Genome fractionation and loss of heterozygosity in hybrids and polyploids: mechanisms, consequences for selection and link to gene function

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Abstract:

Hybridization and genome duplication may cause serious damages but may also open unique opportunities to invade new ecological niches or adapt to novel environments better than their parents. Following the initial merging or multiplications, the subgenomes of hybrids and polyploids undergo considerable changes, often eliminating segments of one parental genome, phenomena known as loss of heterozygosity (LOH) and genome fractionation. Mechanisms causing such changes are not well understood, and remain enigmatic particularly when hybridization is linked with asexual (clonal) reproduction that may enforce diverse array of genome evolutionary pathways ranging from long-term stasis to dynamic reformations.

Analysis of genome evolution in diploid and polyploid clonal hybrids between fish *Cobitis elongatoides* and either C. *taenia* or C. *tanaitica* species revealed that clonal genomes remain generally static on chromosome-scale level but undergo small-scale restructurations resulting in genome fractionation and LOH events. These events have complex molecular background in two distinct processes, the hemizygous deletions and conversions between orthologous subgenomes. The impact of both processes on clonal evolution is ploidy-dependent; while deletions frequently accumulated in polyploids, they appeared to be selected against in diploid asexuals where gene conversions prevailed. The incidence of genomic restructuration was not random with respect to individual genes, but it preferentially affected loci with unusually high transcription levels, genes under relatively strong purifying selection and also genes with particular functions, such as those related to endoplasmatic reticulum. Likelihood that given orholog would be retained or lost correlated significantly with its parental origin, GC content (preferential loss of low-GC alleles) and expression (less expressed alleles tended to be replaced by more expressed ones). Contrary to expectations, however, we observed that the preferentially retained subgenome (the one derived from *C. elongatoides*) was not dominant at the transcription level as all hybrids were phenotypically more similar to the other parent whose genes were preferentially lost.

Our data show that the fate of subgenomes in asexual hybrids and polyploids depends on complex interplay of molecular mechanisms and selection that are affected by sequence composition, expression as well as parental ancestry.

Introduction:

The genome of a typical Metazoan possesses two alleles of each gene brought together by merging reduced gametes of two individuals belonging to the same species. Yet, these rules have often been alleviated as traces of whole genome duplications (WGD) and introgressive hybridizations have been documented in many taxa, vertebrates and humans included (Dehal and Boore 2005; Gittelman et al. 2016). Hybridization and polyploidization may cause serious problems, e.g. in transcription regulation leading to deregulation of transposable elements ((Dion-Côté et al. 2014; Lien et al. 2016)), but may also lead to creation of novel traits and acquisition of gene functions via sub-/neofunctionalization of duplicated genes (Yoo et al. 2014; Fridman 2015), potentially facilitating specialization to new niches (Madlung 2013).

Realizing their evolutionary significance and huge practical value to mankind, e.g. (Mason and Batley 2015), the research focus on hybridization and polyploidy intensified and lead to revelation of some prominent patterns. For instance, hybrid's phenotypes may range from intermediate forms to transgressive expression of novel traits (Bell and Travis 2005; Yoo et al. 2014; Bartoš et al. 2019) but often, one parental subgenome is more expressed than the other one (i.e. expression dominance; (Yoo et al. 2014; Alexander-Webber et al. 2016; Cheng et al. 2018). Hybrid's and poyploid's genomes evolve dynamically and often lose orthologous genes from one or the other parental species in processes referred to as loss of heterozygosity (LOH), genome fractionation or rediploidization in polyploids (Yoo et al. 2014; Lien et al. 2016; Du et al. 2020). These are often asymmetrical, e.g. (Alexander-Webber et al. 2016) and it has been proposed that loss of alleles from the less expressed subgenome may cause less severe effects and may therefore be preferred by selection (Yoo et al. 2014). However, the situation is likely more complex as orthologue loss/retention of may also be under the selection for proper dosage of molecular interactors and relative copy number of gene products (i.e. selection for stoichiometry (Birchler and Veitia 2012)) and hybrid populations may selectively filter orthologous genes according to their adaptive value in a given environment, e.g. (Gittelman et al. 2016; Lancaster et al. 2019; Smukowski Heil et al. 2019).

Thus, despite application of modern technologies, the question of why some genes tend to be retained in heterozygous or duplicated states, while others are subject to fractionation, still represents major evolutionary puzzle. It remains particularly unclear whether aforementioned patterns are driven by case-specific mechanisms or whether independent lineages follow similar evolutionary trajectories (Soltis et al. 2010; Deans et al. 2015).

Such a gap in current knowledge partly results from taxonomic bias in knowledge, especially towards plant species, since the incidence of hybridization and polyploidy have traditionally been underrated by zoologists. Moreover, direct tests of adaptive values of genomic rearrangements could be performed only under laboratory conditions in rapidly reproducing organisms (Smukowski Heil et al. 2017; Lancaster et al. 2019) since events like LOH are rather rare (Dukić et al. 2019). For practical reasons, most available data come from natural hybrids and polyploids, making it difficult to discern which patterns are direct consequences of genome merging and which evolved subsequently. In addition, many polyploids are of hybrid origin, which makes it challenging discerning what effects are inherent to polyploidy and which to hybridization itself. Finally, in many investigated hybrid and/or allopolyploid taxa, it is not even clear how they could establish themselves in natural environments since any new form is rare at the time of its emergence and therefore is threatened by frequency-dependent mating disadvantage and backcrossing with dominating ancestral populations (i.e. the ***minority cytotype exclusion principle***; e.g. (Husband 2000)).

As one of the potential explanations for the establishment of such new strains, it has been hypothesized that they may alleviate initial caveats by taking advantage of asexual reproduction via unreduced gametes, which offers immediate reproductive isolation and clonal multiplication of novel genotypes unencumbered by maintaining two sexes (Cunha et al. 2008; Choleva and Janko 2013; Hojsgaard and Hörandl 2015; Dubey et al. 2019). The perception of asexual organisms indeed changed among biologists from being initially considered marginal evolutionary accident (Howell 1933) to current appreciation that they occur in all major Eukaryotic clades (Schön et al. 2009) and form dominant components of some ecosystems (Kearney 2005; Hojsgaard and Hörandl 2015). Emergence of asexual reproduction is tightly linked to hybridization and polyploidy (rev. in (Choleva and Janko 2013)), and may represent an inherent stage of the speciation process, representing a special type of Bateson-Dobzhansky-Muller (Janko et al. 2018). This paradigm shift coincides with increasing interest about the role recombination modification in evolution (Thompson and Jiggins 2014; Ortiz-Barrientos et al. 2016) suggesting that understanding evolutionary processes in asexual organisms may provide important insights into the mechanisms of genome evolution in general.

Unfortunately, there is no consensus about genomic consequences of asexuality. Clonal inheritance has been originally assumed to ensure stasis of genome with gradual accumulation of deleterious mutations (Muller 1964; Keightley and Otto 2006) and heterozygosity levels (Birky 1996; Mark Welch and Meselson 2000; Balloux et al. 2003) or modified dynamics of transposable elements (Hickey 1982). This view is currently challenged by indications of horizontal gene transfers in some asexual lineages (Gladyshev et al. 2008;

Danchin et al. 2010) as well as by accumulating evidence that genomes may extremely quickly acquire aneuploidy or structural changes once the sex is lost, owing to relaxed constraints on the pairing of homologous chromosomes (Triantaphyllou 1981; Sunnucks et al. 1996; Normark 1999; Spence and Blackman 2000; Tucker et al. 2013). Heterozygosity may degrade quickly by hemizygous deletions and specially by gene conversions (Tucker et al. 2013), whose inherent GC bias may lead to increased guanine & cytosine contents in asexual genomes (Bast et al. 2018). The dynamics of asexual's genome may also be determined by its mode of origin since hybrid asexuals generally express high levels of heterozygosity, probably indicating efficient clonal transmission of parental genomes, while nonhybrid asexuals appeared to have lost most of their heterozygosity in consequence of pervasive gene conversions (Jaron et al. 2018).

With such a variety of patterns it is difficult to discern which mechanisms are taxon-specific and which relate to asexual reproduction *per se*. A major complication is that the so-called asexual organisms form very heterogeneous group employing a wide specter of gametogenetic mechanisms, ranging from ameiotic processes (apomixis) to those involving more or less distorted meiotic divisions (automixis) (Stenberg and Saura 2009; Stenberg and Saura 2013). Some types of automixis involve intragenomic recombinations or exclusions of large genomic parts, thereby decreasing heterozygosity among progeny (Bi and Bogart 2006) while other types are theoretically equivalent to apomixis. For example, many hybrid asexuals employ premeiotic endoduplication, which is a mechanism when normal meiosis is preceded by WGD in oogonial cells. In consequence, segregation and recombination presumably takes place on bivalents between sister copies of chromosomes rather than between orthologs, resulting in clonal progeny (Lutes et al. 2010; Arai and Fujimoto 2013) Fig. 1a.

In this study we analyze causes and consequences of allelic recombination, conversion and loss of heterozygosity in a clonally reproducing vertebrate of hybrid origin, *Cobitis.* Actinopterygii. We focus on the so-called *Cobitis taenia* hybrid complex, which arose by hybridization of the species C. *elongatoides* (we denote its haploid genome 'E') with either of its two distant relatives, C. *taenia* ('T') or C. *tanaitica* ('N') (Choleva et al. 2012). Phylogenomic analysis (Janko et al. 2018) revealed that initial E-(TN) divergence occurred ca 9 Mya and was initially followed by intensive gene exchange. However, with ongoing divergence these species lost the capacity to produce sexual hybrids since crossings of current species lead to sterile hybrid males and fertile, but clonally reproducing, hybrid females. Hybrid females form unreduced gametes by premeiotic endoduplication but are gynogenetic, i.e. they require a sperm from sexual species to trigger development of their gametes (Janko et al. 2007; Choleva et al. 2012; Dedukh et al. 2020). Usually, sperm's genome is degraded after fertilization but certain proportion of oocytes fuses with the sperm

cells and consequently, diploid ET or EN females produce certain portion of triploid progeny that may have EET, ETT, EEN or ENN genomic constitution, depending on the sperm donor. Triploids also reproduce clonally. The hybridization between parental species is reciprocal but C. *taenia* is maternal ancestor of most *elongatoides-taenia* hybrids, while C. *elongatoides* is maternal ancestor of *elongatoides-tanaitica* hybrids (Janko et al. 2003). Because parental species lack obvious prezygotic reproductive barriers and glacial-interglacial cycles repeatedly brought their ranges into contact (Fig. 1b), new clones have been dynamically arising throughout much of Pleistocene and subsequently colonized Europe (Janko et al. 2005; Janko et al. 2012). Current asexual populations consist of recent clones with postglacial origin in Central European hybrid zone between C. *elongatoides* and C. *taenia* (these involve diploid ET and triploid EET and ETT forms) but also of ancient *elongatoides-tanaitica* hybrids (the so-called hybrid clade I, consisting of EN and EEN biotypes) which originated in Balkan hybrid zone ca 300 ky ago (Janko et al. 2005).

Cobitis hybrids thus offer excellent opportunity to investigate how genomes of natural asexual organisms evolve through time and across different ploidy levels. (Majtánová et al. 2016) demonstrated by karyotypic analysis that clonal hybrids maintain remarkable integrity of parental chromosomes without traces of large-scale recombinations and restructurations despite more than 300 ky of evolution since the initial hybridization event. This study investigates the dynamics of diploid and polyploid clonal genomes on the fine scale. To achieve this aim, we performed exome sequencing of sexual parental species and their clonal hybrids, and we subsequently compared such data to recently published gene expression profiles (Bartoš et al. 2019). This allowed us to identify mechanisms underlying the fractionation and heterozygosity loss in clonal genomes and to test how they relate to expression and function of affected genes.

