

1 Genomic repeat landscape evolution across the teleost fish lineages

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12

13 Abstract

14 Repetitive DNA make up a considerable fraction of most eukaryotic genomes. In fish, transposable
15 element (TE) activity have coincided with rapid species diversification. Here, we annotated the repetitive
16 content in 100 genome assemblies, covering the major branches of the diverse lineage of teleost fish. We
17 investigated if TE content correlates with family level net diversification rates and found support for a
18 weak negative correlation. Further, we found that TE content, the degree of parental care and short
19 tandem repeat (STR) content contributed to genome size variability. In contrast to TEs, STR content
20 showed a negative relationship with genome size. STR content did not correlate with TE content, which
21 implies independent evolutionary paths. Last, marine and freshwater fish have large differences in STR
22 content. The most extreme propagation was found in the genomes of codfish species and Atlantic herring.
23 Such a high density of STRs is likely to increase the mutational load, which we propose could be
24 counterbalanced by high fecundity as seen in codfishes and herring.

25

26 **Key words:** transposable elements, short tandem repeats, diversification, repetitive DNA, genome size,
27 genome dynamics

28

29 **Introduction**

30 Repetitive sequences including transposable elements (TEs) and short tandem repeats (STRs) comprise
31 large fractions of most eukaryotic genomes. STRs are repetitive stretches of DNA with unit sizes ranging
32 from 1 to 10 bp, increasing and shrinking in size primarily due to replication slippage (Levinson &
33 Gutman 1987). The origin of STRs in genomes can be attributed to processes of unequal crossing over
34 (Smith 1976). New STRs can also originate from parts of active TEs, as insertions of poly-A tails from
35 retrotransposition, or from *de novo* mutations of STR-like patterns (Ellegren 2004; Pasquesi et al. 2018).
36 TEs take advantage of the DNA replication and transcription processes of their hosts to facilitate
37 propagation and are defined into two main classes: DNA transposons, which transpose directly from
38 DNA to DNA, and retrotransposons (RTs) that transpose *via* an RNA intermediate. RTs are further
39 divided into elements containing long terminal repeats (LTRs) and those that do not, the long interspersed
40 nuclear elements (LINEs) and the short interspersed nuclear elements (SINEs) (Kapitonov & Jurka 2008).
41
42 Comparative studies have revealed that TE content to some extent explains genome size variation across
43 vertebrates (Chalopin et al. 2015) and across chordates (Canapa et al. 2015). Within more
44 phylogenetically narrow taxa, differences in repeat content do not necessarily reflect the variation in
45 genome size, such as within reptiles, mammals and birds (Pasquesi et al. 2018; Kapusta et al. 2017). In
46 the largest vertebrate group, teleost fish, the correlation between genome size and repetitive DNA content
47 appears to be modest (Gao et al. 2016; Yuan et al. 2018; Canapa et al. 2015; Chalopin et al. 2015), with
48 the largest study (Yuan et al. 2018) reporting an R of 0.6 (R^2 : 0.36). In contrast, TE content have been
49 suggested to explain 98% of the variation in genome size in angiosperms (Tenailon et al. 2010). Due to
50 the nature of TE propagation it is not surprising that an increase in TE copies may lead to an increase in
51 genome size, but the empirical evidence is less clear for correlations between STR content and genome

52 size. Across eukaryotic domains, the relationship seems to be positive (Hancock 2002; Mayer et al. 2010;
53 Hancock 1996). However, no significant correlation has been reported within domains (Morgante et al.
54 2002). As reported by Hardie and Hebert (2004), genome size is likely linked to differences in egg
55 diameter, parental care and aquatic habitat (saltwater or freshwater). These factors have so far not been
56 taken into account when testing the relationship between genome size and repetitive DNA in teleosts.
57
58 Beyond their contribution to genome size variability, TEs have been postulated to cause deletions,
59 translocations, duplications, and inversions in response to stress conditions (McClintock 1984). A role for
60 TEs in adaptation has been indicated in invasive species of ants, where TE-dense genomic islands were
61 shown to have generated variability in genes deemed important in the adaptation process (Schrader et al.
62 2014). Interestingly, bursts of TE activity coinciding with speciation have been found in studies of a
63 variety of taxa (Rebollo et al. 2010), including mammals (Ricci et al. 2018). Within teleost fish, elevated
64 TE activity has been shown to coincide with species radiations in salmonids (de Boer et al. 2007) and
65 cichlids (Salzburger 2018; Brawand et al. 2014). Beside a potential role in adaptive radiations through
66 generating adaptive mutations, a mechanism of which TEs could influence speciation is by causing
67 chromosomal rearrangements, possibly as a response to epigenetic release due to environmental stress
68 (Rebollo et al. 2010), which in turn can lead to reproductive isolation. For STRs, different length variants
69 present in a population contribute to the genetic variation and have been shown in some cases to be
70 functionally relevant (Gemayel et al. 2015; Press et al. 2018; Gymrek et al. 2016). As with TEs, STR
71 content varies across vertebrates, with frequencies from approximately 100 loci/Mbp to 1000 loci/Mbp
72 and densities from 1000 bp/Mbp to 50 000 bp/Mbp (Adams et al. 2016; Tørresen et al. 2017, 2018). A
73 large proportion of these STRs occur outside genes; however, in humans for instance, around 4500 STRs
74 occur in protein coding regions (Willems et al. 2014). A STR within an open reading frame (ORF) often
75 encodes homo- or di-amino acid tracts that to a large extent overlap with intrinsically unstructured protein
76 regions (Simon & Hancock 2009). Such regions are abundant in proteins that interact with other proteins
77 (Huntley & Clark 2007). On the other hand, STRs occurring in regulatory regions can affect the

78 expression of genes (Vinces et al. 2009; Quilez et al. 2016) and STRs in introns may impact RNA
79 splicing (Hefferon et al. 2004; Press et al. 2018).
80
81 In light of the above-mentioned observations, a key question is to what extent the genomic repeat
82 landscape impacts the evolution of vertebrates, here exemplified by teleost fishes. First, we investigated
83 the interplay between genome size, aquatic habitat, parental care and repetitive DNA content, using
84 comparative methods taking phylogenetic relationships as well as assembly quality into account. Next, we
85 focused on diversification. Our focal group, teleosts, is the most species rich group of all vertebrates and
86 serves as a suitable system to test for associations between the TE/STR landscape and diversification,
87 given the recent genomic sequencing initiatives of multiple teleost species (Malmstrøm et al. 2016, 2017;
88 Musilova et al. 2019) as well as available species richness data. Teleostean families differ widely in
89 species diversity, ranging from monotypic families such as Helostomatidae to the Cyprinidae, with ~3000
90 species. Teleosts display a 10-fold difference in TE content (Gao et al. 2016, 2017; Chalopin et al. 2015;
91 Canapa et al. 2015) and a 13-fold difference in STR content (Tørresen et al. 2018). We annotated the TE
92 and STR content in the genome assemblies of 100 teleost fish and one non-teleost ray-finned fish (spotted
93 gar, *Lepisosteus oculatus*). Our samples cover the major teleost branches, allowing us to describe
94 differences in TE and STR content after ~270 million years of evolution, and to investigate the role of
95 repetitive DNA in teleost genome size evolution and its potential influence on diversification.

