American Fisheries Society Symposium 58:59–69, 2009

Genetic Differentiation of the Japanese Eel

Mei-Chen Tseng*
Institute of Zoology, National Taiwan University, Taipei 10764, Taiwan

Wann-Nian Tzeng
Institute of Fishery Science, National Taiwan University, Taipei 10764, Taiwan

Sin-Che Lee[1]
Institute of Zoology, Academia Sinica, Taipei 11529, Taiwan

Abstract.—Polymorphic microsatellite loci as genetic markers were used to reject the null hypothesis of panmixia for the Japanese eel, *Anguilla japonica*. Observed heterozygosity showed slight heterozygote deficiencies over all loci. One of the eight loci (MS-4) in one sample showed departure from Hardy-Weinberg equilibrium. Unbiased Nei's genetic distances ranged from approximately 0.058 to 0.134. A slight genetic differentiation was determined by F_{ST} and R_{ST} statistics when adjusted with Bonferroni correction. Although isolation by distance is often observed in marine species, its use as a null hypothesis seems questionable. Although the freshwater eel is categorized as a catadromous fish, the value of genetic diversity obtained fell within that of marine fishes. A higher correlation ($P < 0.001$) resulting from AMOVA supports the separation of Japanese eels into two management units: a low-latitude group (Shantou, Tanshui, and Fangliao) and a high-latitude group (Daecheon-myon, Yalu River, Hangzhou, and Mikawa Bay). Such a population subdivision will be useful for further applications of fisheries conservation and management in the northwestern Pacific Ocean.

Introduction

Although population structures of European eel *Anguilla anguilla*, Japanese eel *A. japonica*, and American eel *A. rostrata* have been studied intensively, controversy still exists as to whether these species are made up of single panmixia or multiple geographically based populations (Table 1). For European eel alone, protein electrophoresis and sequences 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 and Pantelouris 1973; Comparini et al. 1977; Lintas et al. 1998). Similar conclusions have been reached for American eel (Williams et al. 1973; Koehn and Williams 1978; Avise et al. 1986). Although results derived from both nuclear and cytoplasmic markers support the panmixia hypothesis for the genetic structure of Atlantic freshwater eels, Wirth and Bernatchez (2001) challenged existing opinion concerning genetic structure. Slight genetic dif-

[1] Corresponding author: sinchelee@yahoo.com.tw
*Current address: Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung 912, Taiwan

Table 1. Studies of population genetics of freshwater eels (1973 to 2004).

Species	Molecular tool	Sampling locations	Result	Reference
American eel, *Anguilla rostrata*	isozyme	Northeastern America	panmictic population	Williams et al. (1973)
	mtDNA	Northeastern America coastline	panmictic population	Avise et al. (1986)
	micro-satellite loci	Northeastern American	Population decline	Wirth and Bernatchez (2003)
European eel, *A. anguilla*	allozyme	Spain, Greece, Holland and Poland	panmictic population	De Ligny and Pantelouris (1973)
	isozyme	Mediterranean Sea and Welsh Atlantic coasts	panmictic population	Comparini et al. (1977)
	mtDNA	Northwestern Atlantic and Mediterranean coastal regions	panmictic population	Lintas et al. (1998)
	micro-satellite loci	North Atlantic, Baltic Sea and Mediterranean Sea basins	differentiation	Wirth and Bernatchez (2001)
	mtDNA and micro-satallite loci	Italy, Ireland, Morocco, Sweden, and U.K.	differentiation	Daemen et al. (2001)
	micro-satellite loci	European	isolation by distance	Maes and Volckaert (2002)
Marble eel, *A. marmorata*	mtDNA	Indo-Pacific Ocean	differentiation	Ishikawa et al. (2004)
Japanese eel, *A. japonica*	isozyme	Taiwan and China	geographic clines	Chen et al. (1997)
	mtDNA	Taiwan, China and Japan	panmictic population	Sang et al. (1994)
		Taiwan, China and Japan	panmictic population	Ishikawa et al. (2001)
	micro-satellite loci	Taiwan	historical decline	Tseng et al. (2003)
	micro-satellite loci	Taiwan, China, Japan and Korea	differentiation	This study

ferentiation among 13 samples of European eels from the North Atlantic, the Baltic Sea, and the Mediterranean Sea basins refutes the panmictic population hypothesis (Wirth and Bernatchez 2001).

