Techniques

Distinct thermal migration behaviors in response to different thermal gradients in *Caenorhabditis elegans*

P. Jurado†, E. Kodama‡, Y. Tanizawa‡ and I. Mori*†

†Laboratory of Molecular Neurobiology, Department of Molecular Biology, Graduate School of Science, Nagoya University, CREST-JST, Nagoya 464-8602, Japan, and
‡Present address: Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK
*Corresponding author: I. Mori, Laboratory of Molecular Neurobiology, Department of Molecular Biology, Graduate School of Science, Nagoya University, CREST-JST, Nagoya 464-8602, Japan. E-mail: m46920a@nucc.cc.nagoya-u.ac.jp

The nematode *Caenorhabditis elegans* exhibits a complex behavior called thermotaxis in response to temperature. This behavior is defined as a form of associative learning, in which temperature pairs with the presence or absence of food. Different interpretations have been drawn from the diverse results obtained by several groups, mainly because of the application of different methodologies for the analysis of thermotaxis. To clarify the discrepancies in behavioral observations and subsequent interpretations by different laboratories, we attempted to systematize several parameters to observe thermotaxis behavior as originally defined by Hedgecock and Russell in 1975. In this study, we show clearly how *C. elegans* can show a conditioned migration toward colder or warmer areas on a thermal gradient, given certain criteria necessary for the observation of thermotaxis. We thus propose to distinguish thermotaxis from other temperature-related behaviors, such as the warm avoidance response displayed at temperature gradients of 1°C/cm and steeper.

Keywords: behavior, *Caenorhabditis elegans*, learning, nematode, temperature, thermotaxis

Received 24 April 2009, revised 10 August 2009, 15 October 2009 and 20 October 2009, accepted for publication 21 October 2009

*Caenorhabditis elegans* responds to a variety of environmental signals such as volatile, water-soluble chemicals and temperature. The animals can remember these cues and associate them with other existing conditions. Thermotaxis (TTX) of *C. elegans* was originally described by Hedgecock and Russell in 1975. When animals had been conditioned to be fed at a certain temperature and were then placed on a temperature gradient without food, they moved toward the remembered cultivation temperature. If, however, the animals were incubated at a constant temperature without food and then placed on a thermal gradient, they moved away from the temperature at which they had been starved (Hedgecock & Russell 1975; Mori & Ohshima 1995). Hedgecock and Russell also proposed a mechanism to explain how animals possessed the ability to actively migrate toward the preferred temperature, regardless of whether it was colder or warmer than the spot where they were initially placed on the thermal gradient. Hedgecock and Russell suggested that migration to a conditioned temperature involves two opposing drives, upward and downward thermal migration, and that both balance at the preferred temperature at which the animals move along isothermally. Only the upward or thermophilic drive was plastic and reset when changing the cultivation temperature, whereas the downward or cryophilic drive was insensitive to the cultivation temperature (Hedgecock & Russell 1975). For this first description of TTX, a temperature gradient of 0.5°C/cm was carefully chosen because of its similarity with the gradient that naturally occurs in moist soil, the native habitat of *C. elegans* (Hedgecock & Russell 1975).

