Aquaculture 422–423 (2014) 218–224

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Production of genetically diversified fish seeds using spermatogonial transplantation

Mana Sato, Tetsuro Morita [1], Naoto Katayama, Goro Yoshizaki *

Department of Marine Bioscience, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form 12 December 2013
Accepted 13 December 2013
Available online 19 December 2013

Keywords:
Seed production
Genetic diversity
Surrogate broodstock
Spermatogonial transplantation

ABSTRACT

Large-scale release of hatchery-produced seeds has been conducted in order to restore worldwide fishery production; however, concerns exist regarding the genetic effects of hatchery stock on wild fish populations, due to the reduced genetic variation often associated with hatchery-reared fish. Therefore, it is important that fish seeds used in stock enhancement possess sufficient genetic diversity to mitigate their genetic impact on wild fish populations. To promote genetic diversity of artificial seeds, seed production should be performed using a sufficiently large broodstock; however, maintenance of large broodstocks requires significant investments with respect to space, labor, and other associated costs. In order to circumvent the need for these investments, the present study proposed a means of producing gametes that possess a large amount of genetic diversity using only a small number of surrogate-broodstock through spermatogonial transplantation. We previously established spermatogonial transplantation methodology capable of producing functional eggs and sperm derived from donor-type A spermatogonia (ASGs). It was expected that if ASGs isolated from testes of different donor individuals were mixed prior to their transplantation into the peritoneal cavity of a single recipient, the resulting recipient would produce gametes derived from several donor individuals. To determine the feasibility of this scenario, mixed donor ASGs derived from *vasa-GFP*, *vasa-DsRed* and *hsc-GFP* transgenic rainbow trout (*Oncorhynchus mykiss*) were transplanted into triploid recipients. At 20 days post-transplantation, approximately 80% of recipients possessed donor ASGs derived from all three strains within their gonads and mean numbers of incorporated ASGs derived from each donor strain were nearly equal. At 100 days post-transplantation, incorporated donor ASGs proliferated within the gonads of both male and female recipients, and some had differentiated into oocytes within recipient ovaries. Furthermore, in order to ascertain whether incorporated donor ASGs derived from the three strains could differentiate into functional gametes, gametes collected from mature male and female recipients were used in artificial insemination with gametes obtained from wild-type fish of the opposite sex. Phenotypic analysis of F1 offspring revealed that functional sperm and eggs derived from three donor strains were successfully produced in the gonads of individual recipients. These results suggested that individual recipients that received ASGs from multiple donors were capable of producing gametes with different genetic backgrounds. Therefore, it was concluded that the method of spermatogonial transplantation outlined in the present study could serve as a novel and efficient method of producing fish seeds with increased genetic diversity for use in stock enhancement.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In order to restore declining wild fish resources, stock enhancement through the release of hatchery-produced seeds into natural environments has been carried out in many countries. These efforts have resulted in growing concerns regarding potential alterations to the genetic structures of wild fish populations. These concerns are based on the fact that hatchery fish populations often exhibit reduced genetic variation caused by seed production involving relatively small broodstocks and/or inbreeding over multiple generations (Hara and Sekino, 2007; Ryman and Ståhl, 1980; Sekino et al., 2002; Verspoor, 1988). This deficiency could eventually lead to a reduction in genetic diversity of local populations resulting from reproductive interaction between released individuals and native individuals. Indeed, several studies have suggested that massive releases of hatchery-produced seeds had the potential to alter genetic structures of local populations (Eldridge and Naish, 2007; Kitada et al., 2009). Therefore, maintaining genetic variation in hatchery-produced seeds is necessary in order to ensure the preservation of the genetic diversity of local fish populations.

In order to achieve a level of genetic diversity equivalent to that of local populations, seed production should be performed using a sufficiently large broodstock; however, several obstacles preventing the use of increased broodstock exist. The maintenance of sufficient

* Corresponding author. Fax: +81 3 5463 0558.
E-mail address: goro@kaiyodai.ac.jp (G. Yoshizaki).
[1] Present address: Central Research Laboratory, Nippon Suisan Kaisha, Ltd., Tokyo, Japan.

0044-8486/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.12.016

numbers of broodstock requires increases in space, labor and associated costs. In addition, parent fish have to simultaneously participate in spawning immediately prior to egg collection; however, it is difficult to synchronize the spawning time of each parent fish. Consequently, the number of parent fish contributing to seed production tends to be much smaller than the total number of parent fish held in a given spawning tank.

In order to overcome the obstacles associated with production of genetically diverse seeds, the authors of the present study focused on development of surrogate broodstock technique. We previously reported the establishment of a spermatogonial transplantation system using Salmonids (Okutsu et al., 2006) and, using this technique, demonstrated that donor-derived type A spermatogonia (ASGs) were capable of being colonized within the gonads of xenogeneic sterile recipients and of differentiating into either functional eggs or sperm depending on the sex of the recipient (Okutsu et al., 2007). This technique has also been applied using several other marine species (Higuchi et al., 2011; Morita et al., 2012; Takeuchi et al., 2009; Yazawa et al., 2010). If smaller-bodied fish were used as recipients for large-bodied donor fish, donor gametes could be produced using a smaller fish tank. This would allow for seed production using a larger number of parent fish, even when using broodstock tanks of limited size. Furthermore, if ASGs are isolated from several donor individuals and mixed prior to their transplantation into a single recipient, the resulting recipient would be expected to produce gametes genetically derived from several donor individuals. If one pair of surrogate parent fish produced using this methodology were to mate, they could produce seeds whose genetic diversity would be equivalent to seeds derived from several pairs of parent fish. Using this surrogate broodstock technique for seed production, it would be possible to produce genetically diverse seeds in a small space with low costs and labor requirements.

