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Population Genetic Structure of the Year-Round Spawning Tropical Eel, *Anguilla reinhardtii*, in Australia

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Kang Ning Shen and Wann Nian Tzeng (2007) Population genetic structure of the year-round spawning tropical eel, *Anguilla reinhardtii*, in Australia. *Zoological Studies* **46**(4): 441-453. The population genetic structure of tropical freshwater eels is less well investigated than that of their temperate counterparts. To understand both the spatial and temporal population genetic structures of the year-round spawning tropical eel, *Anguilla reinhardtii*, variability at 6 microsatellite loci was examined in 799 glass eels. Samples were collected from 5 estuaries across the range of the species in East Australia in 1997-1999. F_{ST}, a measure of differentiation among populations, did not significantly differ among months or estuaries, suggesting that *A. reinhardtii* populations are panmictic. An oceanic and prolonged spawning period, which lasts nearly the entire year, appears to facilitate interbreeding between adult eels from different generations and areas. A short pelagic larval duration decreases larval dispersal, and therefore decreases the effect of latitude-correlated environmental variables on the spawning migration. Mixing of individuals from different areas in ocean gyres may also contribute to genetic homogeneity among streams. http://zoolstud.sincica.edu.tw/Journals/46.4/441.pdf

Key words: Tropical eel, Microsatellites, Panmixia, Spawning behavior.

Freshwater eels of the genus *Anguilla* Schrank, 1798, are catadromous and semelparous fish with long spawning migrations and extended larval durations. *Anguilla* eels are classified into 15 species and 2 subspecies (Ege 1939, Castle and Williamson 1974, Jellyman 1987, Dijkstra and Jellyman 1999). Among these, 12 are tropical and 5 are temperate species. Individuals of most species spawn in tropical oceans, are transported as larvae westward by warm currents at low latitudes, and mature in rivers along the eastern margins of Australia, Asia, Africa, and North America (Schmidt 1922 1925, Ege 1939, Tesch 1977). One exception is the European eel, *A. anguilla*, which occurs in western Europe and adjacent land masses.

Although individuals occupy diverse habitats during their life cycle, most freshwater eels are considered to form randomly mating panmictic populations. The chief mechanism producing panmixia is that individuals from different areas spawn in a common area in the ocean (Avise et al. 1986). However, recent evidence from studies of microsatellite variations has challenged this model for 2 temperate eels, European eel (*A. anguilla*, Daemen et al. 2001, Wirth and Bernatchez 2001) and Japanese eel (*A. japonica*, Tseng et al. 2001 2003 2006). Wirth and Bernatchez (2001) found that populations of European eel show slight but significant genetic differences, and that genetic distances between populations are positively correlated with geographic distance. This implies restricted gene flow and a lack of random mating between eels from different sampled locations. Those authors suggested 3 possible reasons for this pattern: (1) differences in spawning migration timings of adult eels arriving from different latitudes (temporal allopatry); (2) multiple spawning

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areas (spatial allopatry), and (3) assortative mating in a single area and non-random larval dispersal.

The evidence for population subdivision, however, is controversial. For European eels, Dannewitz et al. (2005) emphasized the importance of temporal replication, because they found that the extent of temporal genetic variation within sites exceeded that of geographical variation. Pujolar et al. (2006) also showed temporal genetic variation in the European eel and found highly significant genetic differences among arrival waves within the same cohort. Tseng et al. (2006) found that Japanese eels in the northwestern Pacific Ocean were genetically divided into high- and low-latitude groups. The level of differentiation, however, implied some degree of connection (gene flow) between the populations. This structure was interpreted as supporting the member-vagrant hypothesis (Sinclair 1988). Most studies of population genetic structure of eels have focused on temperate species in the northern hemisphere and few have examined tropical species (Ishikawa et al. 2004).

Differences between tropical and temperate eel species occur in the pelagic larval duration, distribution range, and spawning season and may lead to different population genetic structures. Temperate eels usually have longer pelagic larval durations and greater long-distance dispersals (Cheng and Tzeng 1996, Arai et al. 1999b, Arai et al. 2000, Marui et al. 2001) than tropical eels (Arai et al. 2002, Shiao et al. 2002). Larval duration is a key factor in determining the final estuarine destinations of glass eels (Cheng and Tzeng 1996, Wang and Tzeng 1998 2000). In general, glass eels of temperate anguillid species enter estuaries from early winter to late spring. In contrast, tropical glass eels (i.e., *A. marmorata* and *A. celebesensis*) are found throughout the year in fluctuating abundances in northern Luzon, the Philippines (Tabeta et al. 1976), and in northern Sulawesi I., Indonesia (Arai et al. 1999a 2001, Sugeha et al. 2001). Differences between tropical and temperate species in the timing of inshore arrivals may be due to some combination of differences in spawning duration, transport in ocean currents, and early life history parameters such as the timing of metamorphosis and age at recruitment (Cheng and Tzeng 1996, Wang and Tzeng 1998 2000, Arai et al. 2001).

The Australian longfinned eel, *A. reinhardtii*, is a tropical eel, distributed between 20°S and 34°S in East Australia (Beumer and Sloane 1990). Glass eels of *A. reinhardtii* can be continuously collected in 6 estuaries of New South Wales, Australia throughout the year (Silberschneider 2005). This year-round recruitment suggests that *A. reinhardtii* may also spawn year-round. Hatching dates, back-calculated from daily otolith increments, also support the year-round spawning hypothesis (Arai et al. 1999a 2001, Marui et al. 2001, Shiao et al. 2002). Hatching dates were estimated to run from Mar. through Jan. of the following year (Shiao et al. 2002).