3. Results

3.1. SNP detection and identification of species-specific variants

Exome-capture data were acquired from 46 specimens, including three parental species and their asexual hybrids, which were selected to cover all major phylogroups, ploidy types and hybrid genome compositions (see Fig. 1b and Table S1 for details) and we also included whole genome sequencing of one ET hybrid for control. Reads were mapped against published *C. taenia* reference transcriptome containing 20,600 contigs (Janko et al. 2018) and for simplicity, we restricted our analysis to 189,927 quality filtered bi-allelic SNPs (i.e. occurring in no more than two states across the entire dataset). The greatest genetic divergence was between *C. elongatoides* and the remaining two sister species, *C. taenia* and *C. tanaitica*, while hybrids appeared intermediate (multidimensional scaling (MDS), Fig. 2a). Examination of pairwise genetic distances between hybrid individuals revealed 11 clusters of individuals that were nonrandomly similar to each other, therefore likely representing clonal lineages. We thus refer to them as independent Multilocus Lineages (MLL) *sensu* (Arnaud-Haond et al. 2007), see Fig. 2a and Table S2.

To characterize hybrid's SNP variation relative to their parental species, we followed (Ament-Velásquez et al. 2016) and divided all hybrids' SNPs into 10 categories (Table S2), of which five were particularly important for this paper. First, we detected 16,372 unique positions with variants occurring only among asexuals but not among sexuals (SNP categories "***private-asexual*** 1a and 1b"). These SNPs presumably represent mutations acquired after clonal origin (Ament-Velásquez et al. 2016; Kočí et al. 2020). Notably, the proportions of such SNPs in genome of each hybrid were tightly correlated with its mtDNA distance from the nearest sexual counterpart (Pearson's r=0.971, df=23, p-value=8.66e-16), so that the ancient *elongatoides-tanaitica* hybrids had the highest amount of private asexual SNPs, while experimental F1 hybrids had the least amount of such SNPs, probably representing only rare sequencing errors. Second, we focused on SNP variants that were diagnostic between pairs of parental species, so that their hybrids should possess one or both parental variants, thereby allowing detection of LOH events. Throughout the entire dataset we therefore identified sites diagnosing *C. elongatoides* from *C. taenia* (referred to as E-T diagnostic sites; total of 37,988), *C. elongatoides* from *C. tanaitica* (E-N diagnostic sites; 30,281) and we also found SNPs differentiating *C. elongatoides* from the joint dataset of *C. taenia* and *C. tanaitica* (E-TN diagnostic sites; 27,311). According to the way how hybrids' SNPs were shared with these parental variants, we categorized them as "shared SNPs" of type sh3a (heterozygous for both parental variants), sh3b11 (homozygous for one parent's allele) and sh3b12 (homozygous for the other parent's allele) in Table S2.

3.2. Clonal lineages accumulate Loss of heterozygosity events in their evolution.

Hybrids were considerably more heterozygous than parental species (Fig. 2b; Wilcoxon rank sum test: W = 520, p-value < 1e-9) with no less than 98.5% of private asexual SNPs and the vast majority of diagnostic sites occurring in heterozygous states. Nevertheless, LOH was observed in some portion of diagnostic SNPs of every hybrid (categories sh3b11 and sh3b12 in Table S2). We verified the quality of base-calling and LOH detection by two approaches. We first compared SNP calling from exome-capture technology and whole-genome sequencing of the same ET hybrid (csc067) and found differences in only ~0.17% E-T diagnostic positions. We also compared two F1 hybrids against their parents and found homozygote states only in ~0,16% positions, where both parental individuals differed from each other. This indicates ***high reliability of LOH detection based on exome capture.***

Two patterns were noted in the distribution of LOH SNPs. First, individual LOH sites were significantly more likely to be shared by individuals belonging to the same clonal lineage (MLL) than by individuals from different clones (Wilcoxon rank sum test, W = 4540, p-value < 1e-9; Fig. 2c). Second, the proportion of LOH sites in each individual significantly correlated with its proportion of private asexual mutations (Pearson's r = 0.955, 95% c.i. = 0.902-0.980, p-value = 3.286e-14; Fig. 3a, Table S2). Hence, although we may not rule out existence of somatic mutations (López and Palumbi 2019), our data indicate that ***erosion of heterozygosity is heritable within clonal lineages, accumulates over clone's evolutionary history, and therefore affects the germline.***

We also performed the same analysis on E-TN diagnostic sites, in order to minimize potential effect of ancestral polymorphism when same allele might have been inherited from both parents at time of clonal origin but was subsequently lost in one parental species. Since *C. taenia – C. tanaitica* divergence predates origin of oldest *Cobitis* clones by hundreds of thousands of years (Janko et al. 2018), it may be posited that most such E-TN sites became diagnostic long before the origin of studied clones. Yet, the proportions of LOH at E-TN diagnostic sites also correlated significantly with private asexual SNPs (r = 0.948, 95% c.i. = 0.885-0.977, p-value = 2.145e-13) albeit with slightly less steep slope than in ET- and E-N sites, respectively (Fig. 3a). This suggests that some false-positives might have affected our dataset, but altogether the ***retention of ancestral polymorphism is an unlikely explanation of most observed LOH events***.

3.3. Heterozygosity deteriorates by gene conversions and hemizygous deletions in asexuals.

We next investigated topological context of LOH sites to test if they might have been generated by point mutations. Since we used cDNA reference that excludes introns, we could not simply analyze the physical distance between SNPs in studies loci. Instead, we

tested if LOH sites within individual genes tend to occur in contiguous stretches. We thus compared observed distribution of LOH sites to permuted datasets with LOH sites randomly distributed across all genes (see the Methods for details). We observed that numbers of LOH occurring in contiguous stretches significantly exceeded simulated values, suggesting that LOH sites tend to occur in clusters. This is inconsistent with point mutations being the primary mechanism generating LOH events but instead suggests that most ***LOH events are created by processes affecting contiguous stretches of DNA, like gene conversions and gene deletions***.

To distinguish between both candidate processes, we analyzed the sequencing coverage following (Tucker et al. 2013), who showed that conversions conserve the amount of allelic copies, while allelic deletions would result in coverage drop. To do so, we first ***confirmed that exome capture data are suitable for coverage comparisons of sequencing*** by analyzing among-individual variance in sequencing coverage across loci (Bragg et al. 2016). We found that capture efficiency considerably varied among loci but was highly correlated among individuals, thereby allowing inter-individual coverage comparisons to be made (see Appendix S1).

We than used normalized per-SNP coverages to calculate relative values of coverage for each hybrids' LOH by comparing it to the coverages of the same site in parental species (see Methods). Following (Tucker et al. 2013) we predicted that conversions result in relative coverage ~1, while allelic deletions would result in coverage drop to values ~0.5 in diploids, or ~0.66 (single deletion) and ~0.33 (double deletion) in triploids. To investigate roles of both processes in LOH creation, we constructed for each hybrid biotype the histograms of relative coverages and tested their modality at aforementioned biologically relevant values. Ancient clones (EN and EEN) possessed relatively smooth distributions of relative normalized coverages with peaks close to 1, suggesting gene conversions as the main mechanism causing their LOH events. In contrast, recent clones (ET, EET and ETT) had additional peaks located at lower values (Fig. 4a-e), indicating simultaneous operation of both processes (Tucker et al. 2013).

To formally test whether observed data may be explained by single process or several simultaneously operating processes, we compared the fits of each histogram by single gamma distribution as a proxy for operation of only one process, or mixtures of more distributions with means fixed at aforementioned biologically relevant values. Nonlinear least square method was used to estimate the parameters controlling relative proportions of distributions in combined models (A and B parameters control the rate of conversions / hemizygous deletions in two-gamma distribution model while A, B & C control the rates of conversions & hemizygous & double deletions in three-distribution model). The most complex model assuming the occurrence of conversions and both single and double

deletions (three gamma distributions) did not significantly improve the fit to triploids' data. However, mix of two gamma distributions assuming gene conversions and hemizygous deletions significantly outperformed any single-distribution in all datasets (F test in diploids: EN: df = {40,38}, F=2.583; ET: df = {41,39}, F=7.87; triploids: EEN: df = {44,42}, F=11.01; EET: df = {44,42}, F=112.6; ETT: df = {31,29}, F=57.49), suggesting that joint operation of both processes.

To further validate if deletions indeed occur in hybrids, we used Kolmogorov-Smirnov test to compare distributions of relative coverages at hybrids' LOH sites with the distribution of coverages at the same sites in parental species, where no deletions are expected. This comparison indicated all hybrid biotypes have significant excess of low-coverage LOH sites, again suggesting that deletions cause coverage decrease in hybrids (see Fig. 4e and Fig. S1 for details).

Both tests thus documented the existence of LOH sites with decreased DNA content suggesting that ***LOH events are caused by simultaneous operation of conversions and hemizygous deletions in all biotypes. However, double deletions in triploids are very rare or absent***.

3.4. Accumulation of LOH is biased with respect to parental subgenome, ploidy and hybrid type.

We noted that LOHs were non-randomly distributed among hybrids, and there were several trends behind such unevenness. First, there was a clear bias in retention of parental subgenomes (Fig. 3b). Virtually all LOH sites detected in triploids possessed allele of that parent which contributed two chromosomal sets. This type of bias in triploids is likely methodological, since sites where one allele of the diploid subgenome is lost would still appear heterozygous and hence escape our attention. However, significant bias was observed in diploid hybrids with ***preferential retention of C. elongatoides allele*** at ~80% LOH sites in ET and ~87% in EN hybrids.

Second, the ***hemizygous deletions were significantly more common in triploid hybrids than in their diploid counterparts***. Specifically, the ratio of A/B parameters of combined Gama distributions suggest that deletions accounted for only ~21% LOH events in diploid hybrids between *C. elongatoides and C. taenia*, while their contribution rose to ~ 50% in triploid hybrid forms (EET and ETT); Fig. 4a-e. Similarly, in *elongatoides-tanaitica* hybrids, deletions explained accounted for less than 0.1% LOH events in diploid EN hybrids, while triploid EEN possessed ~18% deletions at LOH sites. These differences appeared highly significant after comparing data fitting by 'free' mixed gamma model using optimized A / B ratios and by 'forced' model with A / B ratio fixed to values estimated from triploids (EN with

A / B estimated from EEN data: df = {40,38}, F=2.33; ET with A / B estimated from EET data: df = {41,39}, F=14.14).

Finally, ***hemizygous deletions were more common among recent asexuals than in ancient clones***, as evident from comparisons of A / B ratios between recent (ET) and ancient (EN) diploid clones (~21% vs. ~0.1%) as well as of recent (EET or ETT) and ancient (EEN) triploid clones (~50% vs ~18%); Fig. 4a-e.

3.5. Occurrence of LOH is related to sequence composition, allelic expression and gene function.

3.5.1. LOH depends on GC content but patterns are complex:

To investigate potential GC bias, we first inspected transcriptome-wide GC contents of sexual and asexual forms, measured either across all positions or only at the relatively neutral third codon positions, but found no significant differences between any biotypes. Next, we performed a more fine-scale analysis on E-TN diagnostic positions and separated all detected LOH sites into E-like or TN-like groups, depending on parental allele retained. Comparing parental sequences with each hybrid we inferred how many LOH sites underwent A/T -> G/C substitutions, G/C -> A/T substitutions or no change in GC content (i.e. A <-> T or G <-> C substitutions) and used contingency tables compared these counts with overall A/T - G/C differences between respective parental species across all E-TN diagnostic positions.

