A Comparison of Growth Patterns between Large- and Small-sized Rana tagoi (Amphibia: Ranidae) from Kitayama, Kyoto, Japan

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A Comparison of Growth Patterns between Large- and Small-sized *Rana tagoi* (Amphibia: Ranidae) from Kitayama, Kyoto, Japan

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Abstract: In the Kitayama-region of Kyoto city, two types of the brown frog *Rana tagoi* occur syntopically. They are populations of small and large frogs that do not overlap in adult body size and proved to be heterospecific genetically. In order to clarify the cause and significance of this body size difference, we studied the growth pattern in the two populations. For comparison, we also examined an allopatric population of *R. tagoi* from Shishiga-tani, Kyoto, which is genetically very close to the large type, and occurs without co-inhabiting different sized individuals. We examined sections of periosteal bones, and counted the number of LAGs in the bone to determine ages of individuals. We used logistic model and estimated instantaneous growth rate and asymptotic size, to describe growth pattern. Each of the two types from Kitayama exhibited unique growth pattern, and the Shishiga-tani population showed a growth pattern intermediate between those of the two types in asymptotic size. Difference in size of two types is discussed with relation to the character displacement.

Key words: Adult body size; Growth pattern; Longevity; Sexual maturity; Syntopic distribution

Introduction

Sugahara (1990) and Sugahara and Matsui (1992, 1993, 1994, 1995, 1996) reported the syntopic occurrence of two types of the brown frog, *Rana tagoi* in the so-called Kitayama-region, a chain of low mountains in the northern part of the city of Kyoto, Japan. These two types are populations of small and large frogs that do not overlap in adult body size (Sugahara and Matsui, 1993, 1996). Actually, they proved to be heterospecific by allozyme analyses (Sugihara, 2002), and by DNA analyses (Eto et al., 2012): individuals of the large population are not those of the small population with exceeding growth, and not vice versa. However, the cause and significance of this difference in body size are still not clear, but the supposed difference in growth pattern between the two populations is expected to resolve one of the causes. In order to clarify the growth pattern, it is ideal to use the method

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of capture-recapture of marked individuals. Such a method, however, is not practical to get consecutive recapture data for a given individual because the chance of its recapture may be not high or constant, and long period is required to detect lifespan. On the other hand, we can estimate average growth pattern of a population if we know accurate age at a given time for each individual.

Age estimations in amphibians have been made by analysis of body size frequency (e.g., Clarke, 1974), capture-mark-recapture (CMR) data (e.g., Ferner, 2010), and skeletochronology (e.g., Hemelaar, 1985; Kusano et al., 1995a). Of these, analysis of body size frequency is most problematical, because amphibians greatly vary in individual growth pattern (Halliday and Verrell, 1988), and this method is not used in recent growth estimation studies (e.g., Ento and Matsui, 2002). CMR of individuals is not practical as noted above. At present, therefore, the most popular method regarded as accurate for estimating ages of amphibians is skeletochronology (Castanet and Smirina, 1990; Smirina, 1994). This technique counts lines of arrested growth (LAGs) formed in the long bones during the period of hibernation or aestivation, and can be applied to a single digital bone, without giving much damage to specimen. We, therefore, applied this technique to estimate ages in *Rana tagoi* from Kyoto.

Materials and Methods

We used digits from preserved specimens, collected between May 1992 and March 1993, and between January 2000 and September 2001. A total of 166 digits, including those from 36 representatives of the Kitayama small population (30 adult males and six adult females) and 69 of the Kitayama large population (52 adult males and 17 adult females). We had little problem in assigning adult individuals to either size group because most of them were collected during the heterochronous breeding season (see below) and the range of body size did not overlap between the size groups. Additionally, 61 digits from the Shishiga-tani population (32 adult males and 29 adult females) were also analyzed. This population is genetically very close to the Kitayama large population (Sugihara, 2002), but occurs geographically distant from Kitayama, without co-inhabiting different sized individuals. For each of the three populations, sizes of 30 just metamorphosed froglets were also measured as shown below.

Outline of life histories related to growth is as follows: the breeding season is between the end of March to mid May in the Kitayama small population, but from early May to mid June in the Kitayama large population (Sugahara and Matsui, 1997). In the small population, metamorphosis begins mid June with the SVL of metamorphs, 6.9–7.3 (mean=7.1) mm, while in the large population metamorphosis takes place later from mid July with a larger body size (6.9–9.3, mean=8.2 mm). In the Shishiga-tani population, the breeding season begins in early May, and eggs hatch by the end of May; Young completes metamorphosis by early July at the SVL of 6.9–11.4 (mean=8.9) mm, grows to 12–25 mm by mid October, and starts hibernation after November.

