Population genetic structure of the Japanese eel *Anguilla japonica* in the northwest Pacific Ocean: evidence of non-panmictic populations

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ABSTRACT: The catadromous Japanese eel has an unusual life history. Controversy remains, however, as to whether its population structure is characterized by panmixia or genetic differentiation. Migration patterns are considered an important factor in determining a species' population structure. Eight polymorphic microsatellite loci were used as genetic markers through which we were able to reject the null hypothesis of panmixia. Slight genetic differentiation between these populations was determined by Wright's and Slatkin's fixation indices, F_{ST} and R_{ST} statistics adjusted using Bonferroni's correction. Although isolation-by-distance is often observed in marine species, the present result is thought to follow the member-vagrant migratory hypothesis. The metapopulation model fits the elver recruitment strategy according to results of the high probability of assignment test. The UPGMA tree showed the Japanese eel populations as being divided into 2 groups: a low-latitude group (Shantou in South China, and Tanshui and Fangliao in Taiwan) and a high-latitude group (Mikawa Bay in Japan, Daecheon-myon in Korea, and the Yalu River and Hangzhou in Northeast China). Temporal genetic variations indicate that most progeny return to similar habitats where their ancestors formerly resided. There was a high correlation (AMOVA; p < 0.05) when the Japanese eel was divided into the 2 management units (high- and low-latitude groups). These units will be useful when applied to fisheries conservation and management in the northwestern Pacific Ocean.

KEY WORDS: Catadromous eel · Fisheries management units · Genetic differentiation · Member-vagrant model · Microsatellites

INTRODUCTION

Diadromous fish move predictably between fresh and saltwater at relatively fixed periods in their life history. Many are commercially important, but their life histories and population structures are still not well known. As the catadromous eels of *Anguilla* have an interesting life history of spawning in the sea and growing up in rivers, many studies of their population genetics have been carried out. During the last 3 decades, these studies have attempted to determine whether conspecific eels from different freshwater localities are panmictic or if they represent different populations.

Although the population genetic structures of *Anguilla anguilla*, *A. japonica*, and *A. rostrata* have been intensively studied, controversy still exists as to whether these species are made up of a single panmictic population or multiple geographically based populations. For *A. anguilla*, protein electrophoresis and sequencing of the mitochondrial DNA (mtDNA) control region support the hypothesis that eels from European and North African rivers belong to a panmictic population (De Ligny & Pantelouris 1973, Lintas et al. 1998,

Avise 2003). Similar conclusions were reached for *A. rostrata* and *A. japonica* (Avise et al. 1986, Sang et al. 1994, Ishikawa et al. 2001). Although results derived from both nuclear and cytoplasmic markers have supported the panmictic hypothesis for the genetic structure of *Anguilla* eels, several studies have challenged this opinion. Slight genetic differentiation among 13 samples of European eels from the North Atlantic, the Baltic Sea, and Mediterranean Sea basins refutes the panmictic population hypothesis (Wirth & Bernatchez 2001). However, *A. japonica* on the western Pacific fringe otherwise exhibits clear geographic clines when using NADP-isocitrate dehydrogenase (IDHP) and 6-phosphogluconate dehydrogenase (PGDH) allozyme loci as markers (Chan et al. 1997). A migration time-lag from various parts of different continents to spawning grounds west of the Mariana Islands is hypothesized as being a reason for the formation of this cline. The same results as applied to aging, using otoliths at metamorphosis and estimation of arrival times at estuaries, were determined from otolith increments; e.g. changes in the growth-line width and the strontium/calcium ratio further subdivide geographic populations of Japanese eel (Cheng & Tzeng 1996).

Anguilla japonica is a temperate freshwater eel distributed in the rivers of northeastern Asian countries including Taiwan, China, Japan, and Korea (Tesch 1977). The leptocephali hatch in spawning grounds presumably located at deep offshore grounds to the west of the Mariana Islands at 15° N, 140° E (Tsukamoto 1992). They then drift westerly with the North Equatorial Current. When approaching the continental shelf of the Philippines, they continue to move northwards to join the Kuroshio Current by the aid of Ekman transport. Once the leptocephali metamorphose into the glass eel stage, they leave the strong Kuroshio Current journeying to estuaries where they continue to grow as elvers. In the course of their upstream migration, elvers grow to yellow eels which spend the next 5 to 10 yr in freshwater rivers (Tzeng 1985). Once eels attain sexual maturity, they migrate downstream toward the deep sea for spawning (Tesch 1977).

The extremely compressed willow-like body shape facilitates the long-distance dispersal of leptocephali by ocean currents. Accordingly, metamorphosis times and migration patterns are considered important factors in determining the population structure of the Japanese eel. Opinions regarding the migratory route of Japanese eels, however, are diverse (Sang et al. 1994, Cheng & Tzeng 1996, Ishikawa et al. 2001).

Two migration models of freshwater eels have been proposed. The panmictic model suggests that adult eels (e.g. the American eel, *Anguilla rostrata*) from different nursery grounds randomly mate in a common spawning ground, with their juveniles reaching estuaries at random after metamorphosis (Avise et al. 1986, Wirth & Bernatchez 2003). The isolation model assumes that adult eels grow in separate freshwater habitats and spawn in separate breeding grounds at different times. These freshly hatched leptocephali are carried back to their respective parents' freshwater nursery habitats by the aid of currents. For *A. marmorata*, this model involves different migration loops over the western Pacific Ocean which may result in genetic differentiation (Ishikawa et al. 2004).

