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Salinity and thermal tolerance of Japanese stream tree frog (*Buergeria japonica*) tadpoles from island populations

Shohei Komaki[1,2], Takeshi Igawa[1,2], Si-Min Lin[3] & Masayuki Sumida[2]

[1]*Division of Developmental Science, Graduate School of International Development and Cooperation, Hiroshima University, Hiroshima, Japan*

[2]*Institute for Amphibian Biology, Graduate School of Science, Hiroshima University, Hiroshima, Japan*

[3]*Department of Life Science, National Taiwan Normal University, Taipei, Taiwan*

Physiological tolerance to variable environmental conditions is essential for species to disperse over habitat boundaries and sustain populations in new habitats. In particular, salinity and temperature are one of the major factors determining species' distributions. The tree frog *Buergeria japonica* is the most widely distributed amphibian species found in the Ryukyu Archipelago in Japan and Taiwan, and uses a wide range of breeding sites. Such characteristics suggest a high salinity and thermal tolerance in *B. japonica* tadpoles. We measured the salinity and thermal tolerance of tadpoles from three islands to determine if physiological tolerance could have contributed to the wide dispersal and survival across different environments. The critical salinity of *B. japonica* was 10–11‰, a value markedly below seawater. We also observed a critical maximum temperature of approximately 40°C, a value which is higher than what is commonly observed for other anuran species. This high thermal tolerance may have favoured island dispersal and survival, particularly in volcanic islands.

Key words: adaptation, geothermal hot springs, physiological tolerances, Ryukyu Archipelago, survival rate, Taiwan, volcanic islands

INTRODUCTION

One of the fundamental factors defining a species' distribution is its physiological tolerances and the connectivity of suitable environment. Among various environmental factors, temperature strongly defines patterns of species distribution and abundance (Somero, 2002, 2005). Distributions of ectotherms in particular are strongly influenced by temperature, as they generally have no mechanisms of thermoregulation (Huang et al., 2006). In addition, the sea is considered the greatest barrier to dispersal of terrestrial and freshwater species because, with the exception of those species adapted to higher salinity, seawater disrupts osmoregulation. Amphibians are particularly sensitive to osmotic stress because of the permeability of their skin, and global amphibian biogeography can be largely explained by sea barriers (e.g. Komaki et al., 2015). Species with wide physiological tolerances are more able to cross environmental boundaries, successfully undergoing range expansions. For example, invasive species often have higher or wider tolerance to environmental factors such as salinity and temperature than either native species or unsuccessful invaders (Moyle & Marchetti, 2006; Pollux & Korosi, 2006; Zerebecki & Sorte, 2011). Studies of invasive species have also emphasised the strong association of environmental tolerance with species' dispersal and survival abilities; thus, studies linking species' physiology and geographical distribution will provide new insights into the formation of patterns of species distributions.

Buergeria japonica (Anura: Rhacophoridae) is a tree frog distributed across the Ryukyu Archipelago in Japan and Taiwan (22–30°N latitude, Maeda & Matsui, 1999). Among various amphibian species occurring in this region, *B. japonica* has the widest range. Presumably as a result of their overseas dispersal (Ota, 1998), *B. japonica* is the only native species inhabiting the volcanic Tokara archipelago (with the also occurring *Microhyla okinavensis* being introduced, Osozawa et al., 2012). *Buergeria japonica* is characterised by breeding in coastal areas, suggesting tolerance to water with high salinity (Maeda & Matsui, 1999); tadpoles have furthermore been recorded from geothermal hot springs, suggesting tolerance to high temperature (Chen et al., 2001; Wu & Kam, 2005). These rather unique ecological characteristics are possible factors to explain why *B. japonica* was able to naturally colonise the Tokara Islands. Although ecological and behavioural characters of *B. japonica* have previously been investigated (Haramura, 2004, 2007a, 2011), little is known about its physiological tolerance levels of tadpoles. Haramura (2007b) found low salinity tolerance of *B. japonica* embryos from Okinawa-Jima. However, tadpoles have not yet been studied, and

Correspondence: Takeshi Igawa (tigawa@hiroshima-u.ac.jp)

salinity tolerance can strongly differ between embryos and larval stages (Gomez-Mestre & Tejedo, 2003). In addition, the wide latitudinal distribution of *B. japonica* may harbour differing degrees of tolerance. While high thermal tolerance of *B. japonica* in geothermal springs was reported in the southern part of their range (Taiwan: Chen et al., 2001; Wu & Kam, 2005), it has yet to be studied whether this also applies to other regions of the species' range. Here, we hypothesise that *B. japonica* has high salinity and thermal tolerances which may have facilitated the formation of the present-day wide distribution of this species. To test this hypothesis, we measured the salinity and thermal tolerances in tadpoles of *B. japonica* from multiple island populations.

