Aquaculture 512 (2019) 734249

Contents lists available at ScienceDirect

Aquaculture

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

Short communication

Inheritance of shell pigmentation in Pacific oyster *Crassostrea gigas*

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ARTICLE INFO

Keywords:
Pacific oyster
Crassostrea gigas
Inheritance
Shell color
Pigmentation

ABSTRACT

The Pacific oyster (*Crassostrea gigas*) is one of the most important species which has the largest production among all cultured shellfish. Recently, several shell color strains of *C. gigas* have been developed to improve the commercial values, however the inheritance of shell pigmentation is still unclear. A total of 29 families cross-mated between different pigmented oysters were produced to investigate the inheritance of the shell pigmentation and purple pigmentation. Random samples from each family were obtained and their shell pigmentation were recorded. Results revealed that shell pigmentation was controlled by two genetic locus, among which one is responsible for secretion of pigmentation and the other is responsible for distribution mode of pigmentation. The locus controlling the secretion of pigmentation has two alleles with the allele for the presence of pigmentation being dominant to the allele for the absence of pigmentation. Similarly, another locus controlling the distribution mode of pigmentation has two alleles with the allele for striped distribution being dominant to the allele for solid distribution. In addition, one independent locus with two alleles was suggested to control the purple-striped pigmentation, which one allele for shell devoid of purple pigmentation is dominant to another allele for shell with purple pigmentation. The findings will provide valuable information for the efficient selective breeding of shell color strains in the Pacific oyster.

1. Introduction

The widespread color polymorphisms of molluscan shell have been appreciated for hundreds of years by collectors and a great deal of scientists. Moreover, the fabulous and diverse colors are not only considered as characters to recognize and distinguish species, but also considered as visual perceptions to affect the consumer preference of seafood. The shellfish products with desirable shell color usually has a premium price, which endows the shell color with product value of great importance. Ranking first in production among all aquaculture shellfish, the Pacific oyster (*Crassostrea gigas*) is usually sold by live-in-shell or half-shell in market, which makes the shell color become an important reference to the consumption of oyster products. For example, the Pacific oysters with golden or orange/bronze shell are rarely seen in the market, resulting a better transaction price than common oysters (Nell, 2001). In Korea, the Pacific oysters with dark pigmented shell are favored by consumers and traded at about 20% higher price (Kang et al., 2013). Obviously, the shell color of the Pacific oyster has been regarded as new high potential traits for a better commercial value, which makes it urgent to develop generalizing ideas on the genetic basis and mechanism of shell color inheritance in the Pacific oyster.

Many studies have demonstrated that the shell color of the Pacific oyster is under a high degree of genetic control (Brake et al., 2004; Evans et al., 2009; Hedgecock et al., 2006; Wan et al., 2017; Xu et al., 2017). However, there are few studies on inheritance of shell color in Pacific oyster. A previous study found that the shell color of the Pacific oyster was determined by background color and foreground pigmentation, and the background color is controlled by one locus with two alleles with the allele for golden being dominant to the allele for white, while the foreground pigmentation is influenced by the epistatic effect of background color (Ge et al., 2015). Nonetheless, there was no further studies on the inheritance of foreground pigmentation.

The shell pigmentation of the Pacific oyster has been viewed as a quantitative trait which is controlled by many genes with small-effects for a long time, according to its continuous distributions from near-white to near-black shells instead of discrete phenotypic classes (Brake et al., 2004; Takeo and Sakai, 1961). However, only a few molecular markers were identified to be well correlated with shell pigmentation using molecular biotechniques, supporting a hypothesis that major genetic locus may exist to control shell pigmentation (Ge et al., 2014; Hedgecock et al., 2006). Up to now, more evidences are found that there are major genes controlling the shell mantle edge pigmentation. Based on genetic map, three shared-QTLs were associated with shell

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https://doi.org/10.1016/j.aquaculture.2019.734249
Received 16 March 2019; Received in revised form 23 June 2019; Accepted 24 June 2019
Available online 26 June 2019
0044-8486/

color traits were identified and one of them was significantly homologous to *C. gigas* calmodulin-like protein (Wang et al., 2018). Based on transcriptional profiling of long non-coding RNAs (lncRNA), Feng et al. (2018) found the chorion peroxidase and its cis-acting lncRNA TCONS_00951105 were implicated in playing an essential role in the melanin synthetic pathway. Using the RNA interference, *tyrosinase* was confirmed to play a vital role in the assembly and maturation of shell matrices and *peroxidase* was essential for black pigmentation in the shell (Feng et al., 2019).