As the indicator of body size, we took measurement of SVL using calipers to the nearest 0.1 mm. To determine sex and maturity, we examined the presence or absence of secondary sexual characteristics such as nuptial pads and vocal sacs in males, and degrees of ovarian development and coiling of oviducts in females by dissection, using a stereoscopic binocular microscope when necessary.

We took out the third phalanx from the third finger of each specimen stored in 70% ethanol. Bones were washed in running water for 24 hrs, decalcified in 5% nitric acid for 25–45 min, and washed again in the running water for 24 hrs. Each digit was cross-sectioned (20–26 μm thick) by a freezing microtome and stained with hematoxylin (Mayer's acid hemalum) for 15–30 min. Sections from the central region of diaphysis were selected, and mounted in glycerin after rinsed in the tap water. We examined sections under a light microscope, took the picture for further analysis, and counted the number of lines of arrested growth (LAGs) present in the periosteal bone. To specify the age of each individual frog, the time of capture in the year was taken into consideration. Because no marked individuals later recaptured were available in this study, we could not ascertain that one additional LAG is formed after one overwintering. However, from the previous studies of other population of *R. tagoi* (Kusano et al., 1995a) and of other ranid species (e.g., Khonsue et al., 2000), we consider that the number of LAGs corresponds to the number of winters experienced.

Logistic model $L=a/(1+e^{c-bt})$, often used to estimate amphibian growth (e.g., Khonsue et al., 2001a, b; Tong et al., 2018; Togane et al., 2018), was used to estimate the growth pattern (Sokal and Rohlf, 1995). In this model, L is the SVL (mm) at time t (yr); a is the asymptotic size; c is the constant determined by putting 0 to t; and b is the instantaneous growth rate (Misawa and Matsui, 1999). The size and date at metamorphosis necessary as default values were determined from the results of observations (see above), each as 7.1 mm and 15 June in the Kitayama-small, 8.2 mm and 15 July in the Kitayama-large, and 8.9 mm and 15 July in the Shishiga-tani populations.

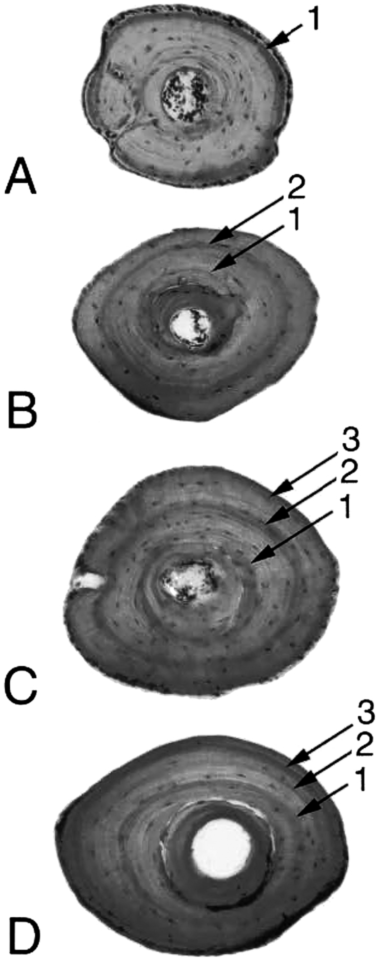

Fig. 1. LAGs detected in phalanges of *Rana tagoi*, Kitayama large type. A: Young collected in June; B: Young collected in August after second overwintering; C: Adult female collected in April soon after the third overwintering; D: Adult male collected in November long after the third overwintering. Not to scale.

Results

Age at sexual maturity and longevity

In the cross-sections of all digit bones examined, there were dense lines each separated by a clear zone and these were interpreted as LAGs (Fig. 1). Destruction of the inner LAGs by a resorption line was found in only a few bones, and it was easy to determine the age.

For each of the three populations, age of each individual was estimated from the date of collection and the number of LAGs, and by assuming average date of metamorphosis as the starting point (t=0). In the Kitayama small population, breeding male was found to have only one LAG. This male was estimated to be 1.83 yr old. In total, ages of breeding males varied from 1.83 to 3.83 yr old, and individuals at 2.83 yr old were most frequently found. In females, all individuals collected in the breeding season were estimated to be 2.83 yr old.