MATERIALS AND METHODS

We collected 20 individuals of adult *B. japonica* including 2–3 females from Amami-Oshima Island (Amami, type locality of the species; Stejnegar, 1907), Tokashiki-Jima Island (Tokashiki) and Yilan and Hualien in Taiwan during the summer of 2013 (Fig. 1). We maintained individuals from each population in separate containers in the laboratory where eggs were laid within a week after collection. Eggs were maintained in aerated containers with a water temperature of 20–25°C and a 9:15 light-dark (LD) cycle. Hatched tadpoles remained in the same containers and were fed with fish food (Kyorin Co., Ltd. Hyogo, Japan).

For experiments we used tadpoles at Gosner stages 26–30 (Gosner, 1960). We moved individuals into 1000 mL glass beakers containing 500 mL of tap water from which chloride had been evaporated overnight. Our pilot study showed 100‰ survival of tadpoles from each island. Hence, we kept 15 tadpoles each from the Amami and Hualien populations in beakers at 25°C under a 9:15 LD cycle as controls for an optimum use of tadpoles for the physiological measurements. All tadpoles in the control groups survived throughout the experiment.

We measured salinity tolerance using 160 tadpoles: two sets of 15 individuals from Amami, five sets of 15 individuals from Tokashiki, one set each of 10 and 15 individuals from Yilan, and two sets of 15 individuals from Hualien (Table 1). We kept tadpoles from the 11 groups in individual beakers. Using incubators, we maintained the water in each beaker at 25°C for one day, after which we added lab-grade NaCl (Nacalai Tesque, Inc. Kyoto, Japan) to each beaker every 12 hours to increase salinity from 0 to 12.5‰ through 6.0, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5 and 12.0‰. Because salinity tolerance is known to correlate with body mass in fish (Parry, 1954), we moderately elevated the salinity level (every 12 hours) to minimise the effect of variation in body mass. We ended experiments when tadpoles no longer displayed a righting response (see also Wu & Kam, 2005), which we checked every 12 hours just before adding NaCl. We moved tadpoles with no righting response first into a beaker with lower-salinity water and then into fresh water.

We measured thermal tolerance in 12 groups of 15 individuals (180 tadpoles): five groups from Amami,
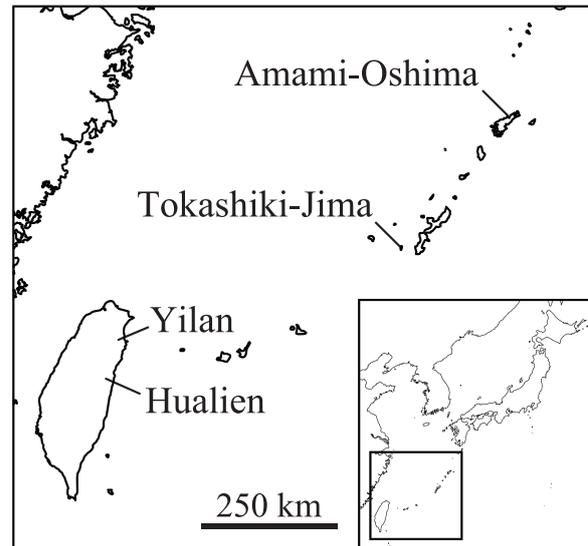
Fig. 1. Collection sites of adult *B. japonica* used in this study.

two groups from Tokashiki, and five groups from Hualien (Table 2). We kept each of the 12 groups in a beaker with the dechlorinated tap water, and after one day at 20°C, we increased the air temperature 1°C every 12 hours using incubators. We monitored water temperatures with electrical thermometers (Japan Pet Drugs Co., Ltd. Tokyo, Japan). As previously, we stopped measuring individuals that no longer showed a righting response. We checked individuals every 12 hours and moved those without a righting response to a beaker maintained in the same incubator that we then placed at room temperature to gently decrease the temperature. Because we aimed to determine the ability of tadpoles of *B. japonica* to live and persist in hot water, we conducted long-term experiments rather than measuring short-term responses as in previous work (Chen et al., 2001; Wu & Kam, 2005). Although the air in the incubator was circulated, we occasionally rearranged beakers at random to minimise any difference in water temperature resulting from their positions in the incubators.

To test if the levels of salinity or thermal tolerances are significantly different among groups, we performed one-way analyses of variance (one-way fixed-effects ANOVA) and pairwise *t*-tests using R (R Core Team, 2015). Tolerance levels of each individual were directly used as input data for the analyses without averaging over groups or populations.

