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Family effect on cultured pearl quality in black-lipped pearl oyster *Pinctada margaritifera* and insights for genetic improvement

Chin-Long KY[1,a], Carole BLAY[1], Manaarii SHAM-KOUA[1], Vincent VANAA[1], Cédrik LO[3] and Philippe CABRAL[2]

[1] Ifremer, UMR EIO241, Labex Corail, Centre du Pacifique, BP 7004, 98719 Taravao, Tahiti, Polynésie Française
[2] Gauguin's Pearl Farm, BP 191, 98776 Avatoru, Rangiroa – Archipel des Tuamotus, Polynésie Française
[3] Direction des Ressources Marines, BP 20, 98713 Papeete, Tahiti, Polynésie Française

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Abstract – Individual *Pinctada margaritifera* molluscs were collected from the Takapoto atoll (Tuamotu Archipelago, French Polynesia) and used to produce ten first generation full-sib families in a hatchery system, following artificial breeding protocols. After three years of culture, these progenies were transferred to Rangiroa atoll (Tuamotu Archipelago, French Polynesia) and tested for their potential as graft donors. A large-scale grafting experiment of 1500 grafts was conducted, in which a single professional grafter used ten individual donor oysters from each of the ten families, grafting 15 recipient oysters from each donor. The recipient oysters were all obtained from wild spat collection in Ahe (Tuamotu Archipelago, French Polynesia). After 18 months of culture, 874 pearls were harvested. Highly significant donor family effects were found for nucleus retention, nacre thickness, nacre weight, pearl colour darkness and visually-perceived colour (bodycolor and overtone), pearl shape categories, surface defects and lustre, the last two of which are components of the Tahitian classification grade. No significant difference was recorded between the ten G1 families for the absence or presence of rings. The progenies could be ranked from "best" (i.e., the donor whose grafts produced the greatest number of grade A pearls) to the "worst". Some progenies had extreme characteristics: family B presented the greatest number of pearls with lustre (98%) and a high proportion of dark gray to black with green overtone pearls (70%). These results have important implications for the selective breeding of donor pearl oysters: it may be possible to reach a point where specific donor lines whose grafts produce pearls with specific quality traits could be identified and maintained as specific breeding lines.

Keywords: Cultured pearl oyster / Pearl quality / Progeny effects / Heritability / Genetic selection / *Pinctada margaritifera* / French Polynesia

1 Introduction

The black-lipped "pearl oyster", *Pinctada margaritifera* (Linnaeus, 1758) (Bivalvia, Pteriidae) is a saltwater pearl-producing mollusc principally cultivated in French Polynesia. *P. margaritifera* is found throughout the coral areas of the Indo-Pacific; it is particularly abundant in the atolls of French Polynesia. Cultured pearls are the top exportation resource of the country: in 2009, the "pearl oyster" industry was worth 66.2 billion euros and about 5000 local people were involved. At the end of 2010 there were 571 pearl farms in activity, which were dispersed among 27 islands, mostly in the Tuamotu Archipelago (Talvard 2010). However, since 2001, this industry has been in decline and is now in a critical situation, due to a combination of several economic factors: slowdown of the world economy, overproduction and poor average pearl quality (Wane 2013). In this context, there is a need to promote measures that will enhance pearl quality, as high quality pearls command premium prices.

Cultured Tahitian pearls are produced by inserting a round bead of nacre (nucleus) and a small rectangular piece of mantle tissue (i.e. graft of ~4 mm^2) dissected from the mantle of a sacrificed mollusc of the same genus ("a.k.a." donor) into the gonad of animal of the same genus ("a.k.a." acceptor). It has been estimated that only 5%–10% of cultured pearls per harvest are of gem quality; however, this small percentage accounts for about 95% of a farm's income (Ellis and Haws 1999).

Producing cultured pearls of high quality with *P. margaritifera* is one of the major challenges for the pearl industry in French Polynesia. Cultured pearls commercial value mainly depends of five factors: shape (which includes the presence of "circles"), size (diameter, weight and nacre thickness – in French Polynesia, cultured pearls for exportation must have a nacreous layer with a minimum thickness of 0.8 mm built up on the nucleus-), colour (bodycolour and overtone), lustre and

[a] Corresponding author: chinky@ifremer.fr

surface condition. Large (with thick nacre thickness), unblemished, lustrous and spherical pearls fetch the highest market prices.

Pinctada margaritifera cultured pearls are still produced using wild populations due to: 1) the abundance of natural oyster resources and 2) the absence of systematic selective breeding. A study on *P. margaritifera* using wild donors showed evidence of a donor effect on cultured pearl quality (Tayale et al. 2012). Among "pearl oyster" species, few breeding programs have been conducted to select for improved donor oysters as a means to improve proportion of high quality cultured pearls and those that have been carried out have focused on the Akoya "pearl oyster" (*P. fucata*) and the silver- or gold-lipped "pearl oyster" *P. maxima* species. To date, knowledge about the genetic basis of important traits in *P. fucata* is mostly restricted to the heritability of shell traits (He et al. 2008) and their phenotypic correlations with pearl weight and colour (Wada 1984, 1986; Velayudan et al. 1996; Wada and Komaru 1996). For *P. maxima*, estimation of the donor-derived heritability and the effects of genotype × environment interactions on the production of pearl quality traits has been recently reported (Jerry et al. 2012). Donor influence on pearl quality traits was definitively demonstrated when a donor was found to have a significant influence on pearl growth, colour and surface complexion using xenografted oysters (McGinty et al. 2010). Since the domestication of *P. margaritifera*, i.e., since the rearing of the species over its entire life cycle – including artificial breeding – became possible, a genetic programme for donor oysters has been initiated at Ifremer (French Research Institute for Exploitation of the Sea) in French Polynesia. Other countries, in where natural spat was not abundant, have started breeding of *Pinctada margaritifera* since several years ago. It is the case for example in Micronesia[1] and in Fiji islands[2]. One part of the Ifremer programme is to establish selected lines through progeny testing for quality traits of interest. We have already bred and reared several first-generation (G1) donor families. The main objective of the present study was to test these G1 families in a standardised single-site experimental grafting trial in order to evaluate family effect on cultured pearl quality traits, and thus their potential for the production of high quality pearls. This study will help the development of breeding programmes for the production of donor oyster lines with desired traits.

2 Materials and methods

2.1 Controlled reproduction and crosses of *Pinctada margaritifera*

Ten bi-parental families were produced in the Ifremer hatchery in Vairao (Tahiti, French Polynesia) in 2007 by spawning ten "non-selected" female and ten male broodstock oysters. Spawning was triggered by thermal shock (Hui et al. 2011): the pearl oysters were placed in cooled seawater at 20 °C for one night before being plunged into seawater at 31−32 °C. Soon after spawning started, male and female oysters were placed in separate containers for gamete collection (Le Moullac et al. 2011). To minimize the risk of contamination, oocytes were thoroughly rinsed out of the mantle cavity of the spawning females. Each female was then placed into an individual spawning tray where it continued releasing fresh oocytes that had not come into contact with sperm. These oocytes were then cleaned through a sieve of 80 μm and washed on a sieve of 25 μm. A verification of sperm motility was carried out for each male. Oocytes were then fertilised at a ratio of 5−10 sperm per oocyte. The fertilisation rate was estimated 3 h later by counting 200 to 300 eggs under a compound microscope.