In this study, 36 families cross-mated between different pigmented oysters were designed to make a further exploration of the inheritance pattern of shell pigmentation, which will provide valuable information for the efficient selective breeding of shell color traits in the Pacific oyster.

2. Material and methods

2.1. Parental source

Adult oysters for cross-mating were selected from the black shell line and the white shell line, respectively. Both two lines were the ninth-generation lines which were selected from the wild populations in Shandong province, China. The selection of the black shell line focused on the black shell color and growth rate (Xu et al., 2017). Similarly, the selection of white shell line focused on the white shell color and growth rate (Xing et al., 2018). Both two lines were with stable heritable shell color and the progenies from each line were fully homogeneous in shell color. Oysters with two shell color were used as parents in this study (Fig. 1), including those with black shell (B) and white shell (W).

2.2. Mating design and rearing

Four types of a full factorial cross-design including WB (W♀ × B♂), BW (B♀ × W♂), BB (B♀ × B♂) and WW (W♀ × W♂) were produced using parents with the two shell color patterns. And a total of 36 families were designed to investigate the inheritance of shell pigmentation, including 15 WB, 15 BW, 3 BB and 3 WW. The detailed mating design are shown in Table 1.

Oysters with each shell color were sexed, and males and females were separated. Gametes were then rinsed into separate buckets by stripping the gonad. Eggs from each dam was divided into two equal portions and fertilized by corresponding sperm according to the mating design, respectively. Twenty hours after fertilization, embryos developed into D-stage larvae and the larvae of each family were transferred into a 70-L plastic bucket. Stocking densities were initially set to 5 larvae mL^{-1}, and decreased with larval growth. The rearing of the larvae was carried out using practices described by Ge et al. (2015). Briefly, larvae were raised for 18–22 days on an algae diet of *Isochrysis galbana* and *Nitzschia closterium*. Half volume of water was replaced with filtrable seawater twice per day. Strings of scallop shells were used to collect the spat metamorphosed from eyed larvae. After being metamorphosed, spat were transported to an outdoor nursery tank for a temporary rearing. Then all spat were transported to Sanggou Bay, Shandong Province placed on nylon ropes and cultured on suspended longlines.

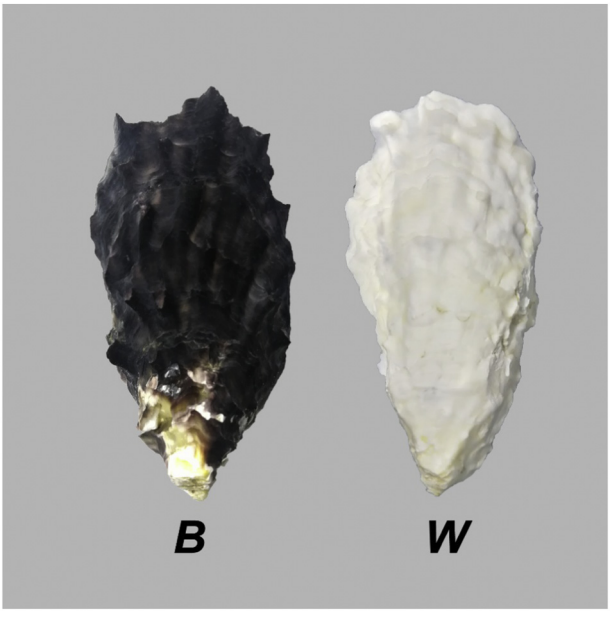

Fig. 1. Representative parents of black shell and white shell of *C. gigas*. B = Black; W = White.