RESULTS AND DISCUSSION

Salinity tolerance

Mean critical salinity in all 11 groups was around 11‰ (Table 1), without significant differences among groups (one-way fixed-effects ANOVA: $F_{10,149}$=1.64, p=0.10) but with significant differences among the four island populations ($F_{3,156}$=3.43, p=0.02). The populations from Amami and Yilan had the lowest and highest salinity tolerances at 10.70±0.82‰ and 11.20±0.41‰ (mean±SD), respectively. Although Hopkins et al. (2013) reported such difference can be caused by maternal identity, the different degree of tolerance observed in

Table 1. Sample sizes and critical salinity levels of *Buergeria japonica* from different populations.

Group/population	*n*	Mean (‰)	SD
Amami 1	15	10.70	1.00
Amami 2	15	10.70	0.62
Tokashiki 1	15	10.97	0.79
Tokashiki 2	15	11.13	0.74
Tokashiki 3	15	10.83	0.52
Tokashiki 4	15	10.67	0.56
Tokashiki 5	15	10.73	0.56
Yilan 1	15	11.10	0.39
Yilan 2	10	11.35	0.41
Hualien 1	15	11.17	0.45
Hualien 2	15	11.00	0.82
Amami (total)	30	10.70	0.82
Tokashiki (total)	75	10.87	0.65
Yilan (total)	25	11.20	0.41
Hualien (total)	30	11.08	0.66

this study may be also caused by population differences linked to the high genetic divergence observed between Taiwan and Amami (Komaki et al., 2014). At ~11‰, the mean salinity tolerance of *B. japonica* was higher than that of *B. japonica* embryos (<5‰: Haramura, 2007b), but substantially lower than that of the seawater-adapted anurans *Bufo viridis* (29‰) and *Fejervarya cancrivora* (39‰, reviewed in Christman, 1974). Several studies found salinity tolerance levels for inland anurans which are similar to our values (e.g., Brown & Walls, 2013; Hua & Pierce, 2013, however with differing methodologies used), for which a certain degree of salt tolerance might be linked to local adaptation to brackish waters and road

de-icing salt (Gomez-Mestre & Tejedo, 2003; Hopkins et al., 2013). While the same might apply to *B. japonica*, it is also known to behaviourally avoid high salinity water (Haramura, 2004).

Thermal tolerance across populations

Across groups, mean critical temperatures ranged from 40.58 to 42.70°C (Table 2). Significant differences were found among groups (one-way fixed-effects ANOVA: $F_{11,168}=75.32$, $p<0.01$) and islands ($F_{2,177}=418$, $p<0.01$). Pairwise *t*-tests with Bonferroni correction indicated significant differences between the Tokashiki population and other populations ($p<0.01$). The lowest and

Table 2. Sample sizes and critical temperatures of *Buergeria japonica* from different populations.

Group/population	*n*	Mean (°C)	SD
Amami 1	15	40.80	0.00
Amami 2	15	40.79	0.32
Amami 3	15	40.65	0.39
Amami 4	15	40.84	0.11
Amami 5	15	40.84	0.11
Tokashiki 1	15	42.49	0.21
Tokashiki 2	15	42.70	0.00
Hualien 1	15	40.58	0.46
Hualien 2	15	40.58	0.46
Hualien 3	15	40.58	0.46
Hualien 4	15	40.58	0.46
Hualien 5	15	40.58	0.46
Amami (total)	75	40.78	0.24
Tokashiki (total)	30	42.60	0.18
Hualien (total)	75	40.58	0.44

highest thermal tolerances were found in individuals from Hualien (40.58°C±0.44 mean±SD) and Tokashiki (42.60°C±0.18), respectively. The two groups from the Tokashiki population independently exhibited high thermal tolerance, suggesting that *B. japonica* is adapted to the high temperatures in Tokashiki.

With means above 40°C, the critical water temperature of each group was relatively high, and similar to values found in subtropical anurans of South America (Duarte et al., 2012). The heating rate is known to negatively affect critical thermal maxima (Rezende et al., 2011), and our slower heating rate and longer experimental duration compared to previous studies may explain why our critical temperatures were below those found by Chen et al. (2001) and Wu and Kam (2005). Our experiment demonstrated that *B. japonica* tadpoles can tolerate unusually high water temperatures on the scale of weeks, as opposed to hours or days. Chen et al. (2001) and Wu & Kam (2005) suggested that high thermal tolerance of *B. japonica* tadpoles may allow its survival in geothermal hot springs in Taiwan. The high thermal tolerance we demonstrated for island populations of *B. japonica* coincides with its sole occurrence on volcanic islands such as the Tokara Archipelago. Thus, high thermal tolerance and the ability to utilise geothermal hot springs as habitat may have facilitated the wide dispersal and survival of *B. japonica* throughout the Ryukyu Archipelago in Japan and Taiwan.

