

Population Genetic Structure among Intra-Annual Arrival Waves of the Japanese Eel *Anguilla japonica* in Northern Taiwan

Kai-Chieh Chang, Yu-San Han, and Wann-Nian Tzeng*

Institute of Fisheries Science, College of Life Science, National Taiwan University, Taipei 106, Taiwan

(Accepted April 18, 2007)

Kai-Chieh Chang, Yu-San Han, and Wann-Nian Tzeng (2007) Population genetic structure among intra-annual arrival waves of the Japanese eel *Anguilla japonica* in northern Taiwan. *Zoological Studies* 46(5): 583-590. A previous study showed that the Japanese eel *Anguilla japonica* Temminck and Schlegel is not a panmictic species, as it is divided into low- and high-latitude groups, but it shows no significant genetic differentiation among year cohorts in the same habitat. However, based on hatching dates back-calculated from otolith of elvers recruited in Taiwan in the winter, the arrival waves of elvers are from different intra-annual cohorts with separate spawning times. To test if temporal genetic differentiation exists within intra-annual cohorts, 7 polymorphic microsatellite DNA loci were examined. Four arrival waves of Japanese eel elvers were collected from a single location in northern Taiwan from Nov. 2000 to Mar. 2001. Deviations from Hardy-Weinberg equilibrium were found in three of the 7 loci after the Bonferroni correction, and five of the 7 loci were used for a genetic variation test. No significant genetic differentiation was observed among the overall sample ($F_{ST} = 0.0029$, $p = 0.173$), and subtle, but significant, differentiation was only found between temporal cohorts of Nov. and Dec. 2000 ($F_{ST} = 0.0111$, $p = 0.008$). The phylogenetic tree indicates patchiness among elver waves with samples of Nov. 2000 significantly separated from the others. The assignment test, however, showed a high mixing rate among each cohorts. These results indicate that arrival waves of elvers in consecutive months at the same habitat exhibit subtle genetic patchiness but overall lack significant temporal genetic variations.
<http://zoolstud.sinica.edu.tw/Journals/46.5/583.pdf>

Key words: Intra-annual cohort, Japanese eel, Genetic differentiation, Microsatellite DNA, Panmixia.

The Japanese eel *Anguilla japonica* Temminck and Schlegel is a temperate catadromous fish, distributed in rivers of the northeastern Asian countries of Taiwan, China, Japan, and Korea (Tesch 2003). The spawning ground of this species is presumed to be in the western Mariana Is., at a salinity front near 15°N, 140°E, as evidenced by the occurrence of newly hatched larvae (leptocephali) in the area (Tsukamoto 2006). Leptocephalus larvae drift from their spawning grounds with the North Equatorial Current (NEC) followed by the Kuroshio Current (KC) for 4-6 mo to reach the coasts of Northeast Asia (Cheng and Tzeng 1996). The translucent glass eels become pigmented elvers during their upstream migration. Eels live in rivers for 4-10 yr before their gonads

begin to mature in late autumn when they metamorphose into silver eels and are ready to migrate a distance of 2000-3500 km back to their birthplace for spawning (Tzeng 1986, Tsukamoto 1992, Tzeng et al. 2000, Han et al. 2003).

The Japanese eel is an important fishery resource in Asia. For cultivation, great numbers of elvers are caught in estuaries, resulting in a serious problem with overfishing (Tzeng 1985, Tseng et al. 2003). For effective management of eel resources, a better understanding of the population structure of the eel is fundamental. In the past, the concept of panmictic populations of Japanese eel, European eel, (*Anguilla anguilla*), and American eel (*A. rostrata*) was accepted due to evidence from allozyme, nuclear markers, and

*To whom correspondence and reprint requests should be addressed. Tel: 886-2-33662887. Fax: 886-2-23639570. E-mail: wnt@ntu.edu.tw. Kai-Chieh Chang and Yu-San Han contributed equally to this work.

