PNAS · 2023 · PLANT BIOLOGY

Non-B-form DNA tends to form in centromeric regions and has undergone changes in polyploid oat subgenomes

Liu Q*, Yi C*, Zhang Z, Su H, Liu C, Huang Y, Li W, Hu X, Liu C, Birchler JA, Liu Y†, Han F.†

doi: 10.1073/pnas.2211683120

Presented by **Sun Yanqing** 2026 / 05 / 08

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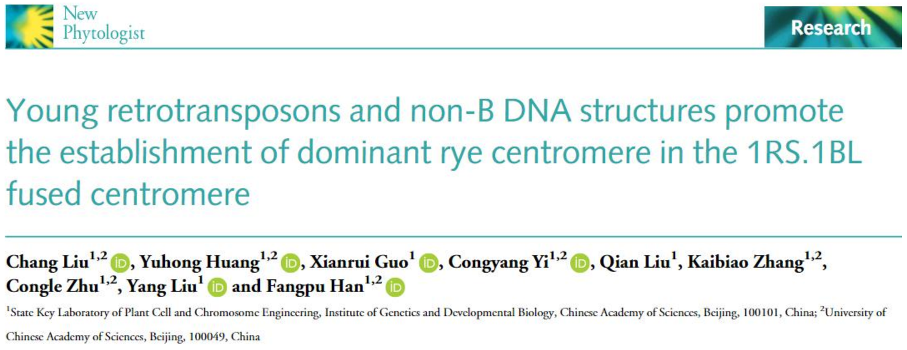
Huang *et al. Genome Biology* (2025) 26:271
https://doi.org/10.1186/s13059-025-03759-4

Genome Biology

BRIEF REPORT **Open Access**

Distinct evolutionary trajectories of subgenomic centromeres in polyploid wheat

Yuhong Huang[1†], Yang Liu[1†], Chang Liu[1†], Congyang Yi[1], Jinsheng Lai[2], Hongqing Ling[1], Handong Su[3*] and Fangpu Han[1*]



New Phytologist

Research

Young retrotransposons and non-B DNA structures promote the establishment of dominant rye centromere in the 1RS.1BL fused centromere

Chang Liu[1,2], Yuhong Huang[1,2], Xianrui Guo[1], Congyang Yi[1,2], Qian Liu[1], Kaibiao Zhang[1,2], Congle Zhu[1,2], Yang Liu[1] and Fangpu Han[1,2]

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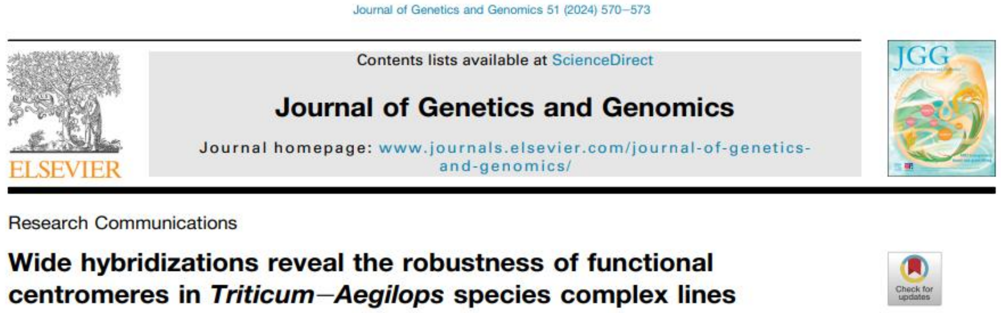


Journal of Genetics and Genomics 51 (2024) 570–573

Contents lists available at ScienceDirect

Journal of Genetics and Genomics

Journal homepage: www.journals.elsevier.com/journal-of-genetics-and-genomics/

ELSEVIER

JGG

Research Communications

Wide hybridizations reveal the robustness of functional centromeres in *Triticum*–*Aegilops* species complex lines

BACKGROUND I

Non-B DNA structures

- DNA can adopt multiple non-canonical secondary structures beyond the strandard right-handed B-form double helix.

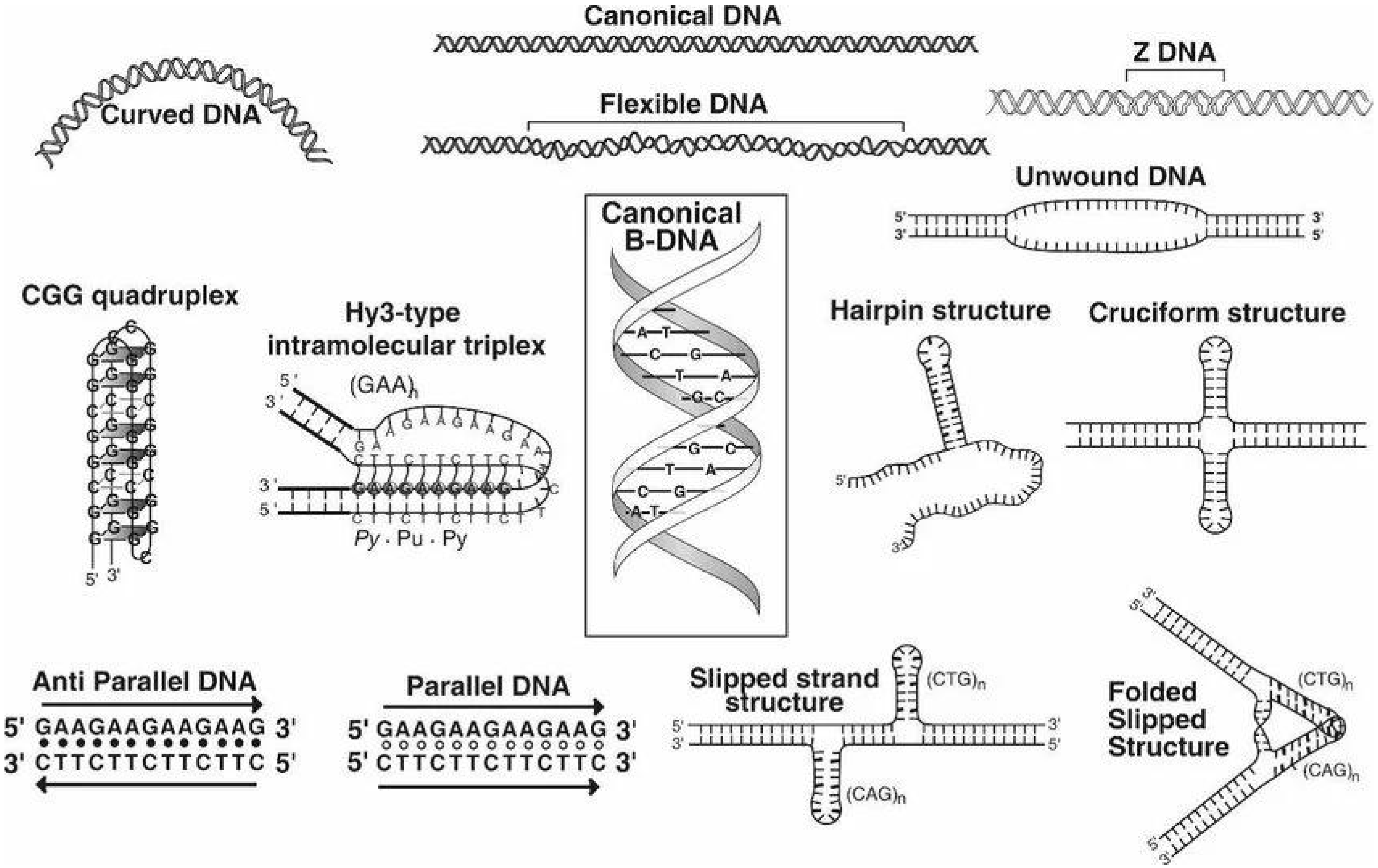
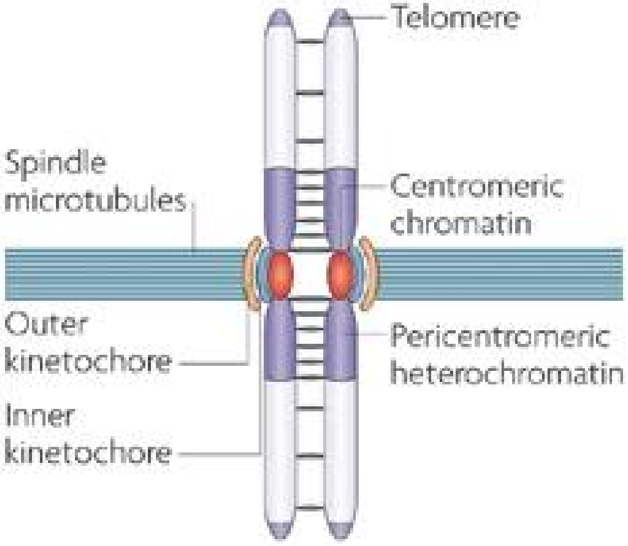


