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Lack of genetic heterogeneity in the Japanese eel based on a spatiotemporal sampling

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Abstract— To determine the population structure of the Japanese eel *Anguilla japonica*, both geographic and temporal genetic variations in a total of 724 of newly recruited glass eels were investigated using six microsatellite loci. *F*-statistics found no significant genetic difference among nine localities covering the species range (F_{ST}=−0.0091–0.0207, R_{ST}=−0.0086–0.0207, P>0.05). No inter- or intra-annual changes in genetic variation were observed in pairwise comparisons of *F*-statistics (F_{ST}=−0.0054–0.0108, R_{ST}=−0.0143–0.0401, P>0.05) and AMOVA (F_{CT}=0.0009, P>0.05), respectively. The results of the present study showed that the Japanese eel consists of a genetically homogeneous population in time and space. This information will be helpful for planning efficient stock management and conservation of this species.

Key words: *Anguilla japonica*, population structure, spatial and temporal variation, stock management

Introduction

The stock of eels appears to have been decreasing globally during the last three decades (Dekker 2003). The European eel *Anguilla anguilla* has been recently described in the red list as a critically endangered species (IUCN 2008), and thus present conservation actions include requiring escapement of at least 40% of silver eels migrating back to their spawning area for reproduction, in addition to regulations of small eel catches and a reduction of fishing effort (Freyhof and Kottelat 2008). The Japanese eel, *A. japonica*, also has been declining, but no concrete efforts for organized management of this species have been initiated yet, although the Japanese eel is one of the most important aquatic resources in East Asian countries.

Appropriate and efficient stock management requires both ecological information about life history and reproduction and identification of management units. The migratory ecology of Japanese eels has been mostly revealed, whereas the population structure has remained unresolved in recent years. A number of studies conducted with allozymes, mitochondrial DNA sequences and microsatellites observed no significant genetic differences over the geographic distribution of the species (Taniguchi and Numachi 1978, Sang et al. 1994, Ishikawa et al. 2001a, Tseng et al. 2003, 2009). However, Tseng et al. (2006) revisited the issue using microsatellites and showed a north-south cline in allelic frequencies, as has been reported by allozyme analyses by Chan et al. (1997).

Slight but non-significant temporal variations were also found in 20-year classes of glass eels (Han et al. 2008). More recently though, Han et al. (2010) analyzed the spatial and temporal genetic structure and provided evidence that the Japanese eel in East Asia forms a panmictic population, and the discrepancies among the results of the previous studies were suggested to be caused by occasional randomly occurring genetic variations among samples. However, only one sampling site was included from Japan and the Korean Peninsula in their study and the temporal genetic variation was examined at a locality near the south limit of the species range. The population structure of Japanese eels therefore should be simultaneously examined in time and space, with a sampling that thoroughly covers the species range and highly sensitive molecular markers, as was found to be the case for population genetic studies on the European eel (Dannewitz et al. 2005).

The aim of this study was to examine the spatial and temporal genetic composition of Japanese eels using six polymorphic microsatellite loci. We evaluated the geographic genetic population structure using glass eels collected from nine localities widely distributed throughout the species range during a single recruitment season. Temporal genetic variation was then characterized using eight consecutive year classes at a single locality. Based on these analyses, we discussed the population structure of the Japanese eel and considered the implications for the conservation of this species.

Materials and Methods

Sampling

A total of 724 glass eels of *Anguilla japonica* were analyzed in the present study (Table 1, Fig. 1). Geographic genetic variations were examined using a total of 500 glass eels collected from the following nine localities throughout the species range: Miyagi, Ibaraki, Wakayama, Tanegashima (Japan), Dandong, Shanghai, Amoy (China), Nakdong-gang (Korea), Taiwan. All specimens belonged to a single year class, recruiting from November in 1998 to April in 1999 except for Nakdong-gang in April 2000.

Annual genetic variations were characterized with a total of 280 glass eels that recruited to Tanegashima Island in Japan, which is located at the center of the geographic distribution of the Japanese eel. 32 specimens were collected every December from 1997 to 2004 except for the sample in 1998 that were the same specimens as those used for the geographic genetic variations as stated above (56 specimens). For comparisons among different spawning seasons in a single year, the above spatial specimens except Nakdong-gang were regrouped into three groups according to the recruitment periods: the early spawning group (November and December; Ibaraki, Tanegashima, Taiwan), middle (January and February; Miyagi and Wakayama), late (March and April; Dandong, Shanghai, Amoy).