mtDNA sequences (De Ligny and Pantelouris 1973, Avise et al. 1986, Sang et al. 1994, Lintas et al. 1998, Ishikawa et al. 2001, Avise 2003). However, recent studies using more-sensitive microsatellite markers indicate that the population genetic structure does not support the panmictic hypothesis in the Japanese eel (Tseng et al. 2006), American eel (Wirth and Bernatchez 2003), or European eel (Wirth and Bernatchez 2001, Maes and Volckaert 2002). The European eel fits the model of isolation-by-distance (IBD) with some level of gene flow occurring between spatially adjacent populations (Wirth and Bernatchez 2001, Maes and Volckaert 2002). However, no IBD was found among eels of different locations in another study of the European eel (Dannewitz et al. 2005). American eel populations have very weak but significant spatial genetic differentiation although an IBD pattern was not observed (Wirth and Bernatchez 2003). The Japanese eel is divided into a low-latitude group (South China and Taiwan) and a high-latitude group (Japan, Korea, and North China) without IBD being detected (Tseng et al. 2006). The "member-vagrant model" hypothesis, which emphasizes the role of stable oceanographic features in retaining larvae in a favorable environment (Sinclair 1988), was modified to explain the population structure of the Japanese eel: most progeny tend to be transported back to similar locations as were their ancestors with some degree of exchange between neighboring populations (Tseng et al. 2006). The spawning site of the Japanese eel appears to be near 3 seamounts in the western Mariana Ridge (Tsukamoto et al. 2003, Tsukamoto 2006). These seamounts are located in the westward flow of the North Equatorial Current (NEC) and are hypothesized to provide cues for migrating silver eels and to serve as possible aggregation sites for spawning. In addition, the eel shows a protractive spawning period induced by different departure times and distances for geographically distant groups (Tesch 2003, Tsukamoto et al. 2003, Maes et al. 2006). The existence of separate spawning sites and spawning migration loops for the southern and northern eel groups is likely to be the reason for the formation of spatial genetic heterogeneity in the Japanese eel. Cheng and Tzeng (1996) determined the ages at metamorphosis from leptocephalus to glass eels of Japanese eels by calculating the daily growth rings of otoliths, which increased from south to north. The difference in larval durations between southern and northern groups of Japanese eel may have contributed to

the separation of these 2 groups.

Despite the spatial differentiation of the genetic structures of the Japanese, American, and European eels, temporal genetic variation is also an important issue for better and accurate understanding of eel population structures. Dannewitz et al. (2005) indicated that the overall genetic differentiation of the European eel is low but significant, with temporal genetic variation within sites exceeding the geographical factor among sites. Pujolar et al. (2006) found highly significant genetic differentiation among arrival waves of European eels either among inter-annual cohorts or among intra-annual samples, and genetic patchiness among recruits was raised to explain the heterogeneous character of the eel that results from a large variance in the contribution of individuals to each annual cohort. Maes et al. (2006) also found significant genetic differentiation among arrival waves of European eel, and evidence of the isolation-by-time (IBT) pattern was found among waves. These studies suggest that both spatial and temporal genetic differentiations occur in the European eel. For Japanese eel, no significant genetic differentiation was observed among annual cohorts in 3 successive years in northern Taiwan with samples randomly selected from intra-annual cohorts for each year, indicating the annual stability of the population structure (Tseng et al. 2003). However, back-calculated birth dates based on otolith microstructure of glass eels indicated that the Japanese eel does not continuously spawn during the long spawning season from Apr. to Nov., but is synchronized to spawn periodically once a month during the new moon (Tsukamoto et al. 2003, Tsukamoto 2006). Glass eels caught in estuaries of Taiwan during the course of their upstream migration between Nov. and the following Mar. have been proven to come from different intra-annual cohorts which spawned in different months (Tzeng 1990). If the spawning time of the Japanese eel is inheritable, then genetic differentiation might occur within intra-annual cohorts due to temporal isolation. Heterogeneity of the genetic composition among arrival waves of intra-annual cohorts of the Japanese eel, however, has not been determined.

The aim of this study is to examine the genetic composition of 7 microsatellite loci among intra-annual cohorts of different arrival waves of Japanese glass eels. The population structure of intra-annual cohorts on a temporal scale is discussed.

MATERIALS AND METHODS

Sample collection

In total, 189 live *Anguilla japonica* glass eels were collected by fyke net from the Tanshui River estuary of northern Taiwan (121°25'E, 25°10'N, Fig. 1) on 25 Nov. and 25 Dec. 2000, and 7 Feb. and 9 Mar. 2001 (Table 1). Glass eels were immediately preserved in 95% ethanol until DNA extraction. The method to separate glass eels of the Japanese eel from those of other 2 eel species, namely *A. marmorata* and *A. bicolor pacifica*, followed Tzeng (1982) by pigmentation of the tail and the distance between the verticals through the anus and origin of the dorsal fin. Total length (± 0.1 mm) was measured before DNA extraction for the 4 different collecting times (Table 1).

