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High macro-collinearity between *Crassostrea angulata* and *C. gigas* genomes was revealed by comparative genetic mapping with transferable EST-SNP markers

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ABSTRACT

Comparative genomics has become an important strategy for transferring genetic information from a well-characterized model species to one that is less described and can facilitate in functional gene mining and genomic evolution. *Crassostrea angulata* and *Crassostrea gigas* are closed related species that they can hybridize to produce fertile offspring, and *C. gigas* has accumulated a considerable number of genetic and genomic resources while *C. angulata* lags far behind. In the current study, 684 single nucleotide polymorphism (SNP) markers developed from *C. gigas* expressed sequence tags (ESTs) were used for transferability test in *C. angulata*, and 635 loci were successfully amplified. Then a genetic linkage map of *C. angulata* was for the first time constructed using these transferable SNP markers. A total of 988 transferable SNP markers were used for polymorphism screening in a mapping family, and 273 loci were distributed on the genetic map which was composed of 10 linkage groups and estimated 1040.29 cM in total length. The average spacing between markers was 3.96 cM. By comparing the shared EST-SNP marker locations on homologous linkage groups, comparative mapping analysis between the *C. angulata* genetic map and an integrated map of *C. gigas* revealed putative macro-collinearity in the two oyster genomes with little difference in markers order. These transferable SNP markers could be applied in genetic diversity and population structure studies, and the genetic map would be useful for quantitative trait loci mapping and genetic improvement through marker-assisted selection for *C. angulata*.

1. Introduction

Oysters play a crucial role in worldwide mariculture industry with the characteristics of fast growth and rich nutrition. China, Japan, Republic of Korea, America and France are the major countries of oyster farming around the world (FAO, 2020). Most of the oyster species with commercial interest belong to the genera *Crassostrea* or *Ostrea* (Ostreidae). *Crassostrea angulata*, also known as the Portuguese oyster or Fujian oyster, is naturally distributed in the Northwest Pacific region and has been introduced to many countries around the world. *C. angulata* was once a vital importance of edible bivalve species in Europe until the 1970s while massive mortality was caused by virus infection (Comps, 1988). In southern China, *C. angulata* is the main oyster species in coastal regions with a wide distribution of ranging from Zhejiang Province to Hainan Province (Qin et al., 2012). To date, abundant studies about *C. angulata* focused on species identification (Wang and Guo, 2008; Wang et al., 2010) and interspecific hybridization (Su et al., 2016; Yan et al., 2018), while there were only few reports on the development and application of molecular markers (Boudry et al., 1998; Cross et al., 2014; Huvet et al., 2001; Vu et al., 2021). The lack of co-dominant simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers has severely restricted studies on genetic diversity analysis, linkage map construction and quantitative trait loci (QTL) mapping for *C. angulata*. As another *Crassostrea* species, the Pacific oyster *Crassostrea gigas* has been used as a model shellfish species for evolutionary and genetic studies, and substantial genomic knowledge and genetic resources have been accumulated (Ge et al., 2014; Qi et al., 2017; Sauvage et al., 2010; Wang and Li, 2017; Wang et al., 2018; Yu and Li, 2008), although some important characteristics of genome structure and inherited mechanism of important economical traits

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remain unclear (Evans et al., 2009; Xu et al., 2017). In particular, the completion of whole-genome sequencing of *C. gigas* offers significant sequences and structure information for comparative genomics and phyletic evolution analysis for oysters (Zhang et al., 2012).

It has been a confusion on the evolution and phylogenetic relationship between *C. angulata* and *C. gigas*. The two oysters can be cross-fertilized in laboratorial and natural environment and some authors suggested that *C. angulata* and *C. gigas* are the same species (Huvet et al., 2004; López-Flores et al., 2004; Reece et al., 2008). Nevertheless, some authors identified *C. angulata* as a subspecies of *C. gigas* based on the findings of genetic analysis (Lapegue et al., 2004; Ren et al., 2010; Wang et al., 2010). On account of the closely genetic relationship between *C. angulata* and *C. gigas*, a considerable proportion of molecular markers would be shared and transferable in both of the two *Crassostrea* species. Especially the markers developed from conservative sequences, expressed sequence tags (ESTs), have a higher transferable probability than the markers developed from genome in theory (Kong et al., 2014; Picoult-Newberg et al., 1999). There have been lots of studies on developing transferable markers from closely related species, which has become an important approach to obtain molecular markers for species with limited genomic resources (Decroocq et al., 2003; Saha et al., 2004; Varshney et al., 2005).

Comparative genomics analysis is a significant approach for understanding genome structure and function from one organism to another organism. Comparative mapping is the major method for comparative genome analysis, containing comparative physical mapping and comparative genetic mapping, and its molecular basis is the conservativeness of DNA sequence especially the coding sequence in genes. Synteny or collinearity can be revealed by comparing the shared molecular markers location and order on physical or genetic maps between different species, which would provide insights into understanding genomic conservation and evolution (Abby and Daubin, 2007; Miller et al., 2004; Rubin et al., 2000; Shimamoto and Kyozuka, 2002; Visel et al., 2007). Comparative mapping studies in aquatic animals mainly focus on the comparation between several economically important fishes and zebrafish, such as *Ctenopharyngodon idella* (Xia et al., 2010), *Cyprinus carpio* L. (Li et al., 2011) and *Oncorhynchus mykiss* (Palti et al., 2012), while there is no report on comparative genetic mapping analysis by using shared and transferable markers in molluscs.