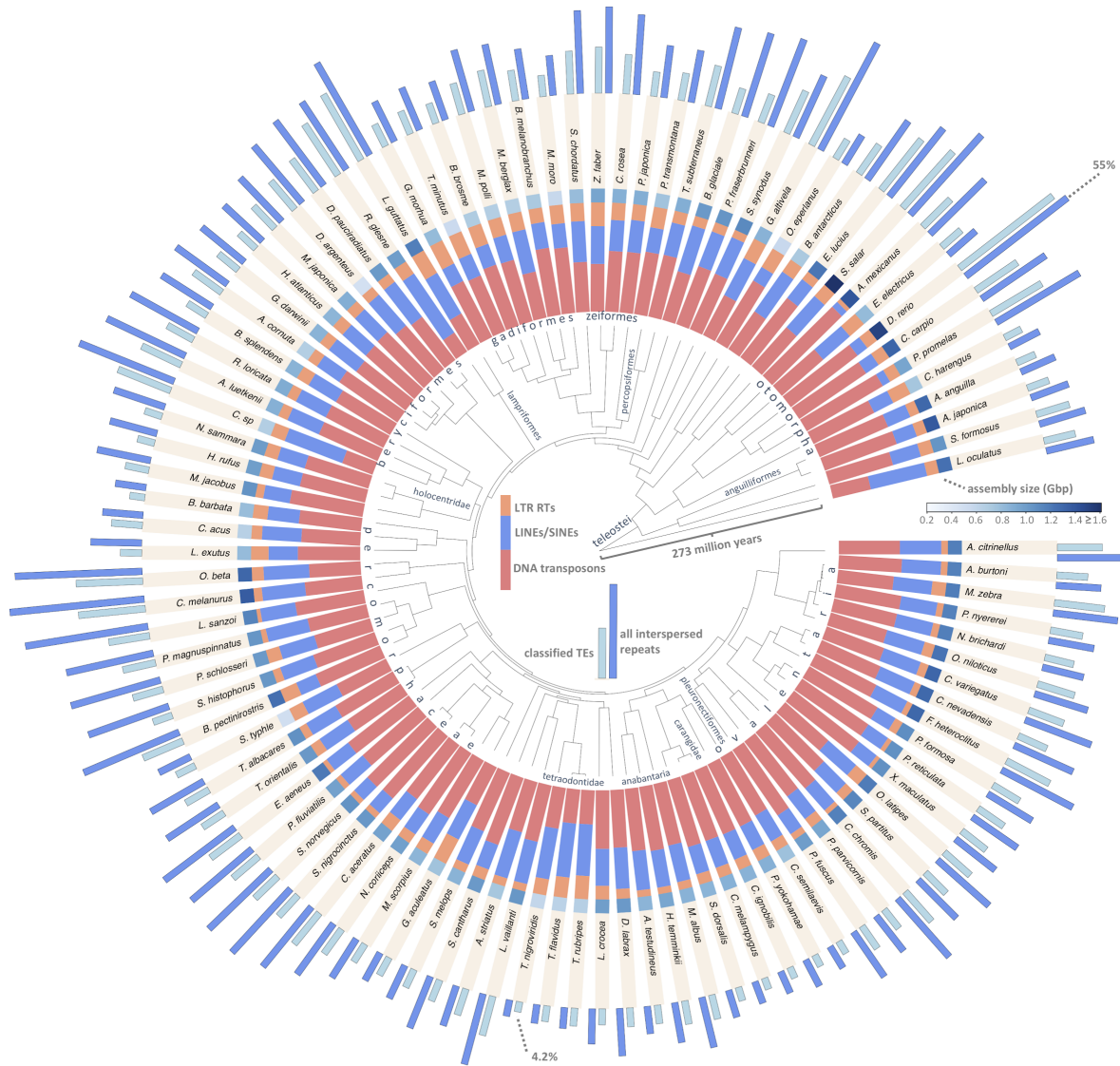
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97 **Results**

98 *Substantial changes in TE content over 270 million years of evolution*

99 Our annotation of TEs (and unclassified interspersed repeats) revealed high variation among species
100 (Figure 1). DNA transposon content varies the most, ranging from 1.6% in the tetraodon (*Tetraodon*
101 *nigroviridis*) genome to 37.1% in the zebrafish (*Danio rerio*) genome. The LTR-RT content ranges from
102 0.48% in bluefin trevally, southern platyfish and climbing perch (*Caranx melampygus*, *Xiphoporus*
103 *maculatus* and *Anabas testudineus*) to 7.4% in opah (*Lampris guttatus*). LINE content varies from 0.89%

104 in blind cavefish (*Astyanax mexicanus*) to 12.6% in giant oarfish (*Regalecus glesne*) and SINE content
105 ranges from 0.02% in electric eel (*Electrophorus electricus*) to 3.6% in giant oarfish (*R. glesne*). We also
106 quantified the proportions of DNA transposons, LTR retrotransposons, LINEs and SINEs relative to the
107 total classified TE content. We find that DNA transposons collectively make up the largest proportion of
108 the TE composition in most teleost fish genomes (94 out of 101 species). However, we find multiple
109 lineage-specific differences in TE composition. The DNA transposon fraction is especially high in the
110 genomes of *Astyanax mexicanus* (89.7%), Cyprinidae (mean: 77.5%), Sebastidae (mean: 76.9%) and
111 Poeciliidae (mean: 74.1%). Of retrotransposons, LINEs are the most prevalent TE subclass, and display
112 the highest relative fractions in *Cetomimus* sp. (51.4%), *Regalecus glesne* (51.0%), Tetraodontidae (mean
113 of 44.9%) and *Lampris guttatus* (40.9%). The LTR-RT fraction is comparably low in most of the
114 genomes studied, but is more prevalent in *Gasterosteus aculeatus* (25.2%), *L. guttatus* (24.2%) and
115 Gadidae (mean: 23.3%). Relative SINE fractions are generally low (mean: 4.2%), with a few exceptions
116 being *Synodus synodus* (16.8%), the non-teleost *Lepisosteus oculatus* (16.5%) and *R. glesne* (14.5%). The
117 Tetraodontidae family (represented by *Takifugu rubripes*, *Takifugu flavidus* and *Tetraodon nigroviridis*)
118 have a particularly small fraction of DNA transposons, a feature shared only with distant relatives such as
119 *L. oculatus*, *R. glesne* and *Cetomimus* sp. The two lampriform fishes (*R. glesne* and *L. guttatus*) stand out
120 from other fishes in TE composition, and from each other as well. Overall, the large differences in TE
121 composition among and sometimes within teleost families highlight the dynamic nature of TEs during
122 teleost evolution.



