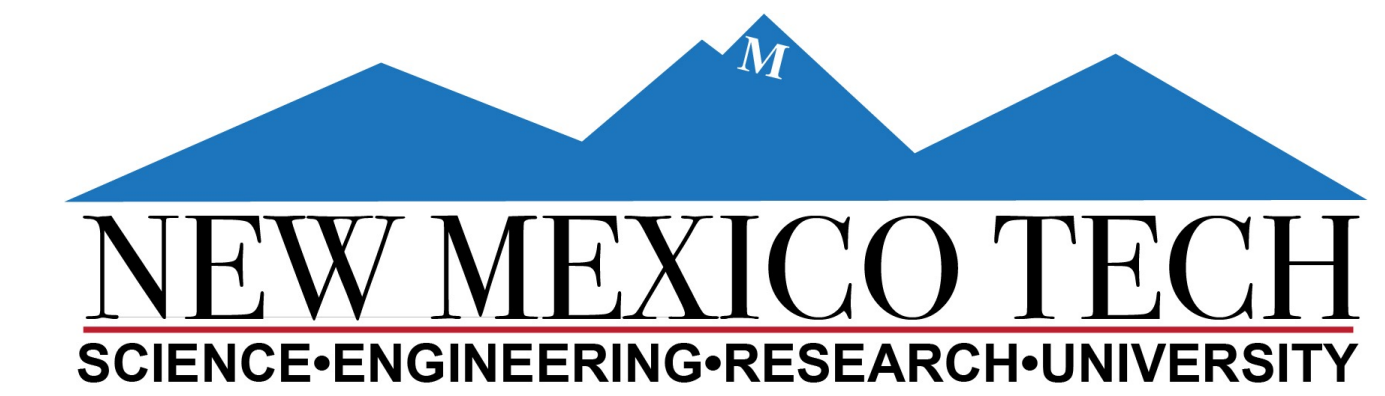




Genomic, Cellular, and Physiological Effects of Whole-Genome Duplications on Organismal Energy Production



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Whole-genome duplications perturb cytonuclear interactions

- Plants genomes are partitioned across three distinct compartments: **nuclear**, **mitochondrial**, and **chloroplast**
- Interactions between these three different genomes are essential for plant function and fitness
- Whole-genome duplications (WGDs) perturb the stoichiometry of mitochondrial and chloroplast vs. nuclear genomes (Figure 1)

