Genetic Relationships of a Morphologically Unique Population of the Genus *Eumeces* (Scincidae: Squamata) from Iotorishima Island, Ryukyu Archipelago, as Revealed by Allozyme Data

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Abstract: The Ryukyu five-lined skink, *Eumeces marginatus*, is an endemic species of the central and northern Ryukyus, and is composed of two morphologically defined subspecies, *E. m. marginatus* from the Okinawa Group, and *E. m. oshimensis* from the Amami and Tokara Groups. A population of the genus *Eumeces*, recently found on Iotorishima Island (an uninhabited islet located north of the main island chain of the Ryukyus), resembles *E. m. oshimensis* in having a relatively short median white line on the tail, but is distinct from any population of *E. marginatus* in having more midbody scale rows. To assess the taxonomic status of the Iotorishima population from a historical viewpoint, we examined its genetic relationships with *E. marginatus* populations. The degree of genetic differentiation between the Iotorishima population and a population of *E. m. oshimensis* from Tokunoshima, northern Amami islands, was fairly low ($D=0.043$). Cluster analysis of genetic data also indicated that the Iotorishima population constitutes one of the two major clusters within *E. marginatus* together with four *E. m. oshimensis* populations from the northern Amami and southern Tokara islands. Thus, it is likely that the number of midbody scale rows in the Iotorishima population has increased rapidly after its relatively recent isolation. Taxonomically, the Iotorishima population seems to be most appropriately assigned to *E. m. oshimensis*.

Key words: *Eumeces marginatus*; Reptilia; Allozyme; Iotorishima Island; Ryukyu Archipelago

INTRODUCTION

Eumeces marginatus (Hallowell, 1861) is a moderate-sized scincine lizard endemic to the Ryukyu Archipelago. The range of this species includes most islands of the Okinawa and Amami Groups, and

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Takarajima, Kodakarajima and Kojima Islands of the Tokara Group (Toyama, 1989; Ota et al., 1994). Two subspecies, *E. m. marginatus* from the Okinawa Group, and *E. m. oshimensis* from the Amami and Tokara Groups, are generally recognized on the basis of scutellation and coloration (e.g., Nakamura and Uéno, 1963). Although some external characters, such as the scale row number and juvenile tail coloration, are known to vary among island populations of *E. marginatus*, variations are not consistent with the geographic arrangement of populations and thus are considered to have little significance in terms of their historical relationships (Toyama, 1989). In fact, Kato et al. (1994), in the phylogenetic analyses of allozyme data for 19 populations representing six species of the East Asian *Eumeces*,

recognized two assemblages of island populations within *E. m. oshimensis*, of which one is genetically closer to *E. m. marginatus* than to the other "conspecific" assemblage.

Recently, Toyama (1995) reported on two *Eumeces* specimens collected for the first time from Iotorishima Island, an uninhabited islet 65 km west of Tokunoshima Island, the Amami Group (Fig. 1). These specimens, while most resembling *E. m. oshimensis* in head scutellation and coloration, differed from *E. marginatus* in having more rows of scales around the midbody (34, vs 24–30; Toyama, 1995).

In May 1997, four additional specimens of *Eumeces* were collected from Iotorishima Island by S. Ikehara and colleagues during a comprehensive scientific survey of the island. We sampled fresh tissues from

FIG. 1. A map of the central Ryukyus, showing sampling localities of *Eumeces marginatus*. 1=Iotorishima, 2=Kodakarajima, 3=Takarajima, 4=Amamioshima, 5=Tokunoshima, 6=Okinoerabujima, 7=Yoronjima, 8=Yona, 9=Bise, 10=Shuri, 11=Kumejima.

these specimens, and examined them electrophoretically together with tissue samples of *E. marginatus* from almost whole of its range. Our purpose was to determine the taxonomic status of the Iotorishima population on the basis of its relationships with *E. marginatus* populations reflected by allozyme variations.

