

1 **Population genomics reveals extensive inbreeding and purging of mutational load in**
2 **wild Amur tigers**

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23 **Abstract**

24 The inbreeding is a big threat for the persistence of genetic diversity in small and isolated populations of
25 endangered species. The homozygous genome could exacerbate inbreeding depression by introducing homozygous
26 deleterious alleles in the population. However, purging of inbreeding loads as they become homozygotes in small
27 populations could alleviate the depression. The Amur tiger (*Panthera tigris altaica*) is typically exists in small
28 population living in forests in Northeast Asia and is among the most endangered animals on the planet with great
29 symbolic significance of conservation. By comparing with captive individuals, we revealed substantially higher
30 and more extensive inbreeding in the wild Amur tiger population ($F_{ROH}=0.51$) than in captive Amur tigers
31 ($F_{ROH}=0.26$). We further found much less mutational loads in wild populations when compared with captive Amur
32 tigers. However, the frequency of loss of function and deleterious nonsynonymous mutations inside ROH regions
33 are much lower than that in non-ROH regions in both wild and captive Amur tigers, indicating the purging may
34 had occurred in both populations but much effective in the wild population. In addition, we found the average
35 frequency of deleterious alleles was much lower than that of neutral alleles in the wild population, indicating that
36 the purifying selection contributed to the purging of mutational loads in the wild Amur tigers. These findings
37 provide valuable genome-wide evidence to support the making of future conservation plans of wild Amur tigers.

38 **1 Introduction**

39 Human-induced habitat fragmentation and environmental changes make a large proportion of biodiversity
40 persisted in small and isolated populations[1-4]. The genetic diversity of a species is of importance for sustaining

41 its evolutionary potentials, which could help it resist the impact from environment changes, thereby reducing the
42 risk of extinction[5, 6]. The genetic viability in small population is highly sensitive to the genetic drift and
43 inbreeding, which could increase the genome homozygosity and then exacerbate the population depression by
44 introducing homozygous mutational loads[7-10]. Genetic rescue is a common strategy to increase genetic diversity
45 and decrease the deleterious effects by building wildlife corridors or reintroduction of captive individuals to the
46 wild to facilitate gene flow[11-13]. However, planning genetic rescue highly depends on the comprehensive
47 investigation of genetic backgrounds, including the inbreeding, outbreeding and introduction of deleterious
48 mutations [14-17], and otherwise may introduce additional genetic loads and/or result in outbreeding depression
49 that further impair the already weak genetic basis.

50 Purifying selection acts on the small population could help purge deleterious alleles as they become homozygotes,
51 and thereby reducing the inbreeding depression in endangered species[14, 18]. Another question is that whether a
52 small population is undergoing the genetic purging? Or, whether the purging in a population is effective enough to
53 remove deleterious alleles to increase the fitness potential? Although current studies showed that the purging in
54 some model organisms could be very effective to reduce inbreeding depression[19, 20], purging is not always
55 existed or strong enough to alleviate the depression[4, 17, 21]. Therefore, the purging is also a key intrinsic genetic
56 factor that was needed to be investigated for small population to help plan the genetic rescue strategies.

57 The Amur tiger (*Panthera tigris altaica*) is among the most endangered big cats on the planet with great symbolic
58 significance of conservation[22] and has been prioritized for conservation for decades. The wild population has
59 fragmented into three subpopulations, including the main one in the Sikhote-Alin Mountain containing ~95%
60 (415~490) individuals, the small one in the southwest Primorsky Krai of Russia with ~20 individuals, and a
61 subpopulation in northeast China connecting with the two ones in Russia with ~60 tigers[3]. Previous investigation
62 using microsatellite markers showed that the China subpopulation has been moderately to highly inbred,
63 suggesting a risk to lose genetic diversity rapidly[23]. This became a big concern for its sustainability. On the other
64 hand, China has established a captive population as *ex situ* conservation resource in northeast area[24]. Population
65 genomic analysis showed captive Amur tigers has slight inbreeding level with no apparent inbreeding
66 depression[25]. It has been proposed recently to apply genetic rescue to ameliorate the inbreeding of the wild
67 tigers by introducing captive genes based on the knowledge that introducing genetic variations from large and
68 health populations can effectively improve genetic diversity and survival of small populations[11, 26, 27] and
69 consideration of implications from successful cases[11, 28, 29]. Thus, the Amur tiger becomes an ideal model to
70 tactically investigate the inbreeding, outbreeding, introduction of deleterious mutation and genetic purging to
71 support future genetic rescue plans.

72 Although molecular markers such as mitochondrial DNA sequence and polymorphic microsatellites have been
73 used to estimate these genetic indices, they are obviously limited in accuracy and informativeness, particularly for
74 evaluation of inbreeding, local adaptation, mutational load and purging, which were necessary to guide reasonable
75 genetic rescue. In contrast, whole genomic analysis can not only substantially improve the accuracy of the above-
76 mentioned genetic indices, but also quantify the extent of inbreeding, outbreeding, purging and accumulation of
77 mutational loads *etc.*[10, 30, 31]. Particularly, evaluating inbreeding by measuring runs of homozygosity (ROH) is
78 highly dependent on the contiguity of reference genome, because the ROH in small populations with high degree
79 of inbreeding is often spanning over several millions of base pairs[10, 31, 32], which can either hardly be detected
80 or bias the data by using fragmented genomes assembled by short reads. Additionally, hidden ROHs that will be
81 introduced into the population by future inbreeding several generations later can be also detected if the hybrid
82 genome could be phased into haploid genomes, which is of vital importance to predict the future inbreeding. Under
83 the background of rapid advancement of sequencing technology, decreasing cost, advanced computational power,

84 as well as the transition from conservation genetics to conservation genomics^{41, 43, 44, [33]}, high-quality and
85 haplotype-resolved reference genomes are now more crucial and urgent than ever before for supporting
86 conservation[34].