2.3. Shell pigmentation record and statistical analysis

Offspring were sampled randomly from each family on 180th day after fertilization in 2018. All sampled oysters were stripped from each other, and washed thoroughly using a brush with freshwater. According to the system of pigmentation categorization employed by Takeo and Sakai (1961), a similar system with five shell pigmentation categories based on pigmentation proportion was designed to describe variation among all samples and parents. The categories used are shown in Fig. 2, and were described as follows:

- 0, whole surface with no pigmentation;
- 1, whole surface with little striped pigmentation;
- 2, half part of whole surface with striped pigmentation;
- 3, most part of whole surface with striped pigmentation;
- 4, whole surface with solid pigmentation.

Two distribution types of pigmentation on shell surface were recorded as solid or striped. And the shell with striped pigmentation was recorded as shell color with purple pigmentation (S_{wp}) or shell color devoid of purple pigmentation (S_{dp}).

An assumption that the two-locus model for shell pigmentation and single locus two-allele model for striped purple pigmentation was used to analyze the proportions in progeny. The fit of the phenotypic frequencies to the expected frequencies under the proposed model of inheritance was tested using the Chi-squared test for goodness-of-fit. Fisher's exact test of independence was used to test the association of shell pigmentation index and shell with purple pigmentation.

3. Results

A total of 29 families were obtained eventually, with 6 families suffered defeat in hatching and 1 family lost during the grow-out stage. The pigmentation index and shell color phenotype of offspring from 29 families were recorded and shown in Table 1. Obviously, segregation of shell pigmentation universally existed in the progeny among the families crossed by pure black and white parents. According to the pigmentation index, there was a large difference among the proportions of the segregation from all families, which the continuous variation was from nearly all white (family 31) to nearly all black (family 35). The observed segregations were unable to be explained by a hypothesis that the shell pigmentation is simply controlled by one single major gene, suggesting that more genes participate in the inheritance of shell pigmentation.

According to the pigmentation index, phenotype of offspring from all families were reclassified into shell with pigmentation (S_w) and shell devoid of pigmentation (S_d). As shown in Table 2, the presence of shell pigmentation was dominant over the absence of shell pigmentation. There were no segregations in the families (families 1–2, 7–12, 14, 18, 20–22, 26–27, 30, 33, 35) cross-mated between shell without

Table 1
Pigmentation index of the parents and offspring from 36 crosses between two shell color patterns of *C. gigas*.

Family no.	Pigmentation index of parents[a]				Pigmentation index of offspring					
	Female		Male		4	3	2	1	0	Total
1	W♀1	0	B♂1	4	0	26	22	40	0	88
2	W♀2	0	B♂1	4	0	60	45	113	0	218
3	W♀3	0	B♂2	4	0	18	11	28	53	110
4	W♀4	1	B♂2	4	0	48	50	60	43	201
5	W♀5	0	B♂3	4	0	11	11	43	67	132
6	W♀6	0	B♂3	4	–					
7	W♀7	0	B♂4	4	0	49	15	65	0	129
8	W♀8	0	B♂4	4	0	46	36	88	0	170
9	W♀9	0	B♂5	4	0	13	17	147	0	177
10	W♀10	0	B♂5	4	0	61	21	113	0	195
11	W♀11	0	B♂6	4	0	49	48	77	0	174
12	W♀12	0	B♂6	4	0	81	79	79	0	239
13	B♀13	4	B♂7	4	81	0	0	0	0	81
14	W♀13	0	B♂7	4	0	32	13	51	0	96
15	B♀14	4	B♂8	4	99	0	0	0	0	99
16	W♀14	0	B♂8	4	–					
17	B♀15	4	B♂9	4	112	0	0	0	0	112
18	W♀15	1	B♂9	4	0	45	21	52	0	118
19	B♀1	4	W♂1	0	–					
20	B♀2	4	W♂1	0	43	33	1	4	0	81
21	B♀3	4	W♂2	0	0	30	28	54	0	112
22	B♀4	4	W♂2	0	0	39	47	77	0	163
23	B♀5	4	W♂3	1	0	10	38	63	0	111
24	B♀6	4	W♂3	0	–					
25	B♀7	4	W♂4	0	–					
26	B♀8	4	W♂4	0	0	43	45	73	0	161
27	B♀9	4	W♂5	0	0	32	29	35	0	96
28	B♀9	4	W♂5	0	–					
29	B♀11	4	W♂6	0	0	22	32	45	88	187
30	B♀12	4	W♂6	0	0	2	2	69	0	73
31	B♀13	4	W♂7	0	0	0	0	47	51	98
32	W♀13	0	W♂7	0	0	0	0	0	70	70
33	B♀14	4	W♂8	0	54	39	2	6	0	101
34	W♀14	0	W♂8	0	–					
35	B♀15	4	W♂9	0	52	51	0	0	0	103
36	W♀15	1	W♂9	0	0	8	3	23	41	75