Several groups followed the work on TTX using the same methodology and obtained results consistent with those in the initial description by Hedgecock and Russell (Gomez et al. 2001; Hobert et al. 1997; Komatsu et al. 1996; Kuhara et al. 2002; Mori & Ohshima 1995; Satterlee et al. 2001). In 2002, Ryu and Samuel proposed a new way to analyze TTX. Rather than scoring the final distribution of the worms after migration on a temperature gradient, they video recorded the movement of *C. elegans* on a thermal gradient during short periods of time (2–10 min) and dissected the movement later into turns and runs. Following this approach, the authors found no evidence of an active thermophilic drive, although they confirmed cryophilic drive and isothermal movement. They proposed that the animals dispersed following a random walk pattern and accumulated at the preferred temperature only when it matched the previous cultivation temperature by the activation of the isothermal movement. A similar method dissecting the movement of the animals in turns and runs...
TTX plate (13.5 × 6 × 2 cm) that contained 11 ml of TTX medium (2% agar, Difco Bacto Agar ref. 214010; 0.3% NaCl and 25 mM potassium phosphate buffer at pH 6.0) was placed over the aluminum platform and the gap between metal and plastic was filled with water. The center of the plate was adjusted to 20°C and the plate was left to stabilize during the 15 min prior to the assay (Fig. 1). The temperatures of the aluminum slab corresponding to the center and the two edges of the TTX plate were measured with a surface digital thermometer (Anritsu HA-100K) before each assay to confirm that the temperature gradient was maintained. The temperatures at the sides of the TTX plates ranged roughly between 18.6 and 21.3°C for the 0.2°C/cm gradient, 17.3 and 22.7°C for the 0.4°C/cm gradient, 16.0 and 24.0°C for the 0.6°C/cm gradient, 14.6 and 25.4°C for the 0.8°C/cm gradient, 13.2 and 28.7°C for the 1°C/cm gradient and 11.9 and 28.1°C for the 1.2°C/cm gradient. The temperatures represented on the horizontal axis in Figs. 2 and 4 are estimations of the gradients measured on the TTX platform. These may present slight variations with the temperatures on the agar. Thermal images and the exact temperature gradients on the agar plates were measured with a thermal camera (Thermo tracer Type TS9230; NEC Avio Infrared Technologies Co., Ltd, Tokyo, Japan). We used Ir Motion collection (Professional 3.13; NEC Avio Infrared Technologies Co., Ltd) for the treatment of the data. To improve ventilation, holed plastic lids were used for the experiments performed as shown in Figs. 3 and 4. Several glass slides placed on top of the TTX plate, instead of the plate lids, were used as a cover during the video recordings to improve the quality of the image.

Uncrowded and well-fed late L4 larvae were placed on plates that contained 14 ml of nematode growth medium (NGM) with 2% agar on which approximately 0.1 ml of a saturated culture of Escherichia coli OP50 had been overlaid and grown. The animals and their progeny were cultivated for 3 days at 23°C or for 5 days at 17°C until most of the population reached the adult stage. For each TTX assay, animals from three of the plates were collected using approximately 5 ml of nematode growth buffer (NG buffer; 0.3% NaCl, 1 mM CaCl2, 1 mM MgSO4 and 25 mM phosphate buffer, pH 6.0) and allowed to settle in a conical tube. The worms were washed two more times with approximately 2 ml of NG buffer. These steps were carried out within 10 min in a water bath maintained at 20°C. The NG buffer used for the collection and washing of the worms was also kept at 20°C. Approximately 100–300 animals were placed in three spots along the center of the plate (20°C) in all the experiments unless otherwise indicated. Excess buffer was removed carefully with a tissue paper immediately after placement. The TTX plates were left undisturbed for 1 h unless otherwise indicated. After that, the animals were killed with chloroform gas and the number of worms in each of the eight equal subdivisions on the TTX plate (from the colder to the warmer section marked as −4, −3, −2, −1, +1, +2, +3, +4) was scored (Ito et al. 2006). The distribution of worms was represented either as a percentage of accumulation on each of the sections of the plate or by using the TTX index (Fig. 1; Ito et al. 2006).

### Statistical analysis

PASW Statistics 17.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Error bars in Fig. 2a–h, m indicate standard deviation (SD). Error bars in Fig. 2g, n indicate standard error of mean (SEM). Non-parametric Kruskall–Wallis test was performed on the data in Fig. 2g, n. Associated degrees of freedom (df) equals 5 in both Fig. 2g ($\chi^2 = 94.3$) and Fig. 2n ($\chi^2 = 126.5$); $P < 0.001$. We analyzed the data in Fig. 2g, n with a Mann–Whitney (MW) test by comparing each variable to ttx-neutral (TTX index = 0). The results from MW supported the rejection of the null hypothesis _Ho_ (thermotactic behavior is not significant) with $P > 0.05$ in all cases, except when the worms were grown at 23°C and assayed on gradients of 1.0 and 1.2°C/cm. Error bars in Fig. 3 indicate SEM. Analysis of variance (_ANOVA_; Fig. 3a, $F_{3,24} = 201.7$; Fig. 3b, $F_{3,21} = 53.8$; Fig. 3c, $F_{3,26} = 152.2$; Fig. 3d, $F_{5,16} = 50.7$; $P < 0.001$) followed by Dunnett’s multiple comparisons using N2 as a control were performed on these data. Asterisks represent $P < 0.05$ for the multiple comparisons. Error bars in Fig. 4d indicate SD. Error bars in Fig. 4a, b...
Thermotaxis in *Caenorhabditis elegans*

