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RESEARCH ARTICLE

Temperature-dependent behaviours are genetically variable in the nematode *Caenorhabditis briggsae*

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SUMMARY

Temperature-dependent behaviours in *Caenorhabditis elegans*, such as thermotaxis and isothermal tracking, are complex behavioural responses that integrate sensation, foraging and learning, and have driven investigations to discover many essential genetic and neural pathways. The ease of manipulation of the *Caenorhabditis* model system also has encouraged its application to comparative analyses of phenotypic evolution, particularly contrasts of the classic model *C. elegans* with *C. briggsae*. And yet few studies have investigated natural genetic variation in behaviour in any nematode. Here we measure thermotaxis and isothermal tracking behaviour in genetically distinct strains of *C. briggsae*, further motivated by the latitudinal differentiation in *C. briggsae* that is associated with temperature-dependent fitness differences in this species. We demonstrate that *C. briggsae* performs thermotaxis and isothermal tracking largely similar to that of *C. elegans*, with a tendency to prefer its rearing temperature. Comparisons of these behaviours among strains reveal substantial heritable natural variation within each species that corresponds to three general patterns of behavioural response. However, intraspecific genetic differences in thermal behaviour often exceed interspecific differences. These patterns of temperature-dependent behaviour motivate further development of *C. briggsae* as a model system for dissecting the genetic underpinnings of complex behavioural traits.

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INTRODUCTION

Temperature-dependent behaviours in the model organism *Caenorhabditis elegans* represent one of the best-understood behavioural neural circuits in biology, with much known about the essential neuronal and molecular components (Mori and Ohshima, 1995; Cassata et al., 2000; Samuel et al., 2003; Colosimo et al., 2004; Satterlee et al., 2004; Inada et al., 2006; Yoshinori Tanizawa et al., 2006; Ohnishi et al., 2011). These behaviours are complex and ecologically important and, when combined with comparative studies within and between species, will help determine the selective pressures and changes that act on ecologically relevant traits over the course of evolution (Bendesky and Bargmann, 2011). Variability in temperature is a universal environmental factor in biology that affects organisms through a wide-range of temperature-dependent mechanisms, from defining biomolecular reaction rates to developmental timing and geographic range distributions. For ectotherms, temperature-dependent behaviours provide an essential means of regulating body temperature within an ideal range for growth and reproduction, especially for smaller animals with little thermal inertia, such as rhabditid nematodes. These behaviours are complex in that they integrate many neurologic components including temperature sensation, locomotion, memory, foraging and decision-making. *Caenorhabditis elegans* has been used extensively to study the biological circuitry involved in temperature-dependent behaviours, starting with the classic work by Hedgecock and Russell (Hedgecock and Russell, 1975). However, most thermotaxis research in *Caenorhabditis* has focused on only a single species, *C. elegans*, and primarily a single genetic background within that species, the laboratory-adapted N2 strain. Here, we expand the scope of this system by explicitly quantifying temperature-dependent behaviours in two genetic backgrounds of *C. elegans* and 15 wild genetic backgrounds of *Caenorhabditis briggsae*.

When removed from their food source and placed on a thermal gradient, *C. elegans* forage using two key behavioural patterns that depend on their prior temperature history: thermotaxis and isothermal tracking (Hedgecock and Russell, 1975). On a thermal gradient, *C. elegans* move towards their cultivation temperature *via* a biased random-walk strategy, a behaviour known as thermotaxis (Hedgecock and Russell, 1975; Mori and Ohshima, 1995; Ryu and Samuel, 2002; Zariwala et al., 2003). *Caenorhabditis elegans* worms are amenable to cryophilic thermotaxis under most experimental conditions, whereas thermophilic thermotaxis depends on the steepness of the gradient and the relative starting position of worms on the gradient (Ramot et al., 2008; Jurado et al., 2010). When close to the preferred temperature on a thermal gradient, individual *C. elegans* animals are capable of long runs with minor heading adjustments to follow isotherms, a behaviour known as isothermal tracking (Mori and Ohshima, 1995; Ryu and Samuel, 2002; Luo et al., 2006).

Temperature plays a crucial role in the ecology and evolution of *C. briggsae*. The fitness of different wild genetic backgrounds of *C. briggsae* is affected differentially by rearing temperature in accord

with the strong phylogeographic patterning of this species in which genotype, phenotype and geographic distribution all are associated (Prasad et al., 2011). By contrast, *C. elegans* shows no comparable association between phenotypes and geographic origin, including differences in temperature-dependent fecundity (Hodgkin and Doniach, 1997; Rockman and Kruglyak, 2009; Anderson et al., 2011). In particular, strains from the two most commonly isolated *C. briggsae* genetic groups suggest a latitudinal divide, and these two groups have been designated as 'temperate' and 'tropical' clades (Cutter et al., 2006). There is evidence of local adaptation for these groups, as strains from the 'tropical' clade have much higher fecundity at high temperatures (30°C) while 'temperate' clade strains have a higher fecundity at low temperatures (14°C) (Prasad et al., 2011). In both *C. briggsae* and *C. elegans*, sperm are sensitive to temperature, with sperm fertility negatively affected by high temperature during sperm development (Murray and Cutter, 2011; Prasad et al., 2011). *Caenorhabditis briggsae* is morphologically very similar to *C. elegans*, sharing a common life cycle and similar androdioecious sexual system (Nigon and Dougherty, 1949; Baird, 2001), but *C. briggsae* exhibits greater molecular differentiation among wild strains that might, in turn, underlie higher phenotypic variation (Cutter et al., 2006). As more genetic tools become available in *C. briggsae*, it is quickly catching up to *C. elegans* as a model system in its own right (Koboldt et al., 2010; Ross et al., 2011).