We found that E-like LOH events were significantly biased in favor of A/T -> G/C substitution in triploid (EET, EEN) and diploid (ET, EN) biotypes. This bias was ~25% on average and proved significant in every individual after FDR correction. However, we observed no such GC bias in TN-like LOH sites of any biotype (Fig. 5a). Our data thus indicate that ***GC-dependence has complex background and occurs only during loss of taenia/tanaitica allele, but not in the opposite direction***.

3.5.2. LOH is affected by gene's transcription:

We evaluated the effects of allele expression on LOH occurrence using recently published transcription profiles of livers and oocytes in sexual (C. *elongatoides*, C. *taenia*) and asexual (ET, EET, and ETT) females (Bartoš et al. 2019). While Bartoš et al. analyzed different individuals, they used the same reference transcriptome and thus we could investigate the transcription profiles of those genes which carried E-T diagnostic SNPs and were LOH-positive (either E-like or T-like LOH bearing), or LOH-negative, according to present exome-capture results. We applied two approaches to test for differences in gene and allele expression between these categories.

First, we found that ***LOH events generally tend to occur in genes with abnormally high expression levels.*** Specifically, we compared the TPM-normalized expression levels of LOH-positive and LOH-negative genes and found that the genes where LOH occurred had

significantly higher expression levels in liver tissue of all biotypes (FDR corrected WMW test p values < 0.02 for all biotypes). Same trends, albeit insignificant, were observed in oocytes. Second, published DeSeq2-normalized expression levels of both parental species were compared to test if less expressed alleles tend to be preferentially lost during LOH events. We adopted such an indirect approach of comparing both parental species instead of directly inspecting allelic expressions in hybrids because (Bartoš et al. 2019)'s data in hybrids might have been affected by undetected allelic deletions. Moreover, the interparental expression divergence is a good predictor of relative allelic expression given pervasive cis-regulation between subgenomes (Bartoš et al. 2019). We found that distributions of C. *elongatoides* / C. *taenia* log2 fold change (log2FC) were very similar for LOH-negative genes and for genes bearing E-like LOH (oocytes: mean log2FC -0.22 vs -0.21; livers: mean log2FC -0.14 vs -0.16; note that prevalence of negative values is in agreement with slight but pervasive under expression of C. *elongatoides* documented by (Bartoš et al. 2019). In contrast, log2FC values were more negative (i.e. deviated towards *C. taenia*) in genes bearing T-like LOH than in LOH-negative genes (oocytes: mean log2FC -0.34 vs -0.22; livers: mean log2FC -0.22 vs -0.14) and Kolmogorov-Smirnov test proved that such a difference was significant in oocytes (p value = 0.022). This suggests that the ***fate of hybrid's alleles is affected by expression levels*** inherited from parental species. However, ***the effect is again not symmetrical with respect to subgenome ancestry,*** since less expressed alleles are preferentially removed during E-like LOH events while no bias was observed in the reciprocal direction.

3.5.3. LOH accumulate in genes with specific functions

Finally, we investigated whether LOH events accumulate in genes with specific functions. For this purpose, we selected only loci with diagnostic SNPs that were successfully annotated and performed two tests. First, as a proxy for selection regime of particular genes, we investigated d_N/d_S values among orthologous sequences of C. *elongatoides*, C. *taenia* and C. *tanaitica* (note that d_N/d_S values for all genes were published by (Kočí et al. 2020)). We found in both *elongatoides-taenia* and *elongatoides-tanaitica* hybrid types that ***LOH-positive genes were characterized by significantly lower dN/dS values than LOH-negative genes*** (Linear mixed effect model with pairs of individuals as random factor; LRT p value for *elongatoides-taenia* = 1.604095e-135; p value for *elongatoides-tanaitica* = 1.518951e-05).

Next, we searched if LOH positive genes are associated with particular Gene Ontology terms using the GO::TermFinder (Boyle et al. 2004). Results are shown in Table 1, which lists top 20 enriched GO terms of each category in each biotype. It shows that GO terms associated with membrane coats and endoplasmatic reticulum were enriched among LOH-positive genes in EET, EN and EEN biotypes with p-values corrected for multiple tests

below alpha level 0.1. It also shows that some GO terms, whose corrected p value exceeded the threshold level, were repeatedly encountered among top enriched GO terms in several hybrid biotypes including independent *elongatoides-taenia* and *elongatoides-tanaitica* hybrid types. These namely contained Cellular compartment type GO terms associated with cell-cell junction and cell-substrate adherence junction and Biologic processes type GO terms of cellular biogenic amine metabolic process and Protein glycosylation.

4. Discussion

Genomes of asexual organisms may evolve in various ways ranging from fast restructuration to long-term conservation of heterozygosity. Such a diversity of patterns may reflect the variety of gametogenetic pathways used by such organisms so that hybrids employing premeiotic endoreplication, like *Cobitis*, can maintain integrity of parental subgenomes without any chromosomal-scale restructurations (Majtánová et al. 2016). However, present study showed that despite apparent stasis on large scale, the heterozygosity gained during original hybridization may gradually deteriorate by small-scale restructurations that affects genomic regions in relation to allelic origin, sequence composition and gene expression.

4.1. Large-scale stasis vs small-scale dynamics of asexual genomes.

After initial merging and duplication, allopolyploid organisms appear to deduplicate their genomes prominently via fractionation and deletions of orthologues (Yoo et al. 2014; Cheng et al. 2018; Du et al. 2020). Yet, we found that in polyploid loaches, or hybrids in general, deletions accounted for rather minor fraction of restructuration events. The majority of LOH sites had relative coverage ~1, thereby indicating higher incidences of recombinations between orthologues. Recombination may be followed by crossover (CO), which is expected to cause long stretches of LOH spanning till another recombination site or until the telomeric ends of paired chromosomes (Fig. 1c). But as (Majtánová et al. 2016)'s cytogenetic study ruled out any large-scale exchanges of chromosomal arms between subgenomes, it appears that most LOH events detected in this study were caused by gene conversions without crossovers.

Interestingly, organisms employing premeiotic endoreplication should form bivalents between sister copies of duplicated homologs, rather than between orthologous chromosomes (Lutes et al. 2010; Arai and Fujimoto 2013; Dedukh et al. 2020) and it is therefore unclear how such conversions may arise (Fig. 1c). Theoretically, they may result from errors in homology search during early meiosis if ExT bivalents form, but this explanation is unlikely for two reasons. First, C. *elongatoides* and C. *taenia* karyotypes are so divergent that most orthologous chromosomes may not form proper bivalents leading to sterility of those hybrid forms, which lack endoreplication, typically males (Dedukh et al. 2020). Hence, even if ectopic ExT pairings occur in asexual females, it would be unlikely to form proper bivalent. Second, (Dedukh et al. 2020) documented the occurrence of true crossovers in ExE and TxT bivalents in female hybrids (Fig. 1c), and hence hypothetical ExT bivalents would result in the exchange of large pieces of chromosomal arms, which have not been observed (Majtánová et al. 2016). An alternative explanation would therefore assume the role of mitotic conversions, which are important in DNA damage repair (Helleday 2003).

Indeed, mitotic conversions have been hypothesized to impact the evolution of asexuals (Omilian et al. 2006; Mandegar and Otto 2007) although, to our knowledge, they haven't been directly observed in any multicellular asexual organism.

Whatever the underlying mechanism, the fact that LOH sites are heritable and shared among clone-mates suggests that LOH events occur in the germline. The genes affected by LOH events also do possess some characteristics typical of loci undergoing conversions. Namely, LOH positive genes have above-average expression levels, which is consistent with the hypothesis that DNA of transcriptionally active loci is more relaxed and hence prone to double strand breaks (DSB) followed by repair cascade including the recombination machinery (González-Barrera et al. 2002; Cummings et al. 2007). *Cobitis* hybrids also tend to replace the less expressed parental allele by the more expressed one, which is in line with growing evidence that more transcribed homoeologs are preferably utilized as templates during double strand break (DSB)-induced gene conversion (Schildkraut et al. 2006). Finally, the prevalence of AT->GC substitutions on some LOH sites conforms to expected GC bias in template preference (Duret and Galtier 2009; Williams et al. 2015).

Interestingly, however, our study revealed that ***processes affecting asexual genomes strongly depend on ancestry of allele acting as a template.*** Namely, the preferential retention of more transcribed allele was apparent only during *elongatoides* -> *taenia* allele replacement (T-like LOHs), while the GC bias was detected only in the opposite direction (E-like LOHs). On the other hand, overall GC contents were not notably affected by these processes as we found no differences between sexual and asexual forms at transcriptome-wide scale. This suggests that predicted increase in GC content (Bast et al. 2018) cannot be generally applied to all types of asexuals and that the ancestry of subgenomes should be taken into account.

4.2. Impact of LOH on evolution of hybrids and polyploids.

Genome rearrangements may bring both, the benefits and their accumulation may be facilitated by the lack of requirement of proper homology for chromosomal pairing in asexuals (Sunnucks et al. 1996). Consequently, conversions & deletions of genes or even chromosomal arms may potentially proceed at faster rates than mutation accumulation in some asexuals (Triantaphyllou 1981; Sunnucks et al. 1996; Normark 1999; Spence and Blackman 2000; Tucker et al. 2013), and hence slow the mutational deterioration (e.g. Muller's ratchet process) by erasing deleterious mutations or increasing the fixation rate of beneficial mutations (Khakhlova and Bock 2006; Mandegar and Otto 2007). On the other hand, recombination may have mutagenic effects on its own (Arbeithuber et al. 2015).

In any case, recent analysis of mutation accumulation and fitness deterioration proposed several reasons why LOH events do not play important role in slowing the Muller's

ratchet in asexual loaches (Kočí et al. 2020). In brief, only less than ~1.5% of private asexual SNPs occurred in homozygous states indicating quite efficient mechanism of clonal reproduction when the majority of newly acquired mutations occur at heterozygous state on one chromosome with little possibility of recombination or conversion. Consequently, observed rate of LOH accumulation was low occurring in only ~10% genes after ~300kya of evolution in the oldest clone. This is orders of magnitude less than in aforementioned asexuals, where such processes have been hypothesized to interfere with the accumulation of deleterious mutations. Finally, efficiency of LOH in mutations erasing should increase with clonal age since in recent clones, the rare LOH events would likely happen on genes, where mutations haven't yet accumulated, while in older clones the emerging LOH event have higher chance to 'heal' previously mutated genes. However, such a process should lead to exponential correlation between the proportions of LOH and the private asexual SNPs, which was not found by (Kočí et al. 2020). This is not to say that LOH events may not counteract the ratchet in some asexuals, however, their role in removal of deleterious mutation is supposedly smaller in organisms with relatively efficient clonal reproduction, like *Cobitis*.

Nevertheless, we found evidence that LOH events considerably impact the evolution of studied organisms by other mechanisms. For example, we noticed that proportions of deletions were higher among young clones and polyploid hybrids than in ancient or diploid clones.

