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Is the geographic variation in size of American eel *Anguilla rostrata* elvers due to genetic differentiation?

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Abstract

Elvers of the American eel *Anguilla rostrata* collected along the east coasts of North America and Haiti exhibited geographic variations in age and size at time of arrival at estuaries and in duration of glass eels as well as their growth rate, based on a previous otolith study. They were able to divide into two groups: the northern large size group and the southern small size group. Thus, this study aims to understand whether the geographic variation in size of elvers is due to genetic differentiation by using microsatellite DNA. A total of 216 elvers of *A.rostrata*, collected from 6 estuaries along the Atlantic coasts of Central and North America,were used for the microsatellite DNA (6 loci) analysis. The genetic analyses indicated that there were no geographical isolation in genetic structures between the northern and southern groups (F_{CT} = -0.00101; P = 0.507), although there was a weak significant difference among sampling locations (F_{ST} = 0.00538; $P < 0.05$). The differences were patchy and did not correspond to the geographic difference in size of elvers. Integrating the preious otolith daily growth increment (ring) analyses and genetic data suggested that the geographic variation in size of the elver at estuarine arrival between these two groups was not due to genetic differentiation but to the distance of the estuaries from the spawning ground and latitudinal difference in coastal water temperatures.

Keywords: Population genetic structure, Life history traits, North America

Introduction

Anguillid eels (*Anguilla* spp.) are diadromous fishes, spawning in ocean and growing in freshwater streams. There are 18 known species and subspecies of the eels in the world (Ege 1939), of them 16 species are found in the Indo-Pacific region and two in the Atlantic region. All of them share a common life history (Tesch 1977), but they differ in the strategies, resulting in genetic divergence among the populations (Chang et al. 2007; Minegishi et al. 2008; Sang et al. 1994; Shen and Tzeng 2007; Shiao et al. 2001; Tseng et al. 2006; Vollestad 1992; Wirth and Bernatchez 2003).

The life history strategy of eels has been diversified during evolution, somehow linked to their population structures that could be a result of adaptation to the wide array of environments they inhabit. *A. marmorata*, the

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most widespread species among the anguillid eels, has differentiated into five populations across the Indo-Pacific Ocean to fit each independent oceanic current system (Ishikawa et al. 2004). The study done by Shen and Tzeng (2007) using microsatellite markers showed that Australian short-finned eels, *A. australis*, has two sub-populations in Oceania, including East Australia and New Zealand. This relates to the differences in life history strategy of two sub-populations due to environmental influences (Shiao et al. 2001).

The American eel, *A. rostrata,* spawns in the Sargasso Sea, south east of Bermuda, and grows in freshwater streams along the east coasts of Central and North America (Schmidt 1925; Kleckner et al. 1983; Kleckner and McCleave 1988). Similar to the other anguillid eel species, geographical variation in life history traits has been reported for American eels (Vladykov 1966; Smith 1968; Haro and Krueger 1988). A previous study carried out based on the otolith daily growth increment analysis by Wang and Tzeng (1998) concluded that elvers geographically vary in age and size at the time of their arrival at estuarine waters along the Atlantic coasts of Central and North America. They are dividable into two groups: the northern group and the southern group. The former group is larger and older while the latter group is smaller and younger, upon arrival at the estuarine area. Thus, it is obvious that the American eel exhibits different life history strategies to survive and successfully disperse to its wide distribution range. However, it is still not clear whether such differentiation is due to genetics or environments. Morphological characters of organisms are combined expression of both genetic characters and environmental adaptation. These may show phenotypic plasticity in different levels of the variations in life history traits among individuals, populations, and species (Robinson and Wilson 1996). The open ocean comprises of various current systems and different temperature and salinity regimes. Eventually, these might isolate marine organisms into different geographical populations with different levels of differentiation in life history traits (Stearns 1983; Stearns and Koella 1986; Reznick 1990; Schlichting and Pigliucci 1995).

The main goal of this study was to use microsatellite DNA of previously collected samples to determine whether there is any population structure in glass eels that differ in both age and size in the northern and southern regions where this species recruits. Specifically, this study aimed to (1) explain the morphological/developmental discrepancies within American eel elvers with previous otolith data set and new microsatellite DNA data, and (2) elucidate the genetic structure of American eel elvers and to hypothesize scenarios that might explain it.

