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Source: Zoological Science, 42(1) : 13-24

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zs240060

ZOOLOGICAL SCIENCE **42**: 13–24 (2025)

[REVIEW]

Evolution of Temperature Receptors and Their Roles in Sensory Diversification and Adaptation

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Among various environmental factors, temperature is one of the critical factors for organisms since it can affect most, if not all, biological processes. Therefore, animals precisely sense ambient and body temperatures and physiologically and behaviorally respond to temperature changes. Taking such nature into consideration, alteration of thermal perception should have played a pivotal role in adaptation to diverse thermal niches. Temperature as well as other physical and chemical stimuli are perceived by the primary afferent neurons where transient receptor potential (TRP) channels are expressed, and these channels serve as multimodal receptors in the somatosensory system. To understand the roles of TRP channels in the evolution of sensory perception, comparative analyses have been performed using various animal species, and their functional diversity has been well documented over the past 2 decades. Furthermore, in recent years, species differences in the thermal responses of TRP channels have been found among closely related species inhabiting different thermal niches, which have uncovered the contributions of TRP channels to environmental adaptation in various vertebrate species. The purpose of this review is to summarize the studies that addressed the functional evolution of TRP channels associated with sensory diversification and environmental adaptation.

Key words: temperature and chemical receptor, thermosensitive TRP channel, behavioral response, thermal tolerance, environmental adaptation, vertebrates

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INTRODUCTION

Environmental adaptation is the process by which organisms alter their traits to better perform in new environments or in response to changes in their current habitats. Sensory systems convey information that is required for animals to respond to changes in environments, thereby playing a pivotal role in adaptive evolution. Various sensory systems, including vision, olfaction, taste, hearing, and somatosensation, have developed during animal evolution, and changes in these systems directly affect the way animals respond to environmental changes (Baldwin and Ko, 2020; Valencia-Montoya et al., 2024).

Among the variety of environmental factors, temperature critically affects most, if not all, biological processes, and thus each animal species evolved physiological and behavioral systems to better survive in their thermal habitats. Indeed, the association between thermal tolerance of species and environmental temperature is well documented in a wide variety of animal species (Araujo et al., 2013; Sunday et al., 2019). In addition to changes in thermal tolerance, thermal perception, which is essential for monitoring ambient and body temperature, should also have changed during the adaptation processes.

Temperature as well as other physical and chemical stimuli are perceived by the primary afferent neurons, and transient receptor potential (TRP) channels, which are activated by temperature, chemical, and other physical stimuli, serve as multimodal receptors in the somatosensory system (Patapoutian et al., 2003; Julius, 2013). To understand the functional diversity of thermosensitive TRP channels, comparative analyses have been performed using various animal species, and their species differences have been well documented over the past 2 decades. Furthermore, associations between the functional variation of thermosensitive TRP channels and environmental adaptation have been recently elucidated in phylogenetically different groups of animal species (Bagriantsev and Gracheva, 2015; Laursen et al., 2015; Saito and Tominaga, 2015, 2017). Moreover, the structural bases for functional changes in TRP channels have also been uncovered in some cases. The purpose of the present review is to summarize the studies that have illuminated the evolutionary aspects of TRP channels associated with sensory diversification and environmental adaptation.

THE PHYSIOLOGICAL ROLES OF TRP CHANNELS IN SENSORY PERCEPTION

In many animal species, changes in ambient temperature are perceived by free nerve endings of sensory neurons

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doi:10.2108/zs240060

located beneath peripheral tissues such as skin. Temperature stimuli are converted to electrical signals, which are transduced to the central nervous system, where sensory signals are processed. The temperature-sensitive TRP channels expressed in peripheral sensory neurons serve as thermal receptors (Patapoutian et al., 2003; Julius, 2013). Most TRP channels are non-selective cation channels that modulate the permeation of sodium and calcium ions. The activation of TRP channels leads to the influx of cations, which causes membrane depolarization and leads to action potential generation in peripheral sensory neurons (Fig. 1A). TRP channels form a gene family that can be classified into multiple subfamilies based on sequence similarity and domain structure. In vertebrates, the TRP superfamily contains seven subfamilies: TRP canonical (TRPC), TRP vanilloid (TRPV), TRP melastatin (TRPM), TRP ankyrin (TRPA), TRP polycystin (TRPP), TRP mucolipin (TRPML), and TRP NOMP (TRPN) (Pedersen et al., 2005). Of note, TRPN is well conserved in a wide variety of animal species except for amniotes (Schuler et al., 2015).

In mammals, the dorsal root ganglion (DRG) and trigeminal ganglion (TG) serve as peripheral sensory neurons, where various TRP channels are expressed (Patapoutian et al., 2003; Julius, 2013). Mice and humans possess 28 and 27 ion channels, respectively, belonging to the TRP superfamily (with TRPC2 pseudogenized in humans), 11 of which are temperature-sensitive in both species (Fig. 1B) (García-Ávila and Islas, 2019; Kashio and Tominaga, 2022). These channels are activated by a varying range of temperatures and roughly cover the entire thermal range perceived by mammals (Fig. 1C). TRP channels are multimodal receptors that are activated by physical stimuli other than temperature, as well as chemical compounds. In addition to the sensory neurons, thermosensitive TRP channels are expressed in various tissues and involved in diverse physiological functions associated with temperature (Guler et al., 2002; Patapoutian et al., 2003; Moqrich et al., 2005; Talavera et al., 2005; Dhaka et al., 2006; Bandell et al., 2007; Uchida and Tominaga, 2011; Uchida et al., 2011; Kashio et al., 2012; Nilius et al., 2012; Julius, 2013; Wang and Siemens, 2015; Laing and Dhaka, 2016; Sun et al., 2016; Bernal et al., 2021; Kashio and Tominaga, 2022).

Recent developments in cryo-EM have facilitated the characterization of the tertiary structures of TRP channels. TRP channels are comprised of four subunits each of which possesses six transmembrane domains and relatively large intracellular N- and C-terminal regions (Pedersen et al., 2005; Cao et al., 2013; Liao et al., 2013; García-Ávila and Islas, 2019). Each subunit possesses a selective filter in the loop region between the fifth and sixth transmembrane domains, and the ion permeation pathway is formed at the center of the four subunits. The composition of the domain structure characterizes each subfamily of TRP channels. Multiple ankyrin repeat domains are found in the channels belonging to the TRPV, TRPC, and TRPA subfamilies. Especially, a long stretch of 16 ankyrin repeat domains is present in the TRPA1 channel (Paulsen et al., 2015). The TRP box located near the sixth transmembrane domain exists in the channels belonging to the TRPC, TRPM, and TRPV subfamilies. These domain structures are related to the regulation of TRP channel activity. For example, intracellular components such as ATP and calmodulin interact with ankyrin repeat domains and/or a part of the C-terminal region of TRP channels to regulate the channel activity (Julius, 2013; Laing and Dhaka, 2016). Phosphatidylinositol 4,5-bisphosphate (PIP_2) binds to the pocket formed by the transmembrane domains and regulates TRP channel activity (Rohacs, 2023). Recent studies have provided insight