2.2 Larval, seed and juvenile rearing of *Pinctada margaritifera*

Fertilized eggs were put in 150-L containers filled with 1-μm filtered seawater held at 28 °C to develop into veliger larvae. After 24 h, the tanks were drained, the larvae collected onto 45 μm screens, and each family transferred to a separate 150-L rearing tank filled with 1-μm filtered seawater held at 28 °C, where they were reared until settlement. Veliger larvae (Fig. 1a) were initially reared at a concentration of 10 larvae ml^{-1}. D-shaped larvae were fed for 10 days on two tropical microalgae species: *Isochrysis galbana* (T-Iso) and *Chaetoceros* var."*minus*". Then, from the 11th day, they were fed on a mix of three microalgae (*I. galbana*, *C. minus,* and *C. gracilis*). During larval rearing, seawater was renewed every two days and larvae were sieved at day 13 and day 19 on 60 μm and 80 μm mesh, respectively, to remove the remains of any dead larvae. On these days, larvae were concentrated in a 5-L graduated cylinder and their number was assessed in a sample ranging from 20 μl to 1 ml in volume. The larval phase lasted approximately 23 days before the pediveliger stage was reached.

The pediveliger larvae (Fig. 1b) were transferred in batches of approximately 10 000 individuals for settlement into downwellers (Ø 40 cm, height 40 cm, mesh 130 μm) set out in flow-through raceways (200 × 50 × 50 cm), with four downwellers per raceway. Filtered sea water (5 μm) was supplied continuously with a mixture of microalgae T-Iso and *C. gracilis* at an average concentration of 20 000 cell ml^{-1}. After one week, the non-settled larvae were eliminated. The meshes were cleaned every day by brushing the external side. After approximately 45 days, the attached seed were detached using a brush and transferred to the nursery.

The seed oysters (Fig. 1c) were reared in raceways of 90 × 20 × 20 cm (corresponding to a volume of 30 L). Unfiltered seawater was added with a suspension of algae produced in outdoor tanks at a renewal rate of 100 L h^{-1}. The juveniles (Fig. 1d) were detached once they reached an average size of 20 mm and were transferred to the natural environment in Aquapurse® plastic trays.

The "pearl oysters" were reared for 2 years in these Aquapurse® trays suspended on long lines located in the

[1] Micronesia: http://www.ctsa.org/files/projects/2003_Final_Report_Black_Pearl_Farming6324467313314891801.pdf

[2] Fiji: http://www.fiji-savusavu.com/jhp/JH%20NEWSLETTER%202013.pdf

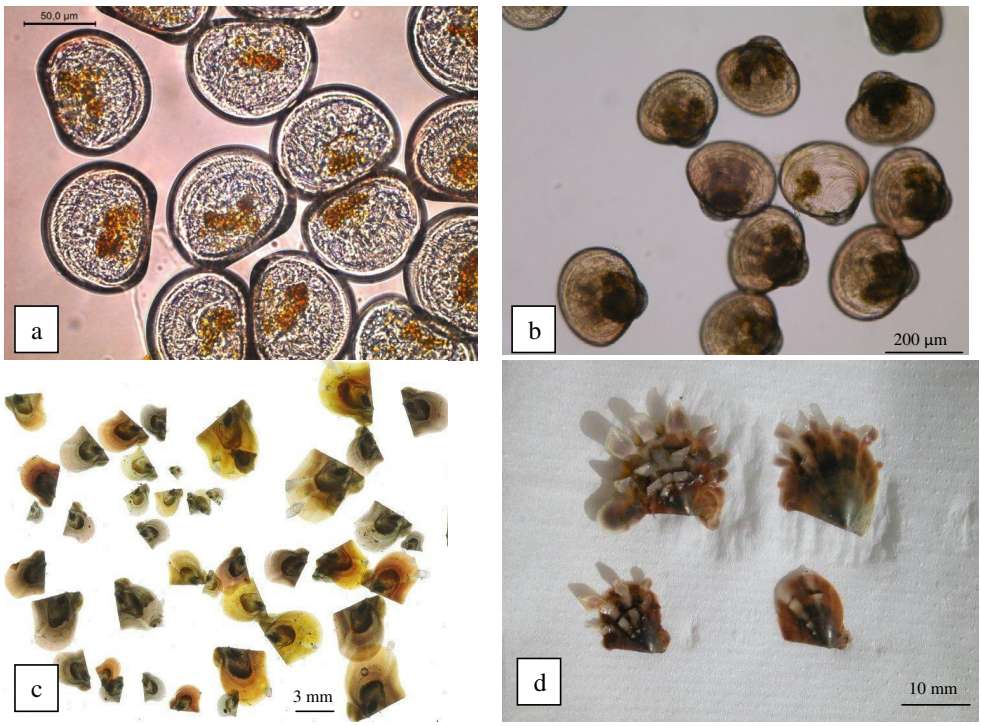

Fig. 1. *Pinctada margaritifera* produced by hatchery system at IFREMER facilities for the generation of full-sib families. Veliger larvae stage (D-shape stage) at 3 days post-fertilisation (a), pediveliger larvae stage at 21 days post-fertilisation (b), early spat at 49 days post-fertilisation (c), and later spat at 120 days post-fertilisation (d).

lagoon of Vairao. They were maintained at a depth ranging between 6 and 10 metres. The mesh of the trays varied according to their size in order to allow a better management of the individuals. The baskets were cleaned every 3 months as well as animals.

2.3 Experimental animals and grafting procedure

At the age of three years (average shell size of 12 cm), ten individuals were randomly selected from each of the ten G1 families (named with the letters from A to J) and labelled. These hundred oysters, which would serve as donors, were transferred to Rangiroa atoll (Tuamotu Archipelago), where they were reared for two months prior to the grafting campaign, which was carried out at Gauguin's Pearl farm facilities in October 2010. A single professional grafter performed 1500 grafts with these donors over 4 days, in a way to minimized grafter effect on pearl quality traits. Twenty five donor oysters were randomly selected per day for the grafting procedure. Fifteen graft pieces were excised from the mantle edge valves of each donor, following standard cultured pearl farm procedures. Each receiving mollusc (issued from natural spat collection) was selected based on visible health status (colour of the visceral mass and gills), shell size and appearance, and muscle resistance when opening the shells. Each receiving mollusc was grafted using a 2.4 BU nucleus (7.304 mm diameter, 0.59 g weight – Nucleus Bio, Hyakusyo Co. Japan). After grafting, the receiving oysters were placed in separate net retention bags with a mesh size that allowed rejected nuclei to be caught. Nucleus rejection (presence of the rejected bead in the bags i.e., outside the molluscs) and receiver mortality were evaluated 45 days after the graft operation. The labelled pearl oysters that retained their grafted nucleus (no bead detected in the bags) were drilled and fixed on long-lines after removing the net retention bags. Cultured pearls were harvested 18 months after grafting (April 2012).

2.4 Measurement of cultured pearl quality traits

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. Surface defects, lustre, darkness and colour categories of the cultured pearls, were evaluated visually (without loupe) by two operators working in cooperation.

Two quantitative variables were measured on the cultured pearls:

- Nacre thickness, using a digital micrometer (for diameter evaluation), nacre thickness = [(cultured pearl diameter)-(nucleus diameter)]/2;
- Nacre weight, using a digital balance (for weight evaluation), nacre weight = (cultured pearl weight-nucleus weight).