[a] W: White shell; B: Black shell.

pigmentation oysters and shell with pigmentation oysters, and all offspring from those families were with pigmentation. Similarly, three black families (families 13, 15, 17) produced all offspring shell with pigmentation. In contrast, one white family (family 32) produced all offspring shell devoid of pigmentation. Families 3, 5, 29, 31, 36, cross-mating between shell with pigmentation oysters and shell devoid of pigmentation oyster, resulted in shell pigmentation of offspring at ratios that were not different from 1:1. Only one family (family 4), mating by both parents with pigmentation, produced offspring shell with pigmentation and shell devoid of pigmentation in a ration closely approaching 3:1. Obviously, these observations suggested one genetic locus controlling the presence of pigmentation in shell, and the one allele for the presence of pigmentation in shell was dominant to the allele for the absence of pigmentation.

The distribution mode of shell pigmentation for offspring in 29 families were recorded and shown in Table 3, and the representative shell color of offspring was shown in Fig. 2. Phenotype of offspring segregated only in three families (families 20, 33, 35), producing both striped and solid pigmentation offspring fit into a 1:1 ratio. All families cross-mated between unknown phenotype of pigmentation oysters and solid pigmentation oysters produced all offspring with striped pigmentation. Offspring with solid pigmentation could only be produced by both parents with solid pigmentation (families 13, 15, 17). According to the observations, one genetic locus could be inferred to control the distribution of shell pigmentation, with one allele for striped pigmentation being dominant to the allele for solid pigmentation.

Offspring with purple-striped pigmentation in shell surface were observed in five families (families 1, 7, 8, 26, 30). The phenotype of purple striped pigmentation is shown in Fig. 2, and the record of offspring is shown in Table 4. The proportions of shell without purple pigmentation to the shell with purple pigmentation fit into a 3:1 ratio, suggesting one genetic locus controlling the presence of purple pigmentation in shell, and the one allele for the presence of purple pigmentation in shell was dominant to the allele for the absence of purple pigmentation. Results of independent test on the association between shell pigmentation index and shell with purple pigmentation are shown in Table 4. The significant correlations between shell pigmentation index and shell with purple pigmentation were only found in families 1, 8 and 27. There was no enough evidence that the inheritance of purple pigmentation was related to pigmentation index.

4. Discussion

Most studies on inheritance of shell color supported a single locus-two-allele model is the major determinant in bivalves, such as in the mussel *Mytilus edulis* (Innes and Haley, 1977), the bay scallop *Argopecten irradians* (Adamkewicz and Castagna, 1988), the noble scallop *Chlamys nobilis* (Zheng et al., 2013). However, the single locus-two-allele model is not applicable for inheritance of shell pigmentation in the Pacific oyster. To explain the observations in this study, we used for reference of two-locus models proposed by previous studies. For example, Peignon et al. (1995) proposed a two-locus model for the observed variation of shell color in Manila clam, with one gene controlling asymmetry and one gene controlling different ornamentations. Kozminsky (2016) proposed the two-locus for the heredity in white longitudinal bands in the snail *Littorina obtusata*, with one gene is