In the Kitayama large population, the youngest male had two LAGs. This individual was captured in non-breeding season, and was estimated to be 2.08 yr old, when date of metamorphosis was taken into account. In individuals collected in non-breeding season, the oldest male and female was 4.67 and 3.75 yr old, respectively. Breeding males consisted of individuals of 2.83 and 3.83 yr old and the former exceeded the latter in number. Breeding females were also 2.83 and 3.83 yr old and the younger exceeded the older in number. An overwintering female estimated to be 2.58 yr old had already mature ova.

In the population from Shishiga-tani, the age of the breeding male varied from 1.83 to 3.83 yr old and individuals at 2.83 yr were most frequent. These results agree with those obtained in the Kitayama small population. Breeding females were 2.83 to 4.83 yr old, including older individual than in the Kitayama populations. However, most frequent was 2.83 yr old, just like the large and small populations from Kitayama. The oldest male and female in the Shishiga-tani population was 4.08 and 4.83 yr old, respectively.

Growth

The growth curves obtained from the logistic model indicate that in the small population from Kitayama, the instantaneous growth rate (b±2SE: 1.823±0.5576 in males and 2.101 ±0.3812 in females) and the asymptotic size (a±2SE: 32.29±1.31 mm in males and 31.93±1.07 mm in females) did not differ between sexes (Fig. 2A). Similarly, sexes of large population from Kitayama did not differ in the instantaneous growth rate (1.947±0.2752 in males and 1.713±0.3319 in females) or the asymptotic size (a±2SE: 51.12±1.27 mm in males and 49.74±3.44 mm in females (Fig. 2B). Likewise, *R. tagoi* from Shishiga-tani showed no sexual dimorphism in the instantaneous growth rate (2.434±0.3773 in males and 2.042±0.3014 in females) and the asymptotic size (42.41±1.70 mm in males and 41.53± 1.47 mm in females) (Fig. 2C).

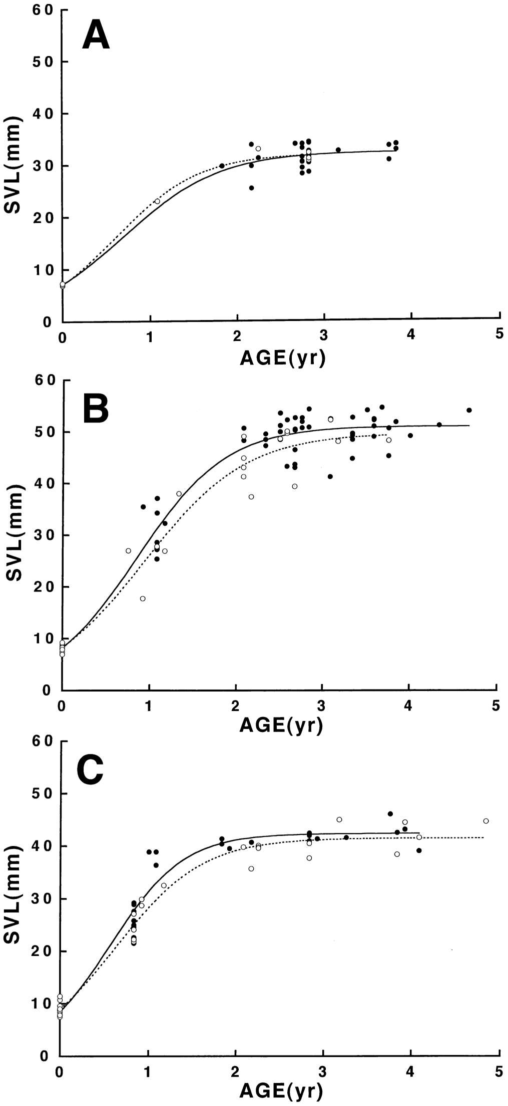

FIG. 2. Growth curve in SVL of *Rana tagoi* estimated by the logistic model. A: Kitayama small type; B: Kitayama large type; C: Shishiga-tani population. Filled circle=male, open circle=female.

