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Differentiation between populations of Japanese grenadier anchovy (*Coilia nasus*) in Northwestern Pacific based on ISSR markers: Implications for biogeography

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ABSTRACT

Northwestern Pacific provides unique scenarios for studying the roles of geography and ecology in driving population divergence and speciation. To elucidate the phylogeographic pattern of *Coilia nasus* in Northwestern Pacific, we examined ninety individuals from five localities along the coastal regions of China and the Ariake Bay of Japan by using seven ISSR markers. Analyses of molecular variance (AMOVA) showed that genetic differentiation among groups is relatively high ($F_{CT} = 0.1904$; $P = 0.000$). Bayesian analysis of ISSR data also revealed significant population structuring between Chinese and Japanese locations. Phylogenetic reconstructions show reciprocal monophyly in populations between China and the Ariake Bay of Japan. We conclude that the present-day phylogeographic pattern is the result of genetic isolation between Japanese and Chinese populations in the Northwestern Pacific following the glacial retreat, and that life-history traits and ecology may play a pivotal role in shaping the realized geographical distribution pattern of this species.

1. Introduction

A continuing challenge in evolutionary biology is to understand the processes by which populations become genetically distinct. In general, genetic drift and local adaptation are counteracted by the unifying effects of gene flow (Riginos and Nachman, 2001). However, marine organisms show low levels of genetic differentiation over large geographic distances (Palumbi, 1994; Grant and Bowen, 1998; Avise, 2000; J.X. Liu et al., 2006). Higher dispersal potential during planktonic egg, larval, or adult history stages coupled with the absence of physical barriers to movement seem to greatly facilitate extensive gene flow among populations of marine species (Grant and Bowen, 1998; Hewitt, 2000; J.X. Liu et al., 2006). Therefore, the high gene flow among populations presents a challenge for understanding how divergence occurs in marine environments. Although the predominant mechanisms leading to population differentiation are not always clear (Palumbi, 1994; Riginos and Nachman, 2001), several factors may be important either singly or in combination, including limited dispersal ability (Wirth and Bernatchez, 2001; Ovenden et al., 2004; Sotka et al., 2004), local adaptation (Grabowski et al., 2009; Libungan, 2009; Nielsen et al., 2009), oceanographic currents (Shaw et al., 1999; Pampoulie et al., 2006; York et al., 2008), deep ocean water (Shaw et al., 1999; Bernardi, 2000; Riginos and Nachman, 2001; Hansen and Hemmer-Hansen, 2007), habitat discontinuities (Riginos and Nachman, 2001; Hansen and Hemmer-Hansen, 2007; Fraser et al., 2010), isolation by distance (Riginos and Nachman, 2001; Hansen and Hemmer-Hansen, 2007) and historic vicariance (Imron et al., 2007; Liu et al., 2007;

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Kenchington et al., 2009). Additionally, the genetic structure of conspecific populations is generally the result of both historical biogeographical factors and ongoing ecological and demographic processes (Carisio et al., 2004).

Biogeographical regions are usually described based on the overlapping ranges of different species, and boundaries between these regions may derive from historical discontinuities or from contemporary environmental differences, such as differences in temperature or salinity (Riginos and Nachman, 2001). Although the underlying causes of such interspecific boundaries are not always well understood, these boundaries represent natural places to find out genetic discontinuities within species as well (Riginos and Nachman, 2001). In the Western Pacific, a series of marginal seas separate Asia from the Pacific, straddling the world's largest subduction zone (Tamaki and Honza, 1991; Liu et al., 2007). Since their formation in the late Cenozoic, the marginal seas have been a unique tectonic and geographical feature of the West Pacific region (Wang, 1999; Liu et al., 2007). Historical and contemporary restrictions to dispersal between the marginal seas are indicated by the confinement of gene flow among marine fish populations. For example, deep intraspecific divisions within *Pennahia argentata* populations were found between two marginal seas (East China Sea and Sea of Japan in Northwestern Pacific) (Han et al., 2008). Similarly, strong genetic breaks between the Japanese populations and Chinese populations have also been reported in redlip mullet *Chelon hematocheilus* (Liu et al., 2007). In the present study, we focus on the impact of biogeographical barriers on genetic differentiation in *Coilia nasus* between Chinese and Japanese coastal waters.