Figure from Smith GR, Genes Dev. 2008

BACKGROUND II

Centromere

- Faithful chromosome segregation in mitosis and meiosis.
- Assembly platform for **kinetochore,** the site connects chromosomes to spindle microtubules.



- Histone H3 variants
  CENH3 (plants)
  CENP-A (animals)

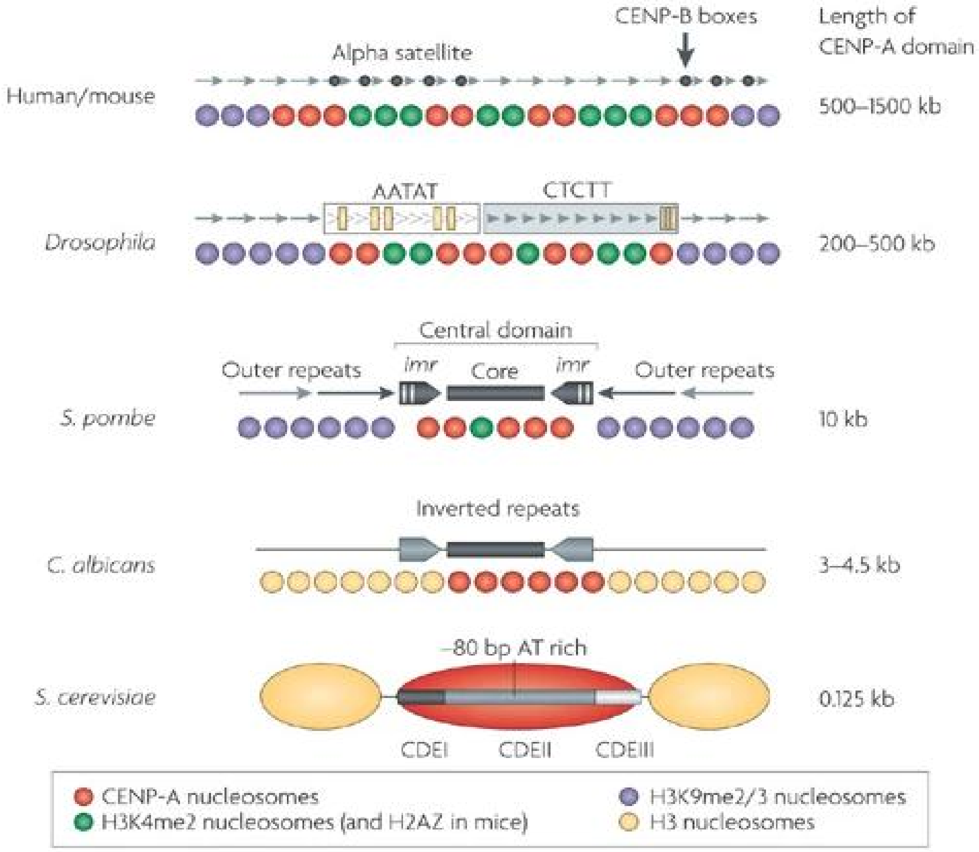
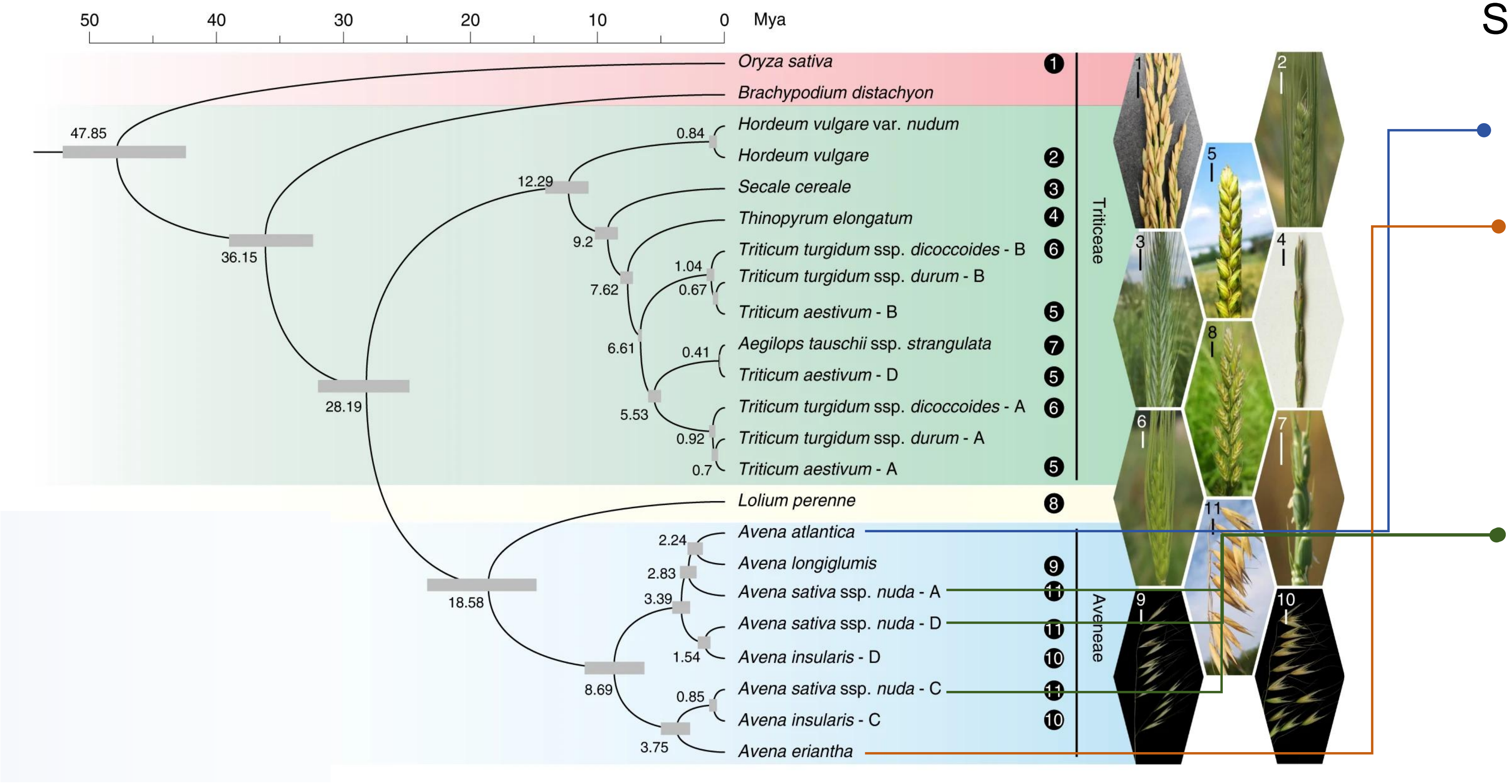