In this study, single nucleotide polymorphism markers developed from expressed sequence tags (EST-SNPs) of *C. gigas* were tested for transferability in *C. angulata*, and a genetic linkage map of *C. angulata* was constructed using the transferable markers. Comparative genetic mapping analysis was carried out between the genetic map of *C. angulata* and an integrated map of *C. gigas*, providing an important foundation for mining the functional genes of *C. angulata* and understanding genome structural evolution of oysters.

2. Materials and methods

2.1. Mapping family of C. angulata

In May 2016, one-year-old *C. angulata* was collected in Fuzhou City, Fujian Province. Six full-sib families of *C. angulata* were constructed by single-pair mating, and cultured on suspended longlines along the coastal of Rongcheng City, Shandong Province. In May 2017, one of the six families was selected for linkage analysis with a population size of 133 individuals. Adductor muscle of the mapping family parents and progenies were stored in 95% ethanol and − 30 °C for genomic DNA extraction. Genomic DNA of the mapping family samples was extracted by using the phenol-chloroform protocol.

2.2. Species identification and paternity testing

For constructing a genetic linkage map with accurate marker location and order, it is necessary to exclude the individuals who do not belong to the mapping family. Species-specific primers as described in Wang and Guo (2008) were used to identify all of the samples of *C. angulata* mapping family to exclude the individuals of *C. gigas*, since the two species were cultured and governed in the same oyster farm. Two microsatellite multiplex PCR systems (Liu et al., 2017, Panel 1 and Panel 2) were used for paternity testing to exclude the individuals that may be mixed during the seed breeding and cultivation stages from other five *C. angulata* families.

2.3. Transferability of EST-SNP markers

A total of 684 SNP markers developed from *C. gigas* EST sequences were used for the transferability test (Wang and Li, 2017; Wang et al., 2018), using DNA templates of sixteen oysters that have been identified to be *C. angulata* (Zhong et al., 2014a). PCR reaction was performed on a Bio-Rad T100™ thermal cycler with a final volume of 10 μl mixture containing 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 μM forward and reverse primers, 10 ng template DNA, 0.25 U *Taq* DNA polymerase (Takara). Cycling conditions were as follows: 95 °C for 5 min, followed by 35 cycles of 95 °C for 10 s, 60 °C for 10 s and 72 °C for 10 s. Then the amplification products were detected by 1.5% agarose gel electrophoresis and polymorphisms screening was performed using high-resolution melting (HRM) analysis. The marker genotyping was carried out using the LightCycler®480 real-time PCR system (Roche) with a final volume of 10 μl reaction mix: 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 μM forward and reverse primers, 10 ng template DNA, 0.25 U *Taq* DNA polymerase (Takara) and 5 μM SYTO®9 (Invitrogen). Data were analyzed using the Gene Scanning and Tm Calling programs within LightCycler®480 Software 1.5 (Roche) (Wang et al., 2018).

2.4. Constructing genetic map of C. angulata and comparative mapping with C. gigas

Previously reported 353 transferable EST-SNP loci (Zhong et al., 2014a) and transferable loci developed in the present study were used for constructing the genetic linkage map of *C. angulata*. Each marker was genotyped by using the HRM technology and the markers showing significant segregation distortion from Mendelian ratios were also considered to be used for linkage analysis. Sex-average genetic map was constructed by JoinMap 4.0 software (Van Ooijen, 2006) according the procedure described in our previous study for constructing an integrated genetic map of *C. gigas* (Wang et al., 2018). Comparative mapping analysis was performed between the genetic map of *C. angulata* (C.an) developed here and the integrated map of *C. gigas* (C.gi) by comparing the shared EST-SNP markers on homologous linkage groups. The comparison results were graphically visualized using Mapchart 2.1 software (Voorrips, 2002), which could provide straightforward view of collinearity between the two oyster genomes.

3. Results

3.1. Species identification and paternity testing

Through species identification, three individuals from the mapping family (number 37, 84, and 120) were identified as *C. gigas*. By paternity testing, four individuals numbered 35, 56, 77, and 102 were suspected to be from other five *C. angulata* families. All the seven individuals above were excluded from linkage analysis and the final mapping population used to construct the *C. angulata* genetic map was 126 individuals.

3.2. Genetic linkage map of C. angulata

Out of the 684 primer pairs tested, 635 loci could produce bright target bands in *C. angulata* and regard as transferable markers. Thus, a total of the 988 transferable EST-SNPs were used for constructing the

genetic map of *C. angulata*, while 486 markers were segregated in the *C. angulata* family. Through linkage analysis, 273 EST-SNP markers were located on the genetic map of *C. angulata*, distributing on 10 linkage groups (LGs, Fig. 1). The map was 1040.29 cM in total length (G_o), with an average spacing of 3.96 cM and a maximum interval of 25.01 cM between markers. The number of SNP markers distributed on each linkage group ranged from 6 SNPs on LG10 to 55 SNPs on LG1, with an average of 27.3 SNPs per linkage group. The longest linkage group LG3 was composed of 37 markers with 121.42 cM in length, and the shortest linkage group LG10 was 71.48 cM in length (Table 1). The estimated genome length (G_e) was 1103.29 cM, and genome coverage for the framework map was 94.29%.

