Accepted Manuscript

Mendelian inheritance of golden shell color in the Pacific oyster *Crassostrea gigas*

Jianlong Ge, Qi Li, Hong Yu, Lingfeng Kong

Mendelian inheritance of golden shell color in the Pacific oyster *Crassostrea gigas*

Jianlong Ge, Qi Li *, Hong Yu, Lingfeng Kong

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

* Corresponding author
Tel and fax: +86-532-82031622
E-mail address: qili66@ouc.edu.cn

ABSTRACT

Shell coloration in many molluscs is highly variable. A shell color segregation study with progenies from a full factorial cross generated among Pacific oysters exhibiting distinct shell colors (golden, white and black) was conducted to investigate the inheritance of the golden shell color and its correlation with dark pigmentation. Random samples from twenty-three full-sib families were obtained and the shell coloration of offspring within each family was recorded. Results revealed that golden coloration was inherited in a different pattern from dark pigmentation, indicating its different genetic basis. Dark pigmentation was identified as a foreground color while golden or white color were background ones. The locus controlling background colors has two alleles with the allele for golden background being dominant to the allele for white background. In addition, the overlying foreground pigmentation of shells with a golden background was significantly lighter than that of shells with a white background, which suggested an epistatic effect of background color on shell foreground pigmentation. All these findings will facilitate the selection of elite oyster lines with desired shell coloration for aquaculture.

Keywords: Pacific oyster; *Crassostrea gigas*; Shell color; Inheritance; epistatic

1. Introduction

Molluscs are a large group of invertebrate animals presenting variable shell shapes and colorations, which have attracted many naturalists and collectors. Moreover, consumers tend to choose live seafood in the market according to their colors, which endows the coloration with product value of great importance (Alfnes et al., 2006). Pacific oyster (*Crassostrea gigas*) has the largest production among all cultured aquatic animals（FAO，2011）and its coloration is of interest to the whole oyster industry (Brake, et al., 2004; Kang, et al., 2013). The oysters with golden shell coloration are rarely seen in the market and are sold at much higher prices than others (Nell, 2001). In our selective breeding practice of Pacific oyster, we obtained a number of golden shell variants, and established a set of full-sib families using these

2

golden shell oysters as parents (Cong et al., 2014). These provided great materials to unravel the genetic basis of the golden coloration that remains unknown at present.

Mollusca shell color is known to be inheritable and affected by environmental factors, such as lights, salinity and substratum (Heath, 1975; Lindberg and Pearse, 1990; Sokolova and Berger, 2000). Several studies investigating the genetic basis of shell coloration with limited data from experimental crosses reported one or two loci with dominance to control shell coloration (Cole, 1975; Innes and Haley, 1977; Adamkewicz and Castagna, 1988; Wada and Komaru, 1990; Winkler, et al., 2001; Kobayashi, et al., 2004; Liu, et al., 2009; Zheng et al., 2012).

Pacific oyster shell pigmentation exhibits a continuous variation from near-white, pigment-free shells to near-black, fully pigmented shells, which was defined as foreground pigmentation. The shell pigmentation has been considered as a quantitative trait that is controlled by many genes with small-effects (Imai and Sakai, 1961; Brake et al., 2004; Evans et al., 2009). However, in some cases, only a small amount of major genes were identified to control shell coloration (Hedgecock et al., 2006; Evans et al., 2009). Our recent study also identified a SCAR marker which was well correlated with Pacific oyster shell pigmentation of black and white, suggesting a major locus that is responsible for foreground pigmentation (Ge et al., 2014). However, the genetic basis and mechanism of shell coloration in Pacific oyster is still inconclusive, especially for specific colors, such as golden coloration, which is clearly different from those dark pigmentation phenotypically. Does golden coloration possess a same genetic basis as the black pigmentation? If not, what are the genetic loci controlling golden coloration and is there any correlation between golden coloration and typical dark pigmentation?

In the present study, we performed controlled crosses between oysters showing different shell colors to determine the inheritance of golden coloration in *C. gigas* and to examine the relationship between golden coloration and typical dark pigmentation, which will facilitate the selection of elite oyster lines with desired coloration patterns.