Materials and methods

A total of 290 elvers of *A. rostrata* were collected from 6 estuaries along the Atlantic coasts of Central and North America from Haiti through the USA to Canada (Fig. 1). They were collected with dip nets during their upstream migration at time of the nighttime flood tide (Wang and Tzeng, 1998). After collection, they were preserved in 95 % alcohol solution. The elvers were collected in 1995, except 50 from Florida in 1997. A number of 125 elvers were used for the otolith microstructure analysis and 216 for microsatellite DNA analysis (Table 1). The elvers from Florida in 1995 were excluded for the microsatellite DNA analysis because of their small sample size.

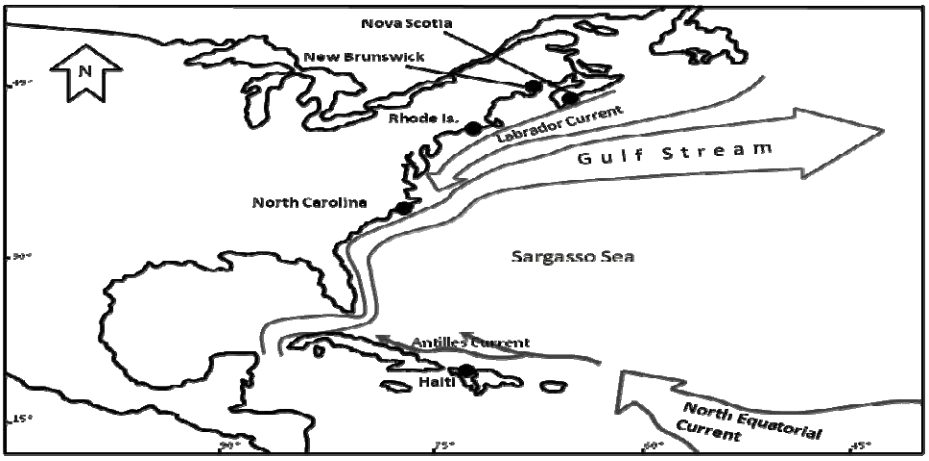

Fig. 1. Collections locations of *A. rostrata* elvers along the east coasts of Central land of North America, the spawning ground of the eel in Sargasso Sea, North Equatorial Current (NEC), and the Gulf Stream transporting eel larvae.

Total lengths (TL) of the preserved elvers were measured to 0.1 mm at the laboratory. The TL of these specimens was not adjusted for shrinkage and therefore was underestimates of the actual lengths of the elvers. The original data of the life history traits such as age (T_t) and total length (TL) of elver at time of arrival at estuaries, age at time of metamorphosis from leptocephalus to glass eel (T_m), duration of glass eel (T_{t-m}) in the coastal waters, and somatic growth rate (GR) were compiled from Wang and Tzeng (1998) (Table 1).The approximate birthdate (T_b) was back calculated from the count of daily growth increment in otolith obtained from the previous study and the date of its sampling.

Genomic DNA was extracted from a small piece of muscle tissue of each of the elvers preserved, using a DNA purification and extraction kit (Bioman Scientific Ltd., Taiwan). They were stored at -20 °C. Six microsatellite loci, one each screened, respectively, from *A. rostrata* (Aro095, Wirth and Bernetchez 2001), *A. Anguilla* (Aan002, Daemen et al. 1997), and *A. japonica* (AjMS-5, AjMS-7, AjTR-12, and AjTR-37; Tseng et al. 2001; Ishikawa et al. 2001), were used for Polymerase Chain Reaction (PCR) amplification (Table 2). PCR amplification was conducted in a 25 µl volume with the following contents: 0.1 ng DNA, 1.25 pmole reverse primer, 1.25 pmole forward primer, 5 mMdNTP, 0.05–0.1 mMMgCl^2, and 0.5 U Taq polymerase (Bioman, Taipei, Taiwan). Reverse primers contained FAM, TAMRA or HEX fluorescence labels for genotyping. PCR amplification procedures were as follows: initial denaturation at 94 °C for 4 min, followed by 36 cycles, each with denaturation at 94 °C for 30 sec, annealing at 55-60 °C for 30 sec, extension at 70 °C for 30 sec, and then final extension at 72 °C for 10 min. The 0.5 µl of PCR product was diluted with 12 µl of ddH_2O. PCR products were used for genotyping with the capillary MegaBACE-500 DNA analysis system (Amersham Biosciences). Scoring of data was made with Genetic Profiling Software 1.5 (Amersham Biosciences) and the sizes of each allele were manually checked.

