Genetic Variation and Differentiation in the Japanese Five-Lined Skink, Eumeces latiscutatus (Reptilia: Squamata)

Authors: Motokawa, Junko, and Hikida, Tsutomu

Source: Zoological Science, 20(1) : 97-106

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.97

ZOOLOGICAL SCIENCE **20**: 97–106 (2003)

Genetic Variation and Differentiation in the Japanese Five-Lined Skink, *Eumeces latiscutatus* (Reptilia: Squamata)

Junko Motokawa* and Tsutomu Hikida

Department of Zoology, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo, Kyoto 606-8502, Japan

ABSTRACT—The genetic variation in *Eumeces latiscutatus* from the main islands of Japan and the northern Ryukyus was investigated and compared with that of its close relatives (*E. okadae* and *E. marginatus*) using allozyme data. All three samples from the northern Tokara Island Group, currently identified as *E. latiscutatus*, were shown to belong to *E. marginatus* and not to *E. latiscutatus*. The non-monophyly of the northern Tokara samples and the great genetic differences within these samples may have resulted from colonization from more than one source population by northeastward overseas dispersal. The samples from the Izu Peninsula were genetically much closer to *E. okadae* than the other conspecific ones. This indicates that the samples from the Izu Peninsula and the other samples of *E. latiscutatus* should be treated as distinct species, and that *E. latiscutatus* from the Izu Peninsula and *E. okadae* from the Izu Island Group may be treated as conspecific. Samples from western Japan were genetically well differentiated from those of eastern Japan. Within the western group, the samples from the Osumi Island Group was genetically distinct from those from the other regions, by possessing unique alleles. Our phenograms also reveal a distant affinity between samples from the Danjo Island Group and the main islands of Japan. This may be the result of long geographic isolation of the Osumi and Danjo Island Groups from Kyushu. By contrast, samples from Sapporo and Aomori were poorly differentiated genetically in spite of the long separation of these two localities by the Tsugaru Strait. This suggests that overseas dispersal of *E. latiscutatus* occurred across this strait after its formation.

Key words: *Eumeces latiscutatus*, Tokara Island Group, Izu Peninsula, geographic variation, allozyme

INTRODUCTION

The Japanese five-lined skink, *Eumeces latiscutatus* (Hallowell, 1861), is a moderate-sized scincine lizard that occurs on the main islands of Japan (Hokkaido, Honshu, Shikoku, Kyushu), the adjacent islands, the northern Ryukyus, and the coastal region of Primorsky Kray, eastern Russia. Within East Asian *Eumeces*, this species is considered most closely related to *E. okadae*, which is distributed in the Izu Island Group (Hikida, 1993; Kato et al., 1994).

Hikida *et al.* (1992) reported that specimens of *E. latiscutatus* collected from the northern Tokara Island Group in the northern Ryukyus differed from those from the main islands of Japan in scale row number. They also noted the presence of remarkable differentiation in a few external characters among insular populations within this region. Based on allozyme data, Kato *et al*. (1994) demonstrated that the Kuchinoshima sample in the northern Tokara Island Group is genetically closer to *E. marginatus* than to *E. latiscutatus* from the main islands of Japan. However, no genetic data are available for the remaining samples from this area.

On the main islands of Japan, *E. latiscutatus* shows variation in scutellation, such as in the postnasal condition and scale row number, even within a population (Stejneger, 1907; Taylor, 1936), but there have been no attempts to study the geographic variation of the morphological characters in this species. Kato *et al*. (1994) found only slight genetic differentiation between two samples, Kyoto and Kagoshima, from western Japan. However, recent genetic studies revealed substantial east-west divergences in other non-volant terrestrial species that show little morphological variation (Saitoh *et al*., 1989; Matsui *et al*., 2000; Harada *et al*., 2001). Considering the wide distribution of *E. latiscutatus*, more comprehensive analyses of the genetic variation in this species are required.

This study analyzed genetic variation and differentiation in *E. latiscutatus* based on allozyme data for a number of samples from throughout its range, and discusses its evolution and taxonomic implications.

* Corresponding author: Tel. +81-75-753-4091;
FAX. +81-75-753-4114.
E-mail: jun@zoo.zool.kyoto-u.ac.jp

MATERIALS AND METHODS

A total of 303 skinks from 20 samples of *E. latiscutatus* (Sample 1–20), a sample of *E. okadae* (Sample 21) and two samples of E. marginatus (Sample 22 and 23) were examined (Fig. 1 and Appendix).

The liver, skeletal muscles and heart were removed from each specimen, and their extracts were subjected to a horizontal starch-gel electrophoretic assay. Sample preparation and electrophoretic procedures followed Kato *et al*. (1994). Voucher specimens were deposited in the collection of the Department of Zoology, Kyoto University (KUZ: see Appendix).

The enzymes examined and their Enzyme Commission (E.C.) numbers, presumptive loci, tissues used, and the buffer systems employed in the electrophoretic assay are given in Table 1. Enzyme nomenclature, E.C. numbers, and abbreviations of enzymes follow Murphy *et al*. (1996). Electromorphs were designated alphabetically in order of their anodal mobility.

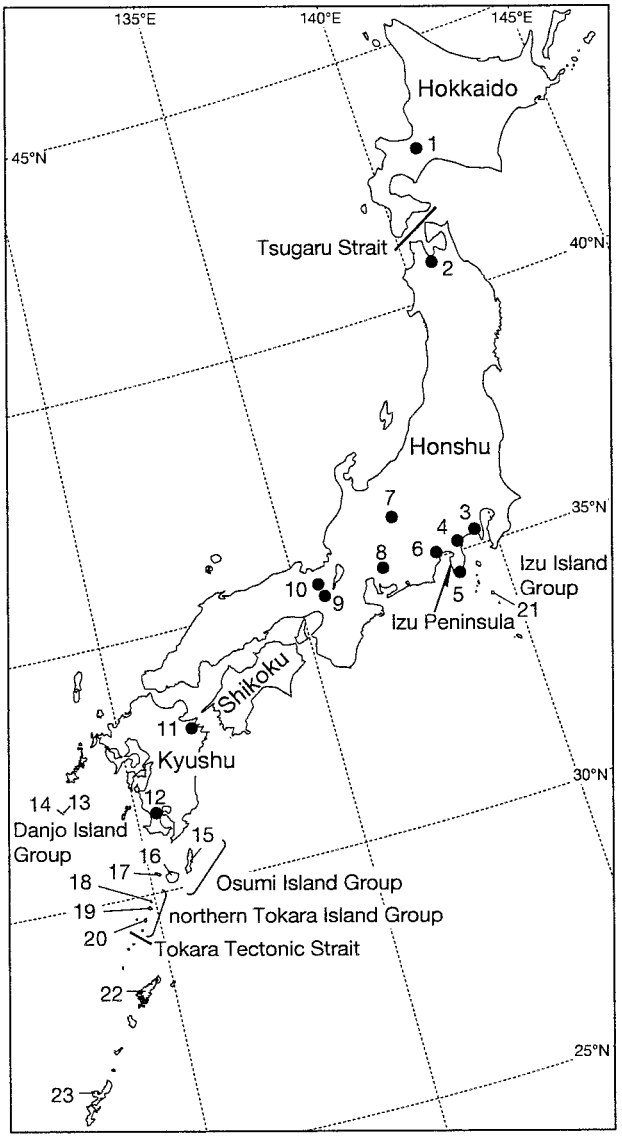

Fig. 1. A map of Japan and the northern Ryukyus, showing the sampling localities of *E. latiscutatus* (Sample 1–20), *E. okadae* (Sample 21), and *E. marginatus* (Sample 22 and 23).