Cultured pearl shape was determined by both of the operators (as were surface defects, lustre, darkness and colour) and was characterized in two ways:

- The presence/ absence of circle on the cultured pearls, shown by regular streaks or concave rings, whatever the shape category;

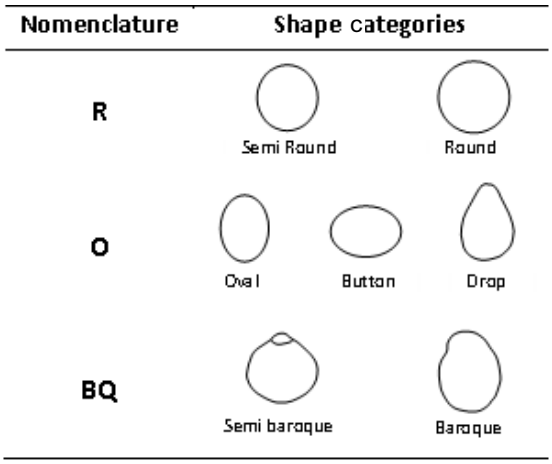

Fig. 2. The three cultured pearl shape categories, as used for the evaluation of quality traits. The nomenclature used is: "R", for cultured pearls with round or semi-round shapes; "O", for those with oval, button and drop shapes; and "BQ", for those with irregular, mostly *baroque* and *semi-baroque* shapes.

- The Shape category (Fig. 2). The three shape categories are: 1) "R", for round or semi-round shapes (diameter variation <5%); 2) "O", for oval, button and drop shapes (diameter variation >5%, with a symmetrical axis); and 3) "BQ", for irregular shapes, mostly baroque and semi-baroque.

Two kinds of colour evaluation (without loupe) were made on the cultured pearls (Tayale et al. 2012):

- The darkness of colour, with three categories depending on its level: high, medium and low;
- The visually-perceived colour category, which is due to pigments (bodycolour), and secondary colour (overtone). Six "colour categories" were detected into which all the harvested pearls could be classified as: 1) bodycolours: grey, white and yellow, and 2) secondary colours: green, aubergine (red / purple), and peacock (a mix of aubergine and green).

Cultured pearl grade for each sample was determined by a single professional expert from *Maison de la Perle*, according to the official Tahitian classification (Journal Officiel 2001 No. 30, 26 July 2001). Cultured pearl grade was thus attributed to each pearl, from the most valuable quality to the least: A, B, C, D and rejects (*rebuts*). Briefly, the four grades are mostly based on surface purity and lustre:

- A, cultured pearls showing no surface defects or small defects confined to less than 10% of their surface and having very good lustre;
- B, cultured pearls showing defects distributed over less than one third of their surface and having good or medium lustre,
- C, cultured pearls showing defects distributed over less than two thirds of their surface and having medium lustre, and
- D, cultured pearls showing many very visible defects over more than two thirds of their surface and having poor lustre. Rejects are cultured pearls that have too many defects to be graded and which are consequently discarded and ultimately destroyed.

Finally, surface defects and lustre (components of cultured pearl grade) were determined separately so that they could be studied independently. Visible sample surface defects including pits, bumps, scratches, deposits or other surface flaws were counted visually (without a magnifier) and each cultured pearl was then classified into one of four categories: no defect, 1 to 5 defect(s), 6 to 10 defects and up to 10 defects. Pearl lustre was evaluated as follows: presence of lustre (glossy and shiny) and absence of lustre (matte appearance).

2.5 Statistical analysis

Differences in nucleus retention rate between ten donor progenies were evaluated using χ^2 tests. Kruskall-Wallis tests were used to test for differences in nacre thickness and weight among the donor progenies (Siegel and Castellan 1988). If the overall test was significant, a Dunn procedure with a Bonferroni correction was performed among all pair of progenies (Winer et al. 1991).

Qualitative classes based on cultured pearl surface defects, lustre, grade, darkness and circles were re-encoded to give quantitative scores that would enable the mean value of progenies to be obtained for each criterion, thus allowing them to be ranked. Scores from 0 to 4 were attributed to the different classes from the least to the most valuable (with grade, surface defects, darkness and lustre), as shown in Table 1. For each criterion, Kruskall-Wallis tests were then applied to compare the progenies.

For the cultured pearl "colour categories" and shape categories, differences and effect of family were evaluated using χ^2 tests.

To test if there were significant relationships between pearl quality traits, Spearman's rank tests, Kendall tests and χ^2 tests were performed (Croux 2005).

In all tests, p-values lower than 0.05 were considered significant (Dagnelie 2007). All analyses were performed using XLSTAT (version 2009.4.02) and R software (version 2.14.1).

3 Results

3.1 Nucleus retention and pearl harvest

Nucleus retention and rejection were evaluated after a period of 45 days post-grafting, during which the pearl oysters were not handled (Table 2). The 1500 grafted molluscs showed an average of 71.5% nucleus retention (N = 1072), with minimum and maximum values of 54.7% (N = 82 nuclei retained among 150 grafts) and 82.5% (N = 121 nuclei retained among 150 grafts), respectively. The 1500 grafted molluscs showed an average of 22.2% rejected nuclei (N = 333), with minimum and maximum values of 13.3% (N = 20) and 35.3% (N = 53), respectively, depending on the family origin of the graft. Mortality rate at 45 days post-grafting operation represented 6.3%

Table 1. *Pinctada margaritifera* cultured pearl surface defects, lustre, grade, darkness and presence/absence of a circle re-encoded into quantitative scores to evaluate the mean value of donor families for each criterion, allowing them to be ranked. Scores from 0 to 4 were attributed to the different classes from the least to the most valuable (for surface defects, lustre, grade, darkness and circle/s) as shown.

Pearl quality	Score				
	0	1	2	3	4
Surface defects	>10 defects	6-10 defects	1-5 defect(s)	no defect	–
Lustre	without lustre	with lustre	–	–	–
Grade	Rebut	D	C	B	A
Darkness	low	moderate	high	–	–
Circle	without circle	with circle(s)	–	–	–

Table 2. Summary of data on grafted *P. margaritifera* oysters: number of oysters (and percentage in brackets): *to*: number of grafted oysters, *t45 days*: oysters retained their nucleus until 45 days post grafting, with percentage of total grafted oysters; oysters that had rejected their nuclei by 45 days post grafting; mortality and/or predation up to 45 days post-grafting*; t18 months: Keshi* at 18 months post grafting that had retained their nuclei, harvested pearls 18 months post-grafting among oysters that retained their nuclei and predation and/or mortality 18 month post grafting among oysters that retained their nuclei.

	t_0	$t_{45\ day}$ post graft			$t_{18\ month}$ post graft		
Donor progenies	Grafted oysters	Oysters that retained their nuclei	Oysters that rejected their nuclei	Mortality and/ or predation	*Keshi*	Harvested pearls	Predation and/ or mortality
A	150	118	27	5	4	100	14
		(78.7)	(18.0)	(3.3)	(3.4)	(84.7)	(11.9)
B	150	82	53	15	11	60	11
		(54.7)	(35.3)	(10.0)	(13.4)	(73.2)	(13.4)
C	150	95	46	9	6	77	12
		(63.3)	(30.7)	(6.0)	(6.3)	(81.1)	(12.6)
D	150	118	22	10	4	103	11
		(78.7)	(14.6)	(6.7)	(3.4)	(87.3)	(9.3)
E	150	109	31	10	6	87	16
		(72.7)	(20.6)	(6.7)	(5.5)	(79.8)	(14.7)
F	150	113	34	3	2	91	20
		(75.3)	(22.7)	(2.0)	(1.8)	(80.5)	(17.7)
G	150	103	40	7	4	79	20
		(68.7)	(26.6)	(4.7)	(3.9)	(76.7)	(19.4)
H	150	107	34	9	3	85	19
		(71.3)	(22.7)	(6.0)	(2.8)	(79.4)	(17.8)
I	150	106	26	18	8	92	6
		(72.5)	(17.9)	(12.0)	(7.5)	(86.8)	(5.7)
J	150	121	20	9	0	100	21
		(80.7)	(13.3)	(6.0)	(0)	(82.6)	(17.4)
Total	1500	1072	333	95	48	874	150
		(71.5)	(22.2)	(6.3)	(4.5)	(81.5)	(14.0)

on average. Highly significant donor family effect was found for both nucleus retention and nucleus rejection, $p < 0.0001$.

