**Materials and Methods**

*Mutation Accumulation Experiment*

The D. magna populations used in this experiment were initially collected from Finland, Germany, and Israel (date?). We used three unique genotypes from each of these three populations (9 genotypes total). The stock cultures for each genotype were maintained in 3L jars containing 2L of the medium AdAM (cite) under a constant 16L:8D photoperiod, and fed the unicellular green alga *Scenedesmus obliquus* ad libitum (2-3 times per week).

We established replicate clonal lineages from each of the 9 genotypes (67 lineages total) between October 2013 and April 2015. The lineages (mutation-accumulation lines [MA lines]) were established and maintained by collecting a single female daphniid from the 3L stock cultures and isolating it in an individual 250mL beaker. Each 250mL beaker contained 100mL of ADaM supplemented with *S. obliquus* at a concentration of 600,000 cells/mL, and was maintained in a Percival environmental chamber under a 16L:8D photoperiod. The media was changed once per week and each line was fed three days after the water replacement to return the algal cell concentration in the beaker back to 600,000 cells/mL.

Each lineage was propagated from generation to generation over the course of the experiment via single offspring descent by taking a single juvenile from the second clutch of the mother. A series of backups were maintained in parallel with the focal lineages in the event that the individual to be used to establish the next generation died prior to reproducing or was male. In the event that the focal lineage and all backups either died before reproduction or were males, the lineage was declared extinct and a new replicate lineage was established from the stock cultures.

*DNA Extraction and Sequencing*

Ten clonal individuals from each MA line and control (1 starting control and 2 existing controls per genotype) were flash frozen for DNA extractions. DNA was extracted (2 extractions per line with 5 daphnia each) using the Zymo Quick-DNA Universal Solid Tissue Prep Kit (No. D4069) following the manufacturer’s protocol (DNA was extracted from a few samples with the Qiagen DNeasy Blood and Tissue Kit, No. 69504). DNA quality was assessed by electrophoresis on 3% agarose gels and DNA concentration was determined by dsDNA HS Qubit Assay (Molecular Probes by Life Technologies, No. Q32851).

The Center for Genome Research and Biocomputing at Oregon State University generated 94 Wafergen DNA 150bp paired-end libraries using the Biosystems Apollo 324 NGS library prep system. Libraries were pooled based on qPCR concentrations across 16 lanes (2 runs) and sequenced on an Illumina Hiseq 3000. The average insert size was approximately 380bp.

*De Novo Assembly*

*Sequence Alignment and Variant Detection*

*Mutation Rate Estimates*

*Variant Validation*