3.3. Comparative mapping analysis between C. angulata and C. gigas

The genetic maps of the two *Crassostrea* oysters were both composed of 10 linkage groups (Fig. 1). A total of 226 SNP markers were shared between the two genetic maps and there were at least 4 shared SNPs between the homologous linkage groups (Table 2). Most of the markers had good collinearity while only a few markers order had changed between the two genetic maps. There was an incomplete one-to-one correspondence between homologous linkage groups of the two oysters. For SNP markers on one linkage group of *C. angulata*, they may be located on two or three linkage groups of the *C. gigas* integration map. For example, the linkage groups Can-1 and Cgi-1, there were 43 homologous and shared markers between them, while three SNP markers on Can-1 were also distributed on the linkage group Cgi-2. There were 17 homologous and shared markers between the linkage groups Can-6 and Cgi-7, while

Table 1
Characteristics of the *C. angulata* genetic map based on EST-SNP markers.

Linkage group	Length (cM)	Marker No.	Maximum distance (cM)	Average distance (cM)
1	115.56	55	4.79	2.14
2	108.44	46	8.48	2.41
3	121.42	37	12.17	3.37
4	117.32	31	10.02	3.91
5	105.43	28	15.83	3.90
6	114.82	25	8.65	4.78
7	103.62	22	13.25	4.93
8	94.38	15	9.61	6.74
9	87.82	8	25.01	12.55
10	71.48	6	24.78	14.30
Total	1040.29	273		3.96

Length means genetic distance in centiMorgans (cM) of each linkage group.
Marker No. means the number of markers on each linkage group.
Maximum distance means the maximum genetic distance between two interval markers on each linkage group.
Average distance means the average genetic distance between two interval markers on each linkage group.

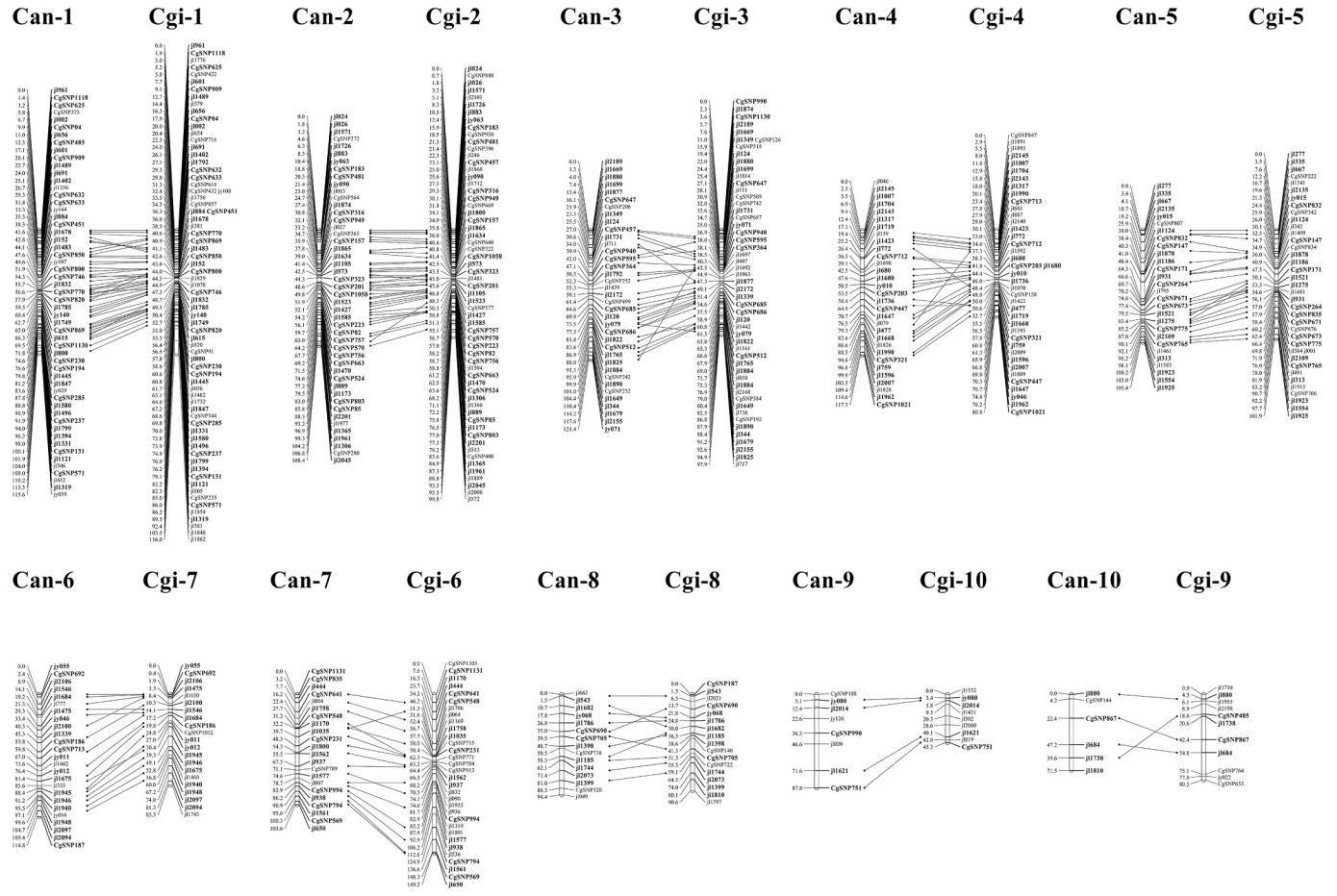

Fig. 1. Comparative mapping and homologous groups between the *C. angulata* and *C. gigas* maps based on the shared EST-SNP markers. Can, represents *Crassostrea angulata*; Cgi, represents *Crassostrea gigas*. Marker distances in Kosambi centi-Morgans and marker names are indicated on the left and right of each linkage group, respectively.

Table 2
Homology between *C. angulata* and *C. gigas* linkage groups.

