploaded to CoGe, and the option to calculate K_s was selected during the SynMap run. All K_s values of coding sequences that overlapped with TE annotation were removed by 'bedtools subtract -A'.

Phylogenetic tree construction

The phylogenetic tree was built based on single-copy orthologues (SCOs) among *R. breviscula*, *R. austrobrasiliensis* and all haplotypes of *R. tenuis*. SCOs were extracted from the output of OrthoFinder (v.2.5.5) in GENESPACE (v.1.3.1). All SCO groups were aligned by MAFFT (v.7.475)⁸⁰ with the parameter '-auto --maxiterate 10'. All alignments were concatenated with a custom Python script available at our project GitHub page (phylogeny_based_on_SCOs/concatenatealn.py). The tree was constructed based on the catenated alignments with RAXML-NG (v.1.2.2)⁸¹, then visualized in FigTree (v.1.4.4) (<https://github.com/rambaut/figtree/>).

F₁ genotyping and analysis

F₁ individuals were obtained from selfed heterozygous mother plants. Genomic DNA from seedlings was extracted and sequenced on the Illumina NovaSeq platform. The F₁ genotyping method was the same as described previously¹⁸. Whole-genome sequencing reads of each F₁ offspring were mapped to the haplotype 1 assembly using bowtie2 (v.2.5.1) paired-end mode and the default parameters, and SNP genotypes were called afterwards using bcftools (v.1.9), whereby we first performed 'bcftools mpileup' then then 'bcftools call -Am'. The resulting SNPs of each individual plant were compared with the defined genotyping markers (see the 'Single-cell analysis' section) to determine the genotypes of all genomic regions along each chromosome. Genotyping results were cross-validated by TIGER and RTIGER (v.2.1.0). RTIGER was implemented under R (v.4.2.0) and Julia (v.1.0.5).

The crossed F₁ individuals were sequenced by Illumina short reads, which were then aligned to the mother genome assembly with haplotype 1 and 2 catenated by bowtie2 (v.2.5.1) in paired read mode with the option '-local --no-mixed --no-discordant'. The alignment file was sorted by samtools sort (v.1.24). SNP calling was performed using the bcftools (v.1.9) mpile up with the option --min-MQ1 and then bcftools call -Avm. The output VCF file was converted by SHOREmap convert

(v.3.6) into a text format file, where the AF of alternative alleles/SNPs can be read. Only alternate alleles with AF > 0.95 and coverage > 3 were extracted as valid SNPs, that is, the alleles called in the cross reads are truly different from the mother alleles. These alleles were compared with the SNPs output by SyRI (v.1.5.3), which mapped father diploid genome assembly to mother diploid genome assembly. The overlapped SNPs indicate they are contributed by father (Fig. 4b).

Gene conversion detection in selfed F₁ plants

To detect gene conversions in selfed individuals, we calculated the alternative (hap2) AF based on the SNP markers used for genotyping. Markers with a combined read depth below 5× were excluded. We then applied binomial test at each marker site with total depth as sample size n and number of reads supporting hap2 as number of successful trials k . The null hypothesis is that the hap2 allele count at a given marker site follows a binomial distribution. To control the false-discovery rate genome wide, every testable marker was assigned a raw P value and the Benjamini–Hochberg procedure was applied globally across all SNP markers on all chromosomes simultaneously. We next merged neighbouring significant SNPs into tracts, and only tracts of 5 bp–5 kb were considered to be potential gene conversions. However, if total depth drops at the same positions relative to flanking sites, we took it as a deletion or hemizyosity rather than conversion, because conversion usually preserves depth but changes the allele fraction. The related R script is available on our project GitHub page (detect_gene_conversion.R). Although the pipeline excluded as many biases and false positives as possible, manual inspection of reported gene conversions with alignments in IGV was performed.

Embryo and endosperm analysis

For extracting the nuclei from embryo and endosperm, frozen seeds were macerated with a plastic rod and resuspended in seed nucleus isolation buffer (100 mM Tris, 5.2 mM MgCl₂, 85.55 mM NaCl, 0.1% Triton X-100, 0.2 U μl⁻¹ Protector RNase Inhibitor, pH 7.5). The obtained solution was filtered through a 20 μm CellTrics cell strainer into a fresh 2 ml tube and stained with DAPI (1 μg μl⁻¹). A BD FACSAriaIII Fusion Flow Cytometer with a 70 μm nozzle, operated with BD FACSDiva software (v.8.0.3), was used for FACS to enrich the 2c (embryo) and 3c (endosperm) nucleus populations; the sequential gating strategy is shown in Supplementary Fig. 16. The separate 2c and 3c nucleus populations were dispensed into a 96-well plate containing collection buffer (1× PBS, 1% BSA, and 0.2 U μl⁻¹ Protector RNase Inhibitor). The quality and number of nuclei were evaluated using the LUNA-FX7 Automated Cell Counter. Nuclei solutions were used for library preparation using Chromium Next GEM Single Cell ATAC according to the manufacturer's instructions. Libraries were sequenced at BGI Genomics according to the Chromium 10x kit recommendations.

Female gametophyte sectioning and imaging

Developing ovaries and whole inflorescences were fixed as described previously³⁴. Dehydration and infiltration into Araldite 502/Embed 812 resin were performed in an EMS LYNX II automated tissue processor (EMS). After resin polymerization, serial semithin sections (1 μm) were produced from ovules at different stages of development, stained with 1% aqueous Toluidine Blue supplemented with 1% sodium tetraborate and imaged on the Zeiss Axio Imager M2 system. Images were stacked and aligned in FIJI/ImageJ (v.2.18.0) using the StackReg plugin (BIG-EPFL). MorphoGraphX (v.2.0.3) was used for 3D rendering of the aligned images and volumetric visualization of the embryo sac tissue⁸². Three-dimensional segmentation of the embryo sac was performed using ITK watershed (<https://www.itk.org>) in MorphoGraphX, taking PlantSeg (v.2.1.1)⁸³ predictions from the 'generic confocal 3DUNET model' as input images for 3D cell segmentation. Immunodetection of β-1,3-glucan to identify pollen tube cell walls was performed as described previously⁸⁴, with the exception that BSA

was omitted from the buffer during all steps. Goat anti-mouse Alexa Fluor 647 (Abcam, ab150119) was used as an alternative secondary antibody at a dilution of 1:200. Imaging was done on the Leica SP8 system using LAS X (v.5.3.3).

For Feulgen staining of siliques, the pericarp was removed from the mature and fertile siliques; for the rest of the siliques, the pericarp was not removed, and the material was fixed in ethanol:acetic acid (3:1) overnight. The samples were washed three times with distilled water for 15 min each wash and then incubated in 5 N HCl for 1 h, followed by another series of three washes with water, as before. The siliques were then incubated in Schiff's reagent for 3–4 h, after which they were washed three times with cold distilled water (4 °C). Next, the siliques were incubated in ethanol series 10 min each, 10%, 30%, 50%, 70%, 95% and then washed several times with 99.5% ethanol until the ethanol came out colourless. The samples were incubated in a 99.5% ethanol:LR White resin series (3:1 then 2:1), 15 min for each solution. The samples were then incubated for 1 h in ethanol:LR White resin (1:1), followed by an overnight incubation in LR White resin. The seeds were next mounted onto microscope slides in LR White resin and polymerized for 24 h at 60 °C. Feulgen-stained samples were imaged using the Leica Stellaris 8 or Leica SP8 FALCON Dive multiphoton microscope using LAS X (v.5.3.3). Samples were excited at either 800 nm or 1,060 nm, and emission was collected from 580 to 700 nm.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Genome assemblies and raw sequencing data have been deposited in the European Nucleotide Archive under accession PRJEB98016. The reference genomes, sequencing data, annotations and all tracks presented in this work are available for download at EDMOND, the Open Research Data Repository of the Max Planck Society (<https://doi.org/10.17617/3.IXRTSY>). The assemblies, annotations and genome-browser tracks hosted at EDMOND are derived products generated from the same sequencing datasets deposited at the European Nucleotide Archive; no primary sequencing data are held exclusively at EDMOND. Both repositories are publicly accessible without restriction.

Code availability

All custom code used in this study is publicly available. Analysis scripts are deposited at https://github.com/Raina-M/Rhynchospora_tenuis_project and the crossover-detection pipeline at https://github.com/Raina-M/detectCO_by_scRNAseq. R scripts used for the cytological measurements are archived at Zenodo⁸⁵ (<https://doi.org/10.5281/zenodo.20847664>).