DNA extraction

Genomic DNA extraction was carried out from a small piece of muscle tissue of the glass eels. Extraction was done using a DNA purification and extraction kit (Bioman Scientific Ltd., Taiwan). Briefly, the ethanol was removed by evaporation before treatment. A piece of muscle weighing 20 mg was digested in 1 ml of lysis buffer (10 mM Tris-HCl, 2 mM EDTA, 10 mM NaCl, 1% sodium dodecyl sulfate, and 10 mg/ml dithiothreitol; pH 8.0) and 55 μ l of proteinase K (0.5 mg/ml) for 1 h at 60°C after grinding. The solution was then applied to the spin column, washed with ethanol

buffer, eluted with 100 μ l of TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH 8.0), and stored at -20°C before the polymerase chain reaction (PCR).

PCR and genotyping

Seven microsatellite loci were selected based on Tseng et al. (2001 2006) including 6 (GT)_n (AJMS-1, AJMS-3, AJMS-5, AJMS-6, AJM-1, and AJM-8) loci and 1 (GA)_n (AJMS-2) locus (Table 2). Microsatellites were amplified via the PCR conducted in a 25 μ l volume with the following contents: 0.5 μ l of DNA template, 0.25 μ l of Taq polymerase, 1 μ l of 10 μ M forward and reverse primers, 2.5 μ l of 10x PCR buffer, 0.5 μ l of 10 mM dNTPs, and 19.25 μ l of double-distilled (dd)H₂O. Reverse primers contained either FAM, TAMRA, or HEX fluorescence labels for genotyping. PCR amplification procedures were as follows: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55-60°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. The PCR product, at 0.5 μ l, was diluted with 12 μ l of ddH₂O for genotyping performed using a capillary MegaBACE-500 DNA analysis system (Amersham Biosciences). Data were scored by Genetic Profiler™ vers. 1.5 (Amersham Biosciences), and the sizes of each allele were manually checked with the naked eye.

Data analysis

The total number of alleles (n_a), effective number of alleles (n_e), and observed (H_o) and expected (H_e) heterozygosities were independently calculated for each locus (Yang and Yeh 1993). Multilocus estimates for each of the 4 samples were calculated as well. Any deviations from

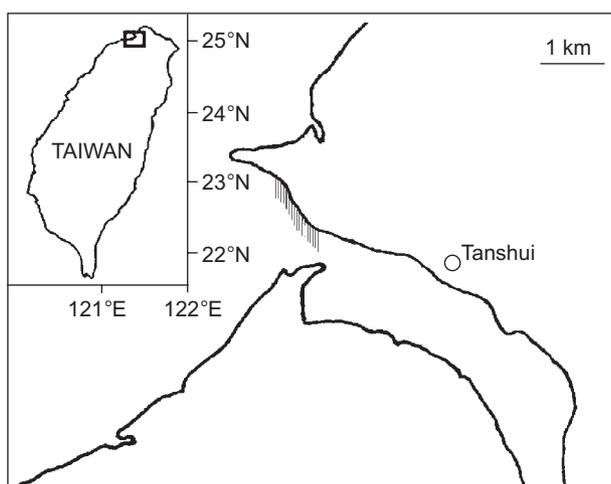


Fig. 1. Map showing the river mouth of the Tanshui River, northern Taiwan where the glass eels were caught. The shaded region indicates the position of the fyke net.

Table 1. Sampling date, number of specimens, and mean total length of Japanese glass eels collected in the Tanshui River estuary

Date	Sample size	Total length (mm)	
		Mean \pm SD	Range
25 Nov. 2000	39	55.25 \pm 2.16	50.68-58.62
25 Dec. 2000	50	54.67 \pm 1.99	50.70-58.90
7 Feb. 2001	50	54.36 \pm 2.58	47.38-60.10
9 Mar. 2001	50	55.23 \pm 2.52	49.39-61.53
Total	189	54.88 \pm 2.34	47.38-61.53

Hardy-Weinberg equilibrium (HWE) were calculated with Genepop vers. 3.1 (Raymond and Rousset 1995). Genetic differentiation was estimated using ARLEQUIN vers. 2.0 software (Schneider et al. 2000). Wright's (Weir and Cockerham 1984) fixation index, F_{ST} , was calculated based on an infinite allele model, and the significance level was adjusted using the Bonferroni correction (Rice 1989). Nei's (1983) unbiased genetic distance (D_s) was used to construct a phylogenetic tree using the Neighbor-joining (NJ) algorithm. Support for the tree nodes was assessed by bootstrapping over loci with 1000 replicates. The tree was built from raw allele frequency data using POPULATIONS 1.2.28. (Langella 2002) and visualized using TreeView (Page 1996). In addition, an individual assignment test based on Bayesian statistics was carried out using the software STRUCTURE 2.1 (Pritchard et al. 2000). This test calculates the number of foreign elvers (vagrants) from residents (members) of a particular group. Differences in the mean total length (\pm SD) between eel cohorts were examined by one-way analysis of variance (ANOVA).