123
 124 **Figure 1.** Transposable element (TE) content in 101 fish genomes. The phylogeny is the same as shown in Musilova et al. 2019. Stacked colored
 125 bars show the relative proportions of TEs; LTR retrotransposons (orange), LINEs and SINEs (blue) and DNA transposons (red). Boxes between
 126 the stacked bars and species names are colored according to assembly size, serving as a proxy for genome size. The light blue and dark blue outer
 127 bars show the genomic percentage of classified TEs and all interspersed repeats (unclassified repetitive elements) respectively, with the longest
 128 bars (*D. rerio*) representing 48.0% classified TEs or 55% interspersed repeats, and the shortest (*T. nigroviridis*) representing 4.2% classified TEs.
 129 Some taxonomic clades are named.

130
 131 *Interplay between genome size, repetitive DNA and other factors*

132 We performed phylogenetic generalized least square (PGLS) regression to test if genome assembly size
 133 was correlated with the TE and STR content of the assemblies, while taking the phylogenetic

134 relationships among samples into account, as well as the aquatic habitat and degree of parental care. The
135 correlation between the number of TEs and genome assembly size (R^2 : 0.72, p value: $< 2.2e^{-16}$, Figure 2a)
136 was stronger than between the genomic proportion of TEs and genome assembly size (R^2 : 0.15, p value:
137 $3.6e^{-5}$, Figure 2c), the latter being a more direct measure of the influence on genome size. The number of
138 STRs displayed a positive correlation (R^2 : 0.41, p value: $5.9e^{-13}$, Figure 2b), but the genomic proportion of
139 STRs appeared to have a negative relationship with genome assembly size. This apparent relationship did
140 not reach a significance threshold of 5% for a linear relationship (R^2 : 0.022, p value: 0.078, Figure 2d).
141 As the local regression resembled a negative exponential relationship, we \log_{10} -transformed the STR
142 content of the assembly and repeated the test, yielding a weak linear negative correlation (R^2 : 0.044, p
143 value: 0.021). We continued using \log_{10} -transformed STR content in all other tests. For all tests with
144 genome assembly size as a response, we omitted the Atlantic salmon (*Salmo salar*) and zebrafish (*Danio*
145 *rerio*), as both drastically impacted the regressions when included (plots including these species can be
146 viewed in Supplementary Figure 1). Although the variation seen in *S. salar* and *D. rerio* is biologically
147 meaningful, *S. salar* is an extreme outlier in terms of genome size (~ 3 Gb) due to a salmonid-specific
148 whole genome duplication and the *D. rerio* assembly has a N50 of > 1 Mb, while no other assembly
149 reached a N50 of 100 Kb. We did not find any correlation between the genomic proportion of TEs and the
150 genomic proportion of STRs. Next, we modelled genome size as a response to TE content, (\log_{10}) STR
151 content, aquatic habitat (marine/freshwater), degree of parental care. To control for differences in
152 assembly quality, we included assembly quality metrics as covariates. We used data on aquatic habitat
153 and parental care from FishBase (Froese & Pauly 06/2018), where the degree of parental care is defined
154 according to (Balon 1990). We grouped fish that carries eggs in their mouth or body (bearers) and that
155 guard their eggs in nests or similar (guarders) together. In the full model (Table 1), aside from TE content
156 having a significant positive effect (p value: $5.5e^{-7}$) on genome assembly size and (\log_{10}) STR content
157 having a significant negative effect (p value: 0.02), non-guarding behavior was positively correlated to
158 genome size (p : 0.007), while the marine habitat did not have a significant contribution (p value: 0.7). The

159 assembly quality metrics N50 and gene completeness had small, but significant effects on the regression.

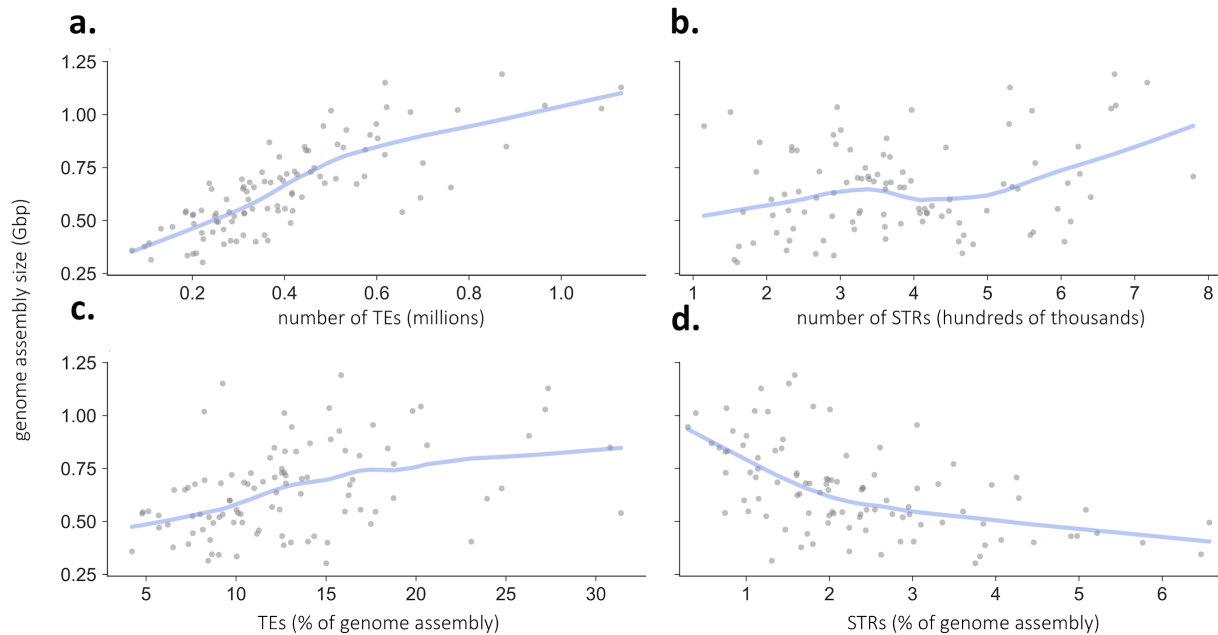
160 In total, the significant variables explained 47.5 % of the variation in genome assembly size.

161

162 **Table 1.** Phylogenetic generalized linear model of genome assembly size. Genome assembly size is modelled as a response to the percentage of
 163 genomic TE, the (\log_{10}) genomic percentage of STRs, whether or not the fish species is guarding its eggs, aquatic habitat, and genome assembly
 164 metrics (N50 and BUSCO single copy gene completeness).