4.2.1. The effects of deletions are less severe in polyploids.

Hemizygous deletions cause aneuploidies on sub-chromosomal levels, and may therefore modify stoichiometry between interacting components of molecular complexes (Birchler and Veitia 2012) or between transcription factors and their binding sites, thereby affecting the gene regulation (Veitia et al. 2013). The magnitude of their effect probably scales with the length of deleted genomic region; long deletions afecting many genes have stronger effect than short-range deletions (Veitia et al. 2013). This may explain why *Cobitis* hybrids retained stable karyotypes with no chromosomal-scale deletions (Majtánová et al. 2016), but small-scale deletions of individual genes do occur and haven't been removed by selection. (Veitia et al. 2013) further postulated that the impact of aneuploidy should depend on allelic dosage. Consequently, hemizygous deletions would have weaker effects in triploids (changing allelic copy numbers from 1 to 2/3 relative to the rest of genome) than in diploids (change from 1 to 1/2) and double deletions in triploids (change from 1 to 1/3) would have the most severe effects. This may explain why triploids possessed higher proportion of hemizygous deletions than their diploid counterparts, but in the same time they had almost no double deletions.

The hypothesis that allelic deletions have mostly negative impact may also explain why young clones possess relatively higher proportion of deletions than old ones. Indeed,

selection-based removal of deleterious mutations requires some time proportional to the selection coefficient, population size and genetic background, and hence, although recent clones acquired lower absolute numbers of LOH events, they would have higher fraction of deletions due to a time-lag necessary to remove these deleterious mutations (Johnson and Howard 2007). In fact, we observed similar differences between young and old clones in loads of nonsynonymous mutations (Kočí et al. 2020).

4.2.2. Biased genome fractionation and template-preference.

Another prominent pattern was the strong preference of *elongatoides* subgenome retention at LOH sites (Fig. 3b). Biased genome fractionation is commonly observed among hybrids and allopolyploids and may have various explanations, ranging from mechanistic reasons when one ortholog induces the other's loss, to natural selection preferring the fixation of one allelic type in hybrid populations. For instance, it has been put in context to expression dominance of one subgenome in hybrids, e.g. (Yoo et al. 2014; Alexander-Webber et al. 2016; Cheng et al. 2018). Mechanisms causing such expression dominance are unclear and may relate to various processes like cis-/trans divergence, unequal content of transposable elements or levels of heterochromatinisation among parental species (Woodhouse et al. 2014; Bottani et al. 2018). In any case, it has been proposed that once expression dominance occurs, the loss of homoeologs from lower-expressed subgenome would be preferred by selection due to less severe consequences (Yoo et al. 2014).

Interestingly, our data contrast this prediction, as the preferentially retained subgenome – *elongatoides* – is clearly not dominant in hybrids. By contrast, recent study of (Bartoš et al. 2019) documented significant bias towards *taenia*-like expression in ecologic and phenotypic traits and an overall expression level dominance of the *taenia* subgenome in hybrids' transcriptomes. In fact, Bartoš et al.' data indicates slight prevalence of *taenia* subgenome transcripts in somatic tissue (~1,5%) and in the germline (~4%) of diploid hybrids, suggesting that the ***expression dominance is not the causal explanation for biased genome fractionation*** in *Cobitis* hybrids.

Selective elimination of one parental subgenome may be particularly adaptive in gynogens by increasing their similarity to that parental species which provides them with sperm, thereby increasing the chance to be fertilized (Beukeboom and Vrijenhoek 1998). Interestingly, though, all investigated ET hybrids coexist with *C. taenia*, ***making it unlikely that preferential loss of C. taenia alleles provides such type of sex-mimicry.***

Our data thus suggest that ***causal link between transcriptome-wide expression dominance and biased genome fractionation is more complex*** than predicted by aforementioned hypotheses. For instance (Bartoš et al. 2019) documented that magnitude of *C. taenia* expression dominance differs between somatic traits and the germline, suggesting

that ***biased genome fractionation may also reflect tissue specific expression characteristics*** and other traits which often escape researcher's attention.

We may also speculate that biased retention of C. *elongatoides* subgenome may reflect its special 'mechanistic' properties. Several mechanisms for biased template preference have been proposed, including different expression levels of orthologous alleles (Schildkraut et al. 2006), different GC contents (Duret and Galtier 2009; Williams et al. 2015) or the effect of maternal ancestry when maternal endonuclease systems may preferentially induce double strand breaks (DSB) on paternal chromosomes thereby causing biased DSB repair and unequal gene conversion (Wang et al. 2010). However, these hypotheses are unlikely to explain the prevalence of E-like LOH as *C. elongatoides* subgenome possesses neither higher expression levels nor different GC content and the maternal ancestor of all studied *elongatoides-taenia* hybrids was *C. taenia*, ruling out major role of maternal bias. But, it is possible that observed bias may reflect specific distribution of epigenetic markings, like methylation, which are known to affect recombination landscape (Mirouze et al. 2012) and may take unexpected and non-additive patterns in hybrids as compared to their parents (Hegarty et al. 2011).

Although we did not identify proximate reason for biased subgenome retention, our data do indicate that both subgenomes differ in their ability to induce LOH events in hybrids. For instance, we observed higher proportion of LOH events in EET triploids than in ETT triploids (Fig. 3). Given that detection of LOH events is limited in triploids by the presence of two conspecific allelic copies, higher fraction of allelic loss/replacements would escape our attention in ETT than in EET triploids if E-like LOH occur more frequently than T-like LOH. Moreover, we already mentioned that E-like and T(N)-like LOH events differ with respect to allelic expression or GC bias, altogether ***suggesting some fundamental differences between hybrid's subgenomes in the ability to induce allelic loss or replacement***.

4.3. Loss of heterozygosity preferentially accumulates in particular gene pathways.

Benefits of heterozygosity loss in hybrids has been directly tested in only a few studies (Smukowski Heil et al. 2017; Smukowski Heil et al. 2019). Even in these cases, the benefits of LOH appeared quite complex and specific for given allele, subgenome, and also for specific environmental conditions (Lancaster et al. 2019). Although we could not directly examine the effects of LOH, our study ***revealed some parallel trends in heterozygosity loss among independent hybrid strains.*** This suggests that LOH tend to accumulate in genes characterized by some common expression and functional properties, such as higher-than-average expression levels and lower-than-average d_N/d_S ratios indicating strong purifying selection maintaining their functionality.

We also found that some gene pathways appear more affected by accumulation of LOH events than others, as apparent from significant enrichment of GO terms associated with endomembrane systems, endoplasmic reticulum and coated vesicles in some biotypes (Table 1). We have no explanation for such observation, but it is worth mentioning that genes with these GO terms often participate in multimeric protein complexes ensuring vesicle tethering, coating and transport to membranes. Since subunits of such complexes are coevolving to maintain proper functionality, it is tempting to speculate that hybrids would profit from removal of heterozygosity because mix of protein interactors from diverged orthologs may negatively impact composition of the entire complex.

Unfortunately, the power of GO analysis was weakened by relatively low number of annotated genes with diagnostic SNPs, so that some GOs appeared insignificant after p-value correction albeit all their genes carried LOH in some biotypes (Table 1). Interestingly, though, same or nested GO terms were sometimes recorded among top-enriched GO terms of different hybrid biotypes, thereby hypothetically indicating LOH accumulation also in other gene pathways which would therefore be worth of further study. For example, this concerned GO terms associated with cell-cell junction and cell-substrate adherence junction, containing genes expressed since the early zygotic embryogenesis that take part in cell migration, and cell-cell communication (Siddiqui et al. 2010; Goonesinghe et al. 2012; Matsui et al. 2015).

Conclusions

There is an increasing evidence that genomes of asexual, hybrid and polyploid taxa evolve dynamically with selective filtering of parts of parental subgenomes. Some common trends have been identified in genome evolution of unrelated hybrid/polyploid taxa, for example, in relation to gene/allelic expression. Although patterns revealed in the present study were generally consistent with several previously proposed mechanisms, some widely cited processes, such as preferential retention of transcriptionally dominant subgenome, were not supported. The lesson from asexual hybrid loaches thus shows that genome fractionation is a very complex process involving simultaneously operating mechanisms that range from a priori bias in template selection to selective fixation of adaptive LOH variants. Relative impact of involved mechanisms thus likely depends on reproductive mode, origin of particular subgenome, allelic sequence composition and transcription activity, properties of involved genes and environmental conditions. In combination with recent advances in understanding the effect of aneuploidies (Birchler and Veitia 2012; Veitia et al. 2013), the data acquired on taxa like asexual hybrid loaches can provide invaluable insight into the role of gene dosage in genome evolution in hybrids and neopolyploids. Investigation of genome

evolution in hybrid and polyploid taxa may also provide important information about basic biological processes related to interaction among genes, meiosis and mitosis.

2. Material & Methods

2.1. Studied specimens

The study is based on exome-capture data from 46 specimens including *C. paludica* as outgroup, thee parental species *Cobitis elongatoides*, *C. tanaitica, C. taenia* and their asexual hybrids of various genomic compositions (see Fig. 1 and Table S1 for details). The specimens were a priori categorized into taxonomical units using flow cytometry and published PCR-RFLP markers (Janko et al. 2007). As in (Janko et al. 2018), we also included two laboratory *elongatoides-taenia* F1 hybrids with their parental individuals as a control of quality of base calling and LOH site detection.

2.2. DNA sequencing, SNP calling and identification of clonal lineages

Isolated gDNA was sheared with Bioruptor™ to proper fragment distribution, tagged by indices, pooled, hybridized to custom-designed exome-capture probes (Janko et al. 2018) and captured fragments were sequenced on Illumina NextSeq in 75 bp paired-end (PE) mode. To verify the robustness of exome-capture data and subsequent interpretation, we also performed whole-genome shotgun sequencing of single ET hybrid using the HiSeq X Ten sequencing platform in pair-end mode (average fragment length 200 bp, library preparation and sequencing performed by Macrogen®). Obtained reads were quality-trimmed by fqtrim tool (Pertea 2015); minimum read length 20 bp; 3' end trimming if quality drops below 15 and aligned to *C. taenia* reference transcriptome that was published and cleaned from potentially paralogous contigs by (Janko et al. 2018). To identify mitochondrial variants, we also mapped the reads to published *C. elongatoides* mitochondrion (accession no. NC_023947.1). Mapping was performed with BWA MEM algorithm (Li and Durbin 2009) and resulting files were processed with Picard tools (Broad Institute 2015). Individuals' variants were called with GATK v3.4 HaplotypeCaller tool and all individuals were jointly genotyped using the GenotypeGVCFs tool (McKenna et al. 2010; DePristo et al. 2011; Van der Auwera et al. 2013). Variant recalibration was based on available database of species-diagnostic positions (Janko et al. 2018) representing learning set for variant quality score recalibration tool VariantRecalibrator. Recalibrated variants were then filtered with ApplyRecalibration tool using 90 % tranche to filter all variants. All resulting highly confident

SNPs with coverage >= 10 and genotype quality >= 20 were transferred into the relational database using our own Python3/SQL scripts.

SNP data of each specimen were subjected to clustering analysis by Plink v1.90b4 (Chang et al. 2015). To simplify the analysis, we focused solely on bi-allelic SNPs with at most two variants throughout the entire dataset. This resulted in removal of ~1‰ of positions. We also removed 103 positions where the two laboratory F1 hybrids differed from their parents, because such variants were suspiciously present in most of the other specimens and suggested potential sequencing or demultiplexing errors rather than real variants.

To identify which hybrid individuals putatively belong to the same clone, we followed (Arnaud-Haond et al. 2007). Specifically, we created a pairwise matrix of distances between all hybrid individuals calculated from SNP mismatches. We then investigated a histogram of such pairwise distances and found a saddle point, which putatively defines a threshold distance between pairs of individuals belonging to the same or different clones.