MATERIALS AND METHODS

We used four specimens from Iotorishima Island and 224 *Eumeces marginatus* specimens from eight islands of the Ryukyu Archipelago: the Tokara Group (Takara-jima and Kodakara-jima), the Amami Group (Amamioshima, Tokunoshima, Okinoerabujima, and Yoronjima), and the Okinawa Group (Okinawajima and Kumejima) (Fig. 1). All specimens from the same island, except for those from Okinawajima, were combined for the analyses under an a priori assumption that they represent a single randomly breeding population. Specimens from three localities within Okinawajima were treated as three separate samples, because the island's large size (120 km in length, 10 km in breadth and 1200 km² in area) may have enhanced subdivisions of its lizard population.

Homogenized tissue extracts were analyzed by horizontal starch gel electrophoresis. Sample preparation and electrophoretic procedures followed Shaw and Prasad (1970) and Harris and Hopkinson (1976) as modified by Kato et al. (1994). Enzymes examined and buffer systems used here were almost same as those in Kato et al. (1994). Nevertheless, Ah-2 (Kato et al., 1994) was excluded from the analyses, because products of this locus were not consistently clear enough to permit reliable scoring. Instead, peptidase was examined for the first time for the Japanese *Eumeces*, and resultant bands from liver extracts were interpreted as being controlled by a single locus, Pep-D. A total of 22 loci of 16 enzymes (i.e., Aat-1, Aat-2, Ada-2,

Ah-1, Ck-2, Est-4, Fh, Gdh, Gpi, Idh-1, Idh-2, Ldh-1, Ldh-2, Ldh-3, Mdh-1, Mdh-2, Mpi, Pep-D, 6Pgdh-1, Pgm-1, Pgm-2, and Sod-1) were thus examined.

Allozyme data were treated as products of codominant alleles at a given locus. For some specimens of *E. marginatus*, allozyme data, taken from our previous study (Kato et al., 1994), were subjected to the analyses. All loci were evaluated for genetic polymorphism (mean number of alleles per locus, proportion of polymorphic loci, and observed mean heterozygosity). In order to estimate overall genetic differentiation among populations, Nei's (1978) unbiased genetic distance (D) was calculated on the basis of population allele frequencies. The resultant pairwise D matrix was further subjected to analyses by the UPGMA algorithm (Sneath and Sokal, 1973) for the purpose of estimating genetic relationships among populations. All computations were carried out using the BIOSYS-1 computer program (Swofford and Selander, 1981).

In addition, we quantified a few external characters (snout-vent length, tail length, extent of the median white line on tail, and the number of midbody scale rows), and also investigated the postnasal condition for the Iotorishima specimens.

RESULTS

Of the 22 presumptive loci analyzed, nine (Aat-2, Ck-2, Fh, Gdh, Ldh-1, Ldh-2, Mpi, Pgm-1 and Sod-1) were fixed in all samples. Allele frequencies at the other 13 polymorphic loci are given in Table 1. At six of these (Idh-1, Idh-2, Ldh-3, Mdh-1, Mdh-2, and Pgm-2), the predominant (frequency of occurrence > 50%) electromorph was identical in all samples. The Iotorishima sample had alleles common to the Tokunoshima sample at all loci, and all but the Est-4 locus were fixed or nearly fixed for these shared alleles. With respect to the latter locus, all of the Iotorishima specimens had a homozygote of an allele,

TABLE 1. Allele frequencies at 13 polymorphic loci and variability estimates for 11 populations of *Eumeces marginatus*. Locality numbers correspond to those in Fig. 1. A=mean number of alleles per locus, P=percentage of polymorphic loci, H=observed mean heterozygosity.