In general, species with wide latitudinal distributions and prolonged spawning are easily differentiated into subpopulations, e.g., the Atlantic herring (*Clupea harengus*) spawning year-round (Sinclair and Tremblay 1984), and *A. marmorata* distributed across the Indian and Pacific Oceans (Tesch 1977, Ishikawa 2004). The tropical eel, A. reinhardtii, spawns year-round but is not as widely distributed as *A. marmorata*, *A. anguilla*, *A. japonica*, or *A. australis* (Ishikawa 2004, Wirth and Bernatchez 2001, Tseng et al. 2006, Shen and Tzeng 2007). The latter species shows genetic differences between populations in Australia and New Zealand (Shen and Tzeng 2007).

This study used *A. reinhardtii* as a model species to understand the effect of spatial and temporal distributions on the genetic structure of tropical eels. We hypothesized that the prolonged spawning season of *A. reinhardtii* is an opportunity for temporal spawning isolation between different spawning groups. Microsatellites, highly sensitive genetic markers, were used to test the population genetic structure of this tropical eel.

MATERIALS AND METHODS

Sampling

In total, 799 *A. reinhardtii* glass eels were collected in 1997-1999 from 5 estuaries, which encompass the species' range on the eastern coast of Australia (Fig. 1). To examine temporal variability, eels in the Albert River estuary were collected in Nov. 1997 and also year-round from Oct. 1998 to Sept. 1999. Sampling locations, sampling dates, and samples sizes used for the microsatellite analysis are shown in table 1. Tissue samples were preserved in 95% ethanol prior to DNA extraction. In addition, some of these specimens were used for age determination in a previous study (Shiao et al. 2002).

DNA extraction, polymerase chain reaction (PCR), and genotyping

Genomic DNA was extracted from muscle tissue using a DNA Purification Kit (Genemark, Tainan, Taiwan) and preserved in TE buffer. Genomic DNA was quantified and diluted to a working concentration of 1 ng/μl for the PCR. Six microsatellite loci including 1 $(GA)_n$ (AJMS-6, EMBL accession no.: AJ297605) and 5 $(GT)_n$ (AJMS-1, -3, -4, -5, and -7, EMBL accession nos.: AJ297599, AJ297601-AJ297603, AM062761) screened from the *A. japonica* genome (Tseng et al. 2001 2006), were used for cross-species amplification. Homologous fragments of these 6 microsatellite loci from *A. reinhardtii* were cloned and sequenced to confirm previous sequences. Reverse primers of each locus were labeled with different fluorescent dyes (FAM, HEX, and TAMRA), and PCR was performed in a volume of 25 μl including 0.1 ng DNA, 1.25 pmole of the reverse primer, 1.25 pmole of the forward primer, 5 mM dNTP, 0.05-0.1 mM $MgCl_2$, and 0.5 U *Taq* polymerase (Bioman, Taipei, Taiwan). Amplification procedures consisted of an initial denaturing at 95°C for 4 min, followed by 35 cycles of successive thermal regimes of 94, 58-60, and 72°C, each at a 30 s duration. Genotypes of microsatellites were detected by an ABI PRISM 377 auto DNA sequencer (Applied Biosystems, Foster City, CA, USA). Allelic sizes of microsatellites were scored by comparing the length of the PCR fragments to the TAMRA-labeled 100 bp standard (Perkin-

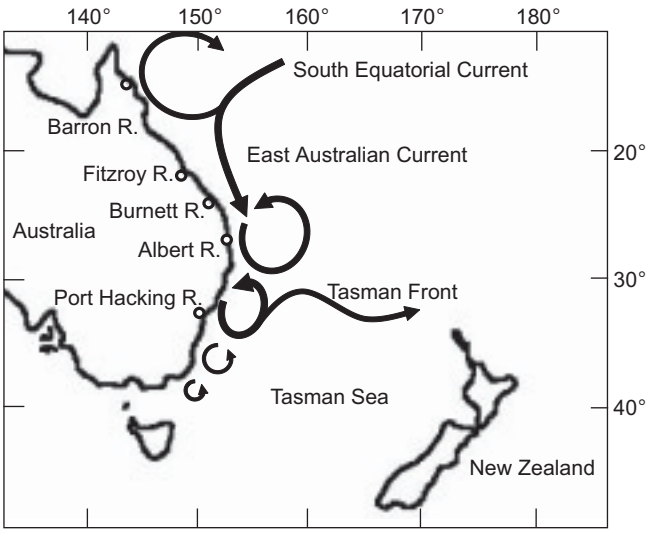

Fig. 1. Sampling locations of the glass eel *Anguilla reinhardtii* and the current system in East Australia. From north to south are the Barron River, Fitzroy River, Burnett River, Albert River, and Port Hacking River. The South Equatorial Current, East Australian Current, Tasman front, and gyres along the eastern coast of Australia are shown.

