Biochemical Systematics and Ecology 66 (2016) 209–215

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Identification of candidate AFLP markers for shell color of the Pacific oyster (*Crassostrea gigas*) under artificial selection

Junlin Song, Qi Li*, Lingfeng Kong, Hong Yu

Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao, 266003, China

ARTICLE INFO

Article history:
Received 7 February 2016
Received in revised form 11 April 2016
Accepted 22 April 2016

Keywords:
Crassostrea gigas
Shell pigmentation
AFLP
Diversity
Outlier loci

ABSTRACT

The shell color of the Pacific oyster (*Crassostrea gigas*) is a desirable trait, but only a few genetic studies on shell color have been documented. Through successive selective breeding, four shell color variants of white (W), gold (G), black (B) and purple (P) *C. gigas* have been developed. The amplified fragment length polymorphism (AFLP) technique was used to scan the genomes of the four variants with different shell colors and one wild population (C) to identify candidate markers for shell polymorphism. Fifteen AFLP primer combinations were used, 1079 loci were scored as polymorphic loci, and the percentage of polymorphic bands was 95.5%. In the gold, white, black, purple and wild populations, the percentages of polymorphic loci were estimated to be 90.5% (G), 90.0% (W), 91.1% (B), 95.3% (P) and 93.2% (C); the expected heterozygosity values were 0.3115 (G), 0.3044 (W), 0.3102 (B), 0.3285 (P) and 0.3105 (C). The white shell variant was observed to have slightly lower genetic diversity than others, with a F_{ST} value of 0.1483. These results indicated that the four different shell color variants had high genetic diversity and that the genetic differentiation of populations mostly results from genetic diversity of individuals within populations. Furthermore, 11 outlier loci were considered candidate markers for shell color. This work provides new insights on relationships among color variants of *C. gigas*.

1. Introduction

Shell color polymorphism is a distinctive feature of the populations of many molluscs and terrestrial gastropods (Sokolova and Berger, 2000). Although shell morphology is typically unique and characteristic for a given species, shell color may vary from mostly single color in some species to a wide array of colors in others (Liu et al., 2009). The shells of molluscs exhibit high diversity in beautiful forms and colors and have always attracted the interest of naturalists and collectors. This suggests an adaptive value to shell color in molluscs and has triggered numerous experimental works on shell coloration (Zheng et al., 2003). In many cases, variation in shell color is related to environmental gradients such as temperature, insolation, ingestion, and salinity (Lindberg and Pearse, 1990; Sokolova and Berger, 2000; Liu et al., 2009). However, some researches have indicated that shell color also has a genetic basis. Kozminsky (2014) proposed that at least two genes are responsible for incorporation of each pigment into the shell of periwinkle (*Littorina obtusata*). The examination of color morphs in Baltic clam (*Macoma balthica*) also suggested shell color intensity is a heritable trait and determined that four alleles at a single locus display a linear hierarchy of dominance (Luttikhuizen and Drent, 2008).

* Corresponding author.
E-mail address: qili66@ouc.edu.cn (Q. Li).

http://dx.doi.org/10.1016/j.bse.2016.04.012
0305-1978/

Shell coloration is an easily recognized trait. Among triangle pearl mussels (*Hyriopsis cumingii*), snail (*Cepaea nemoralis*) and Pacific lion-paw scallop (*Nodipecten subnodosus*), shell color polymorphism is controlled by a few major genes (Petersen et al., 2012; Wen et al., 2013; Richards et al., 2013). Thus, artificial selection was used for shell color polymorphisms in many cultured aquatic animals. For example, strains of the bay scallop (*Argopecten irradians*) with orange, purple, and white shells were selected (Zheng et al., 2003). In pearl oyster (*Pinctada fucata martensii*), white coloration strains were obtained after a few generations of selection (Wada and Komaru, 1996). However, genetic studies on the coloration of molluscs, particularly on the diversity, are still limited. Therefore, it is necessary to evaluate the genetic basis of shell color polymorphisms and the loci linked to shell color.