The homogeneity tests were conducted by Scheffe's multiple comparison method (Zar 1984) for the life history traits, TL, T_t, T_m, Tt-m, GR, and T_B among the sampling locations. Genetic variability and population genetics parameters were calculated for the number of alleles per locus, total and mean number of alleles, observed (H_O) and expected (H_E) heterozygosities of each locus, and multilocus estimates for each of the five populations. The presences of null alleles or scoring errors were tested with MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). The deviation from Hardy-Weinberg equilibrium (HWE) was calculated with Genepop v 3.1 (Raymond and Rousset 1995). The significant level was adjusted by sequential Bonferroni correction (Rice 1989). Genetic differentiation was estimated by calculating Wright's fixation index (F_{ST}) based on infinite allele model (Weir and Cockerham 1984).

The analysis of molecular variance (AMOVA) was done with ARLEQUIN version 3.1 software (Excoffier et al. 2005) to test if the genetic structure of the elvers was different between the northern group (New Brunswick, Nova Scotia and Rhode Island) and the southern group (Haiti and North Carolina), using the size categories, and among locations within groups (Table 1). The fixation indices between groups (F_{CT}), among locations within groups (F_{SC}) and among all samples (F_{ST}) were calculated. Isolation by distance was also analyzed. The D_A distances between locations were then plotted against the corresponding geographic distances for the 5 locations and Mantel's test was used to assess the significance of their correlation.

Table 1. Life history traits of *Anguilla rostrata* elvers compiled from Wang and Tzeng (1998) (N, sample size; TL, total length of elvers at time of arrival at estuaries; n, number of individuals used for otolith analysis; T_t, age at time of arrival at estuaries; T_m, age at metamorphosis from leptocephalus to glass eel; T_{t-m}, duration of glass elvers; GR, average somatic growth rate before arrival to estuaries; T_B, Birth date; the same characters of upper subscripts indicating homogeneity among thesampling locations tested with Scheffe's multiple range comparison.

Location	Sampling Date	*N*	TL (mm)	*n*	T_t (day)	T_m (day)	T_{t-m} (day)	*GR* (mm/day)	T_B (Day/Mo./ Year)
Haiti	17-Dec-95	54	47.79^{a}	21	241.6^{b}	209.3^{ab}	32.3^{a}	1.45^{c}	21-04-95
Florida, USA	28-Feb-95	4	47.32^{a}	4	247.8^{bc}	214.0^{b}	33.8^{a}	1.40^{c}	24-06-94
North Carolina, USA	22-Mar-95	24	48.19^{a}	21	220.4^{a}	188.8^{a}	31.6^{a}	1.52^{b}	14-08-94
Rhode Island, USA	14-Apr-95	50	58.52^{b}	26	251.8^{bcd}	189.5^{a}	62.3^{b}	0.94^{ba}	6-08-94
New Brunswick, Canada	28-Apr-95	55	59.64^{b}	17	272.3^{cde}	192.7^{ab}	79.6^{c}	0.83^{ba}	30-07-94
East River, Nova Scotia, Canada	29-May-95	53	59.99^{b}	32	283.5^{e}	211.4^{b}	71.9^{c}	0.75^{a}	19-08-94
Total		290		125					

Results

The life history traits, T_t, TL, T_m, T_{t-m},GR, and T_b of the elvers are shown in Table 1. Scheffe's multiple comparison tests indicated that TL and T_{t-m} of the 6 sampling locations could be divided into northern (New Bruinswick, Nova Scotia and Rhode Island) and the southern (Haiti, Florida and North Carolina) groups (Table 1). TL in northern group was 58.52 to 59.99 mm, which was significantly larger than that in southern group (47.32 mm to 48.32 mm). T_{t-m} of the northern group was 62.3 - 79.6 days that was about twice longer than that of the southern group with 31.6- 32.3 days. However, the differences in T_t and T_m between these two groups were not consistent with those of TL and T_{t-m}, while the change of GR was almost similar toTL and T_{t-m}.