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Author contributions A.M. conceived the research and supervised the project. M.Z., P.G.H. and A.M. performed the genome assembly, scaffolding and genomics. M.Z., J.A.C., H.S., M.C. and S.S. designed and performed the single-cell experiments and processed the data. K.S. assisted with the single-cell analysis. M.C. performed the immunostaining experiments. L.D. and M.C. performed the functional screening of meiotic genes. T.N. and M. Majka performed FISH experiments. M. Majka performed pollen-FISH genotyping experiments. G. Thangavel and M.Z. performed the ChIP-seq analysis. M. Marek and S.S. performed the flow cytometry measurements and nucleus sorting. N.S., M. Marek and B.H. performed all nuclear sequencing libraries. M.Z., L.A.R., A.L.L.V. and A.M. performed the centromere and repeat characterization. A.L.L.V. collected and provided the plant material. U.P. and M. Majka performed the plant crosses. S.S. performed the pollen viability assay. M.Z., S.D. and A.M. performed the dating analysis. M.Z., T.L. and K.F.X.M. performed the functional annotation. G. Tsipas, U.N., A.V., T.T. and D.D.F. performed and analysed the female gametophyte and embryo sac development microscopy data. M.Z., M.C., S.S. and A.M. wrote the first manuscript draft with input from all of the authors. All of the authors approved the final version of the manuscript.

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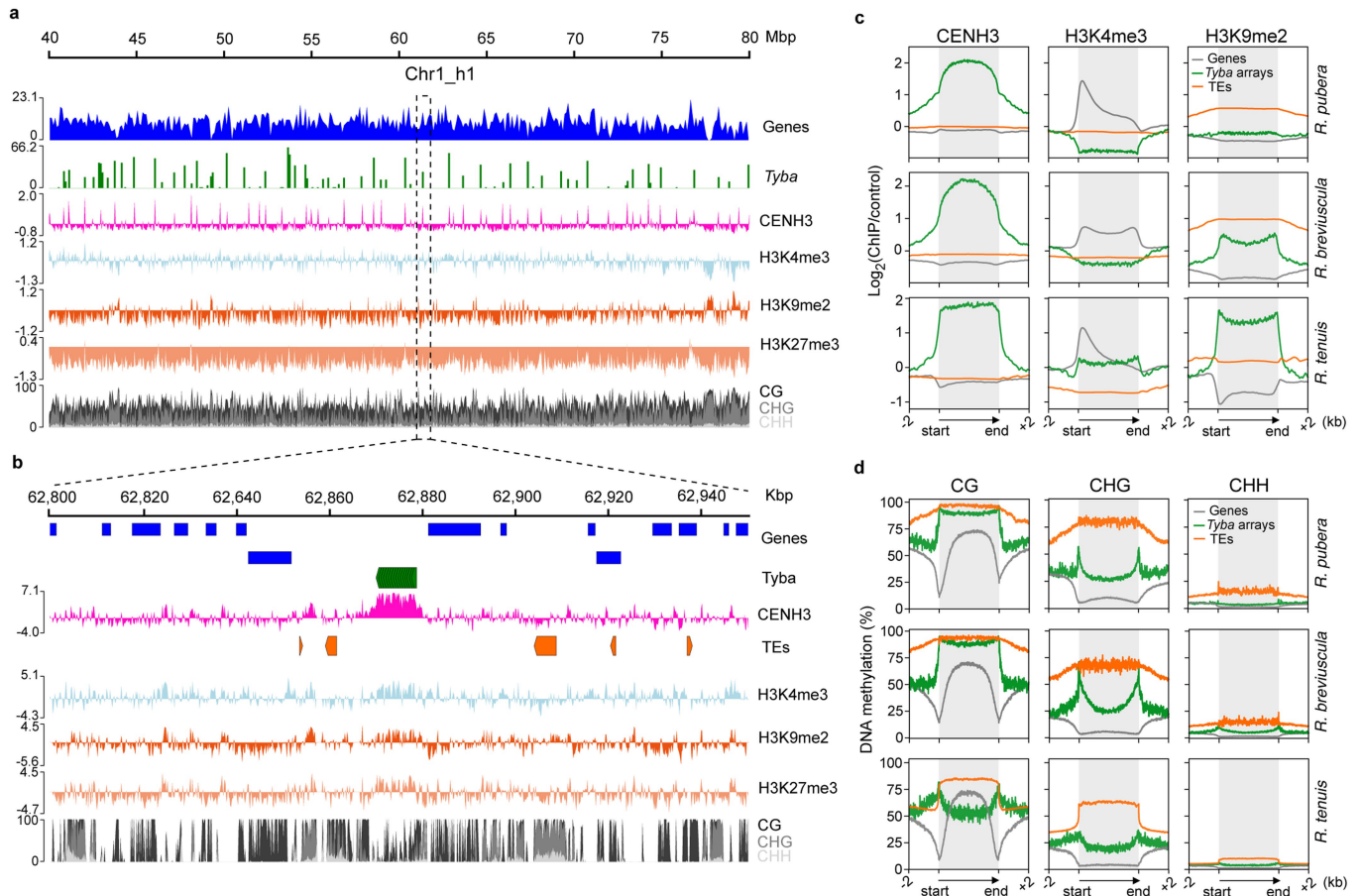
Additional information