2. Materials and methods

2.1 Parental source

One-year-old oysters with desired shell coloration were selected from nine full-sib families to conduct cross mating. All families were the first-generation selective families and were produced using wild oysters with specific shell colors in Rushan, China (Cong et al., 2014). Oysters with three shell coloration patterns were used as parents in this study (Fig. 1), including those with golden shell (*G*) and two extreme foreground pigmentation patterns of solid white shell (*W*) and solid black shell (*B*).

2.2 Mating experiment and rearing

A full factorial cross among oysters with the three shell color patterns was performed to generate 27 families with 9 cross-mating groups and three replicates. To guarantee the survival of offspring, inbreeding between siblings was avoided.

Gametes of oysters with each color were rinsed into separate buckets by stripping the gonad. A suspension of eggs from each dam was divided into three equal portions and fertilized by sperm from the three sires of each color, respectively. Fertilized eggs of all cross groups were hatched at temperature of 24°C and salinity of 30 psu. After 24-h incubation, D-stage larvae of each family were collected and transferred into a 100-L plastic bucket with an initial stocking density of 8 larvae/mL. The larvae stocking density was decreased along with larval growth, and half volume of water was replaced with fresh water twice per day. Veligers were fed with daily rations of *Isochrysis galbana* at early stage (shell length <120 μm), and were fed with *Platymonas helgolandica* and *Chaetoceros calcitrans* at later stage. When oyster eye spots appeared, strings of scallop shells were placed into the bucket for larvae to attach. Within one week, all eyed larvae metamorphosed to spat and then were transferred to an outdoor nursery tank. All spats were spread into lantern nets after 40 days and then deployed to grow-out areas in Rushan, China.

4

2.3 Shell coloration record

Offspring samples were collected randomly from all families at 410 days post-fertilization in summer 2012. All sampled oysters were shucked, with shells washed and brushed thoroughly with freshwater. The shell background color was recorded as golden or not (i.e., white), while the foreground pigmentation was recorded as five categories with five index numbers, according to Imai and Sakai (1961). The categories were described as follow:

   0—Whole surface has no dark coloration;

   1—A small part of shell is dark colored;

   2—Nearly half part of shell is dark colored;

   3—Most part of shell is dark colored;

   4—All part is dark colored.

2.4 Statistical analysis

The proportions of shell background color in the progeny were analyzed with the assumption that the single locus-two-allele model is the major determinant for shell color. 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 foreground pigmentation and shell background color.

3. Results and discussion

Twenty-three full-sib families were obtained eventually, with four families lost during the grow-out stage. Several representative shell color morphs in the offspring were shown in Figure 2. It is clear that dark pigmentation present in the surface of both white and golden shells. Golden coloration tends to be distributed all over the shell, while dark pigmentation is only mottled some area or banded radially in the

5

surface (Fig. 2). All these observations support the hypothesis that golden coloration follow a different pattern from black pigmentation, which is named as the background color (golden or white) to distinguish the foreground dark pigmentation.

As shown in Table 1, the presence of golden background color was dominant over its absence (white). Each of the three golden families (families 1–3) produced both golden and white offspring in a ratio closely approaching 3:1. In contrast, none of the white background families (families 4–9, 19–23) produced golden offspring. Families 10–18, cross matings between golden oysters and different overlying pigmentation oysters, resulted in background color of offspring at ratios that were not different from 1:1. These observations clearly support the hypothesis that the two background colors are controlled by one locus, with one allele for golden background being dominant to the other allele for white. This result is consistent with the observation of Nell (2001) that true breeding oysters with golden shell and mantle were obtained after a few generations of selection. Similar inheritance model has also been reported in the bay scallop (*Argopecten irradians*) that yellow background color versus its absence appears to be inherited as a single gene with absence (white) being recessive (Adamkewicz and Castagna, 1988).

