Bull. Inst. Zool., Academia Sinica 22(1): 91-104 (1983)

BIOCHEMICAL SYSTEMATICS OF THE GENUS *JAPALURA* (SAURIA: AGAMIDAE) IN TAIWAN

SHENG-KUANG LOU AND JUN-YI LIN

Biology Department, Tunghai University, Taichung, Taiwan 400, Republic of China

(Received October 18, 1982)

Sheng-Kuang Lou and Jun-Yi Lin (1983) Biochemical systematics of the genus *Japalura* (Sauria: Agamidae) in Taiwan. *Bull. Inst. Zool., Academia Sinica* 22(1): 91-104. The genus *Japalura* has recently been classified into three subspecies, *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis* in Taiwan and its adjacent islands, Lanyu (蘭嶼) and Green Island (綠島). Because of its morphological variation and color patterns, there exist many systematic disputes over its status. The genetic variation at nine loci are examined to study the systematics of the genus *Japalura*.

Seventeen populations were sampled from 16 localities. The electrophenotypes of muscle proteins and lactate dehyrdrogenase could serve as diagnostic characters in distinguishing *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis*, as far as the Pt-3^e, Pt-3^f, Pt-4^a, Ldh-1^a and Ldh-1^b alleles are concerned. The biochemical phenogram constructed from the matrix of genetic similarity of these 17 populations by using UPGMA (unweighted pair-group method using arithmetic averages), shows three major clusterings, which coincide with the systematics of the three proposed subspecies. The meristic phenogram also shows three major clusterings different in groupings from those of the biochemical phenogram, and reveals that the geographically neighbouring populations are more morphologically identical in spite of their taxonomic status.

The non-overlapping of protein phenotypes at Pt-3, Pt-4 and Ldh-1 loci excludes the possibility of hybridization of these three subspecies at contact areas.

The mean genetic distance between the populations (D=0.081) is within the known ranges at population level; however, the mean genetic distance between the clusterings (D=0.3353) qualifies them for species level.

The classification of the genus *Japalura* into three subspecies, *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis* may be justified until further studies.

The arboreal lizard, *Japalura*, is the only genus of the family Agamidae found in Taiwan and its adjacent islands, Lanyü (蘭嶼) and Green Island (綠島). This genus has been studied by many herpetologists since 1864. Günther (1864) found a new species, *J. swinhonis*, based on the specimens collected from Tansui (淡水), near Taipei (臺北). Later, Stejneger (1898) found another species, *J. mitsukurii*, based on the specimens collected in Lanyü. Van-Denburgh (1912) revised that *J. mitsukurii* Stejneger (1898) was actually a subspecies of *J. swinhonis* Günther (1864) and recognized that there were two subspecies, *J. s. swinhonis* and *J. s. mitsukurii* in Taiwan. Kano (1928) observed that *J. s. swinhonis* confined its distribution to the northern part of Taiwan and *J. s. mitsukurii* to the southern part of Taiwan, especially in Hengchun (恆春). Gressitt (1936) (cit. from Okada, 1937) described a new species *J. breviceps* from the specimens collected from Bukai or Wuchie (武界) (Nantou County, 南投縣). But, Okada (1937) considered that *J. breviceps* was a female specimen of *J. s. swinhonis* with color variation, and followed the

classification of Van-Denburgh (1912). Wang (1962) believed that *J. s. mitsukurii* found in Lanyü was closely related to *J. s. swinhonis* in Taiwan. Liang and Wang (1976) found a new subspecies, *J. s. formosensis*, based on the specimens collected from Puli (埔里) (Nantou County), near the central Taiwan. and suggested that these three subspecies, *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis* are distributed in the northern, south-eastern and central parts of Taiwan, respectively. Since then, three subspecies have been recognized in Taiwan.

The key characters used to classify the genus *Japalura* by the previous authors can be summarized as follows: 1) a longitudinal row of regularly arranged, enlarged scales on each side of dorsal crest; 2) the arrangement of scales in contact with dorsal crest; 3) spot markings on the throat; 4) the size of nuchal; 5) the distance between the outer edges of superciliaries and 6) the length of the third and the fourth toe without claw.

Since these gross morphological characters vary greatly in populations of *Japalura*, the systematics of this genus appears at best confusing and uncertain. The purpose of this study is to examine the genetic variation at non-enzymic soluble muscle proteins, lactate dehydrogenase and pancreatic α-amylase in the populations of *Japalura*, using gel electrophoresis. In addition, we attempt to estimate the genetic similarity and genetic distance in the populations in various parts of their ranges, together with the taxometric characters to discuss the systematic status of the three subspecies in Taiwan.

MATERIALS AND METHODS

A total of two hundred and eighty-eight lizards were collected from the 16 localities (Fig. 1) from April, 1981 to April, 1982. Sample size and other information from each locality are given in Table 1. All individuals were identified by morphological criteria according to Liang and Wang (1976).

After ethered, individuals were dissected; pancreas and white skeletal muscle near the basal part of the tail were excised. Pancreas was homogenized with 10 volumes of Ringer's solution and muscle was homogenized with equal volume of Ringer's solution or phosphate buffer (KH_2PO_4-K_2HPO_4, I=0.05, pH=7.5). The homogenate was centrifuged (15,000 rpm 30 minutes, 4°C) and the clear supernatant was stored at −20°C. Before electrophoresis, the sample was mixed with 40% sucrose and 0.5% bromophenol blue in the volume ratio of 100 μl: 20 μl: 5 μl. Bromophenol blue was added as an indicator.

Electrophoresis was carried out in 7.5% or 10% polyacrylamide gel using a modification of vertical slab gel apparatus (glass plate 16×14 ×0.1 cm with 9 or 13 sample slots) and discontinuous buffer system was modified from Davis (1964), Laemmli (1970) and Ames (1974).