Table 1. Sampling locations, dates, and sample sizes of glass eels of *Anguilla reinhardtii* collected in 5 estuaries of East Australia

Sampling location	Latitude and longitude	Sampling date	Sample size
Barron River (Ba)	145°41'E, 16°52'S	Mar. 1998	40
Fitzroy River (Fi)	150°32'E, 23°21'S	Apr. 1999	47
		Aug. 1999	50
Burnett River (Bu)	152°17'E, 24°53'S	Apr. 1999	50
Albert River (Al)	153°04'E, 27°59'S	Nov. 1997	35
		Oct. 1998	17
		Nov. 1998	50
		Dec. 1998	50
		Jan. 1999	51
		Feb. 1999	49
		Mar. 1999	48
		Apr. 1999	25
		May 1999	50
		June 1999	49
		July 1999	49
		Aug. 1999	49
		Sept. 1999	50
Port Hacking River (Po)	151°50'E, 34°40'S	Apr. 1999	40
Total			799

Elmer, Waltham, MA, USA).

Statistical analyses

Genetic diversities for each sample of glass eels were calculated for each locality using ARLEQUIN vers. 2.0 software (Schneider et al. 2000). The allelic richness of samples was calculated with FSTAT (vers. 2.9.3.2; Goudet 2001). Deviations of observed from expected Hardy-Weinberg genotypic frequencies were tested for each locus-sample combination using an exact test based on the Markov chain method (Guo and Thompson 1992). Significance levels for multiple comparisons of loci were adjusted using a sequential Bonferroni correction (Rice 1989). The presence of null alleles or scoring errors of microsatellite loci was tested with the software MICRO-CHECKER vers. 2.2.0 (Van Oosterhout et al. 2004). Genotypic linkage disequilibrium between loci was examined using the exact test implemented in GENEPOP vers. 3.1 (Raymond and Rousset 1995) to determine if genotypes at 1 locus were independent of genotypes at another locus.

Temporal (by year and month) and geographic (by estuary) allele-frequency differences were analyzed with Wright's *F*-statistics (Weir and Cockerham 1984) as implemented in ARLEQUIN 2.0. The fixation index of genetic differentiation, F_{ST}, and pairwise F_{ST} between estuaries were tested for significance using 10,000 permutations of individual genotypes among samples. Inbreeding was estimated by Wright's F_{IS}. Significant deviations from 0 were tested by the Chi-squared test (Li and Horvitz 1953). Tests of temporal differences among eel cohorts were based on the 2 monthly samples from the Fitzroy River and the 13 monthly samples from the Albert River, including inter-annual and within-year samples. The spatial genetic structure among 5 locations was examined by pooling temporal samples. In these comparisons, however, sample sizes at some locations were much larger than those at others. Nei's genetic distance, D_A (Nei 1983), based on the infinite allele neutral mutation model, was computed between samples with POPULATIONS 1.2.28. (Langella 1999). Genetic relationships among samples were also examined by multidimensional scaling (MDS) (Lessa 1990), as implemented in STATISTICA vers. 6.0 (StatSoft 2001). The fit of samples in the MDS space to genetic distances was measured by "stress" (Kruskal 1964). The smaller the stress value, the better the representation. Genetic relationships of samples among locations and among months were depicted from D_A and the Neighbor-joining (NJ) tree method using MEGA vers. 2.1 (Kumar et al. 2001). Isolation-by-distance and isolation-by-time were tested using Mantel's test in ARLEQUIN 2.0.

RESULTS

Genetic variability and Hardy-Weinberg equilibrium expectations

Summary statistics for 108 combinations of 6 microsatellite loci and 18 temporal and spatial samples of *A. reinhardtii* are given in table 2. The mean H_o for each locus ranged from 0.588 (AJMS-6) to 0.873 (AJMS-4), and the mean H_e ranged from 0.617 (AJMS-3) to 0.881 (AJMS-4). Genotype frequencies in 36 of 108 samples deviated from Hardy-Weinberg expectations. After applying the Bonferroni correction, 26 samples still showed significant deviation. Deviations in these 26 samples reflected deficiencies in heterozygotes at AJMS-1 and AJMS-6. MICRO-CHECKER revealed the presence of null alleles at AJMS-1 and AJMS-6, which also occurred in *A. japonica* (Tseng et al. 2001). These 2 loci were therefore excluded from the following analysis. The number of alleles ranged from 11 in AJMS-3 to 37 in AJMS-6 (Table 2). Allelic richness per sample ranged from 8.499 to 10.590, and did not significantly differ among samples. No linkage disequilibrium between genotypes was found between the 4 microsatellite loci ($p > 0.05$).

Mean values of the population variables for the 4 loci are shown in table 3. The mean number of alleles per locus per sample varied from 8.75 to 12.00. The mean H_o varied from 0.725 to 0.850, and the mean H_e varied from 0.743 to 0.820. The overall mean H_o and He were 0.782 and 0.779, respectively. F_{IS} calculated from the mean H_o and H_e ranged from -0.070 to 0.085, and F_{IS} values of individual samples did not significantly differ from 0 ($p > 0.05$) by the Chi-squared test.

Temporal genetic variation

The fixation index of genetic differentiation among the 13 samples of eels collected from the Albert River from Nov. 1997 to Sept. 1999 was $F_{ST} = 0.00047$ and was not significant ($p > 0.05$). Similarly, the F_{ST} for samples collected from the Fitzroy River between Apr. and Aug. 1999 was 0.00509 which was also not significant ($p > 0.05$).