Table 1. Enzymes, presumptive loci, tissues, and buffer systems used in the analysis of the variation among populations of *E. latiscutatus, E. okadae*, and *E. marginatus*.

Enzyme	E.C.number	Locus	Tissue*	Buffer system**
Aspartate aminotransferase	2.6.1.1	*Aat-1*	L	Poulik
Aspartate aminotransferase	2.6.1.1	*Aat-2*	L	AC6
Aconitate hydratase	4.2.1.3.	*Acoh-1*	L	TC8
Adenosine deaminase	3.5.4.4	*Ada*	L	AC6
Creatine kinase	2.7.3.2	*Ck-2*	M	TC8
Esterase	non-specific	*Est-4*	L	AC6
Fumarate hydratase	4.2.1.2	*Fumh*	L	Poulik
Glutamate dehydrogenase	1.4.1.2	*Gtdh*	L	TC8
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi*	L	Tris-HCl
Isocitrate dehydrogenase	1.1.1.42	*Idh-1*	L	AC6
Isocitrate dehydrogenase	1.1.1.42	*Idh-2*	L	AC6
L-Lacate dehydrogenase	1.1.1.27	*Ldh-1*	L	AC6
Malate dehydrogenase	1.1.1.37	*Mdh-1*	M	TC8
Malate dehydrogenase	1.1.1.37	*Mdh-2*	L	AC6
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi*	M	TBE8.7
Peptidase (leucyl-prolina)	3.4.-.-	*Pep-D*	L	TC8
Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*	L	AC6
Phosphoglucomutase	5.4.2.2	*Pgm-1*	M	TC8
Phosphoglucomutase	5.4.2.2	*Pgm-2*	L	Tris-HCl
Superoxide dismutase	1.15.1.1	*Sod-1*	L	TBE8.7

* Tissue: L=liver, M=mixture of heart and skeletal muscles.

** Buffer system: AC6=Aminopropylmorpholine-Citrate pH6.0 (Clayton and Tretiak, 1972), TC8=Tris-Citrate pH8.0 (Clayton and Tretiak, 1972), TBE8.7=Tris-Borate EDTA pH8.7 (Boyer *et al*., 1963), Poulik=Discontinuous Tris-Ctrate (Poulik, 1957), Tris-HCl=Tris-hydrochloric acid (Selander *et al*., 1971).

All loci were evaluated for genetic polymorphism (mean number of alleles per locus, percentage of polymorphic loci, and mean heterozygosity by direct count) and for genetic structuring using Wright's (1965) F-statistics (F_{st}). In order to estimate the overall genetic differentiation among samples, Nei's (1978) unbiased genetic distance and the modified Rogers' distance (Wright, 1978) were calculated using allele frequencies. Of these, Nei's distance is considered to be well correlated with the divergence time of two given samples, although it is non-metric (Nei, 1987). Estimations of the genetic relationships among samples were obtained using the UPGMA algorithm (Sneath and Sokal, 1973) based on Nei's distance, and the neighbor-joining (NJ) procedure (Saitou and Nei, 1987) based on the modified Rogers' distance. The UPGMA method assumes equal rates of molecular evolution along all branches (Nei, 1987), whereas the NJ analysis does not need such a presumption. The NJ phenogram was rooted at the mid-point of the longest pathway. NJ analysis was applied to the data set using the program included with PHYLIP (Felsenstein, 1993). The remaining computations were made with BIOSYS-1 (Swofford and Selander, 1981). In addition, we conducted a principal component analysis (PCA) using the PRINCOMP procedure (SAS Inst. Inc., 1990) to detect regional patterns of geographical variation. PCA was applied to a correlation matrix based on alleles occurring at frequencies greater than 0.05 in one or more samples. The number of alleles (k) analyzed at a locus was k - 1; therefore, we arbitrarily dropped the most common alleles, for a total of 31 alleles from 12 loci. To render the scale of variation more linear, we used an arcsine-square-root transformation for the allele frequencies (Sokal and Rohlf, 1981). A two-dimensional projection of the samples on the first two PC axes was made to illustrate relationships among the samples.

Table 2. Allele frequencies at 16 polymorphic loci, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and mean hetero zygosity by direct count (*H*) in E. *latiscutatus* (Sample 1-20), *E. okadae* (Sample 21), and *E. marginatus* (Sample 22 and 23). Sample numbers correspond to those in Fig. 1. Sample size is given in parentheses.