2.3. Selection of species-specific SNPs, Detection of Loss of Heterozygosity (LOH) events and evaluation of their topological clustering

All SNPs that passed through aforementioned filters were attributed into one of the 10 categories according to their distribution among biotypes, following (Ament-Velásquez et al. 2016). The most important category of SNPs for this study are the species-specific variants (categories shared sh3a-b), where parental species are fixed (monomorphic) for different alleles, thereby allowing for detection of so-called Loss of Heterozygosity (LOH) events, where hybrids appear homozygous contrary to the expectation. Having detected the LOH variants in hybrids, we test whether observed LOH events tend to be randomly distributed across individual's genes or rather tend to cluster, in which case the SNPs with LOH in given gene occur in tight proximity to each other with no discontinuation by heterozygous diagnostic sites. To do so, we identified within each gene the uninterrupted stretches of diagnostic sites with LOH and assigned each such cluster with a score (***S***) so that if the length of the LOH cluster = ***n***, then ***S=n^2***. Overall clustering score per animal is then simply represented by ***∑S***. Finally, to test whether observed clustering is nonrandom, we permuted for each hybrid individual its LOH sites across all diagnostic sites and genes, calculated ***S*** and compared simulated values to empirical scores.

2.4. Analysis of sequencing coverage

To evaluate whether sequence capture data are suited to compare coverages among loci and across individuals, we investigated the variance in sequencing depth by counting in each sample the number of mapped reads per contig using Bedtools multicov v2.25.0, with default settings (Quinlan and Hall 2010). We then calculated the normalized coverage at each LOH site of every individual using the "total read count" approach (Dillies et al. 2013) and estimated the so-called ‘relative coverage’ by dividing hybrid’s normalized coverage at given site by normalized coverages of the same site in parental species. Since deletions are unlikely in sexual species, this allows detection of hemizygous deletions in hybrids. The expected values then depend on the ploidy of given hybrid: ~1 would indicate the same number of allelic copies indicating a conversion event, while hemizygous deletions would generate relative values ~0.5 and ~0.66 in diploid and triploid hybrids, respectively, while double allelic deletion in triploids would generate values ~0.33.

To reveal whether observed LOH events in hybrids are generated by gene conversion or hemizygous deletions, or their combination, we performed two tests. First, we simulated how the distribution of normalized coverages should look like, were it generated by conversions only. To do so, we generated simulated dataset for each hybrid individual, where coverage values at its LOH sites were sampled from exactly the same number of sites/genes in parental individuals. As such, we obtained realistic null expectation of relative coverages while taking into account used methodology of DNA sequencing and bioinformatic treatment. Such a null distribution simulated for each hybrid biotype has been compared with the empirical one by Kolmogorov-Smirnov test. The simulations have been repeated several times for each biotype, randomizing both the loci considered as well as the specimens used per each locus.

We then constructed histograms of relative coverages of all detected LOH sites for each hybrid biotype and tested their modality at values biologically relevant for gene conversion (~1) or deletion (~0.5 in diploids, or 0.66 and 0.33 in triploids) (Tucker et al. 2013). To test whether observed distributions deviate from unimodality and to evaluate the relative contribution of gene conversion and deletion processes, we applied the nonlinear least square method implemented in Gnuplot software to consecutively fit each histogram by gamma distributions with the shape parameter k optimized by the fitting algorithm and mean ($\mu = \alpha/\beta$) fixed at aforementioned relevant values. In case of diploids, we fitted two distributions (or their mix), centered at 1 and 0.5, while in triploids we fitted three distributions (or their mixes) centered at 1, 0.66 and 0.33. Before fittings, we followed the Freedman-Diaconis rule to select the width of the bins in each histogram (Freedman and Diaconis 1981)

in order to take into account the properties of particular datasets of each biotype. In case of mix of distributions, we let the fitting algorithm estimate the optimal values of A, B and C parameters describing relative contributions of individual distributions.

2.5. Testing the effect of gene/allele expressions on LOH occurrence:

To evaluate potential effects of gene/allelic expression on the occurrence of LOH, we compared the present data with those published by (Bartoš et al. 2019).

Using present gDNA data we categorized loci based on presence or absence of LOH (LOH positive or negative) and its direction (E-like or T-like). We then analyzed the RNA expression of corresponding loci in data of Bartoš et al., (2019) using two tests:

- **Differences in overall gene expression:** To compare the expression levels of LOH-positive and LOH-negative genes in each hybrid biotype, we normalized original read counts by TPM method (Transcripts Per Kilobase Million) instead of DeSeq2 method used by Bartoš et al. (2019), since the TPM allowed for comparisons of multiple loci within each individual. The TPM normalization was performed according to (Mortazavi et al. 2008).
- **Allelic expression:** Because data of hybrids from Bartoš et al. (2019) could be affected by allelic deletions, we investigated expression divergence between parental species as a proxy for relative allelic expression in each locus. Specifically, we extracted the DeSeq2 normalized estimates of *C. elongatoides* and *C. taenia* expression levels and tested whether the direction of LOH event (either E-like or T-like) is related to log2 fold change (FC) between parental species. The test was performed by comparing the distributions of log2FC values in LOH-negative genes with those of either E-like LOH or T-like LOH-positive genes using Kolmogorov-Smirnov test.

2.6. Analysis of Gene Ontology Term enrichment.

The reference transcriptome was annotated with BLAST2GO tool v1.4.4 using GO database as of July 2019). From 20600 sequences, a subset of 13557 received BLASTx hit (e-value < 0.0001), from which 11314 was associated with significant GO Term annotation (default BLAST2GO settings). To identify GO terms potentially associated with LOH-positive genes, we performed GO enrichment analysis restricted to those genes, which possessed

diagnostic sites, thereby technically allowing detection of LOH. P-values were calculated from hypergeometric distribution implemented in GO::TermFinder (Boyle et al. 2004) using the list of LOH-positive genes as a testing dataset for each biotype. Since gene ontologies terms are a part of acyclic directed graphs (parent and child terms are not independent), we also corrected obtained p values with permutation-based correction provided in GO::TermFinder.

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Figures & Tables

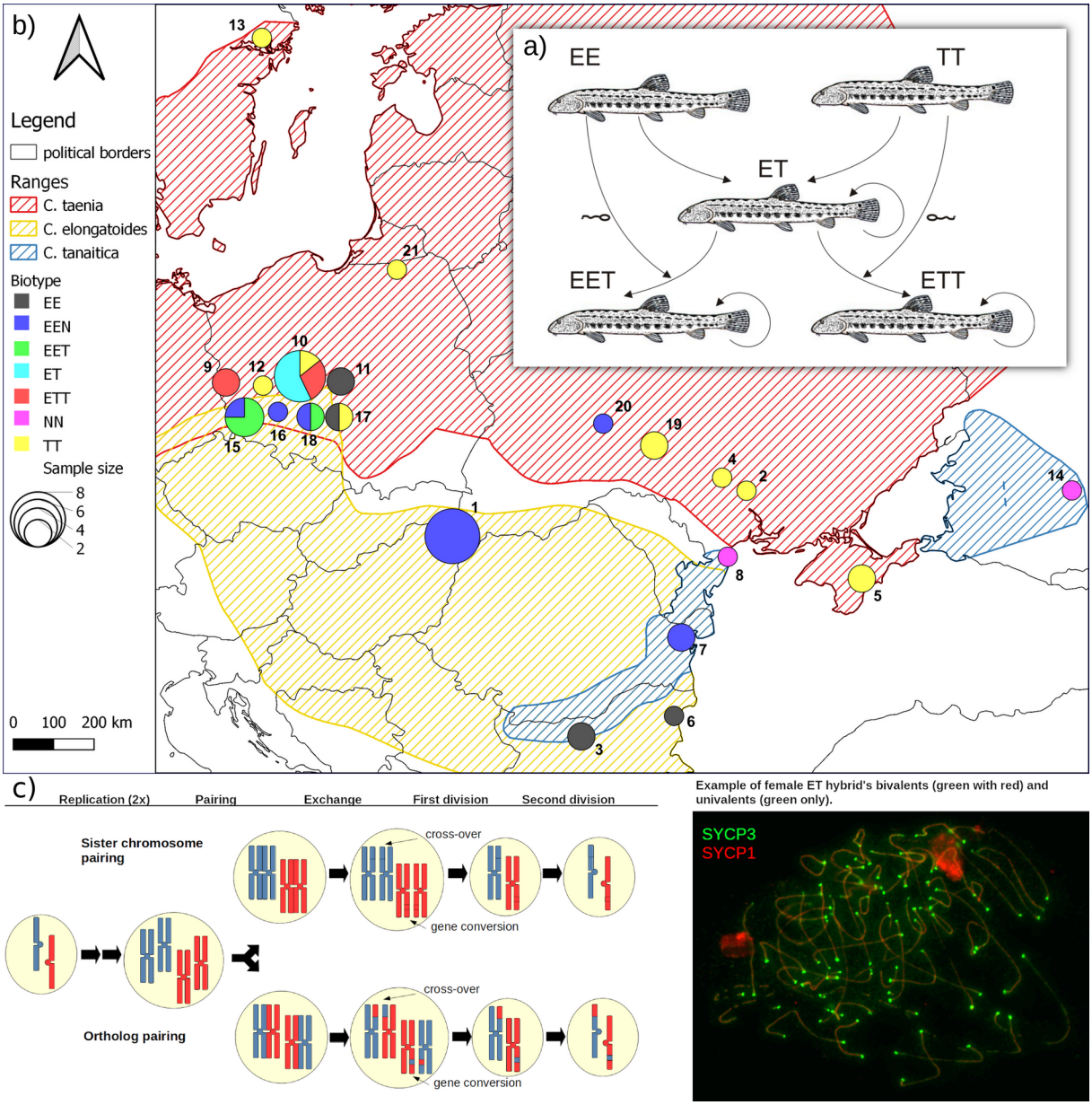

Fig. 1: The *Cobitis taenia* hybrid complex. a) Map of samples' origin; sites' numeric code corresponds to Table S1. b) Reproduction scheme of *Cobitis*; letters correspond to haploid genomes: E = *C. elongatoides*, T = *C. taenia*. c) example of gametogenetic pathways involving endoreplication and followed by meiotic pairing of either sister chromosomes or orthologous chromosomes. Note that the latter case may cause loss of heterozygosity among progeny via crossovers or gene conversions. Insert on the right demonstrates empirical evidence for the presence of bivalents and univalents in hybrid's oocytes as from (Dedukh et al. 2020).