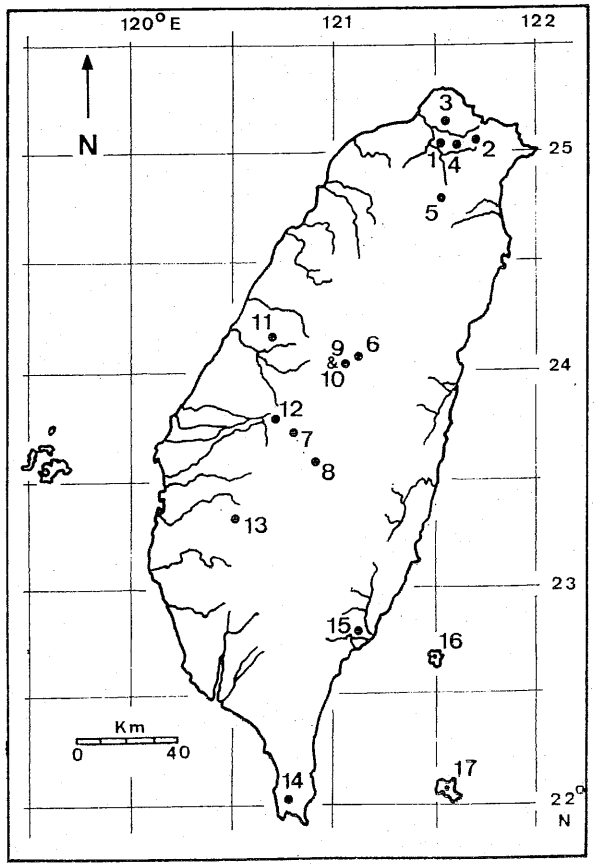

Fig. 1. Localities in which individuals of *Japalura* were collected. The population numbers correspond to those in Table 1.

TABLE 1
Locality information and sample sizes for 17 populations of *Japalura* used in this study

Locality	Subspecies Identified	Sample Number	Elevation (m)	Annual Av. Temperature (°C)	Annual Precipitation (mm)
1. Sungshan (松 山)	*J. s. swinhonis*	37	160.0	21.5	2161.6
2. Nankang (南 港)		16	80.0	22.5	1782.2
3. Yangmingshan (陽明山)		13	430.0	18.3	3906.8
4. Mucha (木 柵)		6	110.0	22.0	2161.6
5. Wulai (烏 來)		8	215.0	20.5	3680.8
6. Lushan (廬 山)		21	1415.0	16.6	2212.2
7. Chitou (溪 頭)		2	1500.0	16.9	2518.0
8. Tungpu (東 埔)		4	1180.0	18.8	1858.8
9. Wushe (霧 社)		8	1148.0	18.6	2140.7
10. Wushe (霧 社)	*J. s. formosensis*	6	1148.0	18.6	2140.7
11. Taichung (臺 中)		33	160.0	23.2	1013.8
12. Chushan (竹 山)		24	156.0	24.1	1711.4
13. Tapu (大 埔)		6	280.0	24.7	1434.1
14. Hengchun (恆 春)	*J. s. mitsukurii*	28	22.0	25.5	988.7
15. Taitung (臺 東)		26	9.0	24.4	817.5
16. Green Island (綠 島)		28	18.0	—	—
17. Lanyu (蘭 嶼)		22	180.0	22.8	2615.5

The 7.5% or 10% (the amount indicated in parenthesis) separating gel was prepared from 6.25 ml (8.4 ml) of a solution containing 30% acrylamide and 0.8% N, N′-bis-acrylamide, 3.0 ml (3.0 ml) of 1.5 M Tris-HCl buffer (pH 8.9), 20 μl (20 μl) of TEMED (tetraethylenemethylenediamine), 15.7 ml (13.5 ml) of distilled water and 0.1 ml (0.1 ml) of 10% ammonium persulfate. The stacking gel was prepared from 3.0 ml of a soultion containing 10% acrylamide and 2.5% N,N′-bis-acrylamide, 1.25 ml of 0.5 M Tris-HCl buffer (pH 6.7), 10 μl of TEMED, 5.7 ml of distilled water and 50 μl of 10% ammonium persulfate; a slot-forming comb was inserted to form sample pockets before it was polymerized. Pancreatic α-amylase was carried out in 10% gel with 8 μl sample mixture in each slot; LDH in 7.5% gel with 8 μl sample mixture in each slot and muscle protein in 7.5% gel with 15 μl sample mixture in each slot. The electrode buffer used for pancreatic α-amylase was 0.05 M Tris-glycine buffer (pH 8.3) (Aquadro and Patton, 1980), and for LDH and muscle proteins was 0.192 M Tris-glycine buffer (pH 8.3) (Morrison and Wright, 1966). Gel was run vertically at 6°C in refrigerator and the current was set up to 10 mA per gel for the first 30 minutes, then changed to 15 mA per gel. Electrophoresis was terminated when the dye band migrated to within 5 mm of the gel end. The entire procedure took about 3 hours. After the gel was removed from the glass plate, it was stained as follows.

Muscle protein staining method

The non-enzymic soluble muscle proteins were stained with 1.25 g of Coomassie brilliant blue in a mixture of 454 ml of 50% methanol and 46 ml of glacial acetic acid (Weber and Osborn, 1969). Gel was stained for 2-8 hours, then destained with a solution composed of 50% of methanol and 7.5% of glacial acid. If the excess dye was not washed out, the gel was destained again with 10% methanol and 7.5% glacial acetic acid for 10-30 minutes. The electromorphs of non-enzymic soluble muscle proteins appeared as blue bands.

LDH staining method

The LDH staining method was modified

from Shaw and Prasad (1970) and Keller and Lyerla (1977). Staining solution was composed of 50 ml of 0.1 M Tris-HCl buffer (pH 8.5), 0.5 ml of DL-lactic acid and 1.0 ml of 1% β-nicotinamide adenine dinucleotide. Gel was incubated in the above solution for 30 minutes in the dark, then added 1.0 ml of 1% nitro blue tetrazolium and 0.5 ml of 0.6% phenazine methosulfate, and kept on incubating in the dark for 1 hour at room temperature (25°C). The LDH electromorphs appeared as dark purple bands on the transparent gel. Gel was then fixed in 7.5% glacial acetic acid for at least 2 hours, and kept in PVC envelope in refrigerator.

Pancreatic α-amylase staining method

An overlay composed of 0.8% of agar, 0.5% of soluble potato starch and 0.02% of sodium chloride at 35°C was poured over the gel. Sequentially, the gel was incubated at room temperature (25°C) for 45 minutes, and then stained with potassium tri-iodine solution (0.1 M iodine, 0.05 M potassium iodine) for 2 minutes. Gel was washed in a mixture of methanol, water and glacial acetic acid (5:5:1), then photographed (Aquadro and Patton, 1980). The pancreatic α-amylase electromorphs appeared as clear bands against a dark blue background.

