

Genomic analysis reveals the influence of climate change and currents on adaptation in an estuarine species

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22 **Abstract**

23 Understanding the evolutionary forces driving adaptive divergence and identifying the
 24 genomic variations, especially those mediating the plastic responses are critical to
 25 evaluate the adaptive capacity of species upon rapidly changing climate. Here we
 26 report a high-quality genome assembly for an estuarine oyster (*Crassostrea ariakensis*)
 27 and 264 resequenced wild individuals from 11 estuaries along Chinese coastline. This
 28 estuarine oyster evolved decreased polymorphism and a clear population structure
 29 than that of marine species. Historical glaciations, ocean currents and environmental
 30 selection play important role in shaping and maintaining their divergence patterns. We
 31 identified genes, especially for expanded genes *solute carrier family*, showing strong
 32 selective signals and most of them responded to temperature and salinity challenges,
 33 suggesting their significance in environmental adaptation. Higher genetic divergence
 34 of environment-responsive genes especially in upstream intergenic regions potentially
 35 regulate their higher plastic changes, providing genomic basis of plasticity upon
 36 climatic selection. Our findings contribute to assess species' vulnerabilities to
 37 climate-driven decline or extinction.

38 Rapidly changing climate threatens the global biodiversity and geographic
 39 distribution of organisms. Accompanying with the rising of global temperature over
 40 the past decades, the dry regions is getting drier, while wet regions wetter¹. The
 41 salinity difference is getting greater in estuaries for increasing of salinity in the north
 42 China, while decreasing in the south². Except for environmental selection, species'
 43 distribution patterns are also influenced by stochastic forces including gene flow and
 44 genetic drift. For marine species living in an open area, gene flow is restricted
 45 compared to previous perspectives, and historical climate (like glaciations) and
 46 tectonic events can shift their geographic distributions, as well as the role of the
 47 natural barrier of diluted water³⁻⁷. Fine-scale local adaptation among different
 48 environmental gradients has been revealed in many marine species, even those with
 49 higher dispersal capacity⁸⁻¹³. Evolutionary adaptation to various environments evolves
 50 plastic changes and accumulation of genetic mutations to alter phenotypes reaching
 51 the fitness optimum over generations¹⁴⁻¹⁶. Most of recent studies independently
 52 revealed the significance of genomic variations^{12,17-19} and plasticity^{13,20-22} especially
 53 for environmentally responsive genes of marine species in adaptation to changing
 54 ocean environments such as challenges from temperature and salinity disturbances.
 55 Their interaction, genomic variations of environmentally responsive genes/traits, is a
 56 critical predictor of organismal adaptive potential to climate-driven challenges^{23,24}.
 57 however, genetic basis of plasticity to environmental heterogeneity remains poorly
 58 understood.

59 Oysters are keystone species in intertidal and estuarine ecology and one of the most
 60 important aquaculture species worldwide. Oyster is sessile species thriving in the
 61 coastal zone with highly environmental variation, and both genetic divergence and
 62 plasticity contribute to its adaptive evolution^{11,25-27}, which suggest that the oyster is
 63 excellent model for studying genomic basis of plastic responses to changing climates.

64 Estuarine oysters (*Crassostrea ariakensis*, Fig. 1a) are broadly distributed in the
 65 estuaries of eastern Asia and experience high extent of environmental gradients in
 66 temperature and salinity²⁸⁻³⁰. A clear genetic structure among different geographic

67 populations by limited neutral markers^{28,31-33}, and differentiation in plastic responses
68 to temperature and salinity stresses between northern and southern populations has
69 been revealed^{2,34}. Understanding whole-genome selective signals and its contribution
70 to plasticity requires exploring genomic variations and plastic changes together at the
71 same gene level. Long-read sequencing platforms provide a chromosome-level
72 assembly and a more informative genome for exploring variations under natural
73 selection³⁵⁻³⁷. Integrating comparative genomics and genome-wide gene expression
74 profiles can not only shed light on selective signals by identifying genomic variations
75 adapted to variable ecotypes, but also provide genomic mechanisms that mediate gene
76 expression plasticity by identifying genomic variations that located around the
77 environmental responsive genes^{11,12,19}.

78 Here, we sequenced the genome of estuarine oyster *C. ariakensis* and re-sequenced
79 264 wild oysters collected from 11 sites across most of Chinese estuaries, to reveal its
80 genetic structure, the potential driven forces and identify genomic regions with
81 selective signals. Transcriptomes under acute thermal and high-salt stresses, and
82 reciprocal transplantation were conducted to explore expression patterns of
83 environmentally responsive genes and further characterized the contribution of
84 genomic variations at different regions to its gene expression plasticity.

Results and Discussion

Genome assembly and annotation. To generate a high-quality reference genome, we sequenced the genome of estuarine oyster *C. ariakensis* using a combination of short-read and long-read sequencing platforms to generate a contig-level assembly. Hi-C libraries were also constructed and sequenced to organize them into a scaffold-level genome assembly. The estimated genome size based on the *k*-mer distribution analysis was 583.41 Mb (Supplementary Fig. 1, Supplementary Table 1). The polymorphism level was 0.58%, which was less than half as that of wild marine oyster species³⁸ (Pacific oyster *Crassostrea gigas*). and this may be resulted from limited gene flow of the oyster that are specifically adapted to estuarine environments. We applied a hierarchical assembly approach using 183.70 Gb of long-reads (299.24-fold coverage), 64.39 Gb of paired-end reads (104.89-fold coverage) and 106.34 Gb (173.22-fold coverage) of Hi-C data. The final, polished and high-contiguity genome assembly spans 613.89 Mb comprising 630 contigs with a contig N50 of 6.97 Mb. Approximately 99.6% of the genome across 416 scaffolds with a scaffold N50 of 62.26 Mb is presented on 10 linkage groups corresponding to 10 chromosomes of the estuarine oysters (Fig. 1b, Supplementary Table 2). To our knowledge, this is the most contiguous assembly among bivalve genomes using long-reads sequencing published to date³⁵⁻³⁷. We mapped pair-end reads to the assembled genome to assess assembly accuracy, resulting in a 97.93% mapping rate. The genome assembly captured 92.54% of the Benchmarking Universal Single Copy Orthologs (BUSCO) datasets (Fig. 1c), indicating a high level of gene region completeness in the genome assembly. Transcripts from the RNA-seq data were used to assess gene coverage rate, and 97.15% of the transcripts could be aligned to the assembly, indicating most of the gene sequences were contained (Supplementary Table 3). The accuracy of genome sequencing data was 98.32% as determined with Sanger sequencing (Supplementary Table 4). These results verified that the accuracy and completeness of the estuarine oyster genome assembly are high and of high quality at the chromosome scale.

114 We predicted 29,631 protein-coding genes in the estuarine oyster genome combining
115 gene evidence from homology annotation, *de novo* annotation and transcripts of
116 mRNA sequencing, and 96.13% of which were functionally annotated
117 (Supplementary Table 5). Various non-coding RNA sequences were also identified
118 and annotated in the genome, including 1,077 transfer RNAs, 20 micro RNAs and 131
119 ribosomal RNAs. A total of 332.40 Mb (54.14%) of repeats and transposable elements
120 were identified (Supplementary Table 6). Generally, the gene density was inversely
121 related to the content of repeat elements across all chromosomes.

122

123 **Genome resequencing, variation calling and population structure.** We
124 resequenced 264 wild oysters of *C. ariakensis* from 11 estuarine areas, representing
125 the major distribution range across north, middle and south geographical regions of
126 Chinese coastlines^{28,29} (Fig. 2a), and obtained 3.81 Tb clean data. The mapping rate
127 averaged 95.33% varied from 86.46% to 96.66%, and the effective mapped read depth
128 averaged 19.89× by aligning reads to the *C. ariakensis* reference genome. We
129 generated 145,271,754 SNPs (range from 487,881 to 640,962 per individual) and
130 103,080,822 indels (range from 342,486 to 443,381 per individual) acrossing genes
131 ranging from 11,383 to 13,321. In addition, we found a considerably lower
132 polymorphism that averaged 0.47 heterozygous SNPs per Kb per individual
133 (Supplementary Table 7), which was more than 35-fold lower than that in Pacific
134 oyster populations¹¹. The number of genomic variations including SNPs and indels
135 (insertions and deletions) was gradually increased from north to south estuarine
136 oysters, and more than half of variations were SNPs (58.45%) that mainly located at
137 intergenic regions (57.38%, Supplementary Fig. 2).

138 Genetic structure analysis based on genome-wide SNPs supported previous findings
139 that differentiations occurred among different geographic populations of *C. ariakensis*
140 in China, using fitness-related traits, neutral markers and transcriptomic analysis^{28,32-34}.
141 The optimal number of population clusters was identified as $k = 3$ (Supplementary Fig.
142 3), exactly representing three main geographic sea areas as north estuaries of China

143 (NEC, 5 sites), middle estuaries of China (MEC, 2 sites) and south estuaries of China
 144 (SEC, 4 sites) (Supplementary Fig. 4). Principle component analysis (PCA),
 145 explaining 15.98% of genetic variance by the two PCs, consistently reveals three
 146 distinct populations of oysters corresponding to NEC, MEC and SEC. A fine-scale
 147 subpopulation was further detected that oysters from Qingdao (QD) site and farther
 148 southern subpopulation (SEC-b) including Taishan (TS) and Qinzhou (QZh) sites
 149 were separated from NEC and SEC, respectively (Fig. 2b). Moreover, Phylogenetic
 150 tree using neighbor-joining (NJ) method supports the above clustering, which first
 151 distinguish the southern population from others and further identified another
 152 subpopulation (NEC-a) within NEC distributed along estuaries of southern Bohai Sea
 153 including oysters from Binzhou (BZ) and Dongying (DY) sites (Fig. 2c). A total of six
 154 subpopulations were identified for 11 wild estuarine sites in China. We calculated the
 155 pairwise F_{ST} for all polymorphic positions among three populations and QD oysters,
 156 and revealed strong divergence between SEC and other populations, ranged from
 157 0.143 to 0.225 (Fig. 2d), which also detected among oysters from different
 158 geographical sites (Supplementary Table 8). Oysters from MEC and NEC were
 159 clustered together in phylogenetic tree with a lower genetic divergence ($F_{ST} < 0.05$),
 160 which is comparable with the divergence among populations of marine oyster species
 161 in north China¹¹. Linkage disequilibrium (LD, measured as r^2) decreased to half of its
 162 maximum value range from 2.54 kb to 3.00 kb among three populations of estuarine
 163 oysters (Supplementary Fig. 5), which is substantially slower than marine oyster
 164 species¹¹. Our findings provided insights into the fine-scale population structure of
 165 estuarine oysters along the coast of China and revealed the stronger genetic
 166 divergence of southern oysters from others.