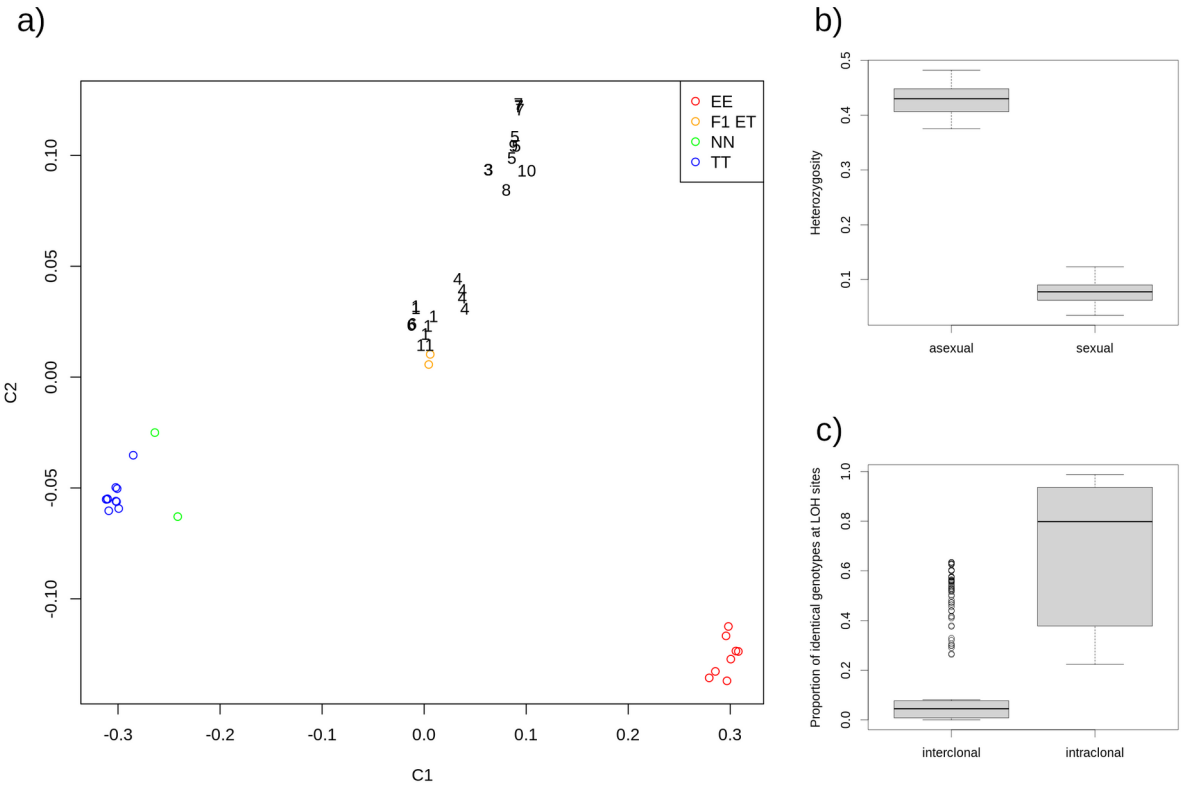

Fig. 2: a) MDS (multidimensional scaling; SVD algorithm) of samples based on filtered recalibrated SNPs; clustering visualization of first two coordinates (Plink v1.9b) to validate sample genetic origin; letters correspond to haplotype genomes as follows: *E* = *C. elongatoides*, *N* = *C. tanaitica*, *T* = *C. taenia*. Hybrid individuals are denoted by black color and numerals, which indicate the ID of determined clonal lineages, as in Table S2 (note that clone-mates tend to be clustered in the MDS plot). b) Heterozygosity of asexual and sexual samples across all filtered variant sites; missing sites are not included. c) Boxplots indicate the proportions of shared genotypes at LOH positions among all pairs of individuals belonging to the same (right) and different (left) clonal lineages. Note that we used the set of E-TN diagnostic sites to maintain compatibility of *elongatoides-taenia* and *elongatoides-tanaitica* hybrids.

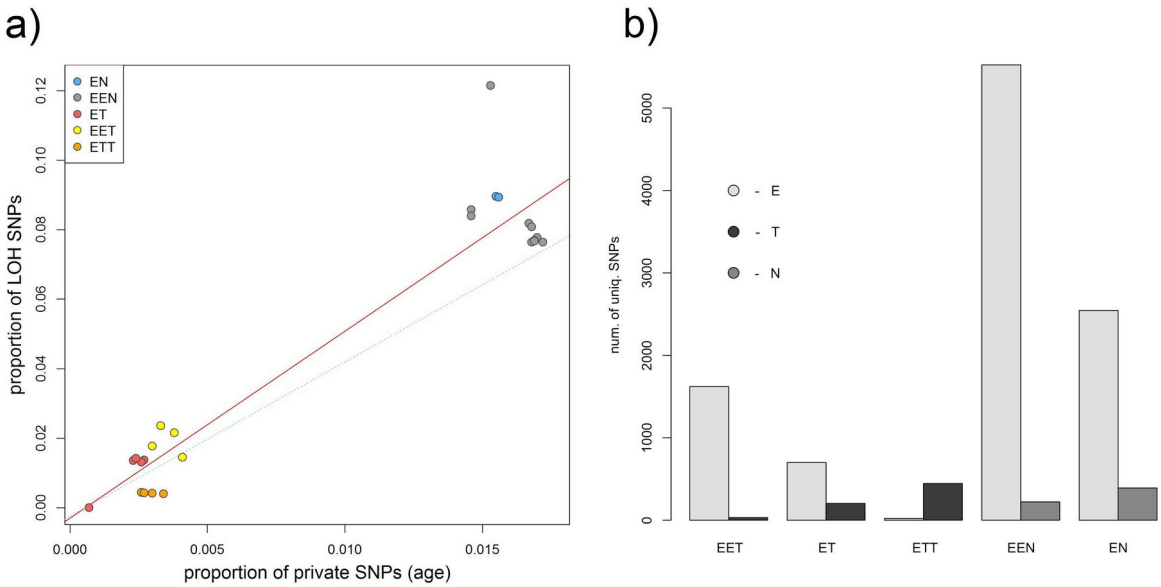

Fig. 3: a) Correlation between proportion of private hybrid SNPs as the proxy for asexual's age, and proportion of LOH loci in each individual. For plotting points and their linear regression (solid line) we used E-T diagnostic sites for *elongatoides-taenia* hybrids and E-N diagnostic sites for *elongatoides-tanaitica* hybrids. Dashed line represents the linear regression fit for data calculated on E-TN diagnostic sites used for all individuals (points not shown). Letters correspond to haplotype genomes as follows: E = *C. elongatoides*, N = *C. tanaitica*, T = *C. taenia*. **b)** Barplot showing proportions of LOH events according to their genomic origin. Height of each bar represents number of unique SNPs that appeared as LOH in a given biotype.

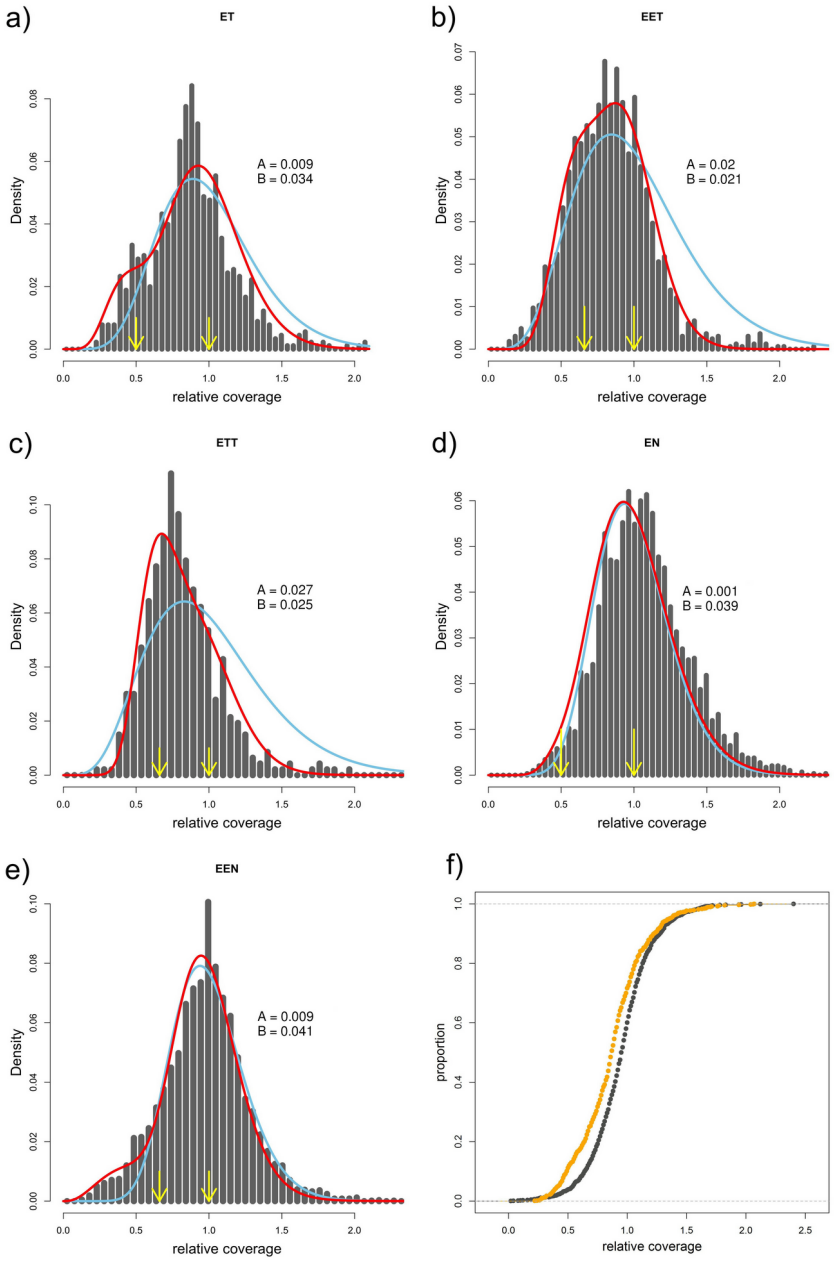

Fig. 4: a-e) Histograms of relative coverage of LOH loci in ET, EET, ETT, EN and EEN biotypes, respectively. Arrows depict biologically meaningful values (for given ploidy); red lines represent mixture of two Gamma distribution; blue lines represent single Gamma distribution with mean centered at value 1. Coefficients A and B indicate proportions of both Gamma distributions in the combined model, A relates to the distribution assuming the mean relative coverage ~1, B to the distribution with the mean ~0.5 or 0.66 (for diploid or triploid biotype, respectively); **f)** orange represents empirical cumulative distribution function (ECDF) of relative coverages at LOH sites of ET biotype, black represents ECDF of relative coverages at the same sites, but taken from parental species, where no deletions are expected. ECDF curves for other biotypes are provided in Fig. S1.

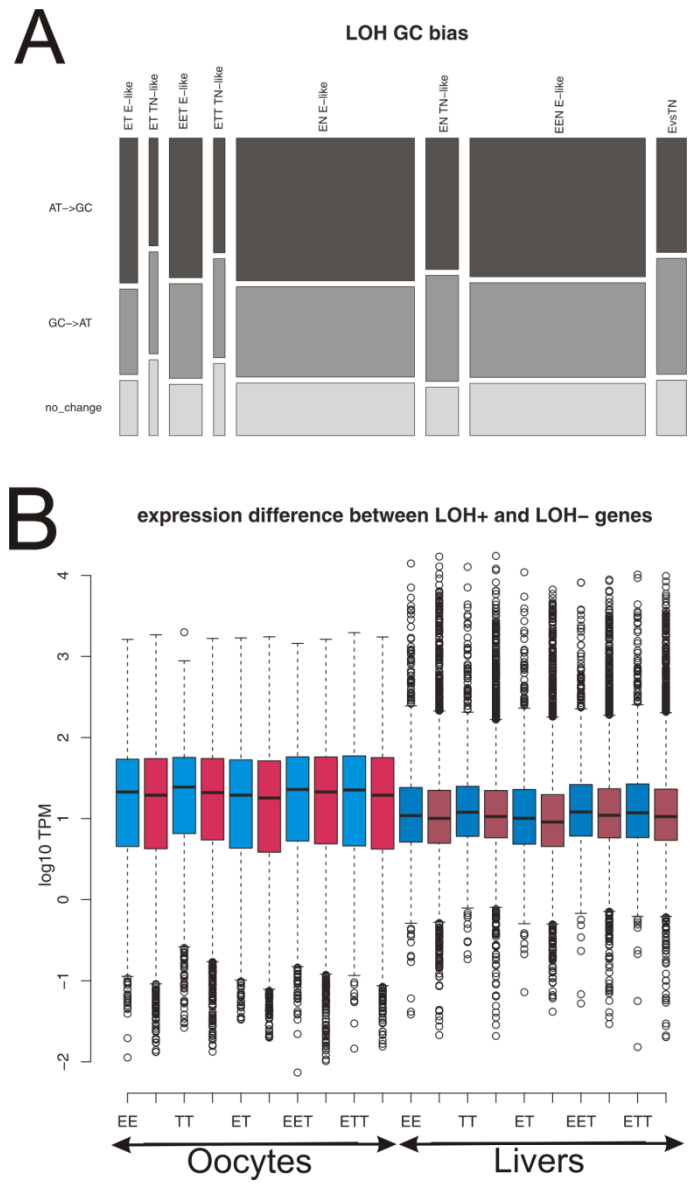

Figure 5:

A: G/C bias at asexuals' LOH sites as demonstrated for one representative of every biotype. Each individual is represented by two columns with E-like (left) and T-like (right) LOH events. Bar widths scale with absolute numbers of observed LOH events in each individual, while heights of color fields demonstrate proportions of LOH causing weak to strong, strong to weak and no GC change. The last bar represents differences between C. *elongatoides* and both other parental species at all E-TN diagnostic sites. Note that in E-like LOH events, all hybrids show consistent and significant increase in weak to strong substitution rates as compared against interparental divergence (no shift has been detected at TN-like LOH sites).