	C. angulata									
C. gigas	Can-1	Can-2	Can-3	Can-4	Can-5	Can-6	Can-7	Can-8	Can-9	Can-10
Cgi-1	43		1	1						
Cgi-2	3	36	1				1			
Cgi-3		2	28			1			1	
Cgi-4				25		2				
Cgi-5					24		1			
Cgi-7						17				
Cgi-6			1				17			
Cgi-8								11		1
Cgi-10									4	
Cgi-9										4
Total	46	38	31	26	24	20	19	11	5	5

Can 1–10 represent linkage group of *C. angulata*; Cgi 1–10 represent linkage group of *C. gigas*.
The figures in the table mean the number of shared markers on linkage groups.

one and two SNP markers on Can-6 were distributed on Cgi-3 and Cgi-4, respectively.

4. Discussion

4.1. Markers

Microsatellites or simple sequence repeat (SSR) and SNP are the most popular molecular markers for aquaculture community in recent years. The different classes of marker systems (genomic SSR, EST-SSR and EST-SNP) have different characteristics, thus may differ in genetic studies they applied. Even though many empirical studies have demonstrated that the level of EST-SSR polymorphisms is lower than that of genomic SSRs, the EST-SSR markers can still reveal sufficient levels of variation for the vast majority of genetic diversity studies (Sharifi Tehrani et al., 2009; Tahan et al., 2009). SNP markers are mainly bi-allelic and the expected heterozygosity value can be expected is only 0.5 for a given SNP locus. These markers are less suitable for routine genetic diversity analysis than the genomic SSRs and EST-SSRs (Kong et al., 2014). However, SNP genotyping can be performed using automated high-throughput methods, the abundance of SNPs in genomes could compensate for the disadvantages of less informative. Furthermore, EST derived SNPs are located within genes and are thus more conserved across species, making them powerful tools for quantitative trait loci (QTLs) mapping and comparative mapping analysis (Ellis and Burke, 2007; Useche et al., 2001).

For aquatic animals, transferable EST-derived SSRs and SNPs have been obtained between different species or genus, but most of these researches only focused on the development of transferable markers and the comparison of markers transferability among different species (Dong et al., 2012; Kang et al., 2013a; Peyran et al., 2020; Wang et al., 2012), without further application. The limited number of markers hinder their utilization in genetic map construction and comparative mapping. Benefitting from the abundant genetic resources of *C. gigas*, more than 600 transferable EST-SNPs could be identified for *C. angulata* in the present study, providing sufficient DNA markers for constructing a primary genetic map and comparative mapping analysis. Here, more than 92% (635 out of 684) of the tested markers were transferable from *C. gigas* to *C. angulata*. Similar results were obtained in previous studies that 93.6% for SNP markers (Zhong et al., 2014a) and 96.5% for SSRs markers (Hedgecock et al., 2004) developed from *C. gigas* could cross amplify in *C. angulata*. The high transferability rate in turn demonstrated that *C. gigas* has a very close relationship with *C. angulata*. In theory, the more phylogenetically closed related species are, the higher possibility of markers transferability success would be between them, and this conclusion has been highlighted in previous studies. For example, Peyran et al. (2020) developed polymorphic microsatellite markers for *Pinna nobilis* and tested cross-species amplification in four *Pinna* species, founding a negative relationship with the genetic distance between the target species and the tested species. However, low transferability success of SSR markers was also observed between closed related species (Kang et al., 2013b; Marín et al., 2012), and widespread PCR-null alleles may significantly affect cross-species amplification (Hedgecock et al., 2004).

4.2. Linkage mapping

As far as we know, the genetic linkage map constructed in the present study was the first for *C. angulata* in a true sense, as only a genetic map was previously constructed based on a hybrid mapping family of *C. gigas* × *C. angulata* to date (Wang et al., 2016). The genetic maps of *C. gigas* were the most well-studied in the genus *Crassostrea* , and yet several first-generation genetic maps were developed mainly using limited number of markers (Guo et al., 2012; Hubert et al., 2009; Hubert and Hedgecock, 2004; Li and Guo, 2004; Plough and Hedgecock, 2011; Sauvage et al., 2010; Song et al., 2018; Zhong et al., 2014b), the low-density of markers on linkage groups limited the resolution for QTL mapping. Then four second-generation high-density genetic maps mainly based on SNP markers were constructed, which were genotyped by GoldenGate assay (Hedgecock et al., 2015) and genotyping-by-sequencing (GBS) (Han et al., 2021; Li et al., 2018; Wang et al., 2016) respectively, increasing the average marker interval up to 0.68 cM. Comparing with the four high-density genetic maps, SNP markers on the medium density integrated map generated in our previous study (Wang et al., 2018) were genotyped by the PCR-based method, HRM analysis, which was technically superior in utilizing the shared and anchored markers for comparing mapping. Here, the genetic map of *C. angulata* was composed of 10 linkage groups, which was corresponding to the haploid chromosome number of oysters ($2n = 20$). The total map length was calculated as 1040.29 cM for *C. angulata*, which was similar to the integrated map of *C. gigas* (947.3 cM) and the high-density map using GBS based on hybrid mapping family of *C. gigas* × *C. angulata* (1084.3 cM), but significantly longer than the high-density maps of *C. gigas* using GoldenGate assay (507.6–689.8 cM) and shorter than another high-density map of *C. gigas* using GBS (1982.1 cM). The major reasons for the high variability of map length in different studies may be the different mapping methods and independence logarithm of the odds (LOD) threshold used in maps construction (Van Ooijen, 2011). The average and maximum intervals between markers on the genetic map of *C. angulata* were higher than that of *C. gigas*, indicating more markers should be added to the genetic map of *C. angulata* for further QTL fine mapping.

