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Age-dependence of cultured pearl grade and color in the black-lipped pearl oyster *Pinctada margaritifera*

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Abstract :

Pinctada margaritifera is an economically important marine bivalve species for cultured pearl production in French Polynesian aquaculture. In order to evaluate the influence of donor oyster age on pearl quality traits, experiments were conducted over 6 years using both grafts and surgreffe operations. At harvest, 6 pearl quality traits were recorded and compared: surface defects, luster, grade, darkness level, and visual color. Analyzing the quality traits of pearls harvested in the initial graft process and those of pearls obtained from surgreffe experiments allowed a comparison of the influence of pearl sac cells originating from the initial mantle graft, which aged together with their recipient oysters. The results demonstrated a significant decrease between these successive grafts in luster, grade (A-B-C,) darkness level, and green color – traits that are of major importance in the pearl market. The duplicated graft experiment allowed the comparison of donor oyster families at 2 and 5 years old, where a mantle graft was inserted into recipient oysters aged 2.5 years old. The results showed the same tendencies to a lesser extent, with 1) an improved pearl grade, predominantly through a most important rate of 0 surface defect category, and 2) a green / grey ratio in favor of the younger donor. A comparison between the graft-surgreffe and the duplicated graft experiments also highlighted: 1) the indirect role played by the younger recipient oysters, which must be optimized for optimal pearl quality realization, and 2) the complex interplay between donor and recipient oysters.

Keywords : Pearl oyster age, *Pinctada margaritifera*, surgreffe, pearl grade, pearl color

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1. Introduction

Pinctada margaritifera (Linnaeus, 1758) (*Mollusca*, *Bivalvia*, *Pteriomorpha*) is a marine pearl-producing mollusk, principally cultivated in French Polynesia. *P. margaritifera* is found throughout the coral areas of the Indo-Pacific, but is particularly abundant in the atolls of French Polynesia. Cultured Tahitian pearl production is based on a surgical operation, which consists of introducing a round nacreous bead (these "nuclei" are made from the shell of a freshwater mussel from the Mississippi River) into the gonad of a "recipient" oyster, together with a small piece of mantle tissue (a graft of ~4 mm²) from a dissected "donor" oyster (Kawakami, 1952; Haws, 2002). A recent report from the *Institut de la Statistique de la Polynésie Française* (Talvard and Challier, 2015) summarized 2013 pearl production data. During this year, pearl production was conducted on 25 islands and atolls, compared with 28 in 2008. The number of maritime concessions was 517, which is 50 more than in 2008. The two most productive archipelagos were the Tuamotu and Gambier Archipelagos, which represented 398 (6300 ha) and 79 (1260 ha) concessions, respectively. Cultured pearl exportation made 65 billion euros for French Polynesia, which constituted the first increase (+10%) for this industry since 2007. The two main places importing Tahitian cultured pearls were Japan (50% of the exportation volume) and Hong Kong (46%). Several auctions also took place in Tahiti in 2013 where the average pearl price reached up to 1000 FCP per unit; this is the first time such a figure has been achieved in the last 5 years.

Tahitian cultured pearl quality is assessed according to an official A–D classification (*Journal Officiel*, 2005). This grading system takes into account two physical parameters: 1) the perfection of the pearl surface and 2) its luster. Overall, there are five grades: 1) Top Gem: a perfect cultured pearl with an excellent luster; 2) Grade A: a surface that is 90% free from imperfections, with a very beautiful luster; 3) Grade B: a smooth surface to 70% of the pearl, with a good luster at minimum; 4) Grade C: a smooth surface to 40% of the pearl, with a medium luster at minimum; and 5) Grade D: weak luster, with small imperfections on more than 60% of the pearl. Under Tahitian Government regulation, cultured pearls of a quality below Grade D cannot be exported from Tahiti, although they can be sold locally. The surface quality is judged by looking at diverse imperfections such as dimples, bumps, stripes, curls, grooves, organic deposits, swellings, growths, and milky, discolored spots. The luster (or shine) refers to the more-or-less perfect reflection of light from the surface of the pearl (Blay *et al*., 2014). Color is generally linked to pearl value for *P. margaritifera* in the Asian market: the darker it is, the more valuable the pearl is. The predominant body colors of *P. margaritifera* cultured pearls are grey, yellow, and white. Overtones (secondary colors) may be present in a variety of combinations, including green, aubergine (reddish purple), and peacock, and are considered a plus factor. A completely black pearl with no overtones is considered less desirable and may be worth 50% less than one of a similar quality with green overtones (Ky *et al*., 2014a).

In the context of a breeding program for pearl quality traits, an understanding of the influence of genetics and the environment, as well as the interactions between the two, is essential to ensure maximum genetic gains in relation to the aquaculture of this particular pearl oyster species, as multiple grow-out locations are used for the end product. To date, studies on the basis of pearl quality traits have mainly focused on the genetics of the donor oyster. Indeed, the influence of the donor on pearl quality traits has been definitively demonstrated using reciprocal xenografts between *P. maxima* and *P. margaritifera* oysters, in which donors were found to have a significant influence on both color and surface complexion (McGinty *et al*. 2010). The xenografts revealed that when a *P. margaritifera* donor is used, the resulting pearls exhibit colors with a black base (consistent with those of *P. margaritifera*), regardless of the host oyster species. Tayale *et al*. (2012) and Ky *et al*. (2013) demonstrated significant donor and family effects on pearl-color darkness and visually perceived color (body color and overtone), pearl surface defects, luster, and grade in relation to *P. margaritifera*, using individual wild donors

and hatchery-bred families. The influence of the environment in the realization of pearl quality traits has also been reported to be particularly important, as shown by the recent study on *P. margaritifera* conducted on Tahaa Island and Rangiroa atoll, where overall inter-site comparison revealed that: 1) all traits were affected by grow-out location, except for luster, and 2) a higher mean rate of valuable pearls was produced in Rangiroa (Ky *et al*., 2015a). In relation to *P. maxima*, significant interactions between cultured pearl color and luster were observed by Jerry *et al*. (2012) at two commercial Indonesian grow-out locations (Bali and Lombok).