Mortality due to post-graft operation and predation were evaluated after a period of 45 days post-grafting (Table 2). The 1500 grafted molluscs showed an average of 6.3% (min 2%, max 18%) mortality and/or predation ($N = 95$). A significant donor family effect was detected for mortality and/or predation: $p = 0.022$.

After 18 months of culture, 874 cultured pearls were harvested. The quality traits of these cultured pearls were recorded, as described in Section 2. The number of pearls harvested (in brackets) per family was as follows: A (100), B (60), C (77), D (103), E (87), F (91), G (79), H (85), I (92) and J (100). No significant family donors for harvested was detected. The number of *keshi* (a.k.a. cultured pearls without bead found in the gonad) harvested (in brackets per family was as follows: A (4), B (11), C (6), D (4), E (6), F (2), G (4), H (3), I (8) and J (0). Highly significant family donor effect for harvested *keshi* was detected: $p = 0.001$. Predation and/or mortality were evaluated after 18 months of culture. Ten families showed an average of 14% ($N = 150$) predation and/or mortality against oysters that retained their nuclei. No significant family donor effect was detected for mortality and/or predation after 18 months.

3.2 Nacre thickness and weight

The average nacre thickness among the 874 harvested pearls was 1.12 mm, with minimum and maximum values of 0.23 mm and 2.98 mm, respectively. A very highly significant family effect was recorded for nacre thickness ($p < 0.0001$). The ten G1 progenies are ranked, from the thickest to the thinnest nacre, as follows: G, B, F, I, H, E, A, C, D and

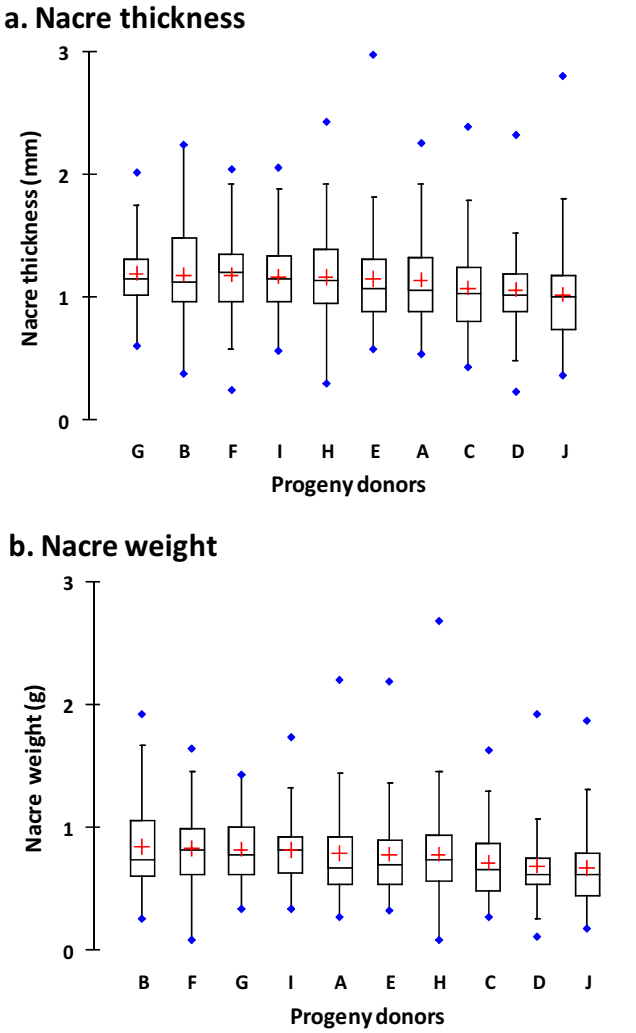

Fig. 3. Nacre thickness (a, in mm) and weight of nacre (b, in g) of cultured pearls produced using graft tissue from each of the *P. margaritifera* donor progenies (A to J). Each box-plot has the following 6 elements: 1) mean ("+" cross in the box-plot); 2) median (solid bar in the box-plot); 3) 25th to 75th percentile (rectangular box); 4) 1.5 × interquartile range (non-outlier range of the box whiskers); 5) minimum and maximum values (extreme dots) and 6) outlier values (outside box whiskers). For nacre thickness (a), the donors are ranked from the family associated with the thickest nacre in the harvested cultured pearls (G) to the family associated with the thinnest nacre (J). For pearl weight (b), the families are ranked from the one associated with the heaviest cultured pearls (B) to the one associated with the lightest cultured pearls (J).

J (Fig. 3). Pearls from family G donors showed the greatest average nacre thickness (1.19 mm) compared with family J (1.02 mm). Family G was 14.3% thicker on average than family J.

The average nacre weight of the 874 harvested pearls was 0.76 g, with minimum and maximum values of 0.08 g and 2.68 g, respectively. A very highly significant family effect was recorded for nacre weight ($p < 0.0001$). The ten G1 families are ranked from the heaviest to the lightest nacre weight: B, F, G, I, A, E, H, C, D and J (Fig. 3). The pearls from progeny B

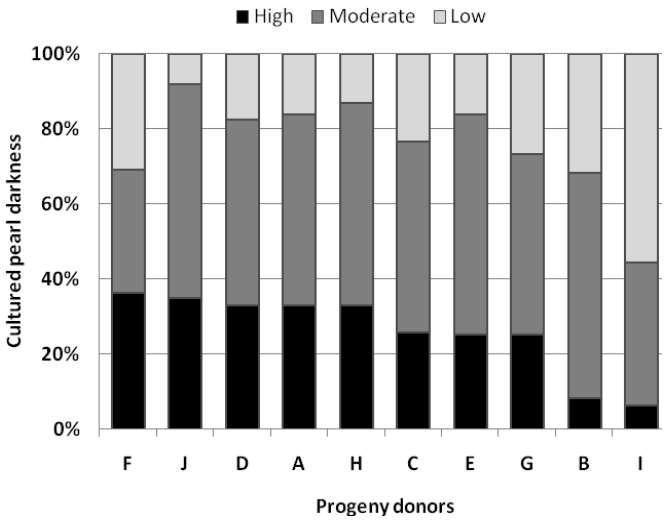

Fig. 4. Colour darkness distribution (% of each of the following categories: low, moderate and high darkness) in cultured pearls from each of the *P. margaritifera* donor families (A to J). Donor families are ranked from the family associated with the darkest cultured pearls harvested (F) to the progeny associated with the palest cultured pearls harvested (I).

donors were the heaviest (0.84 g), compared with those produced by progeny J donors, which were the lightest in weight (0.66 g). Progeny B was 21.4% heavier than progeny J.

3.3 Cultured pearl "colour": darkness and visual perception (bodycolour and overtone)

The darkness of colour in the cultured pearls from the different donor families is illustrated in Figures 4 and 5. The 874 harvested pearls were distributed among each of the darkness levels as follows: 23% ($N = 204$) with low, 50% ($N = 434$) with medium and 27% ($N = 236$) with the high darkness level. A very highly significant G1 family effect was recorded for this trait: $p < 0.0001$. The donor families J and I are at the extremes of darkness level in the pearls produced, with J the darkest and I the lightest in colour (Fig. 4). When the darkness levels was considered independently, the lightest cultured pearls were found in pearls from family I, which had a proportion of 55% of the low darkness level. The darkest cultured pearls were found in the family F, which had 36% of the high darkness level, whereas the greatest number of pearls with the intermediate darkness level was found in pearls from family B grafts, of which 60% had this level of coloration.