From the above results on the three populations from Kyoto, it was estimated that *R. tagoi* has no sexual dimorphism in growth pattern. We therefore combine the sexes to elucidate differences between the three populations (Fig. 3). In the combined sexes in the Kitayama large, the instantaneous growth rate (b±2SE) was 1.764±0.5918, and the asymptotic size (a±2SE) was 51.17±2.13 mm. In the Kitayama small with similarly combined sexes, the instantaneous growth rate was 1.973±0.4983, and the asymptotic size was 32.04±0.90 mm, and the former value did not differ from that in the large population, whereas the latter value

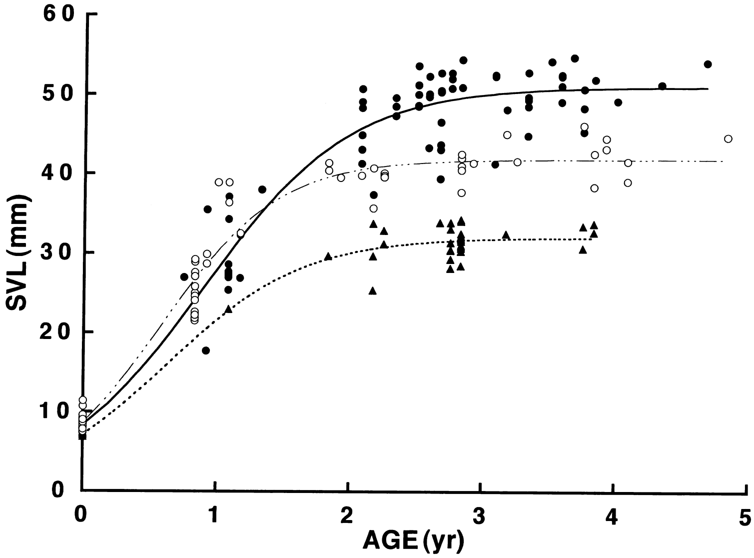

Fig. 3. SVL growth curves estimated by the logistic model of *Rana tagoi*, Kitayama small type (closed triangle), Kitayama large type (closed circle), and Shishiga-tani population (open circle). Sexes combined for calculating growth curves.

greatly differed from that obtained for the large population. In the Shishiga-tani population, combined instantaneous growth rate was 2.292±0.2550, and the asymptotic size was 41.88±1.17 mm. This instantaneous growth rate did not differ from those for the two Kitayama populations. However, the asymptotic size in the Shishiga-tani population was intermediate between those in the latter two populations, differing from both of them.

Discussion

In the Kitayama small population, a part of males sexually matures 1 yr and 10 mo after metamorphosis, but most males are thought to breed in 2 yr and 10 mo, like in the large population. Females were thought to begin breeding at the same age as most males. Survival periods were thought to be three yr, possibly four yr, in males, and two to three yr in females. However, smaller number of females than males examined might have prevented us from detecting older females. Whereas in the large population, many males take two yr after metamorphosis, and first participate in breeding before the LAG indicating the third overwintering appears. Like males, most females sexually mature and ready for breeding by at least 2 yr and 7 mo after metamorphosis. It is also estimated that the survival periods are four years and possibly five years in males, and three to four years in females. Both sexes in the Shishiga-tani population are thought to survive at least four years, and may five years in females.

In this way, most individuals of both sexes in the Kitayama large population seem sexually mature before the third overwintering. This is also the case in the Kitayama small population, although a part of males mature one year earlier. Namely, in these two populations it is not the case that individuals of an identical population with different ages breed in different seasons. Instead, nearly same aged individuals of different populations with different growth rate breed in different seasons.

In the Shishiga-tani population, allopatric with the two Kitayama populations, many breeding individuals were interestingly same in age as in the latter. In *R. tagoi* from Kanto District (Kusano et al., 1995a), the average age of breeding males is 2.9 yr old, nearly identical with the present results. Kusano et al. (1995a) reported the average age of breeding females from Kanto District to be 3.2 yr old, which value is practically not different from those obtained here. In many populations of frogs now collectively called *R. tagoi*, most individuals are thought to sexually mature before the third overwintering, and participate in breeding after emergence from the second hibernation.

This age in breeding *R. tagoi* is not identical with those reported for other Japanese frog species hitherto estimated for ages. Kusano et al. (1995b) reported that males and females of *R. sakuraii* start to breed in 2–4 and 3–5 yr old, later than in *R. tagoi* from Kanto District. In *Pelophylax porosus brevipodus*, males sexually mature in autumn of the year of metamorphosis, while females in the breeding season of the next year (Matsui and Kokuryo, 1984; Khonsue et al., 2002). *Glandirana rugosa* from different environments sexually matures in one year after metamorphosis in both sexes (Khonsue et al., 2001b). Whereas in *P. nigromaculatus*, the age at the first breeding is two yr old in males and three yr old in females (Khonsue et al., 2001a). In this way, ages of sexual maturation and first breeding vary even within Ranidae. *Rana tagoi* seems to be characterized by breeding in older ages and by the absence of sexual dimorphism.