Independence tests indicated that shell foreground pigmentation was significantly related to shell background color in families 1–3 and 10-18, except families 10 and 16 (Table 2). In general, there were more golden offspring with lighter foreground pigmentation (pigmentation index: 0 and 1), whilst more white offspring with darker foreground pigmentation (pigmentation index: 2-4; Table 2). These all point to a tentative hypothesis that the background locus may be epistatic to one or more pigmentation genes which only are permitted to express when the allele is for white background. This has also been reported in the bay scallop as all the offspring with white background color had dark mottling on their shells, whereas none of the orange offspring followed this pattern in that study (Adamkewicz and Castagna, 1988).

In conclusion, our results demonstrated that the shell background color of Pacific oyster is controlled by one locus with two alleles and that the allele for golden background is dominant to the allele for white. Additionally, the oyster shell

background color may have an epistatic effect on its foreground pigmentation. These results suggested major implications for the selection of oyster lines with desired shell coloration.

Acknowledgements

References

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

Alfnes F., Guttormsen A.G., Steine G., Kolstad K., 2006. Consumers' willingness to pay for the color of salmon: a choice experiment with real economic incentives. Am. J. Agr. Econ. 88, 1050-1061.

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.

Cole T.J., 1975. Inheritance of juvenile shell colour of the oyster drill *Urosalpinx cinerea*. Nature 257, 794-795.

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*). Journal of Fishery Sciences of China (in Chinese) 21: 494-502.

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

FAO (Food and Agriculture Organization), 2011. Fishery and aquaculture statistics 2009. Food and Agriculture Organization of the United Nations, Rome.

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.

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.

Imai T., Sakai S., 1961. Study of breeding of Japanese oyster, *Crassostrea gigas*. Tohoku J. Agric. Res. 12, 125-171.

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., et al., 2013. Characterizations of shell and mantle edge pigmentation of a Pacific oyster, *Crassostrea gigas,* in Korean Peninsula. Asian Austral. J. Anim. 26, 1659-1664.

Kobayashi T., Kawahara I., Hasekura O., Kijima A., 2004. Genetic control of bluish shell color variation in the Pacific abalone, *Haliotis discus hannai*. J. Shellfish. Res. 23, 1153-1156.

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., Zhao H., Zhang G., Guo X., 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.

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

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.

Wada K., Komaru A., 1990. Inheritance of white coloration of the prismatic layer of shells in the Japanese pearl oyster *Pinctada fucata martensii* and its importance in the pearl culture industry. Bull. Jap. Soc. Sci. Fish. 56, 1787-1790.

Winkler F., Estevez B., Jollan L., Garrido J., 2001. Inheritance of the general shell

color in the scallop *Argopecten purpuratus* (Bivalvia: Pectinidae). J. Hered. 92, 521-525.

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.

Figure legends

Fig. 1 Representative parents of three shell coloration patterns. G = Golden; W = White; B = Black.

Fig. 2 Representative shell color morphs of offspring. (A) Offspring with white background and different foreground pigmentation (pigmentation index: 0-4); (B) Offspring with golden background and different foreground pigmentation (pigmentation index: 0-3).