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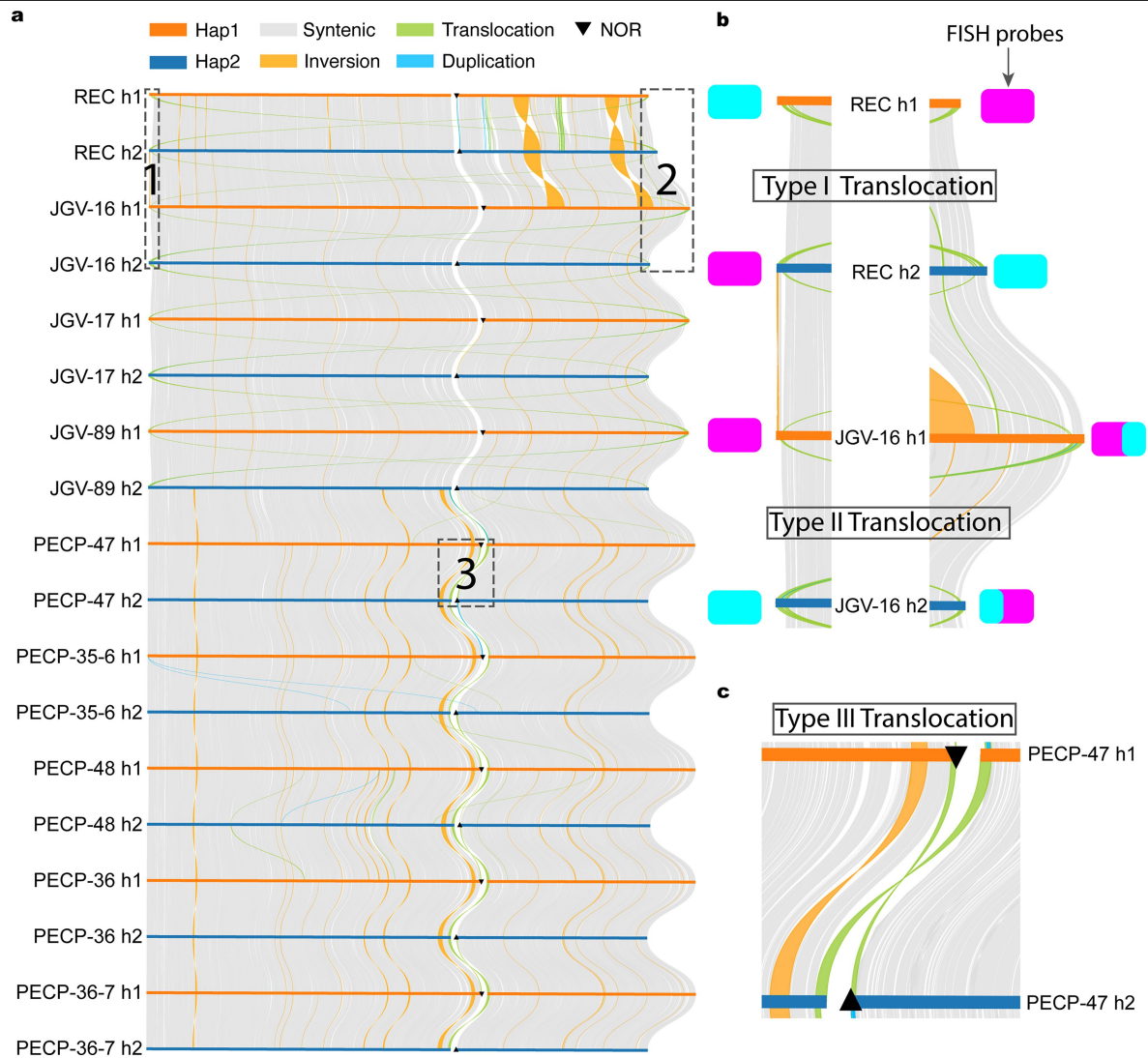
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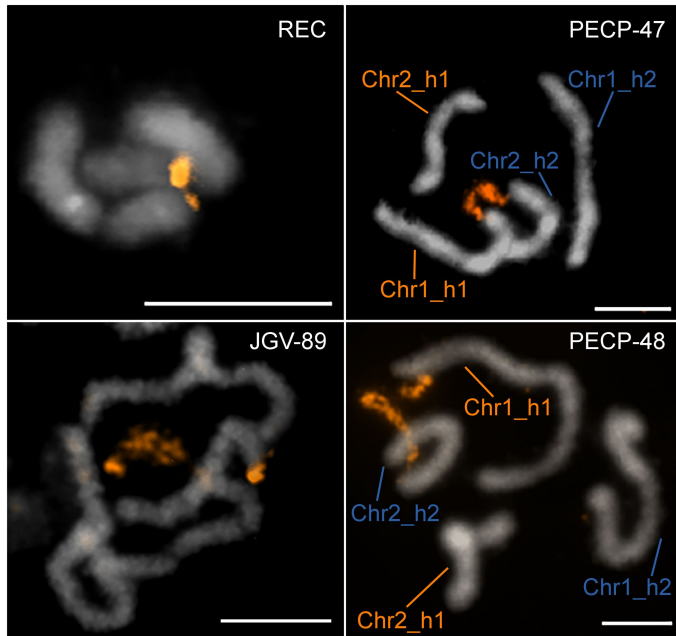
Extended Data Fig. 1 | Divergent epigenetic landscape of holocentromeres in *Rhynchospora tenuis*. (a) Zoomed-in view of *R. tenuis* Chr1_h1 showing a 40-Mb region with multiple CENH3 domains that are closely correlated with *Tyba* repeat distribution. Gene and *Tyba* densities were calculated over 100-kb windows. (b) A detailed view of a representative ~150 kb holocentromeric region on *R. tenuis* Chr1_h1, illustrating the precise correlation between CENH3 binding, *Tyba* arrays and an ambiguous chromatin state, with low CG DNA methylation and accumulation of both repressive and active histone marks. Values in **a** and **b** represent the log₂ ratio of ChIP-seq signal to input control.

(c) Enrichment of CENH3, H3K4me3, and H3K9me2 from the start and end of different types of sequences: genes (grey line), TEs (orange) and *Tyba* repeats (green) for *R. pubera*, *R. breviscula* and *R. tenuis*. ChIP-seq signals are shown as log₂ (normalized RPKM ChIP/input). (d) Enrichment of DNA methylation in the CG, CHG, and CHH contexts for the same sequence types as shown in (c) for *R. pubera*, *R. breviscula* and *R. tenuis*. Grey boxes in (c–d) highlight the modification enrichment over the body of each sequence type. A corresponding correlation analysis of the ChIP-seq and DNA methylation signals are presented in Supplementary Table 2.

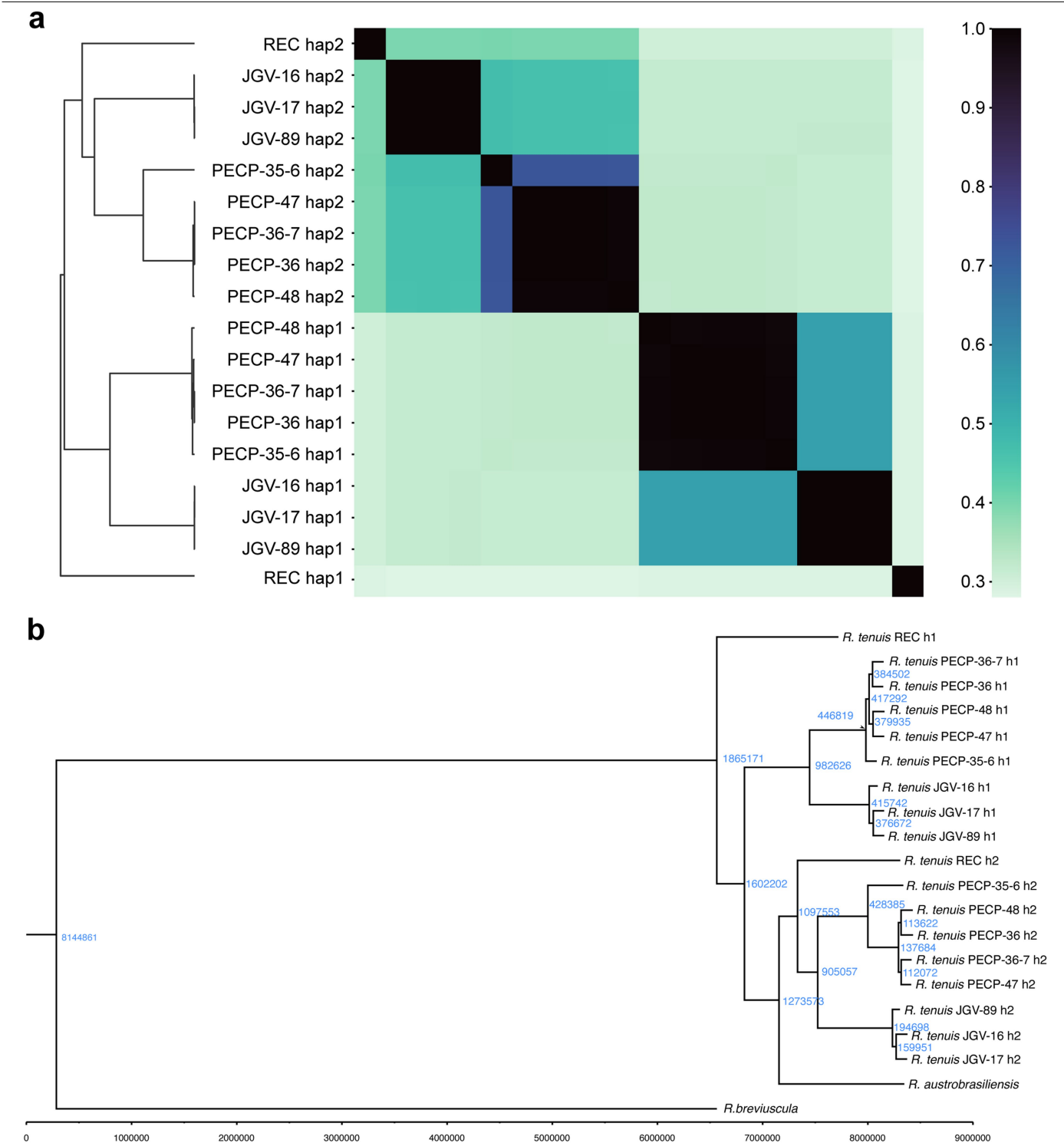


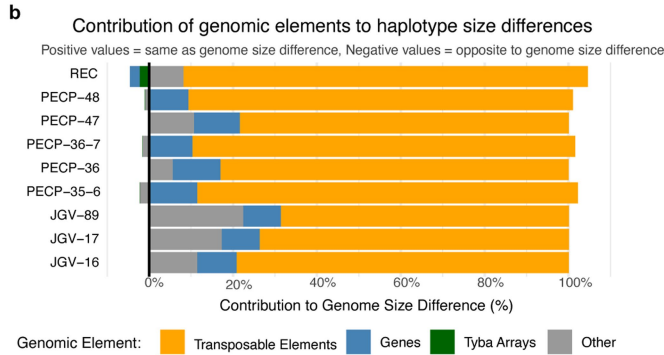
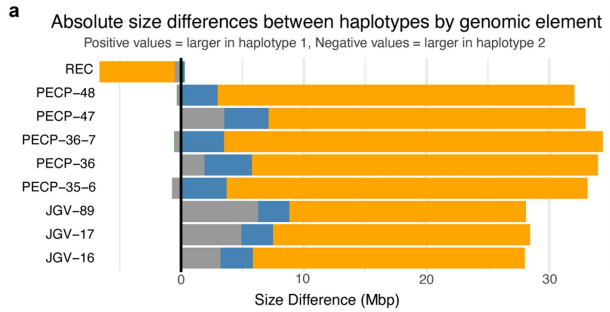
Extended Data Fig. 2 | Colinearity across the two haplotypes of all *R. tenuis* accessions by SyRI. (a) Colinearity based on genomic sequences of all haplotypes of nice *R. tenuis* accessions. (b) Zoom-in view of the regions 1 (left column) and 2 (right column) labelled in a. The translocation FISH probes designed for REC were also shown here, implying the Type I translocation. JGV-16 Chr 2 end (region 2) has a small translocation compared to REC Chr1_h2,