167

168 **Limited gene flow, genetic drift and positive selection shape the distribution**
 169 **patterns.** Population structure of estuarine oysters was largely concordant with the
 170 direction of ocean currents especially during the summer, where the northern or
 171 southern coastal currents are not cross over the middle area nearby the Changjiang

172 estuary (Fig. 2a and Supplementary Fig. 6). To investigate the effects of ocean
 173 currents on gene flow, we examined nucleotide diversity (π) for each population and
 174 found that higher genetic diversity was observed in the middle population (3.56×10^{-4})
 175 and QD oysters (3.59×10^{-4}) where is the joint of more than one ocean currents, than
 176 that of northern (3.33×10^{-4}) and southern (3.43×10^{-4}) populations (Fig. 2d) where
 177 only adjoin one ocean current (Fig. 2a and Supplementary Fig. 6). The same pattern
 178 of nucleotide diversity was also confirmed by oysters from individual geographical
 179 sites that oysters from middle sites showed higher π values, while oysters from
 180 northern and southern sites have lower diversity (Fig. 3a), which is contrast to the
 181 terrestrial vertebrates where wildlife harbored higher genetic diversity in southern
 182 China³⁹. These findings indicate that ocean currents play important role in shaping
 183 and maintaining genetic structure of *C. ariakensis* in China^{11,40}, which contribute to
 184 the homogenization within oyster population by facilitating the mixture of larvae from
 185 sites adjoining the currents with the same direction, but the divergence among
 186 populations adjoining the currents with the different directions such as the NEC and
 187 SEC. However, the MEC oysters showed very limited mixed ancestries from northern
 188 and southern populations (Supplementary Fig. 4), as well as the fine-scale
 189 subpopulations within SEC and NEC even in the same direction of ocean currents
 190 (Fig. 2a), indicating the role of ocean currents in mixing gene pools is restricted as
 191 also found in marine oyster populations¹¹. In addition, Changjiang dilute water (CDW)
 192 is considered as an natural barrier that facilitates divergence between north and south
 193 populations of many marine species in China^{5-7,41,42}. Here, however, we found that
 194 oysters sampled from the north (NT) and south (SH) part of Changjiang River were
 195 clustered together as MEC, indicating CDW is not the barrier and has limited
 196 influence on distribution and divergence patterns for estuarine oysters where
 197 organisms experience wide-range of salinity disturbance.

198 We employed the pairwise sequentially Markovian coalescent (PSMC) to reconstruct
 199 the demographic history and assess fluctuations in effective population size (N_e) of
 200 ancestral estuarine oysters *C. ariakensis* in response to Quaternary climatic change

201 using deep-coverage (25-28×) oyster genomes of two or three individuals from each
 202 of three genetic/geographic populations, as well as three individuals of marine oyster
 203 species from resequencing data with the average coverage of $20\times^{11}$. Both of the
 204 marine and estuarine oysters were severely influenced by glaciation events during the
 205 past million years that the N_e had a peak at ~0.90 mya before the Mindel glaciation
 206 (MG, 0.68~0.80 mya) and then was substantially decreased when subjected to
 207 subsequent three times of glaciations including MG, Riss glaciation (RG, 0.24~0.37
 208 mya) and Würm glaciation (WG, 10,000~120,000 years ago), and a relatively slower
 209 decreasing was found at inter-glaciation period between RG and WG. However, the
 210 N_e of estuarine oyster species was massively lower before historical glaciations and
 211 consistently lower than marine species, and the latter was earlier and more rapidly
 212 increased the population size (Fig. 3b). Moreover, we compared nucleotide diversity
 213 among three populations of estuarine oysters and marine oysters ($n = 26$), and found
 214 that marine species have more than 25-fold higher of π values than that of estuarine
 215 oysters ($\pi_{C. gigas} = 9.27 \times 10^{-3}$, Fig. 3c). Similarly, stickleback fishes adapted to
 216 freshwater areas exhibited lower π and N_e values than counterparts dwelling in
 217 brackish areas⁴³. These findings addressed that the specified life history of adaptation
 218 to restricted estuarine areas affected the N_e and nucleotide diversity of *C. ariakensis*.
 219 Generally, all three oyster populations exhibited similar demographic trajectories until
 220 about 0.2 mya (Fig. 3b). The N_e curves of southern population was the earliest to split
 221 from others that occurred ~0.18 mya corresponding to the isolation period by the land
 222 bridge between Taiwan and Mainland of China from 0.2 mya to 25,000 years ago⁴⁴.
 223 Accordantly, we calculated the putative range of divergence time was 0.14~0.63 mya
 224 between northern and southern populations of *C. ariakensis* using previously reported
 225 pairwise sequence divergence of *COI* gene²⁸, based on the sequence divergence of
 226 *COI* gene⁴¹ and divergence time⁴⁵ between *C. gigas* and *C. angulata*. In addition, the
 227 split of N_e curves between middle and northern populations occurred ~90,000 years
 228 ago, corresponding to the grisly fall of the sea level at the sub-glaciation of WG that
 229 the majority of the Bohai Sea was land⁴⁶. Lower N_e and nucleotide diversity of

northern population (Fig. 3a, b) suggested the stronger recent bottleneck on oysters dwelling in the Bohai Sea. Decreased nucleotide diversity was also found in Bohai populations of marine oyster species¹¹. The role of bottlenecks and geographic isolation resulted from historical glaciations and tectonic events in shaping species' distributions were also demonstrated in other molluscs, such as the divergence between Atlantic coast and Gulf of Mexico populations of eastern oyster³. Our results not only provide putative divergence times among northern, middle and southern estuarine oyster populations, but also pointed out historical glaciation and tectonic event was the critical evolutionary drivers to shape their differentiation.

We further characterized the genomic variations related to natural selection among three oyster populations. The number of SNPs showing heterozygous in northern oysters but homozygous in southern oysters ($n = 14,373$) were 1.89-fold higher than those of SNPs showing homozygous in northern oysters but heterozygous in southern oysters ($n = 7,595$) across 10 chromosomes (Fig. 3d). Correspondingly, homozygous variations in southern oysters ($50.71\% \pm 1.92\%$) were significantly higher than that in middle ($44.35\% \pm 0.39\%$) and northern oysters ($44.53\% \pm 0.86\%$) ($p < 0.01$, Supplementary Fig. 7). Both divergent selection and genetic bottlenecks can result in purified homogeneous variations. Furthermore, southern oysters ($35.67\% \pm 0.35\%$) evolved significantly higher ratio of genes with non-synonymous alleles than these in middle ($33.33\% \pm 0.22\%$) and northern ($31.82\% \pm 0.29\%$) oysters ($p < 0.01$, Fig. 3e), suggesting southern oysters were subjected to stronger natural selection. Our results revealed that stronger natural selection, potentially resulted from strong environmental gradients, preferred to purify the homozygous mutations in southern oysters.

In summary, genetic bottlenecks from historical glaciations and geographic events play important role in shaping geographic distribution patterns of estuarine oysters in China, and gene flow from ocean currents and natural selection from environmental gradients synergistically contribute to maintain their divergent patterns.

Genomic signatures related to environmental adaptation. Oysters inhabiting in northern and southern habitats subjected to significant environmental gradients. Southern habitats are characterized with higher temperature in comparison with northern and middle habitats. Monthly average sea surface temperature (SST) of six sampling sites from each of six subpopulations were collected from satellite remote sensing data during 2000 to 2017. Average SST of southern habitats showed a 10.35 °C higher than that of northern habitats (Supplementary Fig. 8). In addition, we found a 10.98 ‰ higher of salinity at northern (BZ) site than southern (TS) site². And increasing ocean salinity contrast would intensify the salinity increases at north regions but decreases at south regions^{47,48}. Thus, variations in temperature and salinity between northern and southern habitats are two of the critical environmental factors that driven adaptive divergence of northern and southern oyster populations. Combining geographically disconnected distribution and limited gene flow, local adaptation was revealed that southern population evolved higher thermal tolerance and greater sensitivity to high salinity². Observing distinct population structure among three oyster populations, we propose that there were genomic regions under selection contribute to adaptation to higher temperature and lower salinity conditions in the south.

To gain insights into the adaptive genetic basis in adapting to southern environments, we scanned for selection signatures in two population pairs including a) north vs south and b) middle vs south. We calculated the fixation index (F_{ST}) and selection statistics (Tajima's D) between two population pairs, and used an outlier approach (top 1% F_{ST} values, $F_{ST_north\ vs.\ south} = 0.693$, $F_{ST_middle\ vs.\ south} = 0.637$) to identify genomic regions undergoing selective sweeps in these three oyster populations. Only genomic regions surrounding the selective peaks that overlapped between the two population pairs, and further located at the ravines of Tajima's D values along each chromosome in one of the three populations were considered as selective signals. A total of 24 selective regions spanning 51 candidate genes (44 annotated) were identified along chromosomes 2, 3, 4, 6, 8 and 9 among three oyster populations (Fig.

288 4a, b and Supplementary Fig. 9-14, Supplementary Table 9). Most of these candidate
289 genes in other species have also been reported to respond to different environmental
290 gradients of salinity and temperature^{12,19,49-54}.

291 We conducted RNA-seq analysis to examine the responses of genome-wide and
292 selective genes using wild oysters, which were exposed to sublethal conditions of
293 elevated temperature (6 hours under 37 °C) and high-salt (7 days under 60 ‰). Low
294 expressed genes that the aligned read counts were less than 10 in more than 90%
295 samples were removed for subsequent analysis. About 44.17% (10,279 of 23,270) and
296 11.7% (2,757 of 23,650) of highly expressed genes were responsible for high salinity
297 and temperature stresses respectively, and 1,088 genes were both responsive to the
298 two stresses (Supplementary Fig. 15). For candidate genes with strong selective
299 signals, a total of 29 genes were expressed under high salinity and temperature stress
300 conditions, and 75.9% (22 of 29) and 58.6% (17 of 29) of all expressed genes were
301 significantly increased or repressed their expression levels ($p < 0.05$, Fig. 4c),
302 indicating genes under selection preferred to respond to these two environmental
303 changes. Thirteen genes were responsive to both thermal and low salinity stresses,
304 while three genes were not sensitive to these two stresses. Nine and four genes
305 specifically responded to high salinity or temperature exposure, respectively. Our
306 findings reveal that most of genes with strong selective signals were responsible for
307 adaptation of estuarine oysters to environmental gradients especially for temperature
308 and salinity.