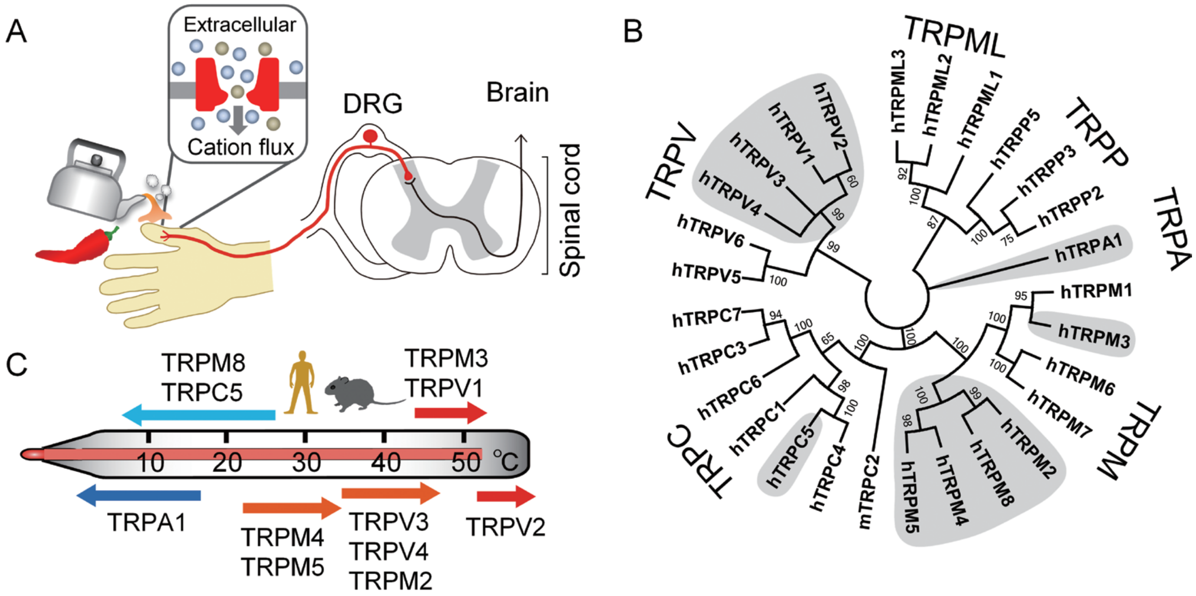


Fig. 1. Physiological roles of TRP channels in thermal perception. **(A)** Schematic structures of the thermosensory system. The activation of TRP channels leads to the cation flux that triggers the action potential generation in sensory neurons such as dorsal root ganglia (DRG). **(B)** Phylogeny of human TRP channels. Eleven thermosensitive TRP channels that have been identified in humans and rodents are shaded. Note that mouse TRPC2 was included in the phylogenetic tree because the orthologous gene in humans is pseudogenized. **(C)** Thermal ranges that activate each TRP channel in humans and rodents. Directions of temperature stimuli are indicated by arrows.

into the molecular basis of temperature-dependent gating of TRP channels. Large conformational changes have been observed in several regions of TRP channels, including the linker regions of transmembrane domains, the ankyrin repeat domains, and intracellular N- and C-terminal regions in rat TRPV1, mouse TRPV3, and *Drosophila* TRPA1. It has been proposed that conformational changes in intracellular regions along with structural alterations in the TRP helix, pore-forming transmembrane domains, and pore-loop, lead to the opening of the channels (Kwon et al., 2021; Nadezhdin et al., 2021; Wang et al., 2023).

EVOLUTIONARY PROCESSES OF TRP CHANNEL GENES

The evolutionary origin of TRP channels can be traced back to the ancestors of eukaryotes. TRP channels have been categorized into multiple subfamilies according to their phylogenetic relationships, and approximately 12 subfamilies are currently recognized (Himmel and Cox, 2020). Although widely shared across various species, the gene repertoires of TRP channels vary extensively among different groups of organisms. Among eukaryotes, animals possess the largest number of TRP subfamilies (TRPC, TRPV, TRPM, TRPA, TRPP, TRPML, TRPN, etc.).

The functional characterization and identification of thermosensitive channels have been mainly performed in model species such as rodents and fruit flies. As mentioned earlier, rodents and humans possess 11 thermosensitive TRP channels (TRPV1-TRPV4, TRPM2-TRPM5, TRPM8, TRPA1, and TRPC5) belonging to four different subfamilies (García-Ávila and Islas, 2019). In fruit flies (*Drosophila melanogaster*), channels belonging to different subfamilies are involved in thermal perception, including TRPC (TRP and TRPL), TRPA (TRPA1, painless, and pyrexia), and TRPP (biribido-1, -2, and -3) (Bellemer, 2015). Therefore, TRP genes involved in thermal perception are largely different between mammals and insects.

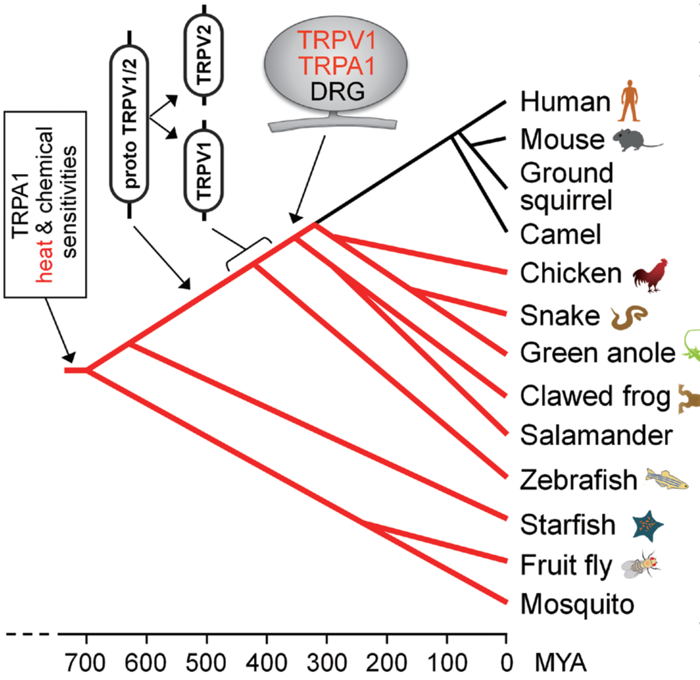
TRPA1 is the only orthologous gene that is likely to be shared between vertebrates and invertebrates (Nilius et al., 2012). Phylogenetic analyses revealed that most thermosensitive TRP channels found in mammals newly emerged no later than in the most recent common ancestor between ray-finned fishes and tetrapods, suggesting that a rearrangement of channels involved in thermal perception occurred during that evolutionary period (Saito and Shingai, 2006; Saito et al., 2011). Variation in the gene repertoire can also be found among vertebrates. For instance, TRPV2, TRPV3, and TRPM8 genes have been lost from several species of ray-finned fishes, while the genes for TRPV1, TRPM2, TRPM4, and TRPA1 have increased in some ray-finned fishes (Saito and Tominaga, 2015). Moreover, several ray-finned fishes possess two copies of TRPV1 and TRPA1 paralogs that potentially emerged through gene duplication events in the most recent common ancestors of ray-finned fishes. The difference in the TRP channel repertoires between ray-finned fishes and tetrapods might be related to adaptation to aquatic or terrestrial environments, respectively. TRPV3, which is predominantly expressed in skin in rodents and humans, is reported to be pseudogenized in aquatic cetaceans, and the loss of this gene is assumed to be related to the land-to-sea transition (Wu et al., 2022). Variation in the gene repertoires has also been found in arthropod lineages. Arthropods generally possess multiple genes in the TRPA subfamily, though the types of TRPA genes vary among arthropod species. For instance, TRPA1, which functions as a heat receptor in *Drosophila*, is absent in several arthropod species including those in the order Hymenoptera, *Daphnia pulex* (water flea), and *Tetranychus urticae* (spider mite), suggesting that it has been independently lost multiple times in arthropod lineages (Matsuura et al., 2009; Peng et al., 2015). These findings suggest that TRP channels evolved by a birth and death process, resembling the genes encoding receptors for other sensory systems such as vision, taste, and olfaction (Baldwin and Ko, 2020; Valencia-Montoya et al., 2024).

FUNCTIONAL DIVERSITY OF TRPA1 AND TRPV1 IN VERTEBRATES

The alteration in the TRP channel properties can directly affect sensory perceptions as they play a pivotal role in signal transductions. Therefore, functional characterization of TRP channels from various species provides informative clues for understanding the diversity in sensory perception. As mentioned above, TRPA1 is widely shared among various animal species ranging from insects to mammals. In addition, it serves as a noxious sensor that is essential for survival in most animals. For these reasons, TRPA1 has been cloned and functionally characterized from a wide variety of animal species using in vitro expression systems (Laursen et al., 2015; Saito and Tominaga, 2017).

TRPA1 was first reported as a cold-sensitive channel found in rats (Story et al., 2003). However, contradictory results have been reported regarding the TRPA1 thermal sensitivity in rodents and humans. Several studies found that TRPA1 is insensitive to cold, whereas others found that it has bimodal activation by both cold and heat, leading to ongoing debates about its role in thermal perception (Bautista et al., 2006; Nilius et al., 2012; Talavera et al., 2020). TRPA1 has also been shown to be activated by chemicals contained in spicy plants such as mustard oil and cinnamaldehyde, as well as environmental irritants such as acrolein and hydrogen sulfide (Bandell et al., 2004; Jordt et al., 2004; Bautista et al., 2005, 2006; Macpherson et al., 2005; McNamara et al., 2007). It is expressed in peripheral sensory neurons derived from DRG and TG that convey noxious signals upon activation.