Genetic similarity and genetic distance between the populations are estimated using Nei's (1972) statistics (I), which is based on Malecot's concept of identity of genes within and between populations. This statistics is computed using the observed allelic frequencies, and the formulas are:

$$I_k=\sum x_i y_i/\sqrt{\sum x_i^2 \sum y_i^2} \quad (1)$$

$$I=J_{xy}/\sqrt{J_x J_y} \quad (2)$$

x_i, y_i=frequency of the ith allele in population X and Y

k=a given locus

J_x, J_y=arithmetic means over all loci, x_i^2 and y_i^2, respectively

J_{xy}=arithmetic mean over all loci, $x_i y_i$

I_k=genetic similarity of a given locus

I=genetic similarity of all loci

The average genetic divergence per locus, or "genetic distance" between the two populations is given by

$$D=-\log_e I \quad (3)$$

The values of I range from 0 to 1; the value 1 indicates the identical distribution of allelic frequencies in the two populations, and the value 0 indicates the non-overlapping distribution of allelic frequencies. The values of D range from 0 to ∞; the value 0 indicates the identical distribution of allelic frequencies in the two populations.

The taxometric characters used are as follows:

1) Snout-vent length (SVL)—the length from tip of snout to the ventral opening.
2) Tail length (TL)—the length from the ventral opening to the end of tail.
3) Head length (HL)—the length from the tip of snout to the posterior margin of ear, distinguished by the shallow depression of the skin, along a line parallel to the median axis of head.
4) Head width (HW)—the distance between the outer edges of superciliaries.
5) Foreleg length (AF)—the length from the axilla to the fourth toe, without claw.
6) Hindleg length (GH)—the length from the groin to the fourth toe, without claw.
7) Axilla-groin distance (AG)—the distance from axilla to groin.
8) Eye-snout length (ESL)—the length from the center of eye to the tip of snout.
9) Fourth toe length, without claw (FTL).
10) Third toe length, without claw (TTL).

The taxometric characters are standardized with the formula given by Sokal and Sneath (1963)

$$X'_{ij}=(X_{ij}-\overline{X}_i)/s_i \quad (4)$$

X_{ij}=the row value

X'_{ij}=the transformed value of character i for form j
$\bar{X}_i$=the mean of character i
s_i=the standard deviation of character i

The mean square distance (MSD) is calculated from the formula given by Sokal and Sneath (1963)

$$C_H^2=\frac{1}{n}\sum d_i^2 \qquad (5)$$

d_i=difference, with respect to character i, between the two forms under comparison
n=number of characters used in the comparison between the two forms

A biochemical phenogram and meristic phenogram are constructed from the clustering of the matrices of genetic similarity (I) and mean square distance (MSD), respectively, by using the UPGMA (unweighted pair-group method using arithmetic averages) given by Sneath and Sokal (1973).

RESULTS

More than thirty discrete bands were observed from the white skeletal muscle proteins in gel electrophoretic assay. Of these, 23 could be routinely demonstrated (Fig. 2); these were presumably derived largely from the contractile proteins, actin and myosin. None of the LDH showed up with the Coomassie brilliant blue stain. The complexity of the banding patterns and the non-specificity of the protein stain prevent safe interpretation in terms of specific gene loci; however, six loci were determined according to their electrophoretic mobility and the clustering positions under defined condition. Each locus is designated Protein (Pt) 1 through 6, and numbered from anode to cathode. The alleles at each locus are designated alphabetically in order of decreasing distance from the anodal end of the gel, except at Pt-4 locus that the null allele represents a bandless phenotype. In absence of cross test, the interpretation is arbitrary, but the variation of the muscle proteins provide information on their genetic relationship.

Ten of the 23 bands identified in the muscle proteins are common to each population. Pt-3^e allele appears only in population 14; Pt-3^f allele appears in every population of *J. s. swinhonis* except population 7; Pt-4^a allele appears in every population of *J. s. formosensis* and three populations of *J. s. mitsukurii* (15, 16 and 17) except population 14; interestingly, the *J. s. swinhonis*-like population 7 possesses the Pt-4^a but not Pt-3^f band character.

LDH is a product of two genetic loci which may be termed as H (heart) and M (muscle), the former is designated as Ldh-1 and the latter as Ldh-2. The active enzyme is a tetrameric molecule and the products of Ldh-1 and Ldh-2 loci can be polymerized to form five tetrameric isozymes: H_4, H_3M_1, H_2M_2, H_1M_3 and M_4 (Markert, 1964).

The isozyme patterns of LDH of *Japalura* are similar to those of amphibians observed by

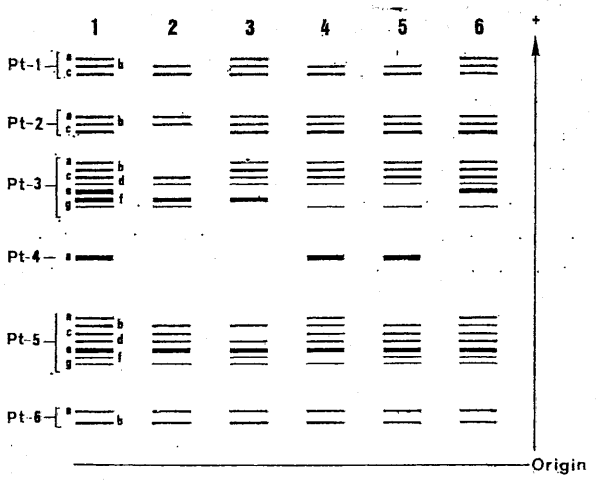

Fig. 2. Electrophoretic variations in soluble muscle proteins. The phenotypes of the individuals illustrated here are as follows (from left to right): (1) the total number of variants found in the genus *Japalura*; (2) *J. s. swinhonis* from Sungshan; (3) *J. s. swinhonis* from Lushan; (4) *J. s. formosensis* from Taichung; (5) *J. s. mitsukurii* from Taitung; (6) *J. s. mitsukurii* from Hengchun.

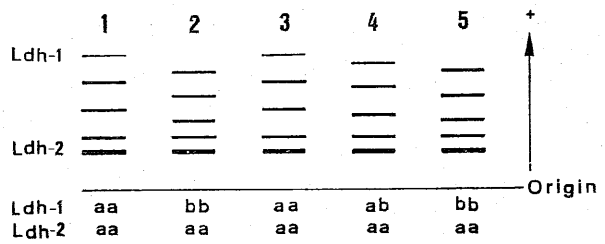

Fig. 3. Electrophoretic variations in lactate dehydrogenase. The phenotypes of the individuals illustrated here are as follows (from left to right): (1) *J. s. swinhonis* from Sungshan; (2) *J. s. formosensis* from Taichung; (3), (4) and (5) *J. s. mitsukurii* from Taitung.

