

博士論文（要約）

Genetic basis for the adaptation of *Anolis* lizards to different thermal microhabitats

(アノールトカゲにおける異なる温度環境への適応の遺伝的基盤の解明)

平成二十八年度

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Introduction

Ecological niche partitioning that allows phenotypic segregation is one of the most important factors promoting ecological specialization, and results in the coexistence of locally adapted phenotypes (Ravigne *et al.* 2009). Species habitats are composed of multidimensional niche axes, including temperature (Hutchinson 1978). Although temperature is a primary factor limiting performances and survival of organisms, particularly ectotherms (Stevenson 1985), some ectotherms coexist by partitioning thermal habitats using temperature as their niche axis (Ruibal 1961; Cádiz *et al.* 2013; Hertz *et al.* 2013). While the traits associated with thermal biology, such as thermal tolerances, are phylogenetically conserved across lineages (Kellermann *et al.* 2012; Araújo *et al.* 2013; Hoffmann *et al.* 2013; Buckley *et al.* 2015). The mechanisms that explain how these species are able to inhabit distinct thermal microhabitats are not well understood. In addition to an important habitat axis, temperature is one of the major determinants for the geographic range of species (Bozinovic *et al.* 2011) as well as the fates of species in response to recent global warming (Hoffmann & Sgrò 2011). Therefore, revealing the genetic basis underlying mechanisms of adaptation to temperature environments is important not only to understand evolutionary adaptation of ectotherms to thermal environments but also how biodiversity has been or will be maintained locally and globally through time.

Anolis lizards have diversified in the Greater Antilles through the divergence and convergence of arboreal habitat specialists, or ecomorph, across the islands (Williams 1972; Losos 2009). Nevertheless, the concept of ecomorphs alone, which have lead to the production of six types of habitat specialists across four islands, can explain *Anolis* diversity only partially. Several studies indicated the thermal properties of *Anolis* lizards to be evolutionarily labile (Hertz *et al.*, 2013; Cádiz *et al.* submitted), the emergence of thermal habitat specialists might be another important mechanism contributing to *Anolis* diversification. In fact, phylogenetically closely related species in Cuba, *Anolis allogus*, *A. homolechis*, and *A. sagrei*, select the distinct thermal microhabitats among the species, and coexist in the same community throughout Cuban island (Ruibal, 1961; Cádiz *et al.* 2013). *A. allogus* inhabit shaded forests, where direct sunlight barely reaches the forest floor. *A. homolechis* live along the edge of the forests, and *A. sagrei* inhabit open forests or human-made architectures. The latter two species have the access to the sunlight, and their habitats become hotter than the habitat of *A. allogus*. Because of the distinct characteristics in their habitats and behaviors, *A. allogus*, *A. homolechis*, and *A. sagrei* achieve different average body temperatures in the wild, having relatively low, intermediate, and high body temperatures, respectively (Ruibal 1961). In contrast to their thermal ecology, how these species have adapted to different thermal microhabitats and, particularly, its underlying genetic basis are not well understood.

In order to understand the genetic basis of adaptation to different thermal habitats, I studied these *Anolis*

lizards based on the two perspectives: behavioral thermoregulations (**chapter I**), and regulations by gene expressions changes (**chapter II**). In this thesis, I have comprehensively studied the genetic basis of thermal adaptation, and demonstrated the divergence in the genetic basis associated with thermal adaptation in the three *Anolis* lizards.

Chapter I

Among thermoregulatory behaviors, heat avoidance is important for ectotherms to avoid overheating particularly in tropics, where the ambient temperatures stay warm through out the year (Price-Rees *et al.* 2013). The activation of a molecular heat sensor, transient receptor potential ion channel ankyrin 1 (TRPA1), is suggested to elicit heat avoidance behaviors (Rosenzweig *et al.* 2008; Kohno *et al.* 2010; Saito *et al.* 2012). These studies have been conducted only on phylogenetically distant taxa, and how the divergence in the function of TRPA1 and heat avoidance behaviors influence species' thermal habitat selection in phylogenetically closely related species has been unexplored. In chapter I, I have investigated whether behavioral and molecular heat sensitivities have diverged among the three

Anolis lizards, which inhabit distinct thermal microhabitats. First, I have conducted the behavioral experiments to examine whether heat avoidance behavior has diverged among the three Cuban *Anolis* lizards. As an index of heat avoidance behavior, we compared the experimental voluntary maximum (EVM), which is defined as the temperature elicit the first attempts at escaping from heat (Ruibal 1961; Hertz 1981). For the heat source to measure the EVM, we used a hotplate (AS One Digital Hot Plate NINOS ND-3L) that the plate temperature and the rate of raising the temperature are programmable. On the 30 x 40 cm hotplate arena, we placed a box (height 25 cm × width 25 cm × depth 33 cm) to enclose the lizards. The tube floor was made open to let the lizards directly contact with the hotplate. The tube roof was made by metallic mesh (height