RESULTS

Genetic diversity

The mean total lengths among the 4 intra-annual cohorts did not significantly differ from each other ($p = 0.173$) (Table 1). Values of n_a , n_e , H_o , H_e , and the Hardy-Weinberg equilibrium (HWE) test of the 7 microsatellites loci from the 4 intra-

annual eel groups are shown in table 3. All loci were polymorphic such that the total number of alleles ranged from 8 in locus AJMS-3 to 32 in locus AJM-8 with a total mean of 19.9. Three, 4, 5, and 6 private alleles were found in samples of Nov. 2000, Dec. 2000, Feb. 2001, and Mar. 2001, respectively. The private alleles may have been deduced from new genealogical mutations, slipped strand mispairings during DNA duplication, or crossovers during DNA recombinations (Goldstein and Schlotterer 1999). Observed and expected heterozygosities of each sample ranged 0.3077-0.9000 and 0.5949-0.9552 with means of 0.6765 and 0.8579, respectively. The HWE test showed that significant deviations occurred at the AJMS-1 and AJMS-2 loci for all eel groups and on 25 Nov. 2000 at locus AJMS-6 after applying the Bonferroni correction (Table 3). Loci AJMS-1 and AJMS-2 were thus excluded from the subsequent analyses.

Genetic distances and genetic differentiation among samples

The overall genetic differentiation among all samples was very low and not significant ($F_{ST} = 0.0029$, $p = 0.173$). Pair-wise F_{ST} values were smallest at -0.0010 between Feb. 2001 and Mar. 2001, and largest at 0.0111 between Nov. 2000 and Dec. 2000 (Table 4). No combinations were found to be significant except between samples of Nov. and Dec. 2000 ($p = 0.008$). Analysis of molecular variance (AMOVA) within and among the 4 intra-annual eel cohorts indicated that most of the total genetic variation was present within samples

Table 2. Characteristics of 7 microsatellite DNA loci of the Japanese eel

Locus	Primer sequences	Accession no.	Fluorescence tag
AJMS-1	5': TCGAGACACCAGATAGTCAC 3': ACATCCTAGGCTCACACC	AJ297599	FAM
AJMS-2	5': ATTTACGTCATCGGACCTGC 3': GCTGGGAGCGACGCTTTATC	AJ297600	TAMRA
AJMS-3	5': GGTATGAATGCAGGCGTTTATG 3': GCAACCGATTTGATCTCCAG	AJ297601	TAMRA
AJMS-5	5': CCTTCAGATTGCTAGCAC 3': CGGAGTCTAATTGTCTCCTC	AJ297602	HEX
AJMS-6	5': ACAGAGCCAGACAAACAGAC 3': GGTCAGCAAGCAAACGAAC	AJ297603	HEX
AJM-1	5': AGTAAAGAGTCCCACGCATTG 3': AAGGTGGATTTTTGCTGGCTC	AM062762	TAMRA
AJM-8	5': AGGCTGAAGTGAGTATGCTCAG 3': AGATATGGAAGCAGGATGGAG	AM062761	FAM

(98.99%), and only 1.01% was present among samples ($p < 0.01$).

Genetic structure and assignment test

Figure 2 shows the NJ tree employing Nei's unbiased genetic distances (D_s) between eel populations (Table 4) with robust bootstrap values

occurring between the samples of Nov. 2000 and all others. The glass eels from samples other than Nov. 2000 were phylogenetically close to each other with low bootstrap values.