The identification of the loci responsible for different traits plays a crucial role of understanding the importance of genetic adaptation (Tang et al., 2007). A genomic approach can be used for detecting candidate genes. The benefits of outlier analyses include the ability to screen numerous markers in genome scans to identify candidate genes for further investigation. Genome scans are a powerful tool to detect natural selection in natural populations among a larger sample of marker loci (Oetjenk and Reusch, 2007). This approach is also commonly employed in ecological and population genetic studies to detect outlier loci that are putatively under selection (Narum and Hess, 2011). There has been recent interest in using genome scans to identify loci that might be targets for selection in many populations of evolutionary interest (Beaumont and Dbaldingi, 2004). The amplified fragment length polymorphism (AFLP) technique is a powerful marker system which can generate a large number of genome-wide markers within a short time (Vos et al., 1995). AFLP genome scans is also an effective technique for detecting outlier loci potentially undergoing selection at the genome scale without DNA sequence (Zou et al., 2014). Therefore, AFLP genome scan has been successfully used to obtain outlier loci under selective pressure, especially suitable for species in which no other genetic information was available (Mattersdorfer et al., 2012).

The Pacific oyster (*Crassostrea gigas*) has the largest production among all cultured aquatic animals and its coloration is of interest to the whole industry. Thus, selective breeding was implemented for the shell color of *C. gigas*, and four variants characterized by shell colors which are gold shell, white shell, black shell, and purple shell were developed after successive four generations of selection (Cong et al., 2014). These particular variants permit us to analyze specific pigmentation in shell of *C. gigas*. In this study, AFLP analysis was used to investigate the extent of genetic diversity and genetic differentiation, and to identify potentially genetic markers in the four shell color variants. This study will provide new insights on relationships among color variants of *C. gigas*, which would be beneficial to conservation of genetic diversity in *C. gigas*.

2. Materials and methods

2.1. Sample collection and DNA extraction

We surveyed one wild population (C) and four shell color variants of *C. gigas*, separately named the gold shell variant (G), white shell variant (W), black shell variant (B), and purple shell variant (P) (Fig. 1). Samples of wild *C. gigas* were collected from city of Weihai in China in 2013, while the samples of four shell color variants of one-year-old *C. gigas* were collected in 2014 from an oyster farm in Rushan in Shandong province, China. The four shell color variants (G, W, B, and P) were the fourth generation offspring produced by successive selection, and exhibited steadily hereditary shell color traits. In July 2013, 92 (47 females and 45 males), 86 (41 females and 45 males), 107 (50 females and 57 males), and 96 (50 females and 46 males) individuals were respectively selected from the third-generation strains selected for gold, white, black and purple shell traits to serve as parents for the fourth generation selected strains. For each variant, oysters were sexed and males and females were separated. Gametes were then rinsed into separate buckets by stripping the gonad. Equal gametes were well mixed after

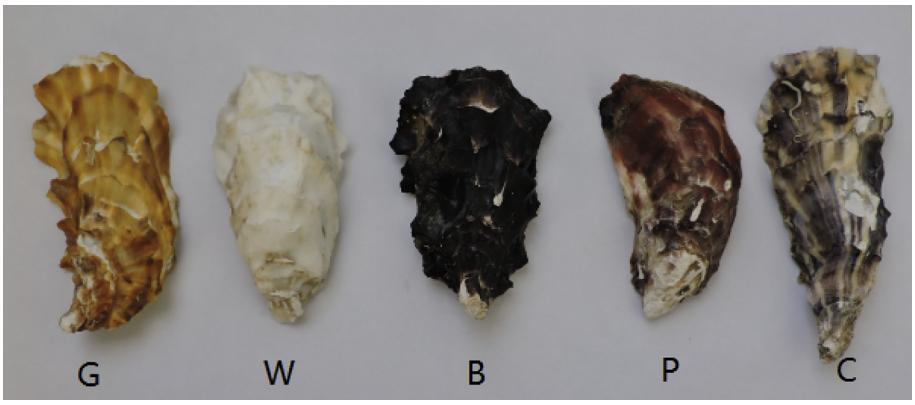

Fig. 1. Five Pacific oyster populations with gold (G), white (W), black (B), purple (P), and common (C) shells in the study. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

estimating concentrations using a microscope. The rearing of the larvae, spat, and adults were conducted using standard practices, and the rearing conditions were kept the same for the four shell color variants.

