

# Origin and Genetic Uniformity of Introduced Population of *Cynops pyrrhogaster* (Amphibia: Urodela) on Hachijojima Island

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**Abstract:** Using mitochondrial DNA sequences, we surveyed genetic features of 12 individuals of *Cynops pyrrhogaster* introduced into Hachijojima Island, in order to estimate their possible origin in the main islands of Japan. The phylogenetic analysis revealed that the 12 examined newts of the introduced population display no genetic diversity and share characteristics with the conspecific population of Shikoku and thus belong to the WESTERN Clade proposed by earlier studies. Our results suggest that the Hachijojima population, having experienced a drastic founder effect, stems from very few individuals from the Shikoku District approximately 650 km to the west.

Key words: *Cynops pyrrhogaster*; Introduced population; Hachijojima; Mitochondrial DNA

## INTRODUCTION

Developments of molecular techniques and accumulations of DNA data of many individuals of a species from various localities allow

us to identify the origin of introduced populations. This information can be useful for the future biological control of exotic species because it might specify their actual transport vector (Lopez et al., 2014). In addition to contributing to the clarification of transportation routes and vectors, the investigation of genetic features of exotic populations might be useful for elucidation of general traits of the exotic

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species because genetic diversity of introduced populations is thought to be an important factor for their potential for self-sustaining establishment in new environments (Suarez and Tsutsui, 2008).

The Japanese red-bellied newt, *Cynops pyrrhogaster*, is naturally widely distributed in the main islands of Japan. Tominaga et al. (2013, 2015) showed that this species displays distinctive genetic variations, and is composed of four distinct clades (NORTHERN, CENTRAL, WESTERN, and SOUTHERN clades) and one additional subclade (Southern Izu Lineage).

This species was introduced into Hachijojima Island (N33°6', E139°47'), ca. 180 km south of the Izu Peninsula of central Honshu, in the 1970s and is now common on the island. Hayashi et al. (1992) surveyed the allozymic features of this population and showed that the allelic composition of this population was similar to those of the populations from Chubu and eastern Chugoku districts of Honshu. The authors also reported the ventral color pattern to be more similar to that of the Chubu population than that present in the eastern Chugoku District. Thus, they tentatively concluded the origin of this introduced population to be somewhere in the Chubu District, which corresponds to the range of the CENTRAL Clade of Tominaga et al. (2013). This time, seeking to reassess the conclusion reached earlier by Hayashi et al. (1992) on the place of origin of *C. pyrrhogaster* population on Hachijojima Island, we surveyed the Hachijojima population through their mitochondrial (mt) DNA sequences as they are deemed very useful in identifying original localities.

#### MATERIALS AND METHODS

We obtained altogether 12 individuals of the newt from three localities on Hachijojima Island. None of the individuals had any morphological abnormalities, sometimes present in individuals from mainland Japan (Meyer-Rochow and Asashima, 1988). We sequenced

a total of 1366 bp of partial mtDNA containing NADH dehydrogenase subunit 6 (ND6), tRNA<sup>Glu</sup>, and cytochrome b (cyt b) genes. Detailed experimental methods are given by Tominaga et al. (2013, 2015). We deposited the newly obtained sequences into DDBJ.

For phylogenetic analysis, we added a total of 174 sequences from DDBJ (AB754594–AB754805, LC016764–LC016765, EU880324–EU880326, AY458597, HQ697272–HQ697273, and HQ697279–HQ697280), including most of the mtDNA dataset in Tominaga et al. (2013, 2015). We constructed phylogenetic trees by maximum likelihood (ML) method also following Tominaga et al. (2013, 2015).

#### RESULTS AND DISCUSSION

The Hachijojima population showed no genetic diversity and only one haplotype (DDBJ Accession number: LC100117) was obtained from the 12 individuals studied.

The optimum substitution models for each partition were selected by Kakusan4 (Tanabe, 2011), based on the Akaike information criterion. For the ML analysis of the mtDNA datasets, J2 (Jobb, 2008) +I+G, TVM (Posada, 2003) +I, and J1 (Jobb, 2008) +I+G were selected as the optimal models for ND6, tRNA<sup>Glu</sup>, and cyt b genes, respectively. The maximum likelihood analysis based on mtDNA sequences generated a topology with  $\ln L = -12,007.02$  and yielded phylogenetic relationships essentially identical to those reported by Tominaga et al. (2013, 2015) who detected four main clades (NORTHERN, CENTRAL, WESTERN, and SOUTHERN clades) and one subclade (Southern Izu Lineage) in *C. pyrrhogaster* (Fig. 1). The phylogenetic analysis revealed that the haplotype detected from Hachijojima Island was included in the WESTERN Clade, which is distributed in Southern Kinki, Western Chugoku, Shikoku, and Northeastern to Central Kyushu districts. The Hachijojima haplotype is genetically especially close to those of Shikoku individuals and different from the others (Fig. 1).