DNA extraction and genotyping

Genomic DNA extraction was performed using a 5% w/v Chelex extraction solution (BioRad) after proteinase *K*

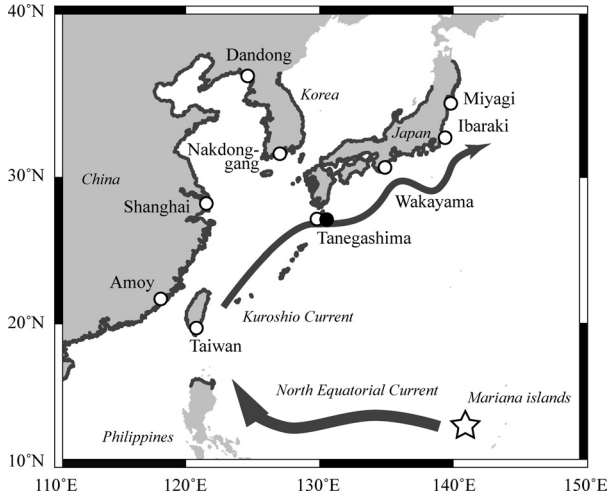

Fig. 1. The geographic distribution of the Japanese eel (bold lines) and sampling localities in this study. White and black circles show the sampling sites for the spatial and temporal analyses, respectively. The star west of the Mariana Islands indicates the spawning area of the Japanese eel (Tsukamoto 2006). See Table 1 for the detailed information about the samples.

Table 1. Summary of the specimens used in the present study.

Locality	Sampling date	N	*Na*	H_E	H_O
Spatial analysis					
Miyagi	22 Feb 1999	56	18.2	0.903	0.762
Ibaraki	1 Dec 1998	56	16.8	0.903	0.794
Wakayama	9 Jan 1999	56	17.8	0.904	0.804
Tanegashima*	18 Dec 1998	56	17.2	0.901	0.771
Dandong	23 Apr 1999	56	17.7	0.909	0.816
Shanghai	15 Apr 1999	52	17.5	0.909	0.814
Amoy	19 Apr 1999	56	16.3	0.906	0.791
Nakdong-gang	9 Apr 2000	56	17.7	0.906	0.816
Taiwan	19 Nov 1998	56	18.2	0.906	0.798
Annual analysis					
Tanegashima	29 Dec 1997	32	16.0	0.904	0.80
	18 Dec 1998	56	17.2	0.901	0.77
	9 Dec 1999	32	15.5	0.904	0.79
	25 Dec 2000	32	16.7	0.913	0.83
	14 Dec 2001	32	15.7	0.910	0.82
	4 Dec 2002	32	15.8	0.905	0.82
	15–25 Dec 2003	32	15.8	0.908	0.79
	15–25 Dec 2004	32	16.0	0.904	0.78
Seasonal analysis (the spatial samples were regrouped)					
Early	Nov–Dec 1998				
Middle	Jan–Feb 1999				
Late	Mar–Apr 1999				

Number of individuals (N), Number of alleles (*Na*), and expected and observed heterozygosities (H_E and H_O) shown in the table were averaged over six microsatellite loci in each cohort.

* the same samples were also used for the temporal analysis as the 1998 year-class.

digestion. All specimens were genotyped at six microsatellite loci that had been previously published (*AjTR-05*, *AjTR-12*, *AjTR-17*, *AjTR-37*, Ishikawa et al. 2001b; *AJMS-5*, Tseng et al. 2001; *Aro121*, Wirth and Bernatchez 2001) and were amplified using fluorescently labeled primers. PCRs were done in a Model 9700 thermal cycler (Applied Biosystems) or a Model TP600 thermal cycler Dice (Takara) with a total of 15 μl reaction volume containing 0.2 mM each of dNTP, 1.5 μl of 10×PCR buffer (Takara), 0.5 μM each of forward and reverse primers, 0.5 units of *Taq* DNA polymerase (Takara), and 1.0 μl of template DNA. Typical amplification parameters were 35 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 15 s and extension at 72°C for 30 s after heating at 94°C for 2 min. The labeled PCR products were electrophoresed on a Model 3130*xl* genetic analyzer (Applied Biosystems) and the fragment sizes were determined by Gene Scan HD500 [LIZ] size standards and GeneMapper software version 3.1 (Applied Biosystems).