Genomic DNA was extracted from the adductor muscle tissue using proteinase-K digestion and DNA extraction buffer. The quality and quantity of extracted DNA were assayed by 1% agarose gel electrophoresis and Thermo NanoDrop-2000, respectively. DNA concentration was adjusted to 100 ng/ml for the following experiments.

2.2. AFLP procedure

The AFLP procedure was performed as described by Vos et al. (1995), with some modifications. Digestion of genomic DNA was processed in 10 μl of mixture containing 100 ng of genomic DNA, 1 U of *Eco*RI and *Mse*I, 2 × Tango™ buffer (MBI Fermentas) at 37 °C for 3 h, and then 65 °C for 3 h. After digestion, 10 μl of ligation mixture including 5 pmol *Eco*RI adaptor, 50 pmol *Mse*I adaptor, 10 × buffer, 1 U T_4 DNA ligase (MBI Fermentas), and 50% PEG was added. The reaction mixture was incubated at 16 °C overnight and then diluted by addition of 180 μl $TE_{0.1}$ (10 mM Tris−HCl, 0.1 mM EDTA, pH 8.0). Pre-amplification PCR was conducted in 10 μl reactions containing 2 μl of diluted ligation mixture and 8 μl of preselective amplification solution (0.25 pmol of pre-amplification primers with a single selective base, 200 μm dNTPs, 1 × PCR buffer, and 0.25 U *Taq* DNA polymerase), and cycled as follows: 72 °C for 2 min, 20 PCR cycles of 20 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C, followed by a final 30-min extension at 60 °C. The pre-amplification PCR product was diluted 20 times with $TE_{0.1}$. Selective amplification was also performed in a 10-μl volume using 1.5 μl of diluted ligation and 8.5 μl of selective amplification solution (0.25 pmol of *EcoR I* and *Mse I*, 0.2 mM dNTPs, 1 × PCR buffer, and 0.25 U *Taq* DNA polymerase). Selective amplification was performed for 2 min of denaturing at 94 °C, then 10 cycles of 20 s at 94 °C, 30 s at 66 °C, and 2 min at 72 °C, with a 1 °C decrease in the annealing temperature each cycle, followed by 20 cycles of 20 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C, with a final extension of 30 min at 60 °C. Amplification products were separated on an ABI 3130 automatic sequencer (Applied Biosystems). Data were analyzed using software GeneMapper® 4.0. Fifteen primer combinations were genotyped (Table 1).

2.3. AFLP data analysis

Polymorphic AFLP fragments ranging from 50 to 500 base pairs were scored by using the presence (1) or absence (0) of a peak as a criterion. The percentages of polymorphic loci (*P*), observed number of alleles (N_a), effective number of alleles (N_e), and the Nei's genetic distance (Nei, 1978) were calculated using POPGENE 1.31 (Yeh et al., 1999). Expected heterozygosity *(He)* and F_{ST} values were analyzed using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). The average expected heterozygosity and percentage of polymorphic loci for cultured populations were tested against local wild populations. Significance was tested using SPSS 14.0 software (Zar, 1999). The significance of F_{ST} estimates was tested with a null distribution created by 10000 random permutations of the data set (Excoffier et al., 1992). An F_{ST} analysis of molecular variance (AMOVA; Excoffier et al., 1992) was employed to define the grouping of genetic variation in hierarchical arrangements using ARLEQUIN 3.5 with a null distribution created by 10,000 random permutations of the data set.