C. nasus, also called Japanese grenadier anchovy, are widely distributed in the Northwest Pacific, including the Yellow Sea and East Sea as well as Ariake Bay (Whitehead et al., 1988; Zhang, 2001). As an anadromous species, it runs several kilometers up the rivers and spawns in the fresh water, and then the spherical eggs float down and hatch near the river mouth (Takita, 1967). However, adults of *C. nasus* spend most their lives living in the marine environment (Yuan, 1987). In fact, the scientific name of the Japanese grenadier anchovy has been contested in the past. In China, the scientific name has always been *Coilia ectenes* (Whitehead et al., 1988; Zhang, 2001). Prior genetic studies of Japanese grenadier anchovy have uncovered population differences between Chinese and Japanese coastal waters but little researches focused on their morphological differences. Yuan and Qin (1985) considered the Japanese individuals of *C. nasus* as a unique population, ecotype or subspecies distinguished from the Chinese population on the basis of morphological differences and *C. ectenes* should therefore be treated as synonyms of *C. nasus*. Recently, genetic and phylogeny studies of *Coilia* also demonstrates that the genetic differentiations, based on AFLP markers, between Chinese and Japanese individuals of *C. nasus* were below the species level (Yang et al., 2010). As a priority, *C. ectenes* (Jordan and Seale, 1905) should therefore be synonymized with *C. nasus* (Temminck and Schlegel, 1846). Additionally, genetic researches on *C. nasus* mainly focused on genetic variations among Chinese populations (Zhang et al., 2006; J.Q. Yang et al., 2008; Ma et al., 2010).

Among various marker systems, inter-simple sequence repeats (ISSRs) employs oligonucleotides based on an SSR motif as anchored primers to initiate the amplification of genomic segments flanked by inversely orientated, closely spaced microsatellite repeats, and it does not require a priori genome sequence information (Zietckiewicz et al., 1994). This technique is similar to that for RAPD, except that ISSR primers consist of a di- or trinucleotide simple sequence repeat with a 5′ or 3′ anchoring sequence of 1–3 nucleotides. Many studies have indicated that ISSR produce more reliable and reproducible bands compared with RAPD because of the higher annealing temperature and longer sequence of ISSR primers (Nagaoka and Ogihara, 1997; Qian et al., 2001; Y.G. Liu et al., 2006).

To investigate geographical patterns of genetic variation in the Japanese grenadier anchovy using ISSR markers we collected individuals from five locations throughout their distribution in the Northwest Pacific. Also, we tested whether the genetic structure of populations from different areas could reflect their biological characteristics and the physical and chemical properties of seawater as well.

2. Materials and methods

2.1. Sample collection

A total of 90 samples of *C. nasus* were collected in five sites along Chinese and Japanese coastal waters used for analysis (showed in Fig. 1). The Chinese individuals were sampled from Yellow River Estuary, Nantong, Ningbo and Wenzhou. In addition, the Japanese individuals were collected from Ariake Bay. Samples were preserved in 95% ethanol or frozen (−20 °C) for DNA extraction.

2.2. DNA extraction

Total DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenolchloroform method. DNA concentration was measured with an UV spectrophotometer. The quality of extracted DNA was assessed by 1.0% agarose gel electrophoresis with ethidium bromide.

2.3. ISSR-PCR amplification

ISSR primers used in this study were synthesized by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., according to the primer set published by Wang et al. (2007), T.Y. Yang et al. (2008) and University of British Columbia (UBC) (http://www.michaelsmith.ubc.ca/services/NAPS/Primer_Sets/Primers_Oct2006.pdf). A total of 60 ISSR primers were