Morphological Variation in *Hynobius naevius* (Caudata: Hynobiidae) in Kyushu, Japan

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Abstract: Color patterns and body sizes of a total of 108 specimens of *Hynobius naevius* from 21 localities of Kyushu were compared. Three distinct morphotypes, which were geographically separated from each other, were recognized. The northern Kyushu type has no white mottling pattern on the dorsal surface of the head or trunk. The central Kyushu type and the southern Kyushu type have a prominent white mottling pattern over the whole body surface, but the former is remarkably larger than the latter. The northern Kyushu type has relatively short limbs and wider head as compared with the central Kyushu type. The three types seem to be different evolutionary lineages which have diverged in connection with the geologic history of Kyushu.

Key words: *Hynobius naevius*; Morphological variation; Color pattern; Kyushu; Japan

Hynobius naevius lives in mountainous regions of southwestern parts of Japan: western Honshu, Shikoku, and Kyushu. Remarkable variations in size and color pattern have been observed in this salamander species. Sato (1943: 203–207) pointed out that specimens from the northern part of Kyushu were similar to those of the Chugoku district in many external characters. On the other hand, specimens from southern Kyushu resembled those from Shikoku and the southern Kinki district. He ascribed the geographic variation of this species to the geologic history of these areas (Sato, 1943: 448–449). That is, the inner and outer zones of the island arch of Japan, divided by the median tectonic line, have a different geologic history and hence the populations of *H. naevius* in the same zone share many characteristics, whereas those in the different zones differ from each other.

The senior author has reported morphological variation of *H. naevius* collected in Kyushu, and suggested that the specimens of Oita district differ from both northern and southern Kyushu specimens (Sato, 1961, 1974). In the present study, we examined a total of 108 specimens from 21 localities and found that three, instead of two, types were recognizable in *H. naevius* of Kyushu. These are easily distinguishable from each other by their size and color pattern, and their distribution and divergence are discussed in connection with the geologic history of Kyushu Island.

MATERIALS AND METHODS

Specimens were collected in the breeding season, mostly late March to late April, at Yokoyama, Munakata-shi (N=6), Mt. Inunaki (N=6), Aka, Aka-mura (N=5), Mt. Sangun (N=4) (Fukuoka Prefecture), Kawachi, Tosu-shi (N=7) (Saga Prefecture), Mt. Futago (N=13), Himetsubaki, Hita-shi (N=3), Yamaura, Kusu-machi (N=8), Yoshiibu, Kokonoe-machi (N=3), Wakasugi, Yufuin-machi (N=6), Yunohira, Yufuin-machi (N=3), Kitaotsuru, Shonai-machi (N=4), Mt. Jinkakuji (N=6), Mt. Sobo (N=4), Mt. Katamuki (N=14) (Oita Prefecture), Mitate, Hinokage-machi (N=3), Mt. Osuzu (N=1) (Miyazaki Prefecture), Kaminomae, Soyo-machi (N=2), Mt. Ohzeki (N=1) (Kumamoto Prefecture), Fuke, Oguchi-shi (N=7), and Mt. Shibi (N=2) (Kagoshima Prefecture) (Fig. 1). In the text, names of populations are indicated by the names of municipalities or mountains, except Yunohira.

Specimens were anesthetized with 10⁻⁴% MS222 (tricaine methanesulfonate), and total length, head length (from tip of snout to gular fold), trunk length (from gular fold to anterior edge of vent), tail length (from anterior edge of vent to tip of tail), head width, forelimb length (from posterior corner of arm-trunk junction to tip of longest finger), and hindlimb length (from posterior corner of leg-trunk junction to tip of longest toe) were measured with calipers. The significance test for mean values followed Student's t-test adjusted by the sequential Bonferroni method (Rice, 1989), and that for

FIG. 1. Map of Kyushu showing the collecting sites of *H. naevius*. a: Munakata. b: Inunaki. c: Aka. d: Sangun. e: Tosu. f: Futago. g: Hita. h: Kusu. i: Kokonoe. j: Yufuin. k: Yunohira. l: Shonai. m: Jinkakuji. n: Sobo. o: Katamuki. p: Hinokage. q: Osuzu. r: Soyo. s: Ohzeki. t: Oguchi. u: Shibi. Broken line A is the median tectonic line and line B is a tentative borderline between the northern Kyushu and central Kyushu type populations. The area between the two lines corresponds to the Beppu-Shimabara graben.

ratios the Mann-Whitney U test.