Among all of the harvested pearls ($N = 874$), six "colour categories" were visually detected. The two main cultured pearl "colour categories" observed were the green secondary colour ($N = 488$; 56%) and the grey bodycolour ($N = 158$; 29%), and four less frequent "colour categories" were also found: peacock ($N = 59$; 7%) and aubergine ($N = 15$; 2%) as secondary colours, and yellow ($N = 37$, i.e. 4%) and white ($N = 26$; 3%) as bodycolour. Figure 5 illustrated cultured pearl bodycolour and secondary colour harvested. A very highly significant G1 family effect was recorded for "colour categories": $p < 0.0001$. The different colour proportions produced by

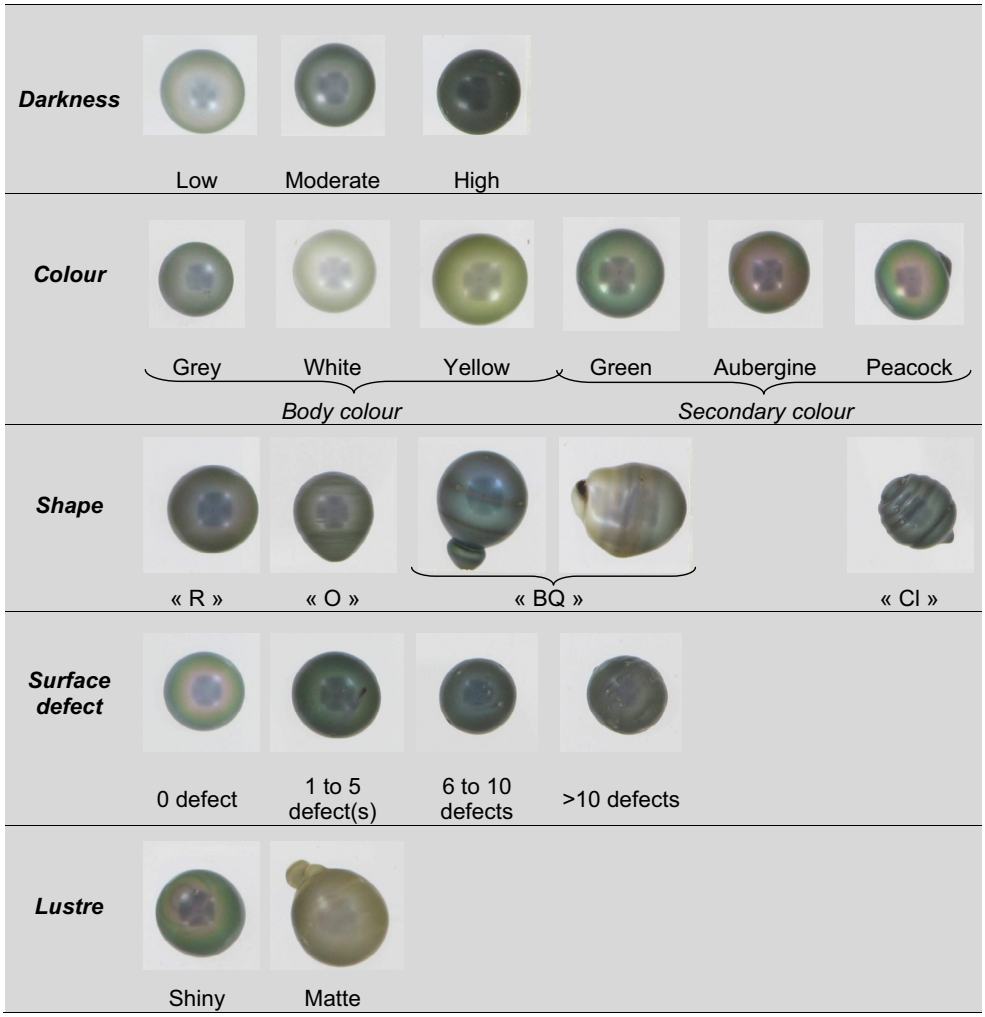

Fig. 5. Variability of cultured pearl quality traits harvested from an experimental graft using full-sib families from hatchery produced oysters. Cultured pearls were visually (without loupe) characterised for: 1) darkness level (low, moderate and high) of colour; 2) colour categories which is due to bodycolour (grey, white and yellow) and secondary colour (green, aubergine and peacock); 3) shape categories with "R" (round or semi-round); "O" (oval, button and drop), "BQ" (*baroque* and *semi-barroque*) shapes (as classified in Fig. 1) and the presence of circles "Cl"; 4) surface defect with 4 categories (no defect, 1 to 5 defect(s), 6 to 10 defects and up to 10 defects) and 5) presence/ absence of lustre.

donors are ranked from each of the G1 progenies, from the family that produced the highest proportion of green secondary colour cultured pearls to the one that lowest proportion: B, G, D, F, C, A, J, H, E and I (Fig. 6). Here, the pearls from progeny B donors showed 70% (N = 42) of green secondary colour pearls in comparison with those produced following grafts from progeny I donors, which showed only 40% (N = 37).

3.4 Cultured pearl circle and shape categories

The 874 harvested pearls included 76% (N = 666) of pearls without circles and 24% with circles (N = 208). No significant difference was recorded between the ten G1 families for the absence or presence of circles: p = 0.434 (data not shown).

The shape categories for each of the studied progenies are illustrated in Figures 5 and 7. The 874 harvested pearls were distributed as follows: 47% (N = 411) of "R" shape, 42% (N = 363) of "BQ" shape and 11% of "O" shape. A highly significant G1 family effect was detected for the proportions of these shape categories: p = 0.007. The families are ordered from the one with the most pearls in the "R" shape category to that with the least: H, D, A, C, I, J, B, E, F and G (Fig. 7). Here, the extremes were represented by pearls from family H donors, which showed 58% (N = 49) of "R" shape, and those from family G donors, which showed only 34% (N = 27).

3.5 Cultured pearl surface defects, lustre and grade

Incidence of cultured pearl surface defects among G1 donor families is illustrated in Figure 8, see Figure 5 too. The 874 harvested pearls were distributed among each of the surface defect classes as follows: 7% (N = 62) without any defects, 49% (N = 425) with 1 to 5 defect(s), 33% (N = 284) with 6 to 10 defects and 12% (N = 103) more than 10 defects. A very highly significant G1 family effect was detected for

Fig. 6. Perceived visual colour (bodycolor and overtone) distribution of cultured pearls (% of each of the following categories: green, grey, peacock (a mix of aubergine and green), yellow, white, and aubergine, from each of the *P. margaritifera* donor families (A to J). Donor families are ranked from the one containing the greatest proportion of green cultured pearls (B) to the one with the least (I).

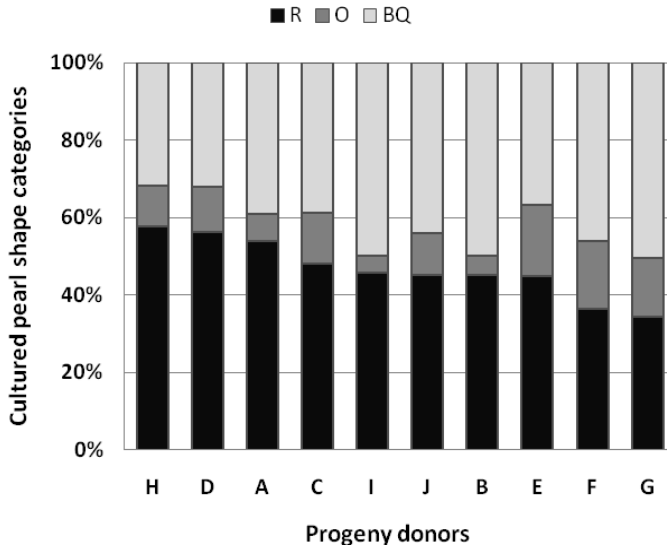

Fig. 7. Cultured pearl shape distribution (%) between the three following categories: 1) "R" shape, corresponding to round or semi-round; 2) "O" shape, corresponding to oval, button and drop; and 3) "BQ" shape, corresponding to irregular shapes, mostly baroque and semi-baroque, in pearls produced with *P. margaritifera* donors from each of the families (to J). The families are ranked from the one from that produced the highest proportion of "R"-shaped pearls (H) to the one that produced the lowest proportion (G).