4.3. Comparative mapping

Construction of high-quality genetic maps is the foundation for

comparative genetic mapping analysis. The rapid development of comparative genomic research in plants is due to the fact that genetic mapping studies of various plants are more adequate. Animals are not as easy as plants to establish and maintain mapping groups because of their biological characteristics, making the comparative genomic research in animals has lagged behind. In aquatic animals, extensive comparisons of syntenic relationships between chromosomes and linkage groups have been focused between genera within the Salmoninae (Danzmann et al., 2005; Phillips et al., 2009) and between some important economic fish and model fish. For example, Zheng et al. (2011) constructed a consensus genetic map of common carp and conducted comparative genome analysis within five model teleost fish, revealing a high percentage (74.7%) of conserved loci corresponding to zebrafish chromosomes. Most each zebrafish chromosome comprised two common carp linkage groups, indicating that genome doubling (autopolyploidization) occurred in the ancestral diploid of the common carp during the early steps of evolution. For the first time, we constructed a genetic map for *C. angulata* and carried out comparative genetic mapping analysis by transferable markers in molluscs. Macro-collinearity was revealed between the *C. angulata* and *C. gigas* genomes. Most of the shared markers in each homologue were aligned in the same order between the two maps. Few differences of shared markers order and one linkage group of *C. angulata* corresponded to two or three linkage groups of *C. gigas* were also found in the study. Further analysis should be performed to verify if the differences truly exist or caused by lacking of adequate markers. In general, the results showed a high similarity of genome of *C. angulata* and *C. gigas*, supporting the view of *C. angulata* should be considered a subspecies of *C. gigas*.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Abby, S., Daubin, V., 2007. Comparative genomics and the evolution of prokaryotes. Trends Microbiol. 15, 135–141. https://doi.org/10.1016/j.tim.2007.01.007.

Boudry, P., Heurtebise, S., Collet, B., Cornette, F., Gérard, A., 1998. Differentiation between populations of the Portuguese oyster, *Crassostrea angulata* (Lamark) and the Pacific oyster, *Crassostrea gigas* (Thunberg), revealed by mtDNA RFLP analysis. J. Exp. Mar. Biol. Ecol. 226, 279–291. https://doi.org/10.1016/S0022-0981(97)00250-5.

Comps, M., 1988. Epizootic Diseases of Oysters Associated with Viral Infections, 18. American Fisheries Society Special Publication, pp. 23–37. https://archimer.ifremer.fr/doc/00000/5912/.

Cross, I., Merlo, M.A., Rodríguez, M.E., Portela-Bens, S., Rebordinos, L., 2014. Adaptation to abiotic stress in the oyster *Crassostrea angulata* relays on genetic polymorphisms. Fish Shellfish Immun. 41, 618–624. https://doi.org/10.1016/j.fsi.2014.10.011.

Danzmann, R.G., Cairney, M., Davidson, W.S., Ferguson, M.M., Gharbi, K., Guyomard, R., Holm, L.-E., Leder, E., Okamoto, N., Ozaki, A., 2005. A comparative analysis of the rainbow trout genome with 2 other species of fish (Arctic charr and Atlantic salmon) within the tetraploid derivative Salmonidae family (subfamily: Salmoninae). Genome 48, 1037–1051. https://doi.org/10.1139/g05-067.

Decroocq, V., Fave, M., Hagen, L., Bordenave, L., Decroocq, S., 2003. Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor. Appl. Genet. 106, 912–922. https://doi.org/10.1007/s00122-002-1158-z.

Dong, Y., Yao, H., Lin, Z., Zhang, L., Bao, Z., 2012. Characterization of 62 polymorphic EST-SSR markers in the blood clam (*Tegillarca granosa*) and their cross-amplification in *Scapharca subcrenata*. Conserv. Genet. Resour. 4, 991–997. https://doi.org/10.1007/s12686-012-9691-9.

Ellis, J.R., Burke, J.M., 2007. EST-SSRs as a resource for population genetic analyses. Heredity 99, 125–132. https://doi.org/10.1038/sj.hdy.6801001.

Evans, S., Camara, M.D., Langdon, C.J., 2009. Heritability of shell pigmentation in the Pacific oyster, *Crassostrea gigas*. Aquaculture 286, 211–216. https://doi.org/10.1016/j.aquaculture.2008.09.022.

FAO, 2020. Fishery and Aquaculture Statistics. Global production by production source 1950–2018 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2020. www.fao.org/fishery/statistics/software/fishstatj/en.

Ge, J., Li, Q., Yu, H., Kong, L., 2014. Identification and mapping of a SCAR marker linked to a locus involved in shell pigmentation of the Pacific oyster (*Crassostrea gigas*). Aquaculture 434, 249–253. https://doi.org/10.1016/j.aquaculture.2014.08.027.

Guo, X., Li, Q., Wang, Q.Z., Kong, L.F., 2012. Genetic mapping and QTL analysis of growth-related traits in the Pacific oyster. Mar. Biotechnol. 14, 218–226. https://doi.org/10.1007/s10126-011-9405-4.

Han, Z., Li, Q., Xu, C., Liu, S., Yu, H., Kong, L., 2021. QTL mapping for orange shell color and sex in the Pacific oyster (*Crassostrea gigas*). Aquaculture 530, 735–781. https://doi.org/10.1016/j.aquaculture.2020.735781.