Pairwise values of D_A between temporal samples ranged from 0.033 (Nov. 1998 and Aug. 1999 in the Albert River) to 0.108 (Oct. and Dec. 1998 in the Albert River). The MDS plot indicated that eels were genetically closer between neighboring months in the Albert River (June and July 1999; Dec. 1998 and Jan. and Feb. 1999; Mar. and May 1999). However, samples collected in Oct. 1998 and Apr. 1999 in the Albert River were separated from other samples due to the smaller sample sizes and the appearance of private alleles (Fig. 2). Therefore, samples collected from the Albert River over 11 months were used to construct an unrooted NJ tree. These temporal samples were roughly divided into 3 groups: summer (Nov. 1997, Dec. 1998, Feb. 1999, Jan. 1999, and exceptionally July 1999), winter (June and Sept. 1999), and autumn samples (May and Mar. 1999, and exceptionally Nov. 1998 and Aug. 1999). However, these groups did not have bootstrap support in the NJ tree of D_A (Fig. 3). No correlation appeared between the time of sample collection and D_A (Mantel test, $p > 0.05$).

Spatial genetic variation

As no significant temporal variability appeared among samples, the geographic genetic structure was examined by pooling temporal samples. The F_{ST} of samples from the 5 estuaries was 0.00094. A permutation test indicated that the F_{ST} among these samples was not significant ($p > 0.05$). Values of D_A between geographic samples were generally less than those among temporal samples. In the MDS plot, the 1st and 2nd axes respectively explained 61.15% and 26.63% of the variance in genetic distances between samples (Fig. 2). The stress value of MDS was 0.11, indi-

Table 2. Genetic variability of population parameters at 6 microsatellite loci in Anguilla reinhardtii collected from 5 river estuaries in East Australia. Table-wide significance levels were adjusted by the sequential Bonferroni technique (Rice 1989)

Microsatellite locus		Barron R. Mar. 1998 (n = 40)	Fitzroy R. Apr. 1999 (n = 47)	Fitzroy R. Aug. 1999 (n = 50)	Burnett R. Apr. 1999 (n = 50)	Albert R. Nov. 1997 (n = 35)
AJMS-1	No. of alleles	14	11	11	13	12
	Allele range	194-220	194-214	194-216	192-218	194-216
	H_o	0.475	0.617	0.6	0.46	0.457
	H_e	0.884	0.876	0.86	0.864	0.867
	H.-W. test	**	**	**	**	**
AJMS-3	No. of alleles	7	8	7	7	7
	Allele range	78-94	80-96	80-94	80-96	80-94
	H_o	0.575	0.702	0.64	0.6	0.714
	H_e	0.7	0.601	0.716	0.609	0.653
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-4	No. of alleles	15	15	12	15	15
	Allele range	123-161	127-161	129-151	119-157	121-155
	H_o	0.875	0.979	0.88	0.9	0.886
	H_e	0.904	0.912	0.861	0.879	0.888
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-5	No. of alleles	11	11	15	14	9
	Allele range	90-114	72-110	72-116	78-122	90-106
	H_o	0.775	0.787	0.8	0.9	0.8
	H_e	0.773	0.777	0.789	0.828	0.792
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-6	No. of alleles	19	20	18	16	12
	Allele range	152-210	150-206	152-208	150-202	154-194
	H_o	0.525	0.617	0.42	0.52	0.6
	H_e	0.902	0.9	0.862	0.86	0.745
	H.-W. test	**	**	**	**	n.s.
AJMS-7	No. of alleles	10	11	11	11	10
	Allele range	79-101	85-105	85-107	85-107	85-105
	H_o	0.675	0.809	0.9	0.84	0.771

Table 2. (Cont.)

	H_e	0.807	0.785	0.858	0.845	0.832
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.

Microsatellite locus		Albert R. Oct. 1998 (*n* = 17)	Albert R. Nov. 1998 (*n* = 50)	Albert R. Dec. 1998 (*n* = 50)	Albert R. Jan. 1999 (*n* = 51)	Albert R. Feb. 1999 (*n* = 49)
AJMS-1	No. of alleles	9	15	14	14	13
	Allele range	190-216	178-216	186-214	190-222	186-212
	H_o	0.647	0.76	0.6	0.588	0.612
	H_e	0.87	0.861	0.899	0.877	0.874
	H.-W. test	n.s.	**	**	**	**
AJMS-3	No. of alleles	5	6	9	5	8
	Allele range	80-90	80-90	80-96	80-88	80-94
	H_o	0.471	0.64	0.5	0.627	0.612
	H_e	0.661	0.545	0.615	0.534	0.59
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-4	No. of alleles	13	15	13	13	13
	Allele range	123-149	123-161	121-149	125-149	123-149
	H_o	0.941	0.82	0.86	0.863	0.837
	H_e	0.923	0.871	0.876	0.878	0.865
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-5	No. of alleles	9	10	12	11	14
	Allele range	88-106	88-110	72-122	88-116	90-124
	H_o	0.706	0.82	0.8	0.804	0.816
	H_e	0.752	0.741	0.752	0.768	0.821
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-6	No. of alleles	10	14	15	13	18
	Allele range	154-184	152-212	152-196	148-182	150-194
	H_o	0.588	0.68	0.66	0.647	0.694
	H_e	0.854	0.832	0.844	0.861	0.898
	H.-W. test	*	**	**	**	**
AJMS-7	No. of alleles	8	10	10	9	12
	Allele range	79-101	85-103	85-105	87-105	77-105
	H_o	0.824	0.84	0.9	0.843	0.857
	H_e	0.886	0.839	0.838	0.805	0.861
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.