Functional characterizations of TRPA1 have been conducted in various animal species, both invertebrates and vertebrates. TRPA1 channels in several vertebrates, such as reptiles, birds, and amphibians, have been reported to be activated by heat rather than cold stimulation (Fig. 2) (Saito et al., 2012, 2014). The thermal sensitivity of TRPA1 among teleost fish is relatively complicated. For instance, zebrafish possess two paralogous TRPA1 genes, TRPA1a and TRPA1b (Saito and Shingai, 2006). TRPA1a is predominantly activated by chemical stimulation, whereas TRPA1b is activated by thermal stimulation in a bimodal manner (both heat and cold) (Oda et al., 2016). Pufferfish also possess TRPA1 exhibiting dual activation by both cold and heat stimuli, while medaka TRPA1 is solely activated by heat (Oda et al., 2017, 2018). These findings show that TRPA1 thermal sensitivity varies among vertebrate species. In invertebrates such as



	Thermosensitivity	
	TRPA1	TRPV1
Human	No ?	Heat
Mouse	Cold ? Heat ?	Heat
Ground squirrel	—	No
Camel	—	No
Chicken	Heat	Heat
Snake	Heat	Heat
Green anole	Heat	—
Clawed frog	Heat	Heat
Salamander	Heat	Heat
Zebrafish	Heat & Cold	Heat (TRPV1/2)
Starfish	Heat	
Fruit fly	Heat	
Mosquito	Heat	

— : Not examined

Fig. 2. Evolutionary processes of TRPA1 and TRPV1 and their thermal sensitivity. Thermal sensitivities of TRPA1 and TRPV1 in representative animal species. Note that the thermal sensitivity of TRPA1 in humans and rodents is under debate. Each evolutionary event is indicated along the phylogenetic tree. TRPA1 likely emerged during the early stages of animal evolution as a heat- and chemical-sensitive channel. The gene duplication producing TRPV1 and TRPV2 occurred before the divergence of tetrapods; however, the precise timing has not been estimated. MYA: million years ago.

starfish and insects, TRPA1 is activated by warmth or heat as well as chemical compounds that also activate vertebrate TRPA1 (Viswanath et al., 2003; Sato et al., 2014; Saito et al., 2017). The fact that TRPA1 is activated by thermal and chemical stimulation among various species indicates that it arose as a noxious receptor in the ancestor of animals approximately 700 million years ago (Fig. 2).

TRPV1 has also been characterized from various species. Phylogenetic analyses revealed that TRPV1 emerged in vertebrate lineages (Saito and Shingai, 2006; Saito et al., 2011). TRPV1 is found to be activated by heat in various vertebrate species, including rodents, chickens, snakes, amphibians, and teleosts (Jordt and Julius, 2002; Gracheva et al., 2010; Gau et al., 2013; Yoshimura et al., 2022), with some exceptions mentioned in a later section. In rodents and humans, TRPV1 has been shown to be highly co-expressed with TRPA1 in a subset of DRG and TG neurons (Story et al., 2003). The overlap in expression of the two channels is also found in several vertebrate species such as chickens, clawed frogs, and zebrafish (Prober et al., 2008; Saito et al., 2012). Therefore, the acquisition of TRPV1 in the ancestor of vertebrates resulted in the redundancy of heat receptors in primary sensory neurons (Saito and Tominaga, 2015, 2017), which likely facilitated the functional plasticity of both TRPA1 and TRPV1 in vertebrate lineages (Fig. 2).

TRPV1 was originally identified as a receptor for capsaicin in rodents (Caterina et al., 1997), and the orthologous channels in several mammalian species, including humans, rodents, and dogs, are also reported to possess high sensitivity to capsaicin. In contrast, rabbits, tropical clawed frogs, chickens, and zebrafish possess TRPV1 with lower sensitivity to capsaicin (Jordt and Julius, 2002; Gavva et al., 2004; Phelps et al., 2005; Ohkita et al., 2012; Gau et al., 2013). The structural basis of capsaicin binding to TRPV1 is well clarified and important amino acids have been identified (Cao et al., 2013; Liao et al., 2013; Gao et al., 2016). Among them, several amino acid residues that interact with capsaicin are involved in the species differences in TRPV1 sensitivity to capsaicin. These amino acids are located in the third and fourth transmembrane domains in TRPV1 (positions 512, 550, and 570 in rat TRPV1) (Jordt and Julius, 2002; Gavva et al., 2004; Ohkita et al., 2012; Saito and Tominaga, 2015; Chu et al., 2020).

Production of capsaicin in *Capsicum* species (chili peppers) helps to deter herbivorous mammals such as rodents, and potentially confers a selective advantage of protecting seeds. Meanwhile, the reduced sensitivity of bird TRPV1 to capsaicin may facilitate seed dispersal of capsicum species (Tewksbury and Nabhan, 2001). Occasional reductions in the TRPV1 capsaicin sensitivity in several vertebrate lineages seem to have occurred in a neutral manner. However, the altered TRPV1 sensitivity to capsaicin in tree shrews is likely to be one exception. Tree shrews (*Tupaia belangeri chinensis*) can consume chili peppers as this species possesses TRPV1 with reduced sensitivity to capsaicin (Han et al., 2018). The distribution of *T. belangeri chinensis*, which inhabit South Asia, is largely overlapped with that of *Piper boehmeriaefolium*, a spicy plant that produces a capsaicin analog. It is hypothesized that the reduced TRPV1 sensitivity to capsaicin in *T. belangeri chinensis* confers a dietary advantage to these animals.

THE ROLE OF THERMOSENSITIVE TRP CHANNELS IN A UNIQUE PHYSIOLOGICAL SYSTEM

Some species acquired unique physiological traits that potentially help them to better adapt to thermal environments or confer a specific sensory perception. In some reptilian species, sex is determined by environmental temperature, although sex is genetically determined in most animals. In American alligators (*Alligator mississippiensis*), all offspring become male when eggs are incubated at 33°C, whereas only females are produced from eggs incubated at 30°C. TRPV4, which is activated by warmth, is expressed during a critical temperature-sensitive period that determines the sexual fate in embryonic stages. Notably, treatment of eggs with chemical compounds that regulate TRPV4 activation alter gene expression patterns associated with male differentiation, suggesting that TRPV4 is involved in temperature-dependent sex determination in American alligators (Yatsu et al., 2015).

Evolutionary changes in TRP channels are involved in the independent acquisition of a unique sensory system for detecting infrared radiation in reptiles and mammals. Some snakes, including pit vipers, boas, and pythons, possess the ability to sense infrared radiation through the pit organ located in the head. The pit membrane, which is innervated by TG neurons, detects infrared radiation. Transcriptomic analysis between TG and DRG, which are involved in somatosensation in the head and trunk, respectively, revealed that TRPA1 is highly expressed in the TG in pit-harboring species (Gracheva et al., 2010). In contrast, the expression level of TRPA1 is similar between TG and DRG in snake species without pit organs. Thermal activation thresholds of TRPA1 tend to be lower in pit-harboring snakes compared to snake species that do not possess a pit. Moreover, molecular evolutionary analysis of snake TRPA1 identified a signature of positive selection in the lineage of pit-harboring snakes (Geng et al., 2011; Yokoyama et al., 2011). All these findings suggest that changes in both the expression and thermal sensitivity of TRPA1 are associated with the acquisition of infrared detection in snakes.

Infrared-sensing organs have also been acquired in the mammalian lineage. Vampire bats, which are obligate blood feeders, also sense infrared radiation. They possess leaf pits that are innervated by heat-sensitive neurons responding to temperatures > 29°C, which enable them to find hotspots on endothermic prey. In vampire bats, TRPV1 is found to be involved in infrared detection in leaf pits. Two types of alternatively spliced (AS) variants of TRPV1 have been found in vampire bats. One AS variant (TRPV1-S) possesses a truncated C-terminal region compared to the canonical AS variant (TRPV1-L). The latter AS variant is activated at around 40°C, while the former AS variant is activated by temperatures around 30°C (Gracheva et al., 2011). Both AS variants are expressed in the TG and DRG of vampire bats, while TRPV1-S is predominantly expressed in the TG. TRPV1-S is also found in the TG of fruit bats, which do not have pits, but its expression is considerably low. These observations suggest that TRPV1-S is involved in the acquisition of infrared detection in vampire bats. It is intriguing that investigation of pit organs in divergent groups of vertebrates (pit snakes and vampire bats) revealed that TRP channels are involved in the acquisition of infrared sensation, but different channels underwent functional alterations with different molecular mechanisms.

TRP channels are also related to seasonal polyphenism in insects. Silk moths, *Bombyx mori*, lay dormant eggs to survive the winter season. They also lay non-diapausing eggs, and the production of egg type is determined by the temperature that the mother is exposed to during the embryonic stages. In Kosetsu type silk moths, embryos exposed to 25°C lay dormant eggs when they become adults, whereas embryos exposed to 15°C lay non-diapause eggs in the adult stage. TRPA1 is expressed in the embryonic stage and involved in the above phenomenon. It is activated at temperatures around 25°C, which then induces a dormancy-inducing peptide hormone (Sato et al., 2014). Knock-out of TRPA1 in *B. mori* resulted in the loss of egg type regulation by environmental temperature (Yokoyama et al., 2021).

