Aquaculture Reports 9 (2018) 62–67

Contents lists available at ScienceDirect

Aquaculture Reports

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

Impact of spat shell colour selection in hatchery-produced *Pinctada margaritifera* on cultured pearl colour

Chin-Long Ky*, Manaari Sham Koua, Gilles Le Moullac

Ifremer, UMR 241, EIO, Labex Corail, Centre du Pacifique, BP 49, 98719 Taravao, Tahiti, French Polynesia

ARTICLE INFO

Keywords:
Pinctada margaritifera
Phenotypes
Spat shell colour
Earlier selection
Pearl colour

ABSTRACT

Beaded cultured pearl farming is a lengthy aquaculture process, particularly when the pearl oysters are produced through a hatchery propagation system, and includes the key steps of artificial breeding, larval and spat rearing before graft operations can take place. Within its genus, *Pinctada margaritifera* has the ability to produce the widest range of pearl colours, thanks to the donor colour polymorphism of the inner shell, which is mainly responsible for colour transmission. As hatchery spat production in *P. margaritifera* leads to several colour phenotypes (at 3 months old), the aim of this study was to determine whether a relation exists between the colour of the donors as spat and the final pearl colour. In the experiment, which took place over a four-year period, earlier spat colour selection was applied to two hatchery-produced *P. margaritifera* families. The spat were traced and then used as donors at the adult stage. A total of 1100 experimental grafts were made, using originally grey, green, red and yellow spat phenotypes as donors. The results showed that all spat colour phenotypes mainly produced pearls in the moderately dark (78.4%) and grey colour (56.7%) classes. Differences in darkness level were produced by red and yellow spat, whose pearls were about twice as pale as those from the grey and green phenotypes. Concerning the pearl colour categories, the results showed that the attractive green/blue pearls were obtained twice as often when using grey and green spat phenotypes and that aubergine/peacock pearls were obtained four times more often by using the red and yellow spat phenotypes. This preliminary study suggests that earlier phenotypic colour selection could be applied in *P. margaritifera* spat as a useful indicator in both pearl production cycles and family selection for donor oyster lines of specific colour propagation.

1. Introduction

The black-lip pearl oyster, *Pinctada margaritifera* (Pteriidae), is a marine bivalve mollusc that has a broad Indo-Pacific distribution, and is highly valued for cultured pearl and pearl shell production (Southgate et al., 2008; Wada and Tëmkin, 2008). Aquaculture of this species represents a valuable industry and an important source of coastal community livelihood across almost the entire extent of its distribution. In French Polynesia, the culture of *P. margaritifera* is the most important industry in Polynesian mariculture and the second highest source of income, after tourism. It produces the highest volume of black pearls in the international market. Currently, *P. margaritifera* aquaculture involves 536 pearl farms, distributed on 26 islands and atolls, covering 7800 ha of marine exploitation and concentrated in Tuamotu, Gambier and Society archipelagos (Talvard, 2015). Grafting for cultured pearl production involves insertion of a piece of mantle tissue (*saibo*) from a donor pearl oyster and a round inorganic nucleus into the gonad of a host pearl oyster (Gervis and Sims, 1992; Taylor and Strack, 2008; Cochennec-Laureau et al., 2010; Gueguen et al., 2013).

The donor pearl oyster has been shown to have a major influence on the quality of the resulting pearls (Alagarswami, 1987), using both individual wild donors (Tayale et al., 2012) and hatchery-bred families (Ky et al., 2013) in *P. margaritifera*. Xenograft studies have reported traces of DNA from *saibo* tissue in the pearl-sac during pearl formation, further confirming the influence of donor oyster tissue on pearl formation (McGinty et al., 2010, 2011). *P. margaritifera* can produce a wider range of pearl colours, from the purest white to the deepest black, including numerous shades of silver, peacock, green, aubergine, purple, golden brown and even rainbow colours, than the two other pearl oyster species, *P. fucata martenseii* (pink, white or silver, cream and yellow pearls) and *P. maxima* (golden, silver-white, yellow or cream pearls) (Tong and Shen, 2001; Taylor and Strack, 2002). In fact, *P. margaritifera* pearls can have an overtone colour (secondary colour such as green, blue, aubergine and peacock), which is considered to increase their value (Karampelas et al., 2011). Recent studies in *P. margaritifera* have shown that pearl colour depends on both the outer (Ky et al., 2017a)

* Corresponding author.
E-mail address: chinky@ifremer.fr (C.-L. Ky).

https://doi.org/10.1016/j.aqrep.2017.12.002
Received 13 June 2017; Received in revised form 21 September 2017; Accepted 17 December 2017

and inner shell colours of the donors at the adult stage (Ky et al., 2017b). A large amount of evidence has suggested that *P. margaritifera* shell colour is under genetic control (Ky et al., 2016a). Thus, shell colour polymorphisms are amenable to artificial selection.