	E. latiscutatus																				other species		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Locus	(5)	(21)	(7)	(6)	(22)	(5)	(6)	(14)	(31)	(8)	(9)	(27)	(9)	(9)	(11)	(20)	(7)	(10)	(2)	(4)	(20)	(19)	(31)
Aat-1																							
a	.100										.167	.055							1.000				.050
b	.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.833	.630	1.000	1.000	1.000	1.000	1.000			1.000	1.000	1.000	.100
c												.315						1.000					.850
Acoh-1																							
a	1.000	.881	1.000		.071	1.000	.667	.071	.052			.037			.091			1.000	1.000			.053	1.000
b		.119		1.000	.929		.333	.929	.948	1.000	1.000	.963	1.000	1.000	.909	1.000	1.000			1.000	.975	.921	
c																					.025		
d																						.026	
Ada																							
a											.062												
b																				1.000		1.000	
c	1.000	1.000	1.000			.900	1.000	1.000	.914	1.000		.600			.409	.650	.714	1.000	1.000				.172
d				1.000	1.000																1.000		.828
e						.100			0.86		.938	.400	1.000	1.000	.591	.350	.286						
Ck-2																							
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000				1.000		
b																		1.000	1.000	1.000		1.000	1.000
Est-4																							
a						.200			.034		.250	.318			1.000	.868	1.000						
b																					.528		
c	1.000	.925	.250			.400			.966	1.000	.750	.682	1.000	1.000		.132							
d		.075	.750	1.000	1.000	.400	1.000	1.000													.472		
e																		.700	1.000	1.000		1.000	
f																		.300					.100
g																							.900
Gpi																							
a																		.200		.125			
b																		.800	1.000	.875		1.000	1.000
c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.929				1.000		
d																	.071						
Idh-1																							
a									.033														
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.967	1.000	1.000	1.000	1.000	1.000	.684	.800	.643	1.000	1.000	1.000	1.000	1.000	1.000
c															.136	.200	.357						
Idh-2																							
a																				.500			
b	1.000	1.000	1.000			1.000	1.000	1.000										1.000	1.000	.500		1.000	1.000
c				1.000	1.000																1.000		
d									.967	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000						
e									.033														
Ldh-1																							
a	1.000	.952	.571	.857	1.000	.400	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b		.048	.429	.143		.600																	
Mdh-1																							
a			.643				.800	1.000	1.000	1.000	1.000	.981	1.000	1.000	1.000	1.000	1.000						
b	1.000	1.000	.357	1.000	1.000	1.000						.019						1.000	1.000	1.000	1.000	1.000	1.000
c							.200																
Mdh-2																							
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.963	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.750	1.000	1.000	1.000
b												.037								.250			
Mpi																							
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.964	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000
b								.036													.025		
Pep-D																							
a	1.000	1.000	1.000	1.000	1.000	1.000	.833	.542	.891	.750	.750	.219	1.000	1.000	.227	.150					1.000		.032
b							.167	.458	.109	.250	.125	.781			.773	.850	1.000	1.000	1.000	1.000		1.000	.968
c											.125												
Pgdh																							
a								.143					1.000	1.000	.300	.688	.357	.278		1.000	1.000	.059	
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.857	1.000	1.000	1.000	.947			.700	.312	.643	.722				.941	1.000
c												.053							1.000				
d																							
Pgm-2																							
a																							
b		.048							.019														.016
c	1.000	.952	1.000	1.000	1.000	1.000	1.000	1.000	.944	.813	1.000	1.000	1.000	1.000	.318	.600	.333	1.000		1.000	1.000	1.000	.887
d									.037	.188					.682	.400	.667		1.000				.097
Sod-1																							
a																.050							
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000
A	1.0	1.2	1.1	1.0	1.0	1.2	1.1	1.2	1.4	1.1	1.3	1.5	1.0	1.0	1.3	1.4	1.3	1.1	1.0	1.1	1.1	1.1	1.4
P	5.0	10.0	15.0	5.0	5.0	15.0	15.0	15.0	20.0	10.0	20.0	25.0	0.0	0.0	30.0	35.0	25.0	15.0	0.0	15.0	5.0	10.0	20.0
H	0.010	0.027	0.076	0.013	0.007	0.072	0.057	0.049	0.038	0.036	0.068	0.105	0.000	0.000	0.110	0.116	0.103	0.060	0.000	0.084	0.031	0.013	0.051

RESULTS

Allele frequencies

Of the 20 presumptive loci examined, four (*Aat-2, Fumh, Gtdh*, and *Pgm-1*) were fixed for the same allele in all samples, and the remaining 16 loci were polymorphic in at least one sample (Table 2). At four of the 16 variable loci, a single allele was predominant (frequencies of 50% or more) in all samples (*Idh-1, Mdh-2, Mpi*, and *Sod-1*); the predominant allele for the remaining loci differed among the 23 samples.

The samples from the northern Tokara Island Group (Sample 18–20), while showing complete allelic displacement from all other samples of *E. latiscutatus* (Sample 1–17) at three loci (*Ck-2, Est-4*, and *Gpi*), exhibited no such displacements at all with the *E. marginatus* samples (Sample 22 and 23). Among the samples from the northern Tokara Island Group, remarkable allele frequency differences were observed at five (*Aat-1, Acoh-1, Ada, Pgdh*, and *Pgm-2*) of the nine polymorphic loci. Based on the pattern of shared alleles at the *Idh-2* locus, all samples except for the northern Tokara samples of *E. latiscutatus* were clearly divided into three groups: an Izu Peninsula group consisting of two samples from the Izu Peninsula (Sample 4 and 5: Fig. 1) possessing *Idh-2*^{c}, an eastern group consisting of six samples from the eastern parts of the main islands (Sample 1–3, 6–8) possessing *Idh-2*^{b}, a western group consisting of nine samples from the western parts of the main islands, including the Danjo and Osumi Island Groups (Sample 9–17) possessing I*dh-2*^{d}. Samples of the Izu Peninsula group, while showing complete allelic displacement from those of the eastern and western groups at two loci (*Ada* and *Idh-2*), showed no such displacement from the sample of *E. okadae* (Sample 21). At *Est-4*, obvious allele frequency differences were observed between samples from the Osumi Island Group (Sample 15–17) and the other region (Sample 9–14) within the western group. Moreover, the former had a unique allele (*Idh-1*^{c}) and two alleles (*Pgdh*^{c} and *Pgm-2*^{d}) at noticeable frequencies at highly polymorphic loci, which were absent or rare in the latter.

Among the samples of *E. latiscutatus*, the mean number of alleles per locus (*A*) varied from 1.0 to 1.5, the percentage of polymorphic loci (*P*) from 0.0 to 35.0, and the mean heterozygosity (*H*) values from 0.000 to 0.116 (Table 2). The greatest heterozygosity was found for the Yakushima sample. The mean F_{st} calculated for all samples of *E. latiscutatus*, for 17 samples excluding the northern Tokara samples, and 15 samples from eastern and western Japan was 0.806, 0.740 and 0.700, respectively.

Genetic distances

Pairwise comparisons of Nei's (1978) and the modified Rogers' (Wright, 1978) genetic distance coefficients, calculated over all 20 loci, are presented in Table 3. Within *E.*

Table 3. Matrix of Nei's (1978) unbiased genetic distance (below diagonal) and the modified Rogers' distance (Wright, 1978: above diagonal) coefficients for all pairwise comparisons between samples of *E. latiscutatus* (Sample 1–20), *E. okadae* (Sample 21), and *E. marginatus* (Sample 22 and 23).

