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廈門大學

硕士学位论文

杂色鲍杂交后代壳色遗传与抗性特征研究

Studies of Shell Colour Genetics and Resistance

Characteristics of Hybrid Progeny of *Haliotis diversicolor*

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## 摘 要

本文以杂色鲍为研究对象,从贝壳颜色的遗传学、生化组成、免疫防御特性及生态因子胁迫下的抗性四个方面阐述了杂色鲍质量性状遗传、营养与免疫方面的内容。主要的结果如下:

1. 杂色鲍贝壳颜色的遗传学和标记研究:采用家系育种的方法,将贝壳颜色为黄色的突变型杂色鲍与贝壳颜色为褐色的正常表型杂色鲍进行了连续 2 代的交配实验。结果表明,杂色鲍的黄壳色明显受到遗传因素的影响,当至少一方亲本中有黄壳色时,后代中同样出现黄壳色表型的个体。杂色鲍贝壳上的黄壳色不会随生长而消失,可作为一种稳定的形态标记,用于标识黄壳色突变型的个体,该标记技术也可用于杂色鲍的养殖技术和遗传学研究。此外,选用了贝壳颜色遗传学实验中建立的贝壳颜色发生分离的家系,以壳长为指标,分析、比较了来自相同家系的两种壳色个体之间的生长速度。在幼鲍发育至 420 天止的 5-7 个统计时段内,没有在同一家系来源的 2 种贝壳颜色表型个体之间检测到生长速度的显著差异。

2. 杂色鲍不同群体的化学成分比较:对杂色鲍台湾群体、日本群体和杂交群体的基础营养成分、氨基酸、脂肪酸和矿物质元素的含量进行了测定,并对不同群体足肌的营养价值进行了评价。结果表明,通过杂交育种的方法培育的杂色鲍杂交种,继承了父本日本群体肉质优良的特点,足肌的营养价值明显高于母本台湾群体,证明了通过杂交育种手段对杂色鲍的足肌营养起到了很好的种质改良效果。两种不同壳色鲍壳的矿物质元素和氨基酸的含量具有明显的差别,在不同的生长阶段,黄壳色鲍壳氨基酸和矿物质元素的含量变化具有一定的规律性。

3. 杂色鲍不同群体的免疫防御特性比较:采用流式细胞法对杂色鲍台湾群体、日本群体和杂交群体血细胞的吞噬能力、基础的呼吸爆发水平及密度值进行了比较,同时采用南京建成的试剂盒测定了血淋巴中与体液免疫密切相关的酸性磷酸酶(ACP)、碱性磷酸酶(AKP)、超氧化物歧化酶(SOD)和髓过氧化物酶(MPO)这四种酶的活力。结果表明,杂色鲍的杂交种继承了父本日本群体较高的机体免疫力,血细胞吞噬能力、基础的呼吸爆发水平、三种酶(ACP、AKP、SOD)的活

力都显著高于母本台湾群体,证明了通过杂交育种手段对杂色鲍的免疫能力起到了很好的改良效果,培育出的杂交鲍机体免疫力高,抗逆性强,更加适应外界环境的变化。采用 FCM 法对杂色鲍两种壳色群体的血细胞吞噬能力、基础的呼吸爆发水平及密度值进行了比较,结果表明:两种壳色的杂色鲍个体没有表现出免疫应答方面的显著差异。

4. 杂色鲍三个群体在不同生态因子胁迫下的耐受力比较:采用生态因子骤变胁迫的方法,比较了杂色鲍台湾群体、日本群体和杂交群体三个群体在高温、低温、低盐和干露胁迫下的生理表现和存活率情况。结果表明,同样的胁迫条件下,杂色鲍的杂交种比母本台湾群体更加适应环境的急剧变化,具有更高的存活率。同时研究结果显示,在水温突变实验中,8℃、32℃是杂色鲍存活的临界低温和高温。在温度为 25℃,湿度为 30%条件下,5h 是杂色鲍在干露胁迫下存活的临界时间。

5. 杂色鲍三个群体腹足吸力的比较:利用改良的拉力工具,对杂色鲍台湾群体、日本群体和杂交群体三个群体个体正常吸附状态下将腹足拉离吸附底物所用的拉脱力进行了测量,并计算了正常吸附状态下的腹足单位面积吸力。实验结果表明,对于同样的吸附底物,台湾群体个体的腹足吸力和单位面积吸力均显著低于日本群体和杂交群体。日本群体和杂交群体在正常吸附状态下,机体活力强,生理状态好,能够更牢靠地吸附在底物上,不易受环境变化的扰动。