There are several challenges in using outlier analysis. For example, false positive and false negative results make the interpretations complicated. The outlier loci were detected using two methods to limit the number of false negatives. We first used the hierarchical analysis of program ARLEQUIN 3.5 for detecting loci under selection using the F-statistic tab, with the *p*-values of each locus under neutrality of heterozygosities. Any locus with higher F_{ST} values might be considered as outlier because an outlier was expected to generate a high frequency disequilibrium. In this study, the F_{ST} values above simulated quantile distributions were designated as 99% outlier loci. The second method we used to detect outlier loci was the Bayesian approach of BAYESCAN 2.01 that implements the method of Foll and Gaggiotti (2008). Bayescan aims at identifying candidate loci under natural selection from genetic data, using differences in allele frequencies between populations. Loci with probability values that exceeded 0.76 were likely considered as outlier loci.

Table 1
Selective primer combinations and their respective extensions chosen (marked with √) for use in the AFLP assay.

*Eco*RI	*Mse*I							
	M-TCC	M-TGA	M-TCT	M-TCA	M-TGC	M-TCG	M-TAC	M-TAG
E-ACC					√			
E-ACT					√			√
E-AGG			√			√	√	√
E-ACA	√			√				
E-ATC	√				√			
E-AGG	√						√	√

Rows contain selective trinucleotide extensions attached to the 3′ end of the fluorescently labeled *Eco*RI primer 5′-GACTGCGTACCAATTCNNN-3′. Columns contain selective trinucleotide extensions attached to the 3′ end of the *Mse*I primer 5′-GATGAGTCCTGACCGANNN-3′.

3. Results

3.1. Genetic diversity

A total of 1130 loci were identified and transferred into 0/1 matrix using 15 AFLP primer combinations across 190 Pacific oyster individuals (Table 2). A total of 1079 loci were scored as polymorphic loci and the percentage of polymorphic loci was 95.5% (Table 2). The number of loci generated from each primer combination ranged from 63 to 116. The percentage of polymorphic loci was estimated to be 93.2% in the wild population (C) and varied from 90.0% (W) to 95.3% (P) within the four shell color variants. The *He* values were 0.3105 in the wild population and ranged from 0.3044 (W) to 0.3285 (P) within the four shell color variants. The white shell variant was observed to have the lowest genetic diversity ($P = 90.0\%$, $He = 0.3044$) than the other color variants. Between the wild population and cultured four shell color variants, there was no significant difference in the percentage of polymorphic loci ($P = 0.400$) and the *He* values ($P = 1.000$).

3.2. Genetic differentiation

Genetic differentiation across all populations as a whole was moderate significant ($F_{ST} = 0.1483$, $P < 0.05$). The most genetic differentiation was between the purple shell (P) and golden shell (G) variants (0.2112), while the least genetic differentiation was between the purple shell (P) variant and common wild (C) population (0.0941) (Table 3). In concordant with F_{ST} values, the pairwise Nei's (1978) genetic distance values were low (0.0293) between the purple shell (P) variant and common wild (C) populations, but high (0.0730) between the purple shell (P) and golden shell (G) variants (Table 3). The AMOVA analysis revealed only 14.8% of the total variations among populations and the largest level of variation (85.1%) was explained by differences within populations.

3.3. Outlier loci analysis

In comparisons with the hierarchical analysis and the Bayesian likelihood method, 17 loci were selected. Eleven of the 17 loci found in both methods were used for positives, four were only found in the hierarchical analysis (Table 4), and 2 were just found in the Bayesian likelihood method (Table 4). The 6 outliers that were only identified in one analysis method were considered as false positives and were not studied further (Table 4). Taking into account the frequency of the 11 outlier loci in different shell color variants, locus 795 was more likely to present in the white shell variant with a frequency of 0.8421. The frequency of loci 309 and 360 was 0.8947 and 0.9737, respectively in the purple shell variant, respectively, while the loci 601 and 946 were highlighted in the purple shell variant and wild population. The same analysis for other outlier loci revealed that the loci 1085 and 1090 were more prone to be found in the gold shell variant (Table 5). On the basic scale of Foll et al. (2010), the locus 998 supported with statistics of log10 (PO) > 2 was considered 'decisive' evidence for selection, and the loci 494 and 1085 supported with log10 (PO) > 1 were 'strong' evidence for selection (Table 4).