87 In order to precisely reveal the intrinsic genetic factor we mentioned above in wild Amur tigers to improve the
88 protection and conservation for this big cat, we here present a chromosome-scale and haplotype-resolved genome
89 for a wild Amur tiger, representing the highest quality reference genome for the Amur tiger up to date. Based on
90 the genome, we performed comparative population genomic analysis between the wild and captive Amur tigers,
91 providing the first insights into the genetic makeup, inbreeding impacts, local adaptation, genetic load dynamics
92 and purging effect of the wild population, providing strong implications for future genetic rescue of this species.

93 **2 Results**

94 **2.1 Haplotype-resolved genome for the Amur tiger**

95 We generated a high-quality and chromosome-scale HiFi genome (hereafter PtaHapG) for the Amur tiger,
96 representing the first haplotype-resolved reference genome of the tiger. The hybrid genome size of the Amur tiger
97 here is 2.44Gb, representing 98.75% of the estimated genome size. The contiguity of the this genome was very
98 high with the NG50 being of 147.84 Mb, and more than 2.43 Gb (~99.68%) scaffolds were anchored to 19
99 pseudochromosomes (Fig. 1A), which is consistent with the karyotypic results of the tiger and other Felidae
100 animals [35-37]. Besides, we identified the complete X chromosome and a 10.87 Mb Y-linked region in the tiger
101 genome by multiple lines of evidence (Fig. 1B, C). We simultaneously resulted two groups of haplotigs for the
102 tiger genome (here after PtaHapGH1 and PtaHapGH2) (Fig. 1A). Merqury k-mer analysis showed high
103 completeness of the haplotype-resolved genome, and meanwhile with low level of artefactual duplication (Fig. 1D).
104 Base-level quality evaluation with QV scores, Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis,
105 genome mapping rate of short reads and structural-level accuracy evaluation all supported high completeness and
106 accuracy of genome assembled in this study. We also found a high collinearity among the tiger and domestic cat
107 genome, without any fissions and fusions among these three genomes. We are confident that the reference genome
108 for the Amur tiger here is superior to most big cat genomes ever published in contiguity, completeness, and
109 accuracy.

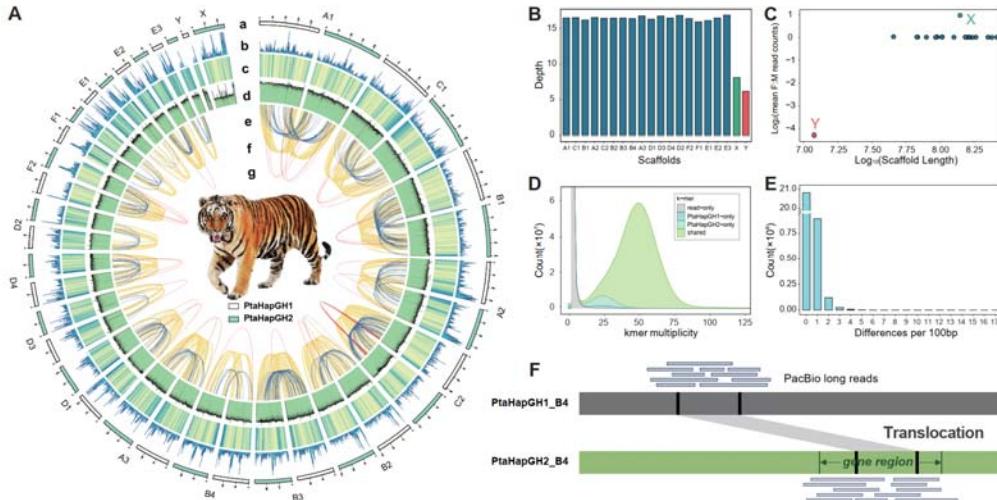
110 We then identified 863.42 Mb (35.46%) repeat elements in the PtaHapG, with the LINE being the most abundant
111 repeat element, making up 28.96% of the genome. We predicted 19,786 gene models in the PtaHapG by
112 integrating *de novo* prediction, homology-based protein alignment and RNA-seq mapping evidence. Gene regions
113 are spanning over 762.06 Mb, making up 31.29% of the genome. The BUSCO analysis showed high completeness
114 of gene sets for all three genomes, with the lowest BUSCO score to be 95.3%. Finally, 19,771 (99.92%) genes
115 were functionally annotated in the PtaHapG. In addition, we predicted 1128 rRNA, 1236 miRNA, 354,836 tRNA,
116 1647 snRNA in this genome.

117 **2.2 Comparison of the two haploid genomes of the Amur tiger**

118 In general, two haploid genomes of the PtaHapG were found to be very similar with nearly the same length, GC
119 content, gene density, *etc.* (Fig. 1A). The single peak k-mer spectra plot and uniform reads mapping patterns to
120 haplotype genomes indicated that we accurately generated haploid assemblies with scarce of haplotype-specific
121 sequences (Fig. 1D). Clear one-to-one synteny blocks between the homologous chromosome pairs between
122 haploid genomes also showed the expected high homozygosity for the PtaHapG. The frequency of sequence
123 differences between the two haplotypes within 100bp windows showed one peak in histogram for the PtaHapG
124 (Fig. 1E), further indicating the high similarity between haploid genomes of the Amur tiger.

