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Population structure and genetic diversity of *Ammodytes personatus* in the Northwestern Pacific revealed by microsatellites markers

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ABSTRACT

Genetic diversity and population structure of *Ammodytes personatus* in the Northwestern Pacific were investigated for 16 collections using eight highly variable microsatellite loci. Microsatellite analyses gave strong support for the presence of two distinct groups of genotypes. Pleistocene glaciations can cause significant geographical differentiation in *A. personatus* populations. However, microsatellite data cannot confirm completely reproductive isolation between north group and south group. About half of comparison values within the first and second cluster were significant after sequential Bonferroni corrections. Routine oceanic currents associated with strong wind condition may provide an excellent chance for connectivity of among populations within clusters. However, gene flow can be restricted by marine gyres due to complex geographical characteristic.

1. Introduction

Ammodytes personatus (Sand lance) is a commercial species which is distributed among the Yellow Sea, East China Sea and Japan (Reay, 1970). Ise Bay, Tohoku area off northeastern Honshu Island and the Seto Inland Sea of western Honshu Island are three major fishing grounds for sand lance in Japan, as described by Tomiyama et al. (2008). The species is regarded as a relatively high-quality forage fish which serves as an important link between zooplankton and marine predatory animal (Hashimoto and Kawasaki, 1981).

The species is generally found associated with sandy bottoms, appearing to avoid rocky, muddy, and coarse gravel bottoms (Tomiyama et al., 2008). The distribution of adult sand lance is highly patchy due to the limit of the substrate (Hashimoto and Kawasaki, 1981). The absence of a swim bladder allows this narrow, elongate fish to spend much time burying themselves in intertidal and shallow subtidal substrates, venturing out only to feed or spawn (Robards et al., 1999). There is only limited movement of post-settled sand lance between habitat areas (Hashimoto and Kawasaki, 1981). The length of the pelagic larval stage is about 30 days, surviving for long periods without food after hatching during the larvae stage (Inoue, 1949). The strategy is probably suitable for dealing with the highly volatile environmental conditions. The characteristic enable sand lance an interesting model for investigating differentiation in marine fishes.

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Previous works have studied the genetic structure and morphological variation in natural populations of the *A. personatus*. Hashimoto and Kawasaki (1981) have demonstrated the differences between subpopulation in Sendai Bay and its neighborhood are distinct across many aspects, i.e, morphology, ecology, and genetics. Subsequently, Okamoto et al. (1988) suggested a distinct genetic differentiation between the north of Iwate Prefecture and the south of Miyagi Prefecture on the basis of allozymes analyses. Kim et al. (2006) found a distinct genetic differentiation between East and West + South population in Korea inferred from mitochondrial DNA control region sequence, a finding subsequently supported by Kim et al. (2008) used multivariate methods to compare meristic and morphological characters. Han et al. (2012) further detected two lineages across 17 populations using mtDNA data, as the same time significant genetic structure was also detected along within Tsushima Current and between the Kuroshio and Oyashio.

Microsatellites are highly polymorphic nuclear loci that have been successfully used to infer fine scale population structure. Compared with mtDNA, the microsatellite mutation rate range from 10^{-5} to 10^{-3}, which is 10^4–10^6 times to mtDNA mutation rate. The mtDNA results in the present study reflected historical divergence between populations. Whereas the microsatellite patterns reflected reduced drift effects on preseparation allele frequencies that have yet to approach migration–drift equilibrium (Shaw et al., 2004). Thus, the addition of microsatellite data will contribute to better understanding of population of *A. personatus*.

The aim of this study was to use microsatellite markers to identify the key evolutionary processes and associated ecological factors shaping genetic population structure of *A. personatus*. Furthermore, a better knowledge of genetic diversity and structure of the sand lance could provide vital suggestions for sustainable exploitation and management of natural populations.

2. Materials and methods

2.1. Sample collection

A total of 372 adult sand lance individuals were collected at 14 locations in coast of Japan and 2 localities in China (Table 1, Fig. 1). Muscle samples were preserved in 95% ethanol or frozen for DNA extraction.

2.2. DNA extraction and PCR amplification

Total DNA was extracted from a piece of muscle tissue using a standard phenol-chloroform method (Sambrook et al., 1989). All individuals were genotyped at all 8 loci. Eight microsatellite loci were amplified by polymerase chain reaction (PCR) using primers developed from *A. personatus* by Ren et al. (2009). PCR amplifications were carried out in 25 μl reaction volumes containing 20 ng template DNA, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 μm of each primer, 1U Taq polymerase (Takara Co., China). The PCRs were performed under the following conditions: 5 min at 94 °C, then 30 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C (Ape104, Ape308, Ape315), 60 °C (Ape302), 54 °C (Ape349), 55 °C (Ape341, Ape313, Ape327) for 30s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min using a Biometra thermal cycler. The size of the alleles was determined according to their migrating distance. The fastest was named allele 1, and those following were named in order.

2.3. Statistical analyses

Using the program MICRO-CHECKER (Van Oosterhout et al., 2004), we check for potential technical problems such as null alleles, stuttering and large allele dropout. Null-allele frequencies were estimated according to Chakraborty et al. (1992).

Table 1
Sampling locations, number of individuals typed and collection date of *A. personatus* populations.

ID	Sampling sites	Sample size	Date of collection
Ish	Ishikari Bay	20	April 2006
Re	Rebun Island	24	June 2006
Sl	Sendai Bay large	24	April 2005
Ss	Sendai Bay small	24	April 2005
Qd	Qingdao	24	April 2005
Ise	Ise Bay	24	May 2005
Hy	Hyogo	24	April 2005
Fuku	Fukuoka	24	April 2005
Ca	Cape Soya	24	June 2006
Fuka	Fukaura	24	March 2006
Mu	Mutsu Bay	24	March 2006
Ot	Otsuko	18	March 2006
Kas	Kashima	22	March 2006
Kag	Kagawa	24	April 2005
Dl	Dalian	24	March 2009
Ha	Hachinohe	24	June 2005