**Figure 1:** Linear population TTX assay. (a) The TTX platform made of aluminum is partially submerged in different temperature water baths to generate a linear temperature gradient. (b) Thermal image of a 0.5 °C/cm temperature gradient. (c) TTX plate (13.5 × 6 cm) to be placed on the TTX platform. After the animals were dropped on the agar surface of the plate, they were permitted to move freely throughout the plate. The number of worms in each of the eight sections ranging from –4 to +4 was counted. Dots represent scored animals in the example. The TTX index was calculated from this score. (d) Plot of a 0.5 °C/cm temperature gradient. Temperature along the gradient was measured at intervals of 10 min. (e) Formula for the linear TTX index.  

\[
TTX\text{index} = \frac{\sum_{i=1}^{N} S_i}{N}
\]

*S*: Score of individual *i* on each plate section (-4 to +4)  
*N*: Total number of animals on the test plate

---

**Results**

Thermotaxis behavior of N2 wild-type animals assayed on different linear temperature gradients

Six different spatial linear temperature gradients ranging from 0.2 to 1.2 °C/cm were built. Populations of 100–300 animals, conditioned to find food at either 17 or 23 °C, were placed at 20 °C (on the center of a TTX plate) on each of these linear gradients and the final distribution of the worms was scored after 1 h. We analyzed two N2 strains used in previously published TTX work. Both were originated from the same CGC (*Caenorhabditis elegans* Genetic Center) stock but maintained in different laboratories (N2.U from USA and N2.J from Japan). We assayed the two N2 strains to discard possible deviations from the original TTX behavior described by Hedgecock and Russell (1975).

The animals cultivated at 17 °C clearly migrated to a lower temperature when placed at 20 °C in all the gradients tested (Fig. 2a–g). As the temperature gradient became steeper, the area of the agar that corresponded to 17 °C moved toward...
Figure 2: Thermal response of the wild-type N2 strain on different linear temperature gradients. Dark diamonds indicate the distribution of N2.J (N2 strain maintained in Japan) and the open squares indicate the distribution of N2.U (N2 strain maintained in USA). (a–f) Distribution of animals conditioned at 17°C and placed on gradients of 0.2, 0.4, 0.6, 0.8, 1.0 or 1.2°C/cm. Error bars indicate SD. (g) TTX index of populations at (a–f). Error bars indicate SEM. (h–m) Distribution of animals conditioned at 23°C and placed on gradients of 0.2, 0.4, 0.6, 0.8, 1.0 or 1.2°C/cm. Error bars indicate SD (n). TTX index of the populations at (h–m). Error bars indicate SEM. Negative TTX values are given for an accumulation of animals at temperatures below 20°C. Positive TTX values represent a general tendency to accumulate at temperatures above 20°C. We performed a non-parametric Kruskall–Wallis test ($\chi^2 = 94.3$ for (g) and $\chi^2 = 126.5$ for (n); $P < 0.001$), followed by Mann–Whitney test to compare each variable with the ttx-neutral (TTX index = 0). The null hypothesis Ho (Ho: thermotactic behavior is not significant) was rejected with $P > 0.05$, except for plus signed cases.
Figure 3: Thermal response of the wild-type strains CB4856, CB4858 and the thermotaxis-defective mutants ttx-1, ttx-3, ttx-4 and tax-4 on shallow and steep temperature gradients. (a) TTX index of populations of worms conditioned at 17°C and assayed during 1 h on a shallow thermal gradient of 0.5°C/cm. (b) TTX index of populations of worms conditioned at 17°C and assayed during 1 h on a steep thermal gradient of 1.2°C/cm. (c) TTX index of populations of worms conditioned at 23°C and assayed during 1 h on a shallow thermal gradient of 0.5°C/cm. (d) TTX index of populations of worms conditioned at 23°C and assayed during 1 h on a steep thermal gradient of 1.2°C/cm. All error bars indicate SEM. ANOVA [(a), \(F_{6,24} = 201.7\); (b), \(F_{6,21} = 53.8\); (c), \(F_{6,26} = 152.2\); (d), \(F_{6,16} = 50.7; P < 0.001\)] followed by Dunnett’s multiple comparisons using N2 as a control were performed on these data. Asterisks represent \(P < 0.05\) for the multiple comparisons.