	E. latiscutatus																				other species		
Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. Sapporo	–	0.041	0.242	0.449	0.440	0.182	0.315	0.394	0.381	0.394	0.450	0.439	0.500	0.500	0.536	0.495	0.551	0.503	0.584	0.557	0.432	0.582	0.558
2. Aomori	0.001	–	0.227	0.427	0.419	0.165	0.298	0.371	0.366	0.379	0.439	0.428	0.481	0.481	0.522	0.480	0.536	0.505	0.587	0.544	0.413	0.569	0.559
3. Kamakura	0.056	0.050	–	0.422	0.420	0.165	0.155	0.271	0.370	0.385	0.434	0.421	0.411	0.411	0.493	0.454	0.510	0.527	0.609	0.575	0.435	0.599	0.580
4. Odawara	0.227	0.205	0.199	–	0.036	0.412	0.408	0.404	0.441	0.454	0.439	0.462	0.389	0.389	0.518	0.482	0.539	0.639	0.708	0.535	0.123	0.588	0.619
5. Shimoda	0.216	0.196	0.196	0.001	–	0.413	0.401	0.402	0.440	0.453	0.438	0.461	0.388	0.388	0.516	0.484	0.538	0.633	0.702	0.534	0.119	0.587	0.613
6. Yui	0.030	0.024	0.020	0.190	0.190	–	0.285	0.370	0.418	0.433	0.466	0.458	0.472	0.472	0.523	0.489	0.542	0.510	0.594	0.555	0.418	0.579	0.560
7. Azumi	0.105	0.094	0.018	0.186	0.178	0.084	–	0.158	0.344	0.355	0.405	0.379	0.354	0.354	0.451	0.404	0.461	0.538	0.618	0.556	0.423	0.582	0.590
8. Kasahara	0.172	0.152	0.075	0.182	0.180	0.152	0.023	–	0.323	0.324	0.380	0.331	0.334	0.334	0.409	0.350	0.406	0.558	0.634	0.526	0.419	0.551	0.607
9. Kyoto	0.160	0.148	0.150	0.222	0.220	0.199	0.129	0.114	–	0.052	0.210	0.195	0.302	0.302	0.356	0.281	0.367	0.624	0.691	0.564	0.426	0.614	0.666
10. Miyama	0.171	0.159	0.163	0.235	0.234	0.214	0.138	0.114	0.001	–	0.233	0.188	0.324	0.324	0.345	0.267	0.348	0.622	0.679	0.561	0.439	0.612	0.667
11. Usuki	0.230	0.219	0.214	0.217	0.216	0.254	0.183	0.160	0.042	0.052	–	0.197	0.211	0.211	0.322	0.278	0.369	0.650	0.711	0.555	0.423	0.606	0.663
12. Kagoshima	0.225	0.215	0.208	0.253	0.251	0.255	0.165	0.123	0.039	0.035	0.037	–	0.307	0.307	0.276	0.187	0.281	0.565	0.653	0.518	0.447	0.569	0.605
13. Oshima	0.289	0.267	0.186	0.164	0.163	0.257	0.135	0.120	0.097	0.112	0.042	0.101	–	0.000	0.371	0.341	0.418	0.676	0.742	0.579	0.405	0.628	0.689
14. Meshima	0.289	0.267	0.186	0.164	0.163	0.257	0.135	0.120	0.097	0.112	0.042	0.101	0.000	–	0.371	0.341	0.418	0.676	0.742	0.579	0.405	0.628	0.689
15. Tanegashima	0.359	0.340	0.301	0.330	0.327	0.349	0.243	0.195	0.143	0.132	0.112	0.085	0.152	0.152	–	0.128	0.103	0.606	0.638	0.570	0.504	0.564	0.614
16. Yakushima	0.299	0.281	0.249	0.285	0.283	0.299	0.191	0.139	0.086	0.076	0.082	0.037	0.128	0.128	0.014	–	0.113	0.593	0.641	0.533	0.471	0.561	0.620
17. Kuchierabujima	0.381	0.360	0.322	0.361	0.359	0.376	0.253	0.190	0.151	0.133	0.150	0.086	0.198	0.198	0.005	0.008	–	0.601	0.633	0.566	0.526	0.565	0.621
18. Kuchinoshima	0.301	0.307	0.344	0.548	0.532	0.319	0.362	0.395	0.524	0.518	0.588	0.423	0.635	0.635	0.505	0.481	0.490	–	0.383	0.443	0.628	0.391	0.274
19. Nakanoshima	0.418	0.430	0.477	0.701	0.683	0.449	0.495	0.529	0.666	0.632	0.732	0.593	0.799	0.799	0.556	0.568	0.541	0.161	–	0.516	0.697	0.491	0.459
20. Suwanosejima	0.384	0.366	0.426	0.348	0.346	0.390	0.390	0.341	0.403	0.396	0.388	0.340	0.420	0.420	0.430	0.367	0.419	0.229	0.317	–	0.522	0.247	0.494
21. Miyakejima	0.210	0.192	0.216	0.015	0.014	0.198	0.203	0.200	0.207	0.220	0.202	0.238	0.182	0.182	0.314	0.270	0.345	0.529	0.680	0.332	–	0.576	0.608
22. Amamioshima	0.418	0.401	0.461	0.430	0.427	0.425	0.428	0.374	0.488	0.482	0.474	0.417	0.506	0.506	0.407	0.405	0.407	0.170	0.277	0.062	0.414	–	0.414
23. Okinawajima	0.386	0.392	0.435	0.503	0.488	0.401	0.454	0.489	0.623	0.621	0.620	0.502	0.665	0.665	0.520	0.540	0.533	0.081	0.243	0.295	0.484	0.194	–

latiscutatus, high values of Nei's genetic distance were found, ranging from 0.001 to 0.799. This is because the northern Tokara samples were genetically highly differentiated from all other samples (D=0.301–0.799). Compared to these values, the values obtained between the northern Tokara samples and *E. marginatus* were relatively small (0.062–0.295). When the three samples from the northern Tokara Island Group were excluded, the D values among 17