so a mixed magenta and cyan probe signal was observed, indicating a different type of reciprocal translocation (Type II translocation). (c) Zoom-in view of the region 3 labelled in a, displaying the Type III translocation that is found in PECP accessions, where the first reciprocal translocation is between Chr1_h1 end and Chr2_h2 start, and the second reciprocal translocation is between Chr1_h2 end and Chr2_h1 start.

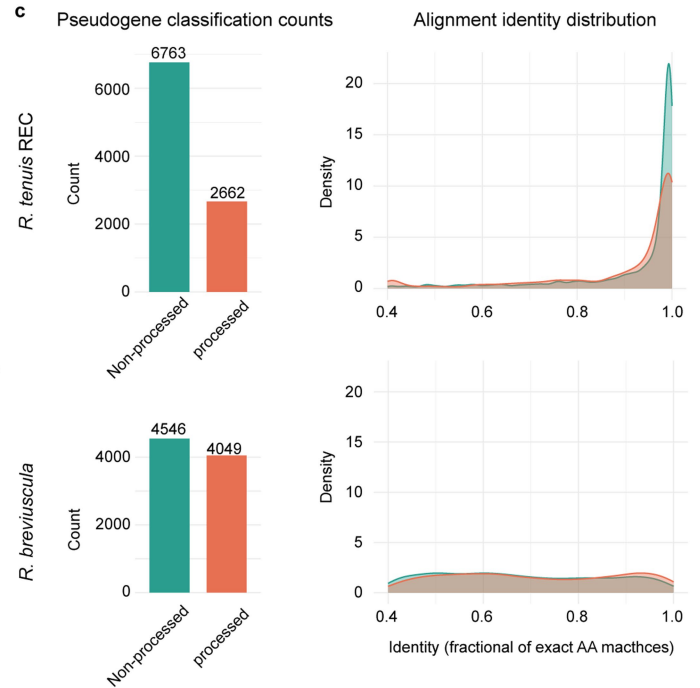


Extended Data Fig. 3 | Distribution of 45S rDNA and identification of a reciprocal translocation in *R. tenuis* genotypes. FISH with a 45S rDNA probe (orange) on mitotic chromosomes of *R. tenuis* genotypes REC, JGV-89, PECP-47 and PECP-48 (n = 11–32 cells per genotype, examined over two independent experiments with similar results; scale bars, 5 μm). Genotypes PECP-47 and PECP-48 exhibit a reciprocal translocation involving 45S rDNA regions. The translocation occurs between chromosomes Chr1_h1 and Chr2_h2 and is absent in genotypes REC and JGV-89. Scale bars correspond to 5 μm.

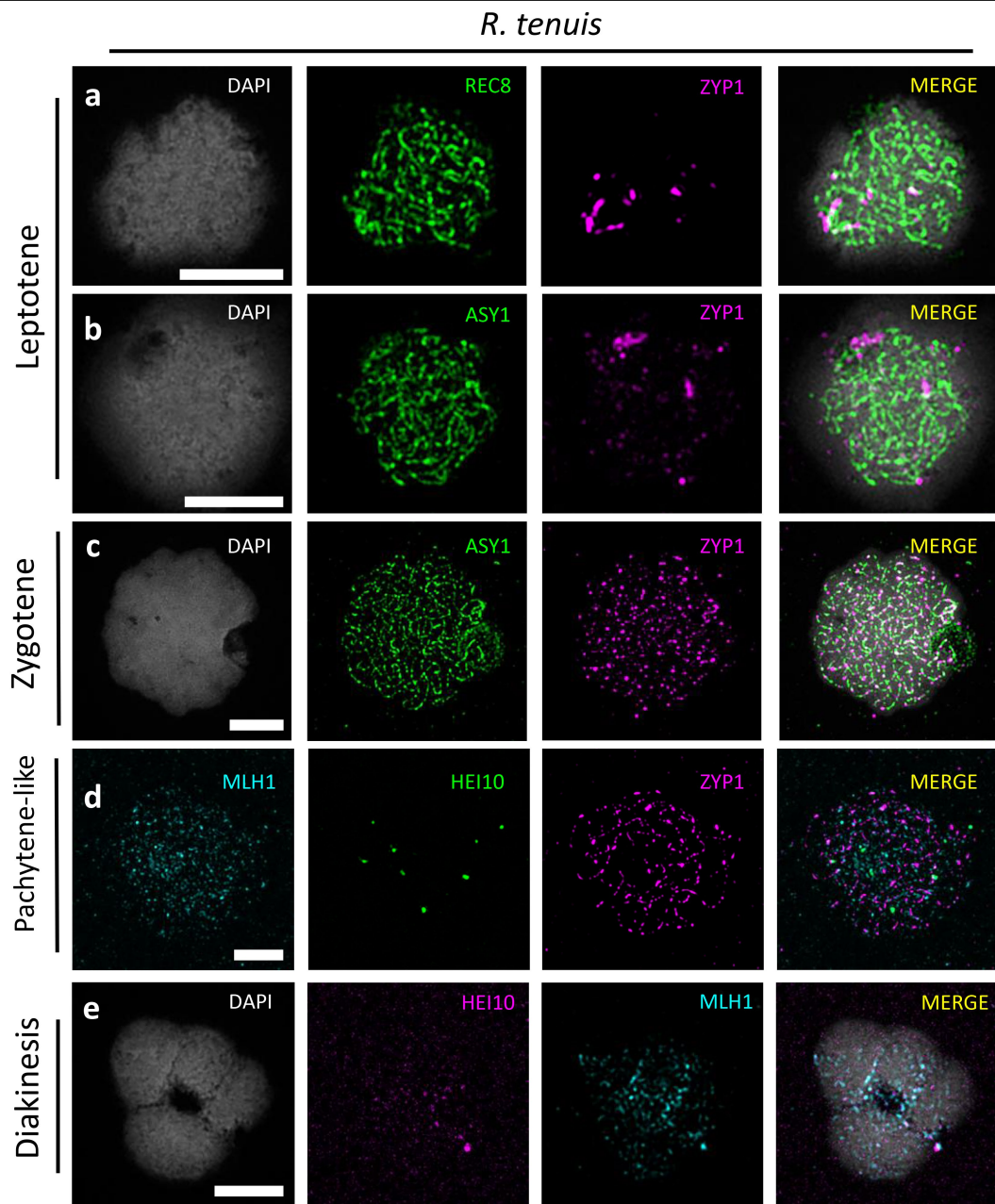




Extended Data Fig. 5 | Haplotype asymmetry, TE expansion, and early signatures of genome divergence in *R. tenuis*. (a) Absolute size differences of different genomic elements between the two haplotypes of all *R. tenuis* accessions. A positive value means the total size of the element is larger in haplotype 1 and a negative value means a larger total size in haplotype 2. (b) Contribution of the genomic elements to the percentage of genome size difference of two haplotypes of *R. tenuis*. A positive value means this element difference is the same direction of genome size difference. For instance, haplotype 1 genome size of REC is smaller than haplotype 2. Transposable elements (TEs) in REC haplotype 1 is also shorter than haplotype 2, so the contribution of TEs has a positive value; while gene total length is longer in