Hedgecock, D., Li, G., Hubert, S., Bucklin, K., Ribes, V., 2004. Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. J. Shellfish Res. 23, 379–386.

Hedgecock, D., Shin, G., Gracey, A.Y., Den Berg, D.V., Samanta, M.P., 2015. Second-generation linkage maps for the Pacific oyster *Crassostrea gigas* reveal errors in assembly of genome scaffolds. G3-Genes Genom. Genet. 5, 2007–2019. https://doi.org/10.1534/g3.115.019570.

Hubert, S., Hedgecock, D., 2004. Linkage maps of microsatellite DNA markers for the Pacific oyster *Crassostrea gigas*. Genetics 168, 351–362. https://doi.org/10.1534/genetics.104.027342.

Hubert, S., Cognard, E., Hedgecock, D., 2009. Centromere mapping in triploid families of the Pacific oyster *Crassostrea gigas* (Thunberg). Aquaculture 288, 172–183. https://doi.org/10.1016/j.aquaculture.2008.12.006.

Huvet, A., Balabaud, K., Bierne, N., Boudry, P., 2001. Microsatellite analysis of 6-hour-old embryos reveals no preferential intraspecific fertilization between cupped oysters *Crassostrea gigas* and *Crassostrea angulata*. Mar. Biotechnol. 3, 448–453. https://doi.org/10.1007/s10126-001-0017-2.

Huvet, A., Fabioux, C., McCombie, H., Lapegue, S., Boudry, P., 2004. Natural hybridization between genetically differentiated populations of *Crassostrea gigas* and *C. angulata* highlighted by sequence variation in flanking regions of a microsatellite locus. Mar. Ecol. Prog. Ser. 272, 141–152. https://doi.org/10.3354/meps272141.

Kang, J., Yang, S., Moon, T., Park, J., Choi, T., 2013a. Development of microsatellite markers for the kelp grouper *Epinephelus bruneus* by 454 pyrosequencing and transfer to related species. Genet. Mol. Res. 12, 5485–5493. https://doi.org/10.4238/2013.november.13.1.

Kang, J.H., Kim, Y.K., Park, J.Y., Noh, E.S., Jeong, J.E., Lee, Y.S., Choi, T.J., 2013b. Development of microsatellite markers for a hard-shelled mussel, *Mytilus coruscus*, and cross-species transfer. Genet. Mol. Res. 12, 4009–4017. https://doi.org/10.4238/2013.september.27.2.

Kong, L., Bai, J., Li, Q., 2014. Comparative assessment of genomic SSR, EST-SSR and EST-SNP markers for evaluation of the genetic diversity of wild and cultured Pacific oyster, *Crassostrea gigas* Thunberg. Aquaculture 420, S85–S91. https://doi.org/10.1016/j.aquaculture.2013.05.037.

Lapegue, S., Batista, F., Heurtebise, S., Yu, Z., Boudry, P., 2004. Evidence for the presence of the Portuguese oyster, *Crassostrea angulata*, in northern China. J. Shellfish Res. 23, 759–763. https://archimer.ifremer.fr/doc/00000/3173/.

Li, L., Guo, X., 2004. AFLP-based genetic linkage maps of the Pacific oyster *Crassostrea gigas* Thunberg. Mar. Biotechnol. 6, 26–36. https://doi.org/10.1007/s10126-003-0001-0.

Li, Y., Xu, P., Zhao, Z., Wang, J., Zhang, Y., Sun, X.-W., 2011. Construction and characterization of the BAC library for common carp *Cyprinus carpio* L. and establishment of microsynteny with zebrafish *Danio rerio*. Mar. Biotechnol. 13, 706–712. https://doi.org/10.1007/s10126-010-9332-9.

Li, C., Wang, J., Song, K., Meng, J., Xu, F., Li, L., Zhang, G., 2018. Construction of a high-density genetic map and fine QTL mapping for growth and nutritional traits of *Crassostrea gigas*. BMC Genomics 19, 626. https://doi.org/10.1186/s12864-018-4996-z.

Liu, T., Li, Q., Song, J., Yu, H., 2017. Development of genomic microsatellite multiplex PCR using dye-labeled universal primer and its validation in pedigree analysis of Pacific oyster (*Crassostrea gigas*). J. Ocean Univ. China 16, 151–160. https://doi.org/10.1007/s11802-017-3121-2.

López-Flores, I., de la Herrán, R., Garrido-Ramos, M.A., Boudry, P., Ruiz-Rejón, C., Ruiz-Rejón, M., 2004. The molecular phylogeny of oysters based on a satellite DNA related to transposons. Gene 339, 181–188. https://doi.org/10.1016/j.gene.2004.06.049.

Marín, A., Fujimoto, T., Arai, K., 2012. Isolation and characterization of 12 microsatellite loci in the Peruvian scallop *Argopecten purpuratus* and cross-species amplification in other scallop species (family Pectinidae). Conserv. Genet. Resour. 4, 179–182, 10.1007%2Fs12686-011-9503-7.

Miller, W., Makova, K.D., Nekrutenko, A., Hardison, R.C., 2004. Comparative genomics. Annu. Rev. Genomics Hum. Genet. 5, 15–56. https://doi.org/10.1146/annurev.genom.5.061903.180057.

Palti, Y., Genet, C., Gao, G., Hu, Y., You, F.M., Boussaha, M., Rexroad, C.E., Luo, M.-C., 2012. A second generation integrated map of the rainbow trout (*Oncorhynchus mykiss*) genome: analysis of conserved synteny with model fish genomes. Mar. Biotechnol. 14, 343–357. https://doi.org/10.1007/s10126-011-9418-z.