Locus	Population (N)										
	1 (4)	2 (18)	3 (12)	4 (19)	5 (21)	6 (22)	7 (22)	8 (30)	9 (31)	10 (24)	11 (25)
Aat-1											
a						.886	.952		.050	.021	
b	1.000	1.000	1.000	1.000	1.000	.023			.100	.109	
c						.091	.048	1.000	.850	.870	.320
d											.680
Ada											
a	1.000	1.000	.958	1.000	1.000						
b			.042					.917	.155	.773	1.000
c						1.000	1.000	.083	.845	.227	
Ah-1											
a	1.000			.053	1.000	.955	.977	1.000	1.000	.861	1.000
b		1.000	.958	.921		.045	.023			.139	
c			.042	.026							
Est-4											
a		.028			.025			.038			
b		.972	1.000	1.000	.950	.929	.955				
c	1.000				.025	.071	.045	.924	.100	.409	.100
d								.038	.900	.591	.900
Gpi											
a	1.000				1.000	.045					
b		1.000	1.000	1.000		.955	1.000	1.000	1.000	1.000	1.000
Idh-1											
a								.017			
b	1.000	1.000	1.000	1.000	.976	1.000	1.000	.983	1.000	1.000	1.000
c					.024						
Idh-2											
a										.159	
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.841	1.000
Ldh-3											
a										.022	.020
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.978	.980
Mdh-1											
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.960
b											.040
Mdh-2											
a								.086			
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.880	1.000	1.000	1.000
c								.034			

TABLE 1. Extended

Locus	Population (N)										
	1 (4)	2 (18)	3 (12)	4 (19)	5 (21)	6 (22)	7 (22)	8 (30)	9 (31)	10 (24)	11 (25)
Pep-D											
a								.786	.016		
b	1.000	1.000	1.000	1.000	1.000	1.000	.969	.214	.984	1.000	1.000
c							.031				
6Pgdh-1											
a			.083	.059							
b	1.000	1.000	.917	.941	1.000	.182	.091	1.000	1.000	.556	1.000
c						.818	.909				
d										.444	
Pgm-2											
a							.023		.016		.040
b	1.000	1.000	1.000	1.000	1.000	1.000	.977	.857	.887	1.000	.960
c								.143	.097		
A	1.0	1.0	1.1	1.1	1.1	1.3	1.3	1.4	1.3	1.4	1.2
(SE)	(0.1)	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.0)
P	0.0	4.5	13.6	9.1	9.1	22.7	27.3	27.3	22.7	31.8	22.7
H	0.000	0.003	0.015	0.002	0.007	0.033	0.015	0.019	0.033	0.034	0.033
(SE)	(0.000)	(0.003)	(0.009)	(0.002)	(0.005)	(0.016)	(0.007)	(0.011)	(0.016)	(0.013)	(0.022)

TABLE 2. Matrix of genetic distance (Nei, 1978) between 11 populations of *Eumeces marginatus*. A negative value set to -0.000.

Population	1	2	3	4	5	6	7	8	9	10
1. Iotorishima	—									
2. Kodakarajima	0.145	—								
3. Takarajima	0.146	-0.000	—							
4. Amamioshima	0.141	0.000	-0.000	—						
5. Tokunoshima	0.043	0.096	0.094	0.090	—					
6. Okinoerabujima	0.223	0.174	0.167	0.166	0.175	—				
7. Yoronjima	0.243	0.188	0.181	0.180	0.191	0.000	—			
8. Yona	0.182	0.233	0.230	0.229	0.231	0.196	0.210	—		
9. Bise	0.174	0.179	0.177	0.174	0.178	0.108	0.122	0.095	—	
10. Shuri	0.162	0.168	0.163	0.163	0.183	0.126	0.139	0.059	0.034	—
11. Kumejima	0.182	0.186	0.182	0.181	0.185	0.161	0.174	0.090	0.053	0.036

which occurred in very low frequency in the Tokunoshima sample.

In the present samples, values of the mean number of alleles per locus (A), proportion of polymorphic loci (P), and observed mean heterozygosity per locus (H)

ranged from 1.0–1.4, 0.0–31.8, and 0.000–0.034, respectively (Table 1). The Iotorishima sample exhibited neither polymorphism nor allelic heterozygosity.