P. margaritifera aquaculture in French Polynesia is almost exclusively dependent on wild oysters, obtained by natural spat collection from wild stocks, mainly from the atolls of Ahe, Takapoto, Takume, Katiu in the Tuamotu Archipelago, and from the lagoons of the Gambier Archipelago. Spat are collected during the reproductive season in these lagoons, where they settle on artificial collectors and are left for up to 6 months for pre-growing. This relative abundance of wild oysters in French Polynesia means that the use of hatchery-produced spat in conjunction with selective breeding has not been developed at a commercial scale, in contrast to Japan, Australia and south-east Asia, where these strategies are used for the other pearl oyster species, *Pinctada fucata* and *Pinctada maxima*, to meet both: 1) the needs of resource production and 2) the development of breeding programs. Colour manipulation through selection in aquaculture has been performed for many species (Lutz, 2001). One example of increasing market demand in the pearl industry is the case of the South Sea pearl, produced by *P. maxima* in the Philippines, which typically produce deep golden pearls through decades of breeding efforts that resulted in a selected gold inner shell strain.

Hatchery-bred *P. margaritifera* display several spat outer shell colourations, among which grey, green, red and yellow are commonly observed. As cultured pearl production is a long process, one production cycle from hatchery to harvest time takes a minimum of four years investment. The objective of the present study was therefore to provide initial information about the potential relation between spat shell colouration (early donor selection) and the final cultured pearl colour, without deliberately checking the colour phenotype of donor oyster at grafting time (late donor selection). If such a colour relation exists, it would be then possible to select donors early, at the spat stage, for the pearl colours demanded by the international market. Grey is clearly a non targeted colour for cultured pearls compared with the more attractive green or peacock overtones. The results will facilitate future selection of black-lipped pearl oyster lines with a defined phenotype for production of specific colours. To respond to this objective, a four-year small scale experiment was conducted, including breeding, rearing, grafting, culture and pearl colour grading (darkness level and colour categories) using randomly selected spat representative of each shell colour as donors, with complete traceability throughout the entire cycle.

2. Materials and methods

2.1. Experimental animals

Two bi-parental families of *P. margaritifera* were produced in the Ifremer hatchery facilities in Vairao (Tahiti, French Polynesia) in 2006 (family 616) and 2009 (family 906) from wild broodstock. These two full-sib families were selected for the experimental design: 1) in a way to reduce the genetic base of the tested individual, *i.e.* making more comparable the phenotypic variation observed, and 2) because among all the families produced, these two exhibited the largest spat shell colour diversity. The parents used for these two crosses originated from Takapoto atoll and were not selected according to their outer or inner shell colouration. Artificial spawning, larval rearing and oyster culture were performed as described in Hui et al. (2011) and Ky et al. (2013). At the age of 80 days post-hatching, spat from the two families were sorted according to their shell colour: red, grey, green or yellow (except in family 906, where no yellow phenotype was found) as shown in Fig. 1. Colour determination was done by two technicians working in cooperation. Each colour phenotype in each family was traced over the entire culture period using plastic labels. Individuals of the two families with representative spat colour phenotypes were randomly selected to be used as donor oysters and transferred by air to Rangiroa atoll (Tuamotu Archipelago) 2 months prior to nucleus implantation to allow the oysters to adapt to local environmental conditions.

Wild *P. margaritifera* were collected as spat in the lagoon of Ahe atoll (Tuamotu Archipelago, French Polynesia) to serve as recipients. Passive techniques were employed for catching spat using commercial spat collectors made from modern synthetic materials, to which planktonic mollusc larvae become attached fifteen to twenty days after their release (Thomas et al., 2012). After nearly one year of subsurface rearing (3–5 m below the surface), the young pearl oysters (4–5 cm in diameter) were then transferred to Rangiroa atoll (where the grafting was performed) and removed from the collectors on which they had developed. These juveniles were pierced and tied together onto a CTN (Cord Technical Nakasai) rearing system, where they were left until grafting. This rearing method involves drilling a small hole through the base of the shell in the dorsal-posterior region. This process does not affect the living tissue. The CTN were protected using plastic mesh to prevent predation in the lagoon. At six months post transfer, the pearl oysters were removed from the water and washed with a high pressure spray, thus removing any parasites (mainly epibionts). This process causes no injury or mortality and maximizes growth rate. Mature oysters, aged approximately 30 months and measuring at least 8 cm in length, were taken from the rearing station, detached and stored ready to be used in the grafting procedure.

2.2. Experimental graft

The experimental graft was performed on Rangiroa atoll in October 2011 (Gauguin's Pearl Farm, Tuamotu Archipelago). Consequently, at the time of graft, donor oysters were 5 years old and 2 years old (family 616 and family 906, respectively). From family 616, a total of 25 donor oysters were randomly selected with "Grey" (N = 7), "Red" (N = 7), "Yellow" (N = 7) and "Green" (N = 4) spat shell colour phenotypes (Table 1). From family 906, 30 donor oysters were randomly selected and used with "Grey", "Red" and "Green" spat shell colour phenotypes (N = 10 for each phenotype; Table 1). The donor pearl oysters (total N = 55) were individually labelled (to trace their family and spat shell colour origins) and randomly attributed to two professional grafters who performed the grafting operations over two days (Table 1). The epithelial cells required for the grafting procedure were excised from the mantle by the expert grafters. Small squares of epithelium ("grafts") measuring approximately 4 mm^2 (N = 20 per donor oyster) were then prepared, before being transplanted into the recipient oysters (issued from a single batch of healthy oysters). The graft process consists of cutting out a hollow in the recipient oyster gonad, into which the grafter places both the nucleus and the graft. The nuclei used were similar and imported from Japan: 2.4 BU nucleus (7.304 mm diameter, 0.59 g weight – Nucleus Bio, Hyakusyo Co., Japan). The whole grafting operation takes approximately 1 min (Ky et al., 2015). The donors were used successively to provide the 20 grafts each, with which the grafts were performed before moving on to the next donor. A total of 1100 grafts were realised over a 2-day period. Traceability of donor oysters was maintained by using numbered plastic labels attached to the chaplets, where the corresponding recipient oysters were reared (Ky et al., 2014a,b). After 18 months of culture (April 2013), the cultured pearls were harvested and assessed for their colour.