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REFERENCES

Brown, M.E. & Walls, S.C. (2013). Variation in salinity tolerance among larval anurans: Implications for community composition and the spread of an invasive, non-native species. *Copeia* 3, 543–551.

Chen, T.-C., Kam, Y.-C., & Lin, Y.-S. (2001). Thermal physiology and reproductive phenology of *Buergeria japonica* (Rhacophoridae) breeding in a stream and a geothermal hotspring in Taiwan. *Zoological Science* 18, 591–596.

Christman, S.P. (1974). Geographic variation for salt water tolerance in the frog *Rana sphenocephala*. *Copeia* 773–778.

Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., et al. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology* 18, 412–421.

Gomez-Mestre, I. & Tejedo, M. (2003). Local adaptation of an anuran amphibian to osmotically stressful environments. *Evolution* 57, 1889–1899.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190.

Haramura, T. (2004). Salinity and other abiotic characteristics of oviposition sites of the rhacophorid frog, *Buergeria japonica*, in coastal habitat. *Current Herpetology* 23, 81–84.

Haramura, T. (2007a). Microhabitat selection by tadpoles of *Buergeria japonica* inhabiting the coastal area. *Journal of Ethology* 25, 3–7.

Haramura, T. (2007b). Salinity tolerance of eggs of *Buergeria japonica* (Amphibia, Anura) inhabiting coastal areas. *Zoological Science* 24, 820–823.

Haramura, T. (2011). Use of oviposition sites by a rhacophorid frog inhabiting a coastal area in Japan. *Journal of Herpetology* 45, 432–437.

Hopkins, G.R., French, S.S., & Brodie, E.D. (2013). Potential for local adaptation in response to an anthropogenic agent of selection: Effects of road deicing salts on amphibian embryonic survival and development. *Evolutionary Applications* 6, 384–392.

Hua, J. & Pierce, B.A. (2013). Lethal and sublethal effects of salinity on three common Texas amphibians. *Copeia* 3, 562–566.

Huang, S.P., Hsu, Y., & Tu, M.C. (2006). Thermal tolerance and altitudinal distribution of two *Sphenomorphus* lizards in Taiwan. *Journal of Thermal Biology* 31, 378–385.

Komaki, S., Igawa, T., Lin, S.-M., Tojo, K., et al. (2015). Robust molecular phylogeny and palaeodistribution modelling resolve a complex evolutionary history: glacial cycling drove recurrent mtDNA introgression among *Pelophylax* frogs in East Asia. *Journal of Biogeography* 42, 2159–2171

Komaki, S., Igawa, T., Nozawa, M., Lin, S.-M., et al. (2014). Development and characterization of 14 microsatellite markers for *Buergeria japonica* (Amphibia, Anura, Rhacophoridae). *Genes & Genetic Systems* 89, 35–39.

Maeda, N. & Matsui, M. (1999). *Frogs and Toads of Japan, Revised edition.* Bun-ichi Sogo Shuppan, Tokyo, Japan.

Moyle, P.B. & Marchetti, M.P. (2006). Predicting Invasion Success: Freshwater Fishes in California as a Model. *BioScience* 56, 515.

Osozawa, S., Shinjo, R., Armid, A., Watanabe, Y., et al. (2012). Palaeogeographic reconstruction of the 1.55 Ma synchronous isolation of the Ryukyu Islands, Japan, and Taiwan and inflow of the Kuroshio warm current. *International Geology Review* 54, 1369–1388.

Ota, H. (1998). Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications. *Researches on Population Ecology* 40, 189–204.

Parry, B.Y.G. (1954). The development of salinity tolerance in the Salmon, *Salmo salar* (L.) and some related species. *Development*, 425–434.

Pollux, B.J.A & Korosi, A. (2006). On the occurrence of the Asiatic cyprinid Pseudorasbora parva in the Netherlands. *Journal of Fish Biology* 69, 1575–1580.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rezende, E.L., Tejedo, M., & Santos, M. (2011). Estimating the adaptive potential of critical thermal limits: Methodological problems and evolutionary implications. *Functional Ecology* 25, 111–121.

Somero, G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living.

Integrative and Comparative Biology 42, 780–789.

Somero, G.N. (2005). Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* 2, 1.

Wu, C.-S. & Kam, Y.-C. (2005). Thermal tolerance and thermoregulation by Taiwanese rhacophorid tadpoles (*Buergeria japonica*) living in geothermal hot springs and streams. *Herpetologica* 61, 35–46.

Zerebecki, R.A. & Sorte, C.J.B. (2011). Temperature tolerance and stress proteins as mechanisms of invasive species success. PLoS ONE, 6, e14806.

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