The Japanese eel is a temperate freshwater fish that is distributed in rivers of northeastern Asian countries, including Taiwan, Japan, China, and Korea (Tesch 1977). As a catadromous fish, it spawns in the sea and grows and matures in freshwater rivers. The spawning grounds are thought to be to the west of the Mariana Islands, at 15°N 140°E (Tsukamoto 1992), evidenced by the occurrence of newly hatched leptocephali in the area. Taking 4 to 6 months to drift passively to the coasts of northeast Asia by the Kuroshio Current, the leptocephali undergo metamorphosis on the way to their freshwater destinations. The metamorphosis into translucent elvers is complete before upstream migration. Eels live in rivers for 5 to 20 years, until they mature. Maturity occurs in late autumn, when they emigrate downstream to the ocean and return to the spawning grounds (Tesch 1977).

The Japanese eel is an important aquaculture species in northeastern Asian countries. Eel farmers in these countries catch large numbers of juveniles in estuaries from November to March during their upstream migration (Tzeng 1985). In these populations, the mitochondrial DNA sequences in the D-loop regions (Sang et al. 1994; Ishikawa et al. 2001) do not show interpopulation genetic differentiation, suggesting panmixia with low levels of genetic structuring due to high levels of gene flow. However, Japanese eels on the western Pacific fringe exhibit clear geographic clines when the IDHP and PGDH allozyme loci are used as genetic markers (Chan et al. 1997). A migration time lag from different parts of continents to the spawning grounds west of the Mariana Islands is hypothesized as a possible reason for the formation of this cline. However, the above results remain somewhat uncertain and controversial.

Previous studies of life cycles and population structures of aquatic animals have been limited due to the lack of proper markers. A possible solution to the question in the Japanese eel is the use of microsatellite DNA markers, which are characterized by high variability. The consistencies of microsatellite sequences include unique DNA sequences and tandem repeats of 1 to 5 bases in length (Beckmann and Weber 1992).

Because of a tendency for higher variability to be inherited in a Mendelian fashion and only the least amounts of tissue are required for PCR assay, microsatellites have been regarded as the most adequate genetic markers for solving many questions of biology (Wright and Bentzen 1994). The purpose of this study was to use eight polymorphic microsatellite loci to test genetic differentiation of Japanese eels in the northwestern Pacific Ocean.

Methods

Sampling

In total, 328 Japanese eel elvers were caught alive from seven distant locations throughout the species' range in northeastern Asia in the winter and spring of 1999 to 2000. The collections came from Tanshui and Fangliao in Taiwan; the Yalu River, Hangzhou, and Shantou in China; Mikawa Bay in Japan; and Daecheon-myon in Korea (Figure 1). All specimens were preserved in 95% ethanol prior to DNA extraction.

DNA Extraction

Genomic DNA was isolated and purified from muscular tissue. Five hundred micrograms of tissue with 1 mL lysis buffer was digested with 55 µL proteinase K solution (10 mM tris-HCl, pH 8.0, 2 mM ethylene diamine tetra-acetic acid (EDTA), 10 mM NaCl, 1% sodium dodecyl sulfate (SDS), 10