Silk moths were domesticated from *Bombyx mandarina* 5000–10,000 years ago. *Bombyx mandarina* moths also lay dormant and non-diapausing eggs, but photoperiod, and not environmental temperature, regulates egg-laying phenotype. TRPA1 is also expressed in *B. mandarina* embryos and activated by temperature around 25°C, resembling *B. mori*. Therefore, during domestication, TRPA1 was recruited as a key molecule to regulate the egg-laying phenotype by environmental temperature (Yokoyama et al., 2021).

EVOLUTIONARY TUNING OF TRP CHANNELS IN THERMAL ADAPTATION

Each species evolved the thermal reaction norms to better fit with environmental changes in habitats or to migrate to a novel habitat with different conditions. It is well documented that thermal tolerance, which is defined by the lower and upper thermal limits of species, is correlated with the lowest and highest environmental temperatures of habitats (Sunday et al., 2011, 2012; Araujo et al., 2013). Combined with the shift in thermal tolerance, thermal perception, which is essential for exerting proper physiological and behavioral responses, must have evolved during adaptation processes. In this respect, investigations on TRP channels in environmental adaptation are of particular interest from the perspective of evolutionary biology. Comparative analyses among closely related species that possess similar physiological and ecological traits but occupy different thermal niches have been performed to investigate the involvement of TRP channels in environmental adaptation in the past several years. These studies focus on different groups of animals including amphibians, reptiles, birds, and mammals.

EVOLUTIONARY CHANGES OF TRP CHANNELS AMONG SPECIES INHABITING DIFFERENT THERMAL NICHES IN AMPHIBIANS AND REPTILES

Amphibians generally spawn eggs in water, although spawning seasons and water environments used for breeding vary among species. Due to the variation in such ecological traits, embryos and larvae develop under different thermal conditions, even among species with similar distributions. To understand the mechanisms of thermal adaptation, comparative analysis among anuran amphibian species endemic to Japan has been performed. *Rana japonica*, *Glandirana rugosa*, *Rhacophorus schlegelii*, and *Buergeria buergeri* are four anuran frog species widely distributed across Japan, while *Buergeria japonica* is distributed in the Nansei Islands.

Buergeria japonica lays eggs in various environments; thus, tadpoles of this species can be found in diverse aquatic habitats. *Buergeria japonica* tadpoles are known to possess extreme heat resistance and inhabit even natural geothermal hot springs where water temperatures reach up to 44°C (Chen et al., 2001; Wu and Kam, 2005; Komaki et al., 2016). More generally, the tadpoles can be found in shallow open puddles. In such habitats, water temperatures can reach as high as 40°C on sunny days (Saito et al., 2022). Therefore, *B. japonica* tadpoles are frequently exposed to heat in natural habitats. *Buergeria buergeri* is a congeneric species to *B. japonica* and inhabits mountain streams. However, *B.*

buergeri tadpoles experience warmth since they are distributed in stream edges where water temperature increases in the daytime during summer. In contrast, *Ra. japonica* tadpoles grow in relatively cool waters because eggs are laid in late winter. Tadpoles of this species metamorphose and move to terrestrial environments before summer. Due to the variation in ecological traits, tadpoles of these anuran species likely adapt to different thermal niches.

The association between thermal tolerance and behavioral responses has been examined among the five anuran species mentioned above. The critical thermal maximum (CTmax), defined as the temperature at which loss of righting reflex upon heating occurs, was determined as an indicator of thermal tolerance. The CTmax values ranged from 38°C to 43°C among the five species in the order *Ra. japonica* < *Rh. schlegelii* < *B. buergeri* < *G. rugosa* < *B. japonica* (Fig. 3). Thermal choice assay of tadpoles revealed that avoidance temperatures of tadpoles were arranged in the same order as the CTmax, suggesting that avoidance behaviors and heat tolerance cooperatively evolved during adaptation to different thermal niches (Fig. 3). It is worth noting that the species difference in avoidance temperatures was 2.6 times larger than that seen for CTmax (Fig. 4A). Thus, avoidance behavior tends to flexibly shift to fit with the thermal niches (Saito et al., 2022).

Finally, TRP channel properties were characterized to examine the link between thermal receptors and behavioral responses. TRPA1, a heat-sensitive channel, was cloned from *Ra. japonica*, *B. buergeri*, and *B. japonica*, which possess low, middle, and high avoidance temperatures, respectively. Two types of AS variants of TRPA1 are expressed in these frog species. One is the canonical AS variant and the other possesses a single valine insertion between the sixth and seventh ankyrin repeat domains, which was not reported previously (Saito et al., 2022). The novel and canonical AS variants of TRPA1 are termed TRPA1(V+) and TRPA1(V–), respectively. In *Ra. japonica*, both TRPA1 AS variants were potently activated by heat stimulation. Meanwhile, TRPA1 of *B. buergeri* exhibited different thermal responses. Faint heat-evoked responses can be seen for TRPA1(V–), and only TRPA1(V+) is activated by heat. The reduction in TRPA1 thermal response was prominent in *B. japonica*, as both AS variants almost completely lack heat sensitivity. In contrast, both TRPA1 AS variants are dose-dependently activated by cinnamaldehyde, a well-known TRPA1 agonist in all three species. Normalization of the heat-evoked current amplitude of TRPA1 by the cinnamaldehyde-evoked current amplitude revealed that TRPA1 heat-evoked activity declines in the order *Ra. japonica*, *B. buergeri*, and *B. japonica* for both AS variants (Fig. 4B) (Saito et al., 2022). Thus, TRPA1 activity to heat is maintained at a higher level in the species susceptible to heat exposure, while it is nearly abolished in the species that acquired extreme heat resistance. In addition to variation in TRPA1 heat activity, the thermal sensitivity of TRPA1 differs between *Ra. japonica* and *B. buergeri*. In *Ra. japonica*, the average thermal activation thresholds of TRPA1(V+) and TRPA1(V–) are approximately 37°C and 26°C, respectively, while that of *B. buergeri* TRPA1(V+) is approximately 39°C (Fig. 3). Thus, *Ra. japonica* harbors TRPA1 AS variants with both high and low thermal sensitivity, whereas *B. buergeri* only harbors a TRPA1 AS variant with low thermal sensitivity. Note that the thermal activation threshold of *B. buergeri* TRPA1(V–) and both AS variants of *B. japonica* cannot be estimated due to the near absence of the heat-evoked responses. These results suggest that the activity and sensitivity of TRPA1 to heat shifted to fit with the thermal niches of each anuran species.

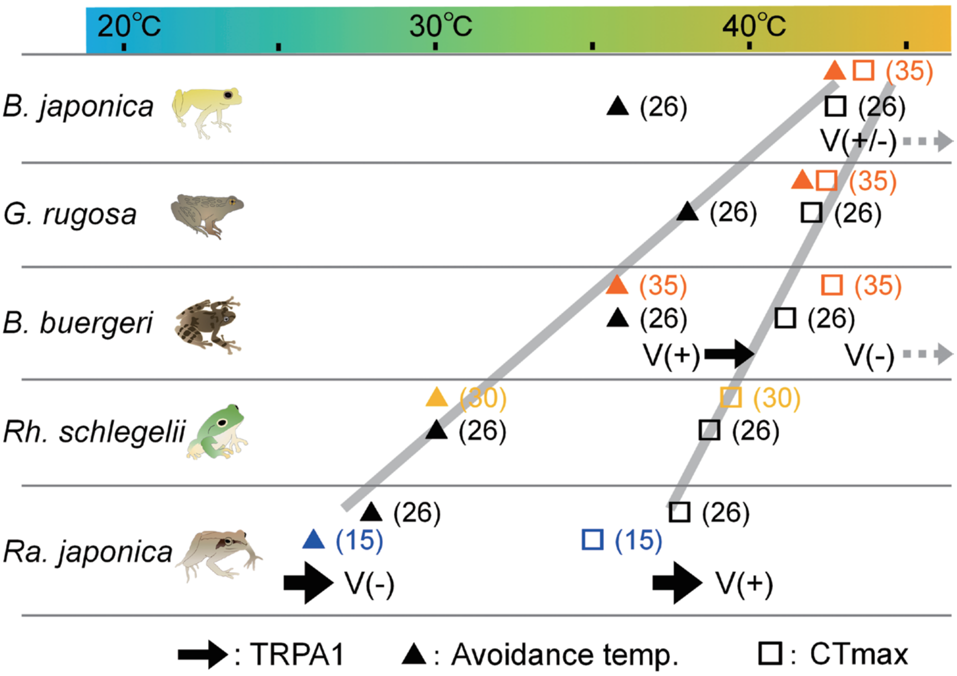


Fig. 3. The relationships between thermal responses of tadpoles and TRPA1 among five frog species. Heat tolerance (CTmax) and avoidance temperature of tadpoles as well as TRPA1 properties of five frog species are summarized. The CTmax and avoidance temperature are indicated by open squares and filled triangles, respectively. Tadpoles were acclimated to different temperatures prior to the behavioral experiment. Acclimation temperatures of tadpoles are shown in parentheses (in °C). The thermal activation thresholds of TRPA1 AS variants are indicated by arrows. The thickness of the arrows represents the activity level of TRPA1 in response to heat, and gray dashed arrows indicate near loss of activity.