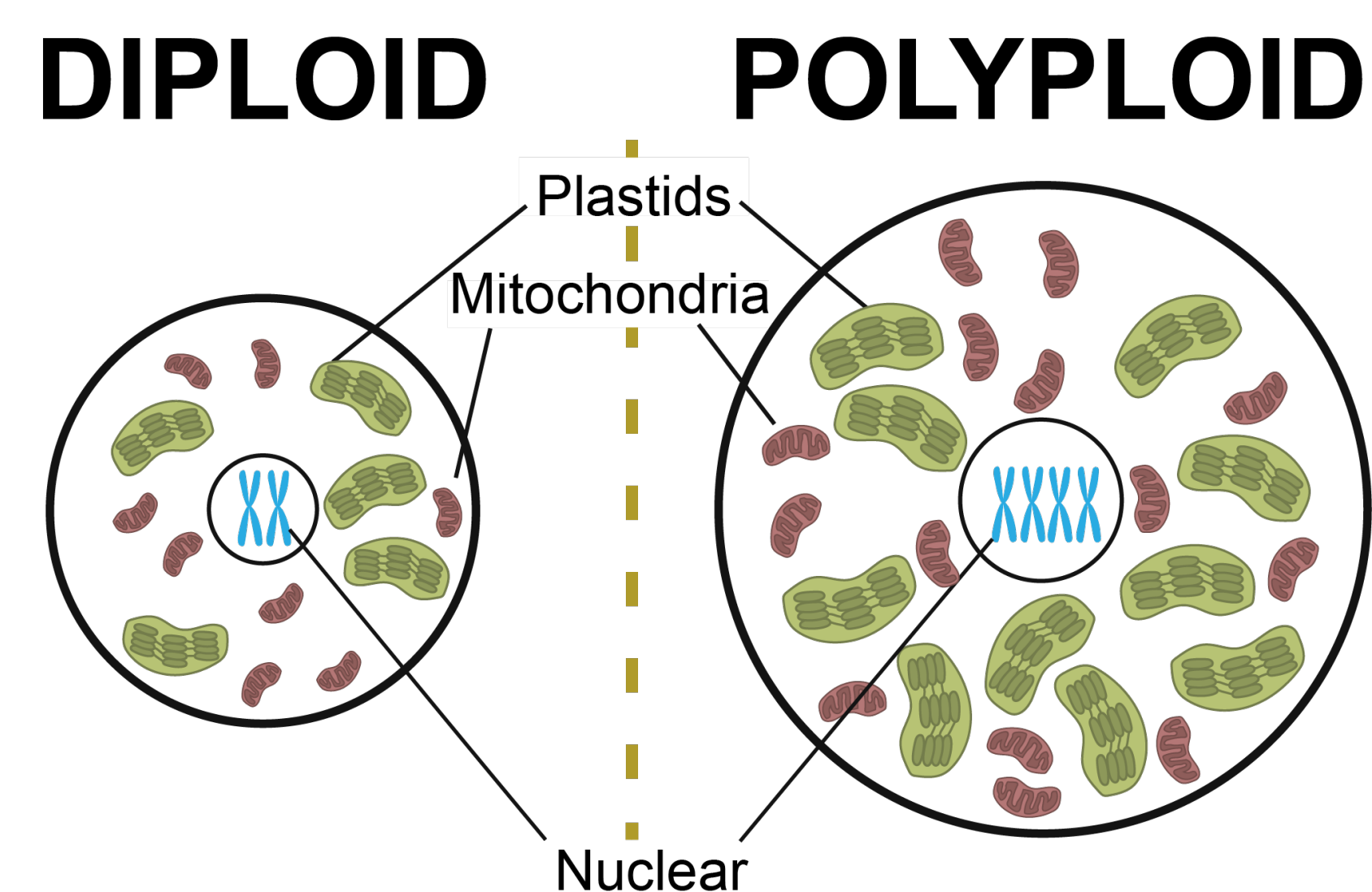


Figure 1. Polyploid cells are larger and have twice as many genes as diploid cells. Whole-genome duplications are therefore expected to alter the number, size, and function of the energy-producing organelles, chloroplasts and mitochondria (modified from Sharbrough et al., 2017).

Polyploids compensate for cytonuclear stoichiometric imbalance

- Wheat and *Arabidopsis* polyploids exhibit elevated cytoplasmic genome copy numbers per cell compared to diploid relatives (Figure 2a-b) (Fernandes Gyorfy et al., 2021)
- Allopolyploids exhibit similar proportions of cytoplasmic transcripts to diploid relatives (Figure 2b) (Forsythe et al., 2022)