Fig. 1

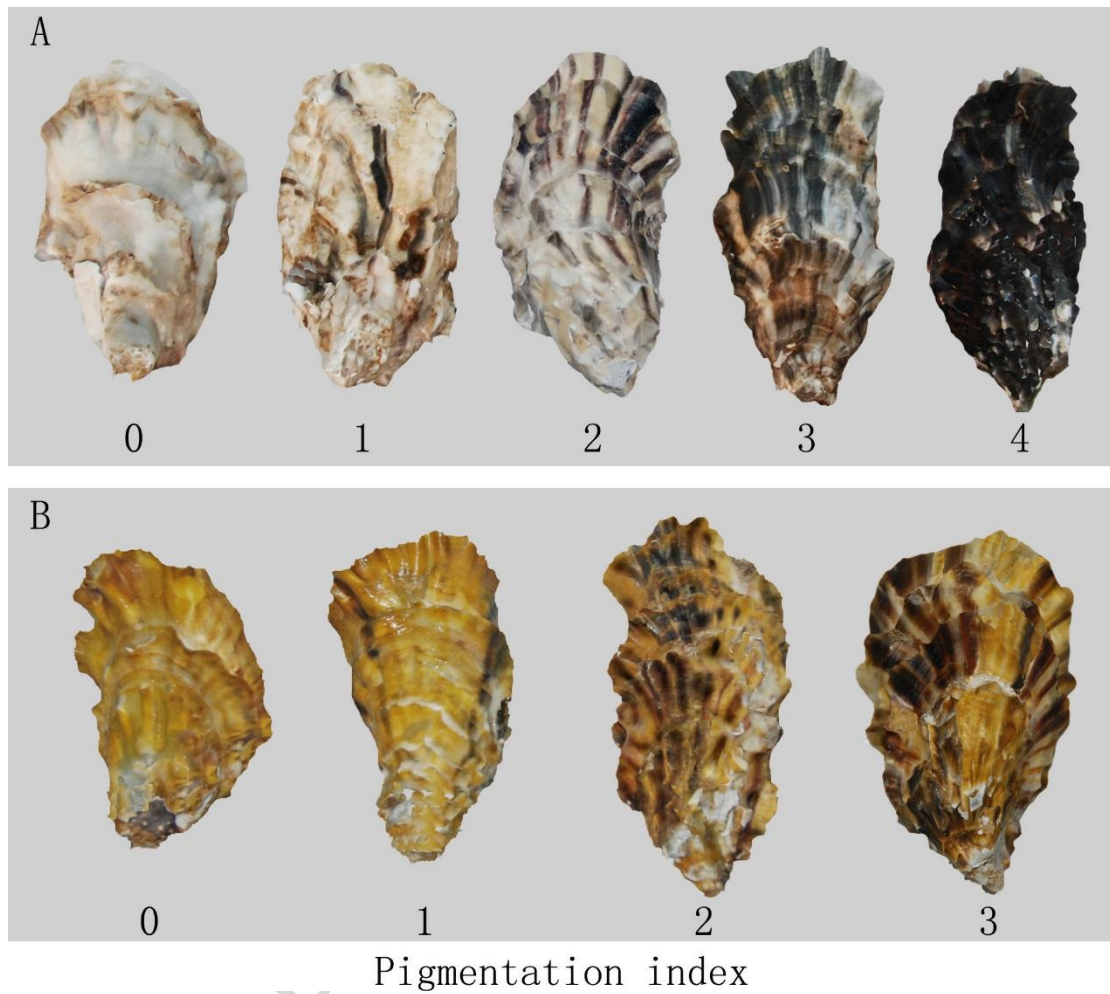G   W   B

Fig. 2

Pigmentation index
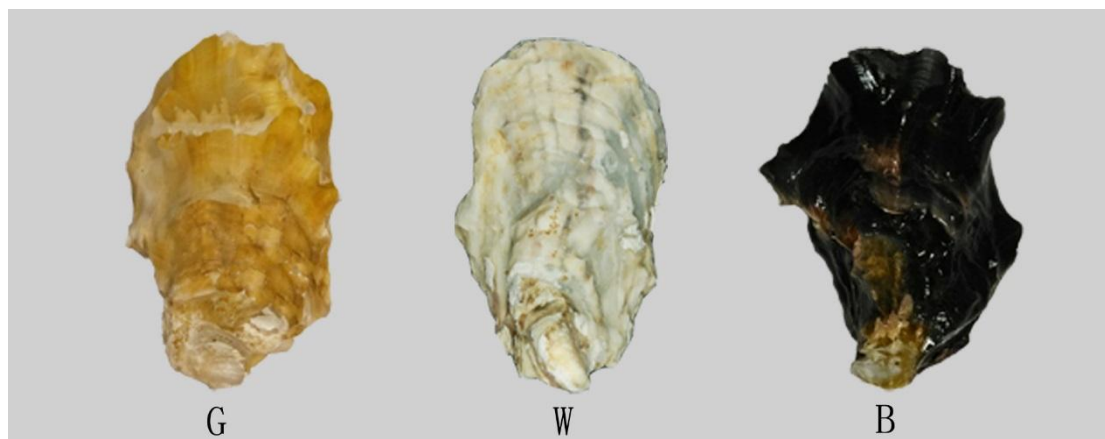
Table 1

Shell background color of the offspring from 23 crosses among three shell color patterns of *C. gigas*.