To investigate whether the functional alterations occurred in other thermal sensors, the properties of TRPV1 channels were also compared between *B. japonica* and *B. buergeri*. TRPV1 from both species did not respond to heat stimulation alone, although combined stimulation with heat and acid

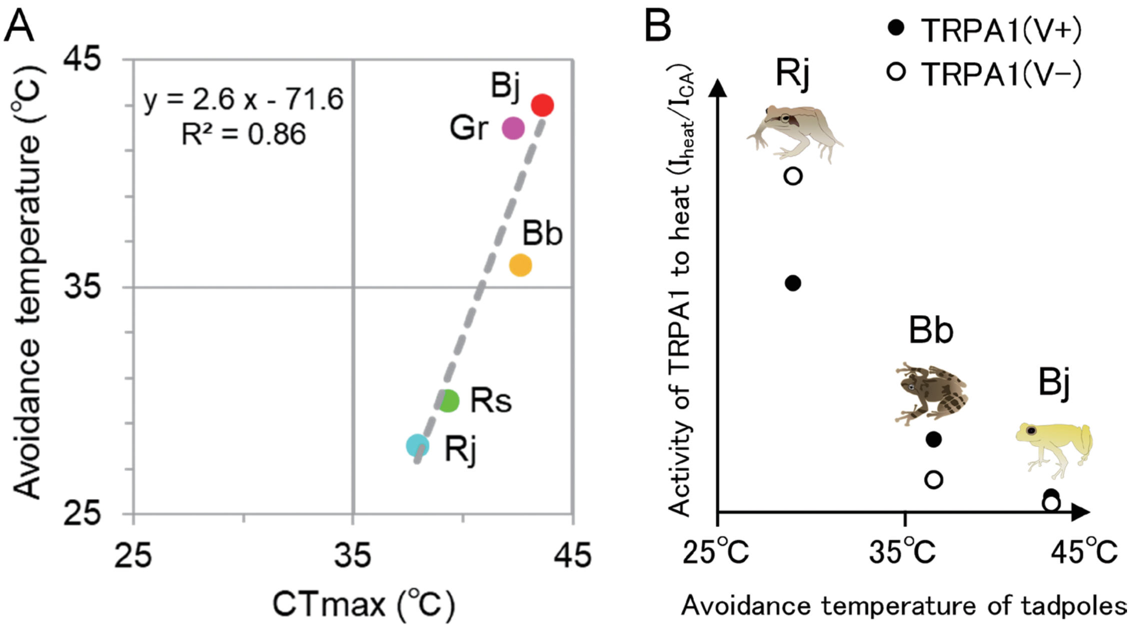


Fig. 4. Correlation between thermal response of tadpoles and TRPA1 among five anuran species. **(A)** Correlation between avoidance temperature and heat tolerance (CTmax) among tadpoles of five anuran species. Rj: *Rana japonica*, Rs: *Rhacophorus schlegelii*, Bb: *Buergeria buergeri*, Gr: *Glandirana rugosa*, Bj: *Buergeria japonica*. **(B)** Relationship between the heat-evoked activity of TRPA1 and avoidance temperatures of tadpoles among three anuran species. The heat-evoked activity was calculated by normalizing the current amplitude induced by heat stimulation (I_{heat}) to that evoked by a saturating concentration (1 mM) of cinnamaldehyde (I_{CA}) in each TRPA1-expressing *Xenopus* oocyte. The heat-evoked activity of TRPA1 declines with avoidance temperatures of species.

evoked apparent responses. No detectable species differences were observed for TRPV1 between *B. japonica* and *B. buergeri*; therefore, TRPV1 may not be related to thermal niche selection in these two species (Saito et al., 2022).

Variation in the TRPA1 thermal responses was also found to be related to the habitat thermal conditions in another group of anuran amphibians that inhabit Africa. *Xenopus tropicalis* and *Xenopus muelleri* are distributed in lowland areas in West Africa with relatively warm conditions. In contrast, *Xenopus laevis* and *Xenopus borealis* are found in relatively cool areas in Africa. A behavioral assay of adult frogs exposed to heat stimulation showed that *X. laevis* started exhibiting nocifensive responses (jumping behaviors) at lower temperatures compared to *X. tropicalis* (Saito et al., 2016). In addition, dissociated DRG neurons of *X. laevis* are also activated by lower temperatures compared to those of *X. tropicalis*. Thermal responses of TRPA1 were compared among the four *Xenopus* species, and TRPA1 heat-evoked activity of *X. laevis* and *X. borealis* was higher than that of *X. tropicalis* and *X. muelleri* (Saito et al., 2019). Therefore, the association between heat-evoked activity of TRPA1 and habitat thermal conditions has been identified in two distantly related groups of anuran amphibians.

The channel properties of TRPV1 have also been compared among four *Xenopus* species, revealing species differences in response to heat. The heat responses of *X. tropicalis* TRPV1 gradually increased with repeated heat stimulation, whereas TRPV1 from the other three species showed maximum responses from the first heat stimulation (Saito et al., 2019). This pattern of species difference in TRPV1 heat response was partly associated with the thermal conditions of their habitats. In contrast, the thermal activation thresholds of both TRPA1 and TRPV1 did not show a clear association with thermal niches (Saito et al., 2019).

Functional alteration of TRPA1 has also been reported among closely related lizard species. In Caribbean islands, three sympatric lizard species (*Anolis allogus*, *Anolis homolechis*, and *Anolis sagrei*) occupy microhabitats with varying thermal conditions. Body temperatures maintained by the three species differ according to the natural habitats. Behavioral responses to heating revealed that the avoidance temperatures of *A. homolechis* and *A. sagrei* are higher than that of *A. allogus* (Akashi et al., 2018). Furthermore, comparative analysis of TRPA1 showed that the TRPA1 thermal activation thresholds of *A. homolechis* and *A. sagrei* are significantly higher than that of *A. allogus*. Therefore, TRPA1 is also likely to be related to thermal adaptation in lizard species.

In contrast to anuran amphibians, TRPV1 is likely to be related with thermal physiology in urodele amphibians generally adapted to cool environments. TRPV1 of several salamander species is activated by lower temperatures (28–34°C), although orthologous channels of mammals and clawed frogs (*Xenopus*) are activated by temperatures over 40°C (Hori and Saitoh, 2020; Hori et al., 2023). In axolotls, the thermal activation thresholds of TRPA1 and TRPV1 are 40°C and 31°C, respectively (Oda et al., 2019; Hori and Saitoh, 2020; Hori et al., 2023). Axolotls are highly susceptible to heat; thus, TRPV1, instead of TRPA1, might serve as a noxious heat sensor in this species.

Comparative analyses of TRP channels also revealed the structural basis of functional alterations. Regarding salamander TRPV1, two single amino acids located in the first ankyrin repeat domain have been identified as responsible sites for the species differences in heat sensitivity (Fig. 5A) (Hori et al., 2023). Detailed analysis showed that the thermal stability of ankyrin repeat domains is correlated with the thermal sensitivity of TRPV1 channels. The amino acids responsible for the species difference in TRPV1 between *X. laevis* and *X. tropicalis* were also identified through chimeric and mutagenesis experiments. Three amino acid substitutions located in the second and third ankyrin repeat domains of TRPV1 are partially responsible for the functional difference observed between these two species (Fig. 5A) (Saito et al., 2016). As mentioned above, among Japanese frog species (*Ra. japonica*, *B. buergeri*, and *B. japonica*), the two TRPA1 AS variants, which differ in a single amino acid insertion between the sixth and seventh ankyrin repeat domains, exhibited altered activity and sensitivity to heat (Saito et al., 2022). Structural studies of TRP channels have revealed that intracellular cytosolic regions, including ankyrin repeat domains, undergo large conformational changes during heat stimulation (Kwon et al., 2021; Nadezhdin et al., 2021; Wang et al., 2023). Additionally, intracellular molecules, such as ATP and calmodulin, interact within these domains to regulate TRP channel activity (Lishko et al., 2007; Phelps et al., 2010; Lau et al., 2012). All these observations suggest that ankyrin repeat domains likely play an important role in the functional alterations of TRP channels, which contribute to the adaptive evolution of thermal perception.