309

310 **Expansion of selective genes contribute to temperature and salinity adaptation.**

311 Among these selective regions, we found two tandem duplications for *Solute carrier*
312 *family* including 10 copies of *Slc23a2* and four copies of *Monocarboxylate*
313 *transporter 12* (*Mct12*, also known as *Slc16a12*) (Supplementary Fig. 14c), which
314 located in the two peaks of chromosome 9 and showed high differentiations among
315 three oyster populations, where average F_{ST} values were 0.81 and 0.76 respectively
316 (Supplementary Fig. 14d). Genomic regions spanning *Slc23a2* gene families exhibited

extremely lower Tajima's D values in northern oysters, while these spanning *Mct12* gene families had extremely lower Tajima's D values in southern oysters (Supplementary Fig. 14e). These findings indicate that *Slc23a2* and *Mct12* genes were under directional selection at northern and southern environments respectively, and highlight the critical role of genes belonging to *Slc* families in adaptation of marine species, such as porpoises and coral, to salinity and temperature gradients^{17,19,55,56}. Moreover, ten copies of *Slc23a2* gene family belong to three orthogroups, where two of them are annotated as purine permease (a: OG0011985 and c: OG0000489) and another is annotated as uric acid transporter (OG0000633). Four copies of *Mct12* gene family belong to one orthogroup annotated as purine efflux pump (OG0000571). All of these orthogroups were extensively expanded in *C. ariakensis*, as well as in other two estuarine oysters of *C. virginica* and *C. hongkongensis* in compare with marine species of *C. gigas* (Supplementary Fig. 16). We found that three copies of *Slc23a2* genes (*Slc23a2_a1*, *Slc23a2_a2* and *Slc23a2_c2*) in two expanded orthogroups responded to both temperature and salinity challenges, while three copies of *Mct12* (*Mct12_1*, *Mct12_2* and *Mct12_3*) genes were responsible for salinity challenges (Fig. 4c). The expanded selective genes of these two gene families responsive to the temperature and salinity challenges mediates adaptive divergence among different oyster populations. This finding highlighted the important role of gene duplication, such as *Heat shock protein (Hsp)* gene family in Pacific oyster dwelling in the intertidal zones^{38,57}, in adapting to species-specifically challenging habitats and further mediate adaptive divergence among different subspecies or populations⁵⁸⁻⁶¹.

Selective preference in upstream intergenic regions of environmentally responsive genes. We explored the divergence patterns of genomic variations, including genic and intergenic (upstream and downstream) regions, and expression responses of these 29 genes under selection to environmental changes by conducting reciprocal transplant experiments. F_1 progenies bred from each of wild northern and southern populations were acclimatized at both northern and southern habitats for

three months. F_{ST} were used to quantify the extent of divergence of different genomic regions and transcriptional changes upon transplantation between two habitats were used to qualify plastic changes of each selective genes.

Expression level of 29 candidate selective genes showed distinct population- and environment-specific patterns that ten and five genes were highly expressed at southern and northern oyster populations respectively ($p < 0.05$), while seven and four genes were highly expressed at northern and southern habitats respectively ($p < 0.05$), as well as three genes exhibited specifically higher or lower expression at native habitats ($p < 0.05$, Fig. 5a). Environment-specific selective genes correspondingly exhibited significant higher expression plasticity (19.15%) than that of population-specific selective genes when oysters were reciprocally transplanted between northern and southern habitats ($p = 0.0255$, Supplementary Fig. 17).

We further characterize the divergence of different genomic regions at genome-wide level, as well as total, population- and environment-specific candidate gene sets. At genome-wide profile, genic regions showed significantly higher F_{ST} values (mean $F_{ST_genic} = 0.163$) than that of intergenic regions ($F_{ST_intergenic} = 0.138$, $p < 0.001$, Wilcoxon signed-rank tests, Fig. 5b), indicating genic regions were substantially preferred to be under selection and evolved higher differentiation between northern and southern oysters. For all of the 29 candidate genes, both genic ($F_{ST} = 0.7745$) and intergenic ($F_{ST} = 0.7585$) regions were under strong selection, but no difference was detected between them ($p > 0.05$). However, at both genic and intergenic regions, environment-specific genes ($F_{ST} = 0.7816$) evolved significantly higher genomic divergence than population-specific genes ($F_{ST} = 0.7274$) between northern and southern oysters ($p = 0.0230$, Fig. 5b). Specifically, we found that the upstream intergenic regions of environment-specific genes exhibited significantly higher divergence than that of population-specific genes between northern and southern oysters ($F_{ST_environment} = 0.7958$, $F_{ST_population} = 0.7402$, $p = 0.01512$), while there was no difference of F_{ST} values at both genic and downstream intergenic regions between two gene sets ($p > 0.05$, Fig. 5c). Further, population-specific genes showed higher

375 divergence at genic region, while environment-specific genes evolved higher
 376 divergence at upstream intergenic region (high divergence at genic region may be
 377 resulted from the higher divergence at upstream intergenic region due to the 100-Kb
 378 sliding window). Although the natural selection preferred to genic regions at
 379 genome-wide level and for population-specific genes, environmentally responsive
 380 genes were preference at upstream intergenic regions and under stronger selection,
 381 which included critical regulatory elements such as promoter and enhancers that
 382 potentially regulate their higher gene expression plasticity²⁴. Our findings provide
 383 insights into the genomic basis of plasticity that can evolve and has higher genetic
 384 divergence by natural selection^{23,24}, facilitating the assessment of species'
 385 vulnerabilities to climate-driven decline or extinction.

386 **Methods**

387 **Genome sequencing.** A wild adult estuarine oyster *Crassostrea ariakensis* was
 388 obtained from the northern China (Binzhou, Bohai Sea, China). Four tissue samples
 389 (gill, mantle, muscle and labial palp) were collected and flash-frozen in the liquid
 390 nitrogen. Genomic DNA extracted from the muscle was used to construct long insert
 391 genomic libraries. Oxford Nanopore Technologies' long-read sequencing platform
 392 generated ~184 Gb of data which corresponding to ~299× coverage. In addition,
 393 libraries with insert size of 350 bp were prepared and sequenced using Illumina HiSeq
 394 4000 platform, which generated ~64 Gb of paired-end reads corresponding to ~105×
 395 coverage of the genome.

396

397 **Genome size and heterozygosity.** Trimmed Illumina short reads were used as input
 398 to calculate the distribution of k-mer copy number (KCN). We selected 21 to obtain
 399 the KCN distribution which showed two distinct peaks (Supplementary Fig. 1). The
 400 first peak (KCN = 45) represents the heterozygous single copy k-mer while the
 401 second peak (KCN = 90) represents the homozygous single copy k-mer in the genome.
 402 Genome size was estimated by the formula $G = K_num / \text{peak depth}$.

403

404 **Contig-level assembly using long-read data.** The Nanopore long reads, with a read
 405 N50 of 33,230 and a mean read length of 23,240 bp, were used for initial genome
 406 assembly. Error correction of clean data was conducted using Canu⁶² v1.5, and then
 407 were assembled using Canu, WTDBG2⁶³ and SMARTdenovo tools. Quickmerge⁶⁴
 408 v0.2.2 was used to join the three assemblies, and then was corrected for 3 cycles using
 409 long reads by Racon⁶⁵ and for 3 cycles using Illumina reads by Pilon⁶⁶ v1.22 with
 410 default parameters. The initial assembly of estuarine oyster genome was 613,892,480
 411 bp in length with a contig N50 of 6,967,240 bp.

412

413 **Chromosome-level assembly with Hi-C.** The same genomic DNA extracted from
 414 the muscle was used to construct Illumina library, which was sequenced on the

415 Illumina HiSeq 4000 platform. A total of 106.34 Gb (173.22-fold coverage) of clean
416 data was obtained, and 41.89% of all reads were truncated that containing enzyme
417 cutting sites. HiC-Pro⁶⁷ was employed to evaluate the alignment efficiency and insert
418 length distribution for valid interaction pairs (75.51%). Furthermore, the genome
419 sequence contigs and scaffolds were interrupted in 50 kb length, which were then
420 sorted and oriented into super scaffolds using LACHESIS⁶⁸ with the following
421 parameters: CLUSTER_MIN_RE_SITES = 47, CLUSTER_MAX_LINK_DENSITY
422 = 2, CLUSTER_NONINFORMATIVE_RATIO = 2,
423 ORDER_MIN_N_RES_IN_TRUN = 40, ORDER_MIN_N_RES_IN_SHREDS = 41.
424

425 **Genome evaluation.** The Hi-C contact heatmap was used to assess the accuracy of
426 the Hi-C assembly. The density of red color represents the number of Hi-C links
427 between 100 kb windows on the pseudochromosomes of the final assembly.
428 Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.0.2 with 978
429 conserved genes were used to assess the completeness and accuracy of estuarine
430 oyster genome. The Illumina genomic reads were also aligned to the oyster genome to
431 assess the completeness using sequence alignment tool BWA⁶⁹.
432

433 **Repeat annotation.** Transposable elements (TEs) were identified and classified using
434 homology-based and *de novo*-based approaches. RepeatScout and LTR_FINDER was
435 used to construct the *de novo* repeat libraries. The *de novo*-based library was further
436 classified by PASTECClassifier⁷⁰ to obtain a consensus library, and combined with the
437 repeat library of Repbase data. RepeatMasker⁷¹ v4.0.5 was used to identify TEs in the
438 estuarine oyster genome with the combined library.
439

440 **Protein-coding genes annotation.** We adopted three methods including *de*
441 *novo*-based predictions, homology-based predictions and RNA-seq-based predictions
442 to annotate the protein-coding genes of estuarine oyster *C. ariakensis*. For the
443 RNA-seq-based prediction, RNA-seq data generated from four tissues (gill, mantle,

muscle and labial palp) using ONT long-reads sequencing platform was filtered to remove adaptors and then trimmed to remove low-quality bases. Clean reads were aligned to reference genome using TopHat⁷² and then assembled using Trinity⁷³. Full transcriptome-based genome annotation was predicted using PASA⁷⁴ v2.2.2 software. For the *de novo* prediction, five *ab initio* gene prediction programs, including Genscan⁷⁵ v1.0, Augustus⁷⁶ v2.4, GlimmerHMM⁷⁷ v3.0.4, GeneID⁷⁸ v1.4 and SNAP⁷⁹, were used to predict genes in the repeat-masked genome. For homolog-based prediction, the protein sequences of 10 well-annotated species, including *Homo sapiens*, *Danio rerio*, *Aplysia californica*, *Strongylocentrotus purpuratus*, *C. gigas*, *C. virginica*, *Biomphalaria glabrata*, *Lingula anatina*, *Octopus bimaculoides* and *Mizuhopecten yessoensis*, were downloaded and aligned to the repeat-masked estuarine oyster genome using tblastn⁸⁰ with E-value $\leq 1E-05$. We employed GeMoMa⁸¹ v1.3.1 to predict gene models based on the alignment sequences. Finally, EVidenceModeler⁸² (EVM) v1.1.1 was used to generate a weighted and non-redundant gene set by integrating all gene models predicted by the above three methods.