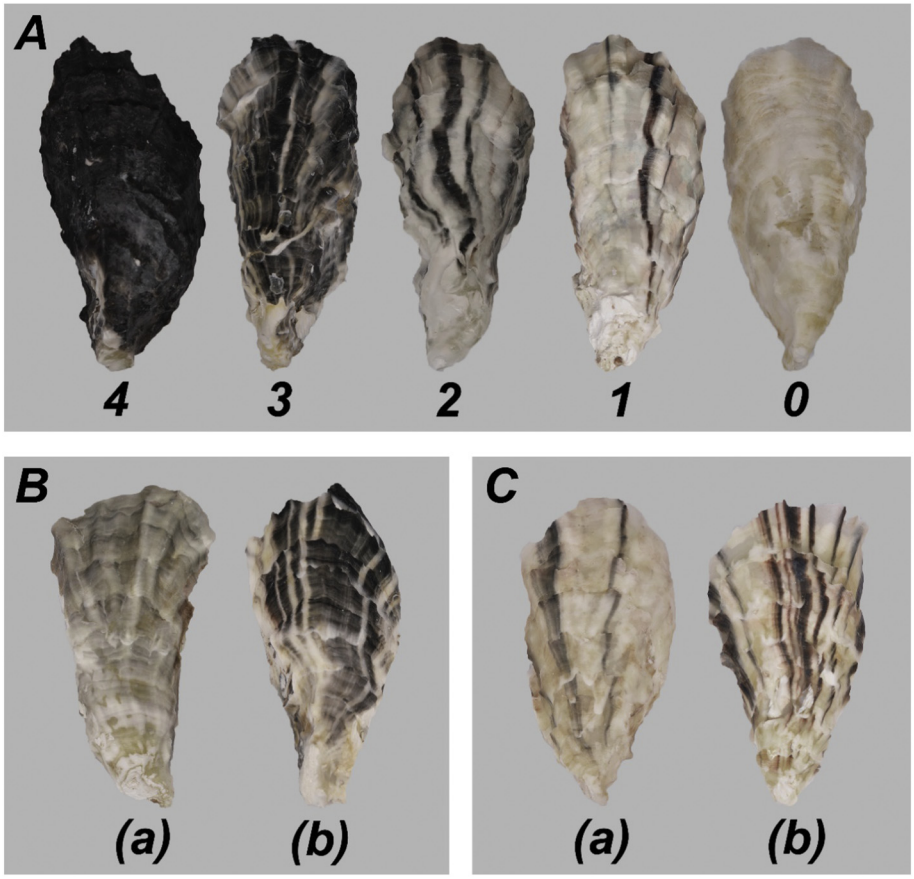

Fig. 2. Representative shell color morphs of offspring. A: Offspring with different pigmentation (pigmentation index: 0–4); B: Offspring with solid pigmentation (a) and with striped pigmentation (b); C: Offspring without purple strips (a) and with purple strips (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Inheritance of shell pigmentation in 29 families.