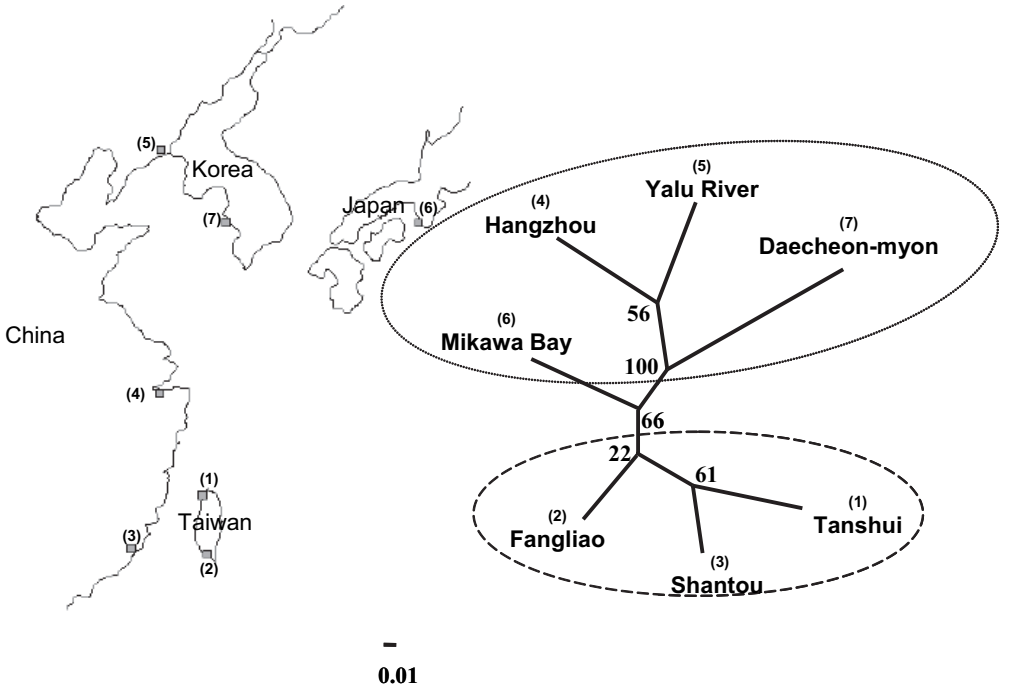

Figure 1. Sampling localities and topological tree for *Anguilla japonica*. The UPGMA tree is drawn using Nei's genetic distance measured for seven samples of Japanese eels.

mg/mL dithiothreitol (DTT), 0.5 mg/mL proteinase K). DNA extraction was carried out twice with phenol, twice with phenol/chloroform/isoamyl alcohol (25:24:1), and once with chloroform/isoamyl (24:1) as described by Kocher et al. (1989). DNA was precipitated once with 95% ethanol and once with 70% ethanol and then dissolved in TE buffer (10 mM tris-HCl, pH 8.0, 1 mM EDTA) following a standard procedure outlined by Sambrook et al. 1989.

Screening and Analysis of Microsatellite Loci

We screened eight microsatellite loci from the Japanese eel genome. The sequences submitted to EMBL under the accession numbers AJ297599-AJ297603, AJ297605, and AM062761-AM062762 (Tseng et al. 2001; Tseng et al. 2006) are listed in Table 2. Microsatellites were amplified via the polymerase chain reaction (PCR) and electrophoreses. Forward primers were labeled with FAM, TAMRA, or HEX flu, and then PCR was performed in a volume of 25 μL, including 1 ng genomic DNA, 2 pmol reverse primer, 2 pmol labeled forward primer, 25 mM dNTP, 0.05 to 0.1 mM $MgCl_2$, 10X buffer, and 0.5 U *Taq* polymerase (supplied by Takara, Shuzo Co., Shiga, Japan 2003). 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; and 38 cycles of 30 s each at successive thermal regimes of 94°C, 56–58°C, and 72°C. Eight microliters of product was precipitated with 95% alcohol. Semiautomated genotyping was performed by a capillary MEGABACE-500 Analysis system. Data were scored by Genetic profiler Software 1.5 (Amersham Biosciences).

Table 2. Locus name, repeat motifs, primer sequence, and PCR conditions of Japanese eel microsatellite loci detected by PCR amplification.

Locus	Repeat motifs	Primer sequence (5'–3')	Annealing temp (°C)(mM)	$MgCl_2$	Range of allele sizes (bp)
MS-1	$(GT)_{16}$	**F**:TCGAGACACCAGATAGTCAC **R**:ACATCCTAGGCTCACACC	58	0.5	188–230
MS-2	$(GA)_{15}$	**F**:ATTTCACGTCATCGGACCTGC **R**:GCTGGGAGCGACGCTTTATC	56	0.5	103–143
MS-3	$(GT)_{10}$	**F**:GGTATGAATGCAGGCGTTTATG **R**:GCAACCGATTTGATCTCCAG	56	0.5	79–97
MS-4	$(GT)_{15}$	**F**:CCTTCAGATTGCTAGCAC **R**:CGGAGTCTAATTGTCTCCTC	58	0.5	117–155
MS-5	$(GT)_{19}$	**F**:ACAGAGCCAGACAAACAGAC **R**:GGTCAGCAAGCAAAACGAAC	56	1.0	83–115
MS-6	$(GA)_{17}$	**F**:TGTCTAACACTAAGAAAAGGAGAGG **R**:GGCTGCCAGTATCTTCTCAAAG	58	1.0	133–181
MS-7	$(GT)_9$	**F**:AGTAAAGAGTCCCACGCATTC **R**:AAGGTGGATTTTTGCTGGCTC	54	0.5	76–90
MS-8	$(GT)_{12}$	**F**:AGGCTGAAGTGAGTATGCTCAG **R**:AGATATGGAAGCAGGATGGAG	56	0.5	100–120

