Biochemical Systematics and Ecology 54 (2014) 53–58

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Genetic variation and an estimation of effective population size in the pearl oyster *Pinctada martensii*

Liu Qingyun, Chen Weiyao, Liu Jialiang, Ronglian Huang, Qingheng Wang, Yu Jiao, Du Xiaodong, Deng Yuewen*, Li Junhui

Fishery College, Guangdong Ocean University, Zhanjiang, Guangdong 524025, PR China

ARTICLE INFO

Article history:
Received 27 June 2013
Accepted 25 December 2013
Available online

Keywords:
Pinctada martensii
Genetic diversity
Effective population size
SSR marker

ABSTRACT

In September 2011, two wild populations of pearl oysters were collected separately from Daya Bay, Shenzhen, and from Beibu Bay, Beihai, China. In April 2012, three cultured stocks were established by sampling breeders from the third-generation selected lines. The genetic variation within the five stocks was analyzed using eight SSR primer pairs. The effective population sizes of the five stocks were analyzed using the sibship reconstruction and multiple summary statistics methods. A total of 22–29 alleles were observed in the five stocks, with two to five alleles per locus and means ranging from 2.750 to 3.625 alleles per locus. The average expected and observed heterozygosities ranged from 0.3387 to 0.5029 and from 0.2917 to 0.4219, respectively. The effective population sizes of the five stocks ranged from 11.0 to 20.0 when estimated using sibship reconstruction and from 13.7 to 179.4 when estimated using multiple summary statistics. The effective population sizes estimated in the three cultured stocks were smaller than the number of breeders used to produce these stocks. Thus, it may be necessary to augment the number of breeders used to produce progeny stocks in selective breeding programs.

1. Introduction

The effective population size (N_e) refers to the size of a theoretical, ideal population with the same genetic characteristics as the actual population. A theoretical, ideal population has no genetic drift, migration, mutation, or natural selection, and mating within such a population is entirely random. However, real populations often deviate from this ideal due to several factors, and N_e is generally smaller than the population's census size (N). Factors contributing to deviations from N are involved in the sex ratio, variations in family size, fluctuations in population size, age structure, mating system, and variability in breeding success within the population (Crow and Kimura, 1970; Martinez et al., 2000; Garcia-Vazquez et al., 2001; Mũnoz and Warner, 2004). Life history is another important factor that can affect the N_e in marine species with sex reversal (Wade and Shuster, 2004; Araki et al., 2007).

N_e is an important parameter in population genetics, conservation biology, and evolutionary genetics, as it reflects the rate of genetic drift and inbreeding. A small N_e will inevitably lead to inbreeding due to the bottleneck effect (B. Evans et al., 2004). Inbreeding will result in a reduction of growth traits, referred to as inbreeding depression (Deng et al., 2005). To avoid

* Corresponding author. Tel.: +86 759 2383346.
E-mail addresses: 1624935761@qq.com (L. Qingyun), 415878754@qq.com (C. Weiyao), 554271896@qq.com (L. Jialiang), huangrl@gdou.edu.cn (R. Huang), wangqingheng@163.com (Q. Wang), jiaoy@gdou.edu.cn (Y. Jiao), duxd@gdou.edu.cn (D. Xiaodong), dengyuewen@sohu.com (D. Yuewen), lijh@gdou.edu.cn (L. Junhui).

0305-1978/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2013.12.033

inbreeding depression and maintain the genetic diversity of cultured stocks, it is greatly important to analyze the genetic structures and estimate the effective population sizes of cultured stocks using molecular marker systems.