In order to achieve this goal, donor ASGs derived from multiple donor individuals have to colonize a single recipient gonad and produce functional eggs and sperm with different genetic backgrounds. In previous studies, we reported that several donor-ASGs were detected in a recipient gonad at 20 days after spermatogonial transplantation (Kise et al., 2012; Lee et al., 2013; Okutsu et al., 2006). However it remained unclear whether these donor cells were derived from clonal proliferation of a single donor ASG or they were originated from multiple donor ASGs. In addition, previous studies reported that competitive confrontation between different germ cell populations exists in mice (Mintz, 1968; Shinohara et al., 2002). Therefore, in this study, in order to examine the feasibility of using surrogate broodstock technology to obtain genetically diversified seeds, germ cells derived from three different donor strains were transplanted into a single recipient in which donor cell fate was analyzed.

2. Materials and methods

2.1. Specimens

All of the fish used in the present study were maintained at the Oizumi Station of the Field Science Center (Yamanashi, Japan), Tokyo University of Marine Science and Technology under a natural photoperiod at a water temperature of 10 °C. Three transgenic rainbow trout, *Oncorhynchus mykiss*, strains that carried different foreign genes encoding fluorescent proteins were used as donors. The *vasa-GFP* strain carried ASGs labeled with strong green fluorescence (Takeuchi et al., 2002; Yoshizaki et al., 2000). The *vasa-DsRed* strain was produced by replacing the *GFP* gene used in the *vasa-GFP* strain with the *DsRed* gene. The *hsc-GFP* strain carried *GFP* gene driven by regulatory elements of the *heat-shock-cognate* gene (Yamamoto et al., 2011; Kawamura and Yoshizaki, unpublished data) and showed green fluorescence throughout the entire body. To prepare the donors having different genetic backgrounds, the *vasa-GFP*, *vasa-DsRed*, and *hsc-GFP* strains were produced using Okutama strain established in Okutama Branch of Tokyo Fisheries Experimental Station (Tokyo, Japan), Oizumi strain established in Oizumi Station of the Field Science Center, and recessive albino-mutant strain obtained from Tochigi Prefectural Fisheries Experimental Stations (Tochigi, Japan) (Boonanuntanasarn et al., 2004), respectively. Non-transgenic triploid rainbow trout (Oizumi strain) were used as recipients. The fertilized eggs were induced to triploidy using the methodology described by Okutsu et al. (2007) in order to prevent them from producing their own gametes. Eggs were reared in water at 10 °C prior to transplantation.

2.2. Preparation of testicular cell suspensions

Testes were collected from 3 to 9 individuals of each hemizygous transgenic strain (*vasa-GFP*/−, *vasa-DsRed*/−, and *hsc-GFP*/−) when they were between 10 and 13 months old. Collected testes were immature and primarily consisted of ASGs. Testes isolated from each strain were pooled for use in subsequent experimentation. Testes were minced and dissociated by enzymatic treatment in accordance with the methodology described by Kise et al. (2012). Dissociated testicular cells were washed three times by centrifugation with Eagle's MEM medium (pH 7.8; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 5% fetal bovine serum (FBS; Invitrogen Co., Grand Island, NY) and 20 mM Hepes (Sigma-Aldrich, Co., St. Louis, MO) in order to eliminate enzyme activity, and filtered through a 42-μm-pore-sized nylon screen (NBC Meshtech Inc., Tokyo, Japan) in order to remove non-dissociated cell clumps. Resulting donor testicular cells were suspended in the previously described medium and kept on ice prior to their transplantation.

2.3. Spermatogonial transplantation

Testicular cells isolated from the *vasa-GFP*, *vasa-DsRed* and *hsc-GFP* transgenic rainbow trout were used as donors for transplantation assay. Since germ cells isolated from the *hsc-GFP* strain did not exhibit strong green fluorescence, they were stained with the red-fluorescent membrane dye, PKH26 (Sigma-Aldrich, Co.) in order to visualize their behavior within recipient gonads (Kise et al., 2012). The same number of ASGs from each strain was mixed together prior to transplantation (Fig. 1A, B). Approximately 1.5×10^4 total ASGs were transplanted into the body cavity of each recipient rainbow trout hatchling (32–36 dpf) using the methodology described by Okutsu et al. (2006). We repeated the transplantations three times using at least 50 recipients for each experiment. At 20 days post-transplantation, recipients were dissected and incorporation rates of donor ASGs within their gonads were analyzed under a fluorescent microscope (model BX-51N-34FL; Olympus, Tokyo, Japan) equipped with a GFP filter set (U-MWIB and U-MNIBA; Olympus) and a DsRed filter set (U-MWIG; Olympus). Incorporation rate was defined as the number of recipients carrying transplanted donor ASGs within their gonads divided by the total number of recipient individuals. Numbers of each type of donor ASGs incorporated into recipient gonads were also counted. Furthermore, at 100 days post-transplantation, proliferation and differentiation of donor ASGs derived from each transgenic strain within the recipient gonads were morphologically observed.

2.4. Progeny test

Recipient fish were reared for 1–2 years until they reached maturity. To ascertain whether mature male recipients produced donor-derived functional sperm, milt was collected from 1- to 2-year-old male recipients and used to fertilize eggs from wild-type females. Additionally, whether or not female recipients produced donor-derived functional eggs was determined by collecting eggs from 2-year-old mature female recipients and fertilizing with sperm from wild-type males. Phenotypic analysis was conducted on all F1 offspring obtained from each male

Download English Version:

https://daneshyari.com/en/article/2421950

Download Persian Version:

https://daneshyari.com/article/2421950

Daneshyari.com