A recent hypothesis, 'the member-vagrant model', examined the role of ecological processes in determining the genetic patterns of marine species (Sinclair & Iles 1988). Members are the fittest individuals, i.e. those juveniles recruited to appropriate habitats shared by adults. Vagrants are individuals which disperse to new habitats with an ancestry different from the nursery. Although larvae have greater dispersal ability to find new habitats, their lives may too short before they become adults. This hypothesis has been corroborated by ecological and genetic studies of populations of the Atlantic herring *Clupea harengus*, anadromous rainbow smelt *Osmerus mordax*, and European eel *Anguilla anguilla* (Iles & Sinclair 1982, Fortier & Gagne 1990, Bernatchez & Martin 1996, Maes & Volckaert 2002).

A metapopulation is described an assemblage of interacting individuals, and it seems an apt term for an assemblage of spatially delimited local populations that are coupled by a few degrees of migration (Hanski 1994, Gaggiotti 1996). However, because the population structure indicates slight but significant genetic differentiation among populations, incomplete isolation among populations may be discovered due to stray recruitment. However, this seems to be suggested for the European eel (Maes & Volckaert 2002).

An approach to studying metapopulations is to analyze patterns of the presence or absence of organisms in relation to landscape characteristics (Sjögren-Gulve 1994). An assignment test was developed to determine the origin of particular individuals. This test assigned individuals to populations on the basis of their genotypes, while simultaneously estimating population allelic frequencies (Pritchard et al. 2000).

The Japanese eel provides a prime model for studying the spatial and temporal population structure of catadromous eels in the northwestern Pacific Ocean. Highly polymorphic microsatellites encoded by selective neutral nuclear loci have recently been used to investigate the genetic structure of populations, including many aquatic species for which it is difficult to tag individuals (Yu et al. 2002). The purpose of this study was to test the panmictic population hypothesis using 8 microsatellite loci and to clarify whether the migration model fits what is known about Japanese eel elvers.

MATERIALS AND METHODS

Sample. In total, 328 *Anguilla japonica* elvers were caught alive in the winter of 1999 and spring of 2000 from 7 locations throughout their range in northeastern Asia. Collecting locations included Tanshui and Fangliao in Taiwan; the Yalu River, Hangzhou, and Shantou in China; Mikawa Bay in Japan; and Daecheon-myon in Korea (Table 1 & Fig. 1). In all, 88 replicate yearly samples were collected separately from Tanshui and Fangliao in 1997 and 1990, respectively. All specimens were preserved in 95% ethanol prior to DNA extraction.

DNA extraction. Genomic DNA was isolated and purified from muscle tissue; the ethanol was removed by evaporation before treatment. A piece of muscle weighing 500 mg was digested in 1 ml of lysis buffer (10 mM Tris-HCl, pH 8.0, 2 mM ethylene diamine tetra-acetic acid [EDTA], 10 mM NaCl, 1% sodium dodecyl sulfate [SDS], 10 mg ml^{-1} dithiothreitol [DTT]) and 55 µl of proteinase K (0.5 mg ml^{-1}) before the DNA extraction (Kocher et al. 1989). DNA was precipitated with 95% ethanol, washed with 70% ethanol, air-dried, and then dissolved in TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) (Sambrook et al. 1989). Genomic DNA was quantified and diluted to a working concentration of 1 ng µl^{-1}.

PCR and genotyping. Eight microsatellite loci were screened from the *Anguilla japonica* genome including 6 (GT)$_n$ (*MS-1*, *MS-3*, *MS-4*, *MS-5*, *MS-7*, and *MS-8*) and 2 (GA)$_n$ (*MS-2* and *MS-6*) loci. The MS-1 to 6 microsatellite sequences on EMBL, under the accession numbers AJ297599~ AJ297603 and AJ297605, were described previously (Tseng et al. 2001). The primers were adjusted based on the flanking regions of 6 microsatellite sequences to avoid null alleles. *MS-7* (forward primer: 5′-AGTAAAGAGTCCCACGCATTC-3′; reverse primer: 5′-AAGGTGGATTTTTGCTGGCTC-3′) and *MS-8* (forward primer: 5′-AGGCTGAAGTGAGTATGCT-CAG-3′; reverse primer: 5′-AGATATGGAAGCAGGAT-GGAG-3′) microsatellite loci were cloned and sequenced first in this study, and their respective accession numbers are AM062761 and AM062762. Microsatellites were amplified via the polymerase chain reaction (PCR). Forward primers were labeled with FAM, TAMRA, or HEX fluorescence. The PCR consisted of approximately 1 ng genomic DNA, 2 pmol reverse primer, 2 pmol labeled forward primer, 25 mM dNTP, 0.05 to 0.1 mM MgCl$_2$, 10× buffer, and 0.5 U *Taq* polymerase (Takara) made up to a 25 µl volume with Milli-Q water. Amplification was performed in a PC-960G Microplate Thermal Cycler (Corbett Research) programmed with the following schedule: initial denaturing at 95°C for 4 min followed by 38 cycles of 30 s each at successive thermal regimes of 94, 52, and 72°C. Eight microliters of PCR products was precipitated with 95% alcohol. Semiauto-
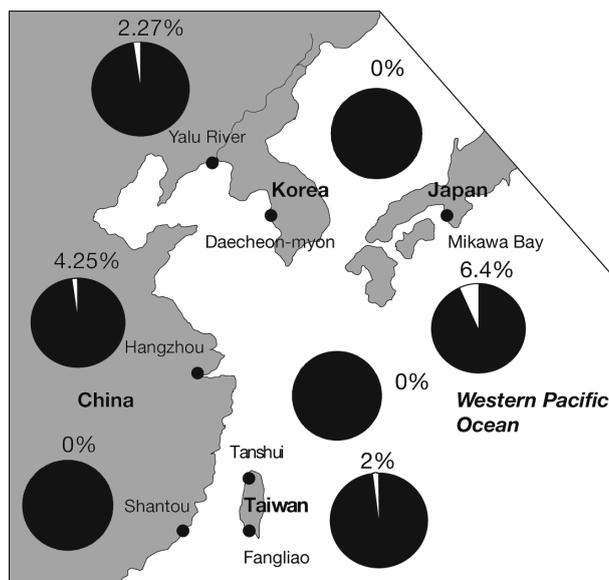