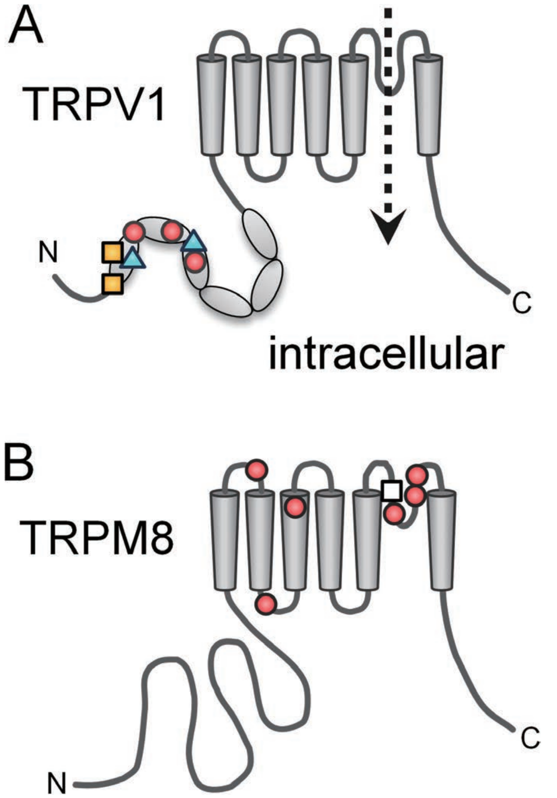


Fig. 5. Locations of amino acids involved in species differences in TRPV1 and TRPM8 thermal responses. **(A)** The locations of amino acids associated with the species difference in TRPV1 between rats and thirteen-lined ground squirrels, *X. laevis* and *X. tropicalis*, or axolotl and other salamanders are indicated by triangles, circles, or squares, respectively. **(B)** The locations of amino acids responsible for TRPM8 species differences between rats and thirteen-lined ground squirrels or emperor penguins and African elephants are indicated by circles and an open square, respectively.

THERMAL ADAPTATION OF TRP CHANNELS IN MAMMALS AND BIRDS

Functional alterations of TRP channels have also been reported in mammals. Investigation of thirteen-lined ground squirrels and camels revealed that these species have TRPV1 channels with nearly no heat sensitivity, although sensitivity to capsaicin and acid is maintained in both species (Laursen et al., 2016). The considerable reduction in TRPV1 thermal sensitivity is thought to be related to the extreme heat resistance of these two species. To identify the amino acid residues that are responsible for the reduction of TRPV1 heat responses, mutagenesis analysis of TRPV1 between thirteen-lined ground squirrels and rats has been performed. As a result, two amino acid substitutions located in the ankyrin repeat domains of TRPV1 were found to be involved in the species differences (Fig. 5A).

A species difference in the cold-activated TRPM8 channel has also been observed among mammalian and avian species that occupy habitats with varying thermal conditions. Comparison of TRPM8 among seven mammalian species and one avian species showed that its cold-evoked activity decreases with a decline in the thermal range of the species' habitats (Yang et al., 2020). Among the species compared, African elephants, which inhabit relatively warm habitats, possess TRPM8 with the highest cold-evoked activity, whereas emperor penguins, which inhabit Antarctica, possess TRPM8 with the lowest activity. A single amino acid substitution located in the pore domain is responsible for the species differences in TRPM8 between African elephants and emperor penguins (Fig. 5B) (Yang et al., 2020). Furthermore, an increase in the pore domain hydrophobicity enhances the cold-evoked activity of TRPM8. TRPM8 of all species examined retained responses to menthol, and thus, it is likely that functional alterations specifically occurred in thermal gating of the channel. Notably, the introduction of emperor penguin TRPM8 into knockout mice, in which TRPM8 had been disrupted, failed to restore the behavioral responses to cold (Yang et al., 2020).

Some mammalian species reduce body temperature and hibernate to survive the winter. To investigate the link between thermal perception and hibernation in mammals, the functional characterization of TRPM8 has been performed in thirteen-lined ground squirrels and Syrian hamsters. As a result, cold-evoked activity of TRPM8 in these two species was found to be considerably lower than that of orthologous channels in non-hibernating rodents (Matos-Cruz et al., 2017). Six amino acids located in the loop and transmembrane domains were found to be involved in the difference in TRPM8 cold responses between rats and thirteen-lined ground squirrels (Fig. 5B). Whether the reduced activity of TRPM8 is indeed related to hibernation awaits further comparative studies between hibernating and non-hibernating species.

FUNCTIONAL DIVERSITY OF THERMOSENSITIVE TRP CHANNELS IN FISH

Functional properties of TRP channels in fish have also been investigated in several studies. As mentioned earlier, the channel properties of TRPA1 have been characterized in teleosts such as zebrafish, pufferfish, and medaka (Oda et al., 2016, 2017, 2018). Recently, TRP channels have been investigated in fish inhabiting cool environments. TRPV1 of masu salmon is activated by temperatures above 28°C, which is close to the upper thermal limits of this species (Yoshimura et al., 2022). TRPV1 and TRPA1 have been characterized from Antarctic notothenioid fish living in extreme cold habitats. Thermal activation thresholds of these channels are considerably higher than the temperatures that these fish experience in the wild (York, 2023). However, detailed functional assays revealed that the thermal sensitivity of TRPA1, but not of TRPV1, increased with changes in cellular conditions such as oxidation and cholesterol reduction (York, 2023). These results suggest that intracellular conditions can influence TRP channel activity and, in some cases, contribute to the species variation in TRP channels.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

The role of TRP channels in environmental adaptation has been investigated by many studies, and the association of their functional divergence with thermal niches has been elucidated in recent years. These studies revealed that changes in the thermal activity of TRP channels occurred in both heat- and cold-activated channels among closely related amphibian, avian, or mammalian species adapted to different thermal niches (Saito et al., 2019; Yang et al., 2020; Saito et al., 2022), suggesting that shifts in the activity of thermal sensors is a widely shared mechanism in environmental adaptation. Similarly, thermal sensitivity of TRPA1, which can be measured by their thermal activation thresholds, has been also reported to be associated with the thermal niches of species (Akashi et al., 2018; Akashi, 2021). However, thermal sensitivity of TRP channels is not always related with the thermal conditions in a habitat (Saito et al., 2019).

Interestingly, altered TRP channel properties are largely confined to thermal responses, since chemical sensitivities of TRP channels are relatively conserved. For example, both TRPV1 and TRPM8 in thirteen-lined ground squirrels retain sensitivity to chemicals such as capsaicin for TRPV1 and menthol for TRPM8, but both channels have nearly abolished thermal sensitivity (Laursen et al., 2016; Matos-Cruz et al., 2017). Similarly, in frog species including *B. japonica* and *X. tropicalis*, TRPA1 maintains sensitivity to cinnamaldehyde, although it exhibits considerable reductions to heat stimulation (Saito et al., 2016, 2019, 2022). These observations suggest that selective pressures have specifically acted on the temperature-related functions of TRP channels in response to thermal environments during evolutionary processes.

Although there are recent advances in our understanding of TRP channels in environmental adaptation, it is still unclear whether the observed species differences in TRP channels revealed by in vitro functional assays are actually associated with sensory perception at the individual level (Gracheva et al., 2010, 2011; Laursen et al., 2016; Saito et al., 2016, 2019, 2022; Matos-Cruz et al., 2017; Akashi et al., 2018). Recent developments in genome editing technologies will facilitate future investigations using genetically modified animals to address the physiological significance of evolutionary changes in TRP channels.

The structural basis of functional differences in TRP channels has been reported in several studies. In most cases, key amino acids responsible for functional alteration of TRP channels have been identified by comparing channels of extant species. However, comparative analyses using extant species do not always highlight the amino acid changes responsible for functional shifts of proteins within an evolutionary context (Laursen et al., 2016; Saito et al., 2019). Ancestral reconstruction of proteins of interest is an effective approach for elucidating the structural basis of functional evolution (Yokoyama, 2013). Investigation of ancestral TRP channels also supplies clues for estimating thermal niches occupied by ancestral species given that their functional properties are tuned with the environmental conditions of habitats in extant species. Further comparative analyses among species inhabiting diverse thermal niches, using a variety of species, are required to deepen our understanding of environmental adaptation in thermal perception.