RESULTS

Two types of geographically segregated mottl-

ing pattern were readily recognized. The ground color of *H. naevius* of Kyushu is dark violet to purplish black on the dorsal side and light purplish gray on the ventral side. All specimens from the five populations of northern Kyushu, Munakata, Inunaki, Aka, Sangun, and Tosu had silverly white mottling pattern on the sides of the trunk, venter, and tail; there were no white marks on the back except on the tail (Fig. 2A). Because the five northern Kyushu populations were indistinguishable from each other by their mottling pattern, we call them the northern Kyushu type. On the other hand, most specimens from Oita Prefecture and southern Kyushu had prominently developed white pattern over the whole surface of their body (Figs. 2B, C). Specimens from southern Kyushu populations tend to be more heavily and finely mottled than those from Oita populations. Our collecting sites ranged from about 200 to 900 m in altitude, and we confirmed that the color pattern was unrelated to altitude.

Although Oita and southern Kyushu populations were similar in their mottling pattern, they differ remarkably in size. The eight populations of Oita Prefecture were decidedly larger than the eight populations from southern Kyushu (Fig. 3). Statistical tests revealed significant differences between Oita and southern Kyushu populations (Table 1). Figure 4 is a scatter diagram of principal component analysis (PCA) based on the correlation matrix. The first principal component (PRN-1) had an eigenvalue of 5.573 (proportion; 0.80) and the second (PRN-2) had a value of 0.502 (proportion; 0.07). In the first component, all of the seven variables had eigenvectors between 0.360 and 0.412, indicating

FIG. 2. Dorsal (left) and ventral view (right) of color pattern of three types of *H. naevius* from Kyushu. A: Northern Kyushu type (specimen from Aka). B: Central Kyushu type (Shonai). C: Southern Kyushu type (Oguchi). Scale 5 cm.

FIG. 3. Hindlimb length (A), trunk length (B), and total length (C) of 21 populations of *H. naevius*. Horizontal line shows the range, vertical line the mean, outer rectangle one standard deviation on either side of the mean, and dotted rectangle one standard error on either side of the mean.

that the X-axis is the vector relating to the body. Forelimb length and hindlimb length had large vectors (0.525 and 0.595, respectively) and head width had a negative vector (-0.516) in the second component, indicating that the Y-axis relates to the shape; relatively longer limbs and

FIG. 4. A scatter diagram showing the distribution of 108 specimens of *H. naevius* in PCA. Squares, circles, and crosses represent the northern, central, and southern Kyushu specimens, respectively.

narrower head give higher scores along the Y-axis.

Specimens from Oita and southern Kyushu overlap only slightly in the scatter diagram, and

FIG. 5. A scatter diagram showing the distribution of 46 specimens of *H. naevius* of the central Kyushu type. Futago (open circle), Hita (plus), Kusu (open square), Kokonoe (triangle), Yufuin (solid circle), Yunohira (cross), Shonai (solid square), and Jinkakuji (reverse triangle).

TABLE 1. Results of significance test on mean values of total length (upper right) and forelimb length (lower left) of 19 populations of *H. naevius* in Kyushu. Levels of significance are shown by symbols **($p < 0.01$), *($p < 0.05$), and — ($p > 0.05$). Names of localities are abbreviated to the first three letters.

	Mun	Inu	Aka	San	Tos	Fut	Hit	Kus	Kok
Mun		—	**	*	**	—	—	—	—
Inu	—		—	—	—	—	—	—	—
Aka	—	—		—	—	—	—	—	*
San	—	—	—		—	—	—	—	—
Tos	—	—	—	—		*	—	**	*
Fut	—	—	—	—	—		—	—	—
Hit	—	—	—	—	—	—		—	—
Kus	—	*	*	—	*	—	—		—
Kok	—	—	—	*	*	—	—	—	
Yuf	—	—	—	—	—	—	—	—	—
Yun	—	—	—	—	—	—	—	—	—
Sho	—	*	—	*	*	—	—	—	—
Jin	—	—	—	—	—	—	—	—	—
Sob	*	*	—	—	—	**	—	**	*
Kat	**	*	*	—	—	**	—	**	**
Hin	—	—	—	—	—	*	—	*	*
Soy	*	—	—	—	—	**	—	**	**
Ogu	**	—	—	—	—	*	—	**	*
Shi	**	*	*	—	*	**	—	**	*