the center of the assay plate. The distribution of the worms accompanied this shift of temperature, resulting in a change in the preferred accumulation area to an area closer to the center of the plate. Animals cultivated at 23°C and placed on shallow gradients (0.2–0.8°C/cm, Fig. 2h–k,n) migrated clearly to a higher temperature when placed at 20°C. The accumulation of worms at the warm area of the TTX plate disappeared at a steepness of 1.0 and 1.2°C/cm (Fig. 2l–n). Although the N2 strain usually used in Japan (N2.J) seemed to have a more dynamic behavior, as shown by its movement to an area slightly further away from the center of the plate, both N2 strains showed the same tendency to move away from the area at 20°C toward colder or warmer areas on the agar surface when previously conditioned at 17 or 23°C, respectively.

We also video recorded TTX on a linear 0.5°C/cm gradient for animals conditioned at 23°C (supporting movies; http://elegans.bio.nagoya-u.ac.jp/~supplement/Jurado_etal_2009/). On the movies, the worms placed at 20°C initially dispersed from the dropping point during the first 10 min of the assay. Then, the whole population gradually biased the movement toward the warmer area of the TTX plate. About 25 min after the assay started, most of the population had gathered in the area between 20 and 23°C.

Thermotaxis behavior of wild-type strains CB4856, CB4858 and several thermotaxis-defective mutants

The migration of strains CB4856 (Hawaiian; Wicks et al. 2001), CB4858 (Californian; Hillier et al. 2008) and four thermotaxis-defective mutants was analyzed at shallow (0.5°C/cm) and steep (1.2°C/cm) temperature gradients. The worms were incubated at either 17 or 23°C and assayed during 1 h (Fig. 3). The strain CB4856 (Hawaiian) and the previously described cryophilic mutants ttx-1 and ttx-3 (Hobert et al. 1997; Perkins et al. 1986; Satterlee et al. 2001) showed a strong tendency to migrate toward the cold area of the temperature gradient, regardless of the conditioning temperature. The recently sequenced strain
CB4858 (Californian), unlike N2, is able to actively migrate toward the warm area of a steep temperature gradient. Otherwise, the behavior of this strain is very similar to the behavior described for N2. The thermophilic mutant ttx-4 (Okochi et al. 2005) showed a consistent migration toward warm temperatures, even on a steep gradient (1.2°C/cm) and regardless of the conditioning temperature. The tax-4 mutant presents an athermotactic response under all conditions tested, consistent with its defect in thermosensation (Komatsu et al. 1996).

**Thermotaxis behavior is affected by the distance that separates the worms from the area at the conditioned temperature**

Wild-type worms (N2) conditioned at either 17 or 23°C were placed at different distances from the area at the conditioned temperature on a 0.5°C/cm linear temperature gradient. The seven divisions that separate the eight sections on the agar plate were chosen as dropping spots (D–3, D–2, D–1, D0, D+1, D+2 and D+3). D0 was always placed at 20°C, and each of the increasing and decreasing divisions corresponded to a distance of 1.7 cm and a temperature difference of 0.8°C (Fig. 4a,c).