Homologous sequences in the genome were identified by genBlastA⁸³ v1.0.4 using the integrated gene set, and GeneWise⁸⁴ was used to identify pseudogenes. Transfer RNAs (tRNAs) were defined using tRNAscan-SE⁸⁵ v1.3.1 software with eukaryote default parameters. Micro RNA and Rna were identified by Infernal BLASTN⁸⁶ against the Rfam⁸⁷ database v12.0.

Functional annotation of protein-coding genes was conducted by aligning them to the NCBI non-redundant protein⁸⁸ (NR), SwissProt⁸⁹, KOG⁹⁰ and TrEMBL⁸⁹ databases using BLAST⁸⁶ v2.2.31 with a maximal e-value of 1e-05. Domains were identified using HMMER⁹¹ v3.0 to search against Pfam⁹² databases. Gene set was mapped to Gene Ontology (GO) terms and KEGG pathway to identify the best match classification for each gene.

Whole-genome resequencing and mapping. We collected 264 wild oysters of *C.*

473 *ariakensis* from 11 estuarine areas (Fig. 3a), representing the major distribution range
 474 across north, middle and south Chinese coastlines^{28,29}. Genomic DNA was isolated
 475 from gill of each oysters following the standard phenol-chloroform extraction
 476 procedure, and then was used to construct a library with an insert size of ~ 350 bp.
 477 Paired-end sequencing libraries were constructed according to the manufacturer's
 478 instructions (Illumina Inc., San Diego, CA, USA) and subsequently sequenced on the
 479 Illumina HiSeq X Ten Sequencer (Illumina Inc.). We obtained ~14.42 Gb of clean
 480 data for each sample, giving an average depth of 19.9× coverage (15-28×)
 481 (Supplementary Table 8). The 150-bp paired-end reads were mapped onto the *C.*
 482 *ariakensis* reference genome (PRJNA715058) with the Burrows-Wheeler Aligner
 483 v.0.7.8⁶⁹ using the default parameters (bwa mem -M -t 10 -T 20). Mapping data were
 484 then converted into the BAM format and sorted by SAMtools v.1.3.1⁹³, which was
 485 further used to remove duplicate reads. Read pair with the highest mapping quality
 486 was retained if multiple read pairs had identical external coordinates.

487

488 **Genomic variation calling and annotation.** The Genome Analysis Toolkit (GATK)
 489 v.3.7⁹⁴ module HaplotypeCaller was used to obtain high-quality variation calling of
 490 each sample. SNPs were further filtered with the parameter 'QD<2.0 || FS>60.0 ||
 491 MQ<40.0'. Similarly, calling of INDELs was conducted and filtered using the
 492 command parameters as 'QD<2.0 || FS>60.0'. Filtered SNPs were annotated by the
 493 SnpEff⁹⁵ based on the *C. ariakensis* genome, and then were classified as variations in
 494 regions of exon, intron, splicing sites, and upstream and downstream intergenic
 495 regions, and in types of heterozygous and homozygous variations. To characterize the
 496 types of variations in northern and southern oysters, Plink⁹⁶ was used to filter the raw
 497 SNPs of each oyster populations using the parameters of MAF > 0.05 and Int > 0.8.
 498 The same SNPs were retrained and then were singly classified as homozygote or
 499 heterozygote in each oyster population (more than half individuals were the same type
 500 of variation in each oyster population). Variations in exons were further categorized as
 501 synonymous or non-synonymous SNPs. Two-sided two-sample Wilcoxon signed-rank

502 tests were conducted to test whether the ratios of genes with nonsynonymous
503 variations were different between northern and southern geographic populations,
504 using the function *wilcoxsign-test* in R package “coin”.

505

506 **Population genetic analysis.** Plink⁹⁶ was used to filter the raw SNPs of all
507 individuals using the parameters of MAF > 0.05 and Int > 0.8. Population structure
508 was investigated using ADMIXTURE v.1.23⁹⁷ with default setting. The number of
509 assumed genetic clusters *K* ranged from 2 to 5, and the optimum number of *K* was
510 assessed by cross-validation (CV) errors. The individual-based neighbor-joining (NJ)
511 phylogenetic tree was constructed using the MEGA⁹⁸ under the Kimura 2-Parameter
512 model with 1000 bootstraps, and was then visualized using FigTree. We performed
513 PCA for whole-genome SNPs of all 264 individuals using Eigensoft⁹⁹. To evaluate
514 linkage disequilibrium decay, the parameter r^2 between any two loci was calculated
515 within each chromosome using Plink v.1.07⁹⁶ with the command (`-ld-window-r2 0`
516 `-ld-window 99999 -ld-window-kb 500`). The average r^2 values were calculated for
517 each length of distance and the whole-genome LD was averaged across all
518 chromosomes. The LD decay plot was depicted against the length of distance.
519 Popgenome R package¹⁰⁰ was used to calculate Tajima’s *D*, global F_{ST} and nucleotide
520 diversity (π) using a 100-kb sliding window with the step size of 10-kb.

521

522 **Demographic history of marine and estuarine oyster species.** We implemented
523 PMSC¹⁰¹ to estimate dynamics of effective population size (N_e) and the possible
524 divergence time over the past several million years ago (mya). A total of eight
525 estuarine oysters (*C. ariakensis*) from northern (n = 3), middle (n = 2) and (n = 3)
526 southern populations and three marine oysters (*C. gigas*)¹¹ with a high sequencing
527 depth (*C. ariakensis*: 25~28×, *C. gigas*: ~20×) were used. To alleviate the probability
528 of false positive, sequencing depth of SNPs was filtered with parameters: MinDepth =
529 average depth/3, MaxDepth = average depth×2. The PSMC parameters were set as:
530 `-N25 -t15 -r5 -p '4 + 25*2 + 4 + 6'` to estimate the historical N_e . The estimated

531 generation time (g) was set as 1 for both species, while mutation rates (μ) were
 532 calculated, following the formula $T_{\text{divergence}} = Ks/2\mu$, as 0.3×10^{-8} and 0.2×10^{-8} for *C.*
 533 *ariakensis* and *C. gigas*, respectively.

534

535 **Detection of selective signals for adaptation to southern environments.** To identify
 536 candidate selective signals potentially contributing to adaptation of oysters to southern
 537 environments, we calculated two pairs of population fixation statistics (F_{ST}) and
 538 selection statistics (Tajima's D), including a) north vs south and b) middle vs south, in
 539 a 100-kb sliding window with a step size of 10-kb. Genomic regions showing strong
 540 selective signals were defined as following: 1) regions showed top 1% F_{ST} values
 541 were overlapped in both comparison pairs; 2) regions located at the ravines of
 542 Tajima's D values along each chromosome in one of the three oyster populations.

543

544 **Exposure to high temperature and salinity.** To investigate environmental responses
 545 of estuarine oysters to challenges of elevated temperature and high salinity, we
 546 collected wild oysters and acutely exposed to different gradients of temperature of 20 °C
 547 and 37 °C for 6 hours and of salinity of 20 ‰ and 60 ‰ for 7 days, respectively. Gills
 548 from five oysters were individually sampled and immediately flash-frozen in liquid
 549 nitrogen for subsequent RNA-seq analysis.

550

551 **Reciprocal transplantation experiments.** Reciprocal transplantation experiments
 552 were described in our previously study². Briefly, wild oysters derived from northern
 553 (Binzhou: BZ, Bohai Sea) and southern (Taishan: TS, East China Sea) environments
 554 were collected and used to reproduce F_1 generation within population. To potentially
 555 maintain an effective population size, a total of 80 mature male and female oysters
 556 were selected as parental individuals after excluding hermaphrodites by microscopic
 557 examination. Eggs were mixed and then divided into 40 beakers. Sperm from each of
 558 40 male oysters were individually crossed with each beaker of mixed eggs, which
 559 warranted each sperm can fertilized with eggs from different female oysters. Zygotes

560 fathered by eight males were combined into one group. Five groups were reared to the
561 D-shaped stage and then cultured in one nursery pond during larvae to spat stages.
562 Two-month-old juvenile oysters from each of two populations were outplanted to two
563 source habitats to test their responses to reciprocal transplantation. After three months
564 of acclimation at northern and southern environments, we sampled gills of five
565 oysters from each of population at both habitats in situ that gills were dissected out
566 immediately on the boat and flash-frozen in liquid nitrogen for subsequent RNA-seq
567 analysis.

568

569 **RNA-seq analysis.** Total RNA was isolated from gills sampled from acute stress
570 experiment (high temperature and salinity) and reciprocal transplant experiment,
571 using the RNAPrep Pure Tissue Kit (Tiangen) following the manufacturer's protocol.
572 The RNA integrity and concentration were examined by 1.2% gel electrophoresis and
573 Nanodrop 2000 spectrophotometer, respectively. DNA contamination was removed
574 with DNase I treatment. RNA integrity was assessed using the RNA Nano 6000
575 Assay Kit of the Agilent Bioanalyzer 2100 system. A total amount of 1 µg RNA per
576 sample was used to construct sequencing libraries using NEBNext Ultra™ RNA
577 Library Prep Kit, and then were sequenced on an Illumina HiSeq 4000 platform to
578 generate 150-bp paired-end raw reads. Clean data were obtained by removing reads
579 containing adapter, reads containing ploy-N and low-quality reads. TopHat⁶⁶ was
580 used to map clean reads to the estuarine oyster *C. ariakensis* reference genome.
581 StringTie v2.0 was used for reads assembly. Only reads with a perfect match or one
582 mismatch were further analyzed and annotated. Gene expression levels were
583 estimated by fragments per kilobase of transcript per million fragments mapped
584 (FPKM). We employed DESeq2 to analysis differentially expressed genes (DEGs)
585 between different populations at northern and southern environments. Genes with an
586 adjusted *p*-value < 0.01 using the Benjamin and Hochberg's approach were assigned
587 as DEGs. A hierarchical cluster analysis was performed to indicate expression level of
588 candidate genes showing strong selective signals, using the *pheatmap* package in R

589 software.

590

591 **Data availability**

592 The genome, whole-genome re-sequencing and transcriptome datasets were deposited
593 in the Sequence Read Archive (SRA) database under the accession number
594 PRJNA715058.