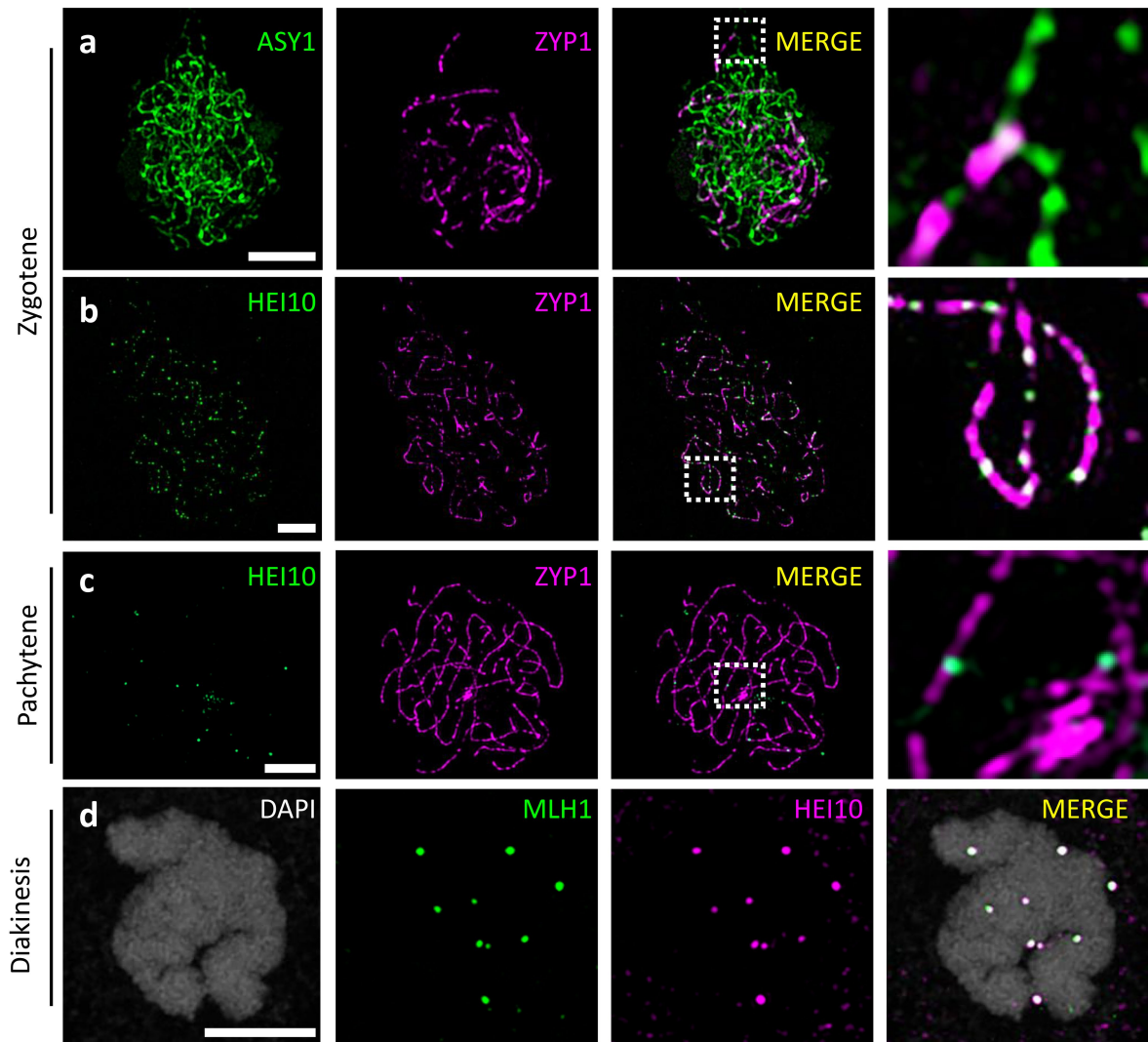
haplotype 1 so the contribution is negative. (c) Pseudogene content and protein divergence between haplotypes in *R. tenuis* and *R. breviscula*. Left panels show the number of processed and non-processed pseudogenes identified in *R. tenuis* (REC) and the recombining relative *R. breviscula*. *R. tenuis* displays a modest increase in pseudogene abundance relative to *R. breviscula*. Right panels show the distribution of amino acid sequence identity between haplotype-resolved orthologous proteins. *R. tenuis* exhibits a highly skewed distribution with a strong peak at near-identical sequences and a secondary tail of divergent proteins, whereas *R. breviscula* shows a broader and more homogeneous identity distribution, consistent with contrasting evolutionary dynamics between recombining and non-recombining genomes.



Extended Data Fig. 6 | Achiasmatic male meiosis of *R. tenuis*. Additional channels and annotations of main Fig. 2, showing the meiotic atlas of *R. tenuis* and major recombination proteins. (a–b) Early recombination proteins ASY1 (n = 41) and REC8 (n = 29) display regular behaviour, localizing as linear signals. (c) At zygotene, ZYP1 shows only fragmented association with the axis (n = 21).

(d) ZYP1 can extend to a more complete signal, but still fragmented. HEI10 foci appear, although not exclusively associated with ZYP1 (n = 11). (e) At diakinesis, HEI10 and MLH1 do not localize as foci and four univalents are observed (n = 15). Scale bars correspond to 5 μ m.

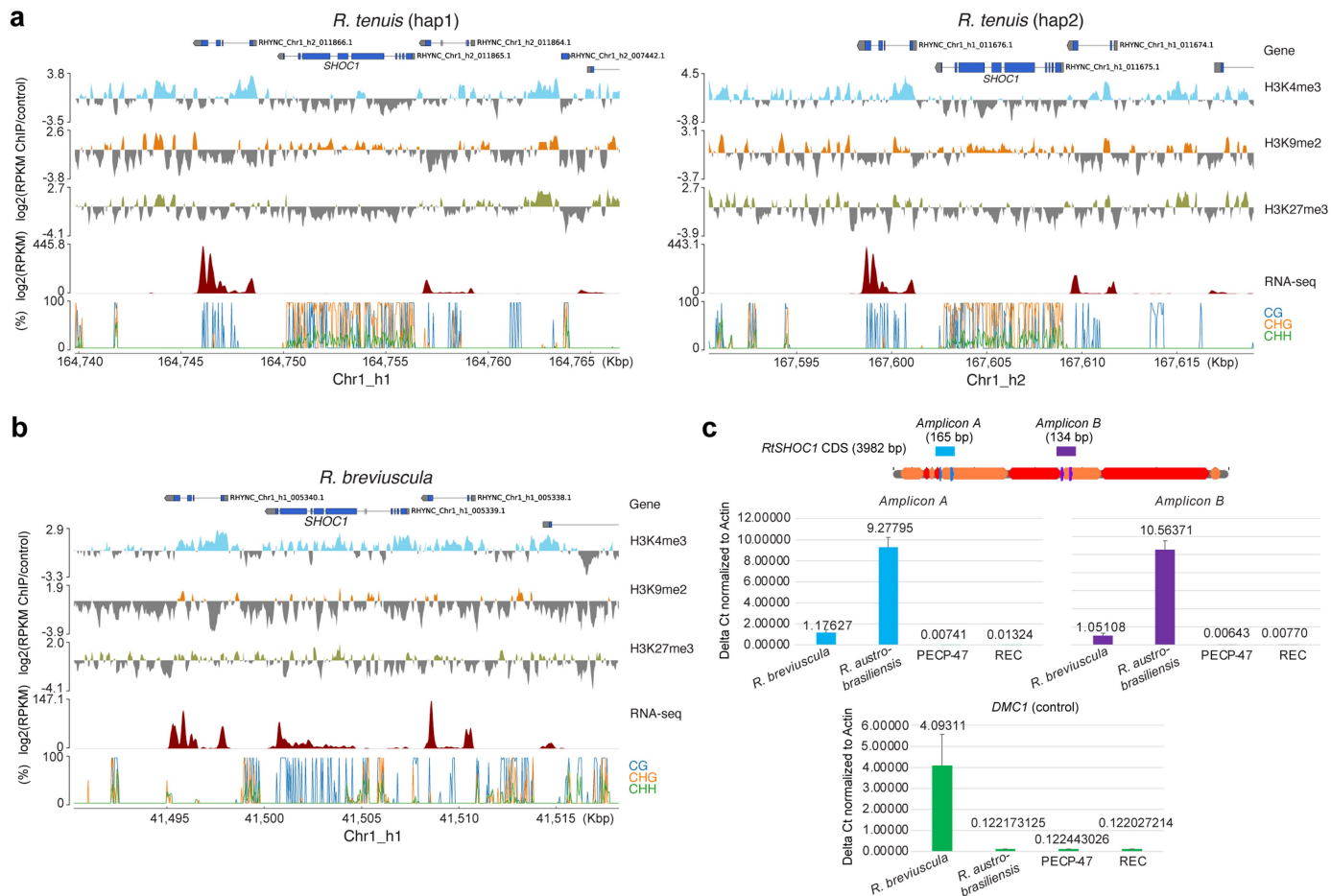
R. austrobrasiliensis



Extended Data Fig. 7 | Chiasmatic male meiosis of *R. austrobrasiliensis*.