The pearl oyster *Pinctada martensii* is an economically important species in the southern provinces of China. However, pearl oyster culture in these provinces has recently suffered from slow growth and mass mortality due to increasingly deteriorating environments. Since 2003, we have initiated mass selective breeding to restore stocks (Deng et al., 2009a,b, 2013; Wang et al., 2011). Determining the N_e of selected lines is very important in the design of mass selection programs. Molecular markers have recently been used to estimate N_e. Compared with other molecular markers, simple sequence repeat markers (SSRs) have many advantages. For example, SSRs are genetically codominant, abundant, dispersed throughout the genome, multi-allelic, and highly reproducible, and they have high levels of polymorphism (He, 1998). In this study, we investigated the genetic structures of two wild populations and three cultured stocks produced by sampling breeders from the third-generation selected lines. We also used two methods to estimate the N_e of the five stocks. The objectives of this study were to provide information on the genetic variation of the three selected stocks relative to the wild population, to understand how N_e deviated from N, and to help determine the number of breeders to use for the production of progeny stocks in mass selective breeding programs.

2. Materials and methods

2.1. Sample sources

A total of five stocks were used in this study. In September 2011, wild animals were collected from Daya Bay (DW), Shenzhen, Guangdong and Beibu Bay (DW), and Biehai, Guangxi. Three stocks designated as S1, S2 and S3 were established in April 2011 by sampling breeders from the third-generation selected lines. These selected lines were previously described by Wang et al. (2011) and Deng et al. (2013). At two years of age, animals were sampled from the three cultured stocks. Information on the experimental stocks used is given in Table 1.

2.2. DNA isolation and PCR assay

We extracted DNA from the adductor muscle of each individual using a Universal Genomic DNAMini-Isolation Kit, provided by Shanghai Biological Engineering Co. Ltd., combined with a chloroform extraction. Genomic DNA was assessed by gel electrophoresis using a 1% agarose gel.

EST-SSR primer pairs with high degrees of amplification success and polymorphism were used in this study. The details of the primer pairs are shown in Table 2. PCR amplification was performed in a 25 μl reaction containing 20 ng of genomic DNA, 250 nmol l^{-1} of each primer, 200 nmol l^{-1} of dNTPs, and 1 U of Taq polymerase. The PCR protocol consisted of a 5 min denaturing step at 95 °C followed by 32 cycles consisting of denaturation at 95 °C for 30 s, annealing at 50, 55 or 60 °C for 30 s, and extension at 72 °C for 1 min. The final PCR cycle was followed by a 10 min extension at 72 °C. The resultant PCR products were tested by 8% polyacrylamide gel electrophoresis (PAGE) with a 20 bp DNA ladder. Following electrophoresis, gels were stained with silver and imaged using a Gel Doc™ XR+ system.

2.3. Statistical analysis

The program Gel-Pro Analyzer v4.5 (Media Cybernetics Inc.) was used to calculate the number of alleles at each locus and to determine the genotype of each individual. Based on the genotype data, we calculated the following genetic parameters for each locus using Popgene version 1.31 (Francis et al., 1999): the number of alleles (N_a), the effective number of alleles (N_f), the allele frequency, the observed heterozygosity (H_o), and the expected heterozygosity (H_e). The polymorphism information content (PIC) of a single locus was calculated based on the allele frequencies at that locus using the program PIC-CALC version 0.6.

N_e of each stock was estimated by both the sibship identification method, using COLONY 2.0 (Jones and Wang, 2010), and by the multiple summary statistics method, using ONeSAMP (Tallmon et al., 2008). Maximum likelihood was used for sibship identification to divide offspring into full-sib and half-sib families according to Mendel's laws. In COLONY 2.0, parameters were designated as follows: males and females were all diploid and polygamous, and the typing error rate of a single locus was 0.025 (Jones and Wang, 2010). The multiple summary statistics method is based on the principle of linkage

Table 1
Information on the experimental stocks used in this study.

Stocks	Breeders	The number of breeders (female/male)	Sample number
DW	–	–	48
BW	–	–	24
S1	The third-generation yellow colored line	18 and 14	48
S2	The third-generation selected line for faster growth	15 and 15	48
S3	The third-generation black colored line	15 and 15	48