595 **Reference**

- 596 1 Chou, C. *et al.* Increase in the range between wet and dry season precipitation. *Nature*
597 *Geoscience* **6**, 263-267, doi:10.1038/ngeo1744 (2013).
- 598 2 Li, A. *et al.* Molecular and Fitness Data Reveal Local Adaptation of Southern and
599 Northern Estuarine Oysters (*Crassostrea ariakensis*). *Frontiers in Marine Science* **7**,
600 doi:10.3389/fmars.2020.589099 (2020).
- 601 3 Buroker, N. E., Hershberger, W. K. & Chew, K. K. Population Genetics of the Family
602 Ostreidae. II. Interspecific Studies of the Genera *Crassostrea* and *Saccostrea*. *Marine*
603 *Biology* **54**, 171-184 (1979).
- 604 4 Liu, J. X., Gao, T. X., Wu, S. F. & Zhang, Y. P. Pleistocene isolation in the
605 Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon*
606 *haematocheilus* (Temminck & Schlegel, 1845). *Molecular ecology* **16**, 275-288,
607 doi:10.1111/j.1365-294X.2006.03140.x (2007).
- 608 5 Dong, Y. *et al.* The Impact of Yangtze River Discharge, Ocean Currents and Historical
609 Events on the Biogeographic Pattern of *Cellana toreuma* along the China Coast. *PLoS*
610 *ONE* **7**, e36178, doi:10.1371/journal.pone.0036178.g001 (2012).
- 611 6 Ni, G., Li, Q., Kong, L. & Zheng, X. D. Phylogeography of bivalve *Cyclina sinensis*.
612 testing the historical glaciations and Changjiang River outflow hypotheses in
613 northwestern Pacific. *PLoS ONE* **7**, e49487, doi:10.1371/journal.pone.0049487.g001
614 (2012).
- 615 7 Ni, G., Kern, E., Dong, Y. W., Li, Q. & Park, J. K. More than meets the eye: The barrier
616 effect of the Yangtze River outflow. *Molecular ecology* **26**, 4591-4602,

doi:10.1111/mec.14235 (2017).

8 Sanford, E. & Kelly, M. W. Local adaptation in marine invertebrates. *Annual review of marine science* **3**, 509-535, doi:10.1146/annurev-marine-120709-142756 (2011).

9 Somero, G. N. The physiology of global change: linking patterns to mechanisms. *Annual review of marine science* **4**, 39-61, doi:10.1146/annurev-marine-120710-100935 (2012).

10 Miller, A. D. *et al.* Local and regional scale habitat heterogeneity contribute to genetic adaptation in a commercially important marine mollusc (*Haliotis rubra*) from southeastern Australia. *Molecular ecology* **28**, 3053-3072, doi:10.1111/mec.15128 (2019).

11 Li, L. *et al.* Divergence and plasticity shape adaptive potential of the Pacific oyster. *Nat Ecol Evol* **2**, 1751-1760, doi:10.1038/s41559-018-0668-2 (2018).

12 Stern, D. B. & Lee, C. E. Evolutionary origins of genomic adaptations in an invasive copepod. *Nat Ecol Evol* **4**, 1084-1094, doi:10.1038/s41559-020-1201-y (2020).

13 Kenkel, C. D. & Matz, M. V. Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Ecology & Evolution* **1**, 0014, doi:10.1038/s41559-016-0014 (2016).

14 Ho, W. C. & Zhang, J. Evolutionary adaptations to new environments generally reverse plastic phenotypic changes. *Nature communications* **9**, 350, doi:10.1038/s41467-017-02724-5 (2018).

15 Gienapp, P., Teplitsky, C., Alho, J. S., Mills, J. A. & Merila, J. Climate change and evolution: disentangling environmental and genetic responses. *Molecular ecology* **17**,

639 167-178, doi:10.1111/j.1365-294X.2007.03413.x (2008).

640 16 Pfennig, D. W. *et al.* Phenotypic plasticity's impacts on diversification and speciation.

641 *Trends in ecology & evolution* **25**, 459-467, doi:10.1016/j.tree.2010.05.006 (2010).

642 17 Barrio, A. M. *et al.* The genetic basis for ecological adaptation of the Atlantic herring

643 revealed by genome sequencing. *eLife* **5**, e12081, doi:10.7554/eLife.12081.001

644 (2016).

645 18 Zong, S. B., Li, Y. L. & Liu, J. X. Genomic architecture of rapid parallel adaptation to

646 fresh water in a wild fish. *Mol Biol Evol*, doi:10.1093/molbev/msaa290 (2020).

647 19 Zhou, X. *et al.* Population genomics of finless porpoises reveal an incipient cetacean

648 species adapted to freshwater. *Nature communications* **9**, 1276,

649 doi:10.1038/s41467-018-03722-x (2018).

650 20 Sandoval-Castillo, J. *et al.* Adaptation of plasticity to projected maximum temperatures

651 and across climatically defined bioregions. *Proceedings of the National Academy of*

652 *Sciences* **117**, 17112-17121, doi:10.1073/pnas.1921124117/-DCSupplemental

653 (2020).

654 21 Eierman, L. E. & Hare, M. P. Reef-Specific Patterns of Gene Expression Plasticity in

655 Eastern Oysters (*Crassostrea virginica*). *The Journal of heredity* **107**, 90-100,

656 doi:10.1093/jhered/esv057 (2016).

657 22 Bernal, M. A. *et al.* Species-specific molecular responses of wild coral reef fishes

658 during a marine heatwave. *Science Advances* **6**, eaay3423 (2020).

659 23 Kelly, M. Adaptation to climate change through genetic accommodation and

660 assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society B:*

- 661 *Biological Sciences* **374**, 20180176, doi:10.1098/rstb.2018.0176 (2019).
- 662 24 Grishkevich, V. & Yanai, I. The genomic determinants of genotype x environment
- 663 interactions in gene expression. *Trends in genetics : TIG* **29**, 479-487,
- 664 doi:10.1016/j.tig.2013.05.006 (2013).
- 665 25 Ahlgren, J., Yang, X., Hansson, L. A. & Bronmark, C. Camouflaged or tanned:
- 666 plasticity in freshwater snail pigmentation. *Biology Letters* **9**, 20130464-20130464,
- 667 doi:10.1098/rsbl.2013.0464 (2013).
- 668 26 Li, A., Li, L., Song, K., Wang, W. & Zhang, G. Temperature, energy metabolism, and
- 669 adaptive divergence in two oyster subspecies. *Ecology and evolution* **7**, 6151-6162,
- 670 doi:10.1002/ece3.3085 (2017).
- 671 27 Gagnaire, P.-A. *et al.* Analysis of Genome-Wide Differentiation between Native and
- 672 Introduced Populations of the Cupped Oysters *Crassostrea gigas* and *Crassostrea*
- 673 *angulata*. *Genome biology and evolution* **10**, 2518-2534,
- 674 doi:10.12770/dbf64e8d-45dd-437f-b734-00b77606430a10.1093/gbe/evy194 (2018).
- 675 28 Wang, H., Guo, X., Zhang, G. & Zhang, F. Classification of jinjiang oysters
- 676 *Crassostrea rivularis* (Gould, 1861) from China, based on morphology and
- 677 phylogenetic analysis. *Aquaculture* **242**, 137-155,
- 678 doi:10.1016/j.aquaculture.2004.09.014 (2004).
- 679 29 Zhou, M. F. & Allen, S. K. A review of published work on *Crassostrea ariakensis*.
- 680 *Journal of Shellfish Research* **22**, 1-20 (2003).
- 681 30 Wang, H. *et al.* Distribution of *Crassostrea ariakensis* in China. *Journal of Shellfish*
- 682 *Research* **25**, 789-790 (2006).

- 683 31 Zhang, Q., Allen, S. K., Jr. & Reece, K. S. Genetic variation in wild and hatchery
684 stocks of Suminoe Oyster (*Crassostrea ariakensis*) assessed by PCR-RFLP and
685 microsatellite markers. *Marine biotechnology* **7**, 588-599,
686 doi:10.1007/s10126-004-5105-7 (2005).
- 687 32 Xiao, J., Cordes, J. F., Wang, H., Guo, X. & Reece, K. S. Population genetics of
688 *Crassostrea ariakensis* in Asia inferred from microsatellite markers. *Marine Biology*
689 **157**, 1767-1781, doi:10.1007/s00227-010-1449-x (2010).
- 690 33 Kim, W.-J. *et al.* Mitochondrial DNA sequence analysis from multiple gene fragments
691 reveals genetic heterogeneity of *Crassostrea ariakensis* in East Asia. *Genes Genom.*
692 **36**, 611-624, doi:10.1007/s13258-014-0198-5 (2014).
- 693 34 Liu, X. *et al.* Transcriptome and Gene Coexpression Network Analyses of Two Wild
694 Populations Provides Insight into the High-Salinity Adaptation Mechanisms of
695 *Crassostrea ariakensis*. *Marine biotechnology* **21**, 596-612,
696 doi:10.1007/s10126-019-09896-9 (2019).
- 697 35 Bai, C. M. *et al.* Chromosomal-level assembly of the blood clam, *Scapharca (Anadara)*
698 *broughtonii*, using long sequence reads and Hi-C. *GigaScience* **8**,
699 doi:10.1093/gigascience/giz067 (2019).
- 700 36 Peng, J. *et al.* Chromosome-level analysis of the *Crassostrea hongkongensis* genome
701 reveals extensive duplication of immune-related genes in bivalves. *Molecular ecology*
702 *resources* **20**, 980-994, doi:10.1111/1755-0998.13157 (2020).
- 703 37 Song, H. *et al.* The hard clam genome reveals massive expansion and diversification
704 of inhibitors of apoptosis in *Bivalvia*. *BMC Biol* **19**, 15,

705 doi:10.1186/s12915-020-00943-9 (2021).

706 38 Zhang, G. *et al.* The oyster genome reveals stress adaptation and complexity of shell
707 formation. *Nature* **490**, 49-54, doi:10.1038/nature11413 (2012).

708 39 Hu, Y. *et al.* Spatial patterns and conservation of genetic and phylogenetic diversity of
709 wildlife in China. *science advances* **7**, eabd5725, doi:10.1126/sciadv.abd5725 (2021).

710 40 Li, C. *et al.* Genome sequences reveal global dispersal routes and suggest convergent
711 genetic adaptations in seahorse evolution. *Nature communications* **12**, 1094,
712 doi:10.1038/s41467-021-21379-x (2021).

713 41 Wang, H., Qian, L., Liu, X., Zhang, G. & Guo, X. Classification of a common cupped
714 oyster from southern China. *Journal of Shellfish Research* **29**, 857-866,
715 doi:10.2983/035.029.0420 (2010).