Family no.	Phenotype of parents[a]		Locus A[b]		Phenotype of offspring				
	Female	Male	Female	Male	S_w	S_d	Total	Expected ratio	X^2 (*P* value)
1	S_d	S_w	aa	AA	88	0	88	1:0	NA
2	S_d	S_w	aa	AA	218	0	218	1:0	NA
3	S_d	S_w	aa	Aa	57	53	110	1:1	0.787
4	S_w	S_w	Aa	Aa	152	49	201	3:1	0.908
5	S_d	S_w	aa	Aa	65	67	132	1:1	0.902
7	S_d	S_w	aa	AA	129	0	129	1:0	NA
8	S_d	S_w	aa	AA	170	0	170	1:0	NA
9	S_d	S_w	aa	AA	177	0	177	1:0	NA
10	S_d	S_w	aa	AA	195	0	195	1:0	NA
11	S_d	S_w	aa	AA	174	0	174	1:0	NA
12	S_d	S_w	aa	AA	239	0	239	1:0	NA
13	S_w	S_w	Aa	AA	81	0	81	1:0	NA
14	S_d	S_w	aa	AA	96	0	96	1:0	NA
15	S_w	S_w	AA	AA or Aa	99	0	99	1:0	NA
17	S_w	S_w	AA	AA	112	0	112	1:0	NA
18	S_w	S_w	Aa	AA	118	0	118	1:0	NA
20	S_w	S_d	AA	aa	81	0	81	1:0	NA
21	S_w	S_d	AA	aa	112	0	112	1:0	NA
22	S_w	S_d	AA	aa	163	0	163	1:0	NA
23	S_w	S_w	AA	Aa	111	0	111	1:0	NA
26	S_w	S_d	AA	aa	161	0	161	1:0	NA
27	S_w	S_d	AA	aa	96	0	96	1:0	NA
29	S_w	S_d	Aa	aa	99	88	187	1:1	0.569
30	S_w	S_d	AA	aa	73	0	73	1:0	NA
31	S_w	S_d	Aa	aa	47	51	98	1:1	0.775
32	S_d	S_d	aa	aa	0	70	70	0:1	NA
33	S_w	S_d	AA	aa	101	0	101	1:0	NA
35	S_w	S_d	AA	aa	103	0	103	1:0	NA
36	S_w	S_d	Aa	aa	34	41	75	1:1	0.566

[a] S_w: Shell with pigmentation; S_d: Shell devoid of pigmentation.
[b] Locus A: Locus which controlling the presence of shell pigmentation; A: the allele for S_w; a: the allele for S_d.

Table 3
Inheritance of distribution mode for shell pigmentation in 29 families.

Family no.	Phenotype of parents		Locus B [a]		Phenotype of offspring based on distribution of shell pigmentation					
	Female	Male	Female	Male	Solid	Striped	Unknown	Total	Expected ratio	X^2 (*P* value)
1	Unknown	Solid	BB	bb	0	88	0	88	0:1	NA
2	Unknown	Solid	BB	bb	0	218	0	218	0:1	NA
3	Unknown	Solid	BB	bb	0	57	53	110	0:1	NA
4	Striped	Solid	BB	bb	0	158	43	201	0:1	NA
5	Unknown	Solid	BB	bb	0	65	67	132	0:1	NA
7	Unknown	Solid	BB	bb	0	129	0	129	0:1	NA
8	Unknown	Solid	BB	bb	0	170	0	170	0:1	NA
9	Unknown	Solid	BB	bb	0	177	0	177	0:1	NA
10	Unknown	Solid	BB	bb	0	195	0	195	0:1	NA
11	Unknown	Solid	BB	bb	0	174	0	174	0:1	NA
12	Unknown	Solid	BB	bb	0	239	0	239	0:1	NA
13	Solid	Solid	bb	bb	81	0	0	81	1:0	NA
14	Unknown	Solid	BB	bb	0	96	0	96	0:1	NA
15	Solid	Solid	bb	bb	99	0	0	99	1:0	NA
17	Solid	Solid	bb	bb	112	0	0	112	1:0	NA
18	Striped	Solid	BB	bb	0	118	0	118	0:1	NA
20	Solid	Unknown	bb	Bb	43	38	0	81	1:1	0.693
21	Solid	Unknown	bb	BB	0	112	0	112	0:1	NA
22	Solid	Unknown	bb	BB	0	163	0	163	0:1	NA
23	Solid	Striped	bb	BB	0	111	0	111	0:1	NA
26	Solid	Unknown	bb	BB	0	161	0	161	0:1	NA
27	Solid	Unknown	bb	BB	0	96	0	96	0:1	NA
29	Solid	Unknown	bb	BB	0	99	88	187	0:1	NA
30	Solid	Unknown	bb	BB	0	73	0	73	0:1	NA
31	Solid	Unknown	bb	BB	0	47	51	98	0:1	NA
32	Unknown	Unknown	Any genotype	Any genotype	0	0	70	70	-:-	NA
33	Solid	Unknown	bb	Bb	54	47	0	96	1:1	0.621
35	Solid	Unknown	bb	Bb	52	51	0	101	1:1	0.944
36	Striped	Unknown	BB or Bb	BB	0	34	41	75	0:1	NA

[a] Locus B: Locus which controlling the distribution mode of shell pigmentation; B: the allele for Striped; b: the allele for Solid.