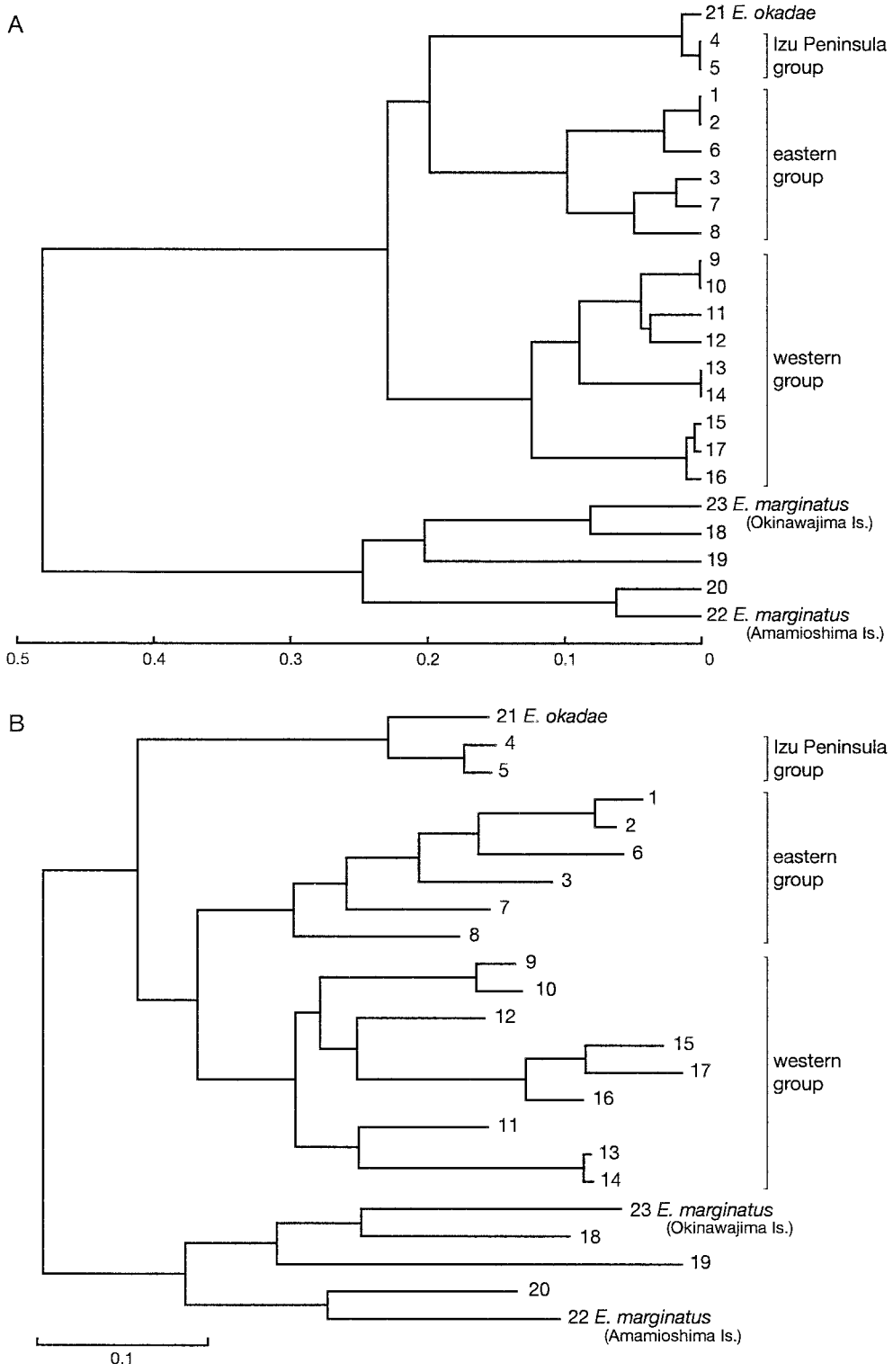

Fig. 2. An UPGMA phenogram A based on the unbiased genetic distances of Nei (1978), and a neighbor-joining phenogram B based on the modified Rogers' distance (Wright, 1978) rooted at the mid-point of the longest path.

samples of *E. latiscutatus* ranged from 0.001 to 0.381, with the highest D value between Sample 1 and 17. The D values of the three groups (Izu Peninsula, eastern, and western) varied considerably, showing 0.178–0.227 between the Izu Peninsula and the eastern groups, 0.163–0.361 between Izu Penisula and western groups, and 0.114–0.381 between eastern and western groups. Two samples from the Izu Peninsula were genetically very similar to each other (D=0.001) and to *E. okadae* (D=0.014–0.015), whereas relatively large D values were observed within the eastern and western groups (0.001–0.172 and 0.001–0.198, respectively). Furthermore, among three samples from the Osumi Island Group in the western group, the D values were very low (0.005–0.014), when compared with values between these samples and the samples in this group from other regions (0.037–0.198). Within the eastern group, an extremely small D value was obtained between the samples from Sapporo and Aomori (0.001).

Cluster analyses

Fig. 2A shows the UPGMA phenogram based on Nei's genetic distance. In this phenogram, the three samples from the northern Tokara Island Group (Sample 18–20) cluster with the *E. marginatus* samples, and are clearly separated from another cluster that includes all the remaining samples. In the latter cluster, three subclusters corresponding to the above-mentioned groups (*i.e.*, Izu Peninsula, eastern and western groups) are recognized. The samples from Izu Penisula and *E. okadae* consititute in a subcluster. Within the western group, three samples from the Osumi Island Group (Sample 15–17) and two samples from the Danjo Island Group (Sample 13 and14) form a compact subgroup.

The topology of the neighbor-joining phenogram (Fig. 2B) is similar to that of the UPGMA one in that two distinct clusters, one consisting of three subclusters, are recognized, although these two phenograms differ slightly in the position of the subcluster consisting of the Izu Peninsula group, and in the internal topology of the two subclusters corresponding to the eastern and western groups.

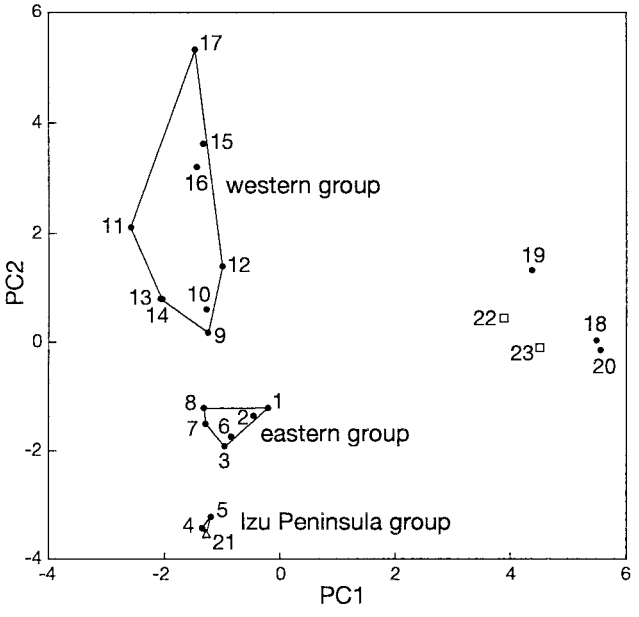

Fig. 3. Scatter plot of the first two principal components based on 31 allele frequencies of *E. latiscutatus* (closed circles), *E. okadae* (open triangle), and *E. marginatus* (open squares). See Fig. 1 for the sample numbers.

Principal component analysis

The relationships among samples were also visualized by projecting the sample positions onto the plane defined by the first two principal components of the PCA, which explain 38.7% of the total variation (Fig. 3 and Table 4). PC1

Table 4. Factor scores of the principal component analysis.

Allele	Factor 1	Factor 2
Aat–1(a)	0.105	0.078
Aat–1(b)	0.211	0.017
Acoh–1(a)	0.162	–0.113
Ada(a)	–0.081	0.093
Ada(b)	0.209	0.010
Ada(d)	–0.009	–0.256
Ada(e)	–0.175	0.218
Ck(b)	0.368	0.033
Est–4(a)	–0.109	0.342
Est–4(b)	–0.040	–0.155
Est–4(d)	–0.144	–0.284
Est–4(e)	0.314	0.041
Est–4(f)	0.223	–0.000
Est–4(g)	0.141	–0.004
Gpi(a)	0.247	–0.002
Gpi(b)	0.357	0.037
Gpi(d)	–0.046	0.233
Idh–1(c)	–0.079	0.327
Idh–2(a)	0.173	–0.006
Idh–2(c)	–0.072	–0.269
Idh–2(d)	–0.194	0.317
Ldh–1(b)	–0.054	–0.158
Mdh–1(b)	0.235	–0.248
Mdh–1(c)	–0.040	–0.064
Mdh–2(b)	0.173	–0.006
Pep–D(b)	0.267	0.268
Pep–D(c)	–0.081	0.093
Pgdh(a)	–0.041	–0.051
Pgdh(c)	0.182	0.203
Pgdh(d)	0.136	0.057
Pgm–2(d)	0.061	0.279
Eigenvalue		
% Variation explained	18.2	13.4

accounted for 22.5% and PC2 for 16.2%. The relationships in the PCA are remarkably similar to those obtained by clustering (Fig. 2). On the first component axis, the northern Tokara samples (Sample 18–20) are completely distinguishable from the remaining samples of *E. latiscutatus* (Sample 1–17) with a high positive score. The second component completely distinguishes the three groups (Izu Peninsula, eastern, and western) from each other. The western group has a positive PC2 score, whereas the Izu Peninsula and eastern groups have negative scores. The PC2 scores in the eastern group are intermediate between those in the western and Izu Peninsula groups. Moreover, within the western group, the samples from the Osumi Island Group (Sample 15–17) were separated from the remainder by fairly large PC2 scores.