Regarding survivorship, our results indicate that in the Kitayama large population, males and females survive four and three years, respectively; longevity is shorter in the Kitayama small population, three years in males and two years in females. These results suggest shorter life span in females than males, although both sexes were thought to survive four years in the Shishiga-tani population. These results are not much different from those reported for Kanto populations (Kusano et al., 1995a). *Rana tagoi* is relatively short-lived because other Japanese ranids are reported to survive longer (four years in both sexes of *P. porosus brevipodus* [Khonsue et al., 2002]; four years in males and five years in females of *Glandirana rugosa* [Khonsue et al., 2001b]; six years in both sexes of *P. nigromaculatus* [Khonsue et al., 2001a]).

When sexes were combined, the asymptotic size was about 51 mm in the large population, contrasting to about 32 mm in the small population. It is interesting to note that in the Shishiga-tani population, which is genetically close to the large population, the size was about 42 mm, because these results suggest that a population genetically corresponding to the large one and originally stopping growth at about 42 mm in SVL, may possibly be increasing the size in Kitayama, where it co-exists with the small population. Contrariwise, in somewhere a population genetically corresponding to the small one alone is present, its asymptotic size may be larger than in Kitayama. Namely, there is a possibility that character displacement in body size has been operating between the two populations from Kitayama.

Among Japanese anurans, presence of character displacement was first suspected in *Pelophylax nigromaculatus* and *P. porosus* (as *Rana*, Matsui and Hikida, 1985). Actually, it is difficult to demonstrate the occurrence of character displacement with certainty, but Matsui (1994) tried to explain co-existence of two allied species in the *Odorrana narina* complex (as *Rana*) from Sakishima Islands of the Ryukyu Archipelago. Matsui (1994) discussed co-evolution on Ishigakijima and Iriomotejima Islands of *O. supranarina* and *O. utsunomiyaorum* by competition based on co-evolution-invasion hypothesis by Roughgarden et al. (1983). Following ideas of Roughgarden et al. (1983) and Matsui (1994), evolution of the two populations of *R. tagoi* in Kitayama, Kyoto, is thought to have occurred in the following way. First the ancestor of the small population entered the region, and then the ancestral large population with slightly larger body size than the former invaded the region. They began competition and the ancestral small population became smaller and the large population larger as seen now. According to Roughgarden et al. (1983), less competitive ancestral small population could not invade the area where the ancestral large population already occupied. In order to test this hypothesis, future studies on many populations from other regions including those in which allies of the two Kitayama populations occur sympatrically.

It is a unique feature of *R. tagoi* that growth pattern is not sexually dimorphic within a population, resulting in the absence of sexual difference in body size. This tendency is not restricted to the three populations treated in the present study, but is also recognized in a population from Kanto region studied by Kusano et al. (1995a). Generally, anurans show sexual dimorphism in body size, and various discussions have long been made to clarify the reasons (cf. Shine, 1979). All the Japanese ranids shown above, whose ages were estimated (*Pelophylax nigromaculatus*, *P. porosus*, and *Glandirana rugosa*: all as *Rana*), are not exceptions: females are always larger than males. In contrast, *R. tagoi* shows no dimorphism, and Matsui and Matsui (1990) discussed the reason for this in relation to the presence of dimorphism in *R. sakuraii*, which is phylogenetically close to *R. tagoi*, but differs in ecology and behavior. According to these authors, in *R. tagoi*, breeding in the closed environments of underground flowing waters, the body size of females is restricted, and the chance of male-male combat is decreased, resulting in lack of selection towards gigantism in both sexes. The two populations from Kitayama have fundamentally the same reproductive pattern as shown above, but breeding environment is subtly different, and thus might provide good material for testing above hypothesis through detailed future studies.

As shown above, the large population of Kitayama proved to be not an exceedingly grown small population. Combined with the genetic knowledge (Eto et al., 2012), this result indicates independent biological/reproductive relationship of the two populations. Further extensive investigations in other regions are strongly desired to extend this result in the context of taxonomy and conservation biology.

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