Table 4
Inheritance of shell purple striped pigmentation trait and independent test of association between "pigmentation index" and "purple striped pigmentation" in five families.

Family no.	Phenotype of parents [a]		Locus C [b]		Phenotype	Pigmentation index of offspring				Expected ratio	X^2	*P* value
	Female	Male	Female	Male		3	2	1	Total			
1	S_{dp}	S_{dp}	Cc	Cc	S_{dp}	19	10	35	64	3:1	0.732	0.002
					S_{wp}	7	12	5	24			
7	S_{dp}	S_{dp}	Cc	Cc	S_{dp}	34	9	55	98	3:1	0.885	0.050
					S_{wp}	15	6	10	31			
8	S_{dp}	S_{dp}	Cc	Cc	S_{dp}	31	23	76	130	3:1	0.8	0.009
					S_{wp}	15	13	12	40			
26	S_{dp}	S_{dp}	Cc	Cc	S_{dp}	23	37	63	123	3:1	0.795	0.000
					S_{wp}	20	8	10	38			
30	S_{dp}	S_{dp}	Cc	Cc	S_{dp}	2	2	50	53	3:1	0.706	0.457
					S_{wp}	0	0	20	20			
Total	–	–	–	–	S_{dp}	109	80	279	468	–	–	–
					S_{wp}	57	40	56	153			

[a] S_{wp}: Shell color with purple pigmentation; S_{dp}: Shell color devoid of purple pigmentation.
[b] Locus C: Locus which controlling the shell purple striped pigmentation; C: the allele for S_{dp}; c: the allele for S_{wp}.

responsible for the appearance of the shell bands and another gene is responsible for the number of forming bands. Similarly, we suggested a two-locus hypothesis which the shell pigmentation is controlled by two major genetic locus, with one is responsible for the secretion of pigmentation and the other one is responsible for the distribution mode of pigmentation.

The inheritance of the secretion of pigmentation was easily explained by the segregations of different crosses. However, the distribution mode could not be directly concluded due to the absence of pigmentation in white color shell. Nonetheless, we could infer the genotypes of parents according to the selection process of two lines. In consideration of the selection effects on the shell color in both black shell color line and white shell color line, it is suggested that the solid allele frequency was increased in black shell color and the striped allele frequency was increased in white shell color line. It was easy to take out the oysters with not completely pigmented in the process of selection on black shell color. The oysters with tiny striped pigmentation were difficult to be eliminated in the process of selection on white shell color, because shell color was influenced by algae attachments and environment factors, such as lights, salinity and substratum (Heath, 1975; Lindberg and Pearse, 1990; Sokolova and Berger, 2000). In contrast, the oysters with solid pigmentation were simply to be removed in the selection of white shell color, resulting in the decrease of the solid allele frequency in white shell color line. This is what indirectly causes the increase of striped allele frequency in white shell color line, suggesting a large proportion of oysters with striped allele in white shell color line.

This model is a good explanation for the observations in our study, however there are still many questions need to be further explored, such as the variety of pigmented strips, the darkness of pigmented strips and so on. In addition, our study was based on progenies from lines selected for several generations. The artificial selection may lead to the loss of polymorphism at other loci besides the two revealed in this study. The genetics of shell pigmentation in the Pacific oysters might be more complex than our assumption. So, further verification experiments need to be designed.

In conclusion, our results demonstrated that the shell pigmentation is controlled by two genetic locus, with one responsible for the secretion of pigment and the other one responsible for the distribution mode of pigment. Furthermore, the results revealed that the allele for presence of pigmentation is dominant to the allele for absence of pigmentation, and the allele for stripped distribution of pigmentation is dominant to the allele for solid distribution of pigmentation. Additionally, a new locus was suggested to control the presence of purple pigmentation in shell surface, and the shell without purple pigmentation is dominant to shell with purple pigmentation. Information obtained in this study will be useful for the selective breeding of the oyster lines with desired shell coloration.