Despite this existing knowledge about the complex interplay between donor, recipient, and environment, no studies have examined the effects that the age of the oysters might have on cultured pearl quality traits in *P. margaritifera*. Presently, most pearl production is realized by using both donor and recipient oysters of approximately 2 to 3 years old. This age range could be exceeded if surgreffe operations were performed. In fact, recipient oysters that produce pearls fitting the criteria for good quality may be seeded with another nucleus to produce larger pearls during a subsequent culture period. Such surgreffe operations can be performed several times (3 to 4 times maximum), over the course of which the recipient oysters will naturally age. The aim of our study, therefore, is to evaluate the possible influence of oyster age on cultured pearl quality traits in *P. margaritifera* – namely, grade, surface defects, luster, and color and its components (darkness level, body color, and secondary colors). The present study was based on experimental grafts and surgreffe methods, in which the grafting process was kept as uniform as possible by using the same expert grafter, nucleus size, graft site, and method (as used for commercial grafting), donor oyster families (hatchery-produced), and recipient oyster source. This study will help with management and propagation of future oyster line breeding programs in hatchery systems.

2. Materials and methods

2.1. Surgreffe experiment: donor and recipient oyster aging

Ten bi-parental families of *P. margaritifera*, produced in the Ifremer hatchery facilities in Vairao (Tahiti, French Polynesia), were used as donors in a previous graft experiment (Ky *et al.* 2013). First, a pool of corresponding recipient oysters aged 2.5 years old were grafted as part of this previous experiment (Ky *et al.* 2013), then they were used in the present surgreffe trial at 4 years old by inserting a second nucleus into the oysters (Figure 1). In order to minimize environmental effects, the surgreffe experiment was undertaken 1) on a single grow-out site on Rangiroa atoll (Tuamotu Archipelago, French Polynesia); 2) on one pearl farm (Gauguin's Pearl Farm), overseen by one professional grafter, so as to minimize differences (the same grafter who performed the initial grafts described in Ky *et al.*, 2013); and 3) with the same nucleus size and brand: 3.5 BU nucleus (10.48 mm diameter and 1.84 g weight – Nucleus Hyakusyo Co., Japan). Figure 1 shows that, of the pearl oysters initially grafted, 711 were not used for the surgreffe experiment. This was due to mortality, rejection during culture, and shell breakage caused by forced opening during harvest of pearls from the initial grafting operation. Consequently, 783 recipient pearl oysters were used in the surgreffe experiment (Figure 1). After the surgreffe operations had been undertaken, the recipient pearl oysters were put on to chaplets in groups of 10 and were covered with a plastic mesh to avoid predation. During the culture time, the pearl oysters were cleaned every 6 months using high pressure seawater (Kärcher®).

2.2. Duplicate graft experiments: donor oyster aging with pools of recipient oysters of fixed age

A bi-parental family named F616 (produced in hatchery system at the Ifremer facilities in Vairao) was used as the donor oyster family for two distinct experimental grafts: at 2 years old (G_{D2}) and 5 years old (G_{D5}) (Figure 1). The two grafts were undertaken by the same professional technician. A total of 30 donors for G_{D2} and 25 donors for G_{D5} were used to perform 600 and 500 grafts, respectively (20 grafts per donor) at Gauguin's Pearl Farm (Rangiroa atoll, Tuamotu Archipelago), under the same conditions as for a commercial graft (Ky *et al*., 2014b). The two batches of 600 and 500 recipient pearl oysters came from natural spat collection from the wild in the same geographic region (Ahe atoll) and were collected during the same spat collection seasonal period (at the end of each year). They were all aged around 2.5 years old and were selected based on visible health status (color of the visceral mass and gills), shell size appearance, and muscle resistance when the shells were pried open. Each recipient was grafted using a 2.4 BU nucleus (7.304 mm diameter – Nucleus Bio, Hyakusyo Co., Japan). Following implantation, the recipient oysters were placed on chaplets in groups of 10 and were protected with plastic mesh to avoid predation. During the culture time of 18 months, the pearl oysters were cleaned every 6 months using high pressure seawater (Kärcher®).

2.3. Measurement of cultured pearl quality traits

The cultured pearls were cleaned in soapy water (hand washed) via ultrasonication, using LEO 801 laboratory cleaner (2-L capacity, 80 W, 46 kHz); they were then rinsed in distilled water. The surface defects, luster, darkness, and colors of the cultured pearls were evaluated visually (without a loupe) by two operators working in cooperation with one another.

The surface defects and luster (both components of the cultured pearl grading system) were determined separately so that they could be studied independently. Visible sample surface defects, including pits, bumps, scratches, deposits, and other surface flaws, were counted visually (without a magnifier), and each cultured pearl was then classified into one of four categories: 1) no defects, 2) 1 to 5 defect(s), 3) 6 to 10 defects, and 4) up to 10 defects (Figure 2a). Pearl luster was evaluated as follows: presence of luster (glossy and shiny) and absence of luster (matte appearance).

The cultured pearl grade for each sample was determined by a single professional expert from *Maison de la Perle*, according to the official Tahitian grading system, from the most valuable to the least: A, B, C, D, and rejects (*rebuts*) (*Journal Officiel*, 2001). Rejects are cultured pearls that have too many defects to be graded. These pearls were discarded and, ultimately, destroyed.