	Explanatory variable	Estimate (Mbp)	Std. error	<i>t</i> value	<i>p</i> value
Repetitive elements	percentage TEs	22.0	3.9	5.6	$5.5e^{-7}$
	\log_{10} percentage STRs	-238.4	102.6	-2.3	0.02
Ecological factors	non-guarding behavior	115.6	41.5	2.8	0.007
	marine habitat	-24.4	60.5	-0.4	0.7
Assembly quality metrics	N50 contig	-0.003	0.001	-2.5	0.01
	gene completeness	0.1	0.03	4.6	$2.5e^{-5}$

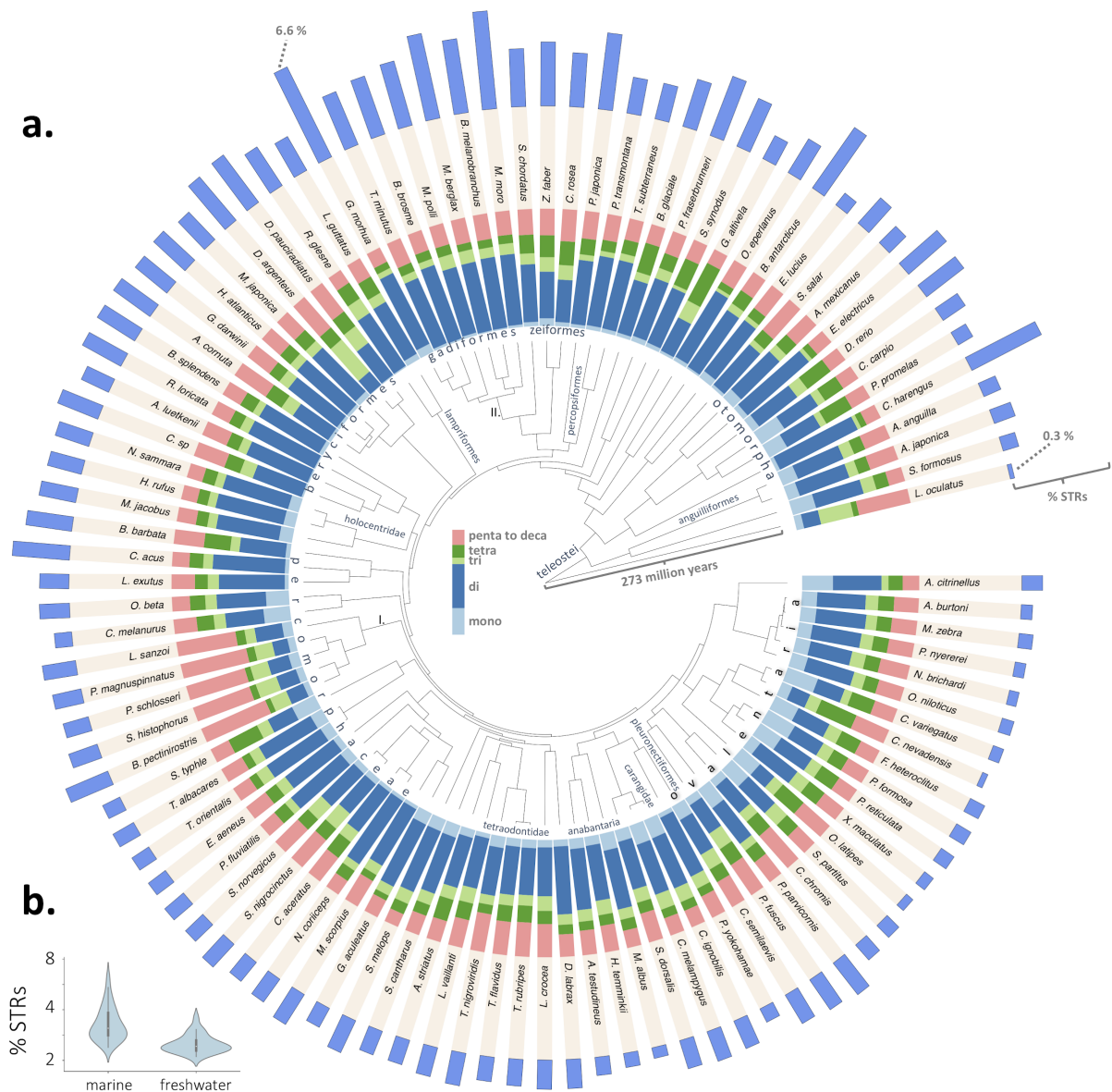
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166

167 **Figure 2.** Correlations between repetitive DNA and genome assembly size. The number of classified transposable elements (TEs) (a), the number
 168 of short tandem repeats (STRs) (b), the proportion of classified transposable elements (c) and the proportion of STRs (d) covering teleost fish

169 genome assemblies. The light blue lines show the local regression. Plots including Atlantic salmon (*S. salar*) and zebrafish (*D. rerio*) can be seen
170 in Supplementary Figure 1
171
172 STR variation across teleost lineages linked to aquatic habitat
173 Our STR annotation efforts show that there is high variability in STR content within teleost fish, both
174 with respect to total STR content and relative differences of STRs with different unit sizes (Figure 3a).
175 One striking pattern is the proportion of STRs with unit size 5-10 in Gobiidae (*Chatrabas melanurus*,
176 *Lesueurigobius cf. sanzoi*, *Periophthalmus magnuspinnatus*, *Periophthalmodon schlosseri*, *Scartelaos*
177 *histophorus* and *Boleophthalmus pectinirostris*), more specifically decanucleotide repeats. Suspecting that
178 this might be an artifact, we looked at Gobiidae tandem repeats with unit sizes from 1 to 20, and found
179 that the high proportion of decamers actually represents a high proportion of k-mers with unit sizes 10-20
180 (mostly 11-mers), which likely confuses the repeat detection algorithm (Phobos) when repeats are
181 interrupted. Why Gobiidae have such a unique STR landscape compared to other teleosts requires further
182 investigation. Using PGLS, we found a significant difference between marine and freshwater fish in STR
183 content ($p: 0.0003$, Figure 3b), supporting the tendency found in Yuan et al. (2018). The association was
184 robust to removal of the whole Ovelentaria clade, which mainly contain freshwater fish. We noted that
185 families within the codfishes (*Gadidae*, *Lodidae*, *Merluccidae*, *Macrouridae*, *Bathygadidae* and *Moridae*)
186 have particularly high STR content, compared to the other species. By annotating additional codfish
187 assemblies (from Malmstrøm et al. 2016, 2017) we found that extreme STR propagation is common
188 within this lineage (Figure 4).
189



190

191 Figure 3. Lineage-specific variation in STRs linked to habitat. (a) Short tandem repeat (STR) content in the genomes of 100 teleost fish and one