4. Discussion

The genetic diversity of a species is related to its ability to adapt to environment, viability and capability of evolution. Diversity can be of great importance to the overall sustainability of populations (Hamrick et al., 1991). In aquaculture, the level of genetic diversity in some species is high under artificial selection such as the pearl oyster (Yu and Chu, 2006) and scallop (Zhao et al., 2009). However, the reduction of genetic diversity in cultured populations was demonstrated in many species (Evans et al., 2004; Liu et al., 2010; Miller et al., 2012). The genetic diversity of hatchery strains is affected by several factors such as a small number of breeders, unequal sex ratios, unequal contribution of parents, and family size variations (Hedgecock and Sly, 1990; Gaffney et al., 1996). In this study, the percentage of polymorphic loci ranged from 90.0% (W) to 95.3% (P), with a mean of 95.5% across all individuals. The *He* values of the five populations varied from 0.3044 (W) to 0.3285 (P), with a mean of 0.3170. There was no significant difference in genetic diversity among the wild population and different shell color variants, demonstrating that the four shell color variants of *C. gigas* maintained a high level of genetic diversity. This could be the result of the relatively large number of the broodstock used and approximately equal sex ratio, which may reduce the risks of

Table 2
Statistical analysis of genetic diversity in one wild population and four shell color variants of *Crassostrea gigas*.

Population	*N*	No. of polymorphic loci	Polymorphic loci (%)	*Na*	*Ne*	*He*
G	38	971	90.5%	1.906	1.503	0.3115
W	38	964	90.0%	1.900	1.466	0.3044
B	38	987	91.1%	1.911	1.526	0.3102
P	38	993	95.3%	1.953	1.515	0.3285
C	38	1039	93.2%	1.932	1.489	0.3105
Overall	190	1079	95.5%	1.950	1.502	0.3170

N = the sample sizes; *Na* = observed number of alleles; *Ne* = effective number of alleles; *He* = average expected heterozygosity.

Table 3
Pairwise estimates of Nei's unbiased measures of genetic distance (above diagonal) and F_{ST} (below diagonal) between all *C. gigas* populations. Significant *Fst* values ($P < 0.05$) are in bold; 10000 permutations were used to test the significance of *Fst* estimates.

Population	G	W	B	P	C
G	****	0.0474	0.0549	0.0730	0.0474
W	**0.1456**	****	0.0505	0.0642	0.0369
B	**0.1639**	**0.1535**	****	0.0611	0.0405
P	**0.2112**	**0.1809**	**0.1763**	****	0.0293
C	**0.1357**	**0.1017**	**0.1111**	**0.0941**	****

inbreeding. Another factor which might account for the stability of genetic diversity was the practice of artificial fertilization. Gametes were rinsed into separate buckets by stripping the gonad and then equally mixed. This ensured that all parental oysters contributed to the next generation and avoided that some parents cannot have offspring. The breeding practices performed in this study appear to have minimized the loss of genetic diversity levels within different shell color variants.

In this study, the white shell variant was observed to have slightly lower genetic diversity than the others, which may be due to the white shell controlled by recessive homozygous model. This result was consistent with the previous observation of Ge et al. (2015) that the white background color types were controlled by a recessive allele in the Pacific oyster. Similar inheritance model was reported in pearl oyster that white coloration might be inherited under the control of recessive gene(s) for shell color (Wada and Komaru, 1996).

As indicated by the pairwise F_{ST} analysis, significant genetic differentiation was detected among the four shell color variants, and between the cultured shell color and wild populations. AMOVA analysis indicated that genetic differentiation of populations mostly resulted from the individuals within populations. Similar phenomena were observed in the Pacific abalone, eastern oyster, and pearl oyster (Li et al., 2004; Yu and Guo, 2004, Yu and Chu, 2006). Significant genetic differentiation between wild and cultured populations is most likely due to random drift, bottleneck effect, and artificial selection during the production of a new generation (Pampoulie et al., 2006). It is well accepted that high genetic differentiation among

Table 4
Outlier loci detected by hierarchical analysis and Bayesian approach.