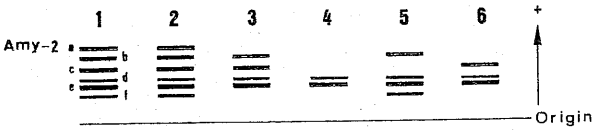

Fig. 4. Electrophoretic variations in pancreatic α-amylase. The phenotypes of the individuals illustrated here are as follows (from left to right): (1) the total number of variants of the genus *Japalura*; (2) *J. s. swinhonis* from Sungshan; (3) *J. s. swinhonis* from Lushan; (4) *J. s. formosensis* from Taichung; (5) *J. s. mitsukurii* from Lanyu; (6) *J. s. mitsukurii* from Hengchun.

Moyer *et al.* (1968) and lizards observed by Montanucci (1974). There are typical five banded patterns as controlled by the two loci, Ldh-1 and Ldh-2, described in all vertebrates. Three electromorphs determined by the mobility of the bands show that *J. s. swinhonis* contains the Ldh-$1^a/1^a$ phenotype, *J. s. formosensis* contains the Ldh-$1^b/1^b$ phenotype and *J. s. mitsukurii* contains the Ldh-$1^a/1^a$, Ldh-$1^b/1^b$ and Ldh-$1^a/1^b$ phenotypes; the Ldh-$1^a/1^b$ phenotype is considered as heterozygous and occurs only in *J. s. mitsukurii* (Fig. 3). Although there were satellite bands and 2 variants at the Ldh-2 locus, they were not included in the analysis.

Six bands of pancreatic α-amylase could be distinguished in gel for pancreatic extracts. They show a high degree of polymorphism in individuals. The six bands are designated for their mobility in alphabetic order from the most anodal a through f, and may be considered as a multiple allele locus or multiple loci (Sick and Nielson, 1964; Nielson, 1974; Evans *et al.*, 1977; Hjorth *et al.*, 1980). However, we tentatively interpret them as a multiple allele locus, Amy-2 (Fig. 4).

Considerable genetic variation was detected from 6 loci in soluble muscle proteins, 2 loci in LDH and 1 locus in pancreatic α-amylase in the genus *Japalura* in Taiwan. The allelic frequencies in the Table 2 are calculated directly by dividing the number of times of a given allele is found by the total number of alleles in each locus in a population. The Ldh-1 locus is consistently in agreement with Hardy-Weinberg equilibrium, and the Ldh-2 locus in monomorphic in most populations except 2 variants in population 14 and 15, respetively.

The genetic distance (D) and genetic similarity (I) between the populations are given in Table 3. The biochemical phenogram constructed from the genetic similarity (I) from the 9 loci shows that there are three major clusterings within the 17 populations studied (Fig. 5). The first clustering consists of population 1, 2, 3, 4, 5, 6, 8 and 9; the second clustering consists of population 7, 14, 15, 16 and 17; and the third clustering consists of population 10, 11, 12 and 13. These three major clusterings derived from the biochemical analysis correspond to the three subspecies, *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis*, respectively, proposed by Liang and Wang (1976), except for the population 7 which according to the morphological characteristics suggested by Liang and Wang should belong to *J. s. swinhonis* However, the population 7 was assigned to *J. s. mitsukurii* according to its biochemical characteristics.

Comparisons of genetic distance were made between the populations of different clusterings and between the clusterings to see if their affinities were within the known ranges of distance. The result is shown in Table 4. The mean genetic distance between the populations of the three clusterings is, D=0.081; the mean

TABLE 2

Allelic frequencies at 9 loci in 17 populations of *Japalura*

Locus	Allele	*J. s. swinhonis*									*J. s. formosenss*				*J. s. mitsukurii*			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pt-1	a	—	0.04	0.24	—	—	—	—	—	0.21	—	0.07	0.25	—	0.17	0.05	0.17	0.02
	b	0.37	0.39	0.31	0.50	0.50	0.25	0.50	0.50	0.37	0.40	0.40	0.29	0.50	0.42	0.57	0.54	0.50
	c	0.63	0.57	0.45	0.50	0.50	0.75	0.50	0.50	0.42	0.60	0.53	0.46	0.50	0.42	0.38	0.29	0.48
Pt-2	a	0.33	0.19	0.24	0.43	0.07	0.40	0.50	—	0.47	0.50	0.45	0.45	0.50	0.15	0.30	0.08	0.30
	b	0.50	0.57	0.53	0.57	0.53	0.50	0.50	1.00	0.47	0.50	0.55	0.43	0.50	0.33	0.49	0.50	0.50
	c	0.17	0.24	0.24	—	0.40	0.10	—	—	0.06	—	—	0.12	—	0.53	0.21	0.42	0.20
Pt-3	a	—	—	—	—	—	—	0.25	—	—	—	0.07	0.04	—	0.07	0.34	0.37	0.30
	b	0.01	—	—	—	—	—	0.25	—	0.07	—	0.17	0.04	0.46	0.13	0.29	0.33	0.29
	c	0.29	0.45	0.14	0.25	0.36	0.30	0.25	0.20	0.29	0.35	0.31	0.44	0.46	0.24	0.05	0.02	0.33
	d	0.24	0.10	0.14	0.25	—	0.20	0.25	0.20	0.21	0.35	0.07	0.13	—	0.12	0.03	0.06	0.03
	e	—	—	—	—	—	—	—	—	—	—	—	—	—	0.27	—	—	—
	f	0.33	0.45	0.62	0.25	0.36	0.33	—	0.60	0.29	—	—	—	—	—	—	—	—
	g	0.13	—	0.10	0.25	0.28	0.17	—	—	0.14	0.30	0.38	0.35	0.08	0.17	0.28	0.22	0.05
Pt-4	a	—	—	—	—	—	—	1.00	—	—	1.00	1.00	1.00	1.00	—	1.00	1.00	1.00
	null	1.00	1.00	1.00	1.00	1.00	1.00	—	1.00	1.00	—	—	—	—	1.00	—	—	—
Pt-5	a	0.01	—	—	—	—	—	—	—	—	—	0.02	—	0.16	0.02	0.07	0.06	—
	b	0.12	0.22	0.15	0.10	0.21	0.14	0.22	—	0.10	0.17	0.24	0.12	0.19	0.17	0.19	0.24	0.24
	c	0.18	0.22	0.27	0.10	0.24	0.17	0.22	—	—	0.25	0.10	0.17	0.19	0.19	0.21	0.19	0.24
	d	0.29	0.28	0.27	0.40	0.28	0.31	0.22	0.50	0.38	0.25	0.31	0.33	0.23	0.27	0.25	0.25	0.24
	e	0.29	0.28	0.27	0.40	0.28	0.31	0.22	0.50	0.38	0.25	0.31	0.33	0.23	0.27	0.25	0.25	0.24
	f	0.07	—	0.04	—	—	—	—	—	0.04	0.08	—	—	—	0.04	—	—	0.01
	g	0.03	—	—	—	—	0.07	0.12	—	0.10	—	0.02	0.05	—	0.04	0.02	0.02	0.03
Pt-6	a	0.50	0.52	0.50	0.50	0.50	0.49	0.50	0.50	0.50	0.40	0.48	0.50	0.55	0.44	0.48	0.53	0.41
	b	0.50	0.48	0.50	0.50	0.50	0.51	0.50	0.50	0.50	0.60	0.52	0.50	0.45	0.56	0.52	0.47	0.59
Amy-2	a	0.18	—	0.09	—	—	0.17	—	—	—	—	—	—	—	—	—	—	—
	b	0.14	0.19	0.23	0.15	0.18	0.04	—	—	0.22	—	—	0.06	—	—	0.10	0.03	0.11
	c	0.15	0.45	0.34	0.15	0.47	—	—	—	—	0.09	0.19	0.02	0.08	0.08	0.08	0.03	0.04
	d	0.08	0.07	0.07	0.15	—	0.38	0.50	—	0.33	0.36	0.33	0.43	0.46	0.40	0.39	0.38	0.36
	e	0.39	0.29	0.27	0.46	0.35	0.42	0.50	1.00	0.44	0.55	0.44	0.49	0.46	0.53	0.43	0.44	0.47
	f	0.06	—	—	0.08	—	—	—	—	—	—	0.04	—	—	—	—	0.11	0.02
Ldh-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—	—	—	0.50	0.48	0.55	0.95
	b	—	—	—	—	—	—	—	—	—	1.00	1.00	1.00	1.00	0.50	0.52	0.45	0.05
Ldh-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