125 Nonetheless, we still found some chromosomal structural variants (>50bp) between PtaHapGH1 and PtaHapGH2, .

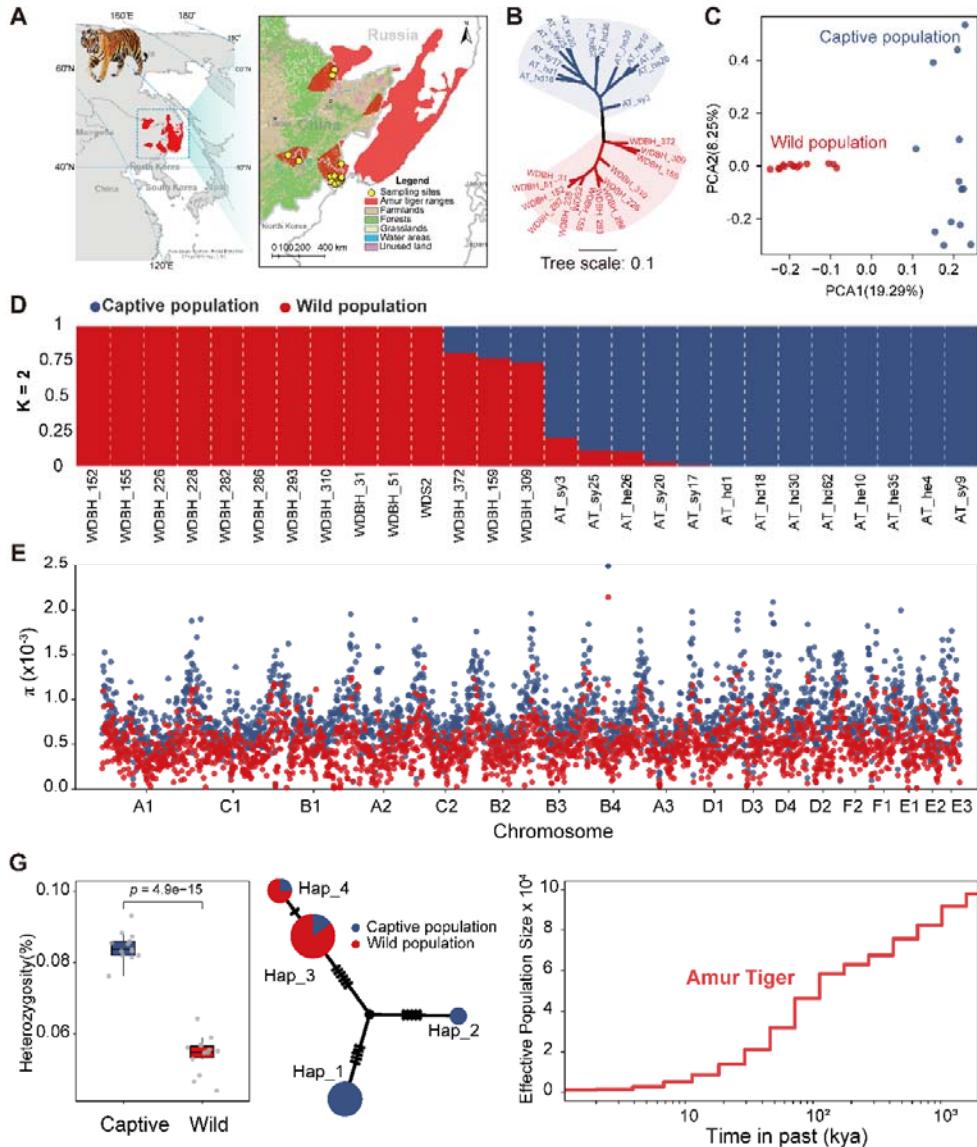
126 In general, we found 4463 (2067 deletions, 424 duplications, 34 translocations and 1938 inversions) structural
127 variants in the PtaHapG (Fig. 1A). All these structural variants were supported by genome mapping of both PacBio
128 long reads and DNBSEQ short reads (Fig. 1F). 2163 genes were distributed in the structural variants of PtaHapG,
129 with 1779 genes spanning over the breakpoints of SVs. However, we didn't find any loss of function genes
130 affected by SVs in the TG, further indicating the high similarity between haploid genomes.



131
132 **Fig. 1| Genomic landscapes and haploid genome characteristics of the Amur tiger.** A. The genomic landscape of the Amur tiger
133 genome. a: schematic diagram of chromosomes (white: PtaHapGH1; green: PtaHapGH2); b: gene density; c: sequencing depth; d: GC
134 contents; e: SV: duplication; f: SV: inversion; g: SV: translocation. B. Sequencing depths of the 18 autosomes, X chromosome
135 (Hic_scaffold_10), and Y chromosome in the PtaHapG. C. Averaged depth ratios of male: female for all scaffolds in the PtaHapG. Each
136 blue plot represents an autosome. D. K-mer spectra plot for haplotype-resolved genomes of Amur tiger produced by Merqury. Almost all
137 haploid specific k-mers presented as single-copy (~24X) in the genome, but the shared k-mers by the two haploid genome presented two-
138 copy (~49X) in the genomes. E. Pairwise differences observed in comparisons between the Amur tiger haploid genomes, the sliding
139 window was set to be 100 bp. F. Schematic diagram of the validation of structural variates with PacBio long reads. False SV was
140 identified if reads cannot map across the breakpoint.

141 **2.3 Genetic differentiation between wild and captive Amur tigers**

142 We performed whole genome resequencing of 14 wild Amur tigers collected from Jilin and Heilongjiang Province,
143 which covers most of distribution areas of the wild Amur tiger in China (Fig. 2A). The average sequencing depth
144 and coverage reached 31.76 folds and 97.64%, respectively. Interestingly, we found the wild and captive Amur
145 tigers formed two distinct clades by phylogenetic tree analysis, and further supported by Admixture and PCA
146 analysis (Fig. 2B-D). This indicated a significant genetic separation between the wild and captive Amur tigers. We
147 further found captive individuals presented a more scattered state in the PCA result, unlike wild tigers showing a
148 more aggregate cluster. The genetic diversity calculated from both whole chromosomal and mitochondrial genome
149 showed a much lower evel in wild Amur tigers than in captive individuals (Fig. 2E-G).