Locus	Bayesian			Hierarchical analysis		
	P	log10(PO)	F_{ST}	Levels	Obs Fs_T	*P*
169				99%	0.50	0.0051
309	0.82	0.65	0.27	99%	0.47	0.009
349	0.86	0.8	0.29	99%	0.46	0.0059
360	0.89	0.92	0.3			
364				99%	0.48	0.0087
483	0.85	0.74	0.3	99%	0.60	0.0049
494	0.95	1.29	0.34	99%	0.63	0.0051
583				99%	0.56	0.00031
601	0.83	0.7	0.28	99%	0.51	0.0028
795	0.77	0.54	0.29	99%	0.71	0.0016
925	0.88	0.88	0.3			
946	0.93	1.12	0.31	99%	0.48	0.0044
985				99%	0.48	0.0051
998	1	2.44	0.37	99%	0.56	0.000700
1085	0.93	1.11	0.34	99%	0.52	0.00069
1090	0.91	0.99	0.29	99%	0.519	0.0024

$P = P$ value of F_{ST}.

Table 5
Frequency of outlier loci in the five populations of *C. gigas*.

Locus	G	W	B	P	C
309	0.02632	0.1842	0.4474	0.9737	0.4737
349	0.92105	1	0.5263	0.1316	0.5789
360	0	0.1053	0.4737	0.8947	0.6053
483	0.9737	1	1	0.2368	0.8421
494	1	1	1	0.2368	0.8421
601	0.08	0.1053	0.5263	0.8684	0.8421
795	0.07900	0.8421	0.0526	0	0
946	0.3158	0.02631	0.4474	0.8947	0.8684
998	0	0.5789	0.8421	0.9474	0.9211
1085	1	0.2632	0.1579	0.1053	0.1316
1090	0.7895	0.6053	0.3947	0.2368	0.1579

cultured strains is due to positive selection, particularly strong artificial selection (Zou et al., 2014). The marked genetic differentiation of the four different shell color variants in this study might be due to strict reproductive isolation by artificial selection.

Two approaches were used to detect outliers in the same data sets in this study. The number of outliers detected by the two approaches were limited, because the two approaches have completely different assumptions and employ separate algorithms. The main difference between Arequin and BayeScan is that the F_{ST} within-populations is variable in BayeScan, but it is assumed to be the same across all populations in the former program (Nunes et al., 2011). We implemented two different outlier programs in order to avoid complications with false-positive loci in outlier tests. It is expected that a strong loci should be picked up by detecting outlier loci in the genome scan. Among 1130 AFLP loci, AFLP genome scans approach detected 11 outlier loci by dual analysis that could be considered as candidates for color artificial selection.

The oyster individuals used in this study was cultured in a common environment which would be critical to minimize the environmentally induced phenotypic and epigenetic effects. Because the outlier analysis was performed with samples from populations with different color and no variables were dependent on environment, it was presumed that outlier loci might have been under the effect of artificial selection for shell colors. Most of the outlier loci were not unique to a single color population (Table 5). One explanation was that some outliers might be linked to the same locus controlling different color. Previous studies have suggested that shell color and shell pigmentation in some shellfish species might be associated with more than one alleles at a single locus (Ge et al., 2015; Luttikhuizen and Drent, 2008). Furthermore, Narum and Hess (2011) suggested that neutral markers were not the actual loci under selection but rather indicators of a nearby quantitative trait locus (QTL), a section of DNA that correlates with variation in a phenotype, or quantitative trait. The small number of loci detected as outliers in this study might not be genes under selection but may represent QTL regions that warrant further investigation and validation as important regions of the genome causing true color variation.

In summary, this study revealed high level genetic diversity and the genetic differentiation of populations mostly resulted from the individuals within populations. Among the different shell color variants, 11 outliers were detected by two methods. The present study provides a solid foundation for the genetic basis of shell color traits in the Pacific oyster. The molecular mechanisms and major gene underlying shell coloration will be required to further develop for addressing shell color polymorphism.