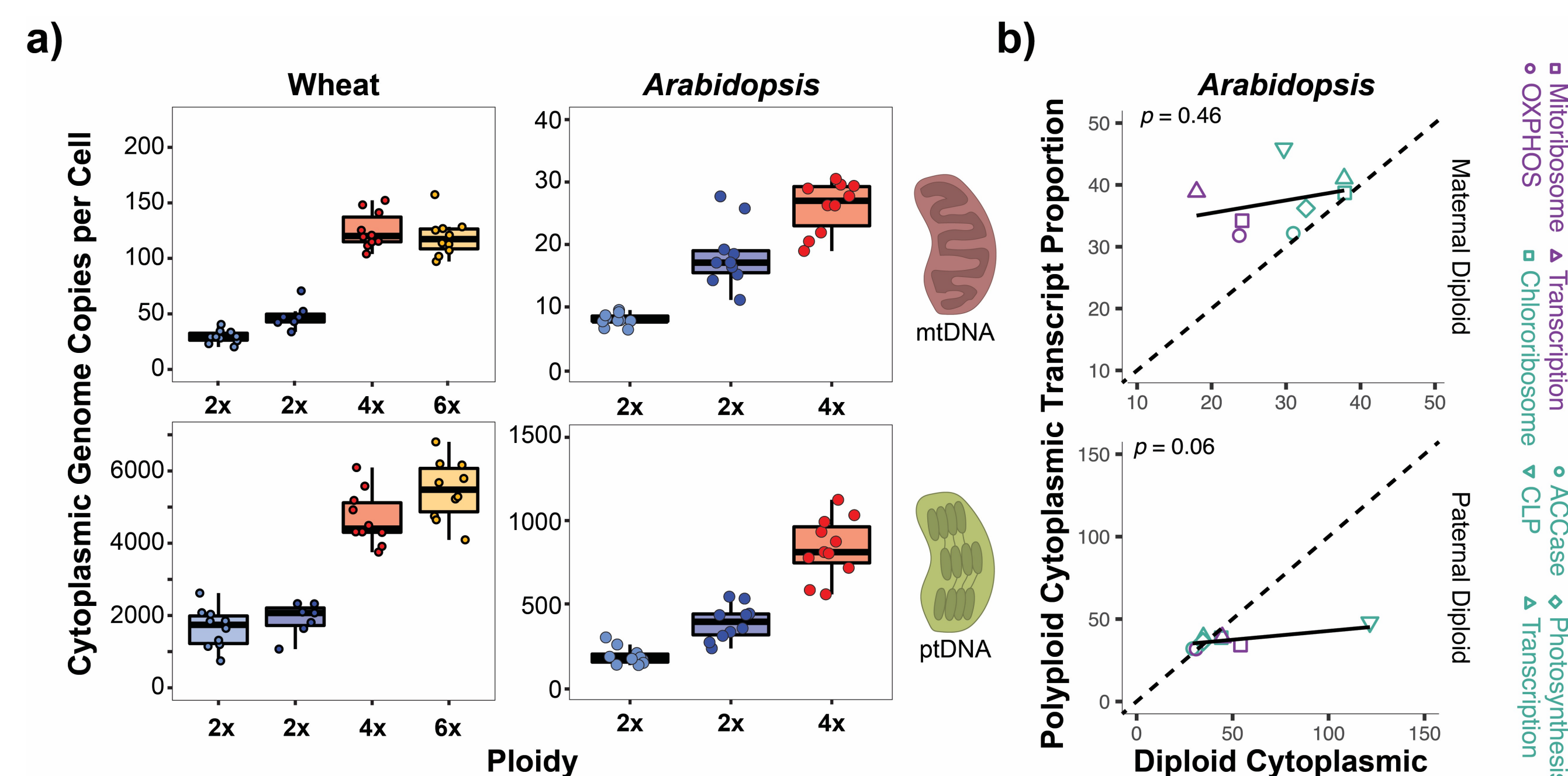


Figure 2. Cytonuclear compensation at the genomic and transcriptomic levels. a) Cytoplasmic genome copy number in Wheat (left) and *Arabidopsis* (right) measured by ddPCR for mitochondria (top) and chloroplast (bottom) genomes (modified from Fernandes Gyorfy et al., 2021). b) Cytoplasmic transcript proportions in polyploid *Arabidopsis* (y-axis) compared to diploid relatives (x-axis) (modified from Forsythe et al., 2022).

What are the consequences of cytonuclear compensation for photosynthesis and respiration?

Genomic architecture of cytonuclear stoichiometry

- Leveraging *Arabidopsis* 1001 genomes project and genetic mapping of F2 inter-crosses to identify candidate gene regions, use knock-outs and knock-ins to confirm molecular function (Figure 3)