Acknowledgments

This study was supported by grants from National Natural Science Foundation of China (31772843), Shandong Province (2017LZGC009), the Fundamental Research Funds for the Central Universities (201762014), and Taishan Scholars Seed Project of Shandong.

References

Adamkewicz, L., Castagna, M., 1988. Genetics of shell color and pattern in the bay scallop *Argopecten irradians*. J. Hered. 79, 14–17.

Brake, J., Evans, F., Langdon, C., 2004. Evidence for genetic control of pigmentation of shell and mantle edge in selected families of Pacific oysters, *Crassostrea gigas*. Aquaculture 229, 89–98.

Evans, S., Camara, M.D., Langdon, C.J., 2009. Heritability of shell pigmentation in the Pacific oyster, *Crassostrea gigas*. Aquaculture 286, 211–216.

Feng, D., Li, Q., Yu, H., Kong, L., Du, S., 2018. Transcriptional profiling of long non-coding RNAs in mantle of *Crassostrea gigas* and their association with shell pigmentation. Sci. Rep. 8 (1), 1436.

Feng, D., Li, Q., Yu, H., 2019. RNA interference by ingested dsRNA-expressing bacteria to study shell biosynthesis and pigmentation in *Crassostrea gigas*. Mar. Biotechnol. 1–11.

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.

Ge, J., Li, Q., Yu, H., Kong, L., 2015. Mendelian inheritance of golden shell color in the Pacific oyster Crassostrea gigas. Aquaculture 441, 21–24.

Heath, D., 1975. Colour, sunlight and internal temperatures in the land-snail Cepaea nemoralis (L.). Oecologia 19, 29–38.

Hedgecock, D., Grupe, P., Voigt, M., 2006. Mapping genes affecting shell color and shape in the Pacific oyster, *Crassostrea gigas*. J. Shellfish Res. 25, 738.

Innes, D.J., Haley, L.E., 1977. Inheritance of a shell-color polymorphism in the mussel. J. Hered. 68, 203–204.

Kang, J.H., Kang, H.S., Lee, J.M., An, C.M., Kim, S.Y., Lee, Y.M., Kim, J.J., 2013. Characterizations of shell and mantle edge pigmentation of a Pacific oyster, *Crassostrea gigas*, in Korean Peninsula. Asian Aust. J. Anim. 26, 1659.

Kozminsky, E., 2016. Inheritance of longitudinal white shell bands in the snail *Littorina obtusata* (Gastropoda, Prosobranchia). Russ. J. Genet. 52, 882–886.

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.

Nell, J.A., 2001. The history of oyster farming in Australia. Mar. Fish. Rev. 63, 14–25.

Peignon, J.M., Gerard, A., Naciri, Y., Ledu, C., Phelipot, P., 1995. Analysis of shell colour determinism in the manila clam *Ruditapes philippinarum*. Aquat. Living Resour. 8, 181–189.

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.

Takeo, I., Sakai, S., 1961. Study of breeding of Japanese oyster, *Crassostrea gigas*. Tohoku J. Agric. Res. 12, 125–171.

Wan, S., Li, Q., Liu, T., Yu, H., Kong, L., 2017. Heritability estimates for shell color-related traits in the golden shell strain of Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. Aquaculture 476, 65–71.

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.

Xing, D., Li, Q., Kong, L., Yu, H., 2018. Heritability estimate for mantle edge pigmentation and correlation with shell pigmentation in the white-shell strain of Pacific oyster, *Crassostrea gigas*. Aquaculture 482, 73–77.

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.

Zheng, H., Zhang, T., Sun, Z., Liu, W., Liu, H., 2013. Inheritance of shell colours in the noble scallop *Chlamys nobilis* (Bivalve: Pectinidae). Aquac. Res. 44, 1229–1235.