The population numbers correspond to those in Table 1.

TABLE 3

Genetic distance (D, upper right) and genetic similarity (I, lower left) between the populations of the three subspecies of *Japalura*

	J. s. swinhonis									*J. s. formosensis*				*J. s. mitsukurii*			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
J. s. swinhonis																	
1. Sungshan		0.069	0.048	0.021	0.070	0.054	0.199	0.073	0.062	0.503	0.508	0.538	0.567	0.166	0.286	0.273	0.169
2. Nankang	0.933		0.016	0.070	0.009	0.158	0.285	0.154	0.132	0.612	0.564	0.655	0.645	0.233	0.351	0.357	0.235
3. Yangmingshan	0.953	0.984		0.065	0.025	0.185	0.287	0.147	0.110	0.618	0.579	0.646	0.663	0.230	0.341	0.345	0.237
4. Mucha	0.979	0.932	0.938		0.072	0.057	0.157	0.057	0.030	0.452	0.451	0.479	0.498	0.141	0.240	0.232	0.135
5. Wulai	0.932	0.991	0.976	0.931		0.175	0.307	0.142	0.149	0.622	0.571	0.669	0.672	0.227	0.353	0.350	0.244
6. Lushan	0.948	0.854	0.831	0.944	0.840		0.120	0.105	0.033	0.443	0.463	0.445	0.458	0.123	0.234	0.222	0.128
7. Chitou	0.819	0.752	0.751	0.854	0.736	0.887		0.211	0.081	0.314	0.326	0.315	0.304	0.216	0.105	0.092	0.014
8. Tungpu	0.929	0.857	0.864	0.945	0.867	0.900	0.810		0.093	0.526	0.550	0.568	0.589	0.194	0.317	0.282	0.190
9. Wushe	0.940	0.876	0.896	0.970	0.861	0.967	0.992	0.911		0.461	0.474	0.444	0.473	0.127	0.227	0.250	0.123
J. s. formosensis																	
10. Wushe	0.604	0.542	0.539	0.636	0.537	0.642	0.730	0.591	0.631		0.013	0.014	0.025	0.209	0.100	0.151	0.302
11. Taichung	0.602	0.569	0.560	0.637	0.565	0.629	0.722	0.577	0.622	0.987		0.019	0.020	0.214	0.091	0.140	0.303
12. Chushan	0.584	0.520	0.524	0.620	0.512	0.641	0.730	0.567	0.641	0.986	0.981		0.021	0.206	0.093	0.138	0.297
13. Tapu	0.573	0.525	0.515	0.608	0.511	0.632	0.737	0.555	0.623	0.975	0.980	0.979		0.216	0.092	0.141	0.294
J. s. mitsukurii																	
14. Hengchun	0.847	0.792	0.794	0.868	0.797	0.884	0.805	0.824	0.880	0.811	0.807	0.813	0.806		0.121	0.120	0.192
15. Taitung	0.751	0.704	0.711	0.787	0.703	0.791	0.900	0.728	0.797	0.905	0.913	0.911	0.912	0.886		0.038	0.093
16. Green Island	0.761	0.700	0.708	0.793	0.704	0.801	0.912	0.755	0.778	0.859	0.869	0.871	0.869	0.887	0.963		0.081
17. Lanyu	0.843	0.790	0.789	0.874	0.783	0.880	0.986	0.827	0.884	0.739	0.739	0.743	0.745	0.825	0.911	0.923	

TABLE 4
Genetic distance (D) between the populations and the clusterings in *Japalura*

Clustering	I	II	III
I	0.085±0.0518*		
II	0.230±0.0762	0.107±0.0614*	
III	0.544±0.0780	0.212±0.0883	0.019±0.0045*

Clustering I consists of population 1, 2, 3, 4, 5, 6, 8 and 9.
Clustering II consists of population 7, 14, 15, 16 and 17.
Clustering III consists of population 10, 11, 12 and 13.
* represents the comparison between the populations in each clustering.

genetic distance between the clusterings is, D= 0.3353.

All the meristic characters are presented in Table 5. The meristic phenogram also shows that there are three major clusterings different in groupings from those of the biochemical phenogram (Fig. 6). The first clustering consists of population 1, 2, 3, 4, 5, 11 and 14; the second clustering consists of population 6, 8, 9, 10, 12 and 13; and the third clustering consists of population 15, 16 and 17. The population 7 appears to be separated from the rest. This analysis shows that geographically neighbouring populations are more morphologically identical in spite of their taxonomic status.