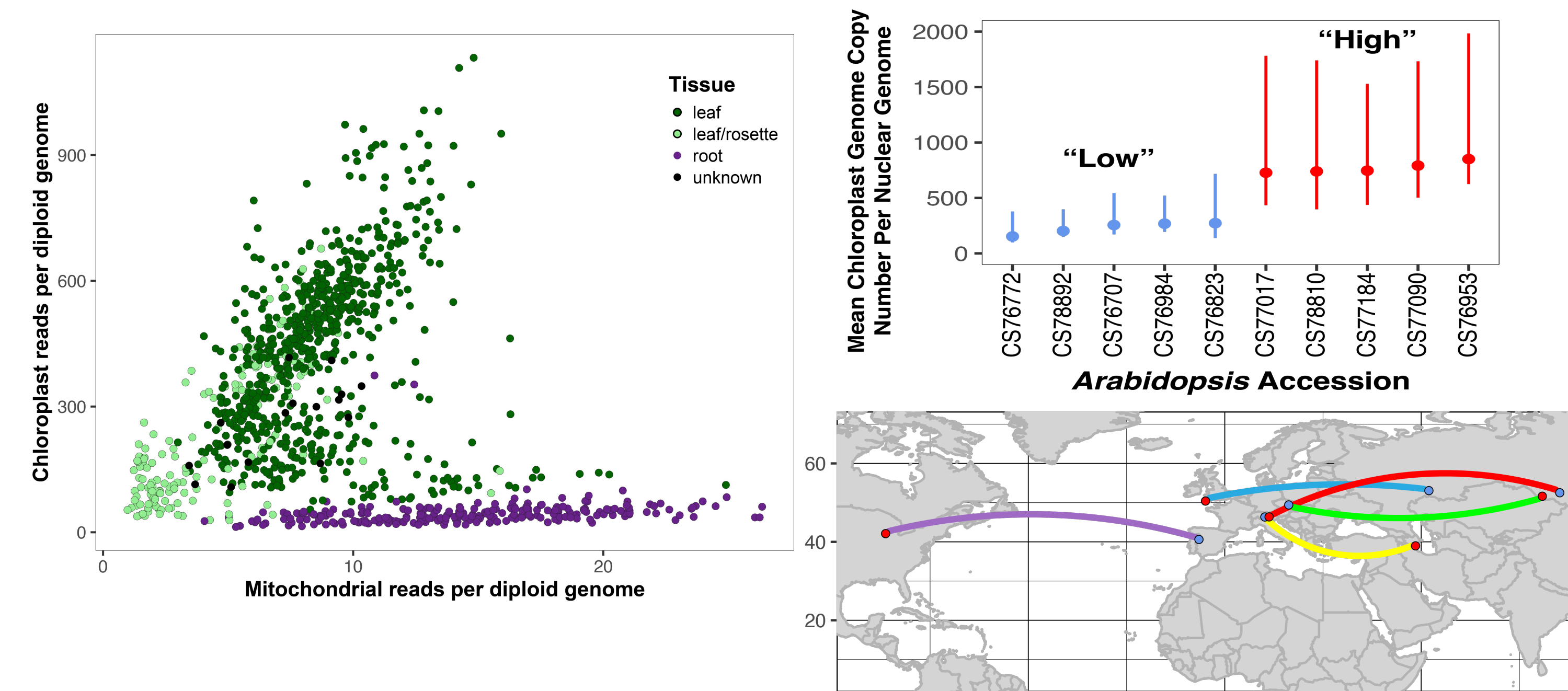


Figure 3. QTL analysis of cytoplasmic genome copy number genetic architecture. Left: Mitochondrial (x-axis) and chloroplast (y-axis) genome sequencing coverage relative to the nuclear genome from total cellular DNA. Top Right: Accessions of *Arabidopsis* with “low” and “high” chloroplast genome copy number from WGS. Bottom right: Map of crosses performed for QTL analysis. F₂ rearing under common garden conditions is ongoing.

- Sequencing diploid, tetraploid, and hexaploid wheat accessions to identify potential targets for optimizing cytonuclear stoichiometry

Cellular cytonuclear responses to whole-genome duplications.

- Count and measure chloroplasts and mitochondria using fluorescent microscopy (Figure 4)
- Quantify photosynthetic activity using MultispeQ spectrophotometer
- Compare relative expression of nuclear vs. organellar transcripts using ribosomal-rRNA-depleted, single-cell sequencing

