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Mono- and polychromatic inner shell phenotype diversity in *Pinctada margaritifera* donor pearl oysters and its relation with cultured pearl colour

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ABSTRACT

The pearl oyster *Pinctada margaritifera* has the specific ability to produce pearls with the widest range of colours among all pearl oyster species. This pearl colour diversity originates from the mantle biomineralising tissue (graft) of the donor oyster, which is originally responsible for the variety of colours of the inner shell surface. This study aimed to: 1) assess the geographic distribution and establish a first stocklist of the colourful oyster phenotypes used as donors in French Polynesia, and 2) investigate the phenotypic relation between inner shell colouration and the corresponding colour of harvested pearls. With the support of a pearl farmers' network, we investigated the different donor phenotype frequencies among five collection sites (Ahe, Apataki, Takaroa, Takume and Mangareva). This donor evaluation was made during grafting of pearl oysters (N = 49,938) obtained from collector stations. Results showed that pearl production is mainly based on six common colourful donor phenotypes classified as monochromatic and polychromatic profiles, which shown different frequencies among the collection sites. Experimental grafts (N = 4640) were then realised and subsequent culture conducted at a single site in order to avoid pearl colour variation due to environmental influences. Traceability between donors (N = 232) and pearls (N = 2776), revealed that: 1) yellow (gold) and aubergine (reddish) pearls could be mostly obtained by using the monochromatic yellow and red donor phenotypes, respectively, and 2) one third to one quarter of grey pearls was inevitably harvested, whatever the polychromatic phenotype chosen as the donor, which leaves at least half the harvest composed of the attractive green and peacock colours. This preliminary stocklist of colour range together with analysis of the colour phenotype transmission between inner shell and pearl provide the basis for producing multiple pearl oyster "colour lines" through hatchery propagation and would be helpful for future selective breeding programs.
Statement of relevance: Donor shell colour selection predict colours of pearls

1. Introduction

The marine mollusc *Pinctada margaritifera* is widely distributed in shallow Indo-Pacific tropical and subtropical waters, and is mostly associated with reef environments (L. Cunha et al., 2010). This pearl oyster is the primary aquaculture species in French Polynesia, where the pearl industry represents the second most important source of income, just after tourism. This aquaculture is based on natural spat collection from wild stocks, that mainly originated from five lagoons located in the atolls Ahe, Apataki, Takaroa and Takume (Tuamotu archipelago), and Mangareva Island (Gambier archipelago). This natural production resource supplied (boat and plane transfers) the 517 pearl farms located on 26 atolls and islands in Tuamotu (398 farms), Gambier (79 farms), and Society (40 farms) (Talvard, 2015). For the cultured pearl production, a grafting process is required, which consists of the introduction of a nucleus (a bead made from the shell of a fresh-water bivalve) and a graft (a piece of mantle tissue from a selected donor oyster) into the gonad of a recipient oyster (Alagarswami, 1970).

A remarkable specificity of the *P. margaritifera* pearl oyster is its ability to produce a very wide range of pearl colours (Ky et al., 2014a). These colours range from the purest white to the deepest black, and include every shade of silver, peacock, green, aubergine, purple, golden brown and even rainbow. Pearl colours are not as diverse in the two other pearl oyster species also used in aquaculture, *P. fucata martenseii* (pink, white or silver, cream and yellow) and *P. maxima* (golden, silver-white, yellow or cream) (Tong and Shen, 2001; Taylor and Strack, 2002). Although only a few studies have been made on transmissions between donor phenotypes and pearl colours in *P. margaritifera*, these relations have been demonstrated through experimental grafting designs using both wild (Tayale et al., 2012) and hatchery-produced (Ky et al., 2013, 2016a) donor oysters. More recently, Ky et al. (2015a)

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also observed correlations between the outer (prismatic layers) and inner (nacreous layers) shell colours of donors and the colour proportions observed in the harvested pearls. In the close related species *P. fucata*, Wada and Komaru (1996) observed an association between donor shell and pearl colour. In xenografts involving *P. maxima* and *P. margaritifera* species, McGinty et al. (2010) demonstrated conclusively that the donor oyster is the primary determinant of pearl colour.

Throughout the distribution of *P. margaritifera* in French Polynesia, only a small proportion of pearl oysters are selected as donors for cultured pearl production. Usually, only the individuals with the most colourful inner shell sides are chosen to be donors; the others commonly have grey-dark coloured bands and are used as recipients. Desirable donor oysters with particular phenotypes show relatively low frequencies at the population level. Phenotype is defined as an observable characteristic of an individual or population that is determined by their genes or a combination of their genes and the environment. A phenotype is the expressed state of a given trait, which may either have distinct categories, as in simple Mendelian qualitative traits or polygenic traits with an underlying threshold that determines the phenotype, or be quantitative, with many possible values along a continuous scale. Some rare colour phenotypes have been recently reported in *P. margaritifera*, with a Mendelian inheritance for orange flesh, and red and white shell colours, compared with the wildtype black flesh and shell commonly found in this species (Ky et al., 2016b). Some phenotypes can be particularly advantageous because of their local adaptation to the constraints of their specific environment (e.g. excessive heat, endemic diseases), which improves not only their survival capacity but also their performance. Understanding the variability in the inheritance and the transmission of the colour phenotype is essential for the future development of the pearl aquaculture.