DISCUSSION

Populations of the northern Tokara Island Group

The distributions of *Eumeces latiscutatus* and *E. marginatus* are separated by the Tokara Tectonic Strait, which is located between the northern and southern Tokara Island Group. Morphologically, this is supported by a few external characters, such as the presence or absence of the postnasals (Nagai, 1928; Toyama, 1989; Hikida *et al*., 1992). However, Kato *et al*. (1994) demonstrated that the sample of *E. latiscutatus* from Kuchinoshima within the northern Tokara Island Group is genetically poorly diverged from the samples of *E. marginatus*, despite their presumed long geographical isolation by the strait. Their result was interpreted as indicative of the northeastward overseas dispersal of *E. marginatus* across the strait after its formation (Kato *et al*., 1994). In our study, the UPGMA and NJ phenograms (Fig. 2) show two distinct genetic groups, samples of *E. latiscutatus* from the northern Tokara Island Group and *E. marginatus*, and samples of *E. latiscutatus* and *E. okadae*. These results strongly suggest that all the northern Tokara populations, formerly assigned to *E. latiscutatus*, actually belong to *E. marginatus*, and support the hypothesized northeastward overseas dispersal of *E. marginatus* (Kato *et al*., 1994; Hikida and Motokawa, 1999).

Very high levels of genetic differentiation were found among samples within the northern Tokara Island Group (D=0.161–0.317). This result coincides with the high morphological differentiation among insular populations of the northern Tokara Island Group (Hikida *et al*., 1992), indicating a large amount of genetic divergence among them. Moreover, the Kuchinoshima sample (Sample 18) is most closely related to *E. marginatus* from Okinawajima (D=0.081), whereas the Suwanosejima sample (Sample 20) is closest to *E. marginatus* from Amamioshima (D=0.062). Compared with these close genetic affinities, the genetic divergence of the Nakanoshima sample (Sample 19) from both samples of *E. marginatus* is relatively large (D=0.243 and 0.277). Kato *et al*. (1994) surmised the founder's effect on a small insular population for explanation of the close genetic affinty between the samples of Kuchinoshima and Okinawajima. However, this is not supported by our data, which indicate that the levels of genetic variability in the samples from Kuchinoshima and Suwanosejima (H=0.060 and 0.084) are higher than those in the samples of *E. marginatus* (H=0.013 and 0.051). To explain our results, it is necessary to postulate that the northern Tokara populations were colonized from more than one source population by rafting or artificial transportation. Further extensive electrophoretic surveys of *E. marginatus* from throughout its range are needed to verify this hypothesis.

Populations of the Izu Peninsula

The high F_{st} values found in all but the northern Tokara samples of *E. latiscutatus* (0.740) indicate that *E. latiscutatus* is highly diverged genetically. Within these samples, the results of our UPGMA and NJ analyses (Fig. 2) and the PC2 values (Fig. 3) reveal three distinct groups (Izu Peninsula, eastern, and western), with a fixed allelic difference at the *Idh-2* locus (Table 2). Of these, the samples of the Izu Peninsula group are genetically similar to *E. okadae*. The samples from the Izu Peninsula possessed alleles unique to *E. okadae*. Nei's D values between them were much lower (0.014–0.015) than any intraspecific values in this genus estimated previously (0.09–0.68: Murphy *et al*., 1983; Kato *et al*., 1994). These results clearly indicate that the samples in the Izu Peninsula belong to the *E. okadae* lineage and not to any lineage of the other groups in *E. latiscutatus*, and that *E. okadae*, now known only from the Izu Isalnd Group, occurs on the Izu Peninsula, at least in part.

Therefore, the samples of *E. latiscutatus* from the Izu Peninsula and from the other areas, currently treated as a single species, should be treated as two different species. The Izu Peninsula population was assigned to *E. latiscutatus* because Hallowell (1861) described this species from Shimoda on the Izu Peninsula (sample 5 in this study). The name *E. japonicus*, which was originally described from Nagasaki in Kyushu (close to sample 11 and 12) by Peters (1864), may be applied to the remaining populations now called *E. latiscutatus*. Further morphological examination of these two species (*E. latiscutatus* and *E. japonicus*) is strongly desired to produce a key for their identification.

By contrast, from the small genetic distance obtained between *E. latiscutatus* from the Izu Peninsula and *E. okadae*, it does not follow that the former population is conspecific with *E. okadae*, because these two species differ in the number of midbody scale rows and the line pattern on the head in juveniles (Taylor, 1936; Nakamura and Uéno, 1963). However, *E. okadae*, which is distributed on most islands throughout the Izu Island Group, is known to be geographically highly variable, even in the key characters mentioned above (Taylor, 1936; Hikida, 1993). Since Stejneger (1907) first described *E. okadae* as a subspecies of *E. latiscutatus* (type locality = Miyakejima), our results seem to imply a conspecific relationship between *E. latiscutatus* from the Izu Peninsula and *E. okadae*. In addition, our results

alone do not seem to justify the taxonomic treatment of Stejneger (1907), because we examined only one sample from the Izu Island Group. In conclusion, we treat them as a single species, and consider *E. okadae* a junior synonym of *E. latiscutatus*. In order to revise the subspecific division of *E. latiscutatus* recognized by Stejneger (1907), the genetic relationships among insular samples throughout the Izu Island Group and the Izu Peninsula samples of this species need to be clarified, with more detailed study of the morphological variation in this species.

Geographic variation in *Eumeces latiscutatus*

As shown above, the samples of *E. latiscutatus* from eastern and western Japan formed two distinct groups in our phenograms (Fig. 2). Phenetic clustering methods such as the UPGMA and NJ methods yield misleading results, such as a large geographic gap in samples exhibiting isolation-by-distance within a single genetically continuous unit, if the distribution of samples was uneven (de Queiroz and Good, 1997). Considering this phenomenon, the apparent existence of two distinct groups (eastern and western) in *E. latiscutatus* might result solely from our sampling, which is not absolutely regular geographically. However, the high F_{st} value (0.700) calculated for 15 samples of this species from eastern and western Japan indicates that the samples show a fairly large interpopulational differentiation, which is largely attributed to the intraspecific subdivision of the eastern and western groups. Moreover, the PC2 values completely discriminated the eastern and western groups (Fig. 3), and complete allelic displacement between them was observed (Table 2). These results suggest that little gene flow occurs between the eastern and western groups. Therefore, we believe that there is fairly large genetic divergence between *E. latiscutatus* from eastern and western Japan.