192 non-teleost (*L. oculatus*). The phylogeny is the same as in Figures 1. The stacked bars show the relative distribution of STRs grouped by unit size

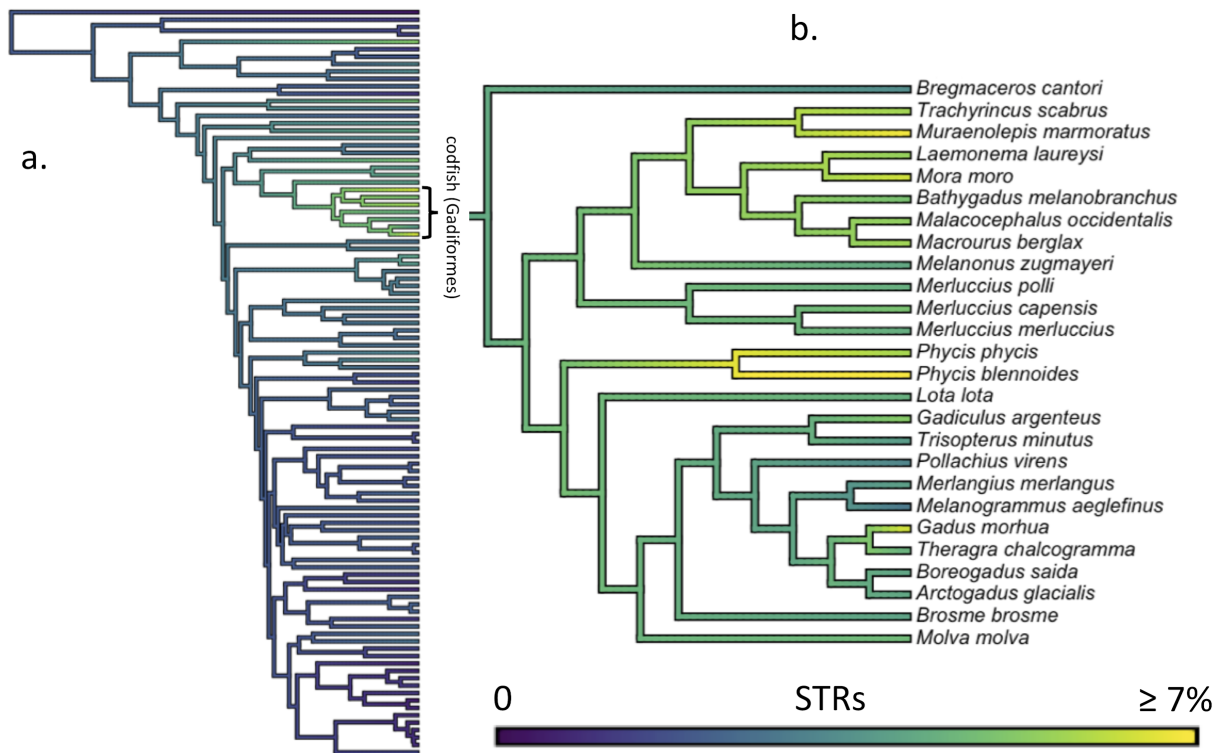
193 (from mononucleotide repeats with unit size of one to four, and for clarity, grouped data for STRs with unit sizes from five to ten). The outermost

194 bars show total STR content, with the highest bar (Atlantic cod, *Gadus morhua*) representing 6.6% STR content (with the Gadiformes in general

195 having high STR content) and the lowest bar (spotted gar, *Lepisosteus oculatus*) indicating 0.3% STR content. Gobiidae (I.) and Gadiformes (II.)

196 are highlighted as they are mentioned in the main text. (b) Violinplots showing the difference in STR content between marine and freshwater

197 genomes.



198

199 Figure 4. STR content in the codfish lineage. (a) The 101 species phylogeny from Musilova et al. 2019, with the codfish (Gadiformes)

200 highlighted. Branches are colored by maximum likelihood ancestral state estimates of genomic STR percentage. (b) Additional codfish species

201 depicting the phylogeny of Gadiformes from Malmstrøm et al. (2016). Extreme STR values are found within this order, exceeding 9.5% in

202 greater forkbeard (*Phycis blennoides*). For clarity, the maximum cutoff was set to 7%.

203

204 *TE content not positively correlated with net diversification*

205 To test if repetitive DNA content within the genomes of teleost families are associated with net

206 diversification rates, we used estimates from Scholl and Wiens (2016) where family-specific net

207 diversification rates were calculated across the tree of life, including 45 out of 71 of our surveyed teleost

208 families (see Material and Methods). Family-specific net diversification estimates were regressed on

209 median TE and STR content per family, as well as median genome assembly size. We used median

210 instead of mean TE proportions per family to avoid that extreme outliers, such as the zebrafish, distort the

211 numbers for individual families. PGLS regressions showed that genome assembly size does not correlate

212 with diversification (Table 2, Figure 5a). We found no significant correlation between net diversification

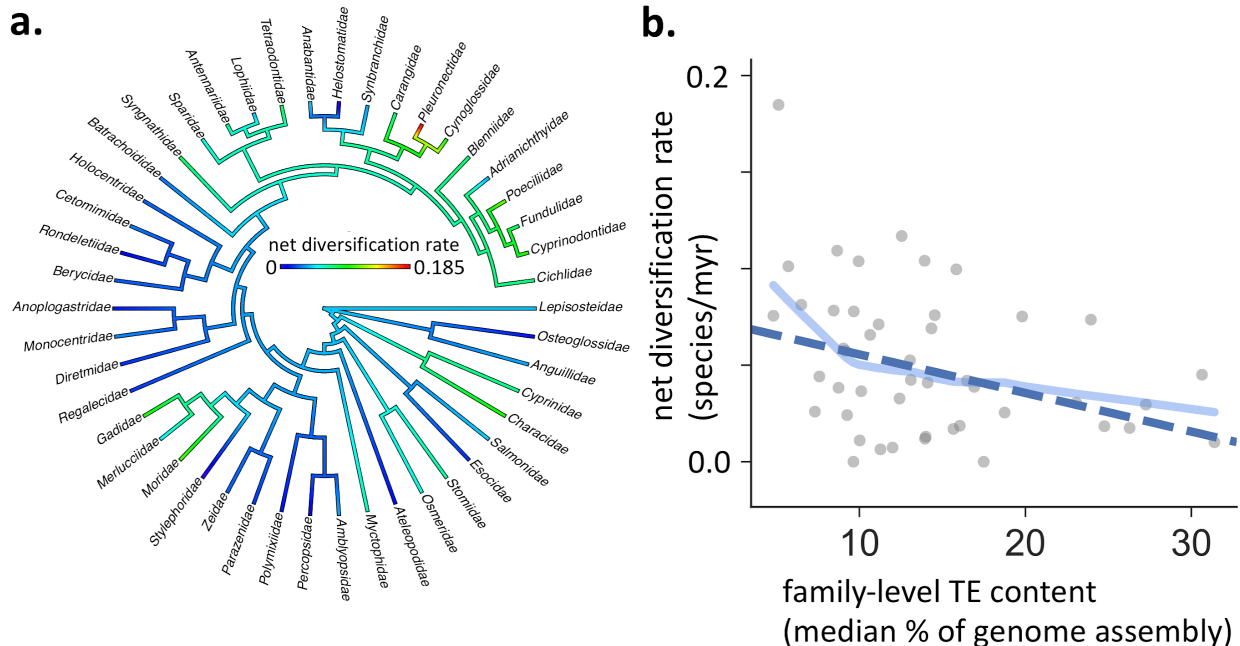
213 and STR content (Table 2, Figure 5b). For TEs, we did not find support for a positive correlation, but