**关键词:** 杂色鲍; 杂交; 壳色; 免疫; 抗性

## Abstract

In this research, quality traits genetics, nutrition and immunology of small abalone *Haliotis diversicolor* were studied in four parts: genetics of shell colour, biochemical compositions, immune defense properties, resistance under the stress of ecological factors. The main results are as follows:

### 1. Shell colour genetics and markers of small abalone

A recently discovered mutation that displays a novel yellow shell coloration that is distinguishable from the normal brown coloration was reported in this study. Cross-mating experiments between the two types abalones were carried out to detect the F<sub>1</sub> phenotypes and F<sub>2</sub> phenotypic segregations. The results showed that the yellow shell colour significantly influenced by genetic factors, If at least one parent were in the yellow shell colour, the offspring also appeared yellow shell colour phenotype. The yellow shell coloration did not disappear with the growth, which can be used as a stable morphological markers to identify the yellow shell colour mutant individuals.

All family lines occurring shell colour segregation were selected and the shell lengths were measured for 5-7 times for the juvenile abalones at aged 2-14 months. Among them, there were no significant differences in all groups of abalones between the two colour type juvenile abalones.

### 2. Chemical compositions of different small abalone groups

The contents of basic nutrients, amino acids, fatty acid and mineral elements in Taiwan group, Japan group, hybrid group and two shell colour groups were measured, and muscle nutritional value of different groups were also evaluated. The results showed that, the hybrid group, which was cultivated in cross breeding method inherited the meat quality of Japan group, the muscle nutritional value of it was significantly higher than Taiwan group. This results indicated that cross breeding method is an effective method to improve meat quality of small abalone. Mineral element and amino acids contents in two colour abalone shell have significant differences, and the amino acid and mineral element contents in yellow colour

abalone shell had a certain regularity at different growth stages.

### 3. Immune defense characteristics of different small abalone groups

Phagocytosis, basic respiratory burst level and density of blood cell in Taiwan group, Japan group and hybrid group were measured by flow cytometry (FCM) method, and the activity of acid phosphatase (ACP), alkaline phosphatase (AKP), superoxide dismutase (SOD) and myeloperoxidase (MPO) was measured using the kits built by Nanjing Jiancheng company. The results showed that the hybrid group, which was cultivated in cross breeding method inherited the excellent immunity of Japan group, phagocytosis, basic respiratory burst level and activity of three enzymes (ACP, AKP, SOD) were significantly higher than Taiwan group. This conclusion proved that cross breeding method is an effective method to improve immunity of small abalone, small abalone hybrids had high immunity and strong resistance to respond to external environmental changes. Phagocytosis, basic respiratory burst level and density of blood cell in two shell colour abalone groups were also measured by flow cytometry (FCM) method. The results showed that two colour small abalone did not show significant differences in immune response.

### 4. Tolerance of small abalone under the stress of different ecological factors

Physiological performance and survival rate of Taiwan group, Japan group and hybrid group under the stress of high temperature, low temperature, low salinity and dry exposure were measured in the method of sudden environmental change stress. The results showed that hybrid group showed a higher survival rate than Taiwan group. They had better ability in adapting to sudden drastic changes in the sea environment. The critical thermal maximum and minimum of small abalone were 32°C and 8°C when the temperature changed suddenly. the critical dry expoure time maximum of small abalone was five hours under the condition of temperature 25°C and humidity 30%.

### 5. Pedal foot suction of different small abalone groups

Pedal foot pull-off force and suction per unit aera of Taiwan group, Japan group and hybrid group was measured in the normal adsorption state. The results showed

that pedal foot pull-off force and suction per unit area of Taiwan group were significantly lower than Japan group and hybrid group. Japan group and hybrid group were in better physiological state and had higher body activity when they were adsorbing on the same substrate, and they were not easily disturbed by environmental changes.

**Keywords:** Small abalone; Cross breeding; Shell colour; Immunity; Resistance

厦门大学博硕士学位论文摘要库

## 第一章 绪论

### 第一节 贝类壳色的研究进展

颜色多态性是指在自然环境中,一个物种的不同群体或同一个群体中,不同个体中呈现两种或两种以上可遗传的分离且不连续的颜色表型,对于阐释微生态选择压力与保持遗传变异的关系,颜色多态性是理想的研究模式(管云雁等,2009)。海产贝类在生长过程中,同种不同群体之间或同一群体中常产生壳色的多态性,一些海产经济贝类不同壳色的个体其生长性状也不同,因此,研究海产贝类壳色的调控机理以及壳色性状和其经济性状的必要性是很有必要的。