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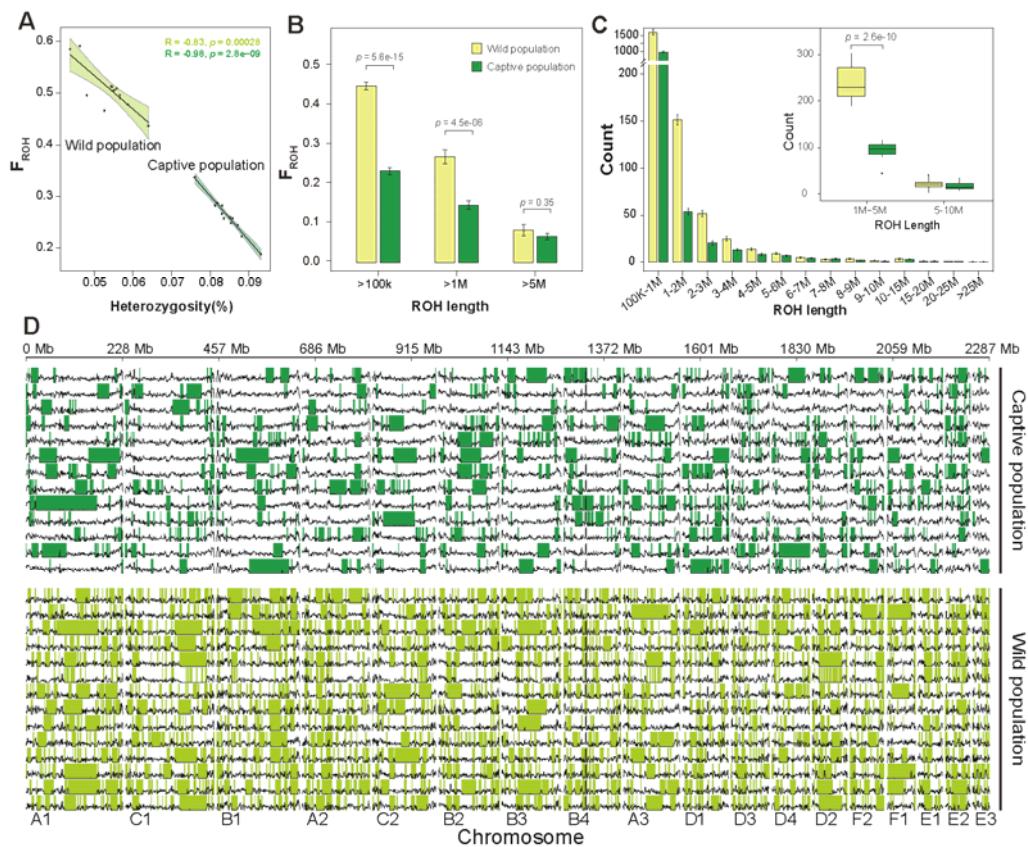
151 **Fig. 2 | Genetic structure, genetic diversity and population demography of Amur tigers.** A. The distribution area and sampling sites
152 of wild Amur tigers in this study. B. Phylogenetic tree constructed using whole genome sequencing data of captive and wild Amur tigers.
153 C. PCA clustering of wild and captive Amur tigers. D. Genome-wide admixture analysis of captive and wild Amur tigers. E. Genome-
154 wide genetic diversity (π) in captive and wild Amur tigers. F. Comparison of genome-wide heterozygosity between captive and wild
155 Amur tigers. G. Network analysis of mitochondrial haplotypes of Amur tigers. H. The dynamic of effective population size in the
156 evolutionary history of wild Amur tiger.

157 2.4 Recent demographic history of the wild Amur tiger

158 To investigate the historical dynamics of population demography of wild Amur tigers, we inferred and described
159 the changes of effective population size (N_e) over time. In general, N_e for the Amur tiger has been declining over
160 its entire evolutionary history (Fig. 2H). However, we did not observe any increase of N_e for wild Amur tiger
161 throughout the recent 10,000 years as for the slight rebound in captive tigers[25]. In addition to low genetic
162 diversity (Fig. 2E, F), the continuous population declining once again reminds us the latent extinction risk of wild
163 Amur tigers.

164 **2.5 Estimates of inbreeding in wild Amur tigers**

165 Here we quantified inbreeding in wild Amur tigers by screening genome-wide ROHs, which is a key genetic
166 indicator to guide possible genetic rescue[25]. We found a total of 26,198 ROHs longer than 100kb, averaged
167 254.19 Kb in length, and the longest ROH reached 35.11 Mb. The averaged number and length per wild individual
168 were 1871 ± 84 Mb and 1160.73 ± 24.80 Mb, respectively. For long ROHs originated from recent inbreeding, we
169 found 3746 ROHs longer than 1Mb in the wild population with averages per-individual of 267 ± 11 in number and
170 696.92 ± 44.56 Mb in length. Similarly, a total of 368 ROHs longer than 5Mb were identified from this population
171 with averages of 26 ± 4 in number and 213.61 ± 37.10 Mb in length.



172

173 **Fig. 3 | Genome-wide inbreeding estimation of Amur tigers.** A. A positive relationship between the heterozygosity and F_{ROH} in Amur
174 tiger. The wild Amur tigers was represented in light green and the captive tigers was in green. B. The comparison of averaged F_{ROH} in
175 wild and captive Amur tigers. C. The length distribution of ROH fragments in Amur tigers. The wild Amur tiger showed a higher level
176 ROH less than 5Mb. D. A fine-scale distribution of ROH larger than 1Mb in wild and captive Amur tigers.