Peyran, C., Planes, S., Tolou, N., Iwankow, G., Boissin, E., 2020. Development of 26 highly polymorphic microsatellite markers for the highly endangered fan mussel *Pinna nobilis* and cross-species amplification. Mol. Biol. Rep. 47, 2551–2559. https://doi.org/10.1007/s11033-020-05338-1.

Phillips, R.B., Keatley, K.A., Morasch, M.R., Ventura, A.B., Lubieniecki, K.P., Koop, B.F., Danzmann, R.G., Davidson, W.S., 2009. Assignment of Atlantic salmon (*Salmo salar*) linkage groups to specific chromosomes: conservation of large syntenic blocks corresponding to whole chromosome arms in rainbow trout (*Oncorhynchus mykiss*). BMC Genet. 10, 46. https://doi.org/10.1186/1471-2156-10-46.

Picoult-Newberg, L., Ideker, T.E., Pohl, M.G., Taylor, S.L., Donaldson, M.A., Nickerson, D.A., Boyce-Jacino, M., 1999. Mining SNPs from EST databases. Genome Res. 9, 167–174. https://doi.org/10.1101/gr.9.2.167.

Plough, L.V., Hedgecock, D., 2011. Quantitative trait locus analysis of stage-specific inbreeding depression in the Pacific Oyster *Crassostrea gigas*. Genetics 189, 1473. https://doi.org/10.1534/genetics.111.131854.

Qi, H., Song, K., Li, C., Wang, W., Li, B., Li, L., Zhang, G., 2017. Construction and evaluation of a high-density SNP array for the Pacific oyster (*Crassostrea gigas*). PLoS One 12, e0174007. https://doi.org/10.1371/journal.pone.0174007.

Qin, J., Huang, Z., Chen, J., Zou, Q., You, W., Ke, C., 2012. Sequencing and de novo analysis of *Crassostrea angulata* (Fujian oyster) from 8 different developing phases using 454 GSFlx. PLoS One 7, e43653. https://doi.org/10.1371/journal.pone.0043653.

Reece, K.S., Cordes, J.F., Stubbs, J.B., Hudson, K.L., Francis, E.A., 2008. Molecular phylogenies help resolve taxonomic confusion with Asian *Crassostrea* oyster species. Mar. Biol. 153, 709–721, 10.1007%2Fs00227-007-0846-2.

Ren, J., Liu, X., Jiang, F., Guo, X., Liu, B., 2010. Unusual conservation of mitochondrial gene order in *Crassostrea* oysters: evidence for recent speciation in Asia. BMC Evol. Biol. 10, 1–14. https://doi.org/10.1186/1471-2148-10-394.

Rubin, G.M., Yandell, M.D., Wortman, J.R., Gabor, G.L., Nelson, C.R., Hariharan, I.K., Fortini, M.E., Li, P.W., Apweiler, R., Fleischmann, W., 2000. Comparative genomics of the eukaryotes. Science 287, 2204–2215. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc2754258/.

Saha, M.C., Mian, M.R., Eujayl, I., Zwonitzer, J.C., Wang, L., May, G.D., 2004. Tall fescue EST-SSR markers with transferability across several grass species. Theor. Appl. Genet. 109, 783–791, 10.1007%2Fs00122-004-1681-1.

Sauvage, C., Boudry, P., De Koning, D.J., Haley, C., Heurtebise, S., Lapegue, S., 2010. QTL for resistance to summer mortality and OsHV-1 load in the Pacific oyster (*Crassostrea gigas*). Anim. Genet. 41, 390–399. https://doi.org/10.1111/j.1365-2052.2009.02018.x.

Sharifi Tehrani, M., Mardi, M., Sahebi, J., Catalán, P., Díaz-Pérez, A., 2009. Genetic diversity and structure among Iranian tall fescue populations based on genomic-SSR and EST-SSR marker analysis. Plant Syst. Evol. 282, 57–70. https://doi.org/10.1007/s00606-009-0207-3.

Shimamoto, K., Kyozuka, J., 2002. Rice as a model for comparative genomics of plants. Annu. Rev. Plant Biol. 53, 399–419. https://doi.org/10.1146/annurev.arplant.53.092401.134447.

Song, J., Li, Q., Yu, Y., Wan, S., Han, L., Du, S., 2018. Mapping genetic loci for quantitative traits of golden shell color, mineral element contents, and growth-related traits in Pacific Oyster (*Crassostrea gigas*). Mar. Biotechnol. 20, 666–675. https://doi.org/10.1007/s10126-018-9837-1.

Su, J., Wang, Z., Zhang, Y., Yan, X., Li, Q., Yu, R., 2016. Early embryo and larval development of inviable intergeneric hybrids derived from *Crassostrea angulata* and *Saccostrea cucullata*. J. Ocean Univ. China 15, 515–522, 10.1007%2Fs11802-016-2877-0.

Tahan, O., Geng, Y., Zeng, L., Dong, S., Chen, F., Chen, J., Song, Z., Zhong, Y., 2009. Assessment of genetic diversity and population structure of Chinese wild almond, *Amygdalus nana*, using EST- and genomic SSRs. Biochem. Syst. Ecol. 37, 146–153. https://doi.org/10.1016/j.bse.2009.02.006.

Useche, F.J., Gao, G., Hanafey, M., Rafalski, A., 2001. High-throughput identification, database storage and analysis of SNPs in EST sequences. Genome Inform. 12, 194–203. https://doi.org/10.11234/gi1990.12.194.

