Short communication

Mendelian inheritance of golden shell color in the Pacific oyster

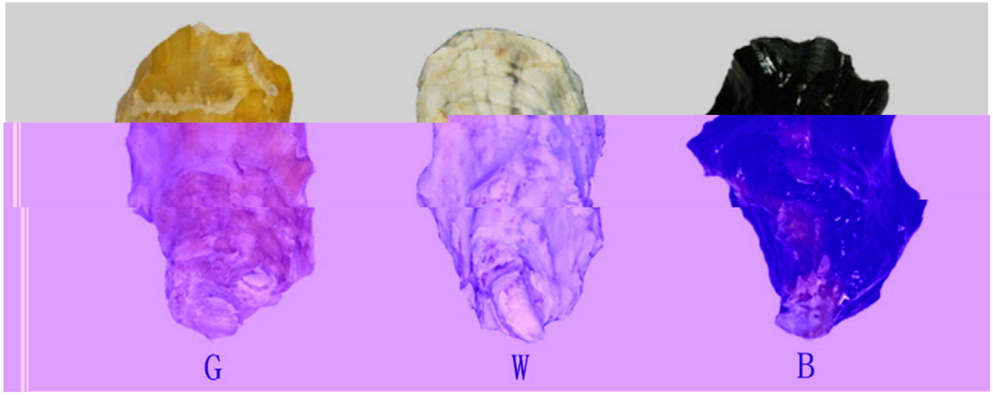

Fig. 1. Representative parents of three shell coloration patterns. G = Golden; W = White; B = Black.

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

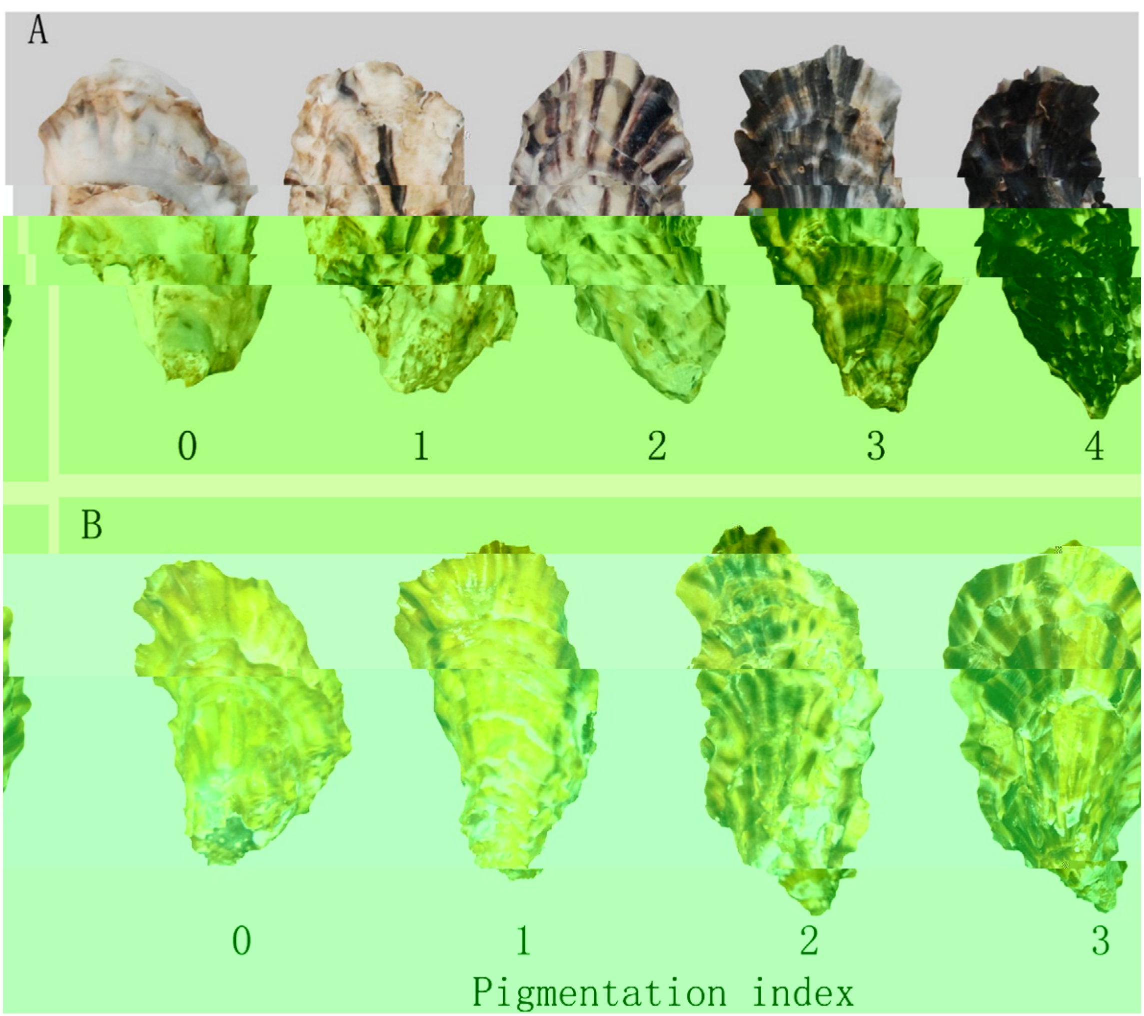

Fig. 2. Representative shell color morphs of offspring. (A) Offspring with white background and different foreground pigmentation (pigmentation index: 0–4); (B) offspring with golden background and different foreground pigmentation (pigmentation index: 0–3).

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				
	Female	Male	Golden	White	Total	Expected ratio	χ^2 (*P* value)
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.

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.

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.

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					Total	*P* value[†]
	♀	♂		0	1	2	3	4		
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.

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 Fig. 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 surface (Fig. 2). All these observations support the hypothesis that golden coloration follows 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 locu9ntr.g()23(as,-1.316TD(w)15(e)15(r)))-25the erarle4(de)25(nhy)28(p)15227whide -2
t-257(go)15(lio)14(y))-262(hy)28(pk)17(gyh)22(esc-227)-271()25(noph)220o(121Tf3.1980Td08(Fi)-19(Au)20(go)13(p-261(ct262(hnho)24(i-248(r)-2(f)161(d