Figure from Allshire, R., Karpen, G. *Nat Rev Genet,2008*

BACKGROUND III

Oat (*Avena sativa* L.)

- Major cereal crop
- Three ploidy levels within one genus (*Avena*)
- Domesticated oat (*Avena sative*, AACCDD, hexaploid, 2n=42)



Species used in this study:

A. atlantica AA Diploid

A. eriantha CC Diploid

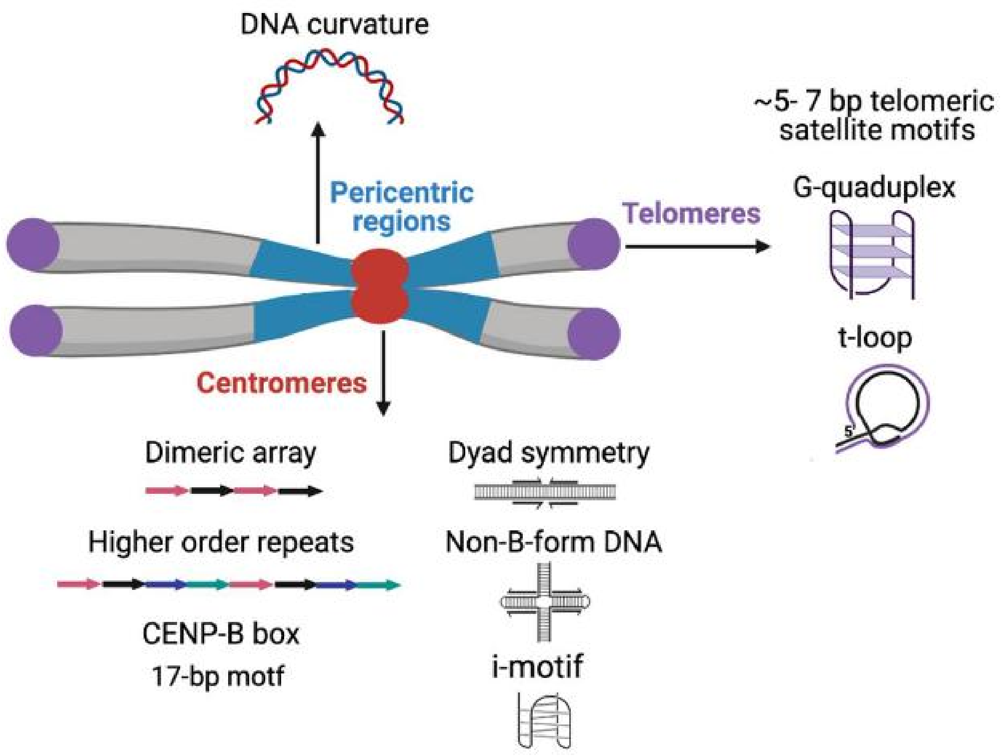
A. maroccana CCDD Tetraploid

A. sativa AACCDD Tetraploid

Figure from Peng, Y., *et al. Nat Genet,2022*

STUDY DESIGN

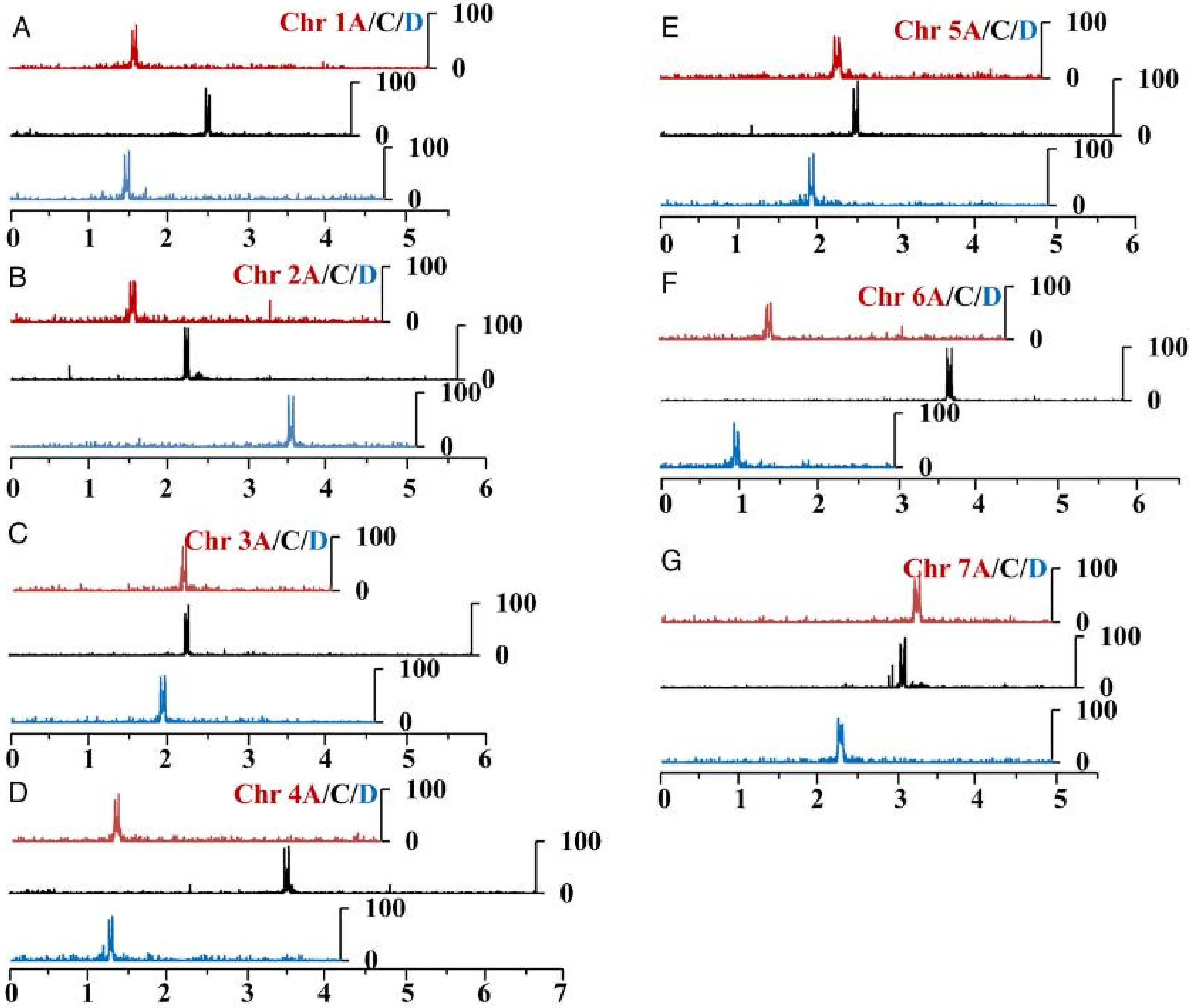
Research Question



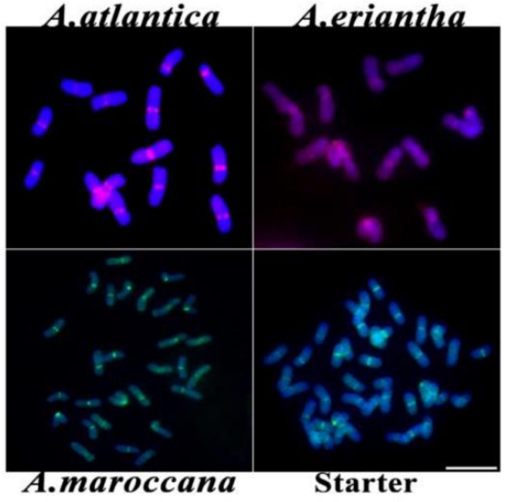
- Do oat centromere harbor non-B DNA structure?
- Which centromeric repeats contribute to non-B DNA formation?
- How do non-B DNA structures change during polyploidization?

RESULT I

Oat Centromeres identified by CENH3 ChIP-seq



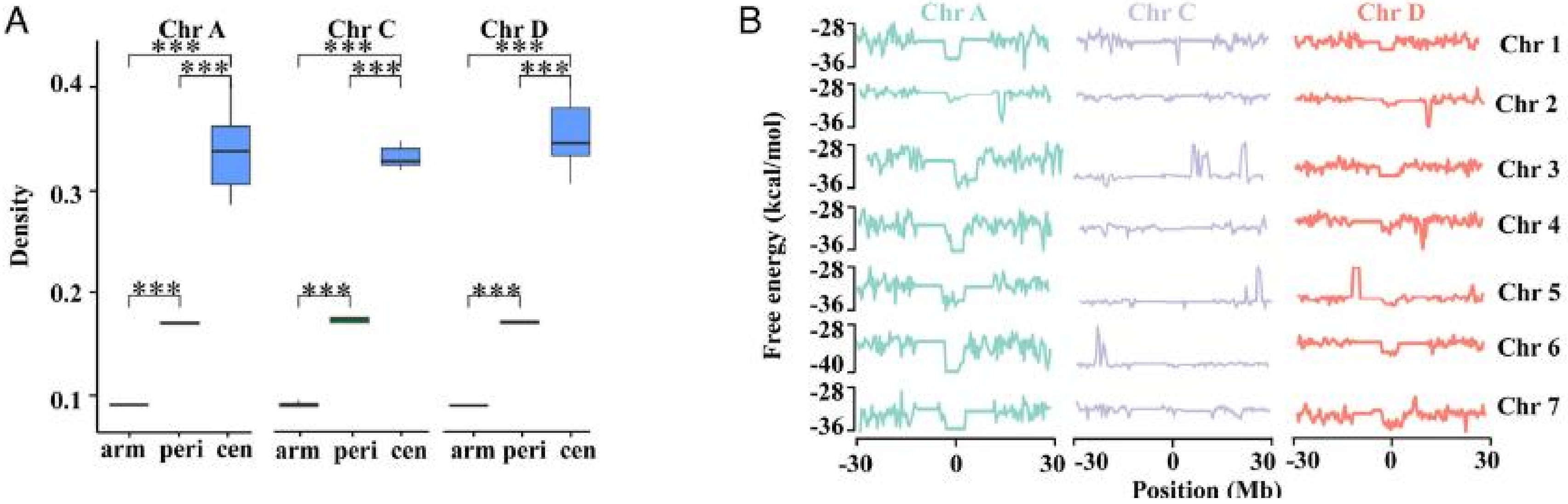
- Clear CENH3 peaks on all 21 chromosomes
- Size: 5.0-9.8 Mb, avg ~6.9 Mb
- AA > DD > CC subgenome
- FISH validates specificity