2.3. Cultured pearl colour

Cultured pearls were cleaned by ultrasonication in soapy water (hand washing) with a LEO 801 laboratory cleaner (2-L capacity, 80 W, 46 kHz). They were then rinsed in distilled water. Colour evaluation (without a jeweller's loupe) was done on the cultured pearls, according to Ky et al. (2013) by two operators working in cooperation, who classed the pearls according to:

Fig. 1. Colour diversity of hatchery-reared *Pinctada margaritifera* at spat stage (80 days post-hatching) with four different shell colour phenotypes.

Table 2
Impact of donor oyster spat phenotypes ("Red", "Grey", "Yellow" and "Green" shell colouration) on cultured pearl colour (darkness level and colour categories) in *P. margaritifera*. Data are expressed as percentages with frequencies in brackets. Values that are significantly different ($p < 0.05$) between the spat shell phenotypes are indicated with letters. Significance is expressed as follows: NS (non-significant results with $p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$) and *** ($p < 0.001$).

		SPAT SHELL COLOUR				
Cultured pearls		Red	Grey	Yellow	Green	Significance
DARKNESS LEVEL	Light	17.7 ^{a} (41)	8.3 ^{b} (18)	17.8 ^{a} (16)	10.1 ^{b} (22)	**
	Moderate	72.7 (168)	79.4 (173)	78.9 (71)	83.0 (181)	**NS**
	Dark	9.5 ^{ab} (22)	12.4 ^{a} (27)	3.3 ^{b} (3)	6.9 ^{ab} (15)	**
COLOUR CATEGORIES	Grey	53.2 (123)	53.7 (117)	66.7 (60)	53.2 (116)	**NS**
	Green/Blue	27.7 ^{b} (64)	40.8 ^{a} (89)	15.6 ^{c} (14)	34.4 ^{ab} (75)	***
	Aubergine/ Peacock	10.8 ^{a} (25)	2.8 ^{b} (6)	10.0 ^{a} (9)	2.3 ^{b} (5)	***
	White/ Yellow	8.2 ^{a} (19)	2.8 ^{b} (6)	7.8 ^{a} (7)	10.1 ^{a} (22)	*

- Darkness of overall colouration, with three levels: light, moderate (medium) and dark (high);
- Visually-perceived colour (due to pigments: bodycolor; and secondary colour: overtone). Four colour categories were established, allowing all the harvested pearls to be classified: 1) samples with dominance of bodycolors (without an overtone): grey and white/yellow, and 2) samples with additional secondary colours (moderate to distinct overtones): green/blue and aubergine/peacock.

2.4. Statistical analysis

All analysis were performed using R© version 3.4.0 software (R foundation for Statistical Computing). The significant threshold was set at $p \leq 0.05$ (Dagnelie, 2007). To determine differences in pearl colour and darkness, χ^2 tests for statistical independence were carried out (Siegel and Castellan, 1988; Winer et al., 1991). To detect differences between families 616 and 906, χ^2 tests of homogeneity were performed by pearl colour and by pearl darkness.

3. Results

3.1. Experimental graft and cultured pearl colour

Of the 1100 grafts, 68.8% led to harvested pearls (N = 757), among which 66.0% (N = 330) came from family 616 donors and 71.2% (N = 427) from family 906 donors. Furthermore, harvested pearl rates for the different spat shell colouration groups were 67.9% (N = 231) for red, 64.1% (N = 218) for grey, 64.3% (N = 90) for yellow and 77.9% (N = 218) for green, when both families (616 and 906) were considered together (Table 1). Neither family nor grafter was found to have a significant effect on harvested pearl rates ($p = 0.065$ and $p = 0.061$ respectively). However, the "green" spat shell colouration group showed a significantly higher harvested pearl rate ($p = 0.001$), compared to the other groups (red, grey and yellow).

The darkness level distribution of the harvested cultured pearls was: 12.8% (N = 97) of light, 78.3% (N = 593) of moderate and 8.9% (N = 67) of dark pearls. Concerning the visual colour categories, the distribution was as follows: 55.0% (N = 416) of grey colour; 32.0% (N = 242) of green and blue colours (*i.e.* multicolour group); 7.1% (N = 54) of white/yellow colour; and 5.9% (N = 45) of aubergine and peacock colours (*i.e.* multicolour group).

