Abstract

Japanese anchovy (Engraulis japonica) is a migratory marine fish of high economic significance in Taiwan. The adult Japanese anchovies migrate from the East China Sea to spawn in coastal waters of Taiwan; the larvae then drift back to the East China Sea to complete their life cycle. We developed six highly polymorphic microsatellites for E. japonica (expected heterozygosity ranging from 0.751 to 0.971) and these microsatellites can be used as genetic markers for identifying stocks to establish regulations in fishing management. Moreover, the markers will be useful in inferring the stock origins and migration routes in the future.

Keywords: Engraulidae, Engraulis japonica, microsatellite, migration, stock

Received 2 August 2001; revision accepted 19 September 2001

Genomic DNA for constructing the partial libraries was prepared according to procedures in Sambrook et al. (1989). Genomic DNA was digested with Sma3AI and fractioned in a 2.5% NuSieve™ GTG gel (FMC). DNA of size range of 300–900 bp was isolated, purified with GeneClean III kit (Bio101, Inc.) and ligated into plasmids, PUC18/BamHI/BAP (Pharmacia) according to the manufacturers’ protocols. Ligated plasmids were transformed into competent SURE cells (Stratagene). Recombinant clones containing inserts were transferred to Hybond-N nylon membranes (Amersham), which were hybridized to a set of oligonucleotide probes, including (AC)\textsubscript{10}, (TC)\textsubscript{10}, (CAC)\textsubscript{5}, CA, CT(ACCT)\textsubscript{6}, (TGTA)\textsubscript{6}, TG, and CT(CCT)\textsubscript{5}. Probes were labelled with DIG Oligonucleotide 3¢-End Labelling Kit (Boehringer Mannheim). Hybridization was performed at 60 °C for 24 h in a standard hybridization buffer, consisting of 5· SSC, 0.1% N-lauroylsarcosine, 0.2% SDS, and 1% Blocking Reagent (Boehringer Mannheim). The membranes were washed twice, each for 5 min at 45 °C with a solution of 2· SSC, 0.1% SDS, and then twice, each for 15 min at 65 °C with a solution of 0.1· SSC, 0.1% SDS. Chemiluminescent detection was performed with DIG Luminescent Detection Kit (Boehringer Mannheim). Exposure time ranged from 20 to 30 min. A total of 46 positive clones was sequenced on an automated sequencer (ABI/Perkin Elmer), in which 31 contained dinucleotide, trinucleotide, or other types of repeats. The online program primer 3.0 (http://www.genome.wi.mit.edu) was used to design primers from flanking regions of microsatellite DNA loci that contain more than 10 repeat units except one locus of pentanucleotide repeat motif. The pentanucleotide locus presumably offers better resolution in discerning alleles.
Individual genotypes were determined by polymerase chain reaction (PCR) with radioactive primers. Each PCR reaction totaled 10 μL, containing 100 ng template DNA, 10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton X-100, 0.25 mM dNTP, 1.5 mM Mg²⁺, 0.25 U Taq DNA polymerase (Promega), and 0.3 μM of each primer, with one end-labelled with [γ³²P]-ATP. Amplification was carried out by the thermal profile: 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, optimal annealing temperature (Table 1) for 1 min, 72°C for 1 min and final extension step at 72°C for 10 min.

PCR products were run on regular denaturing 6% polyacrylamide sequencing gel. Sequence of pUC18 was used as size marker to determine the allele sizes. The allelic PCR products differed in multiples of their repeat motifs. The genotypes were scored by two individuals independently and only consistent results were included for analyses.

Six microsatellites were found to be highly polymorphic among adult anchovies collected from waters near Taiwan (Table 1). The number of alleles per locus ranged from 35 to 53, and the observed and expected heterozygosity ranged from 0.438 to 0.746, and from 0.751 to 0.971, respectively (Table 1). The observed genotypes were deviated from Hardy–Weinberg expectation at five out of the six loci (P < 0.05) resulting from heterozygote deficiency. Should these fishes be representatives of different stocks, the deficiency may be due to the combination of samples from the different localities of their captures (therefore different stocks). However, the presence of null alleles could also cause the heterozygote deficiency. The large numbers of alleles is notable and may reflect the fact of the large effective population sizes of the species. These microsatellites can be used as genetic markers for identifying stocks to establish regulations in fishing management.

Acknowledgements
Yu-Ying Liao, Dr Chu-Fong Lo and members of her laboratory offered technical support for molecular cloning. Financial aids were granted to TSC and to HTY by the National Science Council of the Republic of China.

References


© 2002 Blackwell Science Ltd, Molecular Ecology Notes, 2, 49–50