214 rather a weak negative correlation between net diversification rates and both only classified TE content
 215 (R^2 : 0.07, p value: 0.015, Figure 5c). The correlation remained significant after removing Pleuronectidae,
 216 which in our dataset is an outlier in terms of net diversification rate (classified TE content: R^2 : 0.07, p
 217 value: 0.019). Tests were repeated using net diversification rates based on different assumed extinction
 218 rates (0.1, 0.5 and 0.9), and with total amount of interspersed DNA instead of the percentage of classified
 219 TEs, which had negligible impacts on the results.

220

221 **Table 2.** Phylogenetic generalized linear model of family-specific net diversification rates with an assumed extinction rate of 0.5. The median net
 222 diversification rate per family is modelled as a response to the median family-specific genomic percentage of TEs, the (\log_{10}) genomic proportion
 223 of STRs and genome assembly size.

Explanatory variable	Estimate	Std. error	t value	p value
Percentage TEs	-0.0024	$9.4e^{-4}$	-2.54	0.015
\log_{10} percentage STRs	-0.0063	0.025	-0.25	0.80
Genome assembly size	$1.6e^{-5}$	$1.6e^{-5}$	0.96	0.34



224

225 Figure 5. Net diversification rates of teleost fish families ($n = 45$) do not show a positive correlation with median TE content. (a) Family-specific
 226 net diversification rates as estimated by Scholl and Wiens 2016. (b) Phylogenetic generalized between median TE content and median net
 227 diversification rate. (d) The light blue lines show the local regression and blue dashed lines show the PGLS regressions.

228

229 **Discussion**

230 Using a time-calibrated phylogeny we have investigated the genomic repeat landscape across the teleost
231 radiation. Overall TE content was not positively associated with net diversification, but significantly
232 contributes to genome size variation. Genome size variation was also explained by the genomic
233 percentage of STRs and the degree of parental care. High STR content was associated with smaller
234 genomes, marine habitat and high fecundity (such as codfish and Atlantic herring). We do not observe the
235 same patterns of STR and TE propagation in teleost lineages, pointing towards independent evolutionary
236 paths for these types of repeats.

237

238 Our results on the contribution of TEs to genome size variation (Figure 3a-c) support the general tendency
239 observed in chordates (Canapa et al. 2015), vertebrates (Chalopin et al. 2015) and previous studies of
240 teleosts (Yuan et al. 2018). There was, however, large variance, resulting in a fairly low R^2 of 0.15 (for
241 classified TEs only) and 0.25 (for all interspersed repeats, Supplementary Figure 3). This shows that in
242 teleosts, differential abundance of TEs alone might explain 15% to 25% of the variance in genome size,
243 when the phylogenetic relationship between samples is taken into account. Our model that included STR
244 content, genome assembly quality metrics, and also parental care, which previously have been linked to
245 genome size differences in teleosts (Hardie & Hebert 2004), explained 47.5% of the genome size
246 differences in our samples. Given that the extent of parental care and egg size are positively correlated
247 (Kolm & Ahnesjö 2005), and egg size is positively correlated with genome size (Hardie & Hebert 2004),
248 we expected to find a positive correlation between parental care and genome size. Contrary to
249 expectation, non-guarding behavior had an overall positive contribution to genome size variability.
250 Further, a marine environment did not explain any difference in genome size when the phylogenetic
251 relationship was taken into account.

252

253 In comparison, STR content was significantly higher in marine fish (Figure 4), with the most extreme
254 being the codfish (Figure 5). Given the current understanding of STRs as hypervariable regions with
255 occasional functional impact, we speculate that marine species with high fecundity and high mortality of
256 eggs (Duarte & Alcaraz 1989), more robustly tolerate the mutational load of STRs, which is likely
257 substantial. Theory predicts (Nei 2013; Graur 2017) that the number of offspring an individual on average
258 needs to produce to keep the population size constant is a function of the deleterious mutation rate and the
259 number of functional mutable sites. It is likely that STRs increase the deleterious mutation rate and that
260 the extent would depend on the STR mutation rate and the fraction of STRs in functional regions. This
261 could serve as an explanation for why we see elevated STR propagation in marine clades, i.e., fish with
262 more numerous eggs, compared with freshwater fish. In particular, of species with available fecundity
263 estimates (scaled for body size), *G. morhua* and *C. harengus* have the highest fecundity in our dataset
264 (Barneche et al. 2018) and also stand out as having high STR content.

265
266 We show that DNA transposons are the most common TEs in teleosts (Figure 2), confirming the pattern
267 observed in other studies. Overall, variation is high across lineages and indicates substantial TE activity
268 over 270 million years of evolution. As elevated TE activity has been shown to coincide with teleost
269 species radiations, such as in salmonids (de Boer et al. 2007) and cichlids (Salzburger 2018; Brawand et
270 al. 2014), and in light of the ongoing discussion of the role of TEs in evolution (Brunet & Doolittle 2015;
271 Doolittle & Brunet 2017; Doolittle & Sapienza 1980), a main objective of this study was to investigate if
272 clades with high TE content have had comparably high net diversification. The test relies on the
273 assumption that a fish family with a high proportion of repetitive elements in their genomes is likely to
274 have had more propagation of repetitive elements than a fish family with a low proportion. Our results do
275 not support that high TE content is linked to higher net diversification rates, but rather show mild support
276 for the contrary (Figure 4a), and we see no apparent pattern with regard to the effect of genomic STR
277 proportions or genome size, at least across our broad selection of teleostean families. This does not rule
278 out that TE insertions can lead to novel adaptive traits, and might facilitate diversification in certain