On a 0.5°C/cm temperature gradient, animals showed a strong tendency (represented by a high TTX index) to migrate toward the remembered temperature (17 or 23°C) when placed between the area at 20°C (D0) and the area at the conditioned temperature (Fig. 4a,b). The TTX index became weaker as the worms were placed further from the preferred temperature. If the animals were dropped as far as 5°C or 11 cm from the conditioning temperature (D+3 for the 17°C grown animals and D–3 for the 23°C grown animals), no accumulation at the conditioned temperature was observed after 1 h in the TTX assay. Nevertheless, the total distribution of worms (Fig. 4d) showed that, while the 23°C conditioned animals placed on the colder area of the agar did not show a predominant thermophilic movement, the animals grown at 17°C (and placed on the warmer area of the assay plate) showed a strong migration tendency toward the cold area of the gradient. Although the worms previously cultivated at 17°C did not reach that temperature in the 1-h TTX assay, they successfully accumulated at 17°C after a 2-h assay (Fig. 4d).

**Discussion**

A gradient of approximately 0.5°C/cm occurs in moist soil, the native habitat of *C. elegans* (Hedgecock & Russell 1975), and rarely exceeds 1°C/cm (Ramot et al. 2008; Robinson et al. 2009).
Thermotaxis in *Caenorhabditis elegans*

To estimate the influence of temperature gradients on TTX, we performed TTX assays with linear temperature gradients of increasing steepness and ranging in temperature from 0.2 to 1.2 °C/cm. We chose this range as it is the most likely to occur in the *C. elegans* habitat. Worms conditioned to be fed at 23 °C avoided moving toward 23 °C at gradients of 1.0 °C/cm and above. Under natural conditions, the soil thermal gradients are presumably vertical; being steeper, warmer and more variable near the surface heated by the sun than below the surface. This reluctance to migrate toward a warm area on a steep thermal gradient may represent natural cautionary behavior designed to avoid the proximity of the soil warm area on a steep thermal gradient may represent natural cautionary behavior designed to avoid the proximity of the soil surface, where sudden temperature changes and desiccation are prone to occur. Among the several TTX mutants tested in this work, the behavior of the thermophilic strain *txt*-4 is most notable. Unlike N2, *txt*-4 was able to migrate up a gradient of 1.2 °C/cm. Hence, we believe that the absence of TTX behavior by the N2 strain on steep gradients is not because of a defect in the recognition of the steep gradient *per se* but to the different thermal responsiveness of this strain. These extreme thermal conditions would give rise to a behavior that counterbalances the migration of the animals toward the conditioned temperature, which we refer to as the ‘warm avoidance response’. The fact that TTX-4 is involved in avoiding migration toward extreme gradients that lead to warm temperatures is consistent with its function in the AFD thermosensory neuron (Okochi et al. 2005). This suggests a memory independent role for TTX-4 that is more likely to take place at the interneuron level rather than at the sensory neurons (Mori et al. 2007).

Our results indicate that several of the differences observed in TTX behavior are direct consequences of using gradients steeper than 0.5 °C/cm (Anderson et al. 2007; Ramot et al. 2008; Yamada & Ohshima 2003). A convergent result has been obtained in a recent theoretical mathematical model that analyses the thermal movement of *C. elegans* (Nakazato & Mochizuki 2009). These considerations suggest that a steep thermal gradient will stimulate the ‘warm avoidance response’, which differs from and counterbalances the typical temperature memory-based TTX behavior. In an analogous way, the use of temporal temperature ramps or blunt temperature shifts (Ryu & Samuel 2002; Zariwala et al. 2003) may cause the same effect on animals as navigating in an abrupt spatial temperature gradient, which could also boost the warm avoidance response to induce worm distribution over colder and safer areas. As the steepness of the thermal gradient has been shown to be a crucial factor in thermal behavior (Ramot et al. 2008, this study), we suggest that if temporal temperature gradients or shifts are used, then the steepness of the temperature progression vs. time and crawling speed of the worms before and during the application of the temporal thermal gradient should be taken into account. Otherwise, applying a temporal temperature gradient to an animal can result in the perception of a different overall temperature steepness, depending on whether the animal was initially moving and at what speed it was moving.