Individual assignment tests based on Bayesian statistics with a 95% score were used to identify the origin of individuals. Among the 189 individuals in the 4 eel samples, the assignment

Table 3. Levels of genetic variation observed at 7 microsatellite loci: sample size (n), observed (H_O) and expected (H_E) heterozygosity within samples, and observed (na) and effective (ne) number of alleles. An asterisk (*) indicates a significant Hardy-Weinberg disequilibrium level at 5% adjusted by Bonferroni's correction

Locus	25 Nov. 2000 $n = 39$	25 Dec. 2000 $n = 50$	7 Feb. 2001 $n = 50$	9 Mar. 2001 $n = 50$	Total	Mean
AJMS-1						
na	13	12	13	16	19	
ne	6.79	6.58	6.59	8.85		7.83
H_O	0.3077*	0.4800*	0.3200*	0.4400*		0.3869
H_E	0.8638	0.8566	0.8568	0.8960		0.8683
AJMS-2						
na	19	19	16	18	22	
ne	11.39	11.04	9.67	10.62		11.43
H_O	0.6667*	0.5400*	0.6200*	0.7400*		0.6417
H_E	0.9241	0.9186	0.9057	0.9149		0.9158
AJMS-3						
na	4	7	5	6	8	
ne	2.87	2.43	2.51	2.79		2.70
H_O	0.5385	0.6400	0.5800	0.5800		0.5846
H_E	0.6597	0.5949	0.6077	0.6477		0.6275
AJMS-5						
na	19	18	15	16	24	
ne	9.72	11.06	8.49	9.02		10.34
H_O	0.8974	0.8600	0.7600	0.8600		0.8444
H_E	0.9201	0.9190	0.8935	0.8982		0.9077
AJMS-6						
na	14	12	15	15	17	
ne	7.51	5.59	7.23	6.66		7.09
H_O	0.5128*	0.5800	0.7400	0.7400		0.6432
H_E	0.8781	0.8293	0.8703	0.8584		0.8590
AJM-1						
na	11	15	14	14	17	
ne	7.51	7.96	8.17	7.55		8.16
H_O	0.7949	0.7800	0.8400	0.8000		0.8037
H_E	0.8781	0.8832	0.8865	0.8764		0.8811
AJM-8						
na	25	26	25	26	32	
ne	12.46	18.80	16.45	18.38		19.57
H_O	0.7436	0.8800	0.8000	0.9000		0.8309
H_E	0.9304	0.9545	0.9436	0.9552		0.9459
Mean						
H_O	0.6374	0.6800	0.6657	0.7229		0.6765
H_E	0.8649	0.8509	0.8520	0.8638		0.8579

test indicated that a mean 14.3% of individuals were members of each cohort, while 85.7% of individuals were foreign recruits (vagrants) (Fig. 3). Each sample contained a high mixing rate (60%-92%) of foreign recruits.

DISCUSSION

Values of H_O for genetic variability of the Japanese eel among spatial (Tseng et al. 2006), inter-annual (Tseng et al. 2003), and intra-annual (this study) samples were 0.758, 0.708, and 0.677, respectively. This indicates that genetic differentiation was highest among samples from different locations, intermediate among annual cohorts of the same habitats, and lowest among intra-annual cohorts of the same habitats. Several lines of evidence support the Japanese eel, although exhibiting spatial genetic differentiation, still being genetically stable temporally at the same location. First, the overall temporal genetic variation among arrival waves of elvers in consecutive months of the same habitat was not significant. Pair-wise F_{ST} values showed that only the pair of samples between Nov. and Dec. 2000 exhibited subtle but significant genetic variation. This might have been due to the phenomenon of genetic patchiness, in which random variations in parental contributions

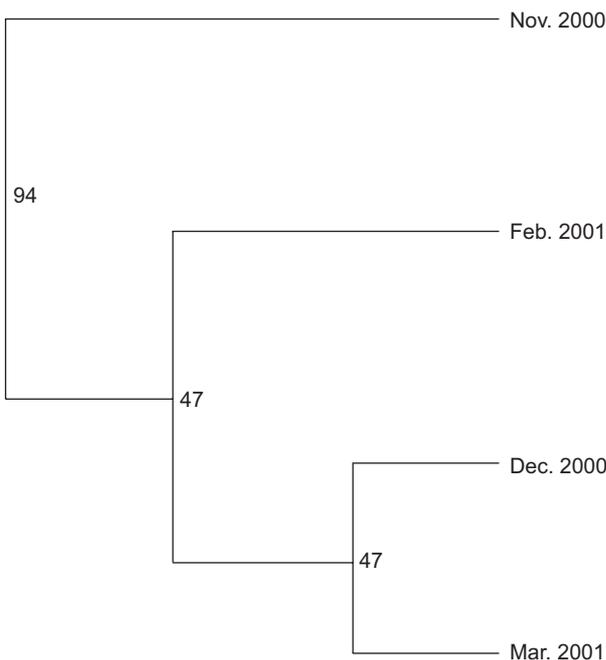


Fig. 2. Neighbor-Joining tree depicting genetic relationships based on Nei's unbiased genetic distances among the 4 intra-annual cohorts.

of reproductive activity result in a heterogeneous genetic composition of offspring (Maes et al. 2006). Second, based on the individual assignment test, high percentages (60%-92%) of elvers were not correctly assigned to their original samples, indicating the high mixing rate among intra-annual cohorts. Third, the phylogenetic tree exhibited low bootstrap values among intra-annual cohorts except samples from Nov. 2000, indicating the generally high genetic identity among arrival waves. These results suggest that there is no overall significant genetic variation among recruits within years.