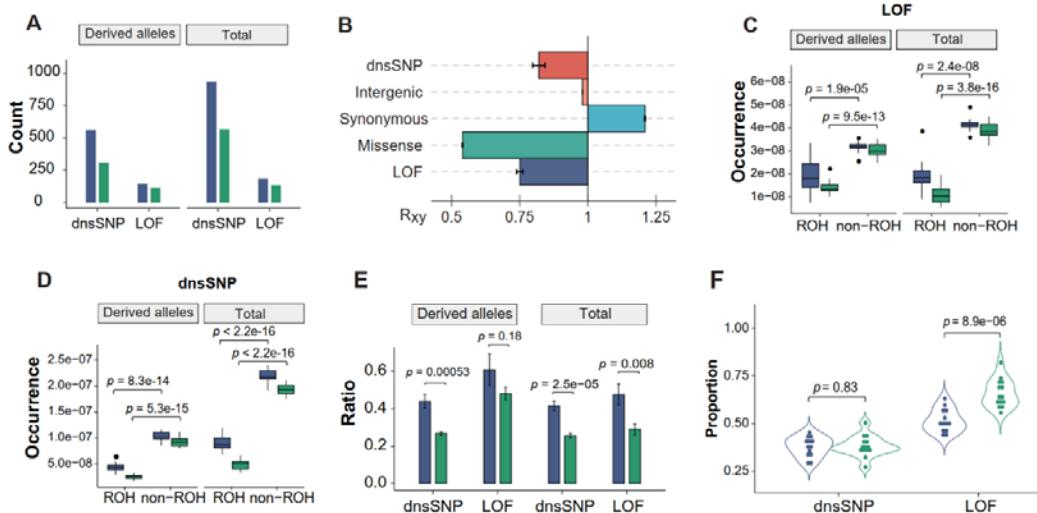
177 We further measured the inbreeding level in wild Amur tigers using F_{ROH} by comparing with captive individuals.
178 In general, the level of F_{ROH} was found to be negatively related with genome-wide heterozygosity in both captive
179 and wild Amur tigers, but the inbreeding level in the wild Amur tiger is higher than captive individuals (Fig. 3A).
180 The $F_{ROH>100kb}$, $F_{ROH>1Mb}$, and $F_{ROH>5Mb}$ were 0.51 ± 0.01 , 0.30 ± 0.02 , and 0.09 ± 0.02 per individual, respectively, in
181 the wild population, while in captive Amur tigers, we showed 0.26 ± 0.01 , 0.17 ± 0.01 , and 0.08 ± 0.01 for the same
182 order of F_{ROH} (Fig. 3B). It is evident that the wild population is more inbred than captive tigers by almost two
183 times as measured with $F_{ROH>100kb}$. The fraction of inbreeding introduced by the recent ancestor (up to 5
184 generations ago)[4] was fairly milder in the wild population, but the $F_{ROH>5Mb}$ was still 1.13 times higher than in
185 captive tigers, although this difference is not significant (Fig. 3C). In addition, 95% and 64% ROH fragments were

186 distributed in the IBD regions in the wild and captive Amur tigers, indicating that most of the ROHs were
187 originated from the inbreeding, especially in the wild population. Overall, the inbreeding level in wild individuals
188 were significantly higher than that in captive Amur tigers ($p=5.6e-15$) (Fig. 3B, D). This was also supported by
189 less mitochondrial haplotype diversity in the wild population (Fig. 2G).

190 To look into detailed inbreeding history of wild Amur tigers, we dissected the ROH distribution by length. We
191 found the large proportion of ROH fragments were restricted to $< 1\text{Mb}$ (Fig. 3C), indicating that inbreeding has
192 occurred since 26 generations ago (based on a recombination rate of 1.9cM/Mb from the domestic cat[39]). We
193 further observed that the number of ROHs shorter than 5Mb was much greater in the wild Amur tigers ($1845 \pm$
194 87.11) than in captive tigers (1071.46 ± 31.55 , $p=2.7e-07$), signifying that the wild population has experienced
195 progressive inbreeding five generations ago. However, the inbreeding levels in the recent five generations (ROH $>$
196 5M) was not significant ($p=0.26$) between the wild (26.29 ± 3.81) and captive tigers (20.69 ± 2.61) although the
197 wild population presented more ROHs. To reveal the inbreeding occurred as recent as the past three generations,
198 we continued focusing on ROH larger than 10Mb . Results showed that the average $F_{\text{ROH}>10\text{Mb}}$ was $3\% \pm 1\%$ in the
199 wild Amur tigers, much lower than that in the South China tiger (*P. t. amoyensis*) (6%)[25] and the small isolated
200 Bengal tiger (*P. t. tigris*) population in India (28%)[4]. This suggests the negative effects signified by recent
201 inbreeding may not be that serious in the wild Amur tigers so far.

202 **2.6 Detection of genome-wide mutational loads in wild Amur tigers**

203 Mutational load is a kind of intrinsic threat to the survival and fitness of endangered species. Here, we firstly
204 investigated the genome-wide mutational loads in protein-coding regions in wild Amur tigers by focusing on
205 deleterious non-synonymous SNPs (dnsSNPs) and loss of function mutations (LOFs), which are both expected to
206 potentially affect the gene functions. Totally, we found 562 and 130 dnsSNPs (Grantham Scores ≥ 150) and LOFs
207 in the wild population, which is lower than that in captive Amur tigers, particularly for the LOF with a much
208 greater difference (Fig. 4A, 5A). We obtained the same result when only considered the derived LOFs and
209 dnsSNPs. Similar result was observed when we used Genomic Evolutionary Rate Profiling (GERP) method to
210 estimate the relative mutational load by measuring the number of derived alleles in the strict evolutionary
211 constraints regions. We further compared the wild and captive population by using the Rxy method to estimate
212 whether the excess of deleterious derived alleles existed in wild Amur tigers. The results showed reduced LOF and
213 missense mutations, but increased synonymous mutations in the wild population (Fig. 4B). In addition, the
214 frequency of LOFs and dnsSNPs inside ROH regions are much lower than that in non-ROH regions in both wild
215 and captive Amur tigers, indicating the purging has occurred in both populations (Fig. 4C, D). Moreover, the
216 frequency ratios of LOFs and dnsSNPs inside ROH to those outside ROH were both much lower in the wild
217 population than in captive Amur tigers (Fig. 4E). This suggests that higher inbreeding in the wild population
218 facilitated purging of deleterious mutations with greater stringency. In addition, we observed a higher proportion of
219 derived LOFs were in homozygous state in the wild populations than that in captive Amur tigers, but we didn't
220 observe significant differences between two populations for the dnsSNPs (Fig. 4F). High proportion homozygous
221 LOFs indicated that fitness cost result from LOFs is higher in the wild population, despite of the existence of
222 purging.
223