Two kinds of color evaluation (without loupe) were made in relation to the cultured pearls (Tayale *et al*., 2012): 1) the darkness of the color, categorized into one of three groups, depending on the level: high, medium, or low; and 2) the visually-perceived color, caused by pigment (body color) and secondary color (overtone). Eight "color categories" (Figure 2b) were detected, into which all the harvested pearls were classified (body colors: grey, white and yellow; secondary colors: green, aubergine, blue, champagne, and peacock – this last being a mixture of aubergine and green).

2.4. Statistical analysis

For the surgreffe experiment, the family effect among the 380 harvested cultured pearls obtained from the ten families was analyzed using a Chi-square test for all variables (Siegel and Castellan, 1988; Winer *et al*. 1991). Of the 380 cultured pearls harvested, 295 could be paired with pearls harvested from the same oysters in the graft experiment (Ky *et al*., 2013) to the present surgreffe experiment. These 295 paired samples made it possible to perform a comparison between graft and surgreffe methods in relation to: 1) luster classes (presence / absence of luster, using a McNemar Chi-square test) and 2) surface defects, grade, darkness level, and color categories (using a Friedman test) (Hutchinson, 1996).

For the duplicated graft experiments, a comparison between G_{D2} (N = 427) and G_{D5} (N = 329) was made using: 1) a Chi-square test for the luster classes and 2) a logistic multinomial regression for surface defects, grade, darkness level and color. The same tests were used to compare the quality traits of pearls obtained from G_{D2} and G_{D5} donors in the surgreffe experiment.

All the tests were performed using XLSTAT (version 2009.4.02), and *p*-values lower than 0.05 were considered significant (Dagnelie, 2007).

3. Results

3.1. The effect of the surgreffe method on cultured pearl luster, surface defects, and grade

For the 380 harvested pearls produced using the surgreffe method, the overall rate of pearls with luster was 45.8% (N = 174), with minimum and maximum values of 34.0% (Family A, N = 18 among 53 harvested pearls) and 57.1 % (Family F, N = 20 among 35 harvested pearls), respectively. The corresponding average rate of pearls without luster was 54.2% (N = 206). A comparison between the surgreffe and graft results (N = 295) revealed a highly significant difference ($p < 0.0001$) in average luster pearl rates of 47.8% (surgreffe) versus 89.1% (initial graft) (Table 1). Thus, there was a -41.3% decrease in the luster pearls obtained from the surgreffe method. Concerning the surface defect trait, the overall rate of pearls (N = 380) with no defects was 1.0% (N = 4), 1 to 5 defect(s) was 9.2% (N = 36), 6 to 10 defects was 18.2% (N = 69), and up to 10 defects was 71.6% (N = 271). For the “up to 10 defects” category, family B showed the highest pearl rate (86.2%), whereas, family I gave the lowest rate (55.9%). A comparison of the surgreffe and graft results (N = 295) revealed a highly significant difference ($p < 0.0001$) between the rates of surface defects. In particular, there was as much as a 7 fold rise in pearls presenting up to 10 defects with the surgreffe method, in comparison with the graft method (Table 1). In addition, the rate of pearls with no defects was 7 times smaller. Using surgreffe, the overall rate of Grade A pearls harvested was 0.5% (N = 2), with Grade B at 1.8% (N = 7), Grade C at 16.0% (N = 61), and Grade D at 47.6 (N = 181). The rate of reject pearls was 33.9% (N = 129). A comparison between the surgreffe and graft results (N = 295) revealed a highly significant difference ($p < 0.0001$) between all the grade categories. In particular, the average of the pearls categorized in classes A + B was nearly 12 times lower using surgreffe than in the corresponding graft experiment (Table 1).

No significant donor family effect was observed for the presence/absence of luster in pearls obtained using the surgreffe method ($p = 0.372$). The family ranking, from the greatest to the smallest amount of pearls with luster, was: F, E, I, C, H, D, J, B, G, A. Considering the families separately, significant differences were found in the numbers of luster pearls

obtained in the surgreffe and graft experiments in all families, except for E (p = 0.206) and G (p = 0.102). In addition, no significant donor family effect was observed for the surface defect category using surgreffe (p = 0.484). Considering the families separately, highly significant differences were found for surface defects between surgreffe and graft methods for all families. By contrast, a significant donor family effect was observed for the grade categories when surgreffe was used (p = 0.005). The family with the highest rate of Grade A pearls was Family F (2.9%), and Family A had the highest level of reject pearls (56.6%). In terms of the individual donor families, very highly significant differences were found between surgreffe and graft methods across all grade categories for all the families (Figure 3).

3.2. Surgreffe effect on cultured pearl darkness level and color

Looking at the pearl darkness level obtained using surgreffe, the overall rate among the 380 harvested pearls was 20.3% high darkness-level pearls (N = 77), 54.5% moderate darkness-level pearls (N = 207), and 25.3% low darkness-level pearls (N = 96). Comparing the surgreffe and graft results (N = 295) revealed that there was a significant difference (p = 0.04) in high darkness levels, whereas no significant effect was observed for the moderate and low levels (Table 2). Thus, there was a -9% decrease in the high darkness levels achieved with the surgreffe method. In terms of the pearl colors obtained by surgreffe, the overall rates among the 380 harvested pearls were 40.8% green (N = 155), 33.7% grey (N = 128), 9.7% aubergine (N = 37), 7.9% champagne (N = 30), 5), 3.7% yellow (N = 14), 3.4% white (N = 26), and 0.8% peacock (N = 3). A comparison between the surgreffe and graft results (N = 295) revealed significant inter-family differences for the green (p = 0.002) and grey (p = 0.042) color categories, whereas no significant effect was observed for the other colors. Thus, the trend exhibited was a decrease in green pearl from the graft to the surgreffe method (-12%) and an increase in grey pearls (+7.5%) (Table 2).