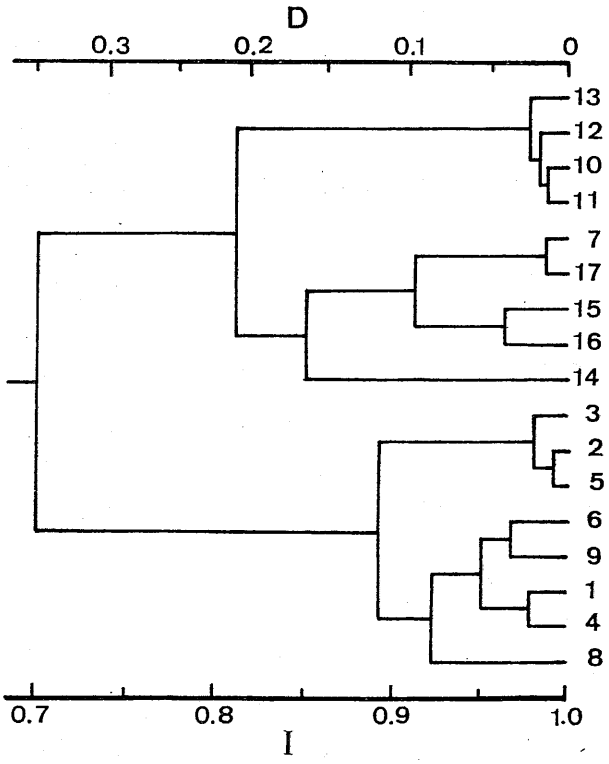

Fig. 5. Biochemical phenogram of genetic relationship of the *Japalura* populations derived from the genetic similarity (I) in Table 3. The population numbers correspond to those in Table 1.

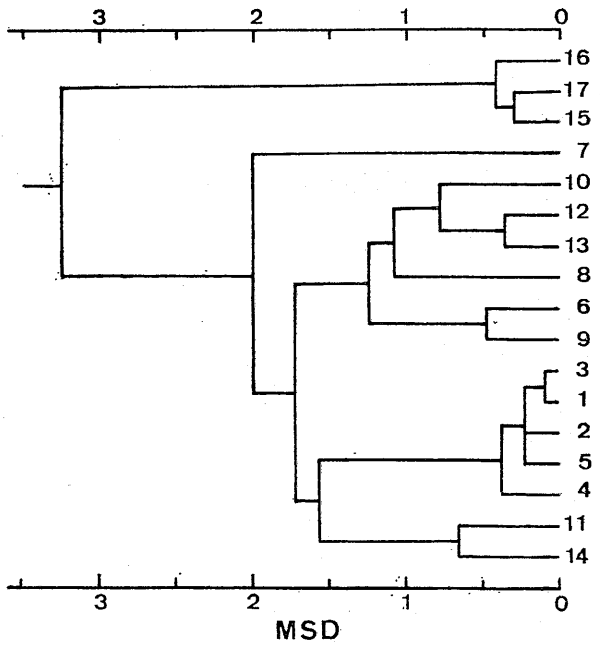

Fig. 6. Meristic phenogram of the *Japalura* populations derived from the mean square distance (MSD). Calculations are based on the standardized external meristic characters in Table 5. The population numbers correspond to those in Table 1.

TABLE 5
External meristic characters in populations of *Japalura*

Population	SVL ($\bar{X}$: mm)	TL/SVL	HL/SVL	HW/SVL	AF/SVL	GH/SVL	ESL/SVL	TTL/SVL	FTL/SVL	AG/SVL
1	61.78	2.136	0.258	0.162	0.435	0.685	0.147	0.136	0.172	0.505
2	60.99	2.178	0.259	0.161	0.418	0.670	0.143	0.141	0.177	0.482
3	61.90	2.042	0.259	0.162	0.425	0.704	0.146	0.137	0.172	0.519
4	63.00	2.194	0.257	0.159	0.445	0.696	0.139	0.138	0.172	0.505
5	60.94	2.069	0.261	0.164	0.413	0.687	0.148	0.144	0.180	0.528
6	61.53	2.448	0.266	0.162	0.456	0.730	0.148	0.160	0.195	0.535
7	65.80	2.207	0.292	0.169	0.450	0.665	0.141	0.152	0.196	0.536
8	65.90	2.261	0.263	0.157	0.456	0.726	0.148	0.157	0.200	0.444
9	59.58	2.414	0.263	0.162	0.449	0.721	0.159	0.154	0.187	0.500
10	68.07	2.240	0.260	0.154	0.474	0.796	0.142	0.155	0.212	0.520
11	74.20	2.163	0.269	0.159	0.439	0.743	0.137	0.129	0.173	0.480
12	69.87	2.423	0.260	0.153	0.444	0.787	0.142	0.149	0.204	0.477
13	76.67	2.430	0.265	0.154	0.452	0.811	0.138	0.140	0.183	0.490
14	74.43	2.153	0.258	0.144	0.434	0.762	0.136	0.133	0.175	0.478
15	71.99	2.427	0.284	0.156	0.485	0.835	0.137	0.154	0.205	0.519
16	73.91	2.404	0.274	0.146	0.487	0.846	0.130	0.150	0.199	0.506
17	73.40	2.520	0.279	0.150	0.504	0.852	0.138	0.156	0.209	0.500

All the meristic characters are presented as proportions of SVL.
The population numbers correspond to those in Table 1.

DISCUSSION

Although this study was not designed to allow direct evaluation of allele frequencies in natural populations, some inferences concerning intrasubspecific and intersubspecific variation can be made, particularly among populations represented by the proposed three subspecies, *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis* by Liang and Wang (1976).

The protein phenotypes are treated as genotypes, although this commonly accepted transformation has not been tested from breeding tests. Lewontin and Hubby (1966) and Selander *et al.* (1971) gave evidence supporting that the electrophoretic patterns for most proteins were similar to those of their homologues in studies that have included breeding tests. Therefore, those individuals possess the same protein phenotype may be regarded as the same genotype. Since the electrophenotypes of the protein at Pt-3, Pt-4 and Ldh-1 loci in this study show little or no overlapping, they can serve as diagnostic characters to distinguish *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis* (Tables 2 and 6). Populations 7 and 14 are puzzling to interpret because they are not consistent with the populations to which they should belong, as far as the Pt-3^e, Pt-3^f and Pt-4^a alleles are concerned. Individuals in population 7 along with populations 6, 8 and 9 were captured at the elevation above 1100 m (Table 1). Morphologically, the individuals in these populations show distinct taxometric distance (MSD) from the individuals captured in the low land areas (Fig. 6). It is possible that the individuals at the high elevation have begun morphological as well as biochemical divergence, and population 7 is an example. However, population 7 is most closely related genetically to populations 15, 16 and 17. Until further information about the phylogenetic lineage and distributional patterns of the genus *Japalura* in Taiwan is available, the relationship of population 7 with populations 15, 16 and 17 can not be determined. According to Kano (1928), and Liang and Wang (1976), Hengchun (恆春) where the individuals (population 14) were collected is the major locality in which *J. s.*