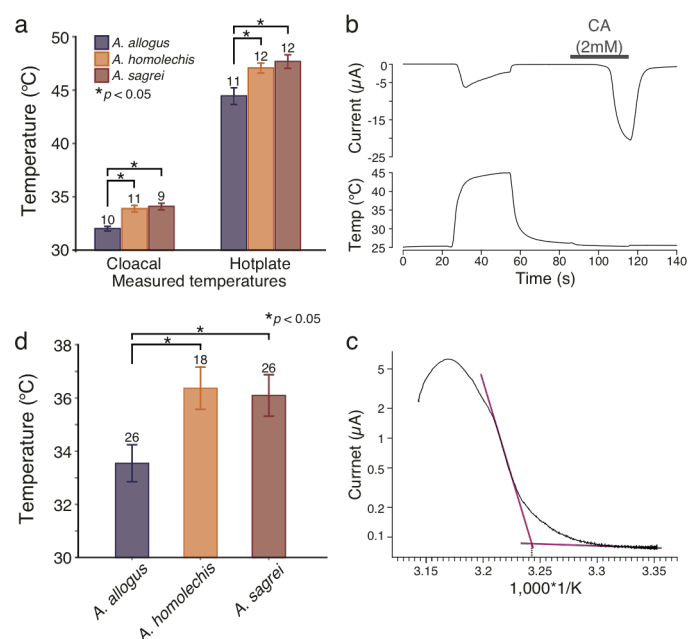


Fig. 1 The behavioral and molecular heat sensitivities of *Anolis* TRPA1. **a**, a comparison of behavioral heat sensitivities, representing the temperatures measured at cloaca of the lizard and at the surface of hotplate. **b**, a representative current and temperature traces for heat and an agonist, CA, stimuli in *A. allogus* TRPA1. **c**, an Arrhenius plot shows thermal threshold of *A. allogus* TRPA1 for the heat stimulus shown in (b). **d**, the mean thermal activation threshold of *Anolis* TRPA1 as estimated by the Arrhenius breakpoints. Sample sizes are indicated on each bar.

20 cm × width 20 cm) with 2.5 cm thick sponge rim so as to be detachable for handling the lizards. The plate temperature was set at 29°C for the first 15 min. to acclimate the lizards to the apparatus. Then, the hotplate was programmed to automatically raise the plate temperature at a rate of approximately 5°C per minute up to 50°C. All the lizard behaviors were videotaped (JVC GZ-EX370). We defined the EVM at which both fore- and hindlimbs of the lizard were off from the hotplate by jumping or climbing the side of the box. We recorded both the hotplate and body temperatures as the EVM. The body temperature of the lizard was measured immediately at the EVM by using a thermometer (Digital Thermometers TX10-02, Yokogawa Meters & Instruments Corporation) with a probe (Yokogawa 245907 Beads T/C Probe Type K, Yokogawa Meters & Instruments Corporation). Rubber gloves were worn. For all the experiments, one individual was tested at a time. Sample sizes for the EVM measurements were from nine to ten per species. The data analyses were performed using R (<http://www.R-project.org/>), with the statistically significant level of $P < 0.05$. In order to examine the EVM among the species, we used analysis of variance (ANOVA). When the differences were statistically significant, we used Tukey's Honest Significant Difference test to identify the comparison(s) that produced significant differences. The result showed that the temperatures eliciting the heat avoidance behavior were significantly lower in *A. allogus* than *A. homolechis* and *A. sagrei*.

Furthermore, after confirming the divergence in TRPA1 amino acid sequences, I heterologously expressed TRPA1 of each *Anolis* species in *Xenopus laevis* oocytes. Then, I recorded ionic currents using two-electrode voltage clamp method to examine the heat sensitivities of *Anolis* TRPA1. For each species, I determined the average temperature threshold for TRPA1 activation by the first heat stimulation, as calculated with Arrhenius plots (Fig. 1b,c). The result showed that the activation temperatures of TRPA1 were significantly lower in *A. allogus* than *A. homolechis* and *A. sagrei* (Fig. 1d).

These results showed the trend, i.e. *A. allogus* as the most heat sensitive species among the three species, as it explains the relationships of thermal microhabitat selections in the wild. This is the first study to show the correlation between behavioral and molecular heat sensitivities in phylogenetically closely related species from distinct thermal habitats.

Chapter II

The genetic basis of physiological mechanisms

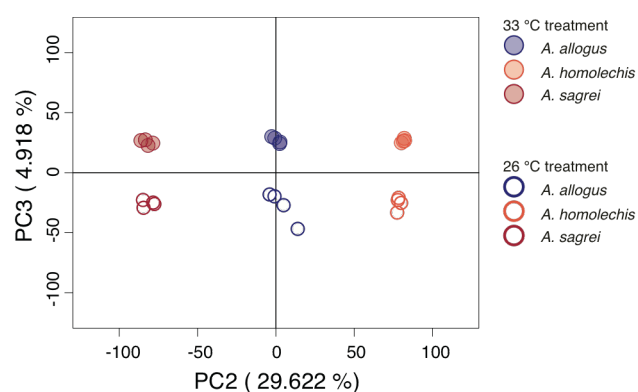


Fig. 2 The principal component analysis for gene expression levels in the brain tissues of the three *Anolis* species. The global gene expression patterns are affected by the incubation treatments at two temperature conditions.