While there was poor genetic differentiation among the Osumi Island Group samples, they were genetically well diverged from the remaining samples within the western group (Figs. 2 and 3). This genetic divergence is also supported by obvious allele frequency differences and the possession of characteristic alleles. Ota *et al*. (1993) postulated that the Osumi Island Group has been connected to Kyushu by a narrow land bridge during the most recent glaciation, when the sea level dropped by no less than 120 m. However, the mean D value between samples from the Osumi Island Group and Kagoshima in Kyushu (0.069) seems to be too large to reflect this paleogeographical hypothesis. Based on the evidence of submarine topography, Ohshima (1990) estimated that the separation of the Osumi Island Group from Kyushu occurred during the Riss-Würm interglacial period in the late Pleistocene (120,000 years ago). He also estimated that the separation of the islands in the Osumi Island Group followed the most recent glaciation (*i.e*., the Würm: 15,000 years ago). If these estimates are correct, the divergence time between samples from the Osumi Island Group and Kyushu might be roughly eight times as long as that within the Osumi Island Group. The mean D value between samples from the Osumi Island Group and Kagoshima (0.069) is eight times as large as that among the Osumi Island Group samples (0.009). Assuming a gross correlation between the genetic distance value and the time of population divergence (Nei, 1987), it is likely that the genetic relationship among samples in the western group reflects the paleogeography in this area proposed by Ohshima (1990).

The populations of several species, including *Amphiesma vibakari* from the Danjo Island Group, are reported to be very divergent morphologically from conspecific populations from the main islands of Japan (Toriba, 1986; Shibata *et al*., 1988; Nakanishi and Ejima, 1989). Therefore, we expected the samples from the Danjo Island Group to be genetically distinct from those from the main islands within the western group. However, the PCA did not detect marked differences between samples from the Danjo Island Group and the main islands (Fig. 3). This is attributed to the absence of alleles unique to the samples from the Danjo Island Group. Conversely, our phenograms reveal the relatively distant affinity between samples from the Danjo Island Group and the main islands, supporting their relatively long isolation (Fig. 2). The mean D value observed between samples from the Danjo Island Group and Usuki, Kyushu (0.042) is nearly as large as that between samples from the Osumi Island Group. This suggests that, like the Osumi Island Group, the Danjo Island Group was isolated from Kyushu during the Riss-Würm interglacial period.

Hokkaido and Honshu are assumed to have been separated by the formation of the Tsugaru Strait during the Riss-Würm interglacial period (Ohshima, 1990). However, the sample from Sapporo in Hokkaido proved to be almost identical genetically to that from Aomori in northernmost Honshu (D=0.001), which is incompatible with the paleogeographical scenario (Ohshima, 1990). Therefore, it is plausible that there has been of *E. latiscutatus* across the Tsugaru Strait overseas dispersal by rafting. This explanation requires substantial corroboration based on more detailed surveys.

ACKNOWLEDGMENTS

We wish to express our sincere thanks to H. Ota and M. Motokawa for pertinent discussion. We also thank M. Toda for the critical reading of the manuscript, and M. Matsui, H. Ota, A. Mori, K. Araya, Y. Misawa, K. Nishikawa, K. Matsuo, S. Narumi and M. Honda for their help in collecting materials examined. This study was partially supported by a grant from the Fujiwara Natural History Foundation (to J. Motokawa) and a Grant-in-Aid from the Japan Ministry of Education, Science, Sports and Culture (C-10836010 to T. Hikida).

REFERENCES

Boyer SH, Fainer DC, Watson-Williams EJ (1963) Lactate dehydrogenase variant from human blood: evidence for molecular subunits. Science 141: 642–643

Clayton JW, Tretiak DN (1972) Amine-citrate buffers for pH control

in starch gel electrophoresis. J Fish Res Board Can 29: 1169–1172
de Queiroz K, Good DA (1997) Phenetic clustering in biology: a critique. Quarter Rev Biol 72: 3–30
Felsenstein J (1993) PHYLIP (Phylogeny inference package) Ver 3.5c. Distributed by the author, Dept Genet, Univ Washington, Seattle
Hallowell E (1861) [1860] Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S.N. Proc Acad Nat Sci Phila 12: 480–510
Harada M, Ando A, Tsuchiya K, Koyasu K (2001) Geographic variations in Chromosomes of the greater Japanese shrew-mole, *Urotrichus talpoides*. Zool Sci 18: 433–442
Hikida T (1993) Phylogenetic relationships of the skinks of the genus *Eumeces* (Scincidae: Reptilia) from East Asia. Jpn J Herpetol 15: 1–21
Hikida T, Motokawa J (1999) Phylogeographical relationships of the genus *Eumeces* (Reptilia: Scincidae) in East Asia. In "Tropical Island Herpetofauna: Origin, Current Diversity, and Conservation" Ed by Ota H, Elsevier Sci, Amsterdam, pp231–247
Hikida T, Ota H, Toyama M (1992) Herpetofauna of an encounter zone of Oriental and Palearctic elements: Amphibians and reptiles of the Tokara Island Group and adjacent islands in the northern Ryukyus, Japan. Biol Mag Okinawa 30: 29–43
Kato J, Ota H, Hikida T (1994) Biochemical systematics of the *latiscutatus* species-group of the genus *Eumeces* (Scincidae: Reptilia) from East Asian islands. Biochem Syst Ecol 22: 491–500
Matsui M, Misawa Y, Nishikawa K, Tanabe S (2000) Allozymic variation of *Hynobius kimurae* Dunn (Amphibia, Caudata). Comp Bioch Physiol B 125: 115–125
Murphy RW, Cooper Jr WE, Richardson WS (1983) Phylogenetic relationships of the North American five-lined skinks, genus *Eumeces* (Sauria: Scincidae). Herpetologica 39: 200–211
Murphy RW, Site Jr JW, Buth DG, Haufler CH (1996) Proteins: Isozyme electrophoresis. In "Molecular Systematics. 2nd ed" Eds by Hillis DM, Moritz C, Mable BK, Sinauer Assoc, Sunderland, pp51–120
Nagai K (1928) Fauna of the Nansei Islands. Kagoshima-ken Siseki Meisho Tennen-Kinenbutsu Chosa Hokoku. Rep Nat Monuments, Kagoshima Pref 4: 49–52 (in Japanese)
Nakanishi H, Ejima M (1989) Fauna and flora of the Danjo Islands, In "Fauna and Flora of Nagasaki Prefecture" Ed by Ejima M, Nagasaki Prefectural Society of Biology, Nagasaki, pp299–304 (in Japanese)
Nakamura K, Uéno SI (1963) Japanese Reptiles and Amphibian in Colour. Hoikusha, Osaka (in Japanese)
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small sample number of individuals. Genetics 89: 583–590
Nei M (1987) Molecular Evolutionary Genetics. Columbia Univ Press, New York
Ohshima K (1990) The history of straits around the Japanese Islands in the late Quaternary. Quart Res 29: 193–208 (in Japanese with English abstract)
Ota H, Sakaguchi N, Ikehara S, Hikida T (1993) The herpetofauna of the Senkaku Group, Ryukyu Archipelago. Pac Sci 47: 248–255
Peters WCH (1864) Die Eidechsenfamilie der Scincoiden, insbesonder eüber die Schneider'schen, Wiegmann'schen und neue Arten des zoologischen Museums. Monatsber K Akad Wiss Berlin 1864: 44–58
Poulik MD (1957) Starch gel electrophoresis in a discontinuous system of buffers. Nature 180: 1477–1479
Saitoh M, Matsuoka N, Obara Y (1989) Biochemical systematics of three species of the Japanese long-tailed field mice; *Apodemus speciosus, A. giliacus* and *A. argenteus*. Zool Sci 6: 1005–1018
Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol Biol Evol 4: 406–425
SAS Inst Inc (1990) SAS/STAT User's Guide. Sas Inst Inc, Cary, North Carolina
Selander RK, Smith HM, Yang SY, Johnson WE, Gentry JB (1971) Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Stud Genet 6 Univ Texas Publ 7103: 49–90
Shibata Y, Hamano S, Ejima M, Matsuo T (1988) First record of *Dinodon orientalis* from the Danjo Island, Japan (Reptilia: Colubridae). Bull Osaka Mus Nat Hist 4: 25–32 (in Japanese with English abstract)
Sneath PHA, Sokal RR (1973) Numerical Taxonomy. WH Freeman, San Francisco
Sokal RR, Rohlf FJ (1981) Biometry. 2nd ed, WH Freeman, San Francisco
Stejneger L (1907) Herpetology of Japan and adjacent territory. Bull US Nat Mus 58: 1–557
Swofford DL, Selander RB (1981) BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72: 281–283
Taylor EH (1936) [1935] A taxonomic study of the cosmopolitan scincoid lizards of the genus *Eumeces*, with an account of the distribution and relationships of its species. Univ Kansas Sci Bull 24: 1–643
Toriba M (1986) Preliminary study on the systematic status of a Danjo Islands snake. Jpn J Herpetol 11: 124–136
Toyama M (1989) Distribution of the genus *Eumeces* (Scincidae: Lacertilia) in the Ryukyu Archipelago. In "Current Herpetology in East Asia" Eds by Matsui M, Hikida T, Goris RC, Herpetology Society of Japan, Kyoto, pp262–267
Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395–420
Wright S (1978) Evolution and the Genetics of Populations Vol 4, Variability within and among natural populations. Univ Chicago Press, Chicago