we named them the central Kyushu type and the southern Kyushu type, respectively. Although the two types overlap partly in the diagram, the adjacent populations did not overlap, that is, the morphometric variation did not follow a cline from north to south, or from east to west. Correlation coefficient between latitude of collecting site and each body part was not significant in the eight central Kyushu populations or in the eight southern Kyushu populations. Correlations between altitude and body size were not significant, either. Within the central Kyushu type, the eight populations could not be separated clearly from each other by PCA (Fig. 5) or canonical discriminant analysis, and the same was true for the northern and southern Kyushu type populations. Although the northern and central Kyushu type overlap rather widely in Fig. 4, specimens from Aka and those from Hita or Kusu did not overlap.

The northern Kyushu type had relatively short limbs and wider head as indicated by lower distribution along the Y-axis of Fig. 4. The ratios of forelimb length to body length (head-trunk length) in the five northern Kyushu type populations were 0.205–0.230 (median=0.217) and those of hindlimb length 0.239–0.279 (median=0.260), in contrast to the ratio of forelimb length of 0.227–0.254 (median=0.234) and the ratio of hindlimb length of 0.269–0.306 (median=0.288) in the eight central Kyushu type

populations. Mann-Whitney U test revealed a highly significant difference ($p < 0.01$) between northern and central Kyushu populations in both ratios. Regression analyses revealed that the relationships of limb length and head-trunk length differed significantly ($p < 0.01$) between northern and central Kyushu populations, thus eliminating the possibility that the difference was due to allomorphic effects. Similarly, the regressions of head width and head-trunk length differed significantly ($p < 0.01$) between the two types. The southern Kyushu type populations were intermediate in limb length and head width relative to head-trunk length.

DISCUSSION

Sato's (1943) distinction between northern and southern populations of *H. naevius* in Kyushu was reconfirmed in the present study with more numerous specimens. However, the populations from Oita Prefecture, where he could not obtain specimens, are clearly different from both northern and southern populations, and thus three types of *H. naevius* are recognizable in Kyushu. The northern Kyushu type is characterized by the absence of white mottling pattern on the back. It has shorter limbs and wider head relative to head-trunk length. The seven body parts measured and the relative limb length of this type do not differ significantly from those of *H. naevius* of Hiroshima

(TABLE 1. Continued.)

Yuf	Yun	Sho	Jin	Sob	Kat	Hin	Soy	Ogu	Shi
**	*	—	—	**	**	*	**	**	**
—	—	—	—	*	**	—	*	—	*
—	—	*	—	—	*	—	—	—	*
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—	—	**	—	*	*	—	—	—	*
—	—	—	—	**	**	*	**	**	**
—	—	—	—	—	*	—	—	—	—
*	—	—	—	**	**	*	**	**	**
*	*	—	—	*	**	—	**	*	*
—	—	*	—	*	**	—	*	—	*
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—	—	—	—	**	**	*	*	**	*
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**	*	**	**	—	—	—	—	—	—
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*	*	*	*	—	—	—	—	—	—
—	—	**	*	—	—	—	—	—	—
*	—	*	*	—	—	—	—	—	—

(Student's t-test, $p>0.05$; Mann-Whitney U test, $p>0.05$; data for Hiroshima were taken from Sato, 1943). The central Kyushu type is characterized by its large size, relatively long limbs and narrow head. Sato (1943) reported that the largest specimens in 172 specimens he measured was 142 mm in total length. In our central Kyushu type specimens ($N=46$), there were three specimens which were more than 150 mm in total length. Whole body surface of the central Kyushu type is covered with white mottling. The southern Kyushu type is characterized by its small size and fine mottling pattern. Relative limb length and head width are intermediate between the former two types. The body sizes of the Sato's specimen from Mt. Ichifusa (Kumamoto Prefecture), Shikoku and southern Kinki district are within the ranges of the southern Kyushu type populations. Our statistical analyses may be biased by the fact that our specimens were not sexed, because most specimens were released after measurement. However, no significant differences were detected in the body sizes of male and female *H. naevius* of Hiroshima (data from Sato, 1943), and our preserved specimens from Munakata consisting of three males and three females did not show any sexual differences. Because the three types occur separately without coexisting, they evidently represent different evolutionary lineages.