3.2. Effect of overall spat shell colouration on cultured pearl colour

Results showed a highly significant impact of spat shell colouration on harvested pearl darkness ($p = 0.003$). The spat shell phenotypes corresponding to "Yellow" and "Red" colours produced significantly ($p = 0.006$) more light-toned pearls (17.8%, N = 16 and 17.7%, N = 41 respectively) than the "Green" and "Grey" phenotypes (10.1%, N = 22 and 8.3%, N = 18 respectively). No significant difference ($p = 0.064$) was observed for the "moderate" darkness level of pearls among the four phenotypes: "Green" (83.0%, N = 181), "Grey" (79.4%, N = 173), "Yellow" (78.9%, N = 71) and "Red" (72.7%, N = 168). Dark harvested pearl frequencies were significantly different among the spar colour groups ($p = 0.047$), with three distinct groups from the highest rate to the lowest: 1) "Grey" (12.4%, N = 27); "Red" and "Green" (9.5%, N = 22 and 6.9%, N = 15 respectively) and 3) "Yellow" (3.3%, N = 3) (Table 2).

Regarding cultured pearl colour, a significant impact of spat shell colouration was observed ($p < 0.0001$), except for the grey pearls where no significant difference ($p = 0.129$) between the spat shell phenotypes was detected (Table 2). For the blue/green pearl colour, a highly significant difference ($p < 0.0001$) was observed between the 4 spat shell phenotypes. When donor oysters originated from "Grey" spat, the highest rate of green/blue pearls was harvested, and was 2.6 fold higher than when donors originated from the "Yellow" spat phenotype. An intermediate rate of green/blue pearls (average 31%) was observed for the "Red" and "Green" spat phenotypes (Table 2). In order to obtain the highest rate of aubergine/peacock pearls, the use of donors originating from "Red" and "Yellow" spat phenotypes was better than "Grey" and "Green" (4 times higher on average) (Table 2). White/yellow pearls were obtained more frequently by using "Green", "Red" and "Yellow" spat phenotypes, than "Grey" ones (3 times more on average) (Table 2).

3.3. Effect of spat shell colouration on cultured pearl colour at the family scale

For darkness level frequencies, a comparison between families 616 and 906 showed no significant difference ($p = 0.965$). For both families taken together, average rates of light, moderate, and dark pearls were respectively: 12.1%, 78.1% and 9.7%. For the three spat shell phenotypes ("Red", "Grey" and "Green"), the same results were observed: no significant difference was observed between the two families within each spat phenotype and for light, moderate and dark pearls (Fig. 2). A comparison between the rate of darkness levels obtained from the three spat phenotypes was consistent with the previous results (section 3.2.): 1) the "Red" phenotype produced more light toned pearls than the "Green" and the "Grey" phenotypes, 2) both "Green" and "Grey"

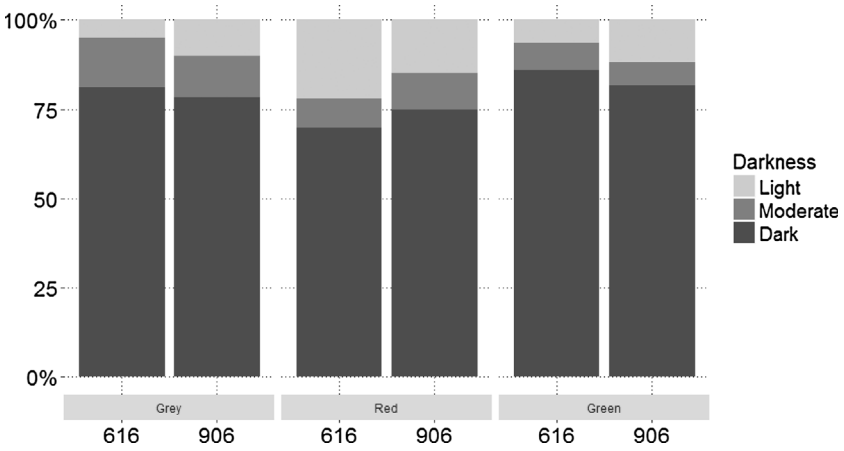

Fig. 2. Darkness level (light, moderate and dark) rate (in%) of cultured pearl from *P. margaritifera*, depending upon the spat shell colour phenotypes (Grey, Red and Green) for families 616 and 906. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phenotypes produced more moderate pearls than the "Red" phenotype and 3) the "Grey" phenotype produced more dark pearls than the "Green" and the "Red" phenotypes (Fig. 2).

For the cultured pearl colour categories, a comparison between families 616 and 906 showed highly significant differences ($p < 0.0001$) for: 1) green/blue pearls (11.2% and 47.1%, respectively); 2) grey pearls (68.7% and 44.7%, respectively) and 3) white/yellow pearls (12.5% and 4.0%, respectively). For aubergine/peacock pearls, no significant difference was observed between the two families, with an average frequency of 5.8%. A comparison of the frequencies of pearl colour categories obtained from the three spat phenotypes was consistent with the previous results obtained (section 3.2.). Grey pearls represented the majority of the samples harvested, especially for family 906, regardless of the colour of the original spat phenotypes (Fig. 3). Green/blue pearls were mostly produced by family 616, using the "Grey" spat phenotype (Fig. 3). The aubergine/peacock pearl category was mainly produced by family 616, using the "Red" spat phenotype (Fig. 3). Finally, the white/yellow pearls were obtained from the "Red" spat phenotype in both families (Fig. 3).

