

## **Mitochondrial cluster-specific genome size variability among sexual and asexual lineages of the ostracod *Eucypris virens* species group**

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Genome size varies considerably among species. Since the discovery that much of the variation in genome size is caused by non-coding, repetitive DNA, studies have focused on the genetic mechanisms and evolutionary importance of increases and decreases in nuclear DNA content. Association between genome size and a wide range of morphological, developmental/reproductive and ecological traits suggest that genome size is under selection, where different evolutionary drivers are favouring either larger or smaller genomes.

The introduction of flow cytometry has improved the detection of small variations in DNA content and can advance our understanding of genome size dynamics and heritability in natural populations. We propose that a correlation between genome size difference and phylogenetic distance, at different scales of divergence, could be employed to infer genome size evolution.

The European freshwater ostracod *Eucypris virens* shows geographical parthenogenesis with sexual reproduction constrained to the Mediterranean region. Previous studies have shown high polymorphism in both nuclear ITS1 and mitochondrial COI sequences across Europe, evidence for multiple origins of asexual lineages. Our initial goal was to determine the different ploidy levels of *E. virens*. We confirmed diploidy and triploidy in an earlier analysis, but surprisingly also discovered a substantial variation in genome size within each ploidy group. Herein, to extend the insufficient classification into these two groups, we map the genome size variation within the different reproductive mode and ploidy groups. The focus lays on how this variability can be explained at a lower taxonomic level, i.e., among mitochondrial COI clusters, describing the heritability of genome size. We also discuss potential genome size changes as a consequence of transitions to asexuality.

Relative genome sizes of 21 populations were determined and linked to nuclear genotypes and mitochondrial clades. We found that the identified groups of this species all showed high variability both, between and within groups in relation to a *Daphnia galeata* clone used as reference. The ratios are for diploid sexual males: 2.15–2.66, diploid sexual females: 2.32–3.99, diploid asexual females: 2.47–3.18 and triploid asexual females: 3.67–5.83 (Fig. 1).

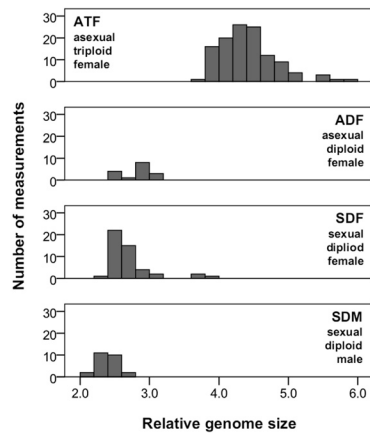


Fig. 1: Histogram of relative genome size in European *E. virens*. Genome size corresponds to DAPI intensity of sample divided by intensity of the control.

These variations were mostly explained on the level of the 8 divergent mitochondrial clades represented in these samples. We also present the first absolute genome sizes in ostracods measured with flow cytometry and the very first at all in *E. virens*.

The genome size of *Eucypris virens* ostracods varies considerably among lineages. In sexual diploid lineages males have a smaller genome than females (Fig. 2), a common pattern in species with male heterogametic sex determination, while genomes of sexual females are typically a bit smaller than asexual diploid females. We further confirmed triploidy in asexual females, and the sizes of triploid genomes were in the range expected based on their least divergent sexual lineage, although size of triploid genomes showed a more irregular deviation pattern than diploid asexuals. Increased deviations from the expected triploid genome size is less surprising if one considers the possibility that these lineages are of independent hybrid origins.

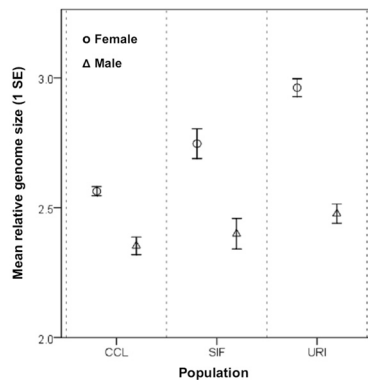


Fig. 2: Separation of mean relative genome sizes ( $\pm 1$  SE) of diploid sexual females and diploid sexual males in 3 sexual population. Genome size corresponds to DAPI intensity of sample divided by intensity of the control.

Our analysis indicates that the clusters defined on the basis of mitochondrial sequence data explain most of the variation in genome size within reproductive mode-ploidy groups. Interestingly, the observed and expected relative genome sizes between reproductive mode and ploidy group, corresponded when phylogenetic distance was short, with a tendency towards an increase in diploid asexual lineages relative to their putative sexual ancestors. This difference in genome size may have been caused by accumulation of e.g., transposons and introns in asexual lineages as they go through bottlenecks during the transition to asexual reproduction. Asexuals also completely lack recombination which reduces the strength of purifying selection.

To summarise, we have shown that the genome size of sexual and asexual *E. virens* ostracods has evolved gradually and that size deviations match the divergence of mitochondrial genes. However, closely related lineages in populations from geographically distinct environments have similar genome sizes suggesting that great decreases or increases, apart from polyploidisations, in genome size due to local selection pressures are unlikely.

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