Acknowledgments

This study was supported by Shandong Seed Project, National Natural Science Foundation of China (31372524), and Shandong Province (2014GHY115002).

References

Beaumont, M.A., Dbaldingi, D.J., 2004. Identifying adaptive genetic divergence among populations from genome scans. Mol. Ecol. 13, 969–980.
Cong, R., Li, Q., Ge, J., Kong, L., Yu, H., 2014. Comparison of phenotypic traits of four shell color families of the Pacific oyster (*Crassostrea gigas*). J. Fish. Sci. China 21, 494–502 (in Chinese).
Evans, B., Bartlett, J., Sweijd, N., Cook, P., Elliott, N.G., 2004. Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). Aquaculture 233, 109–127.
Excoffier, L., Lischer, H.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under linux and windows. Mol. Ecol. Resour. 10, 564–567.
Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes:application to human mitochondrial DNA restriction data. Genetics 131, 479–491.
Foll, M., Gaggiotti, O., 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics 180, 977–993.
Foll, M., Fischer, M.C., Heckel, G., Excoffier, L., 2010. Estimating population structure from AFLP amplification intensity. Mol. Ecol. 19, 4638–4647.
Gaffney, P.M., Rubin, V.P., Hedgecock, D., Powers, D.A., Morris, G., Hereford, L., 1996. Genetic effects of artificial propagation: signals from wild and hatchery populations of red abalone in California. Aquaculture 143, 257–266.
Ge, J.L., Li, Q., Yu, H., Kong, L.F., 2015. Mendelian inheritance of golden shell color in the Pacific oyster *Crassostrea gigas*. Aquaculture 441, 21–24.
Hamrick, J.L., Godt, M.J.W., Murawski, D.A., Loveless, M.D., 1991. Correlations Between Species Traits and Allozyme Diversity: Implications for Conservation Biology. Oxford University Press, Oxford.
Hedgecock, D., Sly, F., 1990. Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster, *Crassostrea gigas*. Aquaculture 88, 21–38.
Kozminsky, E., 2014. Inheritance of the background shell color in the snails *Littorina obtusata* (Gastropoda, Littorinidae). Russ. J. Genet. 50, 1038–1047.
Li, Q., Park, C., Endo, T., Kijima, A., 2004. Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). Aquaculture 235, 207–222.
Lindberg, D.R., Pearse, J.S., 1990. Experimental manipulation of shell color and morphology of the limpets *Lottia asmi* (Middendorff) and *Lottia digitalis* (Rathke) (Mollusca: Patellogastropoda). J. Exp. Mar. Biol. Ecol. 140, 173–185.
Liu, X., Wu, F.C., Zhao, H.G., Zhang, G.F., Guo, X.M., 2009. A novel shell color variant of the pacific abalone *haliotis discus hannai* ino subject to genetic control and dietary influence. J. Shellfish Res. 28, 419–424.
Liu, W.D., Li, H.J., Bao, X.B., Gao, X.G., Li, Y.F., He, ChB., Liu, ZhJ., 2010. Genetic differentiation between natural and hatchery stocks of Japanese scallop (*Mizuhopecten yessoensis*) as revealed by AFLP Analysis. Int. J. Mol. Sci. 11, 3933–3941.
Luttikhuizen, P.C., Drent, J., 2008. Inheritance of predominantly hidden shell colours in *Macoma balthica*. J. Moll. Stud. 74, 363–371.
Mattersdorfer, K., Koblmüller, S., Sefc, K.M., 2012. AFLP genome scans suggest divergent selection on color patterning in allopatric color morphs of a cichlid fish. Mol. Ecol. 21, 3531–3544.
Miller, P.A., et al., 2012. Genetic diversity of cultured, naturalized, and native Pacific oysters, *Crassostrea gigas*, determined from multiplexed microsatellite markers. J. Shellfish. Res. 31, 611–617.