4. Discussion

Shell colour from the donor oyster in the *Pinctada* genus is known to have an effect on the colour of the cultured pearl, particularly in the case of *P. margaritifera.* A xenograft experiment, involving *P. maxima* and *P. margaritifera* species, demonstrated conclusively that the donor oyster is the primary determinant of pearl colour (McGinty et al., 2010). Correlations between the outer shell (prismatic layers) of donors in *P. margaritifera* and the colour proportions have also been observed in harvested pearls (Ky et al., 2015). Recently, Ky et al. (2017b) demonstrated the existence of a clear relation between the inner shell (nacreous layers) colour phenotype of the donor oyster and the colour of the pearl harvested in *P. margaritifera*. These studies investigated colour transmission between different donor phenotypes, visually selected and assessed when they were ready to be used in grafting (when the *saibo* could be cut out, *i.e.* around 2–3 years old), and the resulting pearls, but did not address earlier developmental stages. In hatchery production of large numbers of *P. margaritifera* juveniles, further phenotypic characteristics related to shell colouring have been observed. Depending on the contribution of the number of genitors and their origins, different frequencies of grey, red, yellow, green and white shelled spat can be observed. The present results clearly show that earlier spat colour selection could affect both the darkness and the colour of pearls, although all the spat phenotypes (except for white albinos) were capable of producing similar ratios (non-significant differences) of the moderate darkness level and grey pearls. For the production of darker pearls (targeting the Asian market), selection of grey, and to a lesser extent, red and green spat phenotypes, should be favoured over the yellow spat phenotype. In contrast, for the production of paler pearls (targeting the European market), selection of the red and yellow spat phenotypes would be better than the grey or green ones. An interesting point that supported the previous results obtained at the adult stage (ready to be grafted) (Ky et al., 2017b), is that all the considered spat phenotypes produced around 50% of the non-targeted grey pearl colour. This is a specificity of the so-called "black-lipped" pearl oyster species. The decrease in the number of grey pearls seems to be family dependent, as significant differences were observed between the same spat colour phenotype at the family scale. This family dependence was also observed for the other cultured pearl colours, where differences in frequencies were found for the same spat colour phenotype. Our results confirm that genetic selection of a particular donor oyster line can be performed at the family scale in order to produce a specific colour range (Ky et al., 2013).

The relatively few and simple colour phenotypes displayed at the spat stage in comparison to the numerous and more complex colour phenotypes observed at the adult stage, such as those reflected by the diversity of the monochromatic and polychromatic inner shell phenotypes selected as donors (Ky et al., 2017b), means that during their developmental growth (from spat to adult stage), each spat phenotype can lead to several shell colour phenotypes at the adult stage. The next challenge in understanding phenotypic evolution during the growth process of *P. margaritifera* might be to unravel the detailed relation between spat colour and final shell colour, and particularly the inner face polymorphism. The colour polymorphism observed in mass-produced hatchery progenies suggests that hereditary mechanisms control colour patterns, rather than environmental conditions. In natural systems, organisms face several ecological challenges and often respond with phenotypic shifts (Langerhans et al., 2007). Thus, the interaction between environment and genotype considerably influences the phenotype of the organisms. In the present study, as pearl oyster larvae were fed with the same cultured microalgae in a standardised hatchery system, variation in spat shell colour cannot be attributed to "environmental" variations such as differences in temperature and/or food availability (as these conditions were controlled). Genetic research on inheritance of colour variations concerning the outer shell in *P. margaritifera* was conducted to analyse colour segregation in juveniles produced under controlled conditions, with cross-fertilisation between black, red and white shell phenotypes (Ky et al., 2016a). The results clearly showed a three allele model, where the black wild-type allele is dominant to the red colouration, which is dominant to the white shell (Ky et al., 2016a). Crossing experiments has been previously done in *P. fucata* on different outer shell phenotype (prismatic layer colouration), and have shown relatively simple genetic bases for colour trait inheritance. In this species, the red type may be dominant over the yellow type, which is dominant over other common type (Wada, 1984). In addition, the white type is inherited under the control of recessive gene