Statistical analysis

The number of alleles, expected and observed heterozygosities and the inbreeding coefficient (F_{IS}) were calculated for each locus, each sample, and all samples using GENETIX v4.03 (Belkhir et al. 1999). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested for each locus, each sample, and all samples with 10,000 Markov chain methods using GENEPOP v.3.1 (Raymond and Rousset 1995). Null allele frequency was estimated with software MICRO-CHECKER v.2.2.0 (van Oosterhout et al. 2003) on each locus and each sample.

To examine genetic heterogeneity in the whole spatial and temporal samples, a locus-by-locus hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was conducted with Arlequin ver. 2.001 (Schneider et al. 2000). Pairwise comparisons of hierarchical *F*-statistics θ (Weir and Cockerham 1984, hereafter F_{ST}) and R_{ST} among samples were performed using Arlequin version 2.1 (Schneider et al. 2000) and RST-CALC (Goodman 1997). Statistical significance was evaluated by 10,000 permutation tests.

Results

Microsatellites variability

All six microsatellite loci were polymorphic with the number of alleles in each sampling location and year class ranging from 12 (*AjTR-17* and *AjTR-37*) to 23 (*AjTR-05* and *AjTR-12*), and from 9 (*AjTR-17*) to 23 (*AjTR-05*), respectively. Expected and observed heterozygosities of each sample ranged from 0.824 to 0.946 and from 0.457 to 0.968, respectively (Appendixes 1, 2). Null allele frequency was estimated to be from −5.2 to 21.6%. Significant heterozygote deficits were detected at 11 out of 96 tests (16 samples ×6 loci) (after Bonferroni correction) (Appendixes 1, 2). Since *AjTR-17* showed a significant heterozygote deficit in all of the nine spatial samples and a relatively high null allele frequency in all spatial and temporal samples (9.4–21.6%), further population genetic analysis was performed using five loci excluding *AjTR-17*.

Geographic genetic variations

No significant genetic heterogeneity was found in AMOVA ($P>0.05$). Overall *F*-statistics were very low and not significant ($F_{ST}=0.0003$, $R_{ST}=-0.0097$, $P>0.05$). Pairwise comparisons of F_{ST} and R_{ST} among sampling localities did not demonstrate a significant difference after Bonferroni correction ($F_{ST}=-0.0091$ – 0.0108, $R_{ST}=-0.0086$ – 0.0207, $P>0.05$) (Table 2).

Temporal genetic variations

AMOVA did not detect a significant genetic heterogeneity in the whole annual samples ($P>0.05$). Overall F_{ST} and R_{ST} was 0.0011 and 0.0019, respectively ($P>0.05$). Pairwise comparisons among eight year classes found no significant differences after Bonferroni correction ($F_{ST}=-0.0054$–0.090, $R_{ST}=-0.0143$–0.0401, $P>0.05$) (Table 3). No genetic

Table 2. Pairwise comparisons of *F*-statistics among geographic samples (above, F_{ST}; below, R_{ST}).

	Miyagi	Ibaraki	Wakayama	Tanegashima	Nakdong-gang	Dandong	Shanghai	Amoy	Taiwan
Miyagi		−0.0022	0.0080	−0.0031	−0.0033	0.0009	−0.0061	0.0100	−0.0012
Ibaraki	−0.0043		0.0001	−0.0056	−0.0029	0.0010	−0.0012	0.0021	−0.0087
Wakayama	−0.0064	−0.0062		0.0027	−0.0042	0.0096	0.0057	0.0069	−0.0091
Tanegashima	−0.0057	−0.0029	−0.0046		−0.0029	−0.0009	−0.0031	0.0010	−0.0053
Nakdong-gang	−0.0053	−0.0062	−0.0047	−0.0013		0.0034	−0.0047	0.0073	−0.0072
Dandong	−0.0018	−0.0026	−0.0046	0.0020	−0.0039		−0.0005	0.0016	0.0027
Shanghai	0.0104	0.0207	0.0131	0.0066	0.0122	0.0096		0.0078	0.0002
Amoy	−0.0062	0.0003	−0.0025	−0.0086	−0.0015	0.0006	−0.0011		0.0108*
Taiwan	−0.0079	−0.0008	−0.0045	−0.0057	−0.0033	−0.0025	0.0068	−0.0070	

* $P<0.05$; ** $P<0.05$ after Bonferroni correction

Table 3. Pairwise comparisons of *F*-statistics among annual samples (above, F_{ST}; below, R_{ST})