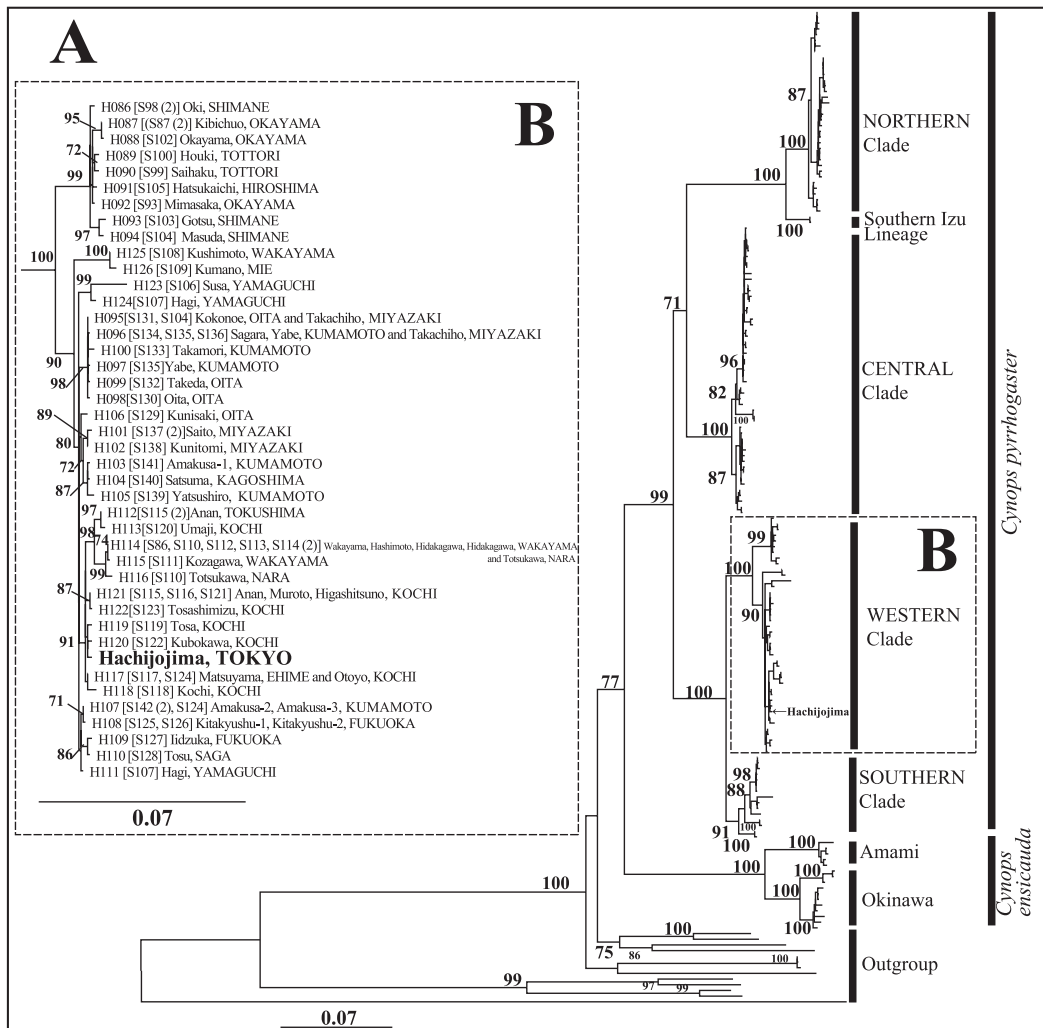


FIG. 1. Maximum likelihood tree of Japanese *Cynops* species and its related species (A) and detailed phylogenetic position of the haplotype of Hachijojima individuals with those belonging to the WESTERN Clade (B) based on 1366 bp of mtDNA sequences. Numbers preceded by “H” indicate haplotype number and those preceded by “S” indicate sample number designated by Tominaga et al. (2013). Nodal numbers represent ML bootstrap supports. Numbers in parentheses after sample number indicate the number of individuals.

Based on these results, we conclude that the population of *C. pyrrhogaster* introduced into Hachijojima Island is derived from individuals of Shikoku populations. Hayashi et al. (1992) reported that the introduced Hachijojima population did not share any alleles in sAat-A, Pgdh-A, and sSod-A with Tohoku, Kanto, or Southern-Kyushu genetic groups, rejecting the possibility of these genetic

groups to be the origin of the Hachijojima population. They further suggested that the Pgm-A allelic composition of the Hachijojima population was similar to that of Chubu and eastern Chugoku populations, but not to that of populations from Western Japan (=populations 9–20 [Hayashi and Matsui, 1988]= Western Japan group [Hayashi, 1993]). They also documented that the ventral coloration of

the introduced Hachijojima individuals was not of the reticulated pattern characteristic of individuals from the eastern Chugoku population (=Sasayama race of Sawada [1963]). Thus, Hayashi et al. (1992) tentatively concluded that the origin of the Hachijojima population was the Chubu District of Honshu, which is inconsistent with our present result.

One could argue that the population surveyed in this study has replaced that surveyed by Hayashi et al. (1992), but it is quite unlikely that multiple independent immigrations and subsequent complete replacements of the populations have occurred in such a remote island. This discordance seems to come from the different analytical methods adopted in the two studies. The conclusion of Hayashi et al. (1992) depended on the allelic composition of allozyme markers, which are highly influenced by the founder effect (bottleneck effect caused by founder event) (Lee, 2002). Our present result indicates that the introduced Hachijojima population had no genetic diversity. This is not at all common for most native populations of this species, which usually show some degree of intrapopulational genetic diversity in mtDNA sequences (Tominaga et al., 2013, 2015) and it leads us to conclude that the introduced Hachijojima population likely originated from small numbers of individuals that subsequently experienced a drastic founder effect. We surmise that this drastic effect had changed their allelic composition in Pgm-A of the introduced population. The ventral color pattern seen in individuals of the WESTERN Clade is not reticulated (Sawada, 1963). Thus, this morphological feature is not inconsistent with our genetic result. A founder event can lead to inbreeding depression (Matute, 2013) and its consequences of lowered resistance and increased abnormalities (Aulstad and Kittelsen, 1971; Lacy and Homer, 1996; Spielman et al., 2004). However, no abnormalities have ever been reported from the now very abundant population of *C. pyrrhogaster* on Hachijojima Island, thought to have arrived there in the 1970s by means still unknown.

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