Current Herpetology 35(2): 128–131, August 2016
doi 10.5358/hsj.35.128

A Note on Natural Triploidy in a Japanese Brown Frog, *Rana neba* (Anura; Ranidae)

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Abstract: An unusual triploid individual (3N=42) was found in a population of *Rana neba* in nature. The individual had triplets of 14 chromosomes, which are apparently identical to those of diploid *R. neba*. This strongly suggests that this individual is autotriploid, although the mechanism of its emergence remains an open question.

Key words: Chromosome; Karyotype; Triploidy; *Rana neba*; Brown frog

Introduction

Extant amphibians show various karyological characteristics, which are informative in estimating their evolutionary histories. Several polyploid species/populations are observed even in nature, and the origin of such unusual karyotypes is often associated with their speciation and/or interspecific interference (e.g., Stöck et al., 1999; Borkin et al., 2007; Holsbeek et al., 2008).

The brown frog *Rana neba* Ryuzaki, Hasegawa et Kuramoto, 2014 is a close relative of *R. tagoi* Okada, 1928, but the former is known to have a unique number of chromosome for the genus, 2N=28, whereas the latter has 2N=26 chromosomes that are most common among congeners or species of other ranid genera (Kuramoto, 1990). Based on morphological and phylogenetic affinity, *R. neba* is suggested to have originated recently from *R. tagoi* by the chromosome reconstruction (Ryuzaki et al., 2014), although karyological variation in each of these species is still insufficiently studied. During karyological survey on a population of *R. neba*, we found an anomalous karyotype, on which we briefly report below.

Materials and Methods

We examined the karyotype for six specimens of *Rana neba* collected from ca. 250 m a.s.l. of Sakuma Town, Shizuoka Prefecture, Japan, on 27 June 2015. All individuals were prepared with injection of 0.1 mL colchicine solution (2 mg/mL) per gram body weight and were kept for 24 hours at ca. 25C before being sacrificed. Cells from femur bone marrow were treated with 0.075 M hypotonic KCl for 30 min, followed by fixation in Carnoy's solution (acetic acid:methanol, 1:3). Conventional air drying method was applied, and the chromosomes were stained with 3% Giemsa solution for 15 min. Voucher specimens are stored

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in the collection of The Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

Results and Discussion

Of the six frogs examined, five specimens had the karyotype typical of *R. neba* (Fig. 1A): 2N=28 chromosomes consisting of four pairs of large biarmed (1–4), two pairs of medium-sized uniarmed (5–6), and eight pairs of small biarmed elements (7–14) (Ryuzaki et al., 2006, 2014). In contrast, all 13 well-spread metaphase cells obtained from the remaining one specimen (KUHE 55183) had 42 chromosomes (Fig. 1B) that were grouped into 14 triplets (Fig. 1C), each of which had an apparently identical chromosome pair in the karyotype previously reported for *R. neba* and also possessed by the other five individuals examined here. Thus we judged KUHE 55183 to be triploid. The frog was small in body size (25.1 mm in snout-vent length [SVL]) and possessed small testes, indicating the specimen a juvenile male. Taking the growth in SVL of the closely related species *R. tagoi* (Kusano et al., 1995) into account, the triploid frog was supposed to be 1 yr old; i.e., born in 2014.

Spontaneous emergence of triploid individuals in nature is known in several anurans. In the case of European pond frogs, genus *Pelophylax*, triploid hybrids are produced by mating of female *P.* kl. (klepton) *esculentus* and male *P. ridibundus* or *P. lessonae* (Uzzell et al., 1975). On the other hand, bisexually reproducing triploid taxa/populations are known in the green toads, genus *Bufotes*, which are thought to be produced at least partially by autotriploidy (Stöck et al., 2002; Borkin et al., 2007). In tetrapod vertebrates other than amphibians, triploid individuals in nature (almost invariably belonging to squamate reptiles) have usually been associated with parthenogenetic assemblages that had originated from hybridization involving more than one sympatric, phylogenetically close but genetically distinct species (Maslin,