Data Analysis

The observed (H_O) and expected (H_E) heterozygosities were independently calculated for each locus (Yang and Yeh 1993). 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 unbiased genetic distances (Nei 1978) were computed between all pairs of populations. The original topology of the phylogenetic tree was inferred from the neighbor-joining method based on Nei's unbiased genetic distances. One thousand bootstrap resamplings were used for evaluating support of the data set for the relationships.

F_{ST} and R_{ST} were used to examine population subdivisions (Weir and Cockerham 1984; Raymond and Rousset 1995). R_{ST} is an analog of F_{ST} that assumes a stepwise mutation model of repeat DNA (Raymond and Rousset 1995). The significance level was adjusted by sequential Bonferroni correction (Holm 1979; Rice 1989; Sankoh et al. 1997).

We tested isolation by distance (IBD) as an indicator of an emerging population structure by a typical regression of geographic distance on Nei's genetic distance. We used Mantel tests for correlation of two parameters to examine the significance of IBD relationships (Mantel 1967; Smouse et al. 1986). The hierarchical genetic structure was investigated by analysis of molecular variance (AMOVA; Excoffier et al. 1992) based on an analysis of the variance of genetic distances.

Results

Primer sequences and PCR conditions, including different concentrations of $MgCl_2$ and annealing temperatures of the eight polymorphic microsatellite loci that were correctly amplified, are given in Table 2. All eight loci were highly variable, with the number of alleles ranging from 10 in MS-3 to 25 in MS-6, with an average of 19.25. The sizes of the

Table 3. Levels of genetic variation observed at eight microsatellite loci within seven 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 locus, observed number of alleles (*na*), and effective number of alleles (*ne*). *Indicates a significant Hardy-Weinberg disequilibrium level at 5%.