Microsatellite locus		Albert R. Mar. 1999 (*n* = 48)	Albert R. Apr. 1999 (*n* = 25)	Albert R. May 1999 (*n* = 50)	Albert R. June 1999 (*n* = 49)	Albert R. July 1999 (*n* = 49)
AJMS-1	No. of alleles	11	13	14	13	12
	Allele range	194-216	194-224	194-222	194-222	194-216
	H_o	0.542	0.76	0.5	0.612	0.571
	H_e	0.869	0.891	0.85	0.893	0.886
	H.-W. test	**	n.s.	**	**	**
AJMS-3	No. of alleles	7	6	7	8	5
	Allele range	80-94	80-94	76-90	80-96	82-96
	H_o	0.667	0.76	0.56	0.633	0.531
	H_e	0.614	0.648	0.621	0.605	0.58
	H.-W. test	n.s.	*	n.s.	n.s.	n.s.
AJMS-4	No. of alleles	14	13	16	15	14
	Allele range	127-161	123-149	121-163	123-153	123-153
	H_o	0.854	0.8	0.86	0.857	0.837
	H_e	0.862	0.882	0.879	0.815	0.902

Table 2. (Cont.)

	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.
AJMS-5	No. of alleles	11	12	13	13	12
	Allele range	72-108	72-114	72-114	88-116	72-112
	H_o	0.875	0.8	0.82	0.776	0.898
	H_e	0.834	0.882	0.806	0.789	0.771
	H.-W. test	n.s.	n.s.	*	n.s.	n.s.
AJMS-6	No. of alleles	16	18	21	24	21
	Allele range	152-190	150-208	150-210	146-208	148-216
	H_o	0.583	0.6	0.44	0.694	0.612
	H_e	0.809	0.868	0.86	0.896	0.88
	H.-W. test	**	**	**	**	**
AJMS-7	No. of alleles	11	10	12	10	12
	Allele range	85-107	79-105	73-109	79-101	79-109
	H_o	0.854	0.8	0.82	0.837	0.816
	H_e	0.861	0.868	0.849	0.854	0.826
	H.-W. test	n.s.	n.s.	n.s.	n.s.	n.s.

Microsatellite locus		Albert R. Aug. 1999 (n = 49)	Albert R. Sept. 1999 (n = 50)	Port Hacking R. Apr. 1999 (n = 40)	Mean (Total)
AJMS-1	No. of alleles	14	16	14	12.9 (21)
	Allele range	186-216	186-226	194-220	
	H_o	0.735	0.68	0.475	0.594
	H_e	0.894	0.908	0.884	0.878
	H.-W. test	**	**	**	
AJMS-3	No. of alleles	7	7	8	6.9 (11)
	Allele range	80-94	82-96	80-96	
	H_o	0.551	0.64	0.675	0.617
	H_e	0.596	0.594	0.623	0.617
	H.-W. test	n.s.	n.s.	n.s.	
AJMS-4	No. of alleles	14	13	14	14 (22)
	Allele range	125-151	127-153	125-153	
	H_o	0.878	0.86	0.925	0.873
	H_e	0.886	0.882	0.9	0.881
	H.-W. test	*	n.s.	n.s.	
AJMS-5	No. of alleles	10	13	13	11.9 (23)
	Allele range	90-122	72-116	72-122	
	H_o	0.694	0.64	0.875	0.799
	H_e	0.819	0.661	0.851	0.789
	H.-W. test	n.s.	n.s.	n.s.	
AJMS-6	No. of alleles	14	20	15	16.6 (37)
	Allele range	148-222	152-224	154-194	
	H_o	0.592	0.52	0.6	0.588
	H_e	0.874	0.885	0.888	0.862
	H.-W. test	**	**	**	
AJMS-7	No. of alleles	10	9	13	10.5 (17)
	Allele range	85-107	85-101	79-105	
	H_o	0.939	0.84	0.925	0.838
	H_e	0.837	0.836	0.855	0.841
	H.-W. test	n.s.	n.s.	n.s.	

n, sample size; H_o, observed heterozygosity; H_e, expected heterozygosity. n.s., not significant; * deviation from Hardy-Weinberg (H.-W.) equilibrium ($p < 0.05$); ** deviation from Hardy-Weinberg equilibrium ($p < 0.01$).

cating that the representation of these samples in the MDS space is acceptable.

Pairwise values of F_{ST} and D_A between locations are shown in table 4. The pairwise fixation index among locations ranged from -0.005 between the Burnett and Port Hacking Rivers to