Fig. 1. *Anguilla japonica*. Sampling localities. The pie charts indicate the percentage of foreign elvers (vagrant)

mated genotyping was performed using a capillary MegaBACE-500 DNA analysis system (Amersham Biosciences). Data were scored by Genetic Profiler software 1.5 (Amersham Biosciences). The sizes of each allele were checked with the naked eye.

Data analysis. The total number of alleles (*na*), effective number of alleles (*ne*), and observed (*H*$_o$) and expected (*H*$_E$) heterozygosities were independently calculated for each locus (Yang & Yeh 1993). Multilocus estimates for each of the 7 samples were calculated as well. Linkage disequilibrium among all pairs of loci and for all samples was determined using Burrows' composite measure and χ^2 values (Ohta 1982). Expected genotypic frequencies under random mating were calculated using the algorithm by Levene (1949), and likelihood ratio (G^2) tests for Hardy-Weinberg equilibrium were performed for each locus.

To examine the relationship among populations, Nei's (1978) unbiased genetic distance (*Ds*) was computed between all pairs of populations. The original topology of the phylogenic tree was inferred using the UPGMA method based on *Ds*. One thousand bootstrap re-samplings were used to evaluate support of the data set for the relationships.

Interpopulational genetic differentiation was estimated by calculating the Slatkin's and Wright's fixation indices, R_{ST} and F_{ST}, based on 2 possible molecular evolution models: the infinite allele model (IAM) and stepwise mutation model (SMM) (Weir & Cockerham 1984, Raymond & Rousset 1995). The significance level was adjusted by sequential Bonferroni corrections (Rice 1989).

Table 1. Levels of genetic variation observed at 8 microsatellite loci within 9 Japanese eel samples: sample size (n), number of alleles detected at each locus, observed (H_o) and expected (H_E) heterozygosity within samples, number of unique alleles (U) in each sample, number of solitary missing alleles (S) in each sample, observed number of alleles (na) and effective number of alleles (ne). *Indicates a significant Hardy-Weinberg disequilibrium level at 5%