	1997	1998	1999	2000	2001	2002	2003	2004
1997		−0.0024	0.0032	0.0005	0.0012	0.0024	0.0002	0.0090*
1998	−0.0028		0.0008	−0.0054	−0.0014	−0.0014	−0.0029	0.0022
1999	−0.0091	−0.0118		0.0018	0.0056	0.0048	0.0000	0.0066
2000	−0.0127	−0.0045	−0.0125		−0.0001	0.0007	0.0014	0.0087*
2001	0.0034	0.0152	0.0019	0.0039		0.0010	−0.0011	0.0050
2002	−0.0016	−0.0023	−0.0143	−0.0085	0.0056		−0.0025	0.0031
2003	0.0157	−0.0009	−0.0055	0.0060	0.0171	0.0020		0.0030
2004	0.0239*	0.0005	−0.0012	0.0143	0.0401*	0.0080	0.0066	

* $P<0.05$; ** $P<0.05$ after Bonferroni correction

structure was observed in AMOVA among three different spawning seasons in a single year ($F_{CT}=0.0009$, $P>0.05$).

Discussion

In order to reveal the population structure of the Japanese eel, we designed sampling so that both the entire species range and several consecutive year classes are covered at the same time. Han et al. (2010) carried out spatial and temporal population genetic analyses, but it still did not fully include the geographic distribution of this species (see Introduction). Despite the fact that the specimens in the present study were not obtained very recently, this comprehensive sample collection has never been achieved in the Japanese eel. Furthermore, the quality of our data in this study was shown to be high (Table 1, Appendixes) although the number of microsatellite loci analyzed might not have been very large comparing to previous studies. These facts indicated that the genetic diversity of the Japanese eel was able to be systematically investigated at spatial and temporal scales based on the thorough sampling and high quality data.

This study found no evidence of population divergence and indicated that the Japanese eel likely forms a genetically homogeneous population in time and space. Chan et al. (1997) and Tseng et al. (2006) described two genetically different groups in the northern and southern regions of the species range of *A. japonica*. However, the north-south cline in allelic frequency found by Chan et al. (1997) was attributed to two allozyme loci, which are known as crucial enzymes for cold acclimation in Japanese eels (Chan et al. 1993). This geographic genetic variation in allozymes is therefore not equally comparable with the present study, potentially due to local adaptation to different environments within the species range. In Tseng et al. (2006), pairwise comparisons of microsatellites *F*-statistics among localities did not always correspond to the north-south difference that they hypothesized to exist. Furthermore, these two geographic groups in their UPGMA tree were supported by only a 34% bootstrap value (Tseng et al. 2006). Accordingly, the presence of the geographic genetic cline has not been fully demonstrated. Indeed the present study found no genetic difference among nine sampling localities throughout the species range of Japanese eels, which was also observed in the recent study on the population structure of this species (Han et al. 2010).

In contrast to the hypotheses that have been proposed about the existence of geographic population structure, no significant annual genetic difference has been reported. This suggests that the genetic composition of the reproductive population of Japanese eels is not temporally variable, at least throughout twenty years. In addition, the genetic homogeneity through a year and a spawning season could be questioned, since the spawning season of Japanese eels extends from late spring to early autumn, and spawning occurs only during the new moon period of each month throughout the whole spawning season (Tsukamoto 2006, 2011). Nonetheless, no genetic variation was observed at an intra-annual scale in the present study. Overall, the results of the present study suggest that Japanese eels compose a genetically homogeneous spawning population through a year, and that all glass eels recruiting to East Asian coasts are generated from a single reproductive population.

In order to maintain a single reproductive population over wide geographic distribution and years, there should be mechanisms that enable gene flow to be kept among localities and year classes. Japanese eels are born in a limited area in the western north Pacific Ocean and their larvae are all transported westward by the North Equatorial Current (Tsukamoto 2006), which actually consist of complicated currents and eddies. Kimura and Tsukamoto (2006) collected Japanese eel larvae ranging from 9.6 to 27.4 mm at different latitudes along 137°E, corresponding to 14 to 50 days old after hatching, which showed that individuals originating from different spawning cohorts were already mixed near the spawning area. Moreover Ishikawa et al. (2001a) found one shared mitochondrial DNA haplotype between Kanagawa (Japan) and Amoy (China), and another between Ibaraki (Japan) and Seto Inland Sea (Japan) in glass eels collected from a total of 14 sites. These showed that larvae of Japanese

eels originating from different spawning cohorts can be mixed well during the oceanic transportation period. Additionally, the oceanic environment is changeable, especially when El Niño occurs (Kim et al. 2008). Therefore, the place and timing of recruitment of Japanese eels can be randomly determined.