Distribution ranges of the three types have not been clearly delimited. Specimens of *H. naevius* from Mt. Tara and Mt. Kunimi (Nagasaki Prefecture) are apparently of the northern Kyushu type in their color pattern (Matsuo et al., 1982; Matsuo, Personal communication). Yunohara (1967) mentioned briefly that *H. naevius* from the northern part of Kumamoto Prefecture corresponds to the central Kyushu type. It is highly probable that the range of the central Kyushu type extends west into the northern part of Kumamoto Prefecture. Although the northern Kyushu type did not differ from the Hiroshima type in body size, they differ in color pattern, according to the description of Sato (1943). Whether the former type occurs in western parts of Honshu should be examined in future studies. Because we have observed that juveniles of the northern Kyushu type just after metamorphosis have a heavily dotted pattern on the back, the further development of white dorsal pattern may be suppressed by some genetic mechanism in this color morph. The color pattern of adults seems to be a stable character; a male from Mt. Sangun that was reared in the laboratory for eight years did not change its color pattern (Kuramoto, Unpublished observation).

Hasumi and Iwasawa (1987) compared morphological characters of *H. lichenatus* of northern Honshu, and found that several characters

varied clinally from east (the Pacific Ocean side) to west (the Japan Sea side). In *Ambystoma tigrinum nebulosum*, Fernandez and Collins (1988) found a correlation between color pattern of animals and color conditions of habitat, substrate color, and water transparency. Geographohic variation in *H. naevius* of Kyushu is not clinal; i.e., it is not correlated with latitudes or altitudes. Every body part measured showed a negative correlation with altitude, although it was not significant ($p > 0.05$). This suggests that the environmental temperature affects growth or longevity of salamanders. Their color patterns do not seem to correlate with the surrounding color conditions, because we could not find any differences in substrate or other environmental colors of habitats.

According to Sato (1943: 446–449), the salamander fauna of Kyushu reflects the geologic history of the island. In the early Tertiary, Kyushu was separated into two islands by an intervening sea which connected with the Seto Inland Sea. The north and south islands corresponded geologically to the inner and outer zone of the median tectonic line. Salamander species in northern Kyushu are related to those of Honshu (*H. nebulosus*, the Hiroshima type *H. naevius*, and *Andrias japonicus*) while those in southern Kyushu are related to those of Shikoku (the Shikoku type *H. naevius*). Later, Sato (1954) found that another species of outer zone elements, *Pachypalaminus boulengeri*, lives on Mt. Sobo. *Hynobius stejnegeri* is a species endemic to the outer zone of Kyushu. The land mass now constituting central Kyushu, which is termed the Beppu-Shimabara graben, rose during the Quaternary period (Karakida et al., 1992). As the graben rose, the newly emerged land mass was colonized by various animals and plants from both sides, and they diverged under new environmental conditions.

From the above scenario, the central Kyushu type *H. naevius* is rather recent in origin. Whether the uniformity of the central Kyushu type populations reflects their single origin (from north or south) or convergence under essentially the same selection pressure prevailing throughout their distribution range remains to be ascertained. The Beppu-Shimabara graben has a unique floral composition. Suzuki (1959), for example, discussed the dominance of *Rhododendron metternichii* (Ericaceae) in the graben in connection with geologic history and

tectonic structures. The origin of *H. dunni*, which lives in Oita Prefecture and a very limited part of Shikoku, may also be related with these geologic changes of Kyushu.

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要旨 九州産ブチサンショウウオの形態的変異**佐藤眞一・倉本 満・小野勇一**

九州の21地点から採集したブチサンショウウオ108個体の斑紋と体の大きさを比較した結果、地理的に隔てられた3型が区別された。九州北部に分布する北九州型は頭胴の背面に白斑をもたない。大分の中九州型と祖母山地以南の南九州型では白斑がよく発達しているが、前者は大形、後者は小形である。北九州型は中九州型に比して相対的に四肢が短く頭幅は大きい。これ

らの3タイプは系統的に異なり、九州の地史と関連して分化したものと考えられる。

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