Van Ooijen, J.W., 2006. JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands.

Van Ooijen, J.W., 2011. Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genet. Res. 93, 343–349. https://doi.org/10.1017/S0016672311000279.

Varshney, R.K., Sigmund, R., Börner, A., Korzun, V., Stein, N., Sorrells, M.E., Langridge, P., Graner, A., 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci. 168, 195–202. https://doi.org/10.1016/j.plantsci.2004.08.001.

Visel, A., Bristow, J., Pennacchio, L.A., 2007. Enhancer identification through comparative genomics. Semin. Cell Dev. Biol. 18, 140–152. https://doi.org/10.1016/j.semcdb.2006.12.014.

Voorrips, R., 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93, 77–78. https://doi.org/10.1093/jhered/93.1.77.

Vu, S.V., Premachandra, H., O'Connor, W., Nguyen, N.T., Dove, M., Van Vu, I., Le, T.S., Vendrami, D.L., Knibb, W., 2021. Development of SNP parentage assignment in the Portuguese oyster *Crassostrea angulata*. Aquacult. Rep. 19, 100615. https://doi.org/10.1016/j.aqrep.2021.100615.

Wang, H., Guo, X., 2008. Identification of *Crassostrea ariakensis* and related oysters by multiplex species-specific PCR. J. Shellfish Res. 27, 481–487. https://doi.org/10.2983/0730-8000(2008)27[481:IOCAAR]2.0.CO;2.

Wang, J., Li, Q., 2017. Characterization of novel EST-SNP markers and their association analysis with growth-related traits in the Pacific oyster *Crassostrea gigas*. Aquac. Int. 25, 1707–1719. https://doi.org/10.1007/s10499-017-0142-1.

Wang, H., Qian, L., Liu, X., Zhang, G., Guo, X., 2010. Classification of a common cupped oyster from southern China. J. Shellfish Res. 29, 857–866. https://doi.org/10.2983/035.029.0420.

Wang, X., Hu, X., Li, J., Li, L., Hou, R., Wang, S., Zhang, L., Bao, Z., 2012. Characterization of 38 EST-derived SNP markers in Zhikong scallop (*Chlamys farreri*) and their cross-species utility in yesso scallop (*Patinopecten yessoensis*). Conserv. Genet. Resour. 4, 747–753. https://doi.org/10.1007/s12686-012-9636-3.

Wang, J., Li, L., Zhang, G., 2016. A high-density SNP genetic linkage map and QTL analysis of growth-related traits in a hybrid family of oysters (*Crassostrea gigas* × *Crassostrea angulata*) using genotyping-by-sequencing. G3-Genes Genom. Genet. 6, 1417–1426. https://doi.org/10.1534/g3.116.026971.

Wang, J., Li, Q., Zhong, X., Song, J., Kong, L., Yu, H., 2018. An integrated genetic map based on EST-SNPs and QTL analysis of shell color traits in Pacific oyster *Crassostrea gigas*. Aquaculture 492, 226–236. https://doi.org/10.1016/j.aquaculture.2018.04.018.

Xia, J.H., Liu, F., Zhu, Z.Y., Fu, J., Feng, J., Li, J., Yue, G.H., 2010. A consensus linkage map of the grass carp (*Ctenopharyngodon idella*) based on microsatellites and SNPs. BMC Genomics 11, 1–16. https://doi.org/10.1186/1471-2164-11-135.

Xu, L., Li, Q., Yu, H., Kong, L., 2017. Estimates of heritability for growth and shell color traits and their genetic correlations in the black shell strain of pacific oyster *Crassostrea gigas*. Mar. Biotechnol. 19, 421–429. https://doi.org/10.1007/s10126-017-9772-6.

Yan, L., Su, J., Wang, Z., Zhang, Y., Yan, X., Yu, R., 2018. Growth performance and biochemical composition of the oysters *Crassostrea sikamea*, *Crassostrea angulata* and their hybrids in southern China. Aquac. Res. 49, 1020–1028. https://doi.org/10.1111/are.13549.

Yu, H., Li, Q., 2008. Exploiting EST databases for the development and characterization of EST–SSRs in the Pacific oyster (*Crassostrea gigas*). J. Hered 99, 208–214. https://doi.org/10.1093/jhered/esm124.

Zhang, G., Fang, X., Guo, X., Li, L., Luo, R., Xu, F., Yang, P., Zhang, L., Wang, X., Qi, H., 2012. The oyster genome reveals stress adaptation and complexity of shell formation. Nature 490, 49–54. https://doi.org/10.1038/nature11413.

Zheng, X., Kuang, Y., Zhang, X., Lu, C., Cao, D., Li, C., Sun, X., 2011. A genetic linkage map and comparative genome analysis of common carp (*Cyprinus carpio* L.) using microsatellites and SNPs. Mol. Gen. Genomics. 286, 261. https://doi.org/10.1007/s00438-011-0644-x.

Zhong, X., Li, Q., Yu, H., Kong, L., 2014a. SNP mining in *Crassostrea gigas* EST data: transferability to four other *Crassostrea* species, phylogenetic inferences and outlier SNPs under selection. PLoS One 9, e108256. https://doi.org/10.1371/journal.pone.0108256.

Zhong, X., Li, Q., Guo, X., Yu, H., Kong, L., 2014b. QTL mapping for glycogen content and shell pigmentation in the Pacific oyster *Crassostrea gigas* using microsatellites and SNPs. Aquac. Int. 22, 1877–1889. https://doi.org/10.1007/s10499-014-9789-z.