ACKNOWLEDGMENTS

We thank Prof. Makoto Tominaga for his kind support throughout the research. This work was supported by Grants-in-aid for Scientific Research (22H02679 to SS) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. We thank Quintin Lau (https://www.kyuedit.com) for editing this manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

SS and CTS wrote the manuscript.

REFERENCES

Akashi H (2021) Thermal sensitivity of heat sensor TRPA1 correlates with temperatures inducing heat avoidance behavior in terrestrial ectotherms. Front Ecol Evol 9: 1–9

Akashi HD, Saito S, Cadiz Diaz A, Makino T, Tominaga M, Kawata M (2018) Comparisons of behavioural and TRPA1 heat sensitivities in three sympatric Cuban *Anolis* lizards. Mol Ecol 27: 2234–2242

Araujo MB, Ferri-Yanez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat freezes niche evolution. Ecol Lett 16: 1206–1219

Bagriantsev SN, Gracheva EO (2015) Molecular mechanisms of temperature adaptation. J Physiol 593: 3483–3491

Baldwin MW, Ko MC (2020) Functional evolution of vertebrate sensory receptors. Horm Behav 124: 104771

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, et al. (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41: 849–857

Bandell M, Macpherson LJ, Patapoutian A (2007) From chills to chilis: mechanisms for thermosensation and chemesthesis via thermoTRPs. Curr Opin Neurobiol 17: 490–497

Bautista DM, Movahed P, Hinman A, Axelsson HE, Sterner O, Hogestatt ED, et al. (2005) Pungent products from garlic activate the sensory ion channel TRPA1. Proc Natl Acad Sci U S A 102: 12248–12252

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, et al. (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124: 1269–1282

Bellemer A (2015) Thermotaxis, circadian rhythms, and TRP channels in Drosophila. Temperature (Austin) 2: 227–243

Bernal L, Sotelo-Hitschfeld P, Konig C, Sinica V, Wyatt A, Winter Z, et al. (2021) Odontoblast TRPC5 channels signal cold pain in teeth. Sci Adv 7: eabf5567

Cao E, Liao M, Cheng Y, Julius D (2013) TRPV1 structures in distinct conformations reveal activation mechanisms. Nature 504: 113–118

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816–824

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. Zool Sci 18: 591–596

Chu Y, Cohen BE, Chuang HH (2020) A single TRPV1 amino acid controls species sensitivity to capsaicin. Sci Rep 10: 8038

Dhaka A, Viswanath V, Patapoutian A (2006) Trp ion channels and temperature sensation. Annu Rev Neurosci 29: 135–161

Gao Y, Cao E, Julius D, Cheng Y (2016) TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. Nature 534: 347–351

García-Ávila M, Islas LD (2019) What is new about mild temperature sensing? A review of recent findings. Temperature 6: 132–141

Gau P, Poon J, Ufret-Vincenty C, Snelson CD, Gordon SE, Raible DW, et al. (2013) The zebrafish ortholog of TRPV1 is required for heat-induced locomotion. J Neurosci 33: 5249–5260

Gavva NR, Klionsky L, Qu Y, Shi L, Tamir R, Edenson S, et al. (2004) Molecular determinants of vanilloid sensitivity in TRPV1. J Biol Chem 279: 20283–20295

Geng J, Liang D, Jiang K, Zhang P (2011) Molecular evolution of the infrared sensory gene TRPA1 in snakes and implications for functional studies. PLOS ONE 6: e28644

Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT, et al. (2010) Molecular basis of infrared detection by snakes. Nature 464: 1006–1011

Gracheva EO, Cordero-Morales JF, Gonzalez-Carcacia JA, Ingolia NT, Manno C, Aranguren CI, et al. (2011) Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. Nature 476: 88–91

Guler AD, Lee H, Iida T, Shimizu I, Tominaga M, Caterina M (2002) Heat-evoked activation of the ion channel, TRPV4. J Neurosci 22: 6408–6414

Han Y, Li B, Yin TT, Xu C, Ombati R, Luo L, et al. (2018) Molecular mechanism of the tree shrew's insensitivity to spiciness. PLOS Biol 16: e2004921

Himmel NJ, Cox DN (2020) Transient receptor potential channels: current perspectives on evolution, structure, function and nomenclature. Proc R Soc B 287: 20201309

Hori S, Saitoh O (2020) Unique high sensitivity to heat of axolotl TRPV1 revealed by the heterologous expression system. Biochem Biophys Res Commun 521: 914–920

Hori S, Tateyama M, Shirai T, Kubo Y, Saitoh O (2023) Two single-point mutations in Ankyrin Repeat one drastically change the threshold temperature of TRPV1. Nat Commun 14: 2415

Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108: 421–430

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, et al. (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427: 260–265

Julius D (2013) TRP channels and pain. Annu Rev Cell Dev Biol 29: 355–384

Kashio M, Tominaga M (2022) TRP channels in thermosensation. Curr Opin Neurobiol 75: 102591

Kashio M, Sokabe T, Shintaku K, Uematsu T, Fukuta N, Kobayashi N, et al. (2012) Redox signal-mediated sensitization of transient receptor potential melastatin 2 (TRPM2) to temperature affects macrophage functions. Proc Natl Acad Sci U S A 109: 6745–6750

Komaki S, Lau Q, Igawa T (2016) Living in a Japanese onsen: field observations and physiological measurements of hot spring amphibian tadpoles, *Buergeria japonica*. Amphibia-Reptilia 37: 311

Kwon DH, Zhang F, Suo Y, Bouvette J, Borgnia MJ, Lee SY (2021) Heat-dependent opening of TRPV1 in the presence of capsaicin. Nat Struct Mol Biol 28: 554–563

Laing RJ, Dhaka A (2016) ThermoTRPs and Pain. Neuroscientist 22: 171–187

Lau SY, Procko E, Gaudet R (2012) Distinct properties of Ca^{2+}-calmodulin binding to N- and C-terminal regulatory regions of the TRPV1 channel. J Gen Physiol 140: 541–555

Laursen WJ, Anderson EO, Hoffstaetter LJ, Bagriantsev SN, Gracheva EO (2015) Species-specific temperature sensitivity of TRPA1. Temperature (Austin) 2: 214–226

Laursen WJ, Schneider ER, Merriman DK, Bagriantsev SN, Gracheva EO (2016) Low-cost functional plasticity of TRPV1 supports heat tolerance in squirrels and camels. Proc Natl Acad Sci U S A 113: 11342–11347

Liao M, Cao E, Julius D, Cheng Y (2013) Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature 504: 107–112

Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R (2007) The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 54: 905–918

Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, et al. (2005) The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol 15: 929–934

Matos-Cruz V, Schneider ER, Mastrotto M, Merriman DK, Bagriantsev SN, Gracheva EO (2017) Molecular prerequisites for diminished cold sensitivity in ground squirrels and hamsters. Cell Rep 21: 3329–3337

Matsuura H, Sokabe T, Kohno K, Tominaga M, Kadowaki T (2009) Evolutionary conservation and changes in insect TRP channels. BMC Evol Biol 9: 228

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, et al. (2007) TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A 104: 13525–13530

Moqrich A, Hwang SW, Earley TJ, Petrus MJ, Murray AN, Spencer KS, et al. (2005) Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 307: 1468–1472

Nadezhdin KD, Neuberger A, Trofimov YA, Krylov NA, Sinica V, Kupko N, et al. (2021) Structural mechanism of heat-induced opening of a temperature-sensitive TRP channel. Nat Struct Mol Biol 28: 564–572

Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Arch 464: 425–458

Oda M, Kurogi M, Kubo Y, Saitoh O (2016) Sensitivities of two zebrafish TRPA1 paralogs to chemical and thermal stimuli analyzed in heterologous expression systems. Chem Senses 41: 261–272

Oda M, Saito K, Hatta S, Kubo Y, Saitoh O (2017) Chemical and thermal sensitivity of medaka TRPA1 analyzed in heterologous expression system. Biochem Biophys Res Commun 494: 194–201

Oda M, Kubo Y, Saitoh O (2018) Sensitivity of Takifugu TRPA1 to thermal stimulations analyzed in oocytes expression system. Neuroreport 29: 280–285

Oda M, Ogino H, Kubo Y, Saitoh O (2019) Functional properties of axolotl transient receptor potential ankyrin 1 revealed by the heterologous expression system. Neuroreport 30: 323–330