279 teleost clades, as indicated in studies of African cichlids (Brawand et al. 2014; Santos et al. 2014;
280 Salzburger 2018). However, a general speciation promoting role for TEs is not reflected in our results.
281
282 Throughout the study, we assessed TE content to be the sum of interspersed repetitive elements judged by
283 our tools (BLAST, BLASTX and HMMR) to be a TE. The classification process is limited by the extent
284 of prior annotated TEs, which in teleosts are biased towards *D. rerio*. This is illustrated by the values
285 obtained from zebrafish, that has the most extensive prior annotation, and the percentage of classified TEs
286 is (48.0 %) is very close to the total interspersed repeats (52.2 %), which is not the case for most other
287 surveyed fish (see Figure 1). The total amount of repetitive elements includes all classified TEs, non-
288 classified TEs, and some sequences that may not be TEs (but occur in multiple loci). Including this
289 measure, as we did in the test with diversification rates, could serve as a less biased estimate of total TE
290 content. Either way, the amount of detected sequences is strongly correlated with the amount of classified
291 sequences (PGLS R^2 : 0.69, Supplementary Figure 2). The detection of interspersed repeats is not biased
292 by *a priori* information, but can be influenced by assembly quality (Treangen & Salzberg 2011; Simpson
293 & Pop 2015). However, in our models that include multiple covariates, we found that two common
294 assembly quality metrics; contig N50 and gene completeness did not impact our conclusions. It should
295 further be noted that genomes inhabiting high numbers of identical TEs (i.e. families that recently
296 expanded) are expected to be harder to assemble, as identical sequences create collapsed repeats. This can
297 lead to an underreporting of elements in genomes with recent expansions. It is also known that high STR
298 content in combination with short read sequencing can produce assemblies of lower quality, reported in
299 the sequencing efforts of the Atlantic cod (*G. morhua*) genome (Tørresen et al. 2017). This implies that
300 assemblies with low assembly quality likely are underestimated with regards to STR content.
301
302 Regardless of some limitations, our results suggest that high proportions of TEs are not positively
303 correlated with net diversification rates in teleost clades, and that elevated levels of STRs are linked to
304 and must thus be tolerated by marine teleosts, potentially due to higher fecundity. Such a link would be

305 very important for understanding genome evolution, but needs to be further investigated within teleosts,
306 as well as in other organism groups.

307

308 **Material and Methods**

309 *Genome assemblies and phylogenies*

310 Most genome assemblies were retrieved from a recent teleost genome data release (Malmstrøm et al.
311 2017), and additional assemblies were sequenced and assembled by Musilova et al. 2019, which also
312 released the 101-species phylogeny. Some genome assemblies were retrieved from ENSEMBL and
313 NCBI. For an overview of assembly origins, see Musilova et al. 2019 and Supplementary Table 1. The
314 codfish phylogeny was taken from Malmstrøm et al. 2016. Details regarding the phylogeny construction
315 can be found in these respective studies.

316

317 *TE and STR annotation*

318 For TE annotation, we used a variant of the computational pipeline that is more thoroughly described in
319 (Tørresen et al. 2017), available at <https://github.com/uio-cels/Repeats>. The pipeline includes multiple TE
320 detection steps using different tools, steps for removing non-TEs from the detected sequences and steps
321 for classifying the elements. For the initial detection step, we used RepeatModeler (v. 1.0.8) (Smit &
322 Hubley 2008-2015) and LTRharvest (part of GenomeTools v. 1.5.7) (Ellinghaus et al. 2008).

323 RepeatModeler detects all sorts of repetitive sequences and LTRharvest is specialized for detecting LTR-
324 RTs. Using BLASTX, TEs with sequences matching known non-TEs in UniProtKB/Swiss-Prot were
325 removed. To classify the TEs, we used RepeatClassifier, which is a part of the RepeatModeler software.

326 As the tool did not manage to classify all of the remaining sequences, additional similarity searches were
327 performed between the sequences and a curated library of TE sequences (RepBase v. 20150807), using
328 nucleotide BLAST. Finally, we built Hidden Markov Model profiles from the detected sequences using

329 HMMER (v. 3.1b1) (Wheeler & Eddy 2013) and compared the profiles with HMM profiles from

330 databases downloaded from GyDB.org (Llorens et al. 2011) and dfam.org (Hubley et al. 2016), using the

331 nhmmer feature included in HMMER. This resulted in additional sequences being classified at the class
332 and subclass level. We merged the *de novo* TE library with a library of known eukaryotic TEs (RepBase)
333 and used this as input for RepeatMasker (v. 4.0.6), run with the -s (sensitive) option. The .out and .tbl
334 files produced by RepeatMasker served as the basis for the downstream analysis, performed using custom
335 Python scripts. For detection of short tandem repeats we used Phobos v3.3.12 (Mayer et al. 2010) to
336 detect all STRs with unit size 1–10 bp in the genome assemblies. The output was in Phobos native format
337 which was further processed with the sat-stat v1.3.12 program, yielding files with statistics and a GFF
338 file. Other options were set as in Tørresen et al. (2017). For the gobiidae genomes, we ran Phobos with
339 unit sizes 1-20 bp.

340

341 *Diversification rates*

342 We retrieved estimates of net diversification rates from Scholl and Wiens (Scholl & Wiens 2016), who
343 calculated diversification rates based on the stem ages of teleost families from the teleost phylogenetic
344 tree produced by Betancur-R et al. (Betancur-R. et al. 2013). They used the method-of-moments estimator
345 as described by Magallon and Sanderson (Magallón & Sanderson 2001);

346

$$347 \quad r = \frac{1}{t} \log(n(1 - \varepsilon) + \varepsilon) \quad (1)$$

348

349 where r is the net diversification rate estimate, t is the family stem age, n is the number of extant species
350 and ε is the relative extinction rate. ε is included to correct for unsampled, extinct clades. The estimates
351 used in this study are based on the r values when ε was set to 0.1, 0.5 and 0.9. Note that more recent
352 diversification estimates are available (Rabosky et al. 2018), but cover only marine fish.

353

354 *Comparative phylogenetic analyses*

355 Statistical analysis was performed using phylogenetic least-squares (PGLS) regressions using the R
356 package ‘caper’ v. 1.1.0 (Orme et al. 2012). PGLS is a commonly used method for incorporating
357 phylogenetic information in the modelling of associations between traits. PGLS assumes that more
358 closely related species have more similar traits and uses the expected covariance structure to modify the
359 slope and intercept estimates. For tests with net diversification rates we used a pruned phylogeny
360 containing tips representing teleost family stem ages, and used median values per family for all
361 covariates. In all tests, we optimized branch length transformations using maximum likelihood. LOWESS
362 (locally weighted linear regressions) lines were created using the ‘seaborn’ Python package with the
363 ‘regplot’ function and standard parameters.

364

365 *Code and data availability*

366 Summaries of the annotation of TEs and STRs, along with the ecological data, are in Supplementary
367 Table 1. Species-specific annotations of TEs and TE-derived DNA can be found at:
368 <https://doi.org/10.6084/m9.figshare.8280800> (~4.6 Gb). The R script used for statistical analysis, can be
369 found at <https://github.com/uio-cels/teleost-repeats>.