	Location	*Tanshui*	Fangliao	Shantou	Hangzhou	Yalu River	Mikawa Bay	Daecheon-myon	Mean	*na*	*ne*
Locus		*n* = 49	*n* = 50	*n* = 46	*n* = 47	*n* = 44	*n* = 47	*n* = 45			
MS-1	No. alleles (U/S)	12 (0/0)	14 (2/0)	14 (0/1)	14 (2/1)	15 (0/0)	11 (0/1)	16 (0/0)	13.71	21	9.82
	H_o	0.774	0.759	0.758	0.767	0.778	0.731	0.800	0767		
	H_E	0.868	0.899	0.880	0.871	0.897	0.866	0.927	0.887		
MS-2	No. alleles (U/S)	15 (0/0)	16 (0/1)	18 (0/0)	15 (0/0)	15 (0/1)	17 (0/0)	15 (0/0)	15.86	22	13.55
	H_o	0.742	0.828	0.818	0.833	0.833	0.846	0.829	0.818		
	H_E	0.909	0.936	0.925	0.906	0.921	0.929	0.913	0.920		
MS-3	No. alleles (U/S)	5 (1/0)	5 (0/0)	7 (1/0)	6 (0/0)	6 (0/0)	7 (1/0)	4 (0/0)	5.714	10	2.99
	H_o	0.516	0.586	0.667	0.567	0.528	0.654	0.514	0.576		
	H_E	0.619	0.693	0.698	0.672	0.635	0.706	0.628	0.664		
MS-4	No. alleles (U/S)	11 (0/0)	12 (0/0)	12 (0/0)	11 (1/0)	10 (0/0)	11 (0/0)	10 (0/0)	11	17	9.49
	H_o	0.742	0.759	0.788*	0.767	0.778	0.769	0.743	0.764		
	H_E	0.881	0.883	0.898	0.898	0.857	0.903	0.880	0.886		
MS-5	No. alleles (U/S)	12 (0/0)	11 (1/0)	12 (0/0)	9 (0/1)	9 (0/0)	11 (0/0)	14 (1/0)	11.14	17	6.72
	H_o	0.774	0.724	0.758	0.667	0.667	0.731	0.800	0.731		
	H_E	0.868	0.838	0.841	0.793	0.791	0.864	0.905	0.843		
MS-6	No. alleles (U/S)	17 (0/0)	19 (0/0)	21 (0/0)	16 (0/1)	19 (1/0)	15 (0/0)	18 (1/1)	17.86	25	17.10
	H_o	0.871	0.828	0.848	0.833	0.833	0.846	0.829	0.841		
	H_E	0.936	0.947	0.947	0.936	0.946	0.939	0.938	0.941		
MS-7	No. alleles (U/S)	13 (0/0)	12 (1/0)	12 (0/1)	13 (0/1)	11 (1/0)	9 (0/1)	13 (0/1)	11.86	22	7.95
	H_o	0.742	0.793	0.758	0.767	0.750	0.808	0.771	0.770		
	H_E	0.879	0.863	0.878	0.895	0.847	0.871	0.889	0.875		
MS-8	No. alleles (U/S)	16 (1/1)	13 (0/0)	16 (1/0)	18 (1/0)	16 (1/0)	12 (0/2)	15 (0/1)	15.14	20	12.83
	H_o	0.839	0.759	0.849	0.800	0.694	0.769	0.771	0.783		
	H_E	0.918	0.893	0.915	0.933	0.768	0.876	0.915	0.888		
Mean	No. alleles	12.62	12.75	14	12.75	12.62	11.62	13.12	12.78	19.25	10.06
	H_o	0.750	0.754	0.780	0.75	0.733	0.769	0.757	0.756		
	H_E	0.860	0.869	0.873	0.863	0.833	0.869	0.874	0.863		
Total	U/S	2/1	4/1	2/2	4/4	3/1	1/4	2/3			

Table 4. Nei's unbiased genetic distance for Japanese eel samples from seven locations.

Location	Daecheon-myon	Mikawa Bay	Yalu River	Hangzhou	Shantou	Tanshui	Fangliao
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	0.096	0.105	0.0894	0.116	0.078	0	
Fangliao	0.092	0.069	0.134	0.103	0.088	0.101	0

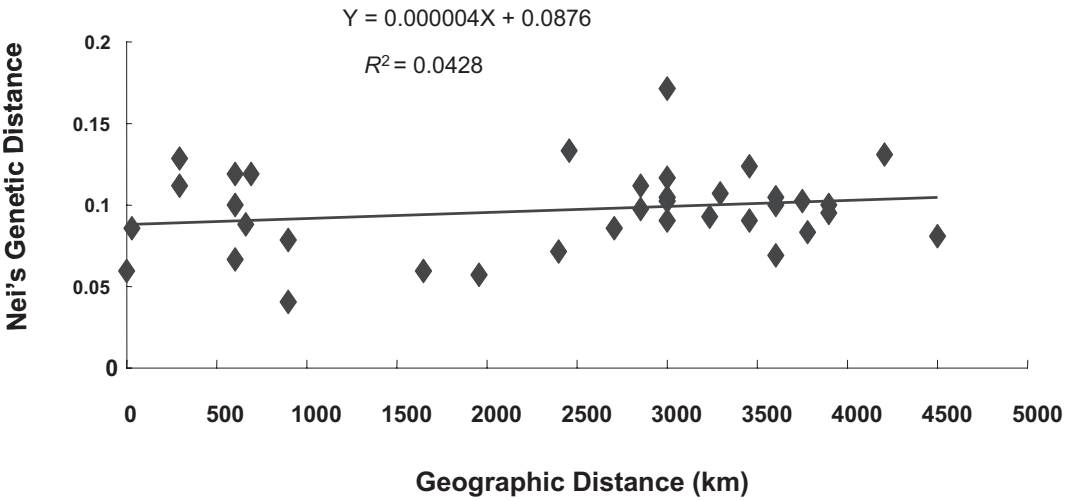

Figure 2. Relationships between Nei's genetic distance and geographic distance (km). Pearson correlation coefficients (*r*) and significance of the correlations (*P*) are given in text.