A significant donor family effect was observed for pearl darkness levels obtained with the surgreffe method (p < 0.0001). Family G demonstrated the highest number of dark pearls on average (nearly 40%), in comparison to Family I, where no dark pearls were found. The family ranking, from highest to lowest amount of dark pearls, was: G, H, A, F, J, C, D, B, E, I. Considering the families separately, no significant differences were found between surgreffe and graft methods for pearl darkness levels, except for Family B (p = 0.040) and Family J (p = 0.013). In addition, a significant donor family effect was observed for pearl colors obtained using surgreffe (p < 0.0001). Family B had the highest average number of green pearls (65.5%), whereas Family H had the lowest number, with only 17.8% green pearls. Considering the families separately, no significant differences were found between the surgreffe and graft methods for the pearl color statistics, except for in Families H, B, and A (Figure 4).

3.3. Duplicate graft effect on cultured pearl luster, surface defects, and grade

For the luster pearl trait, a comparison between G_{D2} and G_{D5} revealed no significant difference (p = 0.140) between the average rate of pearls with luster obtained in the two experiments (Table 3). Thus, the age of the donor (2 years old vs. 5 years old) does not seem to affect the luster trait.

In contrast, in terms of pearl surface defects, a comparison between pearls harvested from G_{D2} and G_{D5} revealed a very highly significant difference (p < 0.0001) for the “no defect” category, where the rate was nearly double with a 2-year-old donor than with a 5-year-old donor. In addition, for the “6 to 10” category, a very highly significant difference (p < 0.0001) was observed, with many more defects appearing in the 5-year-old group. However, no

significant differences were observed for the “1 to 5” (p = 0.924) and “> 10” (p = 0.140) defects categories.

With regard to the grades of the pearls, a comparison between the G_{D2} and G_{D5} groups revealed no significant difference (p = 0.871) for Grade A. The grade category B demonstrated a very highly significant difference (p = 0.001), with nearly three times more Grade B pearls being produced from 5-year-old donors than from 2-year-old donors. Lastly, grade categories C, D, and R exhibited significant differences between 2-year-old donors and 5-year-old donors (p = 0.011, p = 0.033, and p = 0.030 respectively). Indeed, 2-year-old donors produced, on average, 10%, more Grade C pearls and 10% fewer Grade D and Grade R pearls, than 5-year-old donors.

3.4. Duplicate graft effect on cultured pearl darkness level and color

No difference was found between the pearls harvested from the G_{D2} and G_{D5} donors in terms of darkness level (Table 4): a similar range of darkness level was observed, with the most pearls in the moderate category, accounting for nearly 80%.

Where visual pearl color is concerned, significant differences for pearls harvested from G_{D2} and G_{D5} donors were observed for the two most abundant colors, green and grey, (Table 4). Indeed, the rate of green pearls obtained was 3.6 times greater when using grafts from 2-year-old donor oysters than when using 5-year-old ones. For grey colored pearls, the amount obtained was 1.5 times greater when using grafts from 5-year-old donor oysters than when using 2-year-old donors.

For the minority colors, no significance differences between the donor groups were observed for the peacock and white categories (p = 0.702 and p = 0.618, respectively). In contrast, a very highly significant difference was seen in relation to the aubergine and blue color categories (p < 0.0001 and p = 0.001 respectively), with the average for aubergine pearls being 35 times lower and the average for blue harvested pearls being 7 times higher for the 2-year-old donor than for the 5-year-old donor. Moreover, a highly significant difference was found between the 2-year-old donor and the 5-year-old donor for yellow pearls (p = 0.007), with the 5-year-old donor giving 4 times more yellow pearls on average.

4. Discussion

The present study is the first to research the impact of pearl oyster age on pearl grade (including luster and surface defects) and color (in terms of darkness level and visual pigment) in *P. margaritifera*. As the cultured pearl production cycle in French Polynesia usually uses oysters of a common age (around 2.5 years old) for graft operations, a better understanding of how age could modulate pearl quality traits is important for managing resource inputs (pearl oysters) and outcomes (cultured pearls). During the weeks following a graft operation, a pearl sac is formed in the gonad of the recipient oyster via cellular multiplication in the graft cells originating from the donor oyster (Machii, 1968; Inoue *et al*., 2011). Studies of the ultrastructures of pearl sacs from *Pinctada fucata martensii* have shown that they develop from the epithelial cells of mantle graft tissues (Du *et al*., 2010). Microsatellite analysis suggests that DNA originating from the donor oyster can still be detected in the pearl sac of pearl oysters (Arnaud-Haond *et al*. 2007). The biomineralisation process is the property of the epithelial cells of the external mantle of *P. margaritifera* (and by extent in mollusc with shells) to produced an organic matrix that controls nucleation, orientation, growth and the polymorphism of the calcium carbonate formed as aragonite and/or calcite from shell (Belcher et al., 1996). matrix proteins play a major role in shell

biomineralization process. Genes encoding some matrix proteins have been identified and are known to be specifically involved in the formation of the nacreous layer and/or the prismatic layer (Joubert *et al*., 2010; Montagnani *et al*., 2011; Marie *et al*., 2012). Moreover, studies of the expression of two species-specific biomineralization genes (N66 and N44) in two pearl oyster species (*Pinctada maxima* and *Pinctada margaritifera*) have revealed that donor oyster biomineralization genes are transcriptionally active in the pearl sac at the time of pearl harvest (McGinty *et al*. 2011). In the present study, the age of the cells derived from donor oysters was analyzed in two ways: 1) through aging together with the recipient oyster, studied via the surgreffe operation, and 2) by using fixed-age recipients in two separate graft operations, where the ages of the donors were 2 and 5 years, respectively.