The high rate of larval mixing among recruits may be related to the hydrographic features of Pacific Ocean currents. Although the spawning of the Japanese eel is synchronized once a month around the new moon (Tsukamoto et al. 2003, Tsukamoto 2006), the long dispersal time (4-6 mo) of the leptocephali, which may be due to the larval behavior and their diverse positions in the NEC where current velocities differ, provide the conditions needed for larval mixing between intra-annual cohorts. Indeed, the birthdates of previous arrival waves of glass eels caught in Taiwanese estuaries partially overlapped with those of the next arrival wave (Tzeng 1990), supporting the phenomenon of potential larval mixing. The lack of an IBT pattern from either inter-annual (Tseng et al. 2003) or among intra-annual arrival waves (this study) indicates the stable temporal genetic structure between progenies and ancestors in the same geographic region. Plasticity of the maturation age and spawning time together with the high larval mixing in the Japanese eel may hinder the occurrence of genetic differentiation in the same habitat.

Significant spatial genetic differentiation was found in the European eel, which either fits the IBD hypothesis (Wirth and Bernatchez 2001, Maes and Volckaert 2002) or does not (Dannewitz et al.

Table 4. Pairwise F_{ST} (below the diagonal) and Nei's unbiased genetic distance (D_s) (above the diagonal) values between the 4 eel samples. *The significance level was 0.05

Date	Nov. 2000	Dec. 2000	Feb. 2001	Mar. 2001
Nov. 2000		0.1223	0.0905	0.0884
Dec. 2000	0.0111*		0.0569	0.0468
Feb. 2001	0.0052	0.0012		0.0475
Mar. 2001	0.0044	-0.0009	-0.0010	

Overall $F_{ST} = 0.0029$, $p = 0.173$.

2005). The Japanese eel is clearly spatially distinguished into 2 groups. However, this does not support the IBD migration model. For American eels, Wirth and Bernatchez (2003) found weak but significant spatial genetic differentiation which did not support IBD, which meant that the panmixia hypothesis could not be fully rejected. These results infer that spatial genetic variation seems to be highest in the European eel, intermediate in the Japanese eel, and lowest in the American eel. On the other hand, IBT was found in inter-annual cohorts of the European eel (Maes et al. 2006), and genetic patchiness was observed among inter- and intra-annual cohorts (Pujolar et al. 2006). No significant temporal genetic differentiation was suggested in the Japanese eel (Tseng et al. 2003, this study). This further supports higher genetic differentiation in the European eel than in the Japanese eel, both spatially and temporally. The reason may be due to the longer times and distances for the spawning migration of adults and dispersal of larvae in the European eel than in the Japanese or American eels (Wang and Tzeng 1998). The migration distances are longest for the European eel (4000-8000 km), intermediate for the

Japanese eel (2000-3500 km), and shortest for the American eel (900-3000 km) (Aoyama et al. 2003, Tesch 2003). The longer migration distances are coincident with higher genetic differentiation in the 3 eel species. The oceanic factors and climatic variations that the European eel faces are more complicated than those faced by the other 2 eels, causing higher isolation and lower gene flow between spawning groups. This may have promoted genetic differentiation of the European eel. Consequently, the Japanese, American, and European eels have evolved their own particular genetic structures based on their own adaptations to the unique oceanic current systems of the respective Pacific and Atlantic Oceans.

In conclusion, the population genetic structure of the Japanese eels examined, based on microsatellite loci, exhibited no significant genetic differentiation among intra-annual cohorts. Although the Japanese eel can be spatially divided into 2 management units, the genetic structures of inter- and intra-annual cohorts in each habitat were temporally stable.

Acknowledgments: The authors express their gratitude to Ms. H.Y. Teng and Drs. C.W. Chang and Y.T. Wang for collecting specimens from Taiwan. Research funding was provided to W.N. Tzeng by the National Science Council, Executive Yuan, Taiwan (NSC 94-2313-B-002-070) and the Council of Agriculture, Executive Yuan, Taiwan (94 AS-14.12-FA-F1(5)).