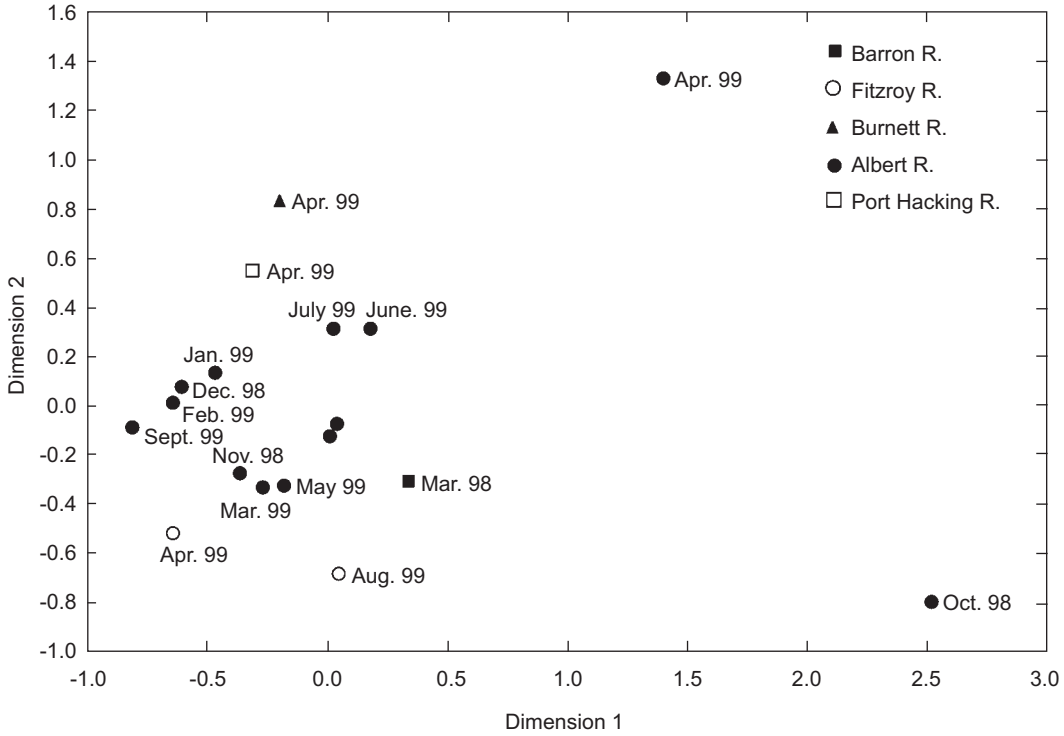

Fig. 2. Genetic relationship among 18 samples of *Anguilla reinhardtii* collected from 5 rivers by 2-dimensional scaling with the D_A distance.

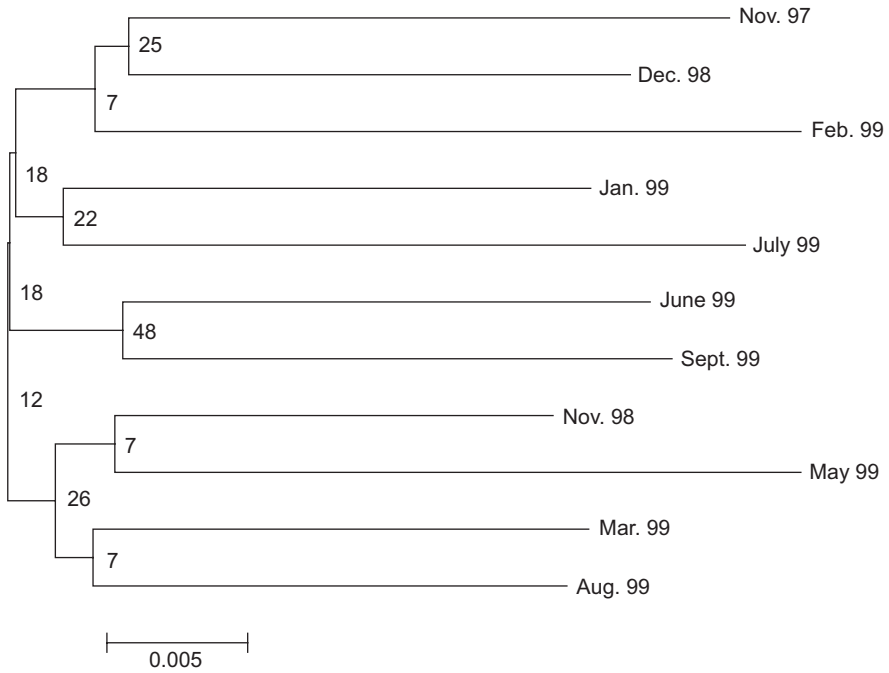

Fig. 3. Unrooted Neighbor-joining tree constructed from Nei's D_A distance of *Anguilla reinhardtii* among 11 samples from the Albert River.

0.004 between the Albert River and both the Barron and Fitzroy Rivers. No significant pairwise values of F_{ST} were detected between samples. D_A between samples ranged from 0.033 between the Barron and Burnett Rivers to 0.071 between the Fitzroy and Albert Rivers. An unrooted NJ tree of D_A is shown in figure 4. Samples were roughly divided into northern (Barron and Fitzroy Rivers) and southern groups (Burnett and Port Hacking Rivers), except for the Albert River sample, which clustered with the northern samples. The Albert River sample occupied a basal position in the unrooted NJ tree, which may have been due to the larger numbers of alleles resulting from pooling 13 temporal samples. Regression of D_A on geographic distance between samples was not significant (Mantel test, $p > 0.05$).