alleles over the eight loci ranged from 79 bp in MS-3 to 230 bp in MS-1. Among them, locus MS-3 was the shortest (<100 bp, Table 2). The observed heterozygosity (H_O) over all loci ranged from 0.514 ± 0.068 (MS-3) to 0.871 ± 0.031(MS-6), with an average of 0.756 ± 0.075. The expected heterozygosity (H_E) ranged from 0.619 (MS-3) to 0.947 (MS-6), with an average of 0.863 (Table 2). The result showed slight heterozygote deficiencies when tested with Hardy-Weinberg equilibrium.

For studying evolutionary relationships among seven spatial samples, unbiased Nei's genetic distances were examined and shown to range from approximately 0.058 (Yalu River/Hangzhou) to 0.134 (Yalu River/Fangliao), with an average of 0.09 ± 0.021 (Table 3). For further determination of population subdivisions, F_{ST} and R_{ST} were used to obtain respective values of 0.002 to 0.015 (mean, 0.008) and –0.009 to 0.05 (mean, 0.016) (Table 4).

Nei's unbiased genetic distance versus coastal geographical distances for all possible paired combinations of seven Japanese eel samples showed no significant correlation (Figure 2). The Mantel test for correlation of param-

Table 5. 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.

Location	Daecheon-myon	Mikawa Bay	Yalu River	Hangzhou	Shantou	Tanshui	Fangliao
Daecheon-myon	-----	0.006*	0.005	0.002	0.006**	0.009**	0.007*
Mikawa Bay	0.011	-----	0.008**	0.007*	0.006*	0.015**[a]	0.004
Yalu River	0.007	0.048**[a]	-----	0.003	0.007**[a]	0.009**	0.015**[a]
Hangzhou	0.010	0.050**[a]	0.019*	-----	0.013**[a]	0.012**[a]	0.009*
Shantou	–0.002	0.016	0.023**	0.005	-----	0.005	0.006
Tanshui	0.005	0.036**[a]	0.028**	0.011**	–0.009	-----	0.009**
Fungliao	0.017	0.022*	0.030**	0.024	–0.008	–0.003	-----

* $p < 0.05$; ** $p < 0.01$; [a] $p < 0.0014$.

eters excludes a possible isolation-by-distance principle in Japanese eel samples ($r = 0.123$, $P = 0.258$). When Nei's genetic distances were substituted by F_{ST} and R_{ST} values, results were similar. The neighbor-joining tree constructed on the basis of the genetic distance data set can be grouped into two clades.

When Japanese eels were grouped into two clades including high-latitude groups (Daecheon-myon, Yalu River, Hangzhou, and Mikawa Bay) and low-latitude groups (Shantou, Tanshui, and Fangliao), the hierarchical genetic structure tested by AMOVA indicated that 1.95% of the total genetic variation partitioned in the among-groups category ($P < 0.05$), 95.73% was within populations ($P < 0.001$), and 2.32% was among populations within a group. Nei's genetic distance ranged from 0.058 to 0.091 in the high-latitude group, 0.078 to 0.101 in the low-latitude group, and 0.069 to 0.134 between high- and low-latitude populations. The tree topology suggested that dispersion of Japanese eel elvers to the coast does not occur randomly.

Discussion

As a catadromous fish, the Japanese eel has more alleles per unit of microsatellite locus (mean 19.25) than do anadromous (mean 10.8) and freshwater fishes (mean 9.1) but slightly fewer than marine fishes (mean 19.9) (DeWoody and Avise 2000). The mean number of alleles per locus per sample varied between 11.62 and 14.00 in Japanese eels and between 9.6 and 12.4 (Daemen et al. 2001) or 12.9 and 21.2 (Wirth and Bernatchez 2001) in European eels, all of which are within the range for teleosts. These results imply a progressive change in genetic variability among freshwater, anadromous, catadromous, and marine fishes. In addition, the significantly low mean heterozygosity of 0.54 assayed in freshwater fishes and the high value of 0.77 in marine fishes (DeWoody and Avise 2000) reveal that Japanese eels (0.75) are intermediate with respect to mean levels of microsatellite variation. Although the freshwater eel is categorized as a catadromous fish, its genetic diversity is close to that of marine fishes.