Table 2. Genetic variability of *A. rostrata* by loci and by locations (significance levels of Hardy-Weinberg Equilibrium (HWE) test: $*P < 0.05$, $**P < 0.001$, NS=not significant)

Locus	Haiti	North Carolina	Rhode Island	New Brunswick	Nova Scotia	Mean/Total alleles
Aro095 number of alleles	13	10	15	15	15	13.600/18
Observed heterozygosity (Ho)	0.646	0.625	0.875	0.708	0.854	0.742
Expected heterozygosity (He)	0.895	0.877	0.904	0.925	0.906	0.901
Allele range	105 - 141	109 – 133	105 – 135	105 - 135	105 – 137	
HWE test	NS	NS	NS	NS	NS	
Aan02 number of alleles	17	15	16	19	21	17.600/30
Observed heterozygosity (Ho)	0.313	0.292	0.208	0.417	0.417	0.329
Expected heterozygosity (He)	0.911	0.953	0.919	0.947	0.935	0.933
Allele range	169 - 215	137 – 217	173 – 223	173 - 211	172 – 251	
HWE test	NS	**	*	*	NS	
AjMS-5 number of alleles	16	10	13	11	13	12.600/19
Observed heterozygosity (Ho)	0.563	0.417	0.646	0.604	0.604	0.567
Expected heterozygosity (He)	0.859	0.897	0.890	0.845	0.868	0.872
Allele range	83 - 115	79 – 103	85 – 129	85 - 107	85 – 111	
HWE test	*	NS	NS	*	NS	
AjMS-7 number of alleles	8	8	6	10	9	8.200/10
Observed heterozygosity (Ho)	0.500	0.583	0.396	0.563	0.375	0.483
Expected heterozygosity (He)	0.598	0.795	0.497	0.742	0.703	0.667
Allele range	77 - 91	75 – 89	79 – 89	75 - 93	77 – 93	
HWE test	NS	NS	NS	NS	NS	
AjTR-12 number of alleles	13	10	13	9	14	11.800/20
Observed heterozygosity (Ho)	0.625	0.667	0.583	0.458	0.708	0.608
Expected heterozygosity (He)	0.809	0.813	0.767	0.643	0.725	0.751
Allele range	149 - 201	149 – 179	147 – 179	155 - 175	143 – 175	
HWE test	NS	NS	NS	NS	NS	
AjTR-37 number of alleles	15	12	15	15	16	14.600/20
Observed heterozygosity (Ho)	0.833	0.958	0.833	0.729	0.917	0.854
Expected heterozygosity (He)	0.899	0.897	0.909	0.899	0.887	0.898
Allele range	181 - 211	187 – 211	185 – 225	181 - 211	179 - 215	
HWE test	NS	NS	NS	NS	NS	

The number of alleles, allele range, H_O, H_E, and HWE test of the six microsatellite loci compared for the elvers from the five sampling locations are given in Table 2. All loci were polymorphic with the total number of alleles ranged from 10 for AjMS-7 to 30 for Aan02. Average alleles ranged from about 8 to 17. H_O and H_E per population ranged between 0.208 and 0.917 and between 0.497 and 0.953, respectively. Mean H_O and H_E also ranged between 0.329 and 0.854 and between 0.667 and 0.933, respectively.

Table 3. Pair-wise F_{ST} estimates with significance test (below diagonal) and Nei's unbiased D_A distances (above diagonal) between sampling locations of *A. rostrata* elvers (* $P > 0.05$)

	Haiti	North Carolina	Rhode Island	New Brunswick	Nova Scotia
Haiti	0	0.0523	0.1056	0.0531	0.0647
North Carolina	0.0022	0	0.0867	0.0877	0.0867
Rhode Island	0.0106*	0.0051	0	0.0338	0.1135
New Brunswick	-0.0013	0.0035	0.0119*	0	0.0908
Nova Scotia	0.0051	0.0071	0.0045	0.0122*	0