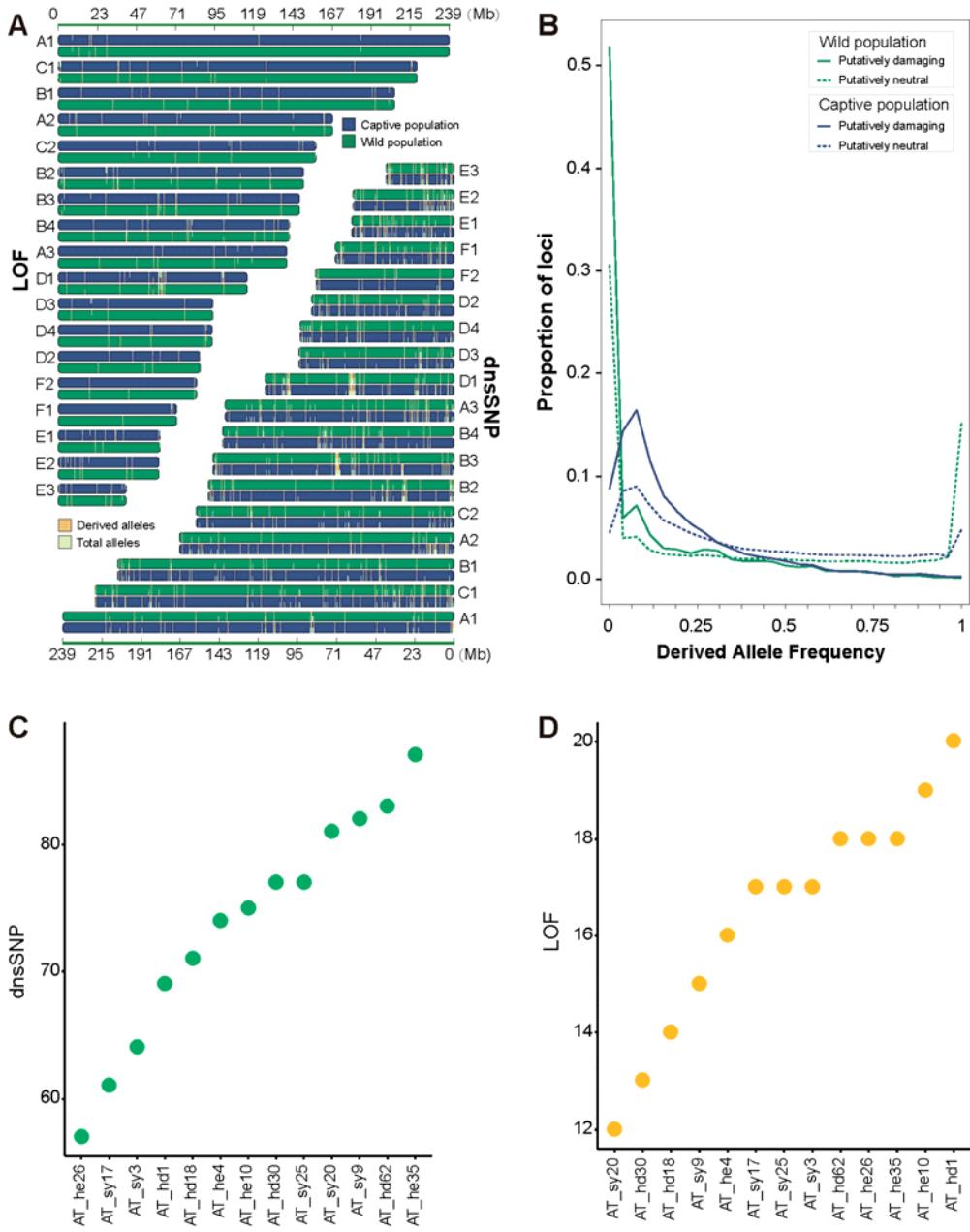
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225 **Fig. 4 | Characteristics of mutational load in captive and wild Amur tigers.** A. Comparisons of the number of mutational loads for
 226 both LOF and dnsSNP in captive and wild Amur tigers. B. The R_{xy} ratio between derive alleles in wild (x) and captive (y) for dnsSNP,
 227 intergenic regions, synonymous, missense and LOF. The R_{xy} < 1 indicated the population y has more derived alleles than population x. C.
 228 The comparison of frequency for LOF variants (LOF number / Total base number of ROH or non-ROH genomic regions) between inside
 229 and outside of ROH regions. D. The comparison of frequency for dnsSNP variants between inside and outside of ROH regions. E. The
 230 ratio of frequency for LOF/dnsSNP inside ROH to that outside ROH in wild and captive Amur tigers. This ratio in the wild Amur tigers
 231 was significantly higher than captive Amur tigers. F. The proportion of homozygous LOF and dnsSNP in wild and captive Amur tigers.
 232 For A, C-F, the blue represented the captive Amur tiger and the green presented the wild Amur tiger.