TABLE 6
The comparison of diagnostic characters at Pt-3, Pt-4 and Ldh-1 loci in 17 populations of the three subspecies of *Japalura*

Locus	Allele	*J. s. swinhonis*									*J. s. formosensis*				*J. s. mitsukurii*			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pt-3	e	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
	f	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−
Pt-4	a	−	−	−	−	−	−	+	−	−	+	+	+	+	−	+	+	+
Ldh-1	a	M	M	M	M	M	M	M	M	M	−	−	−	−	P	P	P	P
	b	−	−	−	−	−	−	−	−	−	M*	M*	M*	M*	P	P	P	P

\+ represents a banded phenotype in every individual in a population.
− represents a bandless phenotype in every individual in a population.
M represents monomorphism at Ldh-1^a allele in a population.
M* represents monomorphism at Ldh-1^b allele in a population.
P represents polymorphism at Ldh-1 locus in a population.
The population numbers correspond to those in Table 1.

mitsukurii occurs. However, population 14 is distinct morphologically and biochemically from the other *J. s. mitsukurii* populations (15, 16 and 17). It is suggested that population 14 may be undergoing a differentiating process as indicated by the high mean genetic distance (D=0.144) compared with the other populations of *J. s. mitsukurii.*

The possibility of hybridization is excluded due to the fact that there is no overlapping found in Pt-3, Pt-4 and Ldh-1 loci. Possible hybridization between *J. s. swinhonis* and *J. s. formosensis* was suggested by Liang and Wang (1976) to occur at Wushe (霧社); however, the data from populations 9 and 10 indicate that such a hybridization is not likely. Collections of individuals of *J. s. formosensis* and *J. s. mitsukurii* at the possible contact areas were not made. However, non-overlapping of frequencies at Ldh-1 locus suggests that hybridization between *J. s. formosensis* and *J. s. mitsukurii* is not likely, either (Gorman and Shochat, 1972). Lin and Lu (1982) showed that *J. s. formosensis* has a restricted home range of 33.5 m^2 for males and 11.5 m^2 for females, and the males have a strong territoriality. Thus a japalure population may restrict itself in a very small area without having any contact with other populations. Furthermore, the signatural display which is important as an isolating mechanism, may be unique and distinct for each of these three subspecies of the genus *Japalura* in Taiwan (Wei and Lin, 1981 and pers. comm.—S. Y. Wei). These evidences support the unlikelihood of hybridization in these three subspecies.

However, the three major clusterings derived from the meristic phenogram are quite different from those derived from the biochemical phenogram. It reveals the uncertain nature in using morphometric characters for systematics in this genus *Japalura* in Taiwan.

Genetic distances between populations, subspecies and species have been studied in many genera (Ayala *et al.*, 1974; Hedgecock and Ayala, 1974; Gorman *et al.*, 1975; Adest, 1977), and are given in Table 7. The mean genetic distance between the populations of the three clusterings of the genus *Japalura* (D=0.081) is within the amounts of genetic differentiation found in lacertids and anoles shown in Table 7. The mean genetic distance between the clusterings (D=0.3353) qualifies them for the species level (Table 7).

Morphological variance is the external manifestation of underlying biochemical difference between organisms. Theoretically, a comparison of biochemical similarity and difference should give the taxonomist more sensitive identification of relationship than gross morphology. The present study indicates that minor

TABLE 7
Genetic distance (D) in populations, subspecies and species of various genera (cit. from Adest, 1977)

Genus	D between populations	D between subspecies	D between species	References
Drosophila	0.030	0.230	1.060	Ayala *et al.*, 1974
Taricha	0.005-0.053	0.109-0.253	0.280-0.590	Hedgecock and Ayala, 1974
Anniella	0.003	0.100	0.250	Bezy *et al.*, in prep.
Lacerta	0.001-0.120	—	0.420	Gorman *et al.*, 1975
Anolis	0.000-0.100	—	0.200-0.500	Soule and Gorman, in prep.
Uma	0.0005	—	0.040-0.290	Adest, 1977
Japalura	0.081	0.3353*	—	Present study

* Genetic distance between subspecies of *Japalura* is as high as 0.3353, which may represent the species level.

differences may be detected more precisely by biochemical techniques than by morphological observations.

Biases and sources of error inherent in electrophoretic comparisons of protein polymorphism are well documented (Lewontin, 1974). Because of the uncertainty of the loci and the small number of proteins studied, the genetic variation may be overestimated. It is proposed that the three subspecies, *J. s. swinhonis*, *J. s. mitsukurii* and *J. s. formosensis* be maintained until more studies are made. The populations of the high elevation areas throughout the island should be the targets for detailed studies for the future comparisons.

Acknowledgements: We thank Drs. Jen-lieh Wu and Paul S. Alexander for their critical reading of the manuscript. The study was partially supported by the National Science Council, ROC.

REFERENCES

ADEST, C.A. (1977) Genetic relationships in the genus *Uma* (Iguanidae). *Copeia* **1977**: 47-52.

AMES, G.F.L. (1974) Resolution of bacterial protein by polyacrylamide gel electrophoresis on slabs. *J. Biol. Chem.* **249**: 634-644.

AQUADRO, C.F. and J.C. PATTON (1980) Salivary amylase variation in *Peromyscus*: Use in species identification. *J. Mamm.* **61**: 703-707.

AYALA, F.J., M.L. TRACEY, D. HEDGECOCK and R.C. RICHMOND (1974) Genetic differentiation during the speciation process in *Drosophila*. *Evolution* **28**: 576-592.

DAVIS, B.J. (1964) Disc electrophoresis-II: Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121**: 404-427.

EVANS, M.C., L.L. HUANG and W.D. DAWSON (1977) Genetic variation of amylase in deer mice. *J. Hered.* **68**: 313-316.

GORMAN, G.C. and D. SHOCHAT (1972) Multiple lactate dehydrogenase alleles in the lizard *Agama stellio*. *Experientia* **28**: 351-353.

GORMAN, G.C., D. SHOCHAT, M. SOULE, S.Y. YANG and E. NEVO (1975) Evolutionary genetics of insular Adriatic lizards. *Evolution* **29**: 52-71.

GÜNTHER, A. (1864) *The reptiles of British India*. London.

HEDGECOCK, D. and F.J. AYALA (1974) Evolutionary divergence in the genus *Taricha* (Salamandridae). *Copeia* **1974**: 738-747.