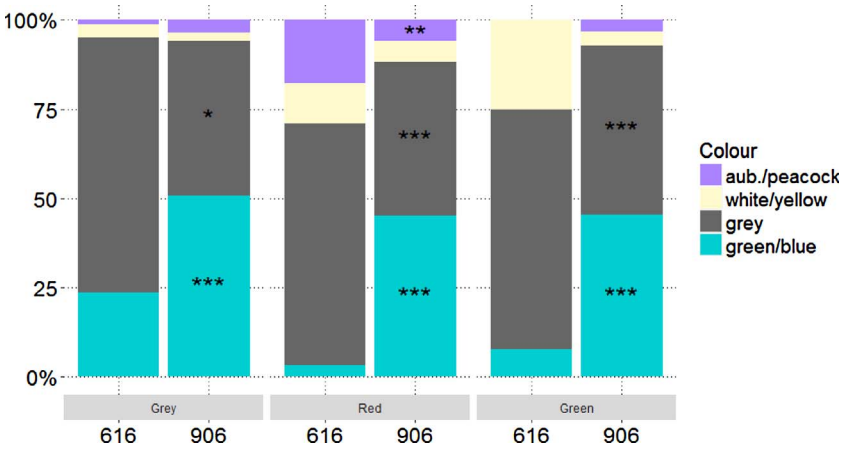

Fig. 3. Cultured pearl colour categories (grey, green/blue, white/yellow and aubergine/peacock) rate (in%) from *P. margaritifera*, depending upon the spat shell colour phenotypes (Grey, Red and Green) for families 616 and 906. Significance is indicated as follows: * (for $p < 0.05$); ** (for $p < 0.01$) and *** (for $p < 0.001$). Table 1. Experimental graft design using two *Pinctada margaritifera* families (616 and 906) as donor oysters. Repartition of the spat shell colour phenotype (red, grey, green and yellow) between the two grafters is indicated with the number of corresponding grafts (in brackets). Rate (%) and number of pearls (in brackets) at 18 months post-graft. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

	Family	616				906		
	Spat phenotype	Red	Grey	Green	Yellow	Red	Grey	Green
Grafter 1	# donors	4	5	1	4	3	4	8
	(grafts)	(80)	(100)	(20)	(80)	(60)	(80)	(160)
	% Pearls	65.0	56.0	80.0	43.7	60.0	65.0	77.5
	(N)	(52)	(56)	(16)	(35)	(36)	(52)	(124)
Grafter 2	# donors	3	2	3	3	4	6	2
	(grafts)	(60)	(40)	(60)	(60)	(80)	(120)	(40)
	% Pearls	73.3	60.0	80.0	68.7	70.7	71.7	75.0
	(N)	(44)	(24)	(48)	(55)	(99)	(86)	(30)
Total	# donors	7	7	4	7	10	10	10
	(grafts)	(140)	(140)	(80)	(140)	(200)	(200)	(200)
	% Pearls	68.6	57.1	80.0	64.3	67.5	69.0	77.0
	(N)	(96)	(80)	(64)	(90)	(135)	(138)	(154)

	Family	616				906		
	Spat phenotype	Red	Grey	Green	Yellow	Red	Grey	Green
Grafter 1	# donors	4	5	1	4	3	4	8
	(grafts)	(80)	(100)	(20)	(80)	(60)	(80)	(160)
	% Pearls	65.0	56.0	80.0	43.7	60.0	65.0	77.5
	(N)	(52)	(56)	(16)	(35)	(36)	(52)	(124)
Grafter 2	# donors	3	2	3	3	4	6	2
	(grafts)	(60)	(40)	(60)	(60)	(80)	(120)	(40)
	% Pearls	73.3	60.0	80.0	68.7	70.7	71.7	75.0
	(N)	(44)	(24)	(48)	(55)	(99)	(86)	(30)
Total	# donors	7	7	4	7	10	10	10
	(grafts)	(140)	(140)	(80)	(140)	(200)	(200)	(200)
	% Pearls	68.6	57.1	80.0	64.3	67.5	69.0	77.0
	(N)	(96)	(80)	(64)	(90)	(135)	(138)	(154)

(Wada and Komaru, 1990, 1996). In the same studies, a relation between outer and inner shell has been observed, with the amount of yellow pigments (inner shell) smaller in white type (outer shell) specimens than in brown wild-type. Much of the pigment-based colouration in invertebrates is under genetic control and results from products of melanin, ommochrome, pteridine, papiliochrome and heme synthesis pathways (Takeuchi et al., 2005). Of these, melanin is the most widespread pigment in nature and consists of two classes: eumelanins, which are black or brown, and pheomelanins, which are red, orange, or yellow (True et al., 1999). The tyrosinase enzyme (phenol oxidase) is essential for all the melanins and even non-pigmented sclerotin (Wittkopp et al., 2003). Indeed, the synthesis of all pigments begins with the conversion of tyrosine to DOPA, a part of which is then converted to DOPA-melanin (black), some of which is then further converted to pigment precursor dopamine. Dopamine can be converted to brown melanin or other pigment precursors N-β-alanyl dopamine (yellow sclerotin) and N-acetyl dopamine (colourless or transparent sclerotin) (Wittkopp et al., 2003). This cascade of conversions probably leads to the spat colour phenotype observed, as for the shell colouration observed in *Crassostrea gigas*, where black and golden phenotypes exist (Feng et al., 2015).

This study is the first to connect the colour of donor spat to that of the resulting pearls with the aim of establishing an earlier selection strategy for the orientation of cultured pearl colour balance in *P. margaritifera*. From an applied point of view, sorting out a specific spat colour phenotype at an earlier stage will reduce the colour diversity of the potential corresponding donor oysters, and thus lead to reduced pearl colour variability for the constitution of specific commercial pearl lots. Selection of an appropriate donor phenotype by incorporating earlier pigmentation traits into a pearl oyster genetic improvement programme would allow the targeting of a generation of donors capable of producing pearls with a green overtone, a characteristic that is associated with high-grade pearl quality (Ky et al., 2014a,b). This earlier selection process could also be incorporated in hatchery-reared spat, which are now used for commercial production in *P. margartifera* in

French Polynesia (Ky and Devaux, 2016). Quantitative measurements of colour could also be done by spectrometric approaches in *P. margaritifera*, as already used on cultured pearls (Dauphin and Cuif, 1995) and on spat shells (Trinkler et al., 2012). The use of such methods would improve the selection of spat based on their non-subjective colour and determine the best developmental age for selection (when the colour is still visible on the spat). In an effort to identify the genetic basis and molecular mechanisms underlying shell colouration in *P. margaritifera* and provide fundamental information to assist selective breeding of superior pearl oyster lines with desired pearl colouration patterns, differentially expressed genes could be identified among the different spat shell colour variants at the transcriptome level by RNA sequencing. Moreover, a complete genome sequence of *P. margaritifera* is under construction. The colour polymorphism at the spat stage was more restricted than in the adult stages in *P. margaritifera*, meaning that the whole animal is a good model for such next generation sequencing approaches.