Ohkita M, Saito S, Imagawa T, Takahashi K, Tominaga M, Ohta T (2012) Molecular cloning and functional characterization of *Xenopus tropicalis* frog transient receptor potential vanilloid 1 reveal its functional evolution for heat, acid, and capsaicin sensitivities in terrestrial vertebrates. J Biol Chem 287: 2388–2397

Patapoutian A, Peier AM, Story GM, Viswanath V (2003) ThermoTRP channels and beyond: mechanisms of temperature sensation. Nat Rev Neurosci 4: 529–539

Paulsen CE, Armache JP, Gao Y, Cheng Y, Julius D (2015) Structure of the TRPA1 ion channel suggests regulatory mechanisms. Nature 520: 511–517

Pedersen SF, Owsianik G, Nilius B (2005) TRP channels: an overview. Cell Calcium 38: 233–252

Peng G, Shi X, Kadowaki T (2015) Evolution of TRP channels inferred by their classification in diverse animal species. Mol Phylogenet Evol 84: 145–157

Phelps CB, Wang RR, Choo SS, Gaudet R (2010) Differential regulation of TRPV1, TRPV3, and TRPV4 sensitivity through a conserved binding site on the ankyrin repeat domain. J Biol Chem 285: 731–740

Phelps PT, Anthes JC, Correll CC (2005) Cloning and functional characterization of dog transient receptor potential vanilloid receptor-1 (TRPV1). Eur J Pharmacol 513: 57–66

Prober DA, Zimmerman S, Myers BR, McDermott BM, Jr., Kim SH, Caron S, et al. (2008) Zebrafish TRPA1 channels are required for chemosensation but not for thermosensation or mechanosensory hair cell function. J Neurosci 28: 10102–10110

Rohacs T (2024) Phosphoinositide regulation of TRP channels: a functional overview in the structural era. Annu Rev Physiol 86: 329–355

Saito S, Shingai R (2006) Evolution of thermoTRP ion channel homologs in vertebrates. Physiol Genomics 27: 219–230

Saito S, Tominaga M (2015) Functional diversity and evolutionary dynamics of thermoTRP channels. Cell Calcium 57: 214–221

Saito S, Tominaga M (2017) Evolutionary tuning of TRPA1 and TRPV1 thermal and chemical sensitivity in vertebrates. Temperature (Austin) 4: 141–152

Saito S, Fukuta N, Shingai R, Tominaga M (2011) Evolution of vertebrate transient receptor potential vanilloid 3 channels: opposite temperature sensitivity between mammals and western clawed frogs. PLoS Genet 7: e1002041

Saito S, Nakatsuka K, Takahashi K, Fukuta N, Imagawa T, Ohta T, et al. (2012) Analysis of transient receptor potential ankyrin 1 (TRPA1) in frogs and lizards illuminates both nociceptive heat and chemical sensitivities and coexpression with TRP vanilloid 1 (TRPV1) in ancestral vertebrates. J Biol Chem 287: 30743–30754

Saito S, Banzawa N, Fukuta N, Saito CT, Takahashi K, Imagawa T, et al. (2014) Heat and noxious chemical sensor, chicken TRPA1, as a target of bird repellents and identification of its structural determinants by multispecies functional comparison. Mol Biol Evol 31: 708–722

Saito S, Ohkita M, Saito CT, Takahashi K, Tominaga M, Ohta T (2016) Evolution of heat sensors drove shifts in thermosensation between *Xenopus* species adapted to different thermal niches. J Biol Chem 291: 11446–11459

Saito S, Hamanaka G, Kawai N, Furukawa R, Gojobori J, Tominaga M, et al. (2017) Characterization of TRPA channels in the starfish *Patiria pectinifera*: involvement of thermally activated TRPA1 in thermotaxis in marine planktonic larvae. Sci Rep 7: 2173

Saito S, Saito CT, Nozawa M, Tominaga M (2019) Elucidating the functional evolution of heat sensors among *Xenopus* species adapted to different thermal niches by ancestral sequence reconstruction. Mol Ecol 28: 3561–3571

Saito S, Saito CT, Igawa T, Takeda N, Komaki S, Ohta T, et al. (2022) Evolutionary tuning of transient receptor potential ankyrin 1 underlies the variation in heat avoidance behaviors among frog species inhabiting diverse thermal niches. Mol Biol Evol 39(9):msac180

Sato A, Sokabe T, Kashio M, Yasukochi Y, Tominaga M, Shiomi K (2014) Embryonic thermosensitive TRPA1 determines transgenerational diapause phenotype of the silkworm, *Bombyx mori*. Proc Natl Acad Sci U S A 111: E1249–1255

Schuler A, Schmitz G, Reft A, Ozbek S, Thurm U, Bornberg-Bauer E (2015) The rise and fall of TRP-N, an ancient family of mechanogated ion channels, in metazoa. Genome Biol Evol 7: 1713–1727

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, et al. (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112: 819–829

Sun W, Uchida K, Suzuki Y, Zhou Y, Kim M, Takayama Y, et al. (2016) Lack of TRPV2 impairs thermogenesis in mouse brown adipose tissue. EMBO Rep 17: 383–399

Sunday J, Bennett JM, Calosi P, Clusella-Trullas S, Gravel S, Hargreaves AL, et al. (2019) Thermal tolerance patterns across latitude and elevation. Philos Trans R Soc B 374: 20190036

Sunday JM, Bates AE, Dulvy NK (2011) Global analysis of thermal tolerance and latitude in ectotherms. Proc R Soc B 278: 1823–1830

Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. Nat Clim Change 2: 686–690

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y, et al. (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438: 1022–1025

Talavera K, Startek JB, Alvarez-Collazo J, Boonen B, Alpizar YA, Sanchez A, et al. (2020) Mammalian transient receptor potential TRPA1 channels: From structure to disease. Physiol Rev 100: 725–803

Tewksbury JJ, Nabhan GP (2001) Seed dispersal. Directed deterrence by capsaicin in chilies. Nature 412: 403–404

Uchida K, Tominaga M (2011) The role of thermosensitive TRP (transient receptor potential) channels in insulin secretion. Endocr J 58: 1021–1028

Uchida K, Dezaki K, Damdindorj B, Inada H, Shiuchi T, Mori Y, et al. (2011) Lack of TRPM2 impaired insulin secretion and glucose metabolisms in mice. Diabetes 60: 119–126

Valencia-Montoya WA, Pierce NE, Bellono NW (2024) Evolution of sensory receptors. Annu Rev Cell Dev Biol 40: 353–379

Viswanath V, Story GM, Peier AM, Petrus MJ, Lee VM, Hwang SW, et al. (2003) Opposite thermosensor in fruitfly and mouse. Nature 423: 822–823

Wang H, Siemens J (2015) TRP ion channels in thermosensation, thermoregulation and metabolism. Temperature (Austin) 2: 178–187

Wang X, Li Y, Wei H, Yang Z, Luo R, Gao Y, et al. (2023) Molecular architecture and gating mechanisms of the *Drosophila* TRPA1 channel. Cell Discov 9: 36

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, 12

Wu T, Deme L, Zhang Z, Huang X, Xu S, Yang G (2022) Decay of TRPV3 as the genomic trace of epidermal structure changes in the land-to-sea transition of mammals. Ecol Evol 12: e8731

Yang S, Lu X, Wang Y, Xu L, Chen X, Yang F, et al. (2020) A paradigm of thermal adaptation in penguins and elephants by tuning cold activation in TRPM8. Proc Natl Acad Sci U S A 117: 8633–8638

Yatsu R, Miyagawa S, Kohno S, Saito S, Lowers RH, Ogino Y, et al. (2015) TRPV4 associates environmental temperature and sex

determination in the American alligator. Sci Rep 5: 18581
Yokoyama S (2013) Synthetic biology of phenotypic adaptation in vertebrates: the next frontier. Mol Biol Evol 30: 1495–1499
Yokoyama S, Altun A, DeNardo DF (2011) Molecular convergence of infrared vision in snakes. Mol Biol Evol 28: 45–48
Yokoyama T, Saito S, Shimoda M, Kobayashi M, Takasu Y, Sezutsu H, et al. (2021) Comparisons in temperature and photoperiodic-dependent diapause induction between domestic and wild mulberry silkworms. Sci Rep 11: 8052
York JM (2023) Temperature activated transient receptor potential ion channels from Antarctic fishes. Open Biol 13: 230215
Yoshimura A, Saito S, Saito CT, Takahashi K, Tominaga M, Ohta T (2022) Functional analysis of thermo-sensitive TRPV1 in an aquatic vertebrate, masu salmon (*Oncorhynchus masou ishikawae*). Biochem Biophys Rep 31: 101315

(Received June 30, 2024 / Accepted November 21, 2024 /
Published online January 30, 2025)