716 42 Wang, H., Zhang, G., Liu, X. & Guo, X. Classification of Common Oysters from North
717 China. *Journal of Shellfish Research* **27**, 495-503,
718 doi:10.2983/0730-8000(2008)27[495:COCOFN]2.0.CO (2008).

719 43 Raeymaekers, J. A. M. *et al.* Adaptive and non-adaptive divergence in a common
720 landscape. *Nature communications* **8**, 267, doi:10.1038/s41467-017-00256-6 (2017).

721 44 Kimura, M. Paleogeography of the Ryukyu Islands. *Tropics* **10**, 5-24 (2000).

722 45 Ren, J., Liu, X., Jiang, F., Guo, X. & Liu, B. Unusual conservation of mitochondrial
723 gene order in *Crassostrea* oysters: evidence for recent speciation in Asia. *BMC*
724 *evolutionary biology* **10**, 394, doi:10.1186/1471-2148-10-394 (2010).

725 46 Qin, Y., Zhao, Y. & Zhao, S. *Geology of the Bohai Sea*. (Science Press, 1985).

726 47 Skliris, N. *et al.* Salinity changes in the World Ocean since 1950 in relation to changing

727 surface freshwater fluxes. *Climate Dynamics* **43**, 709-736,
728 doi:10.1007/s00382-014-2131-7 (2014).

729 48 Mekonnen, M. M. & Hoekstra, A. Y. Four billion people facing severe water scarcity.
730 *Science Advances* **2**, e1500323 (2016).

731 49 Ribeiro, C. A., Balestro, F., Grando, V. & Wajner, M. Isovaleric acid reduces Na⁺,
732 K⁺-ATPase activity in synaptic membranes from cerebral cortex of young rats. *Cellular*
733 *and molecular neurobiology* **27**, 529-540, doi:10.1007/s10571-007-9143-3 (2007).

734 50 Huang, Y., Niwa, J., Sobue, G. & Breitwieser, G. E. Calcium-sensing receptor
735 ubiquitination and degradation mediated by the E3 ubiquitin ligase dorf. *The Journal*
736 *of biological chemistry* **281**, 11610-11617, doi:10.1074/jbc.M513552200 (2006).

737 51 Vienken, H. *et al.* Characterization of cholesterol homeostasis in
738 sphingosine-1-phosphate lyase-deficient fibroblasts reveals a Niemann-Pick disease
739 type C-like phenotype with enhanced lysosomal Ca(2⁺) storage. *Scientific reports* **7**,
740 43575, doi:10.1038/srep43575 (2017).

741 52 Pagano, M. *et al.* Insights into the residence in lipid rafts of adenylyl cyclase AC8 and
742 its regulation by capacitative calcium entry. *Am J Physiol Cell Physiol* **296**,
743 C607–C619, doi:10.1152/ajpcell.00488.2008.-Adenylyl (2009).

744 53 Weinman, E. J., Dubinsky, W. P. & Shenolikar, S. Reconstitution of cAMP-Dependent
745 Protein Kinase Regulated Renal Na⁺-H⁺ Exchanger. *J. Membr. Biol.* **101**, 11-18
746 (1988).

747 54 Fonteles, M. C., Greenberg, R. N., Monteiro, H. S. A., Currie, M. G. & Forte, L. R.
748 Natriuretic and kaliuretic activities of guanylin and uroguanylin in the isolated perfused

749 rat kidney. *The American Journal of Physiology* **F191-F197** (1998).

750 55 Kenkel, C. D., Meyer, E. & Matz, M. V. Gene expression under chronic heat stress in
751 populations of the mustard hill coral (*Porites astreoides*) from different thermal
752 environments. *Molecular ecology* **22**, 4322-4334, doi:10.1111/mec.12390 (2013).

753 56 Hoglund, P. J., Nordstrom, K. J., Schioth, H. B. & Fredriksson, R. The solute carrier
754 families have a remarkably long evolutionary history with the majority of the human
755 families present before divergence of Bilaterian species. *Mol Biol Evol* **28**, 1531-1541,
756 doi:10.1093/molbev/msq350 (2011).

757 57 Guo, X., He, Y., Zhang, L., Lelong, C. & Jouaux, A. Immune and stress responses in
758 oysters with insights on adaptation. *Fish & shellfish immunology* **46**, 107-119,
759 doi:10.1016/j.fsi.2015.05.018 (2015).

760 58 Li, A., Li, L., Wang, W., Song, K. & Zhang, G. Transcriptomics and Fitness Data
761 Reveal Adaptive Plasticity of Thermal Tolerance in Oysters Inhabiting Different Tidal
762 Zones. *Front Physiol* **9**, 825, doi:10.3389/fphys.2018.00825 (2018).

763 59 Zhang, G. *et al.* Molecular Basis for Adaptation of Oysters to Stressful Marine
764 Intertidal Environments. *Annual review of animal biosciences* **4**, 357-381,
765 doi:10.1146/annurev-animal-022114-110903 (2016).

766 60 Li, A., Li, L., Wang, W. & Zhang, G. Evolutionary trade-offs between baseline and
767 plastic gene expression in two congeneric oyster species. *Biology Letters* **15**,
768 20190202, doi:10.1098/rsbl.2019.0202 (2019).

769 61 Ghaffari, H., Wang, W., Li, A., Zhang, G. & Li, L. Thermotolerance Divergence
770 Revealed by the Physiological and Molecular Responses in Two Oyster Subspecies of

771 Crassostrea gigas in China. *Front Physiol* **10**, 1137, doi:10.3389/fphys.2019.01137
772 (2019).

773 62 Koren, S. *et al.* Canu: scalable and accurate long-read assembly via adaptive k-mer
774 weighting and repeat separation. *Genome research* **27**, 722-736,
775 doi:10.1101/gr.215087.116 (2017).

776 63 Jayakumar, V. & Sakakibara, Y. Comprehensive evaluation of non-hybrid genome
777 assembly tools for third-generation PacBio long-read sequence data. *Briefings in*
778 *bioinformatics* **20**, 866-876, doi:10.1093/bib/bbx147 (2019).

779 64 Chakraborty, M., Baldwin-Brown, J. G., Long, A. D. & Emerson, J. J. Contiguous and
780 accurate de novo assembly of metazoan genomes with modest long read coverage.
781 *Nucleic acids research* **44**, e147, doi:10.1093/nar/gkw654 (2016).

782 65 Vaser, R., Sovic, I., Nagarajan, N. & Sikic, M. Fast and accurate de novo genome
783 assembly from long uncorrected reads. *Genome research* **27**, 737-746,
784 doi:10.1101/gr.214270.116 (2017).

785 66 Walker, B. J. *et al.* Pilon: An Integrated Tool for Comprehensive Microbial Variant
786 Detection and Genome Assembly Improvement. *PLoS ONE* **9**, e112963,
787 doi:10.1371/journal.pone.0112963.g001 (2014).

788 67 Servant, N. *et al.* HiC-Pro: an optimized and flexible pipeline for Hi-C data processing.
789 *Genome biology* **16**, 259, doi:10.1186/s13059-015-0831-x (2015).

790 68 Burton, J. N. *et al.* Chromosome-scale scaffolding of de novo genome assemblies
791 based on chromatin interactions. *Nature biotechnology* **31**, 1119-1125,
792 doi:10.1038/nbt.2727 (2013).

793 69 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler
transform. *Bioinformatics* **25**, 1754-1760, doi:10.1093/bioinformatics/btp324 (2009).

794

795 70 Hoede, C. *et al.* PASTEC: An Automatic Transposable Element Classification Tool.
PLoS ONE **9**, e91929, doi:10.1371/journal.pone.0091929.t001 (2014).

796

797 71 Tarailo-Graovac, M. & Chen, N. Using RepeatMasker to identify repetitive elements in
genomic sequences. *Curr Protoc Bioinformatics* **Chapter 4**, Unit 4 10,
doi:10.1002/0471250953.bi0410s25 (2009).

798

799

800 72 Kim, D. *et al.* TopHat2: accurate alignment of transcriptomes in the presence of
insertions, deletions and gene fusions. *Genome biology* **14**, R36 (2013).

801

802 73 Grabherr, M. G. *et al.* Full-length transcriptome assembly from RNA-Seq data without
a reference genome. *Nature biotechnology* **29**, 644-652, doi:10.1038/nbt.1883 (2011).

803

804 74 Campbell, M. A., Haas, B. J., Hamilton, J. P., Mount, S. M. & Buell, C. R.
Comprehensive analysis of alternative splicing in rice and comparative analyses with
Arabidopsis. *BMC genomics* **7**, 327, doi:10.1186/1471-2164-7-327 (2006).

805

806

807 75 Burge, C. & Karlin, S. Prediction of complete gene structures in human genomic DNA.
Journal of molecular biology **268**, 78-94 (1997).

808

809 76 Stanke, M. & Waack, S. Gene prediction with a hidden Markov model and a new intron
submodel. *Bioinformatics* **19 Suppl 2**, ii215-225, doi:10.1093/bioinformatics/btg1080
(2003).

810

811

812 77 Majoros, W. H., Pertea, M. & Salzberg, S. L. TigrScan and GlimmerHMM: two open
source ab initio eukaryotic gene-finders. *Bioinformatics* **20**, 2878-2879,
doi:10.1093/bioinformatics/bth315 (2004).

813

814

815 78 Alioto, T., Blanco, E., Parra, G. & Guigo, R. Using geneid to Identify Genes. *Curr*
816 *Protoc Bioinformatics* **64**, e56, doi:10.1002/cpbi.56 (2018).

817 79 Korf, I. Gene finding in novel genomes. *Bmc Bioinformatics* **5**, 59 (2004).

818 80 Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein
819 database search programs. *Nucleic acids research* **25**, 3389-3402 (1997).

820 81 Keilwagen, J., Hartung, F., Paulini, M., Twardziok, S. O. & Grau, J. Combining
821 RNA-seq data and homology-based gene prediction for plants, animals and fungi.
822 *Bmc Bioinformatics* **19**, 189, doi:10.1186/s12859-018-2203-5 (2018).

823 82 Haas, B. J. *et al.* Automated eukaryotic gene structure annotation using
824 Evidencemodeler and the Program to Assemble Spliced Alignments. *Genome biology*
825 **9**, R7, doi:10.1186/gb-2008-9-1-r7 (2008).

826 83 She, R., Chu, J. S., Wang, K., Pei, J. & Chen, N. GenBlastA: enabling BLAST to
827 identify homologous gene sequences. *Genome research* **19**, 143-149,
828 doi:10.1101/gr.082081.108 (2009).

829 84 Birney, E., Clamp, M. & Durbin, R. GeneWise and Genomewise. *Genome research* **14**,
830 doi:10.1101/ (2004).