Overall, the results clearly demonstrate that there is a tendency for pearl quality traits to decrease in line with the aging of the donor oysters. In the surgreffe experiment, and concerning pearl grade and its components, the grades A, B, or C were obtained in up to 3 times fewer pearls than following the graft operation. This is consistent with most of the surgreffe harvests that have been observed in *P. margaritifera* production, where luster, in particular, has been found to be reduced (farmers' com. pers.). This finding can probably be attributed to the increase in the biological age of both the cells in the pearl sac, originating from the donor oyster (3.5 years old in the graft operation harvest and 5.5 years old in the surgreffe operation harvest), and the recipient oysters themselves (4 years old in the graft operation harvest and 6 years old in the surgreffe operation harvest). Indeed, aging cells are characterized by several detrimental changes that cause differences in gene expression between younger and older individuals in the animal kingdom. For example, the more pronounced changes in expression of stress genes seen in younger individuals of the Antarctic bivalve *Laternulla elliptica* as a response to injury in Husmann *et al.*'s study (2014) were in line with the age-dependent physiological differences witnessed elsewhere in marine bivalves (Philipp and Abele, 2010). Potentially, this might indicate that the oysters are in better physical condition, which is corroborated by the higher mortality rates found in younger individuals following the graft operations in our study (15.9%), compared with older individuals in the surgreffe experiment (8.0%).

In the duplicate graft experiments that used recipient oysters of the same age, but donor oysters of two ages (2 and 5 years old) the grade classification rate was not clearly in favor of younger donor oysters. However, the statistics for surface defects showed that the younger individuals tended to be of a higher quality. This last trait has been shown previously to be influenced by donor oysters, as demonstrated by the xenograft experiments by McGinty (2010) using *P. maxima* and *P. margaritifera* . Here, the effect of the donor species on pearl complexion was found to be highly significant, whereas the host species had no apparent influence on this trait. In fact, implantation with $P.\ maxima$ mantle tissue produced pearls with smoother complexions (i.e., higher grades) than implantation with $P.\ margaritifera$ tissue, regardless of the host oyster species. As grade classifications are based on luster and surface defect assessments, and a comparison between the two grafts in our study revealed similar rates of both pearl luster and surface defects (in the "1 to 5" category), this may help to explain why effects of youth of the donor oyster in the duplicate experiment were not comparable to those in the graft-surgreffe experiment. This highlights the indirect role played by the age of the recipient oyster, which was fixed and young at harvest time in the duplicate graft, in comparison with the graft-surgreffe experiment, in which the recipients were older at harvest time: 4 years old and 6 years old, respectively.

Relations and interactions between recipient oysters and their rearing environments may also play a role, as the pearl luster trait is known to be affected mostly by the environment (Ky *et al*., 2015b). Indeed, Snow *et al*., (2004) hypothesized that pearls with a smooth surface and brilliant luster are produced when consistent and regular crystal formation occurs, with their experiment confirming the hypothesis during winter, when nacre deposition is at its slowest. Nacre-based crystal formation is a complex biomineralization process in the

mantle tissue, which involves numerous genes (Wang *et al*., 2009; Miyazaki *et al*., 2010; Marie *et al*., 2012). Our results indicate that the luster trait is not affected by the donor oyster's age, as no significant difference was observed in the duplicate graft. However, a difference in luster incidence was observed in the graft-surgreffe experiment, where its rate was halved. This finding is supported by the similar pearl luster rate found between the grafts realized before the surgreffe experiment and the average rate obtained in the duplicated graft (using 2 year-old donor oysters), which was 89.1%. Similarly, in McGinty's study (2010), no significant differences in pearl luster grades were evident among the various xenograft combinations, hosts, and donor species used with *P. maxima* and *P. margaritifera*. We should therefore ask how a recipient oyster affects the rate of luster in developing pearls. A reduction in the recipient's vigor with age could be an explanation, as the recipient regulates the metabolism of the pearl sac. Indeed, the pearl sac depends on the recipient oyster for the supply of nutrition throughout the period of pearl formation. A strong host oyster can provide sufficient nutrition and, potentially, a more suitable environment for the pearl sac, resulting in the greater vigor of the pearl sac, promoting nacre secretion rates (Yukihira *et al*., 1998). This has been seen with young oysters, where maximum shell growth was observed, in comparison with older individuals, where the growth rate slowed down (Pouvreau *et al*., 2000). In addition, the genomes of the recipient oysters may regulate the expression of biomineralization genes in the pearl sac. In this way, the expression levels of these genes are controlled by the host oyster and are involved indirectly in pearl luster expression.

Based on our results, the age of the mantle tissue derived from the donor oyster does not affect the pearl darkness level (except for in the graft-surgreffe experiment, where a correlation was found with high darkness).In terms of visual color, the two most common colors were affected in the two experiments (graft-surgreffe and duplicated grafts) as follows: 1) the rate of green pearls was significantly higher with young donor oysters, and 2) the rate of grey pearls was significantly higher in older donor oysters. In the existing literature, xenograft results have shown conclusively that the donor oyster is the primary determinant of pearl color (McGinty *et al*., 2010). The results from a study of *Pintada fucata martensii* has shown that the frequency of yellow pearls was significantly lower in a group produced by grafting mantle tissue from an inbred white line than from the brown lines (Wada & Komaru, 1996). In addition, a study of the digital color of *P. fucata martensii* has shown that the nacre color of the donor oysters contributes to the resulting pearl color (Gu *et al*. 2012). Recently, the pearl color in *P. margaritifera* was demonstrated definitively to be related to the inner shell phenotype coloration of the donor (Ky *et al*., 2015c). For the same wild spat collection location, frequency of colorful inner shells was higher in young individuals than in older ones. This was also confirmed with the older oysters used in successive surgreffe operations, which produced high frequencies of non-attractive, grey inner-shell phenotypes. Variations between inner shell colorations of juveniles and adults may suggest temporal variations in the fitness of the epithelial cells of the mantle, which was expressed as age-related green to grey pearl color variation. The aging of the mantle cells of the donor oysters seemed to alter the coloration in favor of the grey body color. Dark tone is known to be linked to the deposition of black pigment, in which melanins have been implicated (Elen, 2001; Landman et al., 2001). Tyrosinases have been implicated in shell formation and pigmentation (Hofreiter et Schoneberg, 2010; Cieslak *et al*., 2011) and catalyze melanin production (Sanchez-Ferrer *et al*., 1995). Thus the alteration of colour in favor of the grey body color observed could be caused by the high expression levels of tyrosinase genes, which are specific to the mantle tissues of pearl oysters (Aguilera *et al*., 2014) and vary with age.

