

学 位 論 文 の 要 旨

論文題目 Identification of genomic diversity and genes under selection in the hot spring frog (*Buergeria japonica*)
(温泉ガエル (リュウキュウカジカガエル) におけるゲノム多様性と適応関連遺伝子の同定)

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Temperature is the paramount environmental determinant governing species diversity and geographical distribution. A significant proportion of the animal kingdom comprises ectotherms, organisms whose body temperature mirrors the ambient temperature, necessitating continual adaptation to thermal fluctuations. Concurrently, organisms persistently strive to broaden their distribution range. One prevalent strategy to facilitate this expansion involves adaptation to extreme temperatures, circumventing competition for niches with other organisms.

As one of the most diverse groups of ectotherms, amphibians are the first species to have landed from the sea. They evolved various thermal adaptations due to their biphasic life cycle concerning aquatic embryonic larvae and terrestrial adult stages. The tree frogs of the genus *Buergeria* have successfully achieved a latitudinal distribution and a wide range of thermal adaptations. For example, the Japanese stream tree frog (*Buergeria japonica*), also known as the hot spring frog, has remarkably adapted to extremely high temperatures of more than 46°C. Widely distributed on oceanic islands of southwest Japan, it has been reported that this species experienced a drifting migration history from Amami Island to the Tokara Islands. During the stepping-stone colonization of Kuchi Island (the northernmost island in the Tokara Islands), the populations were exposed to harsh environmental conditions in the Seranma hot spring, which were implicated in severe natural selection. This biological phenomenon, which connects distribution expansion and environmental adaptation, is predicted to affect the heat tolerance adaptation of *B. japonica*.

Thermal adaptation and developmental speed from the larval stage to metamorphosis are critical biological processes for the survival of amphibians. A previous study attempted to elucidate the critical maximum temperature of *B. japonica*. However, it could not explain their prolonged survival of continuous heat in their natural habitat. Therefore, using a long-term approach, this study conducted a physiological examination through continuous heat exposure. Heat tolerance experiments were performed on *B. japonica* from three populations, including the Seranma hot spring population and two related species (*Buergeria choui* and *Buergeria buergeri*), across various temperatures until metamorphosis. The findings from the long-term experiments indicated a 10 °C difference in high-temperature tolerance between *B. japonica*, *B. choui* and *B. buergeri*, which are presumed to be influenced by the distribution of climate and environment. This study reveals that Seranma hot spring tadpoles have the highest heat tolerance compared to the Amami and Okinawa populations and other species. However, they cannot survive at temperatures above 35°C, which contradicts findings from previous field studies. Additionally, water temperature plays a significant role in influencing the developmental speed of *B. japonica* tadpoles, with the optimal (fastest) developmental speed observed at warmer temperatures (30°C) and reduced rates at both cooler (25°C) and hotter (35°C) temperatures. The connection between ambient temperature and developmental speed is aligned with the thermal response norm and the conventional thermal performance curve. These results underscore the importance of optimal temperature for promoting efficient growth and facilitating habitat expansion, particularly in *B. japonica*.

In addition to the heat tolerance assessments, a behavioral examination was conducted to understand the thermal preferences of *B. japonica* by using a gradient thermal tank. The behavioral assay found that the thermal tolerance and preference may vary depending on the developmental stage. Notably, the tadpoles tended to prefer lower temperatures as their developmental stage progressed. In the early stages, the tadpoles preferred higher temperatures. However, in the later stage, most tadpoles selected lower temperatures. The behavioral findings are consistent with the heat tolerance test, confirming that *B. japonica* is intolerant of continuous heat exposure. Consequently, the tadpoles are thought to seek their preferred temperatures based on their developmental stage. In addition, various environmental factors, such as weather, water flow, food availability, and microbiome composition, may also influence heat tolerance. In summary, a thermal-adapted species such as *B. japonica* has shown a trade-off trait associated with a specific temperature during metamorphosis. The impact of hot water environments on *B. japonica* is significant, as it may alter the physiological and behavioral responses, which is essential to the

population's fitness.

The physiological assays suggest that the Seranma hot spring population exhibits significant heat tolerance. Presumably, either plasticity responses or adaptation across generations may have altered genetic changes that enhance survival in the hot spring environment. To further explore the evolutionary mechanisms underlying the heat adaptation of *B. japonica*, this study represents the first attempt to investigate these phenomena using the reduced representation genome approach, known as genotyping by random amplicon sequencing-direct (GRAS-Di). The datasets of single nucleotide polymorphisms (SNPs) were generated to investigate the population genomics and identify the candidate genes under selection signatures. The results from the population genomic analyses indicate gene flow among populations originating from a common ancestor in the southern island to the northern island, implying that the Seranma hot spring population may experience a severe genetic drift and/or natural selection, which may lead to genetic differentiation. The F_{ST} -based genomic screening estimated 564 significant loci and identified several potential genes related to developmental processes, stress responses, and cardiac function. Besides that, the GRAS-Di method has a limitation that captures only a subset of SNPs and does not encompass the entire nucleotide sequence of the target genome, leaving unknown the nucleotide substitutions that contribute to phenotypic differentiation. Future research utilizing whole genome resequencing and comprehensive genomic sequence comparisons is necessary to elucidate the molecular mechanisms behind the acquisition of high-temperature tolerance and adaptation to hot spring environments.