(Received June 17, 2002 / Accepted September 30, 2002)

APPENDIX

Sampling locality for each population and catalogue number of specimens examined.

Eumeces latiscutatus

Sample 1: Sapporo, Hokkaido Prefecture, 43°10′N, 141°15′E, KUZ 45151-4, 50355; sample 2: Aomori, Aomori Pref., 40°38′N, 140°50′E, KUZ 28003, 30349-53, 30384, 30674-7, 35199-200, 35222-3; sample 3: Kamakura, Kanagawa Pref., 35°20′N, 139°33′E, KUZ 45679, 45681-5, 45691; sample 4: Odawara, Kanagawa Pref., 35°15′N, 139°10′E, KUZ 36566, 39000-1, 39004, 39009, 39012-3; sample 5: Shimoda, Shizuoka Pref., 34°40′N, 138°58′E, KUZ 35178-83, 36303-7, 36309-12, 36314, 36318-9, 36321-4; sample 6: Yui, Shizuoka Pref., 35°05′N, 138°33′E, KUZ 45650-4; sample 7: Azumi, Nagano Pref., 36°10′N, 137°46′E, KUZ 39023-8; sample 8: Kasahara, Gifu Pref., 35°18′N, 137°08′E, KUZ 36274-5, 36277, 36279-81, 39388-91, 45929-32; sample 9: Kyoto, Kyoto Pref., 35°03′N, 135°50′E, KUZ 28000, 28185, 28573, 28577, 28583, 28586-7, 28887, 28892, 29280, 30185-6, 30316, 30354-6, 30370, 30383, 30667-8, 35923, 35925-6, 36244, 36250, 36257, 36259, 39051-2, 39316, 39376; sample 10: Miyama, Kyoto Pref., 35°20′N, 135°37′E, KUZ 35121, 35201-2, 35257-60, 35922; sample 11: Usuki, Oita Pref., 33°04′N, 131°44′E, KUZ 50377-85; sample 12: Kagoshima, Kagoshima Pref., 31°36′N, 130°34′E, KUZ 8360-1, 8363, 29301, 30346-8, 30360-2, 36295, 36297-301, 36414-5, 36452-3, 36486-91, 46919; sample 13: Oshima Is., Danjo Island Group, Nagasaki Pref., 32°02′N, 128°25′E, KUZ 50002-10; sample 14: Meshima Is., Danjo Island Group, Nagasaki Pref., 32°00′N, 128°21′E, KUZ 21799-801, 46996-9, 50000-1; sample 15: Tanegashima Is., Osumi Island Group, Kagoshima Pref., 30°24′N, 130°26′E, KUZ 27989-99; sample 16: Yakushima Is., Osumi Island Group, Kagoshima Pref., 30°32′N, 130°58′E, KUZ 35870-80, 35882-4, 36523-8; sample 17: Kuchierabujima Is., Osumi Island Group, Kagoshima Pref., 30°28′N, 130°12′E, KUZ 27978-82, 50403-4; sample 18: Kuchinoshima Is., Tokara Island Group, Kagoshima Pref., 29°39′N, 129°58′E, KUZ 8369-73, 28563-4, 28570, 28916-7; sample 19: Nakanoshima Is., Tokara Island Group, Kagoshima Pref., 29°30′N, 129°54′E, KUZ 28582, 28889; sample 20: Suwanosejima Is., Tokara Island Group, Kagoshima Pref., 29°35′N, 129°41′E, KUZ 8374-7.

E. okadae

Sample 21: Miyakejima Is., Izu Island Group, Tokyo Pref., 34°05′N, 139°30′E, KUZ 35123-35, 35295-8, 35747-8, 35751.

E. marginatus

Sample 22: Amamioshima Is., Amami Island Group, Kagoshima Pref., 28°13′N, 129°27′E, KUZ 8379-81, 28165, 28566, 28914, 29295-6, 30333-4, 36421-2, 36454-7, 36459, 36462-3; sample 23: Okinawajima Is., Okinawa Island Group, Okinawa Pref., 26°40′N, 127°54′E, KUZ 27950-1, 27955-60, 28177-84, 28462, 28464, 28466-7, 28478, 28499, 28571, 28908, 30309, 30329, 30357, 30375, 30389, 30679-80.