5. Conclusions

This 6-year-long study into the use of both graft-surgreffe and duplicated graft operations to examine the impact of the cellular age of the graft mantle derived from the donor oyster on pearl traits is the first that seeks to help us understand the complex processes involved in the realization of pearl quality in *P. margaritifera*. Here, the graft-surgreffe experiment demonstrated that aging of the cells originating from the graft and recipient oysters leads to a significant decrease in luster, pearls of grades A,B and C, darkness levels, and rates of green pearls, all of which are traits of major importance in terms of demand in the Asian pearl market. The duplicated graft study highlighted the importance of the youth of both the donor and recipient oysters in relation to the prevalence of pearl surface defects and green and grey pearl color rates. It can be concluded that, although both donor and recipient oysters may be involved in pearl formation in *Pinctada margaritifera*, they probably play different roles. The present study has emphasized the role played by the donor oyster tissue, which influences the green / grey pearl color ratio to a great extent in comparison with the recipient oyster. The latter may play a more significant role in regulating the rate of nacre secretion during pearl development, thus affecting luster and grade in relation, predominantly, to the culture environment.

On a practical level, pearl farmers also appear to be aware of the impact of the age of the donor oyster being used for grafting in the pearl production process. Younger donor oysters, although they have limited mantle size for graft excision, must be considered for their quality. The genetic selection of larger individuals among hatchery progenies would, therefore, be of particular interest. Optimum shell-size selection would be also beneficial for recipient oysters, particularly in relation to surgreffe operations, where younger and larger recipient oysters could be used for initial graft in order to move the graft-surgreffe sequence forward in relation to recipient age and thus increase the rates of luster and color in pearls produced by the surgreffe method. Fundamentally, further studies are needed to trace biomineralization gene expression patterns sequentially, for example, in mantle grafts and pearl sacs at harvest.

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Table 1. Comparison between the graft and surgreffe experiments conducted with *P. margaritifera* in terms of cultured pearl luster (Yes: with luster; No: without luster), surface defects (0: no defects; 1: 1 to 5 defects; 2: 6 to 10 defects and 3: more than 10 defects), and classification grade (A, B, C, D, and R – reject). The first entry in each cell indicates the percentage contribution (%) for each of the quality trait categories in the graft or surgreffe operations. The second entry (in brackets) corresponds to the number of pearls observed in this category. The traits that were found to be significantly different in the graft and surgreffe operations ($p < 0.0001$) are denoted by ***.

Variables	Categories	Graft	Surgreffe	Significance
Luster	Yes	89.1 (263)	47.8 (141)	***
	No	10.8 (32)	52.2 (154)	***
Surface defects	0	7.5 (22)	1.0 (3)	***
	1 to 5	52.2 (154)	9.5 (28)	***
	6 to 10	30.2 (89)	14.6 (43)	***
	> 10	10.2 (30)	74.9 (221)	***
Grade	A	8.1 (24)	0.7 (2)	***
	B	24.1 (71)	2.0 (6)	***
	C	29.5 (87)	15.6 (46)	***
	D	27.8 (82)	50.5 (149)	***
	R	10.5 (31)	31.2 (92)	***

Table 2. Comparison between graft and surgreffe experiments for *P. margaritifera* in terms of cultured pearl visual colors (body colors: grey, white, and yellow; secondary colors: green, aubergine, champagne, and peacock), and darkness levels (high, moderate, and low). The second entry (in brackets) corresponds to the number of pearls observed in the category. The traits that were significantly different between the two variants, at $0.01 < p \leq 0.05$, $0.001 < p \leq 0.01$, and $p \leq 0.001$, are indicated with 1, 2, or 3 asterisk(s) (*), respectively (and NS for 'not significant').

Variables	Category	Graft	Surgreffe	Significance
Darkness	High	27.80 (82)	18.98 (56)	*
	Moderate	48.14 (142)	54.58 (161)	NS
	Low	24.06 (71)	26.44 (78)	NS
Color	Green	56.27 (166)	43.73 (129)	**
	Grey	23.39 (69)	30.85 (91)	*
	Aubergine	5.42 (16)	8.14 (24)	NS
	Champagne	4.75 (14)	7.80 (23)	NS
	Peacock	4.07 (12)	2.37 (7)	NS
	White	4.07 (12)	5.08 (15)	NS
	Yellow	2.03 (6)	2.03 (6)	NS

Table 3. Duplicate graft experiments comparing young (2-year-old) and old (5-year-old) *P. margaritifera* donor oysters in terms of cultured pearl luster (Yes: with luster; No: without luster), surface defects (0: no defects; 1: 1 to 5 defects; 2: 6 to 10 defects and 3: more than 10 defects), and classification grade (A, B, C, D, and R – reject). The second entry (in brackets) corresponds to the number of pearls observed in the category. The traits found to be significantly different between the two variants, at $0.01 < p \leq 0.05$, $0.001 < p \leq 0.01$, and $p \leq 0.001$, are indicated with 1, 2, or 3 asterisk(s) (*) respectively (and NS for 'not significant').