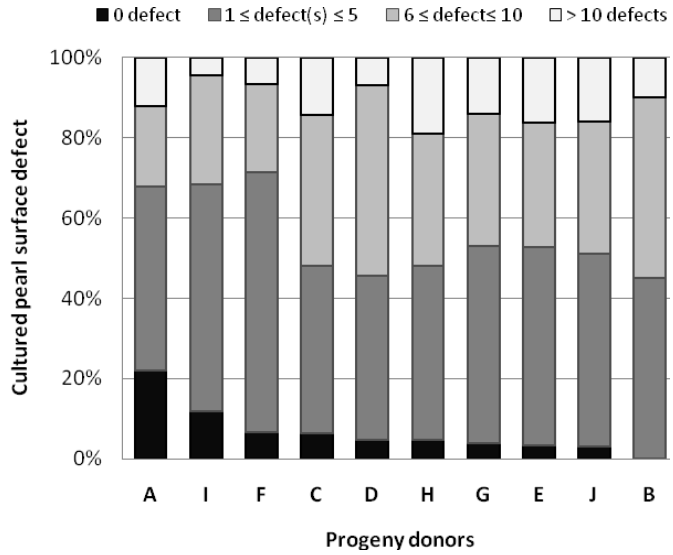

Fig. 8. Surface defects proportions (%) in pearls from grafts obtained from each of the *P. margaritifera* donor families according to the four following categories: no defect, 1 to 5 defects, 6 to 10 defects and more than 10 defects, within each experimental graft, the donor families (A to J) are ranked from those from which the pearls that had the least defects (A) to those from which the pearls had the most defects (B).

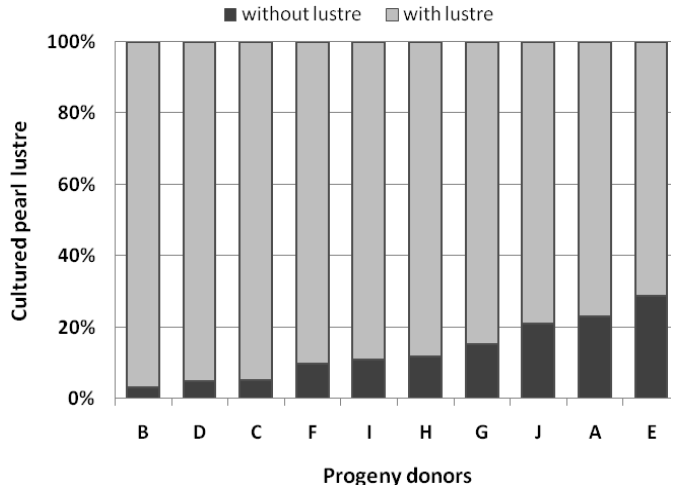

Fig. 9. Pearl lustre distribution (%) of pearls with and without lustre, produced from donors from each of the *P. margaritifera* G1 families (A to J) and are ranked from the one associated with the greatest proportion of pearls with lustre (B) to the progeny associated with the lowest proportion of pearls with lustre (E).

the number of cultured pearl surface defects: $p < 0.0001$. Cultured pearls from family A donors presented the "best" surface quality (Fig. 8), with 68% of cultured pearls having less than 5 defects. A large proportion of cultured pearls from family B (55%) had up to 5 defects, and none of the samples from this family were completely free of defects. The different G1 families could be ranked from the family whose grafts produced the cultured pearls with the minimum number of surface defects to the family with the maximum: A, I, F, G, E, D, C, J, H and B.

Cultured pearl lustre (matte or shiny / glossy) distribution showed 86% ($N = 753$) of the harvested pearls having a shiny / glossy surface (Figs. 5 and 9). Family effect was very highly significant ($p < 0.0001$). In the experiment, pearls from donor families B, D, and C showed significantly better lustre on average compared with families E, A and J ($p < 0.0001$). The different G1 families could be ranked from the families whose grafts produced the most cultured pearls with shiny / glossy lustre to those with the least: B, D, C, F, I, H, G, J, A and E.

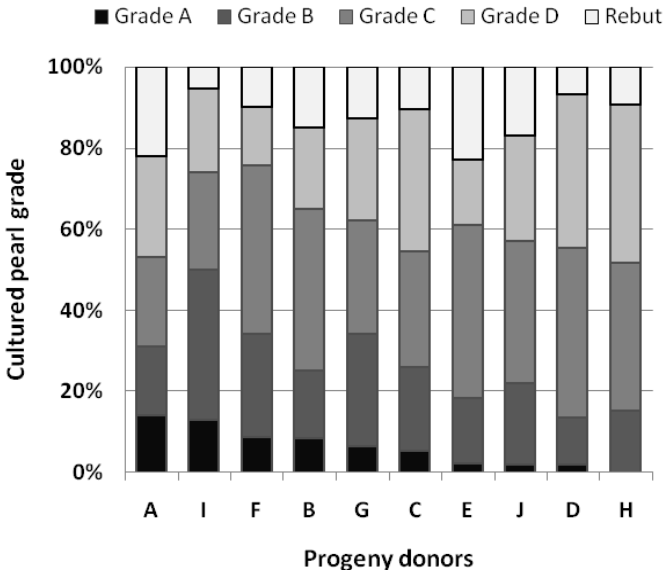

Fig. 10. Cultured pearl grade classification (%, for the grades A, B, C, D and Reject, according to the Tahitian Classification) for cultured pearls from each of the *P. margaritifera* donor families (A to J) and ranked from the family associated with the greatest proportion of the highest grade of harvested cultured pearl (A) to the one associated with the lowest proportion (H).

Cultured pearls (874) are distributed per grade class for each family (Fig. 10) as follows: 6% (N = 54) of grade A, 21% (N = 181) of grade B, 34% (N = 296) of grade C, 26% (N = 228) of grade D and 13% (N = 115) of rejects. Data analysis showed a very highly significant family effect on cultured pearl grade with $p < 0.0001$. In Figure 10, the donor families are ordered from the one that produced the largest proportion of grade A pearls to the one that produced the least: A, I, F, B, G, C, E, J, D and H. Here, the pearls from family A donors showed 14% (N = 14) of grade A pearls, while there were none (N = 0) of this grade in pearls from family H donors.

3.6 Correlations between cultured pearl quality traits

Significant correlations were found between some of the pearl quality traits. As expected, pearl grade was correlated with both lustre (r_s = 0.239, $p < 0.0001$) and surface defects (r_s = 0.435, $p < 0.0001$). Nacre thickness had a strong positive relationship with nacre weight (R^2 = 0.897, $p < 0.0001$). Finally, pearl colour was correlated with pearl darkness level ($p < 0.0001$).