Non-B DNA is Enriched at Oat Centromeres

Computer prediction :

- Dyad symmetry: centromere > arm in all 3 subgenomes
- Folding free energy: centromeric DNA has more stable secondary structures
- nBMST: Z-DNA, Slipped DNA, Bent DNA significantly enriched. (permutation test)



RESULT II

Non-B DNA is Enriched at Oat Centromeres

Computer prediction :

- Dyad symmetry: centromere > arm in all 3 subgenomes
- Folding free energy: centromeric DNA has more stable secondary structures
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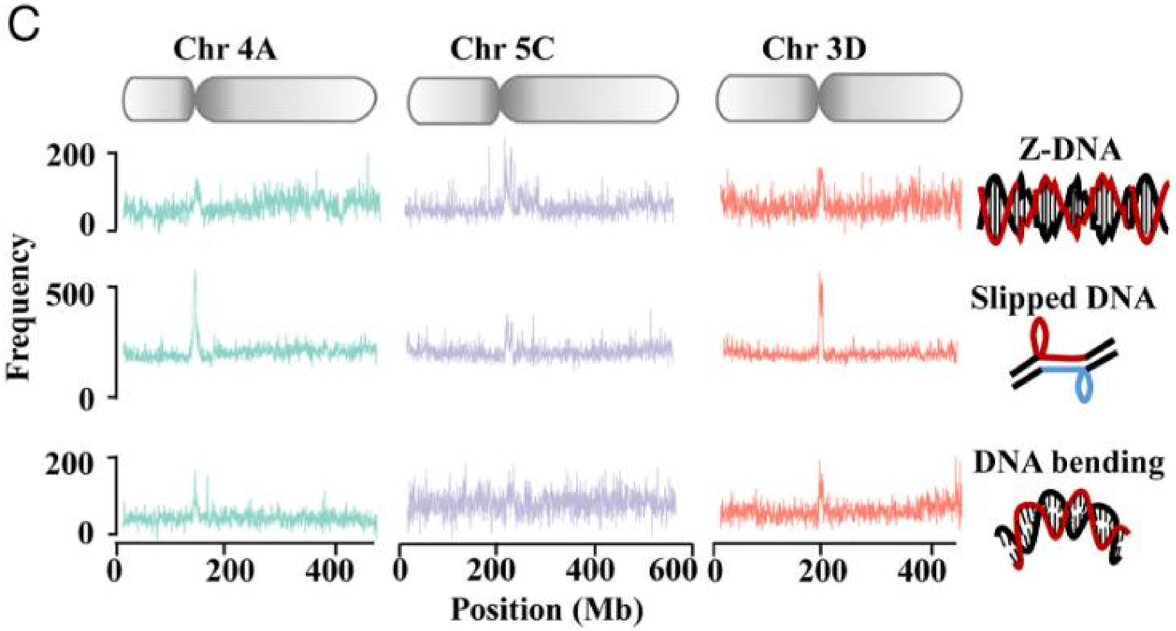
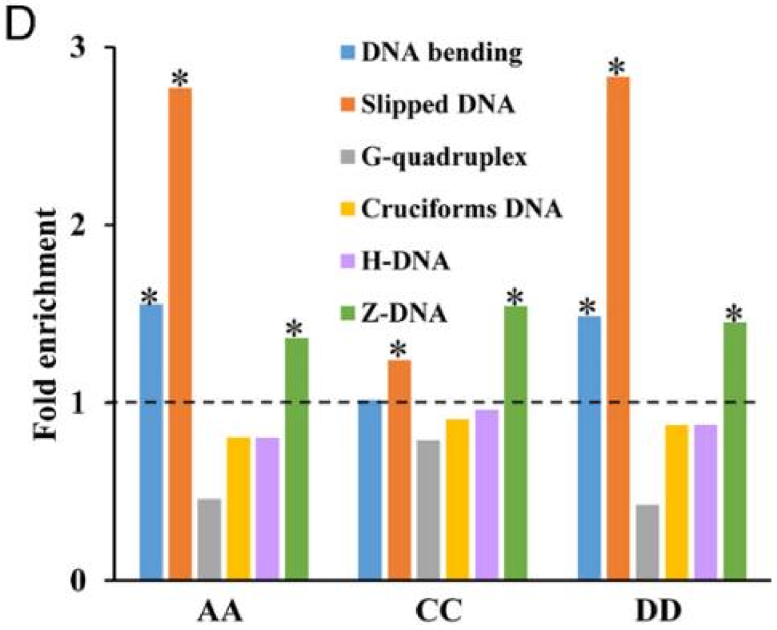