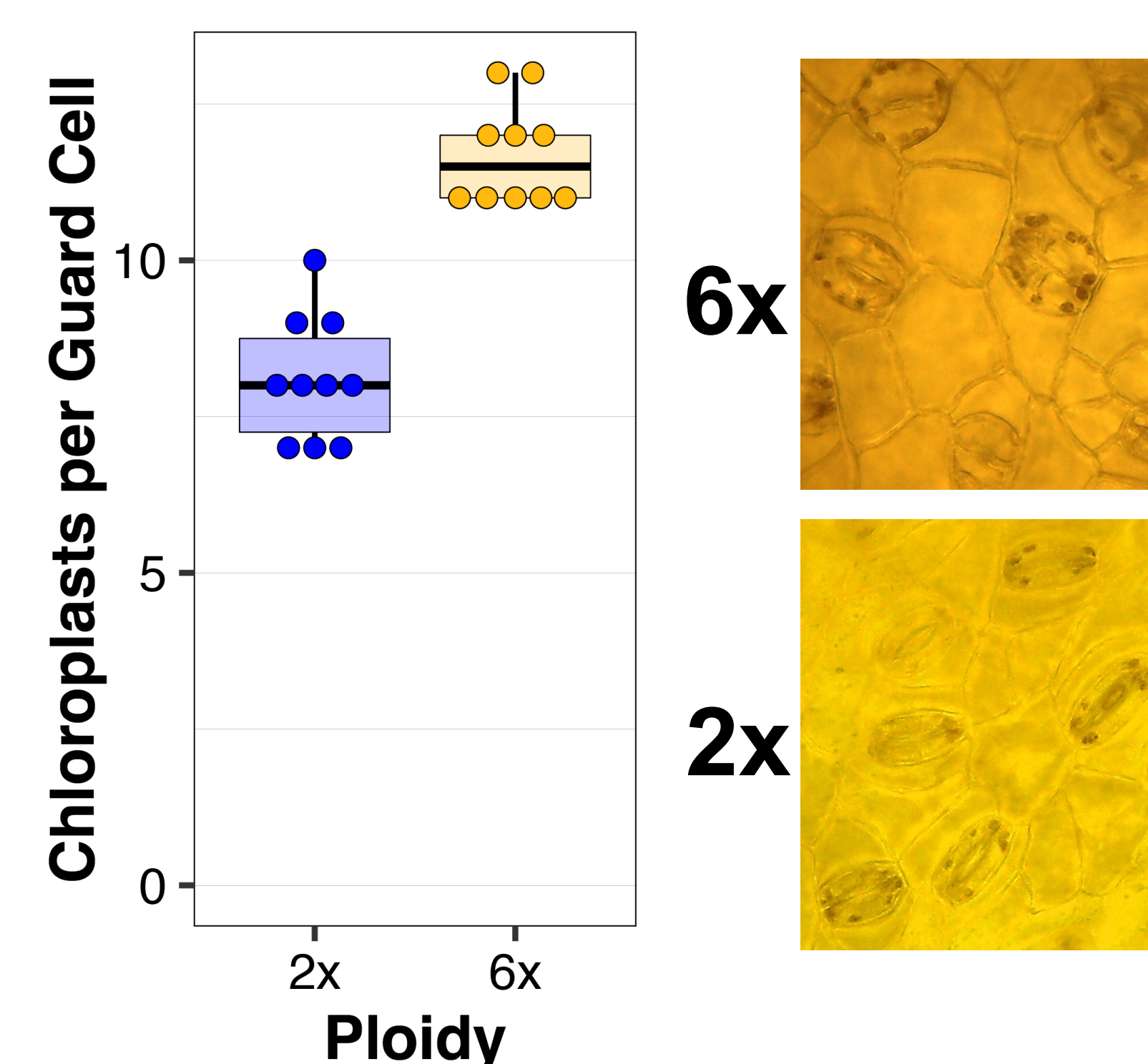


Figure 4. Chloroplast number and size determination in diploid and hexaploid blueberries. Left: Chloroplast numbers per stomata guard cell in young blueberry leaves. Right: Micrographs of diploid (bottom) and hexaploid (top) leaf epidermis stained with Carnoy's solution.

Educational Activities

- Developed and implemented course titled “*The DNA Sequencing Revolution*” as part of the Masters for Science Teachers program <https://github.com/jsharbrough/DNASequencingRevolution>
- Sequenced creosote (*Larrea tridentata*) total cellular DNA extractions using Oxford Nanopore sequencer as part of DNA Sequencing Revolution course
- Developed and implemented Bioinformatics course for senior Undergraduate and junior Graduate students in which students participate in research-based learning projects centered on PI Sharbrough's research https://github.com/jsharbrough/NMT_Bioinformatics_2022
- Designed and implemented high school curriculum to sequence snowflower (non-photosynthetic parasitic plant) live in the classroom in collaboration with Dr. Dave Steakley (North Tahoe High School) (Figure 5)