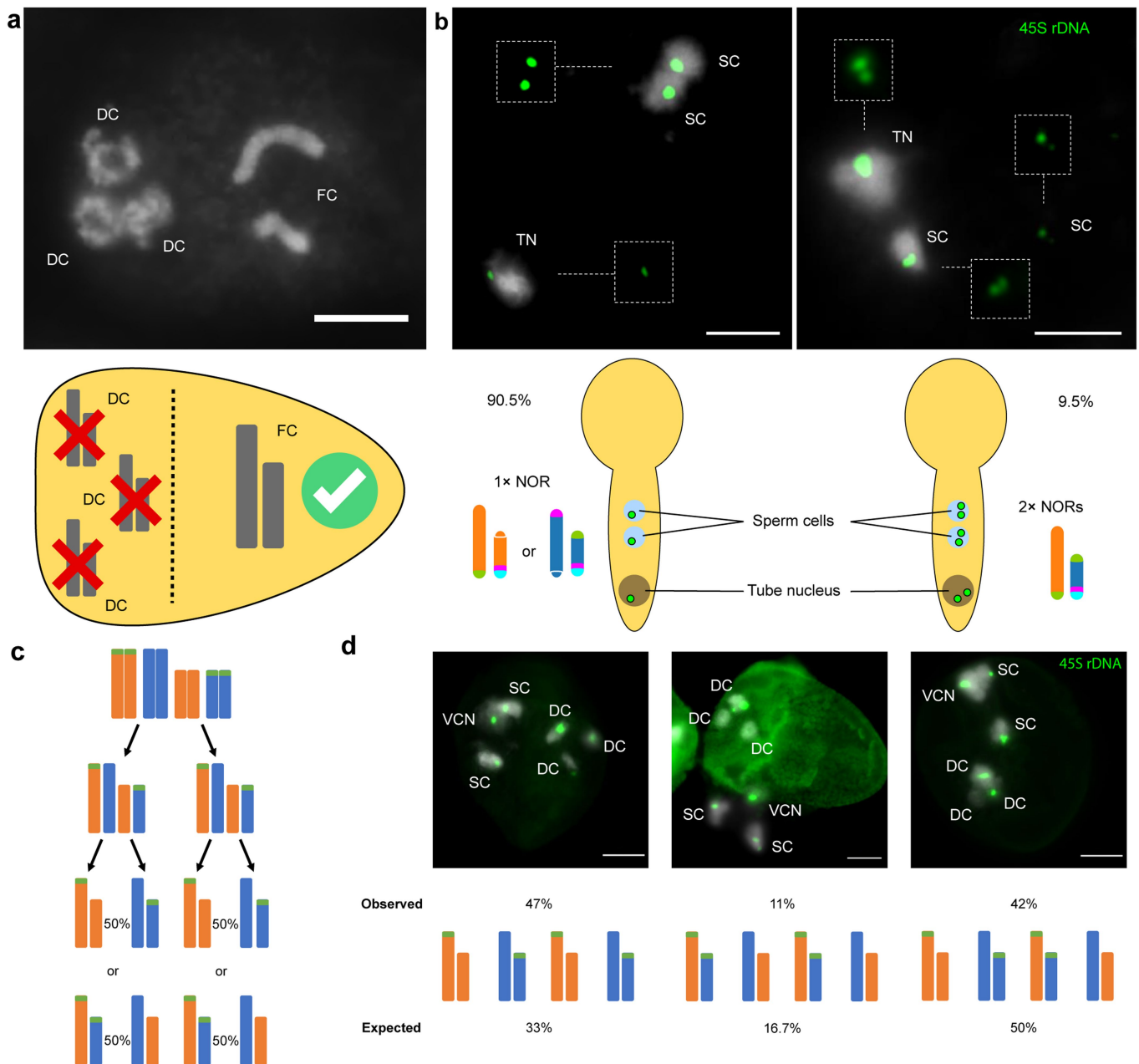
Additional channels and annotations of the main Fig. 2 prophase I of *R. austrobrasiliensis*. **(a)** At zygotene, ZYP1 is loaded on paired chromosomes while ASY1 is unloaded ($n = 24$). **(b)** Simultaneously, HEI10 is loaded as multiple foci along the signal of ZYP1 ($n = 12$). **(c)** At pachytene, ZYP1 forms a continuous

linear signal along the whole chromosome length and HEI10 signal condenses into few high intensity foci ($n = 7$). **(d)** At diakinesis, HEI10 forms foci that colocalize with MLH1 and mark class I crossovers ($n = 56$). Scale bars correspond to $5 \mu\text{m}$.



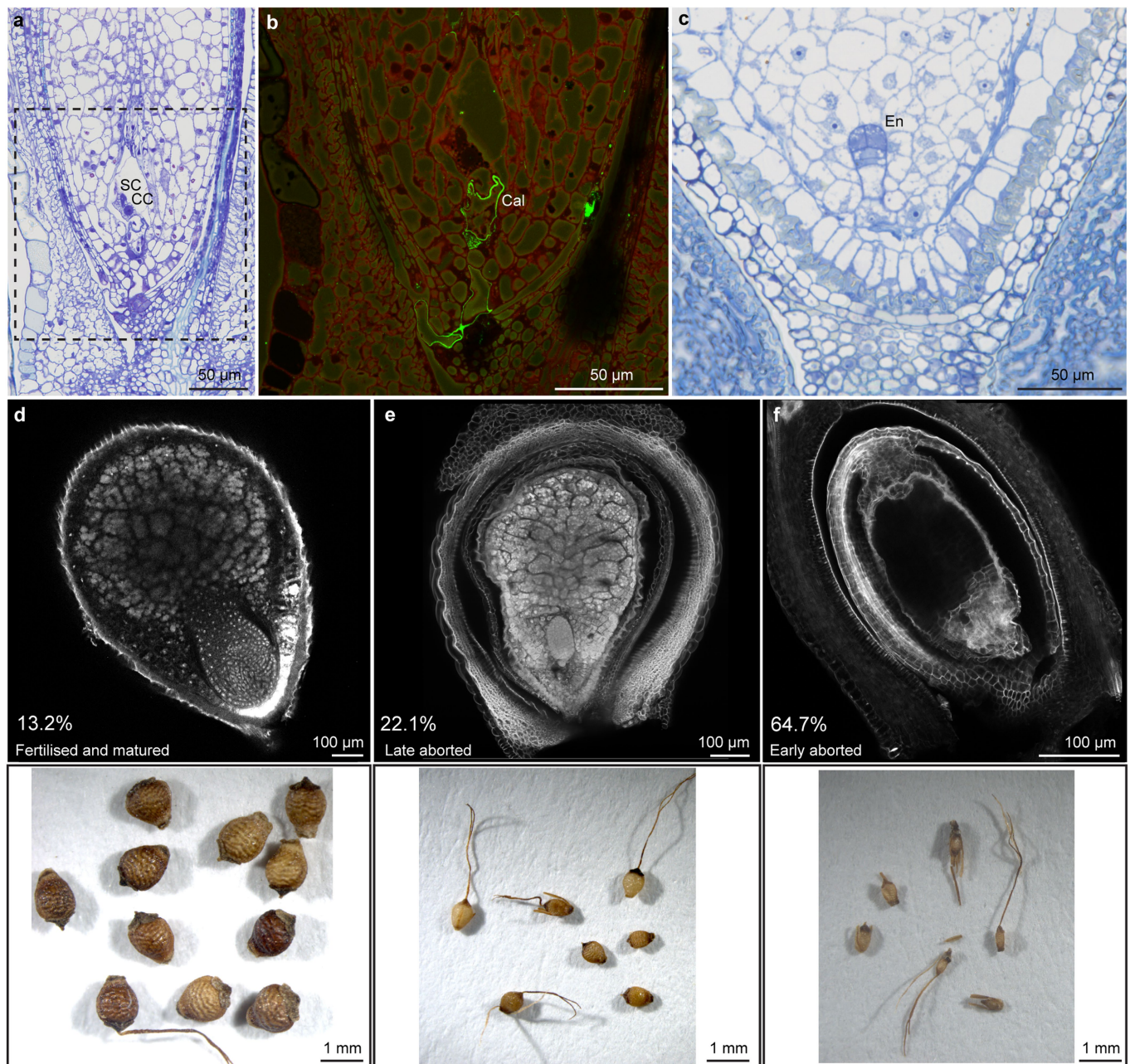
Extended Data Fig. 8 | Epigenetic silencing of the recombination gene *SHOC1* in *R. tenuis*. (a) Genome browser view of the *SHOC1* locus in *R. tenuis* haplotypes 1 and 2. Tracks show gene annotation, histone modifications (H3K4me3, H3K9me2 and H3K27me3), RNA-seq coverage, and DNA methylation levels (CG, CHG and CHH contexts). ChIP-seq signals are shown as log2 (normalized RPKM ChIP/input), RNA-seq signals are shown as log2 (normalized RPKM) and DNA methylation values are shown as percentage (%). In both haplotypes, the *SHOC1* locus shows no detectable RNA-seq signal and strong DNA methylation across the gene body, particularly in the CHG and CHH contexts, consistent with transcriptional silencing. (b) Chromatin and