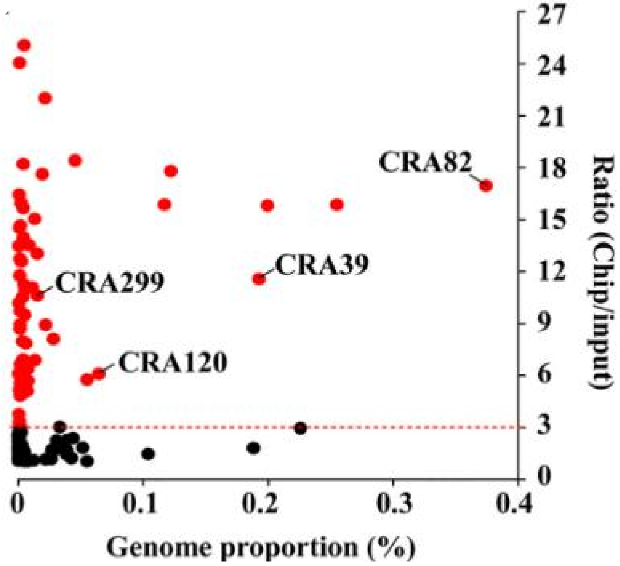


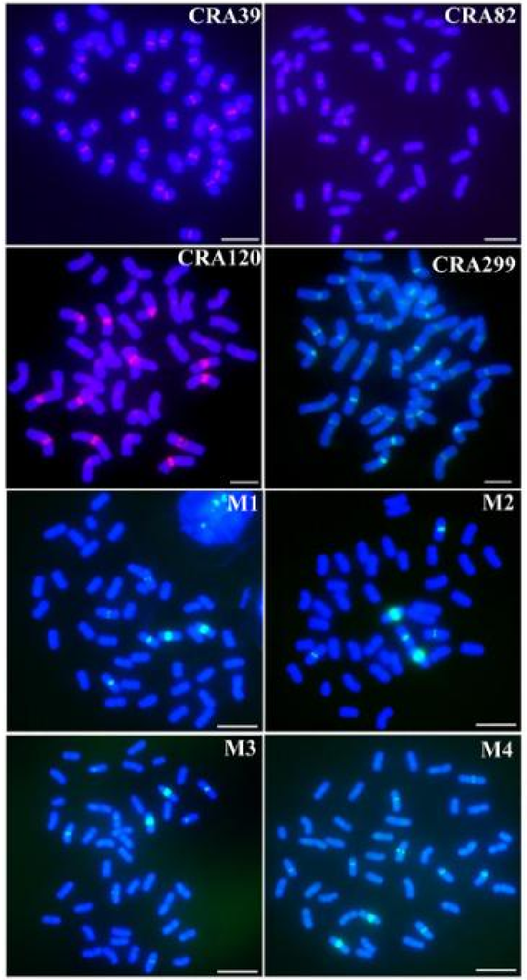
RESULT III

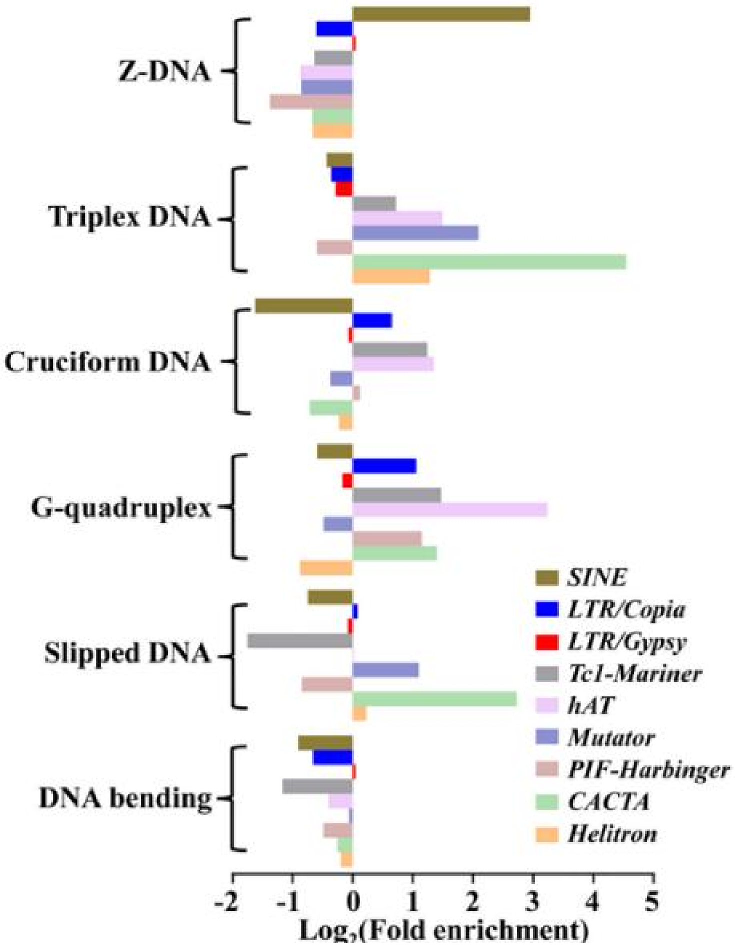
Centromere Repeats Drive Non-B DNA Formation

Centromere repeats in oat:

- LTR retrotransposons (Gypsy and Copia)
- No satellite repeats
- FISH assays validation (4 clusters and 4 minisatellites)