The overall genetic differentiation of the elvers was significant among the five sampling locations (F_{ST} = 0.00538; $P < 0.05$) and among locations within groups (F_{SC} = 0.0064; $P < 0.05$). However, the genetic differentiation was not significant between the northern and southern groups (F_{CT} = -0.00101; P = 0.507). Pairwise D_A distances ranged from 0.0338 between Rhode Island and Haiti to 0.1135 between New Brunswick and North Carolina (Table 3), while pairwise F_{ST}-values ranged from -0.0013 between Haiti and New Brunswick to 0.0122 between New Brunswick and Nova Scotia. Among the 10 pairwise F_{ST} values, only three were significant ($P < 0.05$), indicating that the differences in the life history trait, such as the case of TL between the northern and the southern groups was not due to the genetic differentiation. The regression of $D_A$distances on geographic distances showed that the isolation by distance was not significant (r = 0.086, P = 0.814). The results indicated that the *A. rostrata* elvers were a well-mixing population but with a weak genetic differentiation along the east coasts of Greater Antilles (Haiti) and North America.

Discussion

Based on the life history traits such as *TL*, $T_{t\text{-}m}$, and *GR*, *A. rostrata* elvers in the Atlantic coasts of Central and North America were divided into northern and southern groups. Microsatellite DNA analysis showed that the differences of these traits between these two groups were not due to genetic differentiation. However, there were weak significant genetic differentiations among the sampling locations within groups and within populations.

The distribution of the genetic structures of the elvers along the east coasts of North America and the Greater Antilles appeared to be patchy without geographical pattern. The elvers of Haiti were significantly different only from those of New Brunswick, while the elvers of Nova Scotia were significantly different only from those of New Brunswick. These pairwise differences were not consistent in accordance to the differences in life history traits between the northern and southern groups as indicated by the otolith analysis. This might imply that they had different origins or different spawning time, but the oceanic currents might have remodeled their population structures during the course of oceanic transportation prior to arriving at the estuaries.

TL was longer for the northern group than for the southern group. This might be due to an approximately two fold longer $T_{t\text{-}m}$ for the northern group that can be associated with the distance the leptocephalus will drift from its spawning ground to the corresponding coastal areas which it metamorphose and eventually recruits. Also, the upstream migration of elvers occurs at certain proper temperatures; otherwise, they remain in the estuaries to postpone their upstream migration (Hiyama 1952). Thus, there is a possibility that the cold Labrador Currentin, the north and the warm Gulf current in the south might affect the life history traits of the eel.

No evidence on isolation of genetic characters by geographical distances was found for *A. rostrata* elvers in this study. However, pairwise comparison of their genetic distances showed patchiness, suggesting the presence of significant but weak genetic differentiation among sampling locations, such as the cases between Haiti and Rhode Island, Rhode Island and New Brunswick, and New Brunswick and Nova Scotia. The reason for the patchiness of genetic structures was not clear. One of the reasons was probably due to temporal separation, since T_B of the elvers

were different among five locations. The genetic differentiation among the sampling locations due to sampling date discrepancies might facilitate detection of the genetic differentiation, implying that the elvers might come from different arrival waves or cohorts. This phenomenon was already reported by Dannewitz et al. (2005) for American eel elvers. Similar cases were also found for *A. japonica* (Chang et al. 2007) and *A. anguilla* (Maes et al. 2006).

The hydrographic features in the North Atlantic Ocean are diverse. The Gulf Stream has high velocity (1 - 1.5 m/sec) and sometimes produces meanderings with cold and warm rings or small gyres (Gaskell 1973; Duplessy 1999), mixing leptocephali during their dispersion.The weak genetic differentiation imply that elvers may come from different spawning groups but their mixing may cause difficulty to discriminate them. Oceanic and coastal currents may play an important role in shaping the population structure of *A. rostrata.*

In conclusion, the geographic variation in life history traits of *A. rostrata* elversis caused primarily from environmental differences, not due to genetic differences. Mixing of leptocephali during the course of their long-distant dispersion from the spawning ground to estuaries might result in difficulty for us to discriminate the environmental and genetic aspects of the effects. Further studies are needed using adaptive genetic markers and/or individual-based life history traits.

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