370

371 **Acknowledgments**

372 The authors would like to thank Jostein Starrfelt, Masahito Tsuboi and Kjetil Lysne Voje (CEES,
373 University of Oslo) for conceptual input regarding diversification rates. All computational work was
374 performed on the Abel Supercomputing Cluster (Norwegian metacenter for High Performance Computing
375 (NOTUR) and the University of Oslo) operated by the Research Computing Services group at USIT, the
376 University of Oslo IT-department (<http://www.hpc.uio.no/>). Sequencing library creation and high
377 throughput sequencing was carried out at the Norwegian Sequencing Centre (NSC), University of Oslo,
378 Norway. This research was supported by the Norwegian Research Council under the projects “Functional
379 and comparative immunology of a teleosts world without MHC II (#222378)” and “Evolutionary and
380 functional importance of simple repeats in the genome (#251076)” both led by KSJ. We have adhered to

381 all local, national and international regulations and conventions, and we respected normal scientific
382 ethical practices.

383

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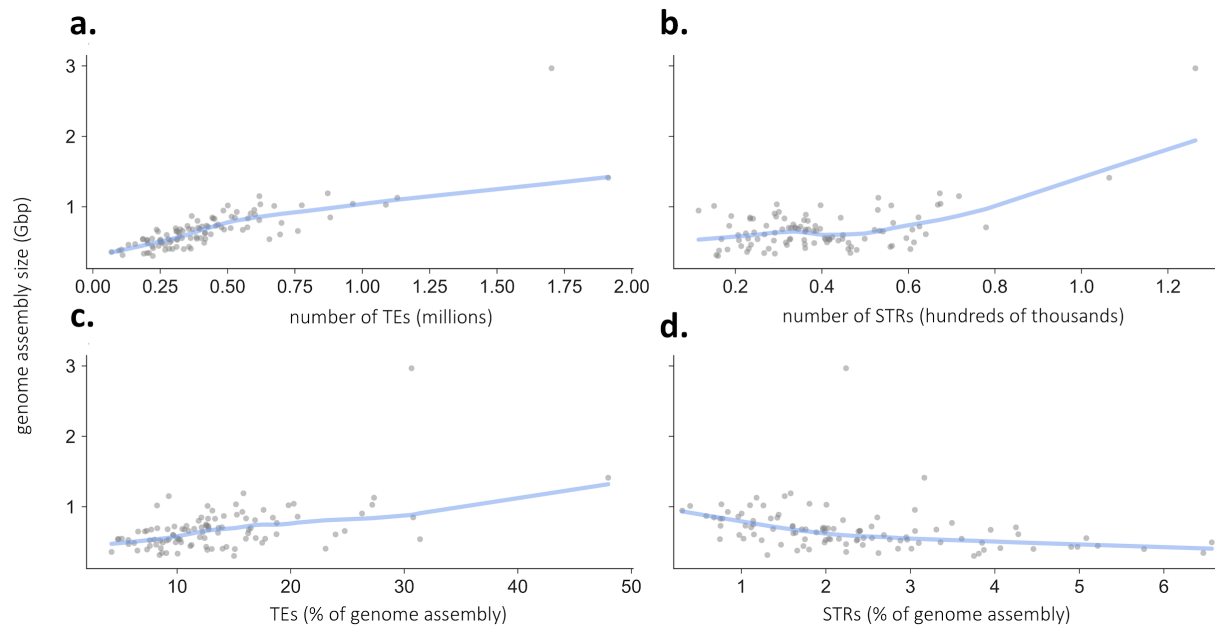
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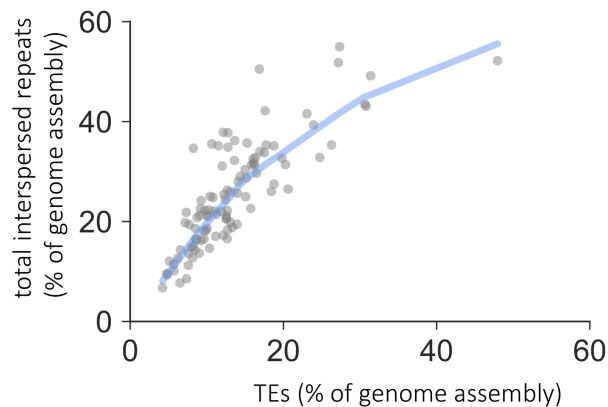
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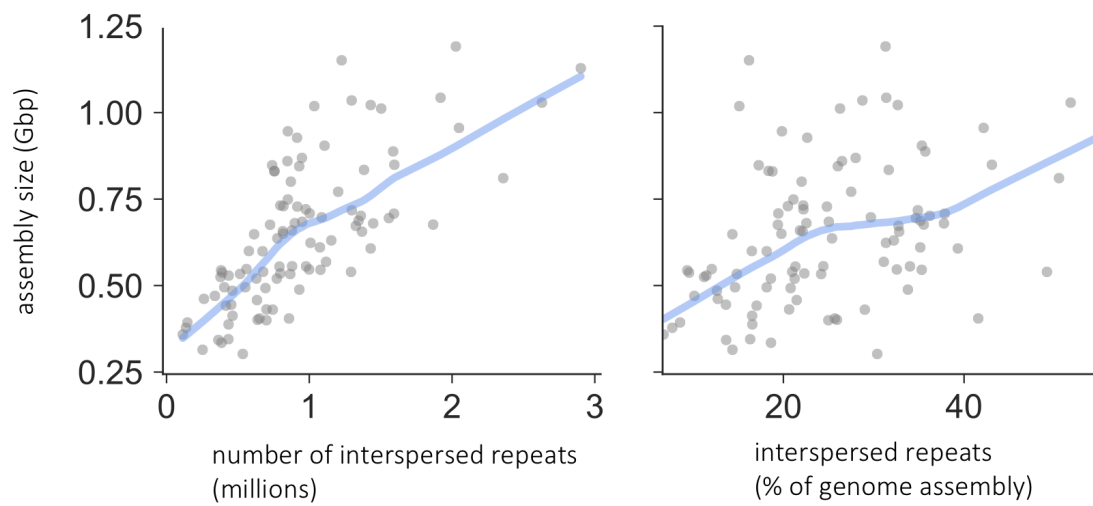
Supplementary Figures



Supplementary Figure 1. As Figure 1, with Atlantic salmon (*S. salar*) and zebrafish (*D. rerio*) included.



Supplementary Figure 2. The relationship between the genomic percentage of total interspersed repeats and the percentage of elements classified as TEs.



Supplementary Figure 3. Associations between the number of interspersed repeats (left) and the genomic percentage of interspersed repeats (right) with genome assembly size.