transcriptional landscape of the orthologous *SHOC1* locus in the recombining species *R. breviscula*, shown for comparison. In contrast to *R. tenuis*, the locus displays detectable transcriptional activity and lower levels of non-CG methylation. (c) Quantitative RT-PCR analysis using two independent amplicons (A and B) confirms the absence of detectable *SHOC1* expression in *R. tenuis* accessions (PECP-47 and REC), whereas expression is detected in the recombining species *R. breviscula* and *R. austrobrasiliensis*. The *RtSHOC1* gene model and positions of the qPCR amplicons are indicated. *DMC1* was used as a positive control. All values are normalized to the housekeeping gene *Actin*.



Extended Data Fig. 9 | Segregation distortion of NOR-bearing chromosomes in PECP-48 during asymmetric male meiosis. (a) Asymmetric microsporogenesis producing one functional microspore (FC) and three degenerating cells (DC) ($n = 25$ tetrads over two independent experiments; scale bar, 5 μm). (b) FISH detection ($n = 21$ germinated pollen tubes over two independent experiments) of 45S rDNA loci (green) in sperm cells and tube nucleus of pollen tubes. Pollen grains exhibit either one (90.5%, left) or two (9.5%, right) 45S rDNA foci. Schematics show corresponding haplotypes (magenta and cyan indicate oligo-probes; green, 45S rDNA; only 45S rDNA was

used in pollen FISH). (c) Predicted segregation outcomes and expected gamete genotype frequencies following achiasmatic inverted meiosis. (d) Expected versus observed ($n = 100$) frequencies of gamete genotypes based on FISH analysis of mature pollen grains hybridised with a 45S rDNA probe (green), illustrating segregation distortion favouring the larger, TE-rich haplotype 1 ($\chi^2 = 9.17$, $\text{df} = 2$, $p = 0.010$). DC = degenerating cell; FC = functional cell; SC = sperm cell; TN = tube nucleus; VCN = vegetative cell nucleus. Scale bars correspond to 5 μm .



Extended Data Fig. 10 | Embryology of *R. tenuis* (PECP-48). (a) Pollen tube entering the mature embryo sac to deliver the sperm cells. SC = sperm cell, CC = central cell. (b) The presence of the pollen tube in the ovule tissue is confirmed by the detection of callose (Cal) in green in the pollen tube cell wall (indirect immunofluorescent labelling of β -1,3-glucan; see Supplementary Fig. 13).

(c) Abnormal development of seed with an embryo (En) but no visible endosperm formation. (d) Viable mature (n = 59), (e) late aborted (n = 9) and (f) early aborted seeds (n = 6) shown by Feulgen staining (top) and respective seed morphology (bottom).

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Data collection

PacBio HiFi, Arima Hi-C, whole-genome short-read, RNA, single-cell RNA/ATAC, ChIP-seq and enzymatic DNA methylation sequencing libraries were prepared at the Max Planck Genome Centre Cologne, Germany; single-cell RNA and whole-genome short-read libraries were also sequenced at BGI Genomics. Microscopy images were acquired with ZEN Blue software from ZEISS (v3.1) on a Zeiss Axio Imager .Z2 with ApoTome.2 for optical sectioning, and on Leica SP8, Leica SP8 Falcon Dive and Leica Stellaris 8 systems using LAS X (v5.3.3). Nuclei were sorted on a BD FACSAriaIII Fusion controlled by BD FACSDiva software (v8.0.3); nuclei were counted on a LUNA-FX7 Automated Cell Counter. Chromium Next GEM Single Cell ATAC and Single Cell 5' kits were used for library preparation. RT-qPCR was performed on a BIO-RAD CFX96 Real-Time PCR Detection System.

Data analysis

ZEN Blue software from ZEISS (v3.1) was used to analyse and process acquired images, including the colocalisation and histogram analyses. FIJI/ImageJ (v2.18.0) with the StackReg plugin was used for skeletonisation and image alignment. MorphoGraphX (v2.0.3) with ITK watershed and PlantSeg (v2.1.1) was used for 3D segmentation and rendering. Adobe Photoshop (v27.0.0) was used to process acquired images. Open-source tools: 3D-DNA (201008); bcftools (v1.9); bedtools (v2.30.0); BLAST (v2.12.0); bowtie2 (v2.5.1); BUSCO (v5.2.2); canu (v2.1); cellranger (v9.0.1); cellranger-atac (v2.1.0); CoGe (version after March 2025); DANTE (v0.2.10) and DANTE_LTR (v0.4.0.5); deeptools (v3.5.5); EDTA (v2.0); FASTK (v1.1); FigTree (v1.4.4); GENESPACE (v1.3.1); GenomeScope (v2.0); Geneious (v2023.0.1); Helixer (v0.3.4); HiCEplorer (v3.7.2); hickr (v2.0.1); hifiasm (v0.25.0); juicer (v2.0); Julia (v1.0.5); LTRharvest (v1.6.1); LTR_FINDER (v1.07); MAFFT (v7.475); MCSanX (v1.0.0); Mercury (v1.3); minigraph (v0.17); minimap2 (v2.28); miniprot (v0.18); OrthoFinder (v2.5.5); PanKmer (v0.20.4); plotsr (v0.5.3); pyGenomeTracks (v3.9); QUASt (v5.3.0); R (v4.2.0); RAXML-NG (v1.2.2); RTIGER (v2.1.0); samtools (v1.24); SHOREmap (v3.6); STAR (v2.7.11b); SubPhaser (v1.2.6); SyRI (v1.5.3); TEsorier (v1.3.0); vg toolkit (v1.40.0). Custom code is available at https://github.com/Raina-M/Rhynchospora_tenuis_project and https://github.com/Raina-M/detectCO_by_scrRNAseq, with archived versions at Zenodo (<https://doi.org/10.5281/zenodo.20847664>), as stated in the Code availability section of the manuscript.

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Genome assemblies and raw sequencing data have been deposited in the European Nucleotide Archive under accession PRJEB98016. The reference genomes, sequencing data, annotations and all tracks presented in this work are made available for download at EDMOND, the Open Research Data Repository of the Max Planck Society, <https://doi.org/10.17617/3.IXRT5Y>. The assemblies, annotations and genome-browser tracks hosted at EDMOND are derived products generated from the same sequencing datasets deposited at the European Nucleotide Archive; no primary sequencing data are held exclusively at EDMOND. Both repositories are publicly accessible without restriction.