233 To further dissect the effects of nature selection and random genetic drift on purging of deleterious mutations in the
 234 tiger populations (Fig. 5A), we performed the Site Frequency Spectra (SFS) analysis. We found the number of
 235 derived neutral alleles fixed in wild (0.15) and captive Amur tigers (0.05) were all much greater than damaging
 236 alleles (wild: 0.0014, captive: 0.0027), while the frequency of fixed alleles was much higher in the wild population
 237 (~0.15) (Fig. 5B). For polymorphic loci, the SFS was flat in the wild population but exhibited a continuing decline
 238 in captive Amur tigers, suggesting captive Amur tigers harbored more rare derived alleles. These above-mentioned
 239 genetic features indicated a strong bottleneck in the wild population compared with the captive individuals.
 240 Furthermore, it could be expected that the deleterious alleles in the population should be at low frequency given
 241 natural selection were more effective to eliminate deleterious alleles than neutral ones. In this study, we found that,
 242 in the wild population, the average frequency of deleterious alleles was 0.14 ± 0.002 , significantly lower than that of
 243 neutral alleles (0.39 ± 0.00021 , $p=2.2e-16$), especially for the deleterious allele with a frequency larger than 0.3. The
 244 frequency spectra were much more flat and lower in the wild population. From results above we inferred that the
 245 purifying selection contributed to the purging of mutational loads in the wild Amur tigers.

246 We finally predicted the possibly introduced deleterious mutations from the captive to the wild population by
 247 simulating pairing regimes between the two populations. We found 315 and 67 derived dnsSNPs and LOFs would
 248 be introduced into the wild population when genetic rescue is applied by using the current captive population.
 249 However, the introduced captive deleterious mutations vary depending on individual gene donors (Fig. 5C, D).

250



251

252 **Fig. 5 | Results of dissecting natural selection on the purging of deleterious mutations and the distribution of deleterious**
 253 **mutations across the whole genome.** A. Distribution of LOF and dnsSNP across each chromosome in wild and captive Amur tiger
 254 population. B. Site-frequency spectrum for damaging and neutral mutations for each population. C. Potentially new dnsSNP mutations
 255 introduced to the wild population by captive individuals if gene flow occurred. D. Potentially new LOF mutations introduced to the wild
 256 population by captive individuals if gene flow occurred.

257 2.7 Genomic signatures of local adaptation in Amur tigers

258 Local adaptation is a key factor should be taken into consideration when making conservation strategies for
 259 endangered species[15, 40, 41]. Here we applied Cross Population Extended Haplotype Homozygosity (XP-EHH)
 260 method to screen the possible signals of recent positive selection in the captive Amur tigers compared to the wild
 261 population to facilitate the genetic rescue by reintroduction of captive individuals to the wild. We found 4559 and

262 4516 SNPs were identified under strong positive selection in the wild and captive Amur tigers, respectively.
263 identified by setting a XP-EHH score in the top 0.1%. Only 56 and 63 genes were found to be under recently
264 positive selection in the wild and captive Amur tigers, respectively. In the wild population, we found many GO
265 terms were closely related with immunity, defense response, and response to environmental pollutants. We also
266 found a KEGG pathway, Toll-like receptor signaling pathway, was significantly enriched in the wild population.
267 However, no evidence was found for overrepresented GO terms and KEGG pathways in captive Amur tigers.

268 **3. Discussions**

269 **3.1 Improved reference genome for the Amur tiger**

270 Currently, the combination of high-fidelity (HiFi) reads and HiFi-specific assembler can generate high-quality and
271 haplotype-resolved *de novo* assembly, representing one of the most promising strategy for genome assembling by
272 far[42, 43]. The reference genome we report here is the first haplotype-resolved reference genome for the tiger[44].
273 There has been reported two Amur tiger genome assemblies, including a second-generation genome (PanTig1.0)
274 [45, 46] and a chromosome-scale genome (PanTig2.0) assembled by PacBio long reads. The Amur tiger genome
275 assembled in this study have a much higher contiguity, with a contig N50 of 26.97 Mb, which is ~692-fold and ~3-
276 fold longer than the PanTig1.0 (N50: 0.039 Mb) and PanTig2.0 (N50: 9.52 Mb), and the contig number of
277 PtaHapG (259) much fewer than PanTig2.0 (3117). This significant improvement in contiguity will greatly
278 improve the evaluation of inbreeding in tigers. In particular, by comparing this genome to our previously published
279 chromosome-scale genome of a captive Amur tiger, we found 37.73 Mb wild individual specific sequences
280 harboring the gene OR56A3 which was missing in the captive genome, further indicating the superiority of the
281 HiFi genome we assembled here.

282 **3.2 Mutational load purging in wild inbred Amur tigers**

283 The wild population of Amur tiger has fragmented into three subpopulations (Fig. 2A). The subpopulation in
284 northeast China connecting with the two ones in Russia restored ~60 tigers at present from ~26 in 2014[3]^[47].
285 Previous study showed a risk of this population to lose genetic diversity rapidly due to moderate to high
286 inbreeding[23]. Thus, inbreeding became a big concern for its sustainability. On the other hand, China has
287 established a captive population as *ex situ* conservation resource in northeast area[24]. Population genomic
288 analysis showed moderate inbreeding level for captive Amur tigers. To draw a general picture of genetic status of
289 the wild population in China, we used advantage of our haplotype-resolved and super high contiguity resolution
290 genome PtaHapG to precisely estimate and predict inbreeding level and genomic consequence for the
291 representatives of the captive and wild Amur tigers (Fig. 3D).

292 We show the inbreeding levels (F_{ROH}) inferred from ROHs longer than 100 kb, 1Mb and 5Mb were all higher in
293 wild tigers than in captive tigers by 1.96, 1.76 and 1.13 times (Fig. 3B). Overwhelming proportion (~95%) of
294 ROHs (>100kb) distributed in IBD regions in the wild population, suggesting majority of ROHs were resulted
295 from inbreeding, although shared bottleneck history may still contribute to the genome homozygosity[48].
296 Furthermore, majority of ROH fragments were restricted to <1Mb, suggesting inbreeding could be dated back to
297 26 generations ago (estimated based on a recombination rate of 1.9cM/Mb from the domestic cat[39]). A relative
298 larger number of ROHs <5Mb were observed in wild tiger genomes than in captive genomes, signifying the wild
299 population has experienced more inbreeding five generations ago. The average $F_{ROH}>10Mb$ was half the South
300 China tiger (*P. t. amoyensis*) (6%) and one tenth the small isolated Bengal tiger (*P. t. tigris*) population in India
301 (28%)[4], suggesting the negative effects signified by recent three generations may not be that serious so far.
302 However, the number of ROHs shared among tigers in the wild population was much higher than that in captive
303 Amur tigers, further suggesting more intensive inbreeding in wild Amur tigers. Inbreeding for such fragmented
304 population[49] will doubtlessly lead to loss of genetic diversity driving it towards extinction without timely human

305 interference.