B: TPM normalized expression characteristics of LOH positive (blue) and LOH negative (red) genes in all *Cobitis* biotypes analyzed by Bartoš et al. 2019. For better orientation, the oocyte

data (left part) are depicted in lighter color tones, while liver data (right part) are in darker tones.

Tables

ET (292 LOH positive genes, 4582 annotated with diagnostic SNP)

GO:0005576	extracellular region pV 0.0028351 (22 vs 184)
GO:0005923	bicellular tight junction pV 0.0161340 (3 vs 9)
GO:0070160	tight junction pV 0.0219788 (3 vs 10)
GO:0000347	THO complex pV 0.0222870 (2 vs 4)
GO:0000445	THO complex part of transcription export complex pV 0.0222870 (2 vs 4)
GO:0016021	integral component of membrane pV 0.0338391 (76 vs 987)
GO:0031224	intrinsic component of membrane pV 0.0353113 (76 vs 989)
GO:0031680	G-protein beta/gamma-subunit complex pV 0.0355939 (2 vs 5)
GO:0097431	mitotic spindle pole pV 0.0355939 (2 vs 5)
GO:0005834	heterotrimeric G-protein complex pV 0.0366592 (3 vs 12)
GO:0043296	apical junction complex pV 0.0366592 (3 vs 12)
GO:1905360	GTPase complex pV 0.0366592 (3 vs 12)
GO:0030687	preribosome, large subunit precursor pV 0.0552198 (3 vs 14)
GO:0000346	transcription export complex pV 0.0686844 (2 vs 7)
GO:0000152	nuclear ubiquitin ligase complex pV 0.0878187 (2 vs 8)
GO:0005911	cell-cell junction pV 0.0891895 (4 vs 27)
GO:0000922	spindle pole pV 0.0897273 (3 vs 17)
GO:0031234	extrinsic component of cytoplasmic side of plasma membrane pV 0.0897273 (3 vs 17)
GO:0043230	extracellular organelle pV 0.1083001 (2 vs 9)
GO:0070062	extracellular exosome pV 0.1083001 (2 vs 9)

ETT (226 LOH positive genes, 4582 annotated with diagnostic SNP)

GO:0005844	polysome pV 0.0287870 (3 vs 14)
GO:0031514	motile cilium pV 0.0318521 (2 vs 6)
GO:0005730	nucleolus pV 0.0390230 (11 vs 123)
GO:0005732	small nucleolar ribonucleoprotein complex pV 0.0431628 (2 vs 7)
GO:0071007	U2-type catalytic step 2 spliceosome pV 0.0431628 (2 vs 7)
GO:0030684	preribosome pV 0.0446155 (6 vs 53)
GO:0044798	nuclear transcription factor complex pV 0.0577484 (4 vs 30)
GO:0090575	RNA polymerase II transcription factor complex pV 0.0577484 (4 vs 30)
GO:0031974	membrane-enclosed lumen pV 0.0808439 (31 vs 488)
GO:0043233	organelle lumen pV 0.0808439 (31 vs 488)
GO:0070013	intracellular organelle lumen pV 0.0808439 (31 vs 488)
GO:0005657	replication fork pV 0.0839463 (2 vs 10)
GO:0034708	methyltransferase complex pV 0.0990699 (4 vs 36)

GO:0071013	catalytic step 2 spliceosome pV 0.1015022 (3 vs 23)
GO:0005793	endoplasmic reticulum-Golgi intermediate compartment pV 0.1228584 (3 vs 25)
GO:0031461	cullin-RING ubiquitin ligase complex pV 0.1456830 (3 vs 27)
GO:0032040	small-subunit processome pV 0.1456830 (3 vs 27)
GO:0071944	cell periphery pV 0.1530158 (23 vs 373)
GO:0035097	histone methyltransferase complex pV 0.1697646 (3 vs 29)
GO:0031981	nuclear lumen pV 0.1815560 (23 vs 382)

EET (662 LOH positive genes, 4582 annotated with diagnostic SNP)

GO:0030117	membrane coat pV 0.0007544 (12 vs 31)
GO:0048475	coated membrane pV 0.0007544 (12 vs 31)
GO:0030120	vesicle coat pV 0.0014970 (9 vs 21)
GO:0030662	coated vesicle membrane pV 0.0137591 (9 vs 28)
GO:0031224	intrinsic component of membrane pV 0.0145280 (165 vs 989)
GO:0016021	integral component of membrane pV 0.0172777 (164 vs 987)
GO:0030126	COPI vesicle coat pV 0.0186441 (4 vs 8)
GO:0031314	extrinsic component of mitochondrial inner membrane pV 0.0186441 (4 vs 8)
GO:0016020	membrane pV 0.0189391 (234 vs 1456)
GO:0005921	gap junction pV 0.0208470 (2 vs 2)
GO:0030057	desmosome pV 0.0208470 (2 vs 2)
GO:0042627	chylomicron pV 0.0208470 (2 vs 2)
GO:0071782	endoplasmic reticulum tubular network pV 0.0208470 (2 vs 2)
GO:0098827	endoplasmic reticulum subcompartment pV 0.0208470 (2 vs 2)
GO:0030663	COPI-coated vesicle membrane pV 0.0297850 (4 vs 9)
GO:0005911	cell-cell junction pV 0.0322002 (8 vs 27)
GO:0012506	vesicle membrane pV 0.0322119 (11 vs 42)
GO:0030659	cytoplasmic vesicle membrane pV 0.0322119 (11 vs 42)
GO:0030660	Golgi-associated vesicle membrane pV 0.0395767 (8 vs 28)
GO:0030687	preribosome, large subunit precursor pV 0.0402965 (5 vs 14)

EN (755 LOH positive genes, 3197 annotated with diagnostic SNP)

GO:0005789	endoplasmic reticulum membrane pV 0.0006197 (46 vs 126)
GO:0072546	ER membrane protein complex pV 0.0007271 (5 vs 5)
GO:0042175	**nuclear outer membrane-endoplasmic reticulum membrane network pV 0.0009193 (46 vs 128)**
GO:0031090	organelle membrane pV 0.0041040 (107 vs 364)
GO:0012505	endomembrane system pV 0.0071884 (116 vs 405)
GO:0005783	**endoplasmic reticulum pV 0.0075373 (53 vs 166)**
GO:0005924	cell-substrate adherens junction pV 0.0098916 (5 vs 7)
GO:0005925	focal adhesion pV0.0098916 (5 vs 7)

GO:0030055	cell-substrate junction pV 0.0098916 (5 vs 7)
GO:0099081	supramolecular polymer pV 0.0121988 (19 vs 49)
GO:0099512	supramolecular fiber pV 0.0121988 (19 vs 49)
GO:0016528	sarcoplasm pV 0.0131308 (3 vs 3)
GO:0016529	sarcoplasmic reticulum pV 0.0131308 (3 vs 3)
GO:0017087	mitochondrial processing peptidase complex pV 0.0131308 (3 vs 3)
GO:0042383	sarcolemma pV 0.0131308 (3 vs 3)
GO:0030176	integral component of endoplasmic reticulum membrane pV 0.0134881 (12 vs 27)
GO:0031227	intrinsic component of endoplasmic reticulum membrane pV 0.0134881 (12 vs 27)
GO:0030016	myofibril pV 0.0147223 (6 vs 10)
GO:0030017	sarcomere pV 0.0147223 (6 vs 10)
GO:0005813	centrosome pV 0.0251815 (12 vs 29)
EEN (1235 LOH positive genes, 3197 annotated with diagnostic SNP)	
GO:0012505	endomembrane system pV 0.00017207 (190 vs 405)
GO:0005783	endoplasmic reticulum pV 0.0008575 (84 vs 166)
GO:0042175	nuclear outer membrane-endoplasmic reticulum membrane network pV 0.0009040 (67 vs 128)
GO:0005789	endoplasmic reticulum membrane pV 0.0009619 (66 vs 126)
GO:0072546	ER membrane protein complex pV 0.0007271 (5 vs 5)
GO:0030176	integral component of endoplasmic reticulum membrane pV 0.0087823 (17 vs 27)
GO:0031227	intrinsic component of endoplasmic reticulum membrane pV 0.0087823 (17 vs 27)
GO:0030496	midbody pV 0.0095585 (8 vs 10)
GO:0005794	Golgi apparatus pV0.0153109 (69 vs 145)
GO:0005924	cell-substrate adherens junction pV 0.0154665 (6 vs 7)
GO:0005925	focal adhesion pV0.0154665 (6 vs 7)
GO:0030055	cell-substrate junction pV0.0154665 (6 vs 7)
GO:0045121	membrane raft pV 0.0201926 (7 vs 9)
GO:0098589	membrane region pV 0.0201926 (7 vs 9)
GO:0098857	membrane microdomain pV 0.0201926 (7 vs 9)
GO:0097525	spliceosomal snRNP complex pV 0.0259453 (10 vs 15)
GO:0031090	organelle membrane pV 0.0351521 (157 vs 364)
GO:0030532	small nuclear ribonucleoprotein complex pV 0.0456027 (10 vs 16)
GO:0031300	intrinsic component of organelle membrane pV0.0534485 (28 vs 56)
GO:0031301	integral component of organelle membrane pV 0.0534485 (28 vs 56)

Table 1: Top 20 enriched GO terms in cellular compartment GO category ranked by p value. For each hybrid biotype we indicate number of genes affected by LOH event and total number of annotated genes with diagnostic SNP relevant for given combination of parental

species. For each GO term, we indicate its ID, description and uncorrected p value, as well as numbers of LOH-positive genes and total number of genes with given GO in parentheses. Underlined GO terms are significant after correction for multiple test at alpha level=0.1. Colors are used to highlight GO terms shared between distinct biotypes so that the same color across biotypes indicates GO terms that are identical or nested.