The aim of this study was to undertake an inventory of the rare pearl oyster inner shell colour phenotypes commonly used as donors in the cultured pearl industry in French Polynesia. For this, a pearl farmers' support network (who obtain their pearl oysters from the collection sites) allowed us to sample and estimate the frequencies of the main donor oyster phenotypes used in French Polynesia. A sample of these donors was used in large experimental graft, grown on in a single culture site as a means to establish a first stocklist detailing the close relation between inner shell and pearl colour phenotypes. This stocklist will provide a basis for breeding multiple donor oyster lines to produce pearls of specific colours that could be established in breeding programme.

2. Materials and methods

2.1. Wild donor pearl oyster collection and selection

Pearl oysters aged around 20 months old (7–9 cm) were selected from collectors from the five main wild seed collection areas located in Ahe, Apataki, Takaroa, Takume atolls (Tuamotu Archipelago) and Mangareva Island (Gambier Archipelago) in July 2013 (Map in Fig. 1). Access to these oysters of broad geographic origin was obtained through a pearl farm network. This network is composed of five pearl farms that get their oyster stock from the five collection sites (Table 1). Indeed, wild saibo donor and recipient were collected as spat in the lagoon of these sites. Passive techniques were employed for catching spat using commercial collectors made from modern synthetic materials, to which planktonic mollusc larvae become attached fifteen to twenty days after their release. The technique consists of immersing a rope at a depth of 3 m, which is stretched out between buoys and moored to pinnacles or dead weights placed on the lagoon floor. Surface buoys keep the 100 to 200 m rope suspended off the bottom. Collectors were attached at roughly one meter intervals. After nearly one year of subsurface rearing (3-5 m below the surface), the collectors were transferred by boat from the collection site to the pearl farm and grown for 8 additional months. During the graft process in each of the pearl farms, the oysters were removed from the collectors on which they had developed.

Selection of the pearl oyster was made by expert grafters and followed a two-step procedure. In the first stage, the grafter selects a healthy pearl oyster, which is identifiable by colour of the visceral mass and gills (brilliant appearance), shell size and appearance (round shape suggesting a regular growth), and muscle resistance when opening the shells. In the second step, each oyster was checked for its inner shell colour phenotype by using a speculum to open the valves from the set of healthy pearl oysters. A dentist's mirror was inserted into the open oyster to be able to see the inner shell colouration, and particularly the contact area (band colour) with the mantle at the edge of the shell. Other inner shell area, that was not in contact with the mantle, exhibited a white colour, characteristic of *P. margaritifera*. Oysters with a yellowish and reddish band colour were selected and classified according to their phenotypes. Among these colourful pearl oyster, six phenotypes has been selected: 1) the yellow band, 2) the yellow band with additional green pigment, 3) the yellow band with green and additional red pigment, 4) the red band, 5) the red band with additional green pigment and 6) the red band with green and additional red pigment (Fig. 2). The un-colourful band colour corresponded to non-selected oysters, which exhibited a grey to black colour. From the five collection sites, a total of 49,938 pearl oysters has been randomly screened for their colours (Fig. 2). The proportions of “selected” vs. “non-selected” phenotypes were evaluated for each of the collection sites to establish a stocklist of donor used for the graft in French Polynesia pearl oyster aquaculture. Based on this stocklist, samples of the donor oysters representing each of the selected phenotypes and originating from each of the collection sites were transferred by plane to Arutua atoll (Pommier pearl farm, Tuamotu Archipelago), where experimental grafts were performed three months later (October 2013) to test the transmission of characters from the donor to the final pearl product.

2.2. Experimental graft

The experimental graft was performed on Arutua atoll (Pommier pearl farm, Tuamotu Archipelago). The donor pearl oysters were opened individually. Where possible, eight donor oysters per phenotype and per collection site ($N = 5$) were used. The epithelial cells required for the grafting procedure were excised from the mantle by an expert grafter. Small squares of epithelium (“grafts”) measuring approximately 4 mm^2 ($N = 20$ per donor oysters) were prepared before being transplanted into the recipient oysters (issued from a single batch of healthy oysters). Another grafter cut out a hollow in the recipient “oyster” gonad into which they then placed the nucleus and graft. The nuclei used were all similar and imported from Japan: 2.4 BU nucleus (7.304 mm diameter, 0.59 g weight - Nucleus Bio, Hyakusyo Co. Japan). The whole grafting operation takes approximately 1 min (Ky et al., 2014b). A total of 4640 grafts were performed over a 6-days period. Traceability of donor oysters was maintained by using numbered plastic labels attached to the chaplets, where the corresponding recipient oysters were reared. After 18 months of culture (April 2015), the cultured pearls were harvested and assessed for their colour.

2.3. Cultured pearl colour

Cultured pearls were cleaned by ultrasonication in soapy water (hand washing) with a LEO 801 laboratory cleaner (2-L capacity, 80 W, 46 kHz); they were then rinsed in distilled water.

Colour evaluation (without a jeweller's loupe) was made on the cultured pearls according to Ky et al. (2013) by two operators working in cooperation. The visually-perceived colour (bodycolor), due to pigments, and secondary colour (overtone) were recorded as categories. Six colour categories were established into which all the harvested pearls could be classified: 1) samples with dominance of bodycolors