Family no.	Parents[a]		Background color of offspring		Total	Expected ratio	χ^2 (*P* value)
	Female	Male	Golden	White			
1	*G1*	*G4*	42	16	58	3:1	0.649
2	*G2*	*G5*	92	30	122	3:1	0.917
3	*G3*	*G6*	51	20	71	3:1	0.537
4	*W1*	*W4*	0	45	45	0:1	*NA*
5	*W2*	*W5*	0	44	44	0:1	*NA*
6	*W3*	*W6*	0	70	70	0:1	*NA*
7	*B1*	*B4*	0	53	53	0:1	*NA*
8	*B2*	*B5*	0	45	45	0:1	*NA*
9	*B3*	*B6*	0	38	38	0:1	*NA*
10	*G1*	*W4*	61	49	110	1:1	0.253
11	*W1*	*G4*	35	34	69	1:1	0.904
12	*G3*	*W6*	29	28	57	1:1	0.895
13	*W3*	*G6*	19	20	39	1:1	0.873
14	*G1*	*B4*	26	28	54	1:1	0.700
15	*G2*	*B5*	25	23	48	1:1	0.773
16	*B2*	*G5*	26	34	60	1:1	0.302
17	*G3*	*B6*	33	30	63	1:1	0.705
18	*B3*	*G6*	46	42	88	1:1	0.670
19	*W1*	*B4*	0	61	61	0:1	*NA*
20	*B1*	*W4*	0	60	60	0:1	*NA*
21	*W2*	*B5*	0	58	58	0:1	*NA*
22	*B2*	*W5*	0	68	68	0:1	*NA*
23	*W3*	*B6*	0	39	39	0:1	*NA*

[a] *G* Golden shell; *W* White shell; *B* Black shell.

Table 2

Independence tests of the association of shell foreground pigmentation (pigmentation index: 0-4) and background color (ie. golden and white) within 12 families.

Family no.	Parents ♀	♂	Background color type	Foreground pigmentation 0	1	2	3	4	Total	*P* value†
1	*G1*	*G4*	Golden	9	17	8	8	0	42	0.001
			White	0	2	12	2	0	16	
2	*G2*	*G5*	Golden	38	52	2	0	0	92	0.000
			White	5	14	9	2	0	30	
3	*G3*	*G6*	Golden	21	21	7	2	0	51	0.000
			White	1	6	6	6	1	20	
10	*G1*	*W4*	Golden	26	31	4	0	0	61	0.067
			White	12	28	7	2	0	49	
11	*W1*	*G4*	Golden	17	11	7	0	0	35	0.005
			White	5	13	12	4	0	34	
12	*G3*	*W6*	Golden	9	10	10	0	0	29	0.011
			White	6	5	9	8	0	28	
13	*W3*	*G6*	Golden	6	9	4	0	0	19	0.000
			White	1	2	12	5	0	20	
14	*G1*	*B4*	Golden	0	4	10	12	0	26	0.001
			White	0	0	6	13	9	28	
15	*G2*	*B5*	Golden	2	6	14	3	0	25	0.000
			White	0	5	3	7	8	23	
16	*B2*	*G5*	Golden	3	8	11	4	0	26	0.250
			White	0	10	14	8	2	34	
17	*G3*	*B6*	Golden	1	12	15	5	0	33	0.000
			White	0	1	8	10	12	31	
18	*B3*	*G6*	Golden	2	22	17	5	0	46	0.000
			White	0	1	22	13	6	42	
Total			Golden	134	203	109	39	0	485	0.000‡
			White	30	87	120	80	38	355	

† *P* values were calculated with the use of Fisher's exact test.

‡ The *P* value was based on Chi-squared test.

Highlights

1. One-locus-two-allele inheritance of shell background coloration was demonstrated.

2. The background allele for golden coloration is dominant to the other allele for white.

3. Epistatic effect of background color on foreground pigmentation was found.