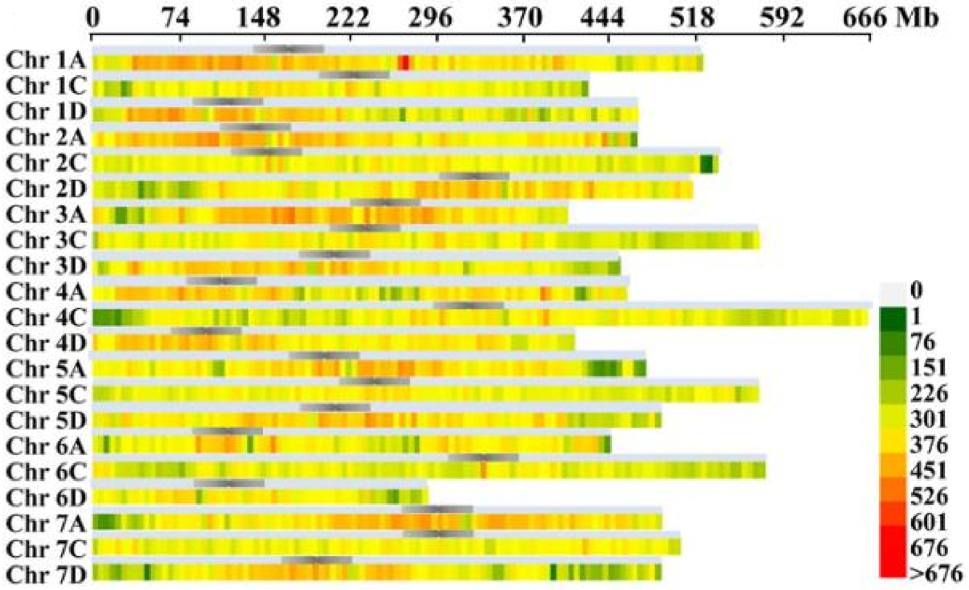


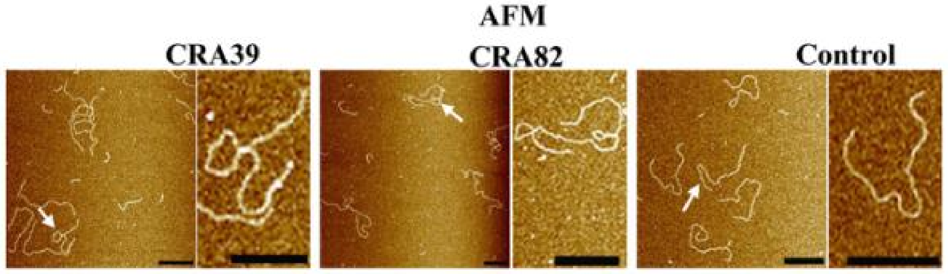


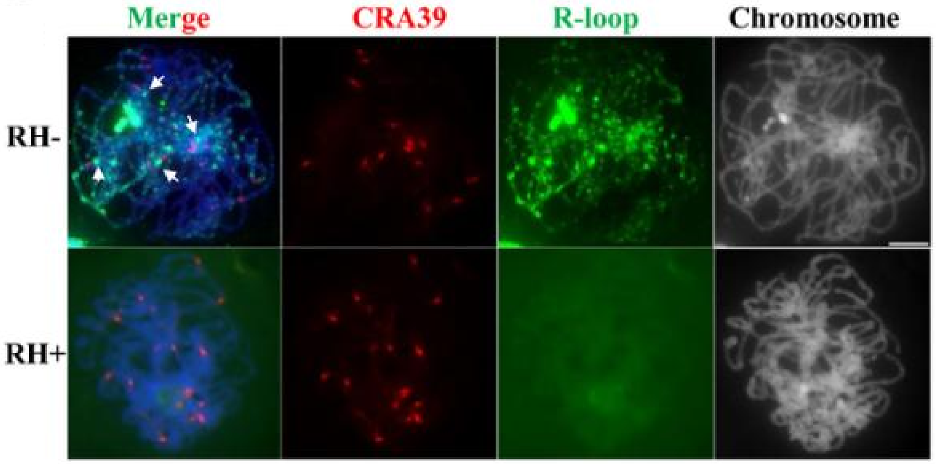
RESULT IV

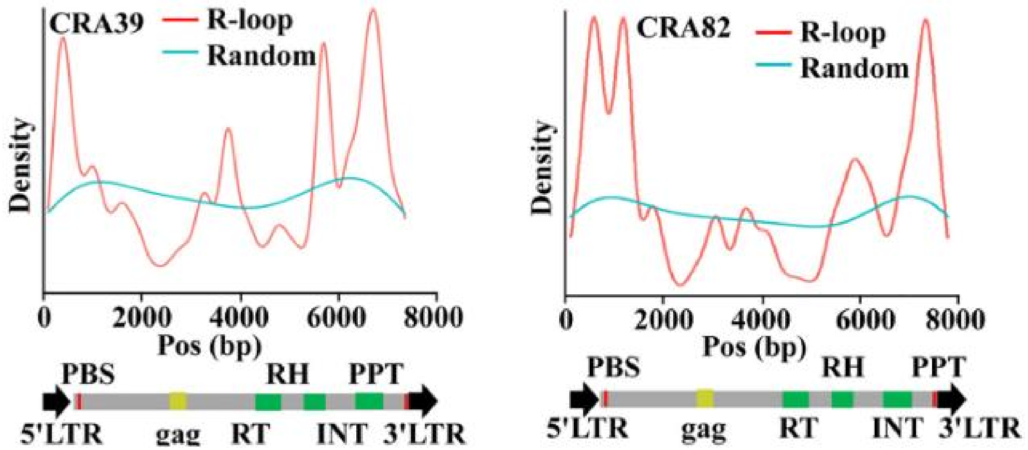
R-loops Are Validated at Oat Centromeres

- R-loops increase toward pericentromeres & centromeres
- R-loops architecture on retrotransposons.