associated with thermal adaptation has been extensively studied with the advent of next-generation sequencing (NGS) technologies (Porcelli *et al.* 2015). The NGS-based studies, particularly transcriptomics or RNA-seq, give insights into the genetic basis of physiological mechanisms from the perspectives of regulatory changes. In chapter II, I have analyzed the gene expression changes in the three *Anolis* lizards to detect genes associated with adaptation to different thermal microhabitats. Prior to perform RNA-seq, I have kept each species under the constant temperatures of 26 °C and 33 °C for five days in the incubators under 12 hours of light and dark cycles. The temperatures of 26 °C and 33 °C were set as they reflect the average body temperatures of *A. allogus* and *A. sagrei*, respectively, in the wild. Poly-A tailed messenger RNA, extracted from the brain, liver, and skin tissues of each species, were analyzed by RNA-seq on the Illumina Hiseq 2000 platform. Then, I analyzed the change in the gene expression levels in comparisons between the samples from 26 °C and 33 °C treatments for each tissue and species. The results showed that the global gene expression patterns were affected by the two temperature treatments (Fig. 2b), and I identified 397, 813, and 774 differentially expressed genes (DEGs), which are the genes whose expression levels were significantly changed, in *A. allogus*, *A. homolechis*, and *A. sagrei*, respectively. Gene ontology (GO) analysis showed that the GO term of translation was enriched in *A. homolechis*, as the large numbers of ribosomal protein genes in the DEGs unique to *A. homolechis* brain were upregulated at 33 °C. The upregulation of ribosomal protein genes under heat stress is suggested as the evidence of upregulating protein synthesis to compensate the damaged proteins and to resist the stress (Quinn *et al.* 2011). Thus, *A. homolechis* may be capable of sustaining functional proteins even under the prolonged exposure to the temperature. The result for *A. sagrei* also implied that *A. sagrei* is capable of sustaining functional proteins but less in degree than *A. homolechis*. On the other hand, *A. allogus* did not show any transcriptional responses that are the indicative of such capability. Furthermore, GO analysis showed that a GO term associated with circadian regulation was enriched by DEGs detected in the brain tissues of all three species. Among the genes in the GO term, a gene, *Nr1d1*, showed the opposite expression patterns between *A. allogus*, a thermoconformer species, and *A. sagrei*, a thermoregulator species. Because the habitat temperature fluctuates more widely in the open habitats than in shaded or closed forests throughout the day, the circadian thermoregulation could also be important for adaptation to distinct thermal habitats. The transcriptomic responses in each species provided several evidences of thermal adaptation in each species. While lizards are considered to be vulnerable to climate warming (Huey *et al.* 2010), the study using NGS to understand the genetic basis of thermal adaptation in terrestrial reptiles has never been conducted as far as reported in the review of Porcelli *et al.* (2015). Thus, this is the first study that provided the information on the genetic basis associated with adaptation to different thermal habitats in the terrestrial reptiles.

Discussion

These findings have demonstrated the genetic divergences associated with thermal adaptation in the three *Anolis* lizards. Both temperatures eliciting heat avoidance behavior and activating TRPA1 were significantly lower in *A. allogus* than in *A. homolechis* and *A. sagrei*. This correlation between behavioral and molecular heat sensitivities in these species suggests that *A. allogus* is genetically constrained to its native or shaded cool forest habitats as opposed to *A. homolechis* and *A. sagrei* that inhabit hotter habitats. In addition, the results from RNA-seq suggest the thermoregulator species, *A. homolechis* and *A. sagrei*, have higher capacity of physiological functions under hotter conditions than the thermoconformer species, *A. allogus*. Therefore, the studies in this thesis provided several evidences of genetic basis important for the adaptation to different thermal habitats.

Hertz *et al.* (2013) indicated thermal biology of *Anolis* species to be evolutionarily labile as various species maintain different body temperatures. While thermal biology is evolutionarily conserved across lineages (Kellermann *et al.* 2012; Araújo *et al.* 2013; Hoffmann *et al.* 2013; Buckley *et al.* 2015), the evolutionarily labile thermal biology is one of the unique characteristics in *Anolis* species. While thermal biology has been extensively studied in *Anolis* lizards (Losos 2009), it has been approached from the studies in the wild or on behavioral experiments, and to what extent the lability is influenced by their genetic background has not been well understood. In this thesis, only three *Anolis* species were studied among the vast diversity in *Anolis* genera, but this is the first study that comprehensively approached the genetic basis of thermal adaptation in *Anolis* lizards. Cádiz *et al.* (submitted) reported that the niche shifting from the closed forest to open habitats has occurred multiple times across Cuban *Anolis* lineages, including the case of the three *Anolis* species studied in this thesis. The studies on other *Anolis* species will provide important evidences to understand the generality of the genetic basis revealed in this thesis, and also to explain the importance of thermal adaptation as a mechanism of *Anolis* diversification. The genetic basis of thermal adaptation unraveled in this thesis will be a foundation stone to understand how biodiversity has been formed, maintained, and respond to the recent climate changes.