5. Conclusions

The study revealed the possibility to orientate pearl colour production by selecting appropriate future donors at an earlier 3-month-old spat, from hatchery-produced or collected from the wild. The attractive green/blue pearls could be obtained twice as often when using grey and green spat phenotypes, whereas aubergine/peacock pearls obtained four times more often by using red and yellow spat phenotypes. Such spat colour phenotype could be used for genetic/genomic selection.

Acknowledgements

This work was supported by grants from the *Direction des Ressources Marines et Minières* through the ColoGEN project (2016-2018). We would also especially like to thank the host site team at Gauguin's Pearl Farm (Rangiroa atoll, Tuamotu Archipelago, French Polynesia) for their generous assistance.

References

Alagarswami, K., 1987. In: Marine Fisheries Research Institute (Ed.), Cultured Pearls-production and Quality. Pearl Culture, Cochin, pp. 105–113.
Cochennec-Laureau, N., Montagnani, C., Saulnier, D., Fougerouse, A., Levy, P., Lo, C., 2010. A histological examination of grafting success in pearl oyster Pinctada margaritifera in French Polynesia. Aquat. Living Res. 23, 131–140.
Dagnelie, P., 2007. Statistique théorique et appliquée, third ed. De Boeck Université, Bruxelles.
Dauphin, Y., Cuif, J.P., 1995. Trichromatic characterization of the black pearls from aquaculture centers of French Polynesia. Aquaculture 133, 113–121.
Feng, D., Li, Q., Yu, H., Zhao, X., Kong, L., 2015. Comparative transcriptome analysis of the pacific oyster crassostrea gigas characterized by shell colors: identification of genetic bases potentially involved in pigmentation. PLoS One 12, e0145257. http://dx.doi.org/10.1371/journal.pone.0145257. 2015 Dec 22;10.
Gervis, M.H., Sims, N.A., 1992. The biology and culture of pearl oysters (Bivalvia: pteriidae). overseas development administration and international centre for living. Aquat. Resourc. Manage. 21, 3–37 ICLARM Studies and reviews, Manila.
Gueguen, Y., Montagnant, C., Joubert, C., Marie, B., Belliard, C., Tayale, A., Fievet, J., Levy, P., Piquemal, D., Marin, F., Le Moullac, G., Ky, C.L., Garen, P., Lo, C., Saulnier, D., 2013. Characterization of molecular processes involved in the pearl formation in pinctada margaritifera for the sustainable development of pearl farming industry in French Polynesia. In: Watabe, S., Maeyama, K., Nagasawa, H. (Eds.), Recent Advances in Pearl Research – Proceedings of the International Symposium on Pearl Research 2011, pp. 183–193 Tokyo.
Hui, B., Vonau, V., Moriceau, J., Tetumu, R., Vanaa, V., Demoy-Schneider, M., Suquet, M., Le Moullac, G., 2011. Hatchery-scale trials using cryopreserved spermatozoa of black-lip pearl oyster (Pinctada margaritifera). Aquat. Living Res. 24, 219–223.
Karampelas, S., Fritsch, E., Gauthier, J.P., Hainschwang, T., 2011. Uv–vis-nir reflectance spectroscopy of natural-colour saltwater cultured pearls from Pinctada margaritifera. Gems and Gemol. 47, 31–37.
Ky, C.L., Devaux, D., 2016. Polynesian pearls. Hatch. Inter. 17, 20–21.
Ky, C.L., Blay, C., Sham-Koua, M., Vanaa, V., Lo, C., Cabral, P., 2013. Family effect on cultured pearl quality in black-lipped pearl oyster Pinctada margaritifera and insights for genetic improvement. Aquat. Living Res. 26, 133–145.
Ky, C.L., Molinari, N., Moe, E., Pommier, S., 2014a. Impact of season and grafter skill on nucleus retention and pearl oyster mortality rate in Pinctada margaritifera aquaculture. Aquac. Int. 22, 1689–1701.
Ky, C.L., Blay, C., Sham Koua, M., Lo, C., Cabral, P., 2014b. Indirect improvement of pearl grade and shape in farmed Pinctada margaritifera by donor oyster selection for green pearls. Aquaculture 432, 154–162.
Ky, C.L., Nakasai, S., Molinari, N., Devaux, D., 2015. Influence of grafter skill and season on cultured pearl shape: circles and rejects in Pinctada margaritifera aquaculture in Mangareva lagoon. Aquaculture 435, 361–370.
Ky, C.L., Nakasai, S., Pommier, S., Sham Koua, M., Devaux, D., 2016a. The Mendelian inheritance of rare flesh and shell colour variants in the black-lipped pearl oyster (Pinctada margaritifera). Anim. Genet. 47, 610–614.