Locus	Tanshui (1999) $n=49$	Tanshui (1997) $n=46$	Fangliao (2000) $n=50$	Fangliao (1990) $n=42$	Shantou $n=46$	Hangzhou $n=47$	Yalu River $n=44$	Mikawa Bay $n=47$	Daecheon -myon $n=45$	Mean	na	ne
MS-1 No. of alleles (U/S)	12 (0/0)	14 (0/1)	14 (2/0)	13 (0/0)	14 (0/1)	14 (2/1)	15 (0/0)	11 (0/1)	16 (0/0)	13.60	21	9.82
H_o	0.774	0.734	0.759	0.815	0.758	0.767	0.778	0.731	0.800	0.768		
H_E	0.868	0.857	0.899	0.890	0.880	0.871	0.897	0.866	0.927	0.884		
Frequency of main allele	0.224	0.262	0.190	0.167	0.22	0.255	0.239	0.234	0.133			
MS-2 No. of alleles (U/S)	15 (0/0)	20 (1/0)	16 (0/1)	14 (0/1)	18 (0/0)	15 (0/0)	15 (0/1)	17 (0/0)	15 (0/0)	16.11	22	13.55
H_o	0.742	0.750	0.828	0.815	0.818	0.833	0.833	0.846	0.829	0.810		
H_E	0.909	0.912	0.936	0.916	0.925	0.906	0.921	0.929	0.913	0.918		
Frequency of main allele	0.204	0.196	0.140	0.202	0.198	0.170	0.160	0.127	0.178			
MS-3 No. of alleles (U/S)	5 (1/0)	6 (0/0)	5 (0/0)	8 (0/0)	7 (1/0)	6 (0/0)	6 (0/0)	7 (1/0)	4 (0/0)	6	10	2.99
H_o	0.516	0.574	0.586	0.667	0.667	0.567	0.528	0.654	0.514	0.586		
H_E	0.619	0.671	0.693	0.735	0.698	0.672	0.635	0.706	0.628	0.673		
Frequency of main allele	0.551	0.469	0.380	0.131	0.396	0.489	0.524	0.319	0.511			
MS-4 No. of alleles (U/S)	11 (0/0)	12 (0/0)	12 (0/0)	14 (3/0)	12 (0/0)	11 (1/0)	10 (0/0)	11 (0/0)	10 (0/0)	11.44	17	9.49
H_o	0.742	0.786	0.759	0.778	0.788*	0.767	0.778	0.769	0.743	0.768		
H_E	0.881	0.902	0.883	0.897	0.898	0.898	0.857	0.903	0.880	0.889		
Frequency of main allele	0.224	0.174	0.246	0.226	0.198	0.149	0.205	0.191	0.200			
MS-5 No. of alleles (U/S)	12 (0/0)	11 (0/0)	11 (1/0)	11 (3/0)	12 (0/0)	9 (0/1)	9 (0/0)	11 (0/0)	14 (1/0)	11.11	17	6.72
H_o	0.774	0.714	0.724	0.778	0.758	0.667	0.667	0.731	0.800	0.735		
H_E	0.868	0.856	0.838	0.860	0.841	0.793	0.791	0.864	0.905	0.846		
Frequency of main allele	0.204	0.229	0.290	0.238	0.286	0.319	0.296	0.276	0.200			
MS-6 No. of alleles (U/S)	17 (0/0)	18 (1/0)	19 (0/0)	16 (0/1)	21 (0/0)	16 (0/1)	19 (1/0)	15 (0/0)	18 (1/1)	17.66	25	17.10
H_o	0.871	0.836	0.828	0.815	0.848	0.833	0.833	0.846	0.829	0.838		
H_E	0.936	0.928	0.947	0.936	0.947	0.936	0.946	0.939	0.938	0.939		
Frequency of main allele	0.133	0.098	0.12	0.131	0.110	0.127	0.114	0.128	0.133			
MS-7 No. of alleles (U/S)	13 (0/0)	12 (0/0)	12 (1/0)	13 (1/0)	12 (0/1)	13 (0/1)	11 (1/0)	9 (0/1)	13 (0/1)	12	23	7.95
H_o	0.742	0.736	0.793	0.822	0.758	0.767	0.750	0.808	0.771	0.772		
H_E	0.879	0.860	0.863	0.902	0.878	0.895	0.847	0.871	0.889	0.876		
Frequency of main allele	0.173	0.174	0.21	0.202	0.242	0.191	0.228	0.213	0.200			
MS-8 No. of alleles (U/S)	16 (1/1)	16 (1/0)	13 (0/0)	14 (1/0)	16 (1/0)	18 (1/0)	16 (1/0)	12 (0/2)	15 (0/1)	15.11	24	12.83
H_o	0.839	0.812	0.759	0.800	0.849	0.800	0.694	0.769	0.771	0.788		
H_E	0.918	0.901	0.893	0.906	0.915	0.933	0.768	0.876	0.915	0.892		
Frequency of main allele	0.163	0.164	0.24	0.250	0.176	0.170	0.183	0.298	0.155			
Mean No. of alleles	12.62	13.62	12.75	14.25	14	12.75	12.62	11.62	13.12	12.88	19.87	10.06
H_o	0.750	0.743	0.754	0.786	0.780	0.75	0.733	0.769	0.757	0.758		
H_E	0.860	0.861	0.869	0.880	0.873	0.863	0.833	0.869	0.874	0.865		
Total U/S	2/1	3/2	4/1	8/2	2/2	4/4	3/1	1/4	2/3			

F statistics of spatial samples following the formula of hierarchical level analysis (Weir & Cockerham 1984) of F_{IT} and F_{IS} were calculated using the jackknife over loci to obtain variance estimates with the TPFGA software (Miller 1998). Wright's overall inbreeding coefficient (F_{IT}) measures the reduction in heterozygosity of an individual relative to the total population. Additionally, Wright's inbreeding coefficient (F_{IS}) measures the reduction in heterozygosity of an individual due to nonrandom mating within its subpopulation. The 95% confidence intervals (CI) for components of the population structure were determined by jackknifing and 5000 bootstraps conducted over loci using the TFPGA program (Miller 1998). The hierarchical genetic structure was investigated here using analysis of molecular variance (AMOVA, Excoffier et al. 1992) based on an analysis of the variance of genetic distances.

In addition, an assignment test was developed to determine the origin of particular individuals. This test assigned individuals to spatial populations on the basis of their genotypes, while simultaneously estimating population allelic frequencies (Pritchard et al. 2000). The high accuracy of assignments (99% probability intervals) was analyzed after 30 000 burn-in periods with 100 000 replications in the 'STRUCTURE' program (Pritchard et al. 2000).

We tested isolation-by-distance (IBD) as an indicator of an emerging population structure by regressing geographic distance on genetic distance (Ds), F_{ST}, and R_{ST}. We used Mantel tests for correlation of 2 parameters to examine the significance of the IBD relationships (Mantel 1967).