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Sample size

No statistical method was used to predetermine sample size. Sample sizes for cytological and histological experiments were set by the number of meiocytes, pollen grains, ovules or seeds at the required developmental stage that could be recovered from the available greenhouse material, and all cells or structures meeting the staging criteria were scored. Pollen viability was scored on thousands of pollen grains pooled from dozens of flowers per species (total counts given in Supplementary Fig. 10). For single-nucleus sequencing, nuclei quality and number were assessed on a LUNA-FX7 Automated Cell Counter and the input was set by the Chromium kit manufacturer's instructions, yielding 10,997 pollen nuclei with complete genotypes across five accessions. Whole-genome sequencing was performed on 49 (REC) and 62 (PECP) F1 individuals. Exact sample sizes are stated in each figure legend.

Data exclusions	No data were excluded from the analysis. All cytological observations obtained under the described experimental conditions were included. In the gene-conversion analysis, marker sites with a combined read depth below 5× were excluded a priori as unreliable, as described in the Methods.
Replication	Each cytological, immunocytochemical, FISH, flow-cytometry and histological experiment was repeated independently at least two times with similar results, and all replication attempts were successful. Single-cell analyses were performed on thousands of independent nuclei. Meiocyte observations and immunolocalisations were repeated across multiple individuals and flowers and gave consistent results; root cytogenetic preparations came from separate root collections and were processed independently; pollen and pollen-tube assays were performed on independent flower-bud collections. The number of independent repetitions is stated in each figure legend.
Randomization	Not applicable, and explained as such in the Methods: all plants of a given accession are clonal propagules of a single field-collected individual and every assay compares fixed genotypes rather than experimental treatments, so samples were not allocated into experimental groups. Within an accession, samples were taken at random from plants maintained under identical growth conditions, and cells and images were selected on predefined developmental-stage criteria to minimise selection bias. The genotype of each accession was verified independently by sequencing and by FISH, controlling the only covariate of interest.
Blinding	Blinding was not applicable because the accessions are distinguished by karyotype and therefore cannot be masked during microscopy. Analyses were nonetheless performed using predefined scoring criteria applied independently of the expected outcome, and the automated measurements (synaptonemal-complex fragmentation coefficient, pixel-intensity skewness, colocalisation coefficients, allele-frequency genotyping) were computed by script without operator input.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems		Methods	
n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☐	☒ ChIP-seq
☒	☐ Eukaryotic cell lines	☐	☒ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		
☐	☒ Plants		

Antibodies

Antibodies used	Previously described: rabbit anti-Arabidopsis thaliana ASY1 (inventory code PAK006; Armstrong et al.); rabbit anti-A. thaliana MLH1 (PAK017; Castellani, Zhang et al., Nat. Plants 2024 ref no. 18); chicken anti-A. thaliana ZYP1-cter (PAK053); rabbit anti-Rhynchospora pubera REC8 (combination of two affinity-purified peptide antibodies, Eurogentec; Castellani, Zhang et al. 2024 ref no. 18); rabbit and rat anti-R. pubera HEI10 (combination of four affinity-purified peptide antibodies, Eurogentec and Lifeprotein; Castellani, Zhang et al. 2024); rabbit anti-CENH3 (custom, as described in the cited reference — supplier and production details: Marquest et al. 2015 ref no. 70). Newly raised: rat anti-R. pubera ZYP1 against peptide KLTAERLVKDQASVKNDLEC, affinity-purified (Lifeprotein). Commercial primaries: anti-H3K4me3 (Abcam, ab8580); anti-H3K9me2 (Abcam, ab1220); anti-H3K27me3 (Merck, 07-449). Commercial secondaries: goat anti-rat STAR ORANGE (Abberior, STORANGE-1007); goat anti-rabbit Alexa Fluor 488 Superclonal recombinant (Thermo Fisher, A27034); goat anti-mouse Alexa Fluor 647 (Abcam, ab150119). Dilutions: all primary antibodies 1:200; STAR ORANGE and Alexa Fluor 488 1:250; Alexa Fluor 647 1:200.
Validation	Commercial histone-modification antibodies are validated by the manufacturers for ChIP in plants and are widely used in the Rhynchospora literature: anti-H3K4me3 (Abcam ab8580), anti-H3K9me2 (Abcam ab1220) and anti-H3K27me3 (Merck 07-449) — manufacturer validation statements, citations and database profiles: https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580 ; https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-di-methyl-k9-antibody-mabcam-1220-chip-grade-ab1220 ; https://www.sigmaaldrich.com/DE/de/product/mm/07449?icid=sharepdp-clipboard-copy-productdetailpage . The previously described ASY1, MLH1, ZYP1, REC8 and HEI10 antibodies were validated in Castellani, Zhang et al., Nat. Plants 10, 423–438 (2024) and earlier studies, where they produce the expected stage-specific meiotic localisation patterns. The newly raised rat anti-R. pubera ZYP1 and the additional HEI10 peptide antibodies were cross-validated by immunocytochemistry in eight further Rhynchospora species against published antibodies raised against the same proteins, and confirmed by signal interpolation; in this study they reproduce canonical synaptonemal-complex and crossover-marker patterns in the chiasmatic control species R. austrobrasiliensis (Fig. 2k–l, o), which serves as an internal positive control for the negative result reported in R. tenuis. Secondary antibodies were validated by the absence of signal in no-primary controls.

Plants

Seed stocks	Rhynchospora tenuis and R. austrobrasiliensis were collected from natural populations in Brazil under the appropriate collection permits (permit numbers: SISGEN AB13824, AF0D72D) and cultivated under controlled greenhouse conditions (>16 h daylight, 26 °C, >70% humidity). Nine geographically distinct R. tenuis accessions were used; all material was propagated clonally from field-collected individuals.
Novel plant genotypes	
Authentication	n/a

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links https://www.ebi.ac.uk/ena/browser/view/PRJEB98016
May remain private before publication.

Files in database submission

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Genome browser session
 (e.g. [UCSC](#))

no longer applicable

Methodology

Replicates	Every ChIP design has two biological replicates.
Sequencing depth	Mean sequencing depth for each single read fastq file is about 4X, so mean for each replicate is about 16X.
Antibodies	CENH3: polyclonal rabbit anti-RpCENH3 (Marques et al., 2015) Rabbit polyclonal to Histone H3 (tri methyl K4) abcam Cat# ab8580; RRID:AB_306649 Mouse monoclonal to Histone H3 (di methyl K9) abcam Cat# ab1220; RRID:AB_449854 Recombinant Rabbit IgG, monoclonal abcam Cat# ab172730; RRID:AB_2687931
Peak calling parameters	No peak calling.
Data quality	No peak calling.
Software	Bowtie2 was used for read mapping with the --very-sensitive-local option. ChIP and control alignments were compared using bamCompare with the parameters --binSize 50 --normalizeUsing RPKM --operation log2. Control reads are library 5650.A (rep1) and 5650.B (rep2) input chromatin.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For pollen nuclei, samples were treated by applying the filter bursting method with scWPB (200mM Tris-HCl; 4mM MgCl ₂ ; 2mM EDTA; 86mM NaCl; 10mM Na ₂ S ₂ O ₅ ; 250mM Sucrose; 0.5mM Spermine; 0.5mM Spermidine; 1% PVP-10; 5mM DTT; 1%BSA; 0.2 U/μL Protector RNase Inhibitor) as an isolation buffer. Pollen was burst in a 5μm CellTrics cell strainer. The obtained nuclei solution was stained with DAPI (1 μg/μl) and run into FACSAriaIII Fusion Flow Cytometer with a 70μm nozzle and 483kPa sheath pressure. For seed nuclei, frozen seeds were macerated with a plastic rod and resuspended in Seed Nuclei Isolation Buffer (100mM Tris, 5.2 mM MgCl ₂ , 85.55 mM NaCl, 0.1% Triton, 0.2 U/μL Protector RNase Inhibitor, pH 7.5). The obtained solution was filtered through a 20μm CellTrics cell strainer into a fresh 2ml tube and stained with DAPI (1 μg/μl). The nuclei solution was sorted as described before.
Instrument	BD FACSAriaIII Fusion Flow Cytometer
Software	BD FACSDiva Version 8.0.3
Cell population abundance	Nuclei were dispensed into a 96-well plate containing 30μl Collection Buffer (1xPBS, 1%BSA, and 0.2 U/μL Protector RNase Inhibitor)
Gating strategy	After identifying the desired nuclear population in a dotplot displaying the DAPI fluorescence signal versus side scatter signal, a sort gate was defined in the corresponding histogram section.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.