Ky, C.L., Le Pabic, L., Sham Koua, M., Molinari, N., Nakasai, S., Devaux, D., 2017a. Is pearl colour produced from Pinctada margaritifera predictable through shell phenotypes and rearing environments selections? Aqua. Res. 48, 1041–1057.
Ky, C.L., Lo, C., Planes, S., 2017b. Mono- and polychromatic inner shell phenotype diversity in Pinctada margaritifera donor pearl oysters and its relation with cultured pearl colour. Aquaculture 468, 199–205.
Langerhans, R.B., Chapman, L.J., Dewitt, T.J., 2007. Complex phenotype-environment associations revealed in an East African cyprinid. J. Evol. Biol. 20, 1171–1181.
Lutz, G.C., 2001. Practical Genetics for Aquaculture. Fishing News Books, Oxford, UK.
McGinty, E.L., Evans, B.S., Taylor, J.U.U., Jerry, D.R., 2010. Xenografts and pearl production in two pearl oyster species, *P. maxima* and *P. margaritifera*: effect on pearl quality and a key to understanding genetic contribution. Aquaculture 302, 175–181.
McGinty, E.L., Zenger, K.R., Taylor, J.J.U., Evans, B.S., Jerry, D.R., 2011. Diagnostic genetic markers unravel the interplay between host and donor oyster contribution in cultured pearl formation. Aquaculture 316, 20–24.
Siegel, S., Castellan, N.J., 1988. Nonparametric Statistics for the Behavioural Sciences, second ed. McGraw-Hill, New-York.
Southgate, P.C., Strack, E., Hart, A., Wada, K.T., Monteforte, M., Cariño, M., Langy, S., Lo, C., Acosta-Salmón, H., Wang, A., 2008. Exploitation and culture of major commercial species. In: Southgate, P.C., Lucas, J.S. (Eds.), The Pearl Oyster, pp. 303–355 Amsterdam.
Takeuchi, K., Satou, Y., Yamamoto, H., Satoh, N., 2005. A genome-wide survey of genes for enzymes involved in pigment synthesis in an ascidian, Ciona intestinalis. Zool. Sci. 22 (7), 723–734.
Talvard, C., 2015. Bilan, la perle en 2013. In: Challier, S. (Ed.), Points Forts De La Polynésie Française, Institut De La Statistique En Polynésie Française. Polynésie Française, Papeete, pp. 1–5.
Tayale, A., Guegen, Y., Treguier, C., Le Grand, J., Cochennec-Laureau, N., Montagnani, C., Ky, C.L., 2012. Evidence of donor effect on cultured pearl quality from a duplicated grafting experiment on Pinctada margaritifera using wild donors. Aquat. Living Res. 25, 269–280.
Taylor, J., Strack, E., 2002. The Pearl Oyster: Biology and Culture (pearl Production). Elsevier, The Netherlands, pp. 273–302 (Ed.).
Thomas, Y., Le Gendre, R., Garen, P., Dumas, F., Andréfouët, S., 2012. Bivalve larvae transport and connectivity within the Ahe atoll lagoon (Tuamotu Archipelago): with application to pearl oyster aquaculture management. Mar. Pollut. Bull. 65, 441–452.
Tong, Y., Shen, H., 2001. Quality Assessment and Testing of Akoya Pearls, vol. 1. China Gems and Jades, pp. 68–69.
Trinkler, N., Le Moullac, G., Cuif, J.P., Cochennec-Laureau, N., Dauphin, Y., 2012. Colour or no colour in the juvenile shell of the black lip pearl oyster, Pinctada margaritifera. Aquat. Living Res. 25, 83–91.
True, J.R., Edwards, K.A., Yamamoto, D., Carroll, S.B., 1999. Drosophila wing melanin patterns form by vein-dependent elaboration of enzymatic prepatterns. Cur. Biol. 9, 1382–1391.
Wada, K.T., Komaru, A., 1990. Inheritance of white colouration of the prismatic layer of shell in the japanese pearl oyster Pinctada fucata martensii and its importance in the pearl culture industry. Nipon Suisan Gakkaishi 56 (11), 1787–1790.
Wada, K.T., Komaru, A., 1996. Color and weight of pearls produced by grafting the mantle tissue from a selected population for white shell color of the japanese pearl oyster Pinctada fucata martensii (dunker). Aquaculture 142, 25–32.
Wada, K.T., Tëmkin, I., 2008. Taxonomy and phylogeny: commercial species. In: Southgate, P.C., Lucas, J.S. (Eds.), The Pearl Oyster, pp. 54–57 Amsterdam.
Wada, K.T., 1984. Breeding study of the pearl oyster, Pinctada fucata. Aquaculture 6, 79–157.
Winer, B.J., Brown, D.R., Michels, K.M., 1991. Statistical Principles in Experimental Design. McGraw-Hill, New-York.
Wittkopp, P.J., Carroll, S.B., Kopp, A., 2003. Evolution in black and white: genetic control of pigment patterns in Drosophila. Trends Genet. 19, 495–504.