RESULTS

Within-population genetic variation

All 8 microsatellite loci scored among the 9 samples of *Anguilla japonica* from northeastern Asian countries were highly polymorphic. The number of alleles per locus ranged from 10 (*MS-3*) to 25 (*MS-6*) ($N = 416$) with an average of 19.87. The mean number of alleles per locus in each sample ranged from 11.62 (Mikawa Bay) to 14.25 (Fangliao 1990) (Table 1). In total, 29 private and 20 solitary missing alleles (only missing in 1 of all samples) were found in the distributions of the 8 loci. The Japanese eel sampled from Fangliao (1990) seemed to have a greater number of private alleles than did the other samples.

The mean observed heterozygosity over all loci ranged from 0.586 ± 0.051 (SE) (*MS-3*) to 0.838 ± 0.012 (*MS-6*), with an average of 0.758 ± 0.049. Samples from Fangliao (1990) exhibited the highest average genetic diversity and had a mean observed heterozygosity of 0.786 ± 0.034. The expected heterozygosity ranged from 0.673 ± 0.031 (*MS-3*) to 0.939 ± 0.005 (*MS-6*), with an average of 0.865 ± 0.052 (Table 1). One of the 8 loci (*MS-4*) in the Shantou sample showed departure from Hardy-Weinberg equilibrium ($p < 0.05$), with a significant heterozygote deficiency. Permutation tests for linkage disequilibrium among the 8 loci for all 9 samples revealed only slight disequilibrium for the entire data set. The total variance of disequilibrium (D_{IT}^2) was estimated to be 0.015, which is a small value.

Among-population genetic variation

To study evolutionary relationships among spatial and temporal samples, unbiased Nei's (1978) genetic distances were examined and shown to range from approximately 0.040 (Shantou vs. Tanshui 1997) to more than 0.171 (Tanshui vs. Hangzhou) with an average of 0.096 ± 0.025.

For further determination of population subdivisions, F_{ST} and R_{ST} were used to obtain respective values of −0.002 to 0.019 and −0.009 to 0.104 (Table 2). Samples from Fangliao (1990) exhibited significant genetic differentiation when compared with other samples (F_{ST},

Table 2. Pair-wise F_{ST} estimates between samples above the diagonal and R_{ST} estimates below the diagonal. The significance level was adjusted by Bonferroni's correction. *$p < 0.05$; **$p < 0.01$; [a]$p < 0.0014$

	Daecheon-myon	Mikawa Bay	Yalu River	Hangzhou	Shantou	Tanshui (1999)	Tanshui (1997)	Fangliao (2000)	Fangliao (1990)
Daecheon-myon	–	0.006*	0.005	0.002	0.006**	0.009**	0.009**	0.007*	0.011[a]
Mikawa Bay	0.011	–	0.008**	0.007*	0.006*	0.015[a]	0.013**	0.004	0.016[a]
Yalu River	0.007	0.048[a]	–	0.003	0.007[a]	0.009**	0.013[a]	0.015[a]	0.011[a]
Hangzhou	0.010	0.050[a]	0.019*	–	0.013[a]	0.012[a]	0.019[a]	0.009*	0.013[a]
Shantou	−0.002	0.016	0.023**	0.005	–	0.005	−0.002	0.006	0.012[a]
Tanshui (1999)	0.005	0.036[a]	0.028**	0.011**	−0.009	–	0.002	0.009**	0.012[a]
Tanshui (1997)	0.029**	0.018	0.059[a]	0.055[a]	0.009	0.011	–	0.010**	0.013[a]
Fungliao (2000)	0.017	0.022*	0.030**	0.024	−0.008	−0.003	-0.001	–	0.005
Fungliao (1990)	0.055[a]	0.104[a]	0.059[a]	0.052[a]	0.067	0.055[a]	0.077[a]	0.012	–

0.005 to 0.016; p < 0.0014) except for Fangliao (2000). In Tanshui alone, samples collected in 1999 revealed no significant genetic differentiation from those in 1997 (F_{ST} = 0.002, p > 0.05). Our overall results indicated moderate genetic divergence between Mikawa Bay and Fangliao 1990 (R_{ST} = 0.104 > 0.1, p < 0.0014), which was the highest among the 9 samples tested (Table 2). Measures of hierarchical genetic structure among spatial populations of *Anguilla japonica* indicated mean values for F_{IT} and F_{IS} of 0.265 and 0.25, respectively.

   The UPGMA tree based on the genetic distance data set showed 2 clades: a low-latitude group (Shantou, Tanshui, and Fangliao) and a high-latitude group (Daecheon-myon, the Yalu River, Hangzhou, and Mikawa Bay) (Fig. 2). Nei's genetic distance ranged from 0.058 to 0.091 (mean 0.072 ± 0.011) in the high-latitude group, and from 0.040 to 0.127 (mean 0.093 ± 0.028) in the low-latitude group (Table 3). The above-tree topology supported by high bootstrap values (100)
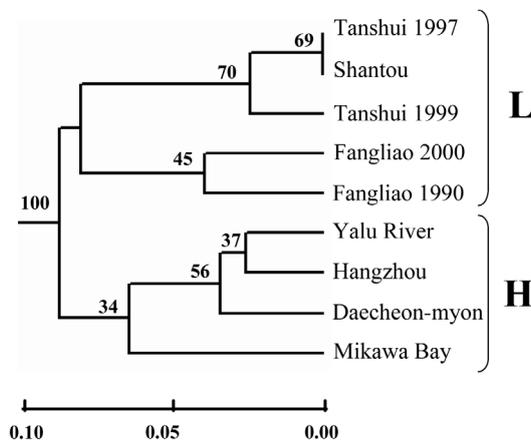