Narum, S., Hess, J., 2011. Comparison of FST outlier tests for SNP loci under selection. Mol. Ecol. Resour. 11 (Suppl. 1), 184–194.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nunes, V.L., Beaumont, M.A., Butlin, R.K., Paulo, O.S., 2011. Multiple approaches to detect outliers in a genome scan for selection in ocellated lizards (*Lacerta lepida*) along an environmental gradient. Mol. Ecol. 20, 193–205.
Oetjenk, K., Reusch, T., 2007. Genome scans detect consistent divergent selection among subtidal vs. intertidal populations of the marine angiosperm Zostera marina. Mol. Ecol. 16, 5156–5167.
Pampoulie, C., Jorundsdottir, T.D., Steinarsson, A., Petursdottir, G., Stefansson, M.O., Danielsdottir, A.K., 2006. Genetic comparison of experimental fanned strains and wild Icelandic populations of Atlantic cod *(Gadus morhua* L.). Aquaculture 261, 556–564.
Petersen, J.L., Baerwald, M.R., Ibarra, A.M., 2012. A first-generation linkage map of the Pacific lion-paw scallop (*Nodipecten subnodosus*): initial evidence of QTL for size traits and markers linked to orange shell color. Aquaculture 350, 200–209.
Richards, P.M., LIU, M.M., Lowe, N., Davey, M.L., Davison, A., 2013. RAD-Seq derived markers flank the shell colour and banding loci of the *Cepaea nemoralis* supergene. Mol. Ecol. 22, 3077–3089.
Sokolova, I., Berger, V.J., 2000. Physiological variation related to shell colour polymorphism in White Sea *Littorina saxatilis*. J. Exp. Mar. Biol. Ecol. 245, 1–23.
Tang, K., Thornton, K.R., Stoneking, M., 2007. A new approach for using genome scans to detect recent positive selection in the human. Plos Biol. 5, 1587–1602.
Vos, P., Hogers, R., Bleeker, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids. Res. 23, 4407–4414.
Wada, K.T., Komaru, A., 1996. Color and weight of pearls produced by grafting themantle tissue from a selected population for white shell color of the Japanese pearl oyster, *Pinctada fucata martensii* (Dunker). Aquaculture 142, 25–32.
Wen, H.B., Gu, R.B., Cao, Z.M., Zhou, X., Nie, Z.J., Ge, X.P., 2013. Variation of color and ray pattern in juvenile shells in hatchery-produced freshwater triangle pearl mussels, *Hyriopsis cumingii*, in China. J. World Aquac. Soc. 44, 154–160.
Yeh, F.C., Yang, R., Boyle, T., 1999. Microsoft Windows-based free Ware for Population Genetic Analysis (Release 1.31). University of Alberta, Edmonton.
Yu, D.H., Chu, K.H., 2006. Genetic variation in wild and cultured populations of the pearl oyster *Pinctada fucata* from southern in China. Aquaculture 258, 220–227.
Yu, Z.N., Guo, X.M., 2004. Genetic analysis of selected strains of eastern oyster (*Crassostrea virginica Gmelin*) using AFLP and microsatellite markers. Mar. Biol. 6, 575–586.
Zar, J.H., 1999. Biostatistical Analysis, fourth ed. Prentice Hall, New Jersey.
Zhao, C., Li, Q., Kong, L.F., 2009. Inheritance of AFLP markers and their use for genetic diversity analysis in wild and farmed scallop (Chlamys farreri). Aquaculture 287, 67–74.
Zheng, H.P., Zhang, G.F., Liu, X., Kan, H.Y., 2003. Establishment of different shell color lines of bay scallop(Argopecten Irradlans Lamarck) and their development. J.Ocean. Limn. China 34, 632–639 (in Chinese).
Zou, K.H., Zhang, D.C., Guo, H.Y., Zhua, C.Y., Li, M., Jiang, S.G., 2014. A preliminary study for identification of candidate AFLP markers under artificial selection for shell color in pearl oyster P*inctada fucata*. Gene 542, 8–15.