306 Fortunately, we found the mutational loads in wild Amur tigers, including both LOF and dnsSNP variants, are
307 much lower than that in the captive tigers (Fig. 4A). Expectedly, this might be resulted from purging promoted and
308 maintained by random genetic drift and/or the combination of inbreeding and purifying selection. The wild Amur
309 tigers has experienced sharp decline within the most recent 1000 years leaving a small N_e (Fig. 2H). Recent
310 decades of isolation would further reduce the N_e . Genetic drift in this case might have effectively acted on this
311 population leading to random loss or fixation of deleterious alleles with equal probability [10]. However, we
312 detected an elevated frequency of fixed neutral alleles comparing to damaging alleles in the wild population,
313 suggesting purifying selection may have been acting on the wild Amur tiger population to reduce the inbreeding
314 depression by purging of damaging alleles. Furthermore, the LOF mutations were much less than dnsSNP (Fig.
315 4A), and the occurrence of LOF and dnsSNP was significantly more frequent outside ROH regions than that inside
316 ROH regions (Fig. 4C, D). This suggests that homozygous deleterious mutations were more likely purged than
317 heterozygous ones, and purging was stronger for LOFs that are more recessive than missense mutations.
318 In general, our genomic analysis showed the inbreeding of the wild Amur tiger population in northeast China could
319 be dated back to at least 26 generations ago. Fortunately, up to the most recent generations inbreeding has not yet
320 reached the level to express severe inbreeding depression. Inbreeding combining with purifying selection has
321 facilitated mutational load purging, but meanwhile presents a risk to lose genetic diversity. In contrast, captive
322 Amur tigers had also been inbred 26 generations ago but experienced relative lower inbreeding in the past five
323 generations. Mutational load purging in the captive Amur tigers is not so effective as in the wild population, very
324 likely due to effective husbandry management[51].

325 **3.3 Implications for genetic rescue of the wild population**

326 The above analysis shows inbreeding had taken place and developed to moderate to high level in the wild Amur
327 tiger. Although the population number of wild Amur tiger has restored successfully in the past decade (National
328 Forestry and Grassland Administration, P. R. China, 2022), the security is still worrying and ameliorating
329 inbreeding should be taken into the main goals of next phase conservation.

330 One immediate and necessary approach is to build ecological corridors to connect up population patches within
331 China and with Russian populations to eliminate landscape resistance of migration and improve genetic
332 communications[3]. This is key infrastructure supporting long-term survival[52]. The second and parallel approach
333 is genetic rescue using the healthier captive population[53]. We found that captive tigers are genetically distant
334 from the wild ones and higher in genetic diversity (Fig. 2B-G), which are very potential to ameliorate ROHs in the
335 wild genomes. However, we also found that captive Amur tigers carries greater number of mutational loads (Fig.
336 4A) and a considerable proportion are absent in the wild population (Fig. 5B). This suggests introducing captive
337 genes into the wild population is risky to simultaneously introduce novel mutational loads that may make further
338 negative impacts. Therefore, cautions should be made to avoid this side-effect.

339 In this study, we established partial list of mutational loads for the wild and captive tigers and predicted 315
340 derived dnsSNPs and 67 LOFs likely to be introduced into the wild population by simulating currently studied
341 captive tigers for genetic rescue (Fig. 5C, D). The introduced mutations vary depending on gene donors, leaving
342 possibilities for selecting the optimal candidate tigers. Our study provides an instance to accurately evaluate the
343 extent to which the ROHs on wild genomes could be ameliorated, mutational loads be introduced, and the
344 likelihood the introduced loads could be subjected to remission by heterozygote under possible reproductive
345 regimes. For planning real genetic rescue, the list of mutational loads should be completed by sampling all, at least
346 majority of wild tigers and all potential captive gene donors. The haplotype resolution reference genome PtaHapG
347 provides a firm platform to carry out elaborate inference in this regard.

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353 Author Contributions

354 These authors contributed equally: Tianming Lan, Haimeng Li, Le Zhang, Minhui Shi, Boyang Liu, Yanchun Xu,
355 Huan Liu, Guangshun Jiang and Tianming Lan initiated and designed the project. Dan Liu, Yue Zhao, Weiyao
356 Kong, Yue Ma, Boyang Liu and Le Zhang collected the samples. Qing Wang, Jiangang Wang, Xinyu Wang, Yuxin
357 Wu, Jiale Fan, Xiaotong Niu and Liangyu Cui performed the RNA/DNA isolation. Haorong Lu, Shaofang Zhang,
358 Jieyao Yu performed the DNA libraries construction and sequencing. Nicolas Dussex and Tianming Lan
359 coordinated the data analysis. Haimeng Li, Minhui Shi, Le Zhang and Boyang Liu carried out the data analysis.
360 Tianming Lan, Haimeng Li, Minhui Shi, Boyang Liu and Yanchun Xu wrote the manuscript. Guangshun Jiang,
361 Shanlin Liu, Love Dalén, Nicolas Dussex and Yanchun Xu revised the manuscript. Guangshun Jiang, Huan Liu,
362 and Yanchun Xu provided the supervision of this project.

363 Competing interests

364 The authors declare no competing interests.

365 Data and Code Availability

366 The data that support the findings in this study have been deposited into CNGB Sequence Archive (CNSA) [54] of
367 China National GeneBank DataBase (CNGBdb) [55] with accession number CNP0003803.

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