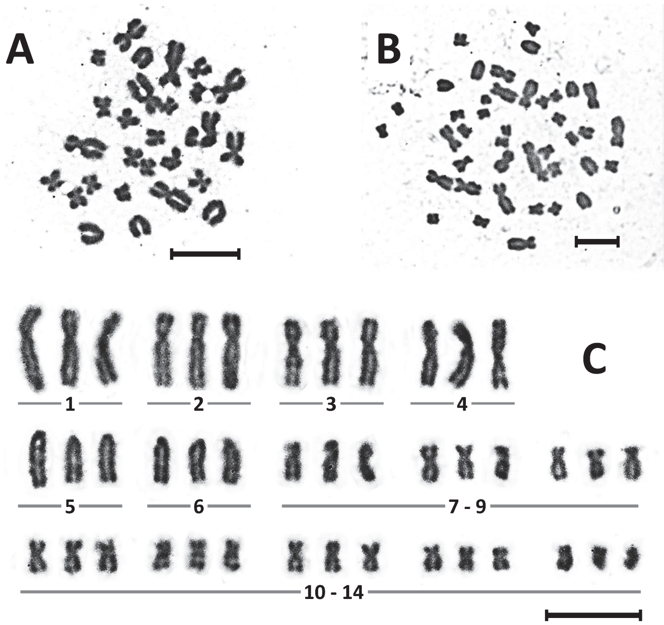

Fig. 1. Chromosome spreads of diploid (A: KUHE 55192) and triploid (B: KUHE 55183) *Rana neba* from Sakuma Town, central Japan. The latter is arranged in triplets following Ryuzaki et al. (2006) (C). In all photographs, scales denote 10 μm.

1971; Darevsky et al., 1985; Dawley and Bogart, 1989). Thus, such individuals almost always exhibit apparent heterogeneity between haploid sets of chromosomes composing a karyotype (e.g., Ota et al., 1991, 1996: but see Moritz and King [1985] for a case of putative autotriploid gecko *Hemidactylus frenatus* found in nature).

In the case of *R. neba*, frequency of triploid individuals around the present site (1/6 in our sample) appears to be low, although the sample size is definitely too small for any way of estimation of such a value with certainty. The karyotype of the present triploid frog seems to share traits of haploid chromosome set with those of diploid *R. neba*. *Rana neba* has a unique karyotype distinct from the karyotype of its close relative *R. tagoi* even in haploid number and gross morphology of chromosomes (2N=28, four large biarmed, two medium-sized uniarmed, and eight small biarmed pairs: vs., 2N=26, five large biarmed and eight small biarmed pairs in the latter species: Ryuzaki et al., 1999, 2006). The other brown frogs distributed in Japan also exhibit 2N=26, typical chromosome number of the genus, or otherwise, 2N=24 (Maeda and Matsui, 1999). If the triploid hybrid was generated by mating between *R. neba* and other congeners, their karyotypes should have 3N=38 (triplet of 12/12/14), 40 (12/14/14 and 13/13/14), or 41 (13/14/14) chromosomes. Thus the present triploid frog should not be the consequence of hybridization between *R. neba* and any of its congeners, but should have been generated by autotriploidy.

The present triploid individual was a male, and this precludes its parthenogenetic origin. *Rana neba* has XX/XY sex chromosomes (Ryuzaki et al., 2006), so the present triploid male should have a combination of XXY or XYY. However, X and Y chromosomes of *R. neba* differ only slightly in size and arm ratio, and thus are difficult to discriminate without applying banding techniques. In this study we applied only conventional Giemsa staining method and could not determine combination of sex chromosomes in the triploid frog. At present, only one male triploid frog is available for examination, so we cannot examine either the frequency of triploidy or the existence of triploid females. Furthermore, because the triploid individual was a juvenile, its fertility remains unclear.

The origin of autotriploidy found in *R. neba* is also unknown. In the laboratory, Nishioka (1972) artificially obtained autotriploid individuals of the pond frog *P. nigromaculatus* by refrigerating fertilized eggs (at 1.0–2.0C for three hours). Thus, the triploid *R. neba* might have hatched from an egg exposed to unusually low temperature. However, because *R. neba* lays eggs in subterranean stream where water temperature is quite stable throughout the year, it seems unlikely that the eggs experienced such a harsh condition. One possible explanation is that the egg accidentally ran out from subterranean stream and was exposed to colder (but nevertheless not lethal) temperatures. The breeding season of *R. neba* around the surveyed area ranges from April to early May (Eto, unpublished data), and the mean daily minimum air temperature during this season is 4.6C in Sakuma Town (Japan Meteorological Agency: http://www.data.jma.go.jp/). However, it was relatively cool in early April of 2014, with the minimum temperatures less than 1.0C on some days, so the colder-than-usual spring climate might have lead to an emergence of the triploid individual reported above. Further studies are needed to clarify mechanisms of production and abundance of triploidy in natural populations of *R. neba*.

Acknowledgments

We wish to thank Masaaki Oda, Tamiko Kondo, and Kenichi Kato for their support in field survey, and Kanto Nishikawa, Norihiro Kuraishi, and Tomohiko Shimada for their technical advices. We also thank an anonymous reviewer and Hidetoshi Ota for improving the manuscript. This work was supported partially by the Sasakawa Scientific Research Grant from the Japan Science Society to KE.

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Accepted: 25 June 2016