REFERENCES

- Aoyama J, S Wouthuyzen, MJ Miller, T Inagaki, K Tsukamoto. 2003. Short-distance spawning migration of tropical freshwater eels. *Biol. Bull.* **204**: 104-108.
- Avise JC. 2003. Catadromous eels of the North Atlantic: a review of molecular genetic findings relevant to natural history, population structure, speciation, and phylogeny. *In* Aida K, Tsukamoto K, Yamauchi K, eds. *Eel Biology*. Tokyo: Springer-Verlag, pp. 31-48.
- Avise JC, GS Helfman, NC Saunders, LS Hales. 1986. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. *Proc. Natl. Acad. Sci. USA* **83**: 4350-4353.
- Cheng PW, WN Tzeng. 1996. Timing of metamorphosis and estuarine arrival across the dispersal range of the Japanese eel *Anguilla japonica*. *Mar. Ecol.-Prog. Ser.* **131**: 87-96.
- Dannewitz J, GE Maes, L Johansson, H Wickström, FAM Volckaert. 2005. Panmixia in the European eel: a matter of time. *Proc. R. Soc. Lond. B* **272**: 1129-1137.
- De Ligny W, EM Pantelouris. 1973. Origin of the European eel. *Nature* **246**: 518-519.
- Goldstein DB, C Schlotterer. 1999. Mechanistic basis for

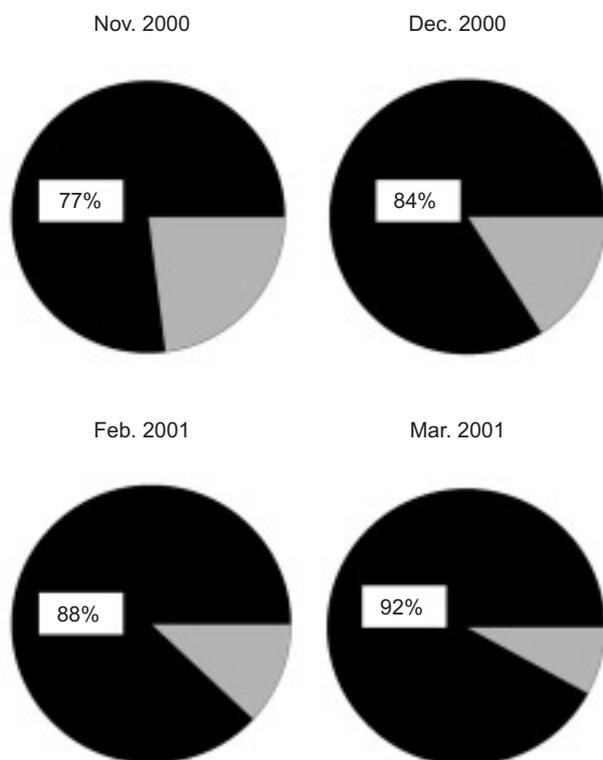


Fig. 3. Assignment test (95% score) among eel groups in the 4 mo sampled. The black and gray pies indicate the percentage of foreign (vagrant) and resident (member) eels, respectively.