Variables	Categories	2 years	5 years	Significance
Luster	Yes	90.9 (388)	87.3 (288)	NS
	No	9.1 (39)	12.7 (41)	NS
Surface defects	0	34.2 (146)	16.1 (53)	***
	1 to 5	37.2 (159)	37.9 (124)	NS
	6 to 10	26.0 (111)	41.5 (137)	***
	> 10	2.5 (11)	4.5 (15)	NS
Grade	A	0.7 (3)	0.6 (2)	NS
	B	3.3 (14)	9.1 (30)	***
	C	27.2 (116)	36.1 (118)	*
	D	35.8 (153)	25.5 (94)	*
	R	33.0 (141)	25.8 (85)	*

Table 4. Duplicate graft experiments comparing young (2-year-old) and old (5-year-old) *P. margaritifera* donor oysters in terms of cultured pearl visual color (body colors: grey, white, and yellow; secondary colors: green, aubergine, champagne, and peacock), and darkness level (high, moderate, and low). The second entry (in brackets) corresponds to the number of pearls observed in the category. The traits found to be significantly different between the two variants, at $0.01 < p \leq 0.05$, $0.001 < p \leq 0.01$, and $p \leq 0.001$, are indicated with 1, 2, or 3 asterisk(s) (*) respectively (and NS for 'not significant').

Variables	Category	2 years	5 years	Significance
Darkness	High	9.4 (40)	8.2 (27)	NS
	Moderate	78.5 (335)	78.2 (257)	NS
	Low	12.2 (52)	13.6 (45)	NS
Color	Green	42.6 (182)	11.8 (39)	***
	Grey	44.7 (191)	68.2 (225)	***
	Aubergine	0.2 (1)	7.3 (24)	***
	Blue	4,4 (19)	0.6 (2)	***
	Peacock	4.0 (17)	4.5 (15)	NS
	White	2.8 (12)	3.3 (11)	NS
	Yellow	1.2 (5)	4.2 (14)	**

Figure 1. Experimental designs for the surgreffe method (following the experimental graft conducted by Ky *et al*., 2013) and the duplicated grafts. All operations were performed on Rangiroa atoll (Tuamotu Archipelago).

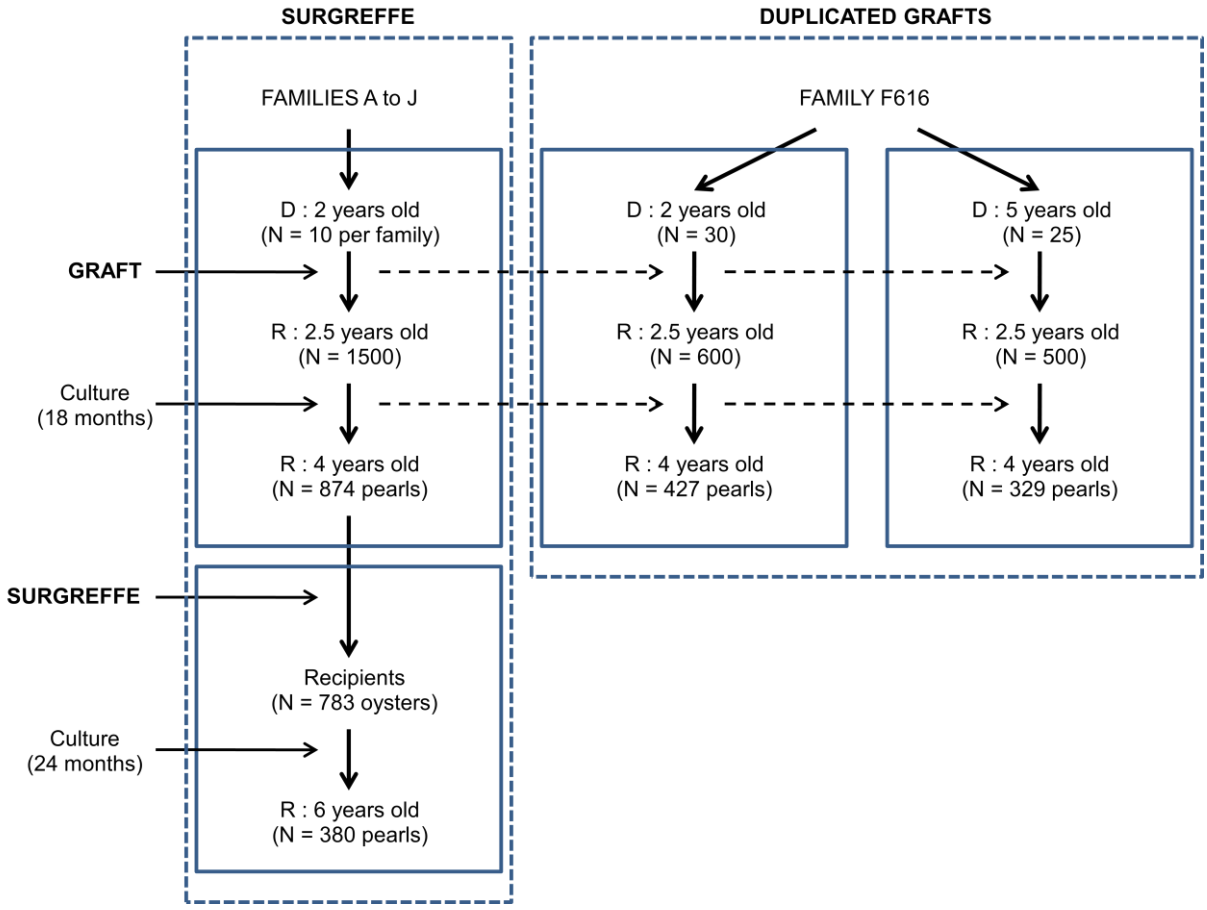
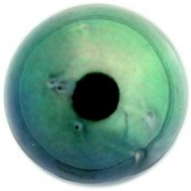