One of eight loci showed a slight departure from expected heterozygosity (H_E) in one sample (Table 2). This may be associated with the Wahlund (1928) effect, where the decrease in heterozygosity is caused by a mixing of differentiated subsamples, and to the null allele effect, where the increase in homozygosity is caused by failure of amplification of an allele mutated in the primer region.

Genetic differentiation indices (F_{ST}, R_{ST}) estimated at the time when glass eels approach the estuaries indicate a weak but significant spatial divergence among samples. Recent studies have argued for a random mating system in anguillid eels, but Wirth and Bernatchez (2001) viewed the European eel differently. However, we support the nonpanmictic hypothesis in Japanese eels because of the significant genetic differentiation index. The magnitude of spatial differentiation in Japanese eels (F_{ST} = 0.002 to 0.015, mean 0.008) is higher than that of the European eel (0.0017 in Daemen et al. 2001; 0.004 in Wirth and Bernatchez 2001). Each species consists of different genetic structures found in the Pacific and Atlantic habitats due to their own particularly hydrographic systems.

We can reject the isolation-by-distance hypothesis for the eels analyzed in the present study; however, when examining the Mantel relation test, the European eel otherwise conforms to this model (Wirth and Bernatchez 2001). Interspecific differences in genetic structure result from various current systems and evolutionary histories. The inconsistency with the isolation-by-distance model is evidenced by the greater divergence between some closely spaced sites. For example, the genetic distance between Mikawa Bay and Fangliao is smaller than the genetic distance between Mikawa Bay and Daecheon-myon. The different groups of elvers passively drifting to destinations depend on complicated subcurrent systems of the northwestern Pacific Ocean.

The Φ-statistic of AMOVA (hierarchical analysis of molecular variance) shows the genetic variance between two groups (Φ_{CT} = 0.009, $P < 0.05$). Thus, Japanese eel populations can be clearly distinguished into a two-groups model, whose general profile is clearly shown in the relevant tree topology (Figure 1). The older metamorphosis age was determined in the high-latitude group compared with that in the lower-latitude group (Cheng and Tzeng 1996).

Current world fisheries have been threatened by overfishing and habitat destruction through dam construction and industrial pollution. Management efforts are extremely important for commercial fisheries, which, through overharvesting, can become economically extinct well before the exploited species becomes threatened with biological extinction (Botsford et al. 1997). In this case, eel populations in the northwestern Pacific Ocean can be classified into two groups based on significant divergence in nuclear allele frequencies. Furthermore, the major roles that ecology, behavior, and environmental conditions of fish populations play in fisheries management need to be recognized.

Acknowledgments

The authors wish to express their gratitude to H. Y. Teng and C. W. Chang for collecting specimens from Taiwan. Special thanks are due to Profs. Y. H. Xie and H. P. Oka for providing us with the samples from China and Japan. We thank two anonymous reviewers for their constructive comments on the manuscript.

References

Avise, J. C., G. S. Helfman, N. C. Saunders, and L. S. Hales. 1986. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. Proceedings of the National Academy of Sciences of the USA 83:4350–4353.

Beckmann, J. S., and J. L. Weber. 1992. Survey of human and rat micosatellites. Genomics 12:627–631.

Botsford, L. W., J. C. Castilla, and C. H. Peterson. 1997. The management of fisheries and marine ecosystems. Science 277:509–515.

Chan, I. K. K., D. K. O. Chan, S. C. Lee, and K. Tsukamoto. 1997. Genetic variability of the Japanese eel *Anguilla japonica* (Temminck and Schlegel) related to latitude. Ecology of Freshwater Fish 6:45–49.

Cheng, P. W., and W. N. Tzeng. 1996. Timing of meta-

morphosis and estuarine arrival across the dispersal range of the Japanese eel *Anguilla japonica*. Marine Ecology Progress Series 131:87–96.