After recruitment to freshwater and estuarine habitats, the size composition, growth rate, sex ratio and age at downstream migration of eels have been found to be highly variable among river systems within the species range (Tzeng et al. 2000, Aoyama and Miller 2003). Considering random recruitment of glass eels, those high variations in biological characteristics of eels in each area are unlikely based on genetic characters, although genetic variation can be formed by adaptation to local environment. This implies that individuals at various ages that have experienced highly diverged environmental pressures migrate together back to produce a next generation at a single spawning area near the Mariana ridge. Even if some genetic variations can be generated in growth habitats, they can be homogenized again at the spawning area. Then newly born eggs and larvae are randomized once again during their transportation periods. Therefore, the migratory ecology of Japanese eels, namely, a limited single spawning area, dynamic transport during the larval period, random recruitment of glass eels, and a high variation at age of spawning adults, appears to enable the Japanese eel to maintain a single panmictic population.

The findings of the present study and the other recent study by Han et al. (2010) have included various intensive sampling designs and clearly support the conclusion that the Japanese eel is panmictic in time and space. This means that all the Japanese eels distributed across Japan, Korea, China and Taiwan belong to a single reproductive population, and that the remarkable decline in abundance of this species is a common issue to be shared among all East Asian countries. A decline in the genetic diversity of Japanese eels is also an additional concern as shown by Tseng et al. (2003) and Han et al. (2008). International cooperation and consensus for efficient management actions that consider the Japanese eel as a single panmictic population throughout its range are therefore urgently needed.

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Appendix 1. Microsatellites variability of the spatial samples.

	Miyagi	Ibaraki	Wakayama	Tanega-shima	Nakdong-gang	Dandong	Shanghai	Amoy	Taiwan	All
AjTR-05										
Ni	55	52	48	48	55	49	45	48	49	449
Na	20	20	20	18	21	20	18	23	21	31
H_E	0.933	0.937	0.931	0.931	0.936	0.926	0.927	0.940	0.939	0.933
H_O	0.836	0.827	0.771	0.896	0.873	0.857	0.822	0.854	0.776	0.835
F_{IS}	0.10	0.12	0.17*	0.04	0.07	0.08	0.11*	0.09	0.18**	0.10**
r	4.9	5.4	8.2	1.4	2.8	3.4	4.9	4.2	8.1	—
AjTR-12										
Ni	56	52	56	51	55	53	50	51	46	470
Na	18	19	23	21	23	22	21	21	21	30
H_E	0.933	0.927	0.937	0.924	0.943	0.938	0.933	0.932	0.935	0.936
H_O	0.821	0.750	0.857	0.882	0.909	0.830	0.820	0.882	0.783	0.838
F_{IS}	0.12	0.19	0.09*	0.05	0.04	0.12	0.12	0.05	0.16*	0.10*
r	5.7	9.2	3.8	1.8	1.3	6.2	5.7	2.2	7.5	—
AjTR-17										
Ni	52	52	51	46	51	50	48	43	51	444
Na	16	13	13	14	12	14	13	12	16	24
H_E	0.869	0.858	0.869	0.824	0.871	0.873	0.876	0.851	0.858	0.864
H_O	0.500	0.596	0.667	0.457	0.510	0.540	0.500	0.558	0.549	0.543
F_{IS}	0.43**	0.31**	0.23**	0.45**	0.42**	0.38**	0.43**	0.35**	0.36**	0.37**
r	20.6	14.5	11.4	21.6	20.3	18.5	19.6	16.8	17.7	—
AjTR-37										
Ni	54	55	55	53	54	54	48	51	50	474
Na	14	13	14	14	15	13	13	14	12	19
H_E	0.862	0.863	0.852	0.872	0.864	0.889	0.853	0.886	0.840	0.865
H_O	0.796	0.873	0.800	0.736	0.907	0.815	0.896	0.941	0.900	0.850
F_{IS}	0.08	−0.01	0.06	0.16	−0.05	0.08	−0.05	−0.06	−0.08	0.02
r	3.7	−1.2	2.7	7.4	−3.3	3.8	−2.9	−3.7	−5.2	—
AJMS-5										
Ni	56	56	55	48	52	53	47	51	52	470
Na	21	16	17	15	16	19	15	18	17	25
H_E	0.909	0.900	0.905	0.916	0.914	0.919	0.910	0.902	0.923	0.913
H_O	0.821	0.857	0.873	0.854	0.865	0.962	0.872	0.804	0.865	0.864
F_{IS}	0.10	0.05*	0.04	0.07	0.05	−0.05	0.04*	0.11	0.06	0.05
r	4.5	1.9	1.3	3.0	2.1	−2.9	1.5	5.1	2.6	—
Aro121										
Ni	54	51	56	51	53	51	49	49	47	461
Na	20	20	20	21	19	17	18	18	22	25
H_E	0.913	0.935	0.929	0.942	0.925	0.911	0.933	0.927	0.944	0.931
H_O	0.796	0.863	0.857	0.804	0.830	0.882	0.837	0.857	0.915	0.848
F_{IS}	0.13	0.08	0.08	0.15**	0.10	0.03*	0.10	0.08	0.03	0.09**
r	6.3	3.5	3.6	6.9	4.9	1.0	4.8	3.1	1.0	—