We could not observe large variability in TTX behavior between different laboratory strains (maintained in USA or Japan) on different temperature gradients. The two lines used behaved in a similar way in the video-recorded TTX assays (see supporting movies; http://elegans.bio.nagoya-u.ac.jp/~supplement/Jurado_etal2009/). As shown in these movies, the worms conditioned to be fed at 23 °C did not move in a straight line in the direction of the conditioned temperature, but rather with a mixed pattern of zigzag trails and track redirections. This action slowly transfers the whole population toward the area of the gradient with a temperature of 23 °C. We also studied the behavior of two other wild-type *C. elegans* strains: Hawaiian CB4856 and the recently sequenced CB4858 strain isolated in California. Each of these strains showed a different thermal behavior. The Hawaiian animals lack a thermostatic drive and showed an assertive cryophilicity under all conditions tested. Because of this, we suggest the use of the alternative strain CB4858 for single nucleotide polymorphism (SNP) mapping of TTX mutants instead of the classical CB4856 Hawaiian strain. CB4858 presents a TTX behavior resembling that of N2 on a shallow gradient of 0.5 °C/cm, although it does not exhibit the warm avoidance response on a steeper gradient of 1.2 °C/cm. Hence, thermal-related behaviors in *C. elegans* wild populations seem to vary depending on the isolate, perhaps pointing to the diverse ecological niches of these strains.

Several other issues could cause the disruption of TTX behavior in diverse situations as noted by different laboratories working in this field. As shown by Ito et al. (2006) and the supporting videos in this work, animals start migrating toward a conditioned temperature of 23 °C after 15 min of being placed on the TTX assay plate. Several hypotheses can be formulated to explain this behavior. One of them is a dispersal reaction to the crowded conditions at the dropping point. In addition, when the worms are transferred from a lawn of food to a bacteria-free agar plate, the pattern of locomotion changes dramatically to a local search state, which exerts itself only shortly after removal of the worms from the food source (Gray et al. 2005; Zhao et al. 2003). Because of this, we believe that TTX behavior is best observed over a long, 60-min assay (Ito et al. 2006). Shorter assay times could result in immediate dispersal (Zhao et al. 2003) or a food search state (Gray et al. 2005) that differs from temperature seeking behavior. This may be one of the causes of the disparities observed in different TTX studies (Ramot et al. 2008; Ryu & Samuel 2002).

Another fact to consider when assaying TTX is the distance from the conditioned temperature at which the worms are initially placed prior to the behavioral assay (Clark et al. 2007). As expected, the tendency to accumulate near the cultivation temperature is stronger when the worms are placed over or near this cultivation temperature. For all the distances tested in a 1-h TTX assay (up to 5 °C or 11 cm away from the conditioning temperature, in a 0.5 °C/cm linear gradient), the animals cultivated at 17 °C showed a bias to move toward the area at 17 °C (Fig. 4b,c). In contrast, the tendency to migrate toward a cultivation temperature of 23 °C, although maintained at closer distances, disappears when the animals are placed as far as 5 °C or 11 cm away from the area at the conditioned temperature. This difference in conditioning temperature-dependent behavior is consistent with the original description of TTX by Hedgcock and Russell (1975). A possible explanation for this difference is that the...
association of food with a cultivation temperature of 17°C lasts longer than when the association is performed at a conditioning temperature of 23°C (Mohri et al. 2005). The animals that remembered and searched for the area at 17°C needed 2 h to cross the entire assay plate and successfully concentrate around the area at this temperature. However, 2 h may be too long for animals fed at 23°C, resulting perhaps in the loss of conditioning before reaching the cultivation temperature. Therefore, we suggest that when animals are placed on a thermal gradient, it is crucial to take into account the relative distance to the conditioned temperature and the time necessary for the animals to cover this distance. This would greatly facilitate a comparison of the interpretations of TTX results from different groups.

References


Acknowledgments

We thank D. Biron for discussion and P. Sengupta for providing N2.U strain. We are also grateful to Y. Tsukada, T. Shimowada and all other members of the Mori lab for discussion and technical support, as well as to K. Nakazato and stat-help.com for statistical advice. P.J. was supported by the Japan Society for the Promotion of Science and the Spanish Ministry of Education. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (Molecular Brain Science) from MEXT (17024023), Japan (to I.M.). I.M. is a Scholar of the Institute for Advanced Research of Nagoya University.