Comparini, A., M. Rizzotti, and E. Rodino. 1977. Genetic control and variability of phosphoglucose isomerase (PGI) in eels from the Atlantic Ocean and the Mediterranean Sea. Marine Biology 43:109–116.

Daemen, E., T. Cross, F. Ollevier, and F. A. M. Volckaert. 2001. Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. Marine Biology 139:755–764.

De Ligny, W., and E. M. Pantelouris. 1973. Origin of the European eel. Nature (London) 246:518–519.

DeWoody, J. A., and J. C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology 56:461–473.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:65–70.

Ishikawa, S., J. Aoyama, K. Tsukamoto, and M. Nishida. 2001. Population structure of the Japanese eel *Anguilla japonica* as examined by mitochondrial DNA sequencing. Fisheries Science 67:246–253.

Ishkawa, S., K. Tsukamoto, and M. Nishida. 2004. Genetic evidence for multiple geographic populations of the giant mottled *Anguilla marmorata* in the Pacific and Indian oceans. Ichthyological Research 51:343–353.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pabo, F. X. Villablabca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the USA 86:6196–6200.

Koehn, R. K., and G. C. Williams. 1978. Genetic differentiation without isolation in the American eel *Anguilla rostrata*. II: Temporal stability of geographic variation. Evolution 32:624–637.

Levene, H. 1949. On a matching problem in genetics. The annals of mathematical statistics 20:91–94.

Lintas, C., J. Hirano, and S. Archer. 1998. Genetic variation of the European eel *Anguilla anguilla*. Molecular Marine Biology and Biotechnology 7:263–269.

Maes, G. E., and F. A. M. Volckaert. 2002. Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). Biological Journal of the Linnean Society 77:509–521.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Raymond, M., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49:1280–1283.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning, a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press, New York.

Sang, T. K., H. Y. Chang, C. T. Chen, and C. F. Hui. 1994. Population structure of the Japanese eel *Anguilla japonica*. Molecular Biology and Evolution 11:250–260.

Sankoh, A. J., M. F. Huque, and S. D. Dubey. 1997. Some comments on frequently used multiple endpoint adjustments methods in clinical trials. Statistics in Medicine 16:2529–2542.

Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology 35:627–632.

Tesch, F. W. 1977. The eel: biology and management of anguillid eels. Chapman and Hall, London.

Tseng, M. C., C. A. Chen, H. W. Kao, W. N. Tzeng, and S. C. Lee. 2001. Polymorphisms of GA/GT microsatellite loci from *Anguilla japonica*. Marine Biotechnology 3:75–280.

Tseng, M. C., W. N. Tzeng, and S. C. Lee. 2003. Historical decline in the Japanese eel *Anguilla japonica* in northern Taiwan inferred from genetic variations. Zoological Studies 42:556–563.

Tseng, M. C., W. N. Tzeng, and S. C. Lee. 2006. Population genetic structure of the Japanese eel, *Anguilla japonica*, in the northwestern Pacific Ocean: evidence of non-panmictic populations. Marine Ecology Progress Series 308:221–230.

Tsukamoto, K. 1992. Discovery of the spawning area for Japanese eel. Nature (London) 356:789–791.

Tzeng, W. N. 1985. Immigration timing and activity rhythms of the eel, *Anguilla japonica*, elvers in the estuary of northern Taiwan, with emphasis on environmental influences. Bulletin of Japanese Society of Fisheries Oceanography 47/48:11–28.

Wahlund, S. 1928. The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity. Hereditas 11:65–106.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Williams, G. C., R. K. Koehn, and J. B. Mitton. 1973. Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. Evolution 27:192–204.

Wirth, T., and L. Bernatchez. 2001. Genetic evidence against panmixia in the European eel. Nature (London) 409:1037–1040.

Wirth, T., and L. Bernatchez. 2003. Decline of North Atlantic eels: a fatal synergy? Proceedings of the Royal Society of London, Series B 270:681–688.

Wright, J. M., and P. Bentzen. 1994. Microsatellites: genetic markers for the future. Reviews in Fish Biology and Fisheries 4:384–388.

Yang, R. C., and F. C. Yeh. 1993. Multilocus structure in *Pinus contoria* Dougl. Theoretical and Applied Genetics 87:568–576.