- microsatellite instability. In *Microsatellites: evolution and applications*. Oxford, UK: Oxford Univ. Press, pp. 34-48.
- Han YS, IC Liao, YS Huang, JT He, CW Chang, WN Tzeng. 2003. Synchronous changes of morphology and gonadal development of silvering Japanese eel *Anguilla japonica*. *Aquaculture* **219**: 783-796.
- Ishikawa S, J Aoyama, K Tsukamoto, M Nishida. 2001. Population structure of the Japanese eel *Anguilla japonica* as examined by mitochondrial DNA sequencing. *Fish. Sci.* **67**: 246-253.
- Langella O. 2002. POPULATIONS 1.2.28. CNRS UPR9034.
- Lintas C, J Hirano, S Archer. 1998. Genetic variation of the European eel *Anguilla anguilla*. *Mol. Mar. Biol. Biotech.* **7**: 263-269.
- Maes GE, JM Pujolar, B Hellemans, FAM Volckaert. 2006. Evidence for isolation by time in the European eel (*Anguilla anguilla* L.). *Mol. Ecol.* **15**: 2095-2107.
- Maes GE, FAM Volckaert. 2002. Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). *Biol. J. Linn. Soc.* **77**: 509-521.
- Nei M. 1983. Genetic polymorphism and the role of mutation in evolution. In M Nei, R Koehn, eds. *Evolution of genes and proteins*. Sunderland, MA: Sinauer Associates, pp. 165-190.
- Page RDM. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Comp. Appl. Biosci.* **12**: 357-358.
- Pritchard JK, M Stephens, P Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959.
- Pujolar JM, GE Maes, FAM Volckaert. 2006. Genetic patchiness among recruits in the European eel *Anguilla anguilla*. *Mar. Ecol.-Prog. Ser.* **307**: 209-217.
- Raymond M, F Rousset. 1995. An exact test for population differentiation. *Evolution* **49**: 1280-1283.
- Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223-225.
- Sang TK, HY Chang, CT Chen, CF Hui. 1994. Population structure of the Japanese eel *Anguilla japonica*. *Mol. Biol. Evol.* **11**: 250-260.
- Schneider S, D Roessli, L Excoffier. 2000. Arlequin: a software for population genetics data analysis. Vers. 2.000. Geneva, Switzerland: Genetics and Biometry Lab, Department of Anthropology, Univ. of Geneva.
- Sinclair M. 1988. *Marine populations: an assay on population regulation and speciation*. Seattle, WA: Univ. of Washington Press.
- Tesch FW. 2003. *The eel*. Oxford, UK: Blackwell Science.
- Tseng MC, CA Chen, HW Kao, WN Tzeng, SC Lee. 2001. Polymorphisms of GA/GT microsatellite loci from *Anguilla japonica*. *Mar. Biotechnol.* **3**: 275-280.
- Tseng MC, WN Tzeng, SC Lee. 2003. Historical decline in the Japanese eel *Anguilla japonica* in northern Taiwan inferred from genetic variations. *Zool. Stud.* **42**: 556-563.
- Tseng MC, WN Tzeng, SC Lee. 2006. Population genetic structure of the Japanese eel *Anguilla japonica* in the northwest Pacific Ocean: evidence of non-panmictic populations. *Mar. Ecol.-Prog. Ser.* **308**: 221-230.
- Tsukamoto K. 1992. Discovery of the spawning area for Japanese eel. *Nature* **356**: 789-791.
- Tsukamoto K. 2006. Spawning of eels near a seamount. *Nature* **439**: 929.
- Tsukamoto K, T Otake, N Mochioka, TW Lee, H Fricke, T Inagaki, J Aoyama, S Ishikawa, S Kimura, MJ Miller, H Hasumoto, M Oya, Y Suzuki. 2003. Seamounts, new moon and eel spawning: the search for the spawning site of the Japanese eel. *Environ. Biol. Fish.* **66**: 221-229.
- Tzeng WN. 1982. New record of the eel, *Anguilla celebesensis* Kaup, from Taiwan. *Bioscience* **19**: 57-66.
- Tzeng WN. 1985. Immigration timing and activity rhythms of the eel, *Anguilla japonica*, elvers in the estuary of northern Taiwan, with emphasis on environmental influences. *Bull. Jpn. Soc. Fish. Oceanogr.* **47/48**: 11-28.
- Tzeng WN. 1986. Resources and ecology of the Japanese eel *Anguilla japonica* elvers in the coastal waters of Taiwan. *China Fish. Month.* **404**: 19-24. (in Chinese)
- Tzeng WN. 1990. Relationship between growth rate and age at recruitment of *Anguilla japonica* elvers in a Taiwan estuary as inferred from otolith growth increments. *Mar. Biol.* **107**: 75-81.
- Tzeng WN, HR Lin, CH Wang, SN Xu. 2000. Differences in size and growth rates of male and female migrating Japanese eels in Pearl River, China. *J. Fish Biol.* **57**: 1245-1253.
- Wang CH, WN Tzeng. 1998. Interpretation of geographic variation in size of American eel *Anguilla rostrata* elvers on the Atlantic coast of North American using their life history and otolith ageing. *Mar. Ecol.-Prog. Ser.* **168**: 35-43.
- Weir BS, CC Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.
- Wirth T, L Bernatchez. 2001. Genetic evidence against panmixia in the European eel. *Nature* **409**: 1037-1040.
- Wirth T, L Bernatchez. 2003. Decline of North Atlantic eels: a fatal synergy. *Proc. R Soc. Lond.* **270**: 681-688.
- Yang RC, FC Yeh. 1993. Multilocus structure in *Pinus contorta* Dougl. *Theor. Appl. Genet.* **87**: 568-576.