HJORTH, J.P., A.J. LUSIS and J.T. NIELSON (1980) Multiple structural genes for mouse amylase. *Biochem. Genet.* **18**: 281-302.

KANO, T. (1928) *Zoological studies of the Mt. Nitaka of Formosa*. pp. 12-13.

KELLER, A.J. and T.A. LYERLA (1977) Lactate dehydrogenase polymorphism in the spring peeper, *Hyla crucifer*. *Copeia* **1977**: 691-694.

LAEMMLI, U.K. (1974) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.

LEWONTIN, R.C. and J.L. HUBBY (1966) A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics* **54**: 595-609.

LEWONTIN, R. C. and J. L. HUBBY (1974) *The genetic basis of evolutionary change.* Columbia Univ. Press, New York.

LIANG, Y. S. and C. S. WANG (1976) Review of the genus *Japalura* (Lacertilia: Agamidae) found from Taiwan. *Quart. J. Taiwan Mus.* **29**: 153-189.

LIN, J. Y. and K. H. LU (1982) Population ecology of the lizard, *Japalura swinhonis formosensis* (Sauria: Agamidae) in Taiwan. *Copeia* **1982**: 425-434.

MARKERT, C. L. (1964) Cellular differentiation—An expression of differential gene function. In *Congenital malformation.* The International Medical Congress, New York.

MONTANUCCI, R. R. (1974) Convergence, polymorphism or introgressive hybridization? An analysis of interaction between *Crotaphytus collaris* and *C. reticulatus* (Sauria: Iguanidae). *Copeia* **1974**: 87-101.

MORRISON, W. J. and J. E. WRIGHT (1966) Genetic analysis of three lactate dehydrogenase isozyme systems in trout: Evidence for linkage of genes coding subunits A and B^1. *J. Exp. Zool.* **163**: 259-270.

MOYER, F. H., C. B. SPEAKER and D. A. WRIGHT (1968) Characteristics of lactate dehydrogenase isozymes in amphibians. *Ann. N. Y. Acad. Sci.* **151**: 650-669.

NEI, M. (1972) Genetic distance between populations. *Amer. Nat.* **106**: 283-292.

NIELSON, J. T. (1974) Pancreatic amylase polymorphism in the house mouse. A model involving several loci. *Hereditas* **78**: 322.

OKADA, Y. (1937) Studies on the lizards of Japan. Contribution II. Agamidae. *Sci. Rept. Tokyo Bunrika Daigaku,* **51 B**: 83-94.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON and J. B. GENTRY (1971) Biochemical polymorphism and systematics in the genus *Peromyscus.* I. Variation in the old-field mouse (*Peromyscus polionotus*). *Studies in Genetics VI. Univ. Texas Publ.* **7103**: 49-90.

SHAW, C. R. and R. PRASAD (1970) Starch gel electrophoresis of enzymes—A complication of recipes. *Biochem. Genet.* **4**: 297-320.

SICK, K. and J. T. NIELSON (1964) Genetics of amylase isozymes in the mouse. *Hereditas* **51**: 291-296.

SNEATH, P. H. D. and R. R. SOKAL (1973) *Numerical taxonomy.* W. H. Freeman & Co., San Francisco.

SOKAL, R. R. and P. H. D. SNEATH (1963) *Principle of numerical taxonomy.* W. H. Freeman & Co., San Francisco and London.

STEJNEGER, R. (1898) Collection of batrachians and reptiles from Formosa and adjacent islands. *J. Coll. Sci. Imp. Univ. Tokyo* 12, It. (3): 215-225.

VAN-DENBURGH, G. (1912) Concerning certain species of reptiles and amphibians from China, Japan, the Loo-Choo island and Formosa. *Proc. Calif. Acad. Sci.* (ser. 4) **3**: 187-258.

WANG, C. S. (1962) The reptiles of Botel-Tobago. *Quart. J. Taiwan Mus.* **15**: 141-191.

WEBER, K. and M. OSBORN (1969) The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**: 4406-4412.

WEI, S. Y. and J. Y. LIN. (1981) Behaviroal study of *Japalura swinhonis formosensis* (Sauria: Agamidae). *Tunghai J.* **22**: 33-48.

以生化方法探討臺灣攀木蜥蜴屬 (*Japalura*) 的分類

羅 昇 光　林 俊 義

臺灣及其鄰近島嶼，蘭嶼和綠島所產的飛蜥科 (Agamidae) 攀木蜥蜴屬 (*Japalura*) 蜥蜴，近時重被釐訂爲三個亞種，分別爲斯文豪氏攀木蜥蜴 (*J. s. swinhonis*)，箕作氏攀木蜥蜴 (*J. s. mitsukurii*) 和臺灣攀木蜥蜴 (*J. s. formosensis*)。由於本屬蜥蜴個體在外型與體色呈大幅度的變異性，其分類仍存許多的爭論。本文檢視 9 個基因座間的遺傳差異性，以探討本屬的分類問題。

本研究自 16 個地區採得 17 個族羣樣本，它們的肌肉蛋白質 (muscle protein) 與乳酸去氫酶 (lactate dehydrogenase) 的電泳型 (electrophenotype) 中之 Pt-3^c, Pt-3^f, Pt-4^a, Ldh-1^a 與 Ldh-1^b 等對偶基因所表現的電泳帶 (band) 可作爲鑑別特徵，用以區分斯文豪氏攀木蜥蜴、箕作氏攀木蜥蜴和臺灣攀木蜥蜴等三個亞種。根據生化分析所得的遺傳相似值 (I)，經用 UPGMA (unweighted pair-group method using arithmetic averages) 方法所導出的生化系統樹圖，顯示這 17 個族羣可區分爲三大羣聚，此一結果恰和現今所分成的三個亞種相吻合。經由外型測值所導出的外型相似性系統樹圖，亦可將此 17 個族羣區分成三大羣聚，但其組成則與生化系統樹圖有所不同，只顯示出地理上鄰接的族羣在外型上的關係而已。

在 Pt-3, Pt-4 與 Ldh-1 等基因座上發現的蛋白質電泳型的不重疊性，足以排除這三個亞種在一些交會地區產生雜交的可能性。

統計得到此三大羣集的平均族羣間之遺傳距離值 (D=0.081)，介於已知的族羣級遺傳距離值中，但是這三大羣聚間之平均遺傳距離值 (D=0.3353)，則已可劃分在種級上。

本文擬議，攀木蜥蜴屬暫仍維持三個亞種的分類，有關高海拔山區的族羣極待進一步的研究，以確定攀木蜥蜴屬之分類地位。