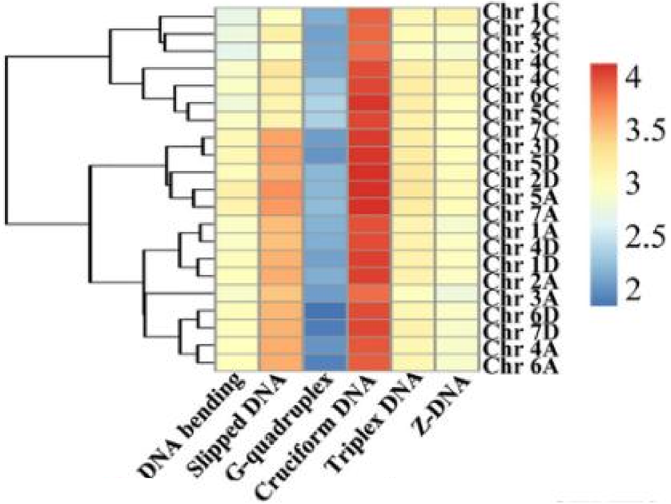


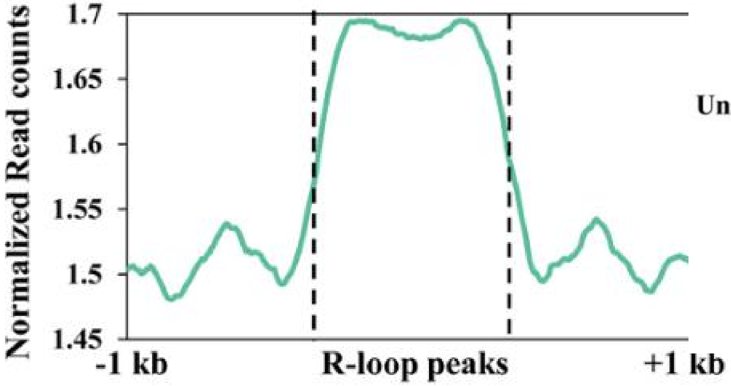


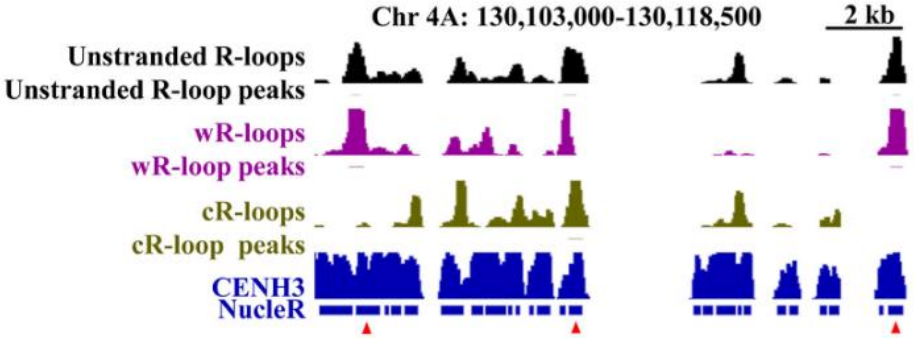
RESULT V

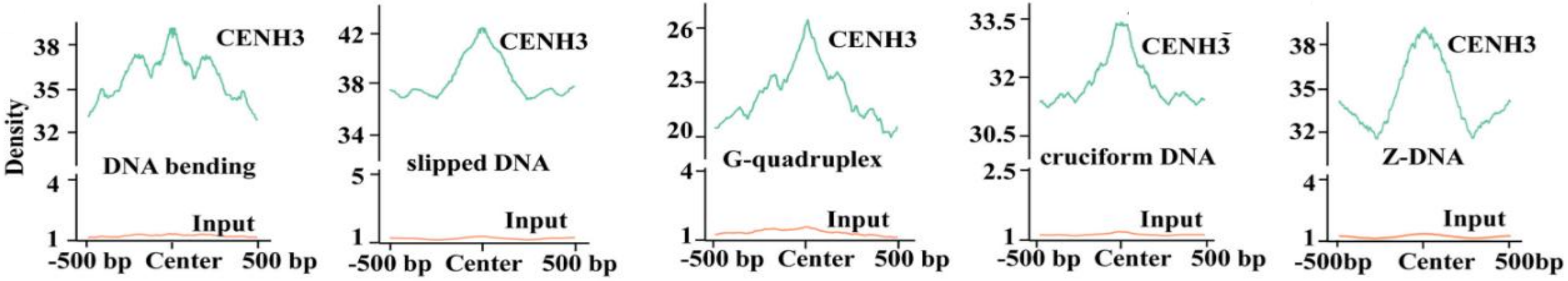
Non-B DNA Co-localizes with CENH3 Nucleosomes

- Bent DNA, Slipped DNA, Z-DNA, Cruciform significantly overlap CENH3 nucleosomes.
- R-loops are highly co-localized with CENH3.