Fig. 2. UPGMA tree drawn using Nei's genetic distance measured for 9 samples of *Anguilla japonica*. Bootstrap values at each node were obtained by 1000 replicates over loci. Local populations on the tree were grouped into 2 major clades, a low-latitude group (L: Shantou, Tanshui, and Fangliao) and a high-latitude group (H: Mikawa Bay, Daecheon-myon, the Yalu River, and Hangzhou)

indicated that the dispersal of Japanese eel elvers to the coast does not occur randomly.

   Results of the hierarchical analysis of molecular variance (AMOVA) within and among the 7 spatial populations of *Anguilla japonica* are shown in Table 4. When genetic variation was tested among the 2 groups (low- and high-latitude groups) from 7 spatial samples, the results of Φ_{ct} = 0.009 (p < 0.05) indicated that 1.95% of the total genetic variation was partitioned in the among-group category, 95.73% within populations (p < 0.001), and 2.32% among populations within a group. The latter also showed significance (p < 0.001) presumably attributable to differences in allelic frequencies among the 7 samples investigated (Table 4).

   Genetic distances versus coastal geographical distances for all possible pairwise combinations of the 7 *Anguilla japonica* samples showed no significant correlation. The Mantel test for correlation of parameters excluded a possible isolation-by-distance principle in the Japanese eel samples (r = 0.123, p = 0.258). When genetic distance was used instead of F_{ST} and R_{ST} values, we found similar results.

   The origin of particular individuals among the 7 spatial samples was calculated. We assigned an accuracy probability of > 0.95. Among the 328 individuals in the 7 samples, assignment tests indicated that 319 individuals (97.26%) obviously belonged to the resident group, while 9 individuals (2.74%) were foreign recruits (Fig. 1). Some local samples contained a few recruits from other populations. In the Mikawa Bay sample alone (n = 47), a rough estimate of foreign-recruited elvers accounted for a total of 3 (6.4%) which included one from the low-latitude region (2.13%) and 2 (4.26%) from the high-latitude region.

DISCUSSION

   As a catadromous fish, the Japanese eel has more-numerous alleles per unit of microsatellite locus (mean,

Table 3. Nei's unbiased genetic distance (below diagonal) of 9 Japanese eel samples

	Daecheon-myon	Mikawa Bay	Yalu River	Hangzhou	Shantou	Tanshui (1999)	Tanshui (1997)	Fangliao (2000)	Fangliao (1990)
Daecheon-myon	0								
Mikawa Bay	0.072	0							
Yalu River	0.066	0.091	0						
Hangzhou	0.060	0.086	0.058	0					
Shantou	0.086	0.083	0.081	0.131	0				
Tanshui (1999)	0.096	0.105	0.089	0.116	0.078	0			
Tanshui (1997)	0.111	0.103	0.125	0.171	0.040	0.059	0		
Fangliao (2000)	0.092	0.069	0.134	0.103	0.088	0.101	0.119	0	
Fangliao (1990)	0.108	0.995	0.099	0.105	0.119	0.111	0.127	0.086	0

Table 4. Hierarchical analysis of molecular variance (AMOVA) within and among 7 populations of *Anguilla japonica* in northeastern Asia. Two groups including the high- and low-latitudinal groups were inferred from the topological tree. The significance level (p) of the Φ-statistic is based on 1000 permutations. *p < 0.05; **p < 0.001

Variance component	Observation–partition		Φ-statistic
	Variance	Percent of total	
Among groups	0.722	1.95	$\Phi_{ct} = 0.009$*
Among populations/ within groups	2.522	2.32	$\Phi_{sc} = 0.029$**
Within populations	72.715	95.73	$\Phi_{st} = 0.036$**

19.87) than do anadromous (mean = 10.8) and freshwater fishes (mean = 9.1), but slightly fewer than do marine fishes (mean = 19.9) (DeWoody & Avise 2000). The mean number of alleles/locus/sample varied between 11.62 (Mikawa Bay) and 14.25 (Fangliao) (mean = 12.88) in Japanese eels, and 9.6 and 12.4 (Daemen et al. 2001) or 12.9 and 21.2 (Wirth & Bernatchez 2001) in European eels. These values are all within the range common for teleosts. These results imply a progressive change in genetic variability among freshwater, anadromous, catadromous, and marine fishes. Although the freshwater eel is categorized as a catadromous fish, its genetic diversity is close to that of marine fishes.