Pairwise comparisons of samples by Nei's (1978) genetic distance coefficient (D)

FIG. 2. Genetic relationships among 11 populations of *Eumeces marginatus* based on contours of Nei's (1978) genetic distance values derived from a UPGMA algorithm.

are presented in Table 2. The D value obtained between the Iotorishima and Tokunoshima samples was very small (0.043), whereas those of the former with other *E. marginatus* samples were larger (0.141–0.243).

Figure 2 shows genetic grouping of samples derived from the UPGMA clustering of pairwise D matrix. Populations currently assigned to *E. m. marginatus* were grouped together with high genetic similarities. By contrast, a great genetic heterogeneity was confirmed for *E. m. oshimensis* in the current delimitation, indi-

cating a relatively distinct differentiation between the southern Amami and the northern Amami-southern Tokara assemblages, as reported previously (Kato et al., 1994). In this analysis, the Iotorishima sample was located in the cluster otherwise consisting of the northern Amami-southern Tokara *E. m. oshimensis*.

The four examined specimens from Iotorishima Island invariably had a medial white line not exceeding the proximal one third of the tail and 34 rows of midbody scales, and lacked the postnasal scale

TABLE 3. Measurements (in mm) and scale characters of the Ryukyu five-lined skink, *Eumeces marginatus*, from Iotorishima Island. SVL=snout-vent length, TL=tail length, EL=extent of the median white line on tail, SR=number of midbody scale rows, PN=postnasal condition.

No.	Date of Collection	Sex	SVL	TL	EL	SR	PN
KUZ34568	10 May 1997	female	48.6	89.4	>1/3	34	absent
KUZ34569	10 May 1997	male	51.5	15.2 + *	—**	34	absent
KUZ34570	10 May 1997	male	49.3	89.1	>1/3	34	absent
KUZ34571	11 May 1997	female	48.8	87.1	>1/3	34	absent

*: + tail broken.

**: — not clear.

(Table 3). These character states are identical to those in the two previously collected specimens reported by Toyama (1995).

DISCUSSION

Toyama (1995), in the description of external characters in the two previous specimens from Iotorishima Island, stated that their head scutellation seemingly fell within the range of variation in *Eumeces marginatus*. He further noted that in these specimens the extent of the median white line on the tail, a character used to discriminate the two subspecies of *E. marginatus*, was consistent with that of *E. m. oshimensis* rather than that of *E. m. marginatus*. However, he also noted that the specimens had many more rows of midbody scales (34) than *E. marginatus* (24–30). Examination of the four additional specimens confirmed these morphological characteristics for the Iotorishima population of *Eumeces*, and this alone may suggest a genealogical distinctness of this population from *E. marginatus*.

However, results of analyses of allozyme data indicate that the Iotorishima population is genetically remarkably similar to *E. marginatus*, and is much closer to the Tokunoshima population of *E. m. oshimensis* than any other consubspecific populations. This suggests an exclusively close

historical affinity of the two populations, and we thus believe that the Iotorishima population should be classified as *E. m. oshimensis*, even though it exhibits a unique state in the number of midbody scale rows. It is probable that in such a small island population as that on Iotorishima Island the midbody scale rows have increased rapidly in response to selection pressure from some environmental factor there.

The Iotorishima population has alleles common to the Tokunoshima population at all loci and the D value between these populations was very small (0.043). Such close genetic resemblance between the Iotorishima and Tokunoshima populations suggests that these populations have been very recently isolated. However, Iotorishima Island is situated 65 km west of Tokunoshima Island, and is separated from the latter, as well as from other islands having *E. marginatus* populations, by straits no less than 500 m in depth (MSAJ, 1978). Since sea level dropped by 120–140 m only during late Pleistocene (Ota et al., 1993), Iotorishima should have remained isolated then. The above result thus suggests a relatively recent overseas dispersal of the lizard between these islands. Otherwise, it necessitates an ad hoc assumption for the formation of a land bridge between them and its recent rapid submergence.

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