Supplementary files

sample	biotype	sex	no. of reads	no. of bases	mapped reads	locality	site ID
csc008	TT	m	9328500	1890943172	7872341	Tnya river, Ukraine	22
csc009	EE	NA	10674754	2200211351	9160050	Kozačevski river, Bulgaria	3
csc011	EET	f	46169330	4546217825	24662190	Odra R. sites RQ, Poland	18
csc012	EEN	f	36325574	3585572296	18876965	Odra R. sites 509, Poland	16
csc013	EEN	f	44514804	4386326838	25987623	Čierna voda, Slovakia	1
csc014	EEN	f	47084588	4630637949	27469787	Čierna voda, Slovakia	1
csc015	EEN	f	50128474	4925558699	25581023	Čierna voda, Slovakia	1
csc016	EEN	f	47466730	4675165684	28108072	Čierna voda, Slovakia	1
csc017	EEN	f	62526316	6142494088	32394947	Čierna voda, Slovakia	1
csc018	EEN	f	55335802	5446458549	30131168	Čierna voda, Slovakia	1
csc019	EE	NA	44761144	4406019008	23043480	Čierna voda, Slovakia	1
csc020	EE	NA	55359494	5451569580	28769818	Čierna voda, Slovakia	1
csc021	TT	m	42119812	2985717253	20350974	South Bug, Ukraine	19
csc022	TT	f	34584512	2448248483	16574306	Kodyma river, Ukraine	4
csc024	EE	NA	38482140	2734317798	18243498	Odra R. sites 801, Poland	11
csc025	EE	NA	37629664	2669330656	18231557	Odra R. sites 801, Poland	11
csc027	EE	m	54542982	3908825666	28305773	Kamcia r., Bulgaria	6
csc028	TT	f	32664314	2308852524	16036910	South Bug, Ukraine	2
csc029	EE	NA	38364748	2723397165	18384671	Kozačevski river, Bulgaria	3
csc033	TT	f	28098246	2010306058	14112070	Krym Alma river, Ukraine	5
csc034	TT	m	27835994	1951741228	13518951	Odra R. sites 705, Poland	12
csc035	TT	m	41334434	2940164538	20820869	Odra R. sites 701, Poland	10
csc036	NN	m	31546234	2250940100	16153019	Sucholužija, Ukraine	8
csc039	NN	NA	22567596	1603680625	10392416	Kuban river, Russia	14
csc046	EEN	f	35381744	2486580209	16471358	Odra R. sites RQ, Poland	18
csc052	ET	f	32020816	2249205626	14937219	Odra R. sites 701, Poland	10
csc055	EET	f	34678947	2432666387	15941455	Odra R. sites 510, Poland	15
csc056	EEN	f	32226860	2270486184	14849216	Sluč Vachovcy, Ukraine	20
csc057	ET	f	31545522	2190478704	14343725	Odra R. sites 701, Poland	10
csc067	ET	f	48483708	3434265969	23877555	Odra R. sites 701, Poland	10
csc069	ET	f	16831044	1632439963	10815080	laboratory hybrid	NA
csc070	EE	m	20691516	2009443636	13090582	Olsztyn, Poland	17
csc071	ET	f	14455828	1399281976	8718131	laboratory hybrid	NA
csc072	TT	f	12540166	1218069246	8555642	Olsztyn, Poland	21
csc073	EN	f	24308246	2377911505	15929335	Ghiol lake, Romania	7
csc074	EN	f	19470508	1898963852	12481576	Ghiol lake, Romania	7
csc076	EET	f	36597710	3585778583	24666067	Odra R. sites 510, Poland	15
csc077	TT	NA	18349390	1778582689	11064568	Malaren lake, Sweden	13
csc078	EET	f	17368108	1693830781	10771162	Odra R. sites 510, Poland	15
csc079	EEN	f	22917494	2231070909	14125361	Odra R. sites 510, Poland	15
csc080	TT	NA	12337404	1200073223	7505927	Crimea, Ukraine	5
csc082	ETT	f	21529004	2091846439	13242462	Odra R. sites 704, Poland	9
csc086	ETT	f	20824472	2036604862	13308483	Odra R. sites 704, Poland	9
csc087	ETT	f	22230674	2174839517	14061887	Odra R. sites 701, Poland	10
csc089	ET	f	50135182	4898480713	27586948	Odra R. sites 701, Poland	10
csc090	ETT	f	34147738	3331600457	19960304	Odra R. sites 701, Poland	10

Table S1: List of samples including sample name, biotype, sex, sequencing statistics and geographical origin.

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dataset	animal	biotype	MLL	pr1a (xy x x)	pr1b (y x x)	prh2a (xy x xy | xy xy x)	prh2b (x x xy | x xy x)	prh2b1 (x y xy | x xy y)	sh3a (xy x y)	sh3b11 (x x y)	sh3b12 (x y x)	sh4a (xy xy xy)	sh4b (x xy xy)	total SNPs	LOH proportion	diagnostic SNPs	private SNPs proportion
elongatoides-tanaitica	csc012	EEN	5	6907	7	20054	23850	254	26346	2527	59	1195	645	81844	0,0894	28932	0,0845
	csc013	EEN	9	6541	10	20704	24035	202	26851	2462	60	1214	639	82718	0,0859	29373	0,0792
	csc014	EEN	7	7193	12	21336	23703	242	27108	2450	48	1231	637	83960	0,0844	29606	0,0858
	csc015	EEN	7	7084	10	21142	23460	247	26870	2460	48	1221	633	83175	0,0854	29378	0,0853
	csc016	EEN	7	7183	11	21291	23712	253	27096	2444	52	1229	636	83907	0,0843	29592	0,0857
	csc017	EEN	7	7134	10	21174	23652	252	27063	2454	60	1237	641	83677	0,0850	29577	0,0854
	csc018	EEN	10	5943	8	20400	24294	511	25820	3748	50	1266	599	82639	0,1282	29618	0,0720
	csc046	EEN	5	6816	7	19987	24922	285	26526	2682	67	1134	669	83095	0,0939	29275	0,0821
	csc056	EEN	8	6149	3	19369	24942	286	26145	2609	109	1108	657	81377	0,0942	28863	0,0756
	csc079	EEN	5	7015	5	20385	24488	255	26926	2563	67	1214	657	83575	0,0890	29556	0,0840
	csc073	EN	3	6423	46	16840	28280	341	26872	2676	377	1086	792	83733	0,1020	29925	0,0773
	csc074	EN	3	6382	36	16798	28192	338	26797	2654	375	1069	774	83415	0,1016	29826	0,0769
elongatoides-taenia	csc011	EET	4	1673	1	28139	25039	109	35107	593	65	2237	798	93761	0,0184	35765	0,0179
	csc055	EET	4	1581	0	27510	24602	152	33638	756	52	1882	717	90890	0,0235	34446	0,0174
	csc076	EET	4	1746	8	28687	26460	100	36596	526	78	2161	835	97197	0,0162	37200	0,0180
	csc078	EET	4	1530	2	26096	24629	131	33012	683	58	1969	740	88850	0,0220	33753	0,0172
	csc052	ET	11	852	4	22318	30595	121	33918	376	179	1559	1008	90930	0,0161	34473	0,0094
	csc057	ET	1	1142	5	22231	30431	87	33495	371	175	1505	1013	90455	0,0160	34041	0,0127
	csc067	ET	1	1233	8	23668	31598	99	35879	387	190	1716	1078	95856	0,0158	36456	0,0129
	csc089	ET	1	1287	11	24245	30160	143	36624	454	177	2055	1085	96241	0,0169	37255	0,0135
	csc082	ETT	6	890	1	24687	28028	33	35045	12	237	2020	901	91854	0,0071	35294	0,0097
	csc086	ETT	6	912	2	24942	28271	27	35290	6	231	1970	912	92563	0,0067	35527	0,0099
	csc087	ETT	1	1238	3	25388	28038	30	35565	12	228	1971	935	93408	0,0067	35805	0,0133
	csc090	ETT	1	1278	3	26137	28426	29	36548	13	232	2153	947	95766	0,0067	36793	0,0134
F1 family only	csc069	ET	F1	40	0	6616	5276	0	43548	12	53	353	45	55943	0,0015	43613	0,0007
	csc071	ET	F1	38	0	6378	5001	1	41845	35	37	340	38	53713	0,0017	41917	0,0007

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Table S2: Counts of SNPs detected per each sample and category. In parentheses, we define each category by demonstrating states at SNP in asexual hybrid (H) and both parental species (P_1, P_2), separated by space (i.e. H P_1, P_2). Note that EN and EEN hybrids were classified against elongatoides—tanaitica comparisons, ET, EET and ETT hybrids against elongatoides—taenia and the two F1 hybrids against their direct parents.

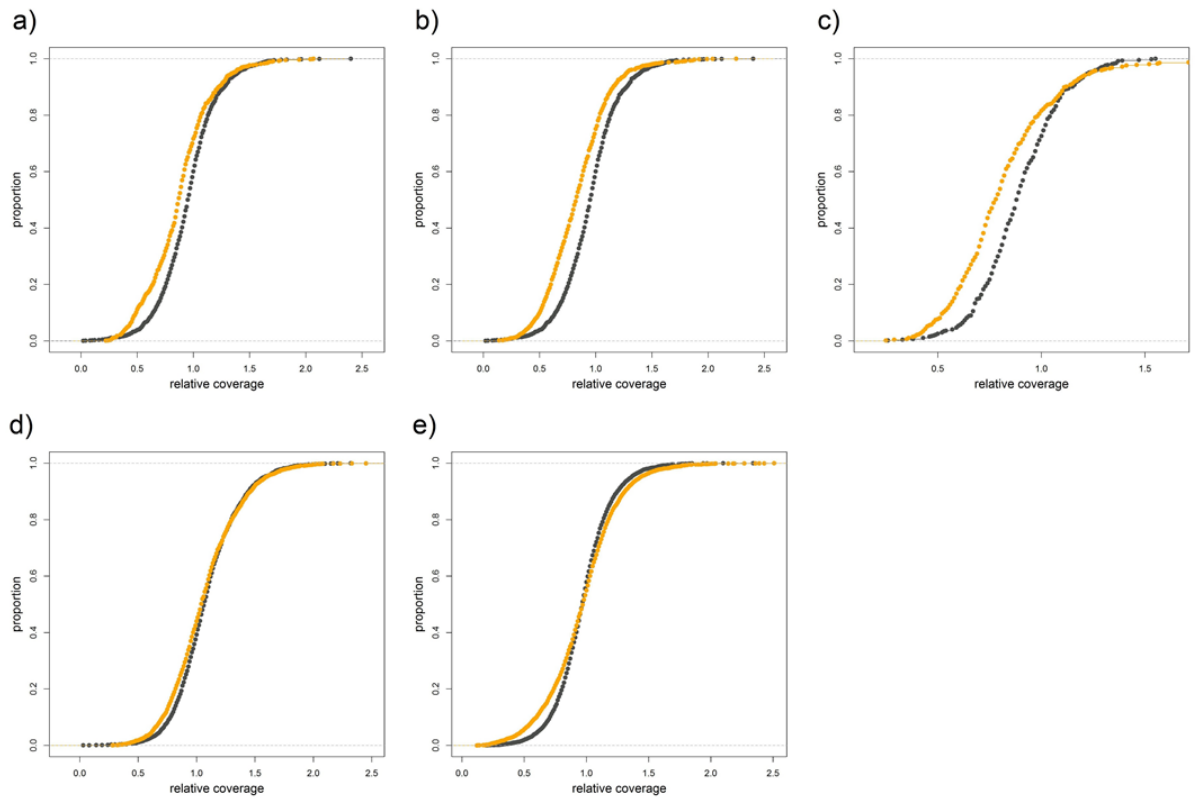

Figure S1: Empirical cumulative distribution functions (ECDF) for individual biotypes. Orange represents empirical cumulative distribution function (ECDF) of relative coverages at LOH sites of given hybrid biotype, black represents ECDF of relative coverages at the same sites, but taken from parental species, where no deletions are expected. Significance of differences between distributions were tested by Kolmogorov-Smirnov test. Hybrid biotypes: **a)** ET biotype, **b)** EET biotype, **c)** ETT biotype, **d)** EN biotype, **e)** EEN biotype.