The average observed heterozygosity over 8 microsatellite loci ranged from 0.586 to 0.838 in Japanese eels, and 0.278 to 0.737 (Daemen et al. 2001) or 0.835 to 0.895 (Wirth & Bernatchez 2001) in European eels. Only 1 locus showed significant departure from Hardy-Weinberg equilibrium in 1 sample. This may have been associated with the null allele effect, where the increase in homozygosity may have been caused by a failure of allelic amplification due to a mutation in the primer region. Among the 8 polymorphic microsatellite loci used, locus *MS-3* exhibited the lowest heterozygosity value and allele number. There were fewer repetitions (6 to 12) at the *MS-3* locus than at other loci, implying a correlation of repetition number with increasing allele number and genetic diversity (Weber 1990).

Population structure

Several pieces of evidence support the population subdivision of Japanese eels using the highly resoluble microsatellites as gene markers. The genetic differentiation indices (F_{ST} and R_{ST}) indicated a weak but significant spatial divergence among samples. Recent studies have argued for a random mating system in *Anguilla* eels, but Wirth & Bernatchez (2001) have a different view of the European eel. The significant ge-

netic differentiation indices obtained in this study support the non-panmictic hypothesis in the Japanese eel. There may have been strong inbreeding within populations ($F_{IT} = 0.265$ and $F_{IS} = 0.25$). The magnitude of spatial differentiation in Japanese eels ($F_{ST} = -0.002$ to 0.019, mean 0.009) is higher than that of the European eel (0.0017 in Daemen et al. 2001; 0.004 in Wirth & Bernatchez 2001).

Since the AMOVA Φ-statistic value of 1.95% (p < 0.05) provides a certain degree of genetic variance between the 2 groups (low and high latitudes), Japanese eel populations are clearly distinguished into a 2-group model, as represented by the general profile shown in the relevant tree topology (Fig. 2). As to their life history, aging, based on otoliths at metamorphosis, is somewhat postponed in the high-latitude group compared to the low-latitude group (Cheng & Tzeng 1996).

Our results thus clearly show that a weak but highly significant genetic structure exists in the Japanese eel. We wanted to determine which one of the population models best explains the observed genetic structure. The timing of metamorphosis of a leptocephalus and transportation by the Kuroshio Current were considered to be important factors determining the ultimate destination of eels. The eel has a lengthy leptocephalus stage during the oceanic passive migration, and there is a geographic cline in age at metamorphosis that increases from south to north (Cheng & Tzeng 1996). Variations in the duration of the larval stage are an important factor in determining the distribution of a fish. In the absence of a suitable habitat for settlement, larvae may delay metamorphosis, a delay which may last for months in several species of invertebrates (Richmond 1985). The age at metamorphosis and the lengthy duration of the leptocephalus stage are important factors determining the long-distance dispersal of the Japanese eel.

Results from this study in conjunction with previous studies (Cheng & Tzeng 1996, Tseng et al. 2003) provide new elements that can refine the current hypotheses including isolation by distance (Maes & Volckaert 2002), spatial differentiation (Ishikawa et al. 2004), temporal reproductive separation (Cheng & Tzeng 1996), and some combination of these.

The first isolation-by-distance hypothesis states that differentiation contributes to genetic structuring at the spawning grounds. The hydrogeographical structure of the spawning area potentially limits contact between spawning groups: a scenario detected using both microsatellite and allozymic markers of *Anguilla anguilla* (Wirth & Bernatchez 2001, Maes & Volckaert 2002). Each

species has its own particular genetic structures based on its own adaptations to the unique hydrographic systems of the respective Pacific and Atlantic habitats. Interspecific differences in genetic structure result from variations in systems of ocean currents and evolutionary histories. When examining the Mantel relation test, we could largely reject the isolation-by-distance hypothesis in the Japanese eel, although the European eel conforms to this hypothesis (Wirth & Bernatchez 2001, Maes & Volckaert 2002). Inconsistencies with the isolation-by-distance model are evidenced by greater divergences between closely spaced sites. This may be because different groups of elvers passively drift to destinations, a process dependent on the complicated sub-current systems of the northwestern Pacific Ocean.

The second hypothesis states that spatial differentiation contributes to genetic structuring at a particular migratory loop: maturing adults may spawn within their subpopulation in a specific area. The existence of 5 geographic populations of *Anguilla marmorata* can be understood by examining the close correspondence of their geographic ranges with the oceanographic divisions of major upper-water masses characterized by salinity and temperature (Ishikawa et al. 2004). The life cycle of each of the 5 populations should be completed within each oceanic division. It can be concluded, therefore, that the general rule of population structure of anguillid eels should be regarded as a 'one-population/one-migration loop within one oceanic division' (Ishikawa et al. 2004). However, *A. japonica* and *A. marmorata* have different life history patterns. *A. marmorata* differs markedly from temperate anguillid eels in having distinct spawning and recruitment seasons.

The third hypothesis suggested by Chan et al. (1997) states that Japanese eel groups of spawning adults originating from different regions are temporally isolated, and this persists from year to year. Offspring fitness increases when individuals stay in close spatiotemporal proximity to their own 'clutch', and offspring return to the habitat of their ancestors due to the system of currents. Tseng et al. (2003) examined temporal genetic variations in Japanese eels in northern Taiwan from 1997 to 1999. The results indicated no significant differentiation among temporal samples. In this study, we analyzed temporal genetic variations from samples of Tanshui in 1997 and 1999 and Fangliao in 1990 and 2000. Lower genetic distances were presented between those respective samples. These results support offspring returning to the habitat of their ancestors.