#### 1. 壳色与环境的关系

在形态学标记中,海水贝类的壳色较为特殊,贝壳的颜色变化范围在不同的种之间,有十分显著的差异。有些种类的贝壳颜色非常稳定,有些种类则贝壳的颜色丰富、多变,如岩栖滨螺(*Littoria saxatilis*)广泛分布于南太平洋、白海和巴伦支海,由于贝壳形态与壳色多样性,先后被分为 35 个种。菲律宾蛤仔(*Ruditapes philippinarum*)由于壳色和壳面花纹各异,也被分类学家定义为许多不同的种。人们发现贝类的壳颜色可以由于环境因素而发生改变,还与贝类本身生态行为、生长存活、生理特性有关,在大多数情况下,壳色与环境紧密相关。早在 20 世纪早期,贝类的壳颜色问题就已开始受到关注,Moore(1936)发现荔枝螺(*Purpura lapillus*)在不同的环境下具有多种壳色。Ino(1949)研究发现马蹄螺(*Turbo cornutus*)摄食 *Eisenia bicyclis* 后贝壳由原来投喂藻类 *Cheilosorum maximum* 时的黑褐色等颜色转变为白色。Turner(1958)研究发现贝壳颜色为深褐色的玉螺(*Polinices duplicatus*)摄食砂海螂(*Mya arenaria*)后转变成为白色。Mitton(1977)研究了不同壳色与壳面条纹贻贝(*Mytilus edulis*)适应环境的重要性。结果发现,在蓝色的贻贝壳面上存在的白色条纹数量与吸收热量的多少有直接关系,是一种对环境适应的结果。Gruneberg(1979)比较了生长于印度斯里兰卡的西奥莱彩螺(western *Clithon oualaniensis*)和马来半岛、香港的东奥莱彩螺(eastern *C. oualaniensis*)的壳色多态性的差异,发现两种螺生活的海区盐度的差异造成了两种螺壳色的不同。

Odile(1998)研究了温度与蜗牛(*Helix aspersa*)壳色和条纹的表型适应的关系, 蜗牛由于表型适应性导致高温 25℃下出现浅色个体, 低温 15℃下出现深色个体, 由于温度条件的不同从而出现了由浅至深的壳色表现型。Komaru(1993)研究发现珠母贝(*Pinctada fucata martensii*)在不同的养殖密度和处于不同的养殖水层下, 不仅生长速度差别很大, 壳色也发生了变化。Gray(1994)比较了 Severn 入海口区的两种有垂直分布差异的滨螺 *L. obtusata* 和 *L. mariae*, 得出地理分隔可造成壳色多态性的结论。Berger et al(1995)在研究滨螺(*L. saxatilis*)壳颜色的影响因素时指出, 日照照射强度不同时, 壳色发生变化。Ekendahl(1997, 1998)采用壳色选择多样性与 5 个环境因素互相作用的模型, 研究了滨螺(*L. saxatilis*)壳色的高度多态性, 发现壳色选择几乎与性别和年龄不相关, 而与环境因素有较高的相关性。另外, Sokolova 和 Berger(2000)在研究俄罗斯白海(White Sea)海口湾地区滨螺(*L. saxatilis*)生理多样性与壳色多态性的关系中发现, 该地区盐度突变对生理选择的影响是导致贝类壳色的多样性的内因。Inouye(2000)选择了日本 17 个地点 19 个群体的斧蛤(*Donax cuneatus*)为研究对象, 结果发现, 在同一地点的斧蛤壳色相对稳定, 有一定地理距离的群体壳色则存在不均一性。Brake et al(2004)对太平洋牡蛎进行家系选择研究, 发现壳的色素沉积与其外套膜缘的色素沉积有一定的遗传相关性, 但体重、成活率及生长速度都与壳色素及外套膜缘色素没有相关性。Goncalves et al(2005)研究了一种潮间带石鳖(*Ischnochiton striolatus*)的壳色多态性和栖息地异质性的关系, 发现生存的岩石背景不同, 产生了不同壳色的变异。

## 2. 贝类壳色的遗传控制

对于壳色的遗传控制方面, 来自杂交实验的结果表明, 有些种类的贝壳颜色受遗传因素控制。Cole(1975)进行了荔枝螺(*Urosalpinx cinerea*)不同贝壳颜色个体之间的杂交实验, 结果表明, 紫色、褐色和白色3种贝壳颜色性状是由一个基因位点控制的3个复等位基因。Innes和Halev(1977)研究了贻贝(*M. edulis*)壳色多态的可遗传性, 发现少见的棕壳色个体受一对显性等位基因控制, 而常见的黑壳色个体受另外一对等位基因控制, 并且认为壳色主要受基因决定, 环境只是起到影响作用。Newkirk(1980)通过杂交手段研究了贻贝壳色的可遗传性, 确认贻贝的壳色是由简单的遗传基因决定的。Gary(1980)对贻贝的研究也证实壳色变异主要是



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