4 Discussion

This paper reports the first complete experiment on *P. margaritifera* that (1) uses G1 progenies produced in a hatchery system and (2) tests their potential influence on cultured pearl quality when they are used as donor oysters. The main result is that highly significant progeny effects exist for several traits of interest: nucleus retention, nacre thickness, nacre weight, pearl darkness, visually perceived pearl colour (bodycolor and overtone), pearl shape categories, surface defects and lustre, the last two of which are components of the Tahitian classification grade. These results are consistent with those obtained by Tayale et al. (2012) where the authors demonstrated in a duplicated experimental graft, that individual wild donors of implanted mantle grafts significantly affect seven of the studied quality trait in *P. margaritifera* cultured pearls (pearl shape categories were not studied in Tayale et al. 2012). In contrast, no significant progeny effect was recorded for oyster mortality or pearl circles. The traits of interest should be included in further genetic selection through progeny testing.

4.1 Nucleus retention and oyster mortalities

Significant differences for nucleus retention rate (and thus nucleus rejection rate) exist between donor families, as shown by the difference we observed between extreme progeny B (with 54.7% retention) and progenies A or D (both with 79% retention). These data are consistent with those of Tayale et al. (2012) on *P. margaritifera* wild donors, where: 1) extreme nucleus retention rate were comparable with 51% to 77% and 2) wild donors had a significant effect on nucleus retention. Cochennec-Laureau et al. (2010) have already suggested that rejection phenomena are linked to a number of causes, among which choice of donor oyster is important.

Oyster mortalities during the period of culture in the lagoon were mainly due to: 1) irreversible trauma after the surgical grafting act (checking at 45 days post-graft), and 2) predation, which is becoming an increasingly serious problem in oyster culture lagoons in French Polynesia, where the diet of some animals has changed because farmed oysters represent a food source that is both easy to find and highly concentrated. Triggerfish (*Balistoides viridescens*, *Pseudobalistes flavimarginatus*), leopard-ray (*Aetobatus narinari*), turtle (*Chelonia mydas*) and diodonfish (*Diodon liturosus*) are the top five predators of *P. margaritifera*. Our results reveal that mortalities detected 45 days post grafting were significantly different between progenies. This could be indirectly correlated with the difference in nucleus retention between the progenies following the surgical grafting operation, as B progeny, which shows the maximum mortality rate (10%) 45 days post grafting, also had the lowest nucleus retention rate (55%) among the 10 progenies. Recipient oysters that received a *saibo* (graft tissue) from a progeny associated with a low nucleus retention rate (like B), seem to have the lowest tolerance to the grafting operation. In contrast, for the mortalities detected after 18 months of culture (harvest time), our results showed no significant difference between the progenies, suggesting that most mortalities during this period were caused by predation, to which receivers grafted with all donor progenies were equally vulnerable. Grafters skill play also an important role in the nucleus retention rate. In our experimentation, a same professional grafter has performed the grafts in a way to minimise grafter effect and highlight the progeny effect.

4.2 Nacre thickness and weight

Nacre weight variation among families was due to difference in nacre thickness, as shown by the correlation between these two dependant variables.

Nacre thickness and weight differed significantly between progenies in our experiment. The average nacre thickness and weight of the cultured pearls produced from extreme progenies in this experiment differed by approximately 14% and 20% respectively between extreme families. Our results showed a strong positive relationship between nacre thickness and nacre weight (R^2 = 0.90 and p < 0.0001). Nevertheless, cultured pearls that exhibited important nacre thickness does not forcibly correspond to heavier nacre weight. Indeed, difference in nacre density and presence of irregular matrix in cultured pearl (that interfered with pearl shape) are possible reasons of this fact. The 14 and 20% ranges are similar to the one found in a duplicated grafting experiment using wild *P. margaritifera* donors (Tayale et al. 2012), suggesting that the present G1 progeny donors have the same genetic potential as wild donors to deposit successive sheets of nacre onto the nucleus, with same range of nacre deposition rates. This implies that that no indirect selection during rearing practices impacted the nacre deposition potential of the hatchery-reared families. Such an effect might have been expected because, during hatchery production, selective sieving is used to discard the smallest larvae. Although this practice offers the advantage of reducing variance in larval size, development rate and time to settlement, it also poses a substantial risk of diversity loss because it increases the variance of reproductive success among parental oysters. Our results show that selective sieving seems to have no effect on nacre deposition potential, as the 20% difference between highest and lowest is equivalent between pearls from the wild donors in this previous study and those in the present hatchery-reared families.

Nacre thickness and weight are directly correlated with the nacre biomineralisation process, which is dependent on calcium metabolism in the epithelial tissue around the pearl. The potential for this metabolism is derived from that of the mantle epithelium of the donor, which varies according to species and environmental conditions (Wada 1972). The genome of the donor oyster is still present in the pearl sac of the recipient at the end of pearl formation. By genotyping the pearl sac and comparing its microsatellite alleles with those in the corresponding host oysters and donor oysters, it was shown that DNA originating from the donor oyster can still be detected in the pearl sac at pearl harvest (Arnaud-Haond et al. 2007). To try to understand the interplay between host and donor genetic contribution in pearl formation, McGinty et al. (2011) produced xenografts between two *Pinctada* species, *P. maxima* and *P. margaritifera,* to examine which species-specific nacreous genes (N66 and N44) were expressed in the pearl sac. The authors showed that the cells originating from the donor oyster actively secrete nacreous shell matrix proteins and likely contribute to the biomineralisation process of pearl development, whereas there is no expression of these same genes in the host oyster itself (McGinty et al. 2012). The persistence of the donor oyster DNA and its activity in the pearl sac supports the observation made by professional grafters that some pearl quality traits are influenced by the donor oyster.

Given the differences in nacreous deposition observed in the present study and the importance of cultured pearl size and weight to the value of a pearl, there may be advantages to the industry of using donor pearl oysters that exhibit rapid nacreous deposition. The greater the nacre deposition rate (as measured by nacre weight) for oysters implanted with a nucleus of the same size, the thicker the resulting pearl should be and the higher the pearl value (given consistency in the other quality traits). Expression studies of candidate gene transcript levels by real-time quantitative polymerase chain reaction (qPCR) implicated in high biomineralisation capabilities should be conducted.

4.3 Cultured pearl colour: darkness and "visual colour categories"

Our results clearly demonstrate a highly significant influence of donor family on both cultured pearl colour darkness and "colour categories".

When used as graft donors, wild and hatchery-bred *P. margaritifera* showed the same capacity to deposit the dark-toned nacre that contributes to cultured pearl darkness. Indeed, pearl characteristics in the present donor family experiment and previous wild donor experiment (Tayale et al. 2012) showed the same mean proportions of pearls of different darkness levels: low and medium/high darkness levels represented 25% and 75%, respectively, in both experiments. The darker levels correspond to the so called "black pearl" produced by using *P. margaritifera* as donors in receiving oysters of the same or other species. This dark tone is due to the production and deposition of pigments (Elen 2001), e.g.; melanin and metalloporphyrins have been implicated (Landman et al. 2001). The xenografts used in the study of McGinty et al. (2010), reveal that when a *P. margaritifera* donor was used, the resulting pearls had colours with a black base, consistent with those of *P. margaritifera*, regardless of the host oyster species. In fact, *P. margaritifera* naturally secretes a serie of pigments, which, depending on their quantity, give predominantly black pearls, with a base colour ranging from black to grey.