831 85 Lowe, T. M. & Eddy, S. R. tRNAscan-SE: a program for improved detection of transfer
832 RNA genes in genomic sequence. *Nucleic acids research* **25**, 955-964 (1997).

833 86 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic Local
834 Alignment Search Tool. *Journal of molecular biology* **215**, 403-410 (1990).

835 87 Griffiths-Jones, S. *et al.* Rfam: annotating non-coding RNAs in complete genomes.
836 *Nucleic acids research* **33**, D121-124, doi:10.1093/nar/gki081 (2005).

837 88 Marchler-Bauer, A. *et al.* CDD: a Conserved Domain Database for the functional
838 annotation of proteins. *Nucleic acids research* **39**, D225-229,
839 doi:10.1093/nar/gkq1189 (2011).

840 89 Boeckmann, B. *et al.* The SWISS-PROT protein knowledgebase and its supplement
841 TrEMBL in 2003. *Nucleic acids research* **31**, 365-370, doi:10.1093/nar/gkg095 (2003).

842 90 Tatusov, R. L. *et al.* The COG database: new developments in phylogenetic
843 classification of proteins from complete genomes. *Nucleic acids research* **29**, 22-28
844 (2001).

845 91 Mistry, J., Finn, R. D., Eddy, S. R., Bateman, A. & Punta, M. Challenges in homology
846 search: HMMER3 and convergent evolution of coiled-coil regions. *Nucleic acids*
847 *research* **41**, e121, doi:10.1093/nar/gkt263 (2013).

848 92 Finn, R. D. *et al.* Pfam: clans, web tools and services. *Nucleic acids research* **34**,
849 D247-251, doi:10.1093/nar/gkj149 (2006).

850 93 Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**,
851 2078-2079, doi:10.1093/bioinformatics/btp352 (2009).

852 94 McKenna, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for
853 analyzing next-generation DNA sequencing data. *Genome research* **20**, 1297-1303,
854 doi:10.1101/gr.107524.110 (2010).

855 95 Cingolani, P. *et al.* A program for annotating and predicting the effects of single
856 nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster*
857 strain w1118; iso-2; iso-3. *Fly* **6**, 80-92, doi:10.4161/fly.19695 (2012).

858 96 Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based

859 linkage analyses. *American journal of human genetics* **81**, 559-575,
860 doi:10.1086/519795 (2007).

861 97 Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry
862 in unrelated individuals. *Genome research* **19**, 1655-1664, doi:10.1101/gr.094052.109
863 (2009).

864 98 Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular
865 Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* **35**,
866 1547-1549, doi:10.1093/molbev/msy096 (2018).

867 99 Price, A. L. *et al.* Principal components analysis corrects for stratification in
868 genome-wide association studies. *Nature genetics* **38**, 904-909, doi:10.1038/ng1847
869 (2006).

870 100 Pfeifer, B., Wittelsburger, U., Ramos-Onsins, S. E. & Lercher, M. J. PopGenome: an
871 efficient Swiss army knife for population genomic analyses in R. *Mol Biol Evol* **31**,
872 1929-1936, doi:10.1093/molbev/msu136 (2014).

873 101 Li, H. & Durbin, R. Inference of human population history from individual
874 whole-genome sequences. *Nature* **475**, 493-496, doi:10.1038/nature10231 (2011).
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890 **Author contributions**

891 L.L., G.Z. and X.G. conceived the study and participated in final data analysis,
892 interpretation and drafting the manuscript. A.L. carried out the data analysis and
893 drafted the manuscript. H.D., A.L., H.C., X.L. and H.Z. contributed to the selective
894 sweep analysis. A.L., Z.Z., K.Z. and C.W. collected and sampled oyster specimens.
895 A.L. and W.W. produced the F₁ progeny. A.L., L.L., X.G. and G.Z. revised the
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899 **Competing interests**

900 The authors declare no competing interests.

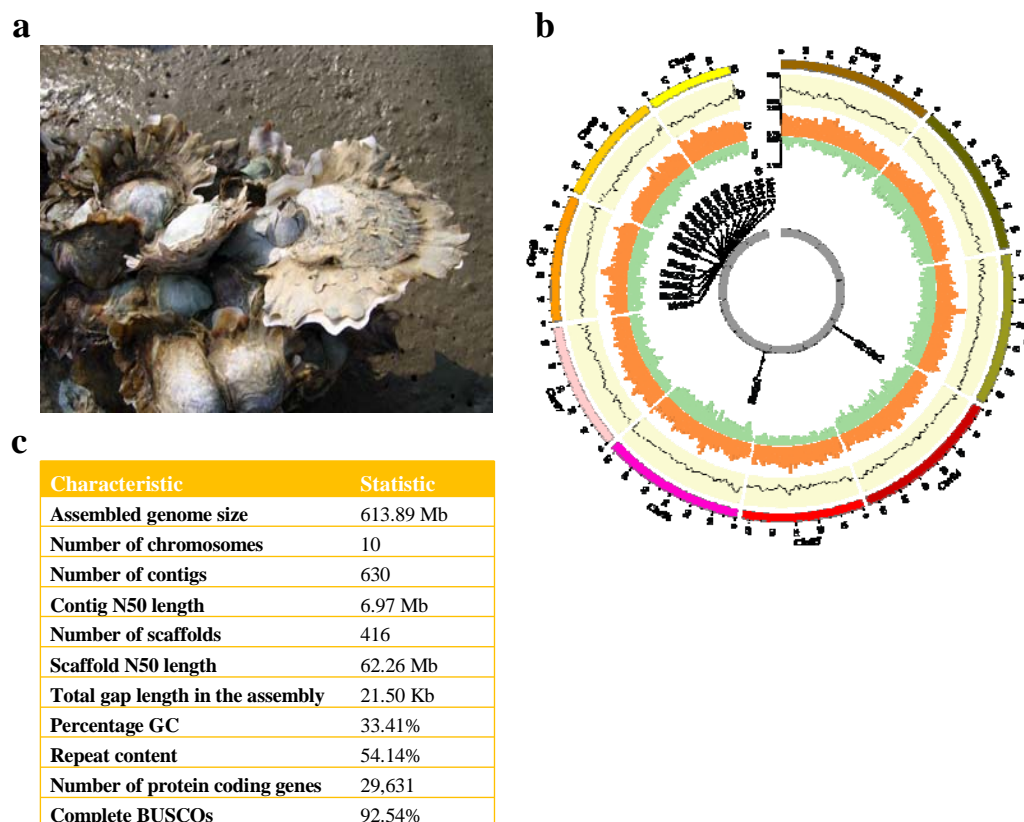


Fig. 1 | High-quality assembly of the genome of estuarine oyster *Crassostrea ariakensis*. **a**, Estuarine oyster (photograph by Lumin Qian). **b**, CIRCOS plot showing the distribution of GC content, transposable elements (TE), coding sequences (CDS) and candidate genes (*solute carrier families*) surrounding selective sweep signals (see **Fig. 4**) in each chromosome of the *C. ariakensis* genome. **c**, Summary of the *C. ariakensis* genome assembly and gene annotation statistics.

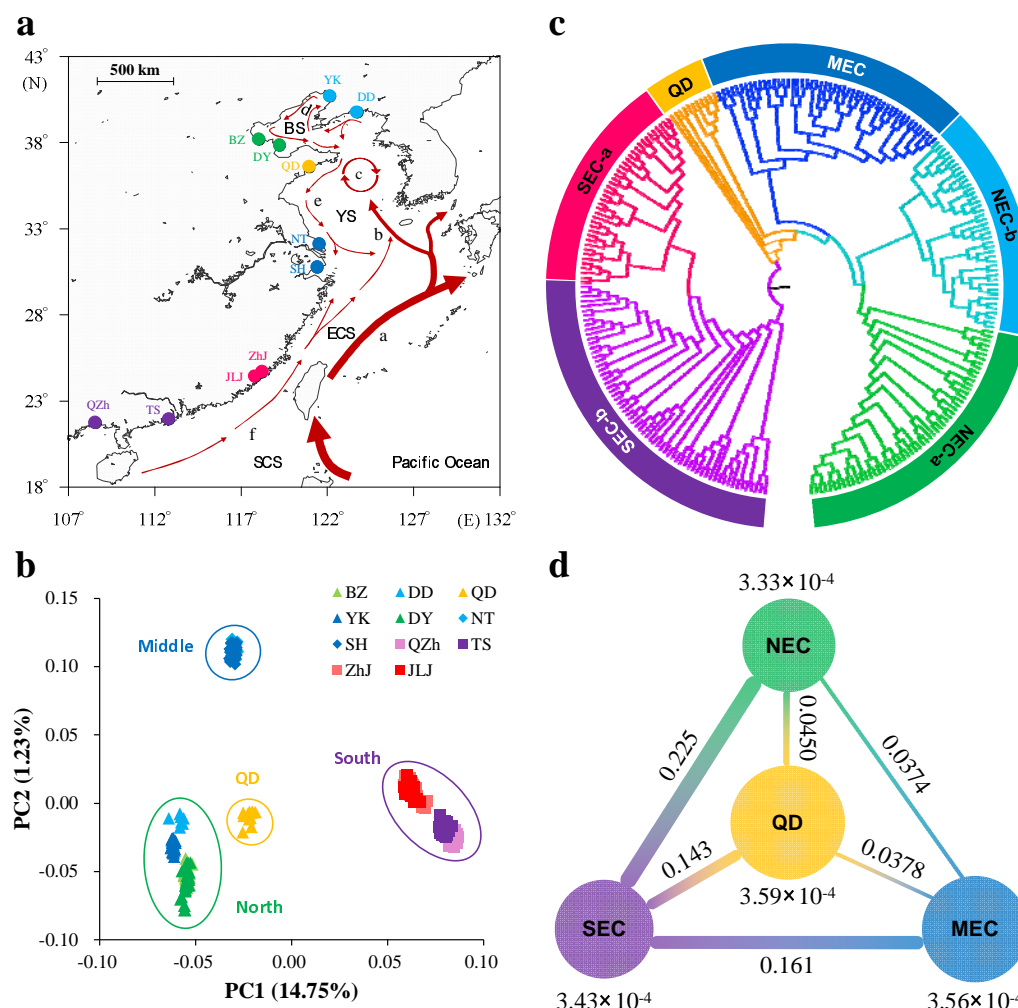


Fig. 2 | Geographic distribution and genetic structure of the *Crassostrea ariakensis* across the estuaries of China. a, Sampling locations of 264 wild estuarine oysters across 11 estuaries in China. The red and arrowed curves represent ocean currents in summer. a: Kuroshio current, b: Yellow Sea Warm Current, c: Yellow Sea Cold Water Mass, d: Bohai Sea Circulation, e: China Coastal Current, f: South China Sea Warm Current. SCS: South China Sea, ECS: East China Sea, YS: Yellow Sea, BS: Bohai Sea. DD: Dandong, YK: Yingkou, BZ: Binzhou, DY: Dongying, QD: Qingdao, NT: Nantong, SH: Shanghai, JLJ: Jiulongjiang, ZhJ: Zhangjiang, TS: Taishan, QZh: Qinzhou. **b**, Plots of principal components 1 and 2 of the 264 oyster individuals. **c**, Phylogenetic tree of estuarine oysters inferred from whole-genome SNPs by the neighbour-joining (NJ) method. NEC-a: north estuaries of China, including BZ and DY, NEC-b: DD and YK, MEC: middle estuaries of China, including NT and SH, SEC-a: south estuaries of China, including JLJ and ZhJ, and SEC-b: TS and QZh. **d**, Nucleotide diversity and genetic divergence across the four populations. The value under the circle is nucleotide diversity (π) for the oyster population, and values between population pairs indicate genetic divergence (F_{ST}).