DISCUSSION

Genetic variability

Understanding the genetic makeup of populations has become increasingly important because environmental degradation and overexploitation

Table 3. Variabilities of mean a (number of alleles), H_o (observed heterozygosity), and H_e (expected heterozygosity) of 18 temporal and spatial samples over 4 loci (AJMS-3, -4, -5, and -7) of *Anguilla reinhardtii* collected from 5 rivers over 3 yr

Sample	a	H_o	H_e	F_{IS}
Barron R. (Mar. 1998)	10.75 ± 3.30	0.725 ± 0.129	0.796 ± 0.085	0.085 n.s.
Fitzroy R. (Apr. 1999)	11.25 ± 2.87	0.819 ± 0.116	0.769 ± 0.128	-0.070 n.s.
Fitzroy R. (Aug. 1999)	11.25 ± 3.30	0.805 ± 0.118	0.806 ± 0.069	0.001 n.s.
Burnett R. (Apr. 1999)	11.75 ± 3.59	0.810 ± 0.143	0.790 ± 0.123	-0.030 n.s.
Albert R. (Nov. 1997)	10.25 ± 3.40	0.793 ± 0.072	0.791 ± 0.100	-0.008 n.s.
Albert R. (Oct. 1998)	8.75 ± 3.30	0.736 ± 0.201	0.806 ± 0.121	0.084 n.s.
Albert R. (Nov. 1998)	10.25 ± 3.69	0.780 ± 0.094	0.749 ± 0.147	-0.042 n.s.
Albert R. (Dec. 1998)	11.00 ± 1.83	0.765 ± 0.181	0.770 ± 0.116	0.001 n.s.
Albert R. (Jan. 1999)	9.50 ± 3.42	0.784 ± 0.108	0.746 ± 0.149	-0.051 n.s.
Albert R. (Feb. 1999)	11.75 ± 2.63	0.781 ± 0.114	0.784 ± 0.131	0.002 n.s.
Albert R. (Mar. 1999)	10.75 ± 2.87	0.813 ± 0.098	0.793 ± 0.120	-0.025 n.s.
Albert R. (Apr. 1999)	10.25 ± 3.10	0.790 ± 0.020	0.820 ± 0.115	0.016 n.s.
Albert R. (May 99)	12.00 ± 3.74	0.765 ± 0.138	0.789 ± 0.116	0.028 n.s.
Albert R. (June 1999)	11.50 ± 3.11	0.776 ± 0.101	0.766 ± 0.110	-0.017 n.s.
Albert R. (July 1999)	10.75 ± 3.95	0.771 ± 0.163	0.770 ± 0.137	-0.002 n.s.
Albert R. (Aug. 1999)	10.25 ± 2.87	0.766 ± 0.177	0.785 ± 0.129	0.023 n.s.
Albert R. (Sept. 1999)	10.50 ± 3.00	0.745 ± 0.122	0.743 ± 0.138	-0.006 n.s.
Port Hacking R. (Apr. 1999)	12.00 ± 2.71	0.850 ± 0.119	0.807 ± 0.125	-0.054 n.s.
Overall mean	10.81	0.782	0.779	-0.001 n.s.

n.s., not significant.

Table 4. Pairwise genetic differentiation index (F_{ST}) (above the diagonal) and Nei's D_A distance (below the diagonal) of *Anguilla reinhardtii* among 5 rivers

	Barron R.	Fitzroy R.	Albert R.	Burnett R.	Port Hacking R.
Barron R.		-0.001	0.004	0.000	0.001
Fitzroy R.	0.047		0.004	0.005	0.001
Albert R.	0.061	0.071		-0.003	-0.002
Burnett R.	0.033	0.049	0.050		-0.005
Port Hacking R.	0.059	0.049	0.049	0.041	

have placed many populations and species at risk. A key character in the identification of population units is the degree of geographically based genetic variation in a species. Freshwater eels are catadromous, but their life history is much less understood than the life history migrations of anadromous salmon, the homing behavior of which is more-easily observed in freshwater streams. Although the locations of spawning areas of many freshwater eel species remain undiscovered, these species are considered to exhibit homing behavior at the silver eel stage using olfactory cues and the earth's magnetic field (Tesch et al. 1992). The population genetic structure of these eels, therefore, is expected to be similar to other marine species that are not constrained by physical barriers to gene flow (DeWoody and Avise 2000).

Genotype data revealed that the 6 microsatellite loci of *A. reinhardtii* were highly polymorphic (with 11-37 alleles per locus). Mean heterozygosities among 4 loci ranged from 0.743 to 0.820 and averaged 0.779. This level of microsatellite variation is similar to that in the coastal sea bream (*Pagrus major*) of 0.810 (0.690-0.869) (Perez-Enriquez et al. 1999) and the highly migratory bluefin tuna (*Thunnus thynnus*) of 0.803 (0.701-0.865) (Takagi et al. 1999). The overall mean of 12 other marine species was 0.77 (DeWoody and Avise 2000). The values were also within the range of other freshwater eels: European eel *A. anguilla* of 0.835-0.895 (Wirth and Bernatchez 2001) and Japanese eel *A. japonica* of 0.670 (0.54-0.89) (Tseng et al. 2001).

Temporal genetic variations

For marine organisms, high fecundity, strong bias in reproductive success among families, and large variations in year class strength may reduce the effective population size by several orders of magnitude below the census size (Hedgecock 1994). This source of variation can lead to significant differences among samples, especially when samples are small or are from different generations (Waples 1989a b). Therefore, long-term studies are needed to understand population genetic structures. The year-round spawning of tropical eels may provide opportunities to understand the origins of temporal genetic variation in semelparous eels. The low F_{IS} values in samples of *A. reinhardtii* indicate the general absence of a heterozygote deficiency within these populations that might be due to inbreeding (Table 3). Spawning groups in a species with year-round spawning may come from different generations and different areas due to the stable temperature environment in tropical areas. Therefore, year-round spawning increases the possibility of mixing among individuals from different stream populations in spawning areas. Accordingly, *A. reinhardtii* in East Australia appears to have a panmictic population despite the year-long spawning. The temporal population variance in European eels (Dannewitz et al. 2005, Pujolar et al. 2006) suggested that the different spawning groups in the spawning areas are not well mixed within a shorter spawning period.