Nevertheless, the larval ecology of the Japanese eel is still poorly known, because of a lack of significant genetic continuity among elvers. Leptocephali of the low-latitude group rarely succeed in drifting to farther destinations due to the postponement of metamorphosis as a result of nutrient shortages. Vagrant larvae may reach the coasts of Japan and northern China with the aid of the speedy Kuroshio Current (2 to 3 knots or 77 to 116 km d^{-1}) after it passes near Taiwan (Nitani 1972). Although the Japanese eel is known to be composed of 2 subgroups, our study does not fully support the isolated migration model. Geographical divergences observed among sites of the present study may be the result of physiological and environmental influences on larval dispersal. For example, 2.27% of the sample in the Yalu River consisted of foreign-recruited elvers (Fig. 1). This suggests that ecological processes inferred by the member-vagrant hypothesis with some degree of exchange between neighboring-populations (metapopulation) explain the present and previous results of the eel's population genetic structure. The member-vagrant model may be a primary factor controlling population genetic diversity in Japanese eel elvers. The population structure of the Japanese eel, however, still remains genetically diverse.

Management

Serious declines in fish stocks in many parts of the world over the past 2 to 3 decades and the collapse of several economically important fisheries have driven many fisheries organizations to reexamine the principles they apply to fisheries management (Richards & Maguire 1998). The gradually decreasing population size of *Anguilla* freshwater eels may be related to several causes, e.g. overfishing of elvers since the 1960s, destruction of adult eel habitats in rivers, and population declines due to long-term climatic fluctuations since the Holocene (Tseng et al. 2003, Wirth & Bernatchez 2003), and suggest that effective management should pay attention to these phenomena. Effective future conservation should incorporate issues of scientific research, legislation, and management. The observed distribution of genotypes in this study are indicative of non-random mating and restricted gene flow among eels from different sampled locations, and are at odds with the concept of a single panmictic stock. In addition to the above, there are many other uncertainties pertaining to the biology and life cycle of the eel. It is thought that this phase which is principally being driven by long-term oceanic and climate changes may be impacting the recruitment of anguillid species throughout the Northern Hemisphere. While such changes may be beyond our control, management actions may be required to reduce exploitation or enhance stocks to counteract the effects of this natural phenomenon. This is particularly pertinent to the management of elver fisheries in parts of the northwestern Pacific Ocean. As to concerns of scientific

research, we have delineated 2 management units (high- and low-latitudinal groups) for the Japanese eel, in order to measure population demographics. A forthcoming work will focus on ecological investigations within these geographic regions and propose constructive polices for the control of overfishing of both elvers and adults.

Microsatellite diversity

The resolution of genetic diversity varies with the genetic markers used. Genetic diversity derived from microsatellite loci of Japanese eels was higher than that from analysis of mitochondria or proteins. Previous studies of genetic differences with the mitochondrial control region revealed similar genetic and haplotypic diversities in Japanese eels (0.16 to 2.60% and 96%) as in sardines (*Sardina* and *Sardinops*, 1 to 3% and 96 to 100%, respectively) (Sang et al. 1994, Bowen & Grant 1997, Ishikawa et al. 2001). Thus, in rapidly expanding populations, low genetic and high haplotypic diversities may result in the accumulation of mutations leading to an increase in the number of haplotypes (Rogers & Harpending 1992). For the Japanese eel, however, population demographics indicate historical declines (Tseng et al. 2003). The high haplotypic diversity probably resulted from the large population size of Japanese eel and the small sample size in mtDNA studies. Isozymic electrophoresis of metabolic IDHP and PGDH loci reveals a slight geographical cline even under such a low Nei's (1972) genetic distance of 0.003 to 0.02 (mean 0.013) (Chan et al. 1997). Nevertheless, Nei's (1978) genetic distances of 0.04 to 0.171 (mean 0.096 ± 0.025) obtained from the present study represent higher genetic variability at microsatellite loci than those determined using mitochondrial and protein studies. There are 2 reasons for such a discrepancy. Protein electrophoresis can detect only one-third of the amino acid substitutions, but microsatellites can resolve many more repetitive sequence variations. In addition, microsatellites have higher mutation rates than mtDNA (Dallas 1992).

Conclusions

The population genetic structure of the Japanese eel examined based on microsatellite loci exhibited slight but significant genetic differentiation among geographic samples. The Japanese eel can be divided into a high-latitude (Daecheon-myon, the Yalu River, Hangzhou, and Mikawa Bay) and a low-latitude (Shantou, Tanshui, and Fangliao) group based on tree topology and AMOVA analysis. The ecological migration patterns of juveniles fit the member-vagrant model within the metapopulation. The genetic evidence including slight but significant genetic differentiation and the topology of the phylogenetic tree confirm that the Japanese eel does not have a panmictic population structure in the northwestern Pacific Ocean.

Current world fisheries have been threatened by overfishing and habitat destruction through water extraction, dam construction, and industrial pollution. Management efforts are extremely important for commercial fisheries, which through overharvesting can become economically unviable well before the exploited species becomes threatened with biological extinction. We propose that the eel populations in the northwestern Pacific Ocean can be classified into 2 management groups based on the divergence in nuclear allele frequencies.

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