Figure 2. Cultured pearl surface defect (a) and visual color (b) categories produced by *Pinctada margaritifera*. The picture samples indicate a round pearl shape.

a) Cultured pearl surface defect categories

No defect	1 to 5	6 to 10	> 10
			

b) Cultured pearl color categories

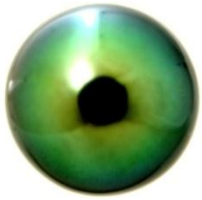
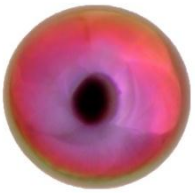
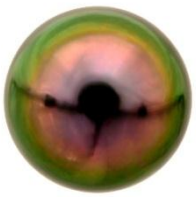
Green	Grey	Aubergine	Champagne
			
Peacock	**Blue**	**Yellow**	**White**
			

Figure 3. Comparison of culture pearl grade (A, B, C, D and R - reject) rate (in %) from graft (G) to surgreffe (SG), for each of the *P. margaritifera* donor families (A to J). The difference between G and SG methods found to be very highly significant ($p < 0.0001$) are denoted by ***.

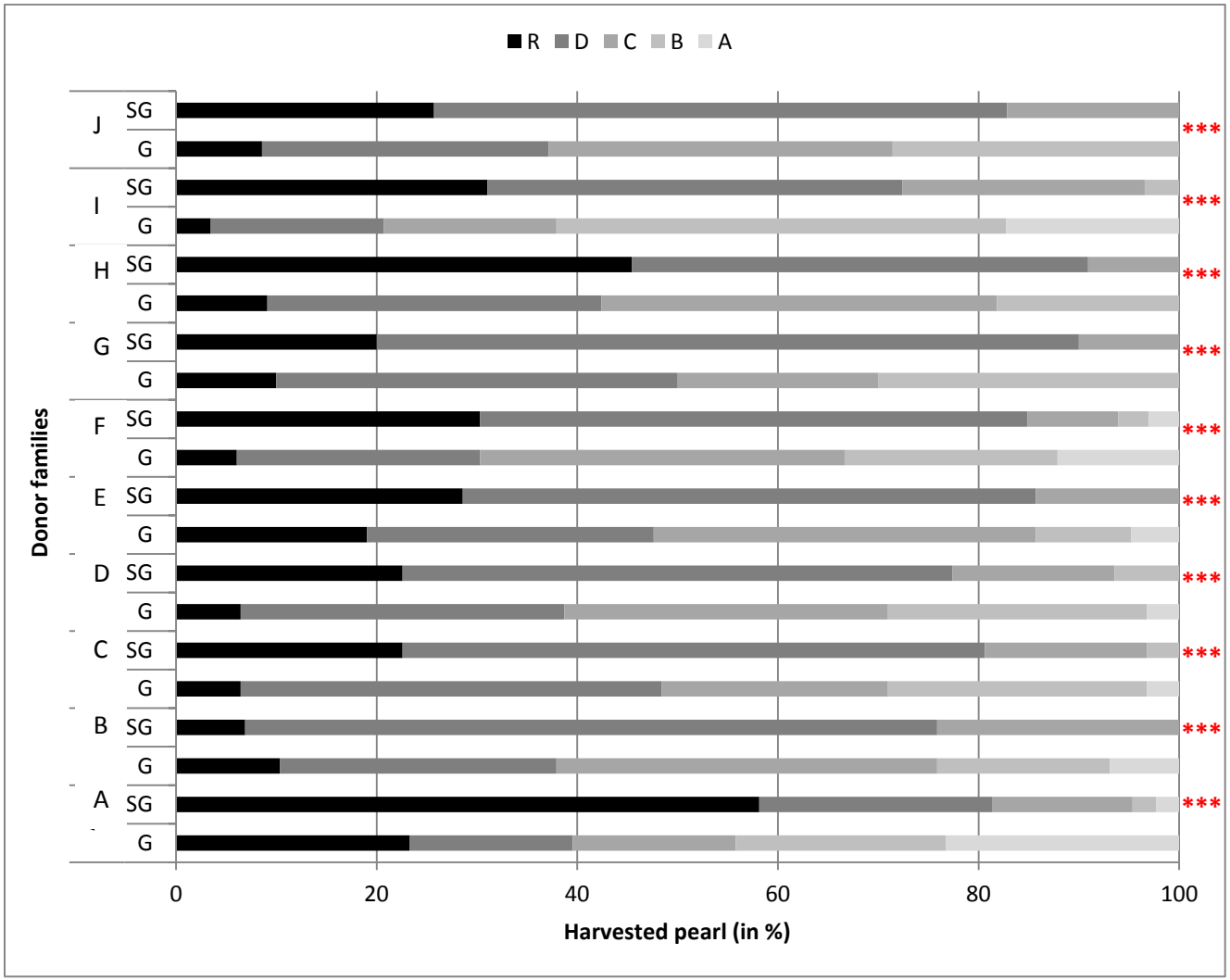


Figure 4. Comparison between the cultured pearl color rates (green; grey; aubergine, champagne, peacock, white, and yellow) (in %) in the graft (G) and surgreffe (SG) experiments for each of the *P. margaritifera* donor families (A to J). Significant differences between the graft and surgreffe methods in terms of green and grey colors are denoted by * ($p < 0.05$).