Spatial genetic variations

The results of recent molecular genetic studies of freshwater eels indicate that some species of temperate eels show low levels of population genetic structure (Wirth and Bernatchez 2001, Daemen et al. 2001, Tseng et al. 2001 2003 2006). The presence of this population differentiation in these species implies that individuals return to parental stream habitats with some degree of fidelity. However, the mechanism for finding

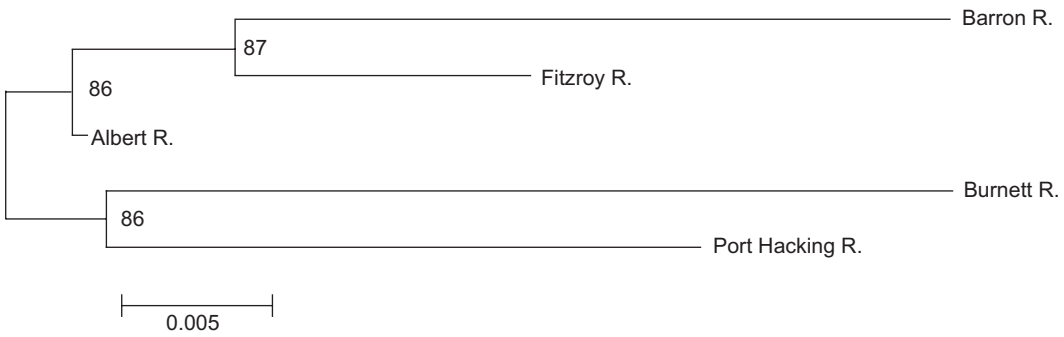

Fig. 4. Unrooted Neighbor-joining tree constructed from Nei's D_A distance of *Anguilla reinhardtii* among 5 rivers.

parental streams is uncertain. One possibility is that the prevailing movements of major ocean currents provide cues for directional swimming (McCleave et al. 1998). A complement to this hypothesis invokes different durations of the pelagic larval stage. The length of the pelagic larval stage may be heritable, so that parents with histories of long pelagic larval periods produce offspring with long pelagic larval stages. Larval duration might then influence the distance larvae passively drift in currents. Individuals with rapid growth rates and early metamorphosis have shorter dispersal distances than larvae that metamorphose later. Another mechanism may be that individuals from different areas go to different spawning grounds or spawn at different times. Larvae from these various groups may be entrained by different currents or ocean circulation systems.

Although the population structure analysis indicated no differentiation among populations of *A. reinhardtii*, the genetic distance between 2 extreme locations (Barron and Port Hacking Rivers) indicated at least a small amount of divergence. The lack of genetic subdivision among populations may be due to complex local ocean current systems. Genetic similarity between the 2 northern locations may be due to ocean currents, which split after meeting the East Australian coast. When the South Equatorial Current (SEC) meets the northeastern coast of Australia, it divides into 2 sub-currents. The northern sub-current, the North Queensland Current, moves north until it reaches the southern coast of Papua New Guinea. The other sub-current, the East Australian Current (EAC), moves south (Fig. 1).

Morphological and life-history data indicate at least some population subdivision. Shiao et al. (2002) found that 2 northern samples (Barron and Fitzroy Rivers) of *A. reinhardtii* had total lengths at recruitment that were similar to each other, but which were shorter than those of samples from southern locations (Albert and Port Hacking Rivers). The pelagic larval duration at recruitment suggests that glass eels arrive at the Albert River first, because these eels have the shortest pelagic larval duration (Shiao et al. 2002). These results are consistent with the genetic findings of the present study. The northern 2 locations have similar genetic compositions, whereas the sample from the Albert River occupied an intermediate position between the northern and southern samples. The information on pelagic larval duration also suggests that glass eels at the 2 northern locations may arrive by way of the North Queensland Current. Ocean gyres in southeastern Australia likely influence the distribution of glass eels and produce a mosaic distribution among some populations.

In addition, the reason for the absence of spatial and temporal genetic structure variations may be due to the narrow dispersal range of the eel along the East Australian coast from approximately 20°S to 34°S, where there is no physical barrier or different current systems to produce population differentiation. For temperate eels, adults coming from the same latitude have a greater chance of meeting due to similar environmental cues that guide migrations to the spawning ground (Tseng et al. 2006). Unlike temperate eels, tropical eels may be more-easily influenced by small geographic differences or changes in temperature in otherwise stable tropical areas.

CONCLUSIONS

The tropical eel, *A. reinhardtii*, appears to consist of a single panmictic population, because no evidence of temporal or spatial genetic differentiation was found. The year-round spawning behavior does not appear to influence the population genetic structure. The unique year-round spawning behavior of this tropical eel in a tropical area may facilitate mating between adult eels from different year classes (cohorts) or from different freshwater areas. The short marine larval stage of *A. reinhardtii* limits its geographic distribution and decreases latitudinal environmental effects on the population structure. Furthermore, gyres along East Australia also increase the opportunity for population mixing. The results of the study indicate that *A. reinhardtii* can be treated and managed as a single population.

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