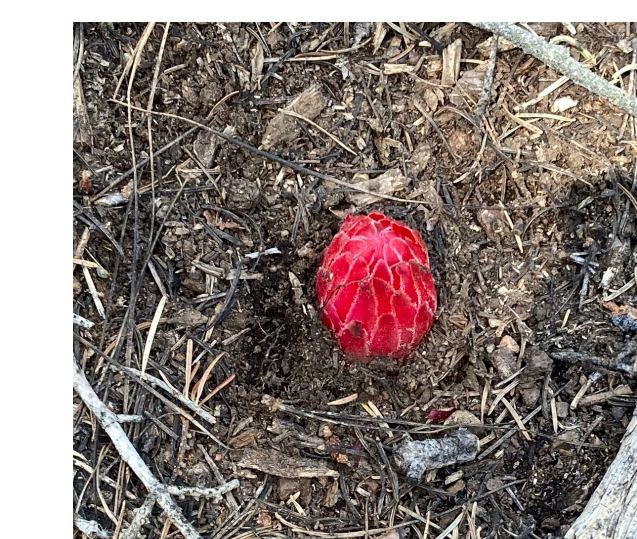


Figure 5. Snowflowers (*Sarcodes sanguinea*) have lost the ability to photosynthesize. High school students at NTHS extracted DNA from snowflowers collected in early June 2023, prepared sequencing libraries, and sequenced them on an Oxford Nanopore MinION in their classroom.

Participant Activities

- Two grant-participant M.S. students defended their theses on cytonuclear interactions in polyploids (R. Castillo, C. Steffensen – Figure 6)

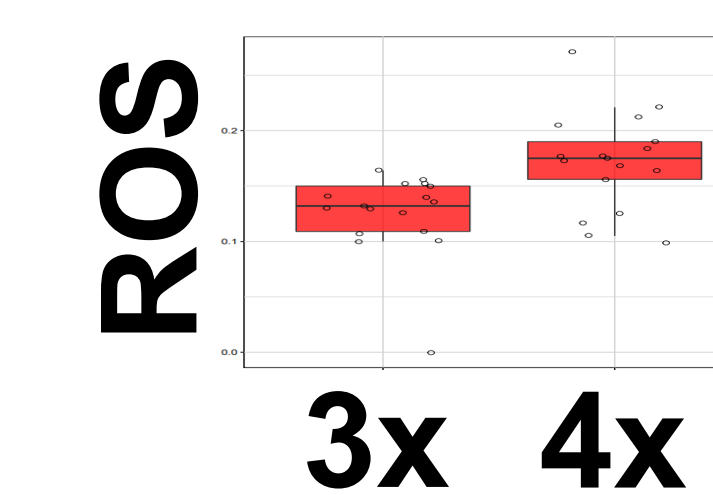


Figure 6. Tetraploids produce more reactive oxygen species (ROS) than diploids. ROS activity was measured after extracting live mitochondria from triploid and tetraploid individuals.

- Five undergraduates and four graduate students are leading their own ongoing projects to investigate the genomic, cellular, and physiological consequences of WGD for cytonuclear stoichiometry (e.g., Figure 7)

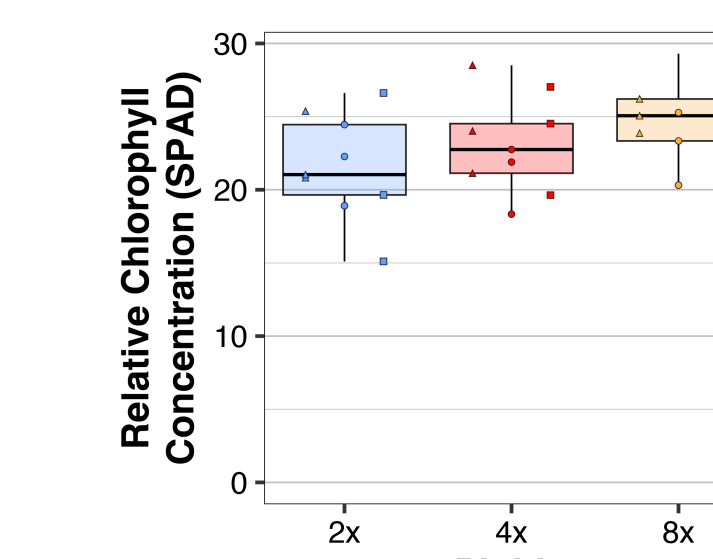


Figure 7. Chlorophyll content appears to scale with ploidy in synthetic *Arabidopsis* polyploids. MultispeQ measurements of chlorophyll content and photosynthetic function are ongoing in common garden experiments.

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