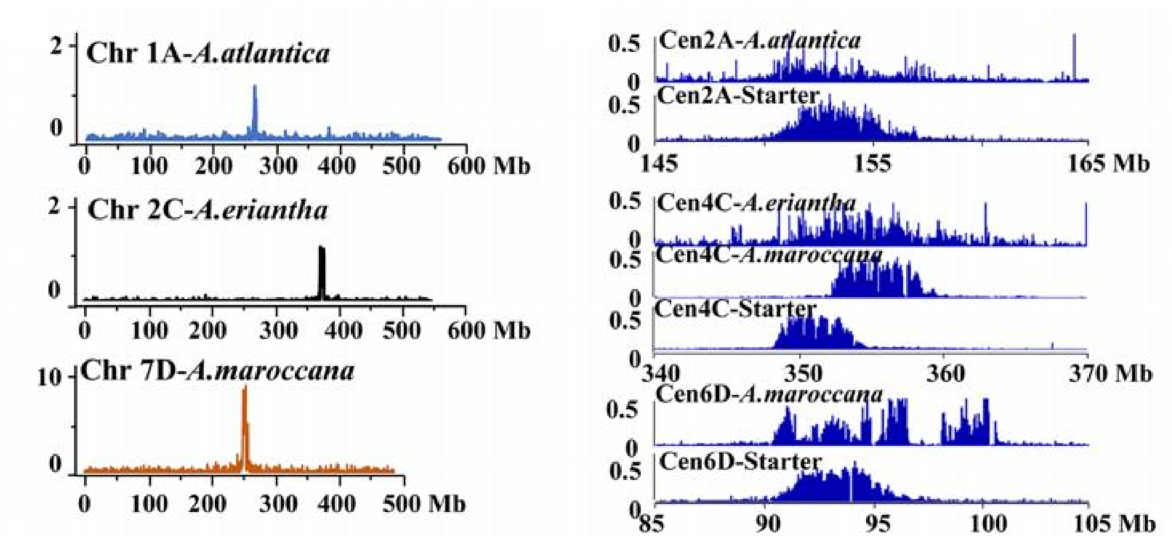




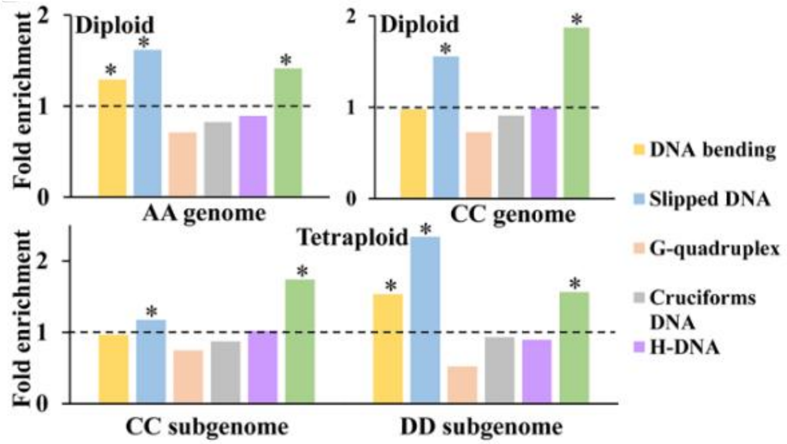
Non-B DNA as a “docking zone” for CENH3 assembly

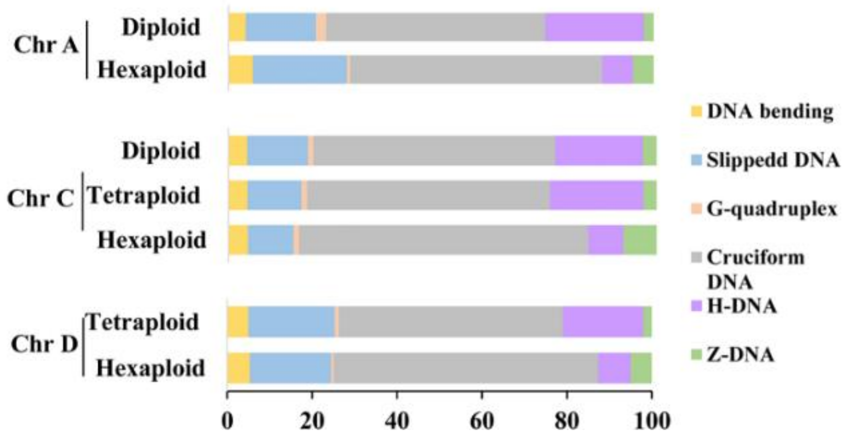
RESULT VI

Non-B DNA Dynamics During Polyploid Evolution



- Only ~37-40% of diploid/tetraploid reads cross-map to hexaploid genome, indicating rapid centromere divergence.





- Despite rapid sequence divergence, the centromeric enrichment of Z-DNA, Slipped DNA, and Bent DNA is conserved across all ploidy levels.

SUMMARY

KEY FINDING

- Centromere mapping across three ploidy levels.
- Z-DNA, Slipped DNA and Bent DNA are enriched at oat centromeres. (Driven by LTR/Gypsy retrotransposons)
- R-loops at centromeres validated by DRIP-seq, S9.6 dot blot, and AFM.
- Non-B DNA enrichment pattern is conserved across polyploidization.

Limitation

- Prediction ≠ in vivo validation. Exception for R-loops, other non-B DNA structures are all computational predictions.

- Correlation, not causation. Non-B DNA co-localizes with CENH3, but no loss-of-fuction experiments shows disrupting non-B DNA-forming sequences affects centromere function.

- Mechanism is unknown. How CENH3 “recognizes” non-B DNA remains unclear.