All, overall sampling localities; Ni, number of individuals; Na, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient (* $P<0.05$; ** $P<0.05$, after Bonferroni correction); *r*, null allele frequency (presented by percentage; underline, $P<0.01$)

Appendix 2. Microsatellites variability of the annual samples.

	1997	1998	1999	2000	2001	2002	2003	2004	All
AjTR-05									
Ni	32	49	31	31	32	32	31	32	270
Na	16	18	20	23	20	17	16	19	31
H_E	0.929	0.931	0.922	0.943	0.929	0.928	0.908	0.925	0.928
H_O	0.781	0.986	0.871	0.839	0.875	0.906	0.774	0.781	0.844
F_{IS}	0.16*	0.04	0.06	0.11	0.06	0.02	0.15	0.16	0.09
r	7.7	1.4	2.0	4.8	2.2	0.2	6.8	10.8	—
AjTR-12									
Ni	32	51	25	29	31	31	32	32	263
Na	18	21	19	17	19	20	19	19	27
H_E	0.927	0.924	0.940	0.921	0.943	0.932	0.940	0.912	0.933
H_O	0.938	0.882	0.840	0.966	0.871	0.935	0.938	0.781	0.894
F_{IS}	-0.01	0.05	0.11	−0.05	0.08	<0.01	<0.01	0.15	0.04
r	−1.1	1.8	4.6	−3.3	3.2	−1.2	−0.7	6.4	—
AjTR-17									
Ni	32	46	30	28	31	29	31	30	257
Na	14	14	9	15	12	11	14	13	21
H_E	0.867	0.824	0.857	0.905	0.897	0.877	0.910	0.869	0.880
H_O	0.594	0.457	0.500	0.714	0.548	0.517	0.581	0.567	0.553
F_{IS}	0.32*	0.45**	0.42*	0.21	0.39*	0.41*	0.37*	0.35*	0.37*
r	14.5	21.6	20.1	9.4	18.8	19.8	17.6	16.6	—
AjTR-37									
Ni	31	53	32	30	31	32	30	32	271
Na	12	14	12	12	13	12	13	14	18
H_E	0.854	0.871	0.839	0.850	0.856	0.863	0.867	0.892	0.860
H_O	0.742	0.736	0.813	0.700	0.871	0.813	0.900	0.875	0.881
F_{IS}	0.13	0.16	0.03	0.18*	−0.02	0.06	−0.04	0.02	0.69
r	4.6	7.4	0.3	8.7	−1.7	2.2	−1.1	−0.2	—
AJMS-5									
Ni	32	48	30	31	31	31	32	31	266
Na	16	15	17	14	15	17	17	12	23
H_E	0.906	0.916	0.932	0.912	0.909	0.916	0.910	0.887	0.916
H_O	0.875	0.854	0.967	0.871	0.871	0.968	0.844	0.903	0.891
F_{IS}	0.03	0.07	−0.04	0.05	0.04	−0.06	0.07	−0.02	0.27
r	1.3	3.0	2.1	1.6	1.4	−3.9	2.9	−2.0	
Aro121									
Ni	32	51	29	31	32	30	32	32	269
Na	20	21	16	19	15	18	16	19	25
H_E	0.944	0.942	0.935	0.946	0.928	0.916	0.916	0.940	0.935
H_O	0.875	0.804	0.759	0.936	0.906	0.833	0.719	0.781	0.825
F_{IS}	0.07	0.15*	0.19	0.01	0.02	0.09	0.22	0.17*	0.12**
r	2.9	6.9	8.7	−0.3	0.3	3.9	10.2	7.7	—

All, all samples over sampling years; Ni, number of individuals; Na, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient (* $P<0.05$; ** $P<0.05$, after Bonferroni correction); *r*, null allele frequency (presented by percentage; underline, $P<0.01$)