A highly significant family effect was detected for pearl colour using our classification into six visually-perceived colours. Some families clearly showed a high proportion of one colour rather than another, which could reach up to 70%. In pearls from family B donors, for example, nearly 70% of the cultured pearls were green but, out of those from family I donors, only 40% were green. These differences in colour among families correspond to the difference in the pigment production (Karampelas et al. 2011); structure of the nacre, which is under genetic control, seems also to play a role (Snow et al. 2004). These findings suggest that selection of a colour tendency for a pearl could be made possible by using individuals from one donor oyster family rather than another. Although research on colour determination in pearls is limited, the few studies that have addressed aspects of pearl colour agree with our findings and suggest that the donor oyster influences the pearl colour (Tayale et al. 2012). The use of reciprocal xenografts involving two *Pinctada* species that produce pearls with distinctively different base-colours (*P. maxima*, the silver-lip pearl oyster, and *P. margaritifera*, the black-lip pearl oyster) showed that the donor oyster is the primary determinant of pearl colour (McGinty et al. 2010). It was observed that in the majority of cases when a *P. maxima* donor was used, the resulting pearl had a white to silver base colour consistent with

that of *P. maxima*, whilst *P. margaritifera* donors produced pearls with a gray to black base colour consistent with that of *P. margaritifera*, regardless of the host oyster species. Our results revealed for the first time, by using farming "pearl oyster" donors, that each of the families have a specific and different "colour signature pattern", that opens the way for genetic selection through progeny testing (Verrier et al. 2009). Colour variation is also likely to be influenced by environmental factors, but in our experiment, these factors were minimised, making the inter-family effect important: all grafted oysters were cultured at the same location, the grafting process were realised by the same grafter, and the receiving pearl oysters provided from a unique spat collection.

Further research is underway to develop an objective and non-destructive colour determination method for routine use and turn this subjective quality trait into quantitative trait. In fact, visual colour perception is a combination of two components: 1) bodycolor, which is a result of pigment deposition; and 2) overtone, which consists of the physical/ secondary colours (Sarikaya and Aksay 1995). Overtone is determined by the way in which light is reflected, diffused and/ or diffracted through the various outer layers of nacre forming the cultured pearl and its colour is a subjective characteristic that may depend on individual perception (Ward 1995). Some techniques are available to help reduce subjectivity in assessing pearl colour, such as UV-visible spectrophotometry (Mamangkey et al. 2010). This technique has yet to be applied for quantification of pearl colour from *P. margaritifera* (Komatsu and Akamatsu 1978; Elen 2001). These studies showed that pearl colour could be clearly characterized by peaks in reflectance spectra, which are correlated with the presence of particular pigments in the nacre layers (Karampelas et al. 2011).

4.4 Cultured pearl shape, grade and their components

Significant differences in pearl shape categories were detected for the first time among pearls produced with donors from the different *P. margaritifera* progenies. Cultured pearl shape is said to be influenced by the ability of the grafter to implant the nucleus into the gonad of the recipient oyster (pearl farmers pers. comm.). This cannot be the case in our experimental graft, as a same professional grafter, who usually operates on this species at the Gauguin's Pearl commercial farm, was employed for all of the grafting operations. For *P. maxima*, heritability estimates indicate that shape exhibits low levels of additive genetic variance, suggesting that other factors such as environmental ones may dramatically influence the shape of the cultured pearl (Jerry et al. 2012). Our results suggest that for *P. margaritifera*, donor oyster selection would be effective in improving the shape of pearls (i.e., increasing the proportion of round and semi-round pearls). In contrast, the presence of circles on cultured pearls was not influenced by donor family. It is not clear what factors could affect the shape of a cultured pearl, and only a few pearls in fact have a round shape. Pearl shape is mostly influenced by the shape and quality of the nucleus, and in order to create a round pearl, a perfectly round nucleus is required, although this does not always guarantee the formation of a round pearl.

Cultured pearl grade and its two components, surface defects and lustre, show significant differences among the progeny tested. When pearls are graded, the appearance of the surface of a pearl is one of the most important characteristics in determining its overall desirability and value. In evaluating the degree of imperfection, the number of defects is taken into account, that is, whether the pearl has clean surface or one or more spots. For cultured pearl surface defects, the proportion of cultured pearls showing no defects or only 1 to 5 defects was different from the proportion obtained by Tayale et al. (2012) on the same species, using wild donors. The proportion of pearls with no defects was 37% on average in the experiment with wild donors but only 7% in the present study with donor families. The proportion of pearls with 1 to 5 defects was 28% on average for the pearls from the wild donors and 48% for those from the progeny donors. In contrast, the proportion of pearls with more than 5 defects was comparable between the two experiments, at nearly 40%. There was clearly a family effect on the number of pearl surface defects and a difference between donors from the families and those from the wild, especially for the most valuable pearls i.e., those with no defects. A pearl's surface lustre is critical in the evaluation of its quality. For lustre, 86% of the cultured pearls produced with the grafts from the families showed lustre, which is consistent with the data previously obtained with wild donors 92% (Tayale et al. 2012). The same tendency was observed for cultured pearl grade, with the A-B grade (the most valuable pearls) representing 27% in the progeny donor study and 23% in the wild donor study. Further research is underway to study correlations between traits as, for example, pearls from family B grafts were never free of defects but this family had the maximum lustre. The lustre of a pearl may be closely related to its homogeneity, light transmittance and nacre thickness (Agatonovic-Krustin and Morton 2012). In this case, family B was also one of the progenies from which the pearls had the thickest nacre.

Quality of cultured pearls therefore depends greatly on the selection of appropriate donor oysters. Among the quality traits, only lustre can be predicted from the appearance of the donor, as selection of the donor oysters on the basis of their nacre lustre may contribute to the quality of the lustre of the resulting pearls (Gervis and Sims 1992). In contrast, selection of donor oysters that could produce high proportions of grade A, or R shape categories remains difficult.

5 Conclusion: Potential genetic improvement for cultured pearl quality

The individual wild *P. margaritifera* donor used was shown to be a primary determinant of seven cultured pearl quality traits: nacre thickness, cultured pearl weight, surface defect, lustre, grade, darkness and colour categories in a previous study (Tayale et al. 2012). The extension of this research made in the present study reveals for the first time that full-sib families produced from wild donors also have significant determinant effects on these seven traits and on the additional cultured pearl quality criteria: nacre weight, circle presence and shape categories. Nucleus retention capabilities were also significantly different between receiver oysters grafted with tissue from the different families. However, further studies are

required to confirm this result by: 1) testing other G1 families and 2) evaluating the impact of environment by rearing the grafted oysters on different farms in contrasted geographic locations. From a molecular point of view, nacre-based crystal formation is a complex biomineralisation process involving numerous genes, some of which could be used for marker-assisted selection.

Attempts must be made improve our understanding of the genetic basis of *P. margaritifera* quantitative traits. The most important genetic parameters of quantitative traits are genetic correlation and heritability. For selective breeding, it will be necessary to quantify parameters such as heritable variance and the ratio of heritable to non-heritable variance (Lynch and Walsh 1998). Sufficient additive genetic variance in a selected trait is a prerequisite for selective breeding and a good breeding efficiency is possible when levels are high (Kvingedal et al. 2010). Research into phenotypic and genetic correlations plays an important role in a breeding program as a whole because genetic correlation gives us an understanding of the genetic relationship between different traits and an accurate estimation of their breeding value.

From an applied point of view, new management perspectives and strategies for the black pearl industry in French Polynesia will require the establishment of hatcheries to produce selected pearl oyster donor lines. Promising G1 families revealed by experimental grafting (progeny testing) could be used as future broodstock for hatchery production. The main advantage of using mass G1 families is that: 1) individuals in families are more genetically homogeneous than wild individuals; and 2) only a sample of individuals per family are sacrificed as part of the experimental grafting process, which allows their siblings to be retained for use as future broodstock. Furthermore, it may be possible to reach a point where specific donors, capable of producing pearls of specific colours, can be identified and their families maintained as specific breeding lines. Farmers wishing to increase their production of pearls of a particular colour, or with a larger size, could thus use the coloured or heavier inbred lines as mantle tissue donors. Such changes would probably not affect the socio-economic structure of the farming activity, as most of the work is devoted to recipient oysters and sustained by wild spat collection.

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