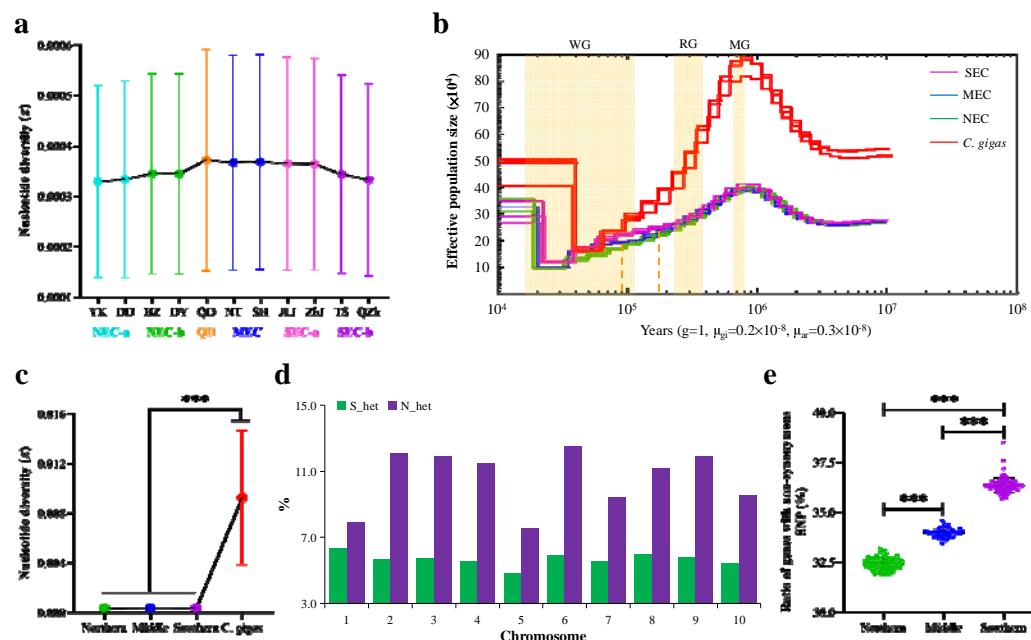


Fig. 3 | Effects of gene flow, historical glaciation and natural selection on population structure of *C. ariakensis*. **a**, Nucleotide diversity (π) of 11 oyster populations of *C. ariakensis*. **b**, Demographic histories of marine oyster species *C. gigas* (gi) and estuarine oyster species *C. ariakensis* (ar) including southern, middle and northern populations (SEC, MEC and NEC), inferred by the PSMC model. The period of the Mindel glaciation (MG, 0.68~0.80 mya), Riss glaciation (MG, 0.24~0.37 mya) and Würm glaciation (WG, 10,000~120,000 years ago) were shaded by pink. **c**, The ratios of SNPs showing heterozygous in northern oysters but homozygous in southern oysters (N_het) and those of SNPs showing homozygous in northern oysters but heterozygous in southern oysters (S_het) across 10 chromosomes. **d**, The ratio of genes with non-synonymous SNPs in three oyster populations. Asterisks indicate significant difference (***) $p < 0.001$.

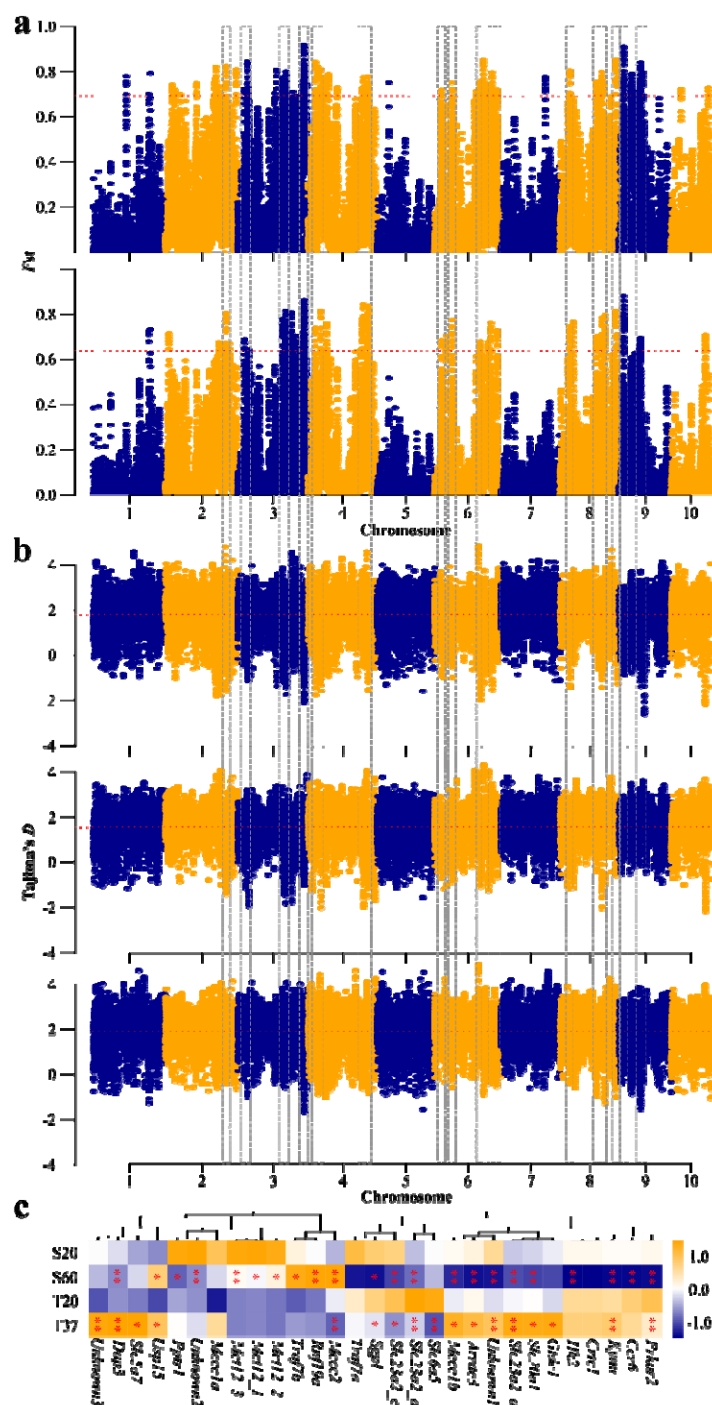


Fig. 4 | Genome-wide distribution of selective sweep signals and transcriptional responses of selective genes to stresses. a, Global F_{ST} values (top 1%, red lines) in two population pairs including northern versus southern (up) and middle versus southern (bottom). **b,** Global Tajima's D values in northern (up), middle (middle) and southern (bottom) oyster populations. **c,** Expression level of genes under selection in estuarine oysters when exposed to thermal (6 hours under 37 °C) and high-salt (7 days under 60 ‰) conditions. Asterisks indicate significant difference (* $p < 0.05$, ** $p < 0.01$).

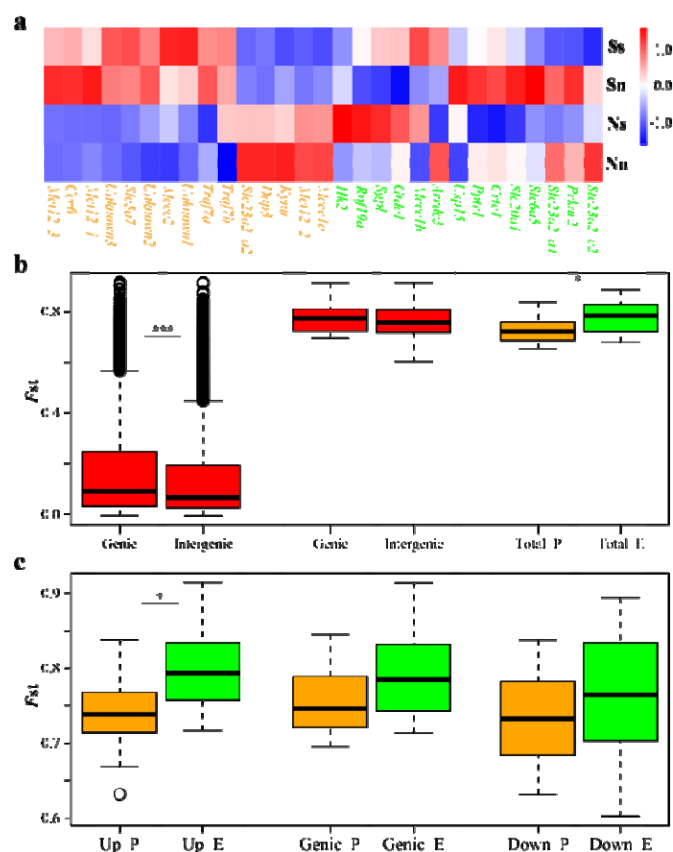


Fig. 5 | Transcriptional and genomic divergence of genes with selective signals. **a**, Expression level of selective genes in northern (N) and southern (S) oyster populations (capital letters) acclimated at northern (n) and southern (s) habitats (lowercase letters), which showed population- (orange) and environment- (green) specific expression patterns. **b**, Genetic divergence (F_{ST}) for genic and intergenic regions at genome-wide level (left) and 29 candidate genes with selective signals (middle), as well as for population- (P) and environment- (E) specific genes at both genic and intergenic regions (Total, right). **c**, Genetic divergence (F_{ST}) for intergenic [Up: upstream (left) and Down: downstream (right)] and genic (middle) regions of population- (P) and environment- (E) specific genes. Asterisks indicate significant difference (* $p < 0.05$, *** $p < 0.001$).