Only a few experiments have assayed temperature-dependent behaviour in wild genetic backgrounds other than the *C. elegans* N2 laboratory strain. *Caenorhabditis elegans* strains from Hawaii (CB4856) and California (CB4858) have different thermotaxis behaviour than the Bristol N2 strain: the Hawaiian strain tends to prefer colder temperatures (Anderson et al., 2007; Jurado et al., 2010; Anderson et al., 2011) and the Californian strain behaves similarly to N2 except when assayed on steep gradients (1.2°C cm^{-1}), where it remains thermophilic even when reared at high temperature (Jurado et al., 2010). Few other differences have been reported among the seven additional *C. elegans* wild isolates that were examined for thermal preferences (Anderson et al., 2011). Hence, the extent to which thermal behaviour is constrained within species and how much it has diverged between species remain open questions. Here, we demonstrate thermotaxis and isothermal tracking in *C. briggsae* and compare these behaviours with those in *C. elegans*. We also quantify striking heritable differences in these temperature-dependent behaviours among strains representing the population genetic diversity of *C. briggsae* from around the world.

MATERIALS AND METHODS

Strains

We cultured strains according to techniques described by Brenner (Brenner, 1974), with minor alterations. *Caenorhabditis elegans* strains N2 and CB4856 and *C. briggsae* strains are all available from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN, USA). These *C. briggsae* strains are wild isolates from distinct isogenic backgrounds (propagated from single self-fertilizing worms) (see Table 1) (Dolgin et al., 2008; Cutter et al., 2010).

Thermotaxis on a linear temperature gradient

Gradient apparatus

This assay replicates classic thermotaxis experiments in *C. elegans* by measuring the accumulation of groups of worms along a temperature gradient on an agar plate. We generated a linear thermal gradient across a 380×220×6.5 mm aluminium plate. Attached along the bottom of the longest edges of this plate were two brass blocks (380×42×11 mm), each bored with U-shaped water channels (diameter 7 mm). To establish a desired temperature gradient, we circulated water through the brass channels from two temperature-controlled water baths (using a Haake DC10 pump and temperature controllers, Themoscientific, Waltham, MA, USA). Using a thermocouple (Fluke 53II thermometer, resolution 0.01°C, Everett, WA, USA; Omega #5SRTC-TT-K-30-36 thermocouple, Stamford, CT, USA) to measure the temperature just below the agar surface of the assay plate, we adjusted the temperature in the water baths to produce assay gradients of 17.0–23.0°C, 20.0–26.0°C or 23.0–29.0°C. This yielded a temperature gradient of ~0.5°C cm^{-1}, consistent with conditions amenable for *C. elegans* thermotaxis (Jurado et al., 2010). Rectangular lids from standard 96-well plates (Greiner 96-well cellstar, external dimensions 127.78×85.48mm, Frickenhausen, Germany), filled with 20 ml of nematode growth medium (NGM: 1.7% agar, 3 g l^{-1} NaCl, 2.5 g l^{-1} peptone, 1 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgSO_4$, 25 mmol l^{-1} kPO_4 and 1 ml of 5 mg ml^{-1} cholesterol in ethanol), were used as assay plates. Before use, the plates were air-dried for 25 min and, to ensure good thermal contact, we spread a layer of glycerol between the assay plates and the aluminium plate. We found that the temperature on the surface of the agar dropped by approximately 1°C within 4 min when the cover was left off the agar assay plate; it took approximately 8 min for the agar surface to return to the original temperature when the lid was replaced. Therefore, we covered assay plates with another 96-well plate lid during both gradient establishment and data collection, then waited 10 min before starting the assay in order to allow the gradient to equilibrate. Experiments were performed at room temperature (~23°C).

Experimental protocol

To ensure that we assayed only young adult worms, we stage-synchronized worms using a standard bleach synchronization protocol (Stiernagle, 2006). Briefly, we used bleach (4% sodium-hypochlorite) and NaOH to isolate eggs, which were left overnight without food so that they would developmentally arrest at the first larval (L1) stage. We then placed these synchronized L1 worms on standard NGM plates with a lawn of *E. coli* (OP50) bacteria and placed these plates of worms in incubators according to their experimental rearing condition (17, 23 or 29°C). When the worms reached the young adult stage we washed them off the plates with M9 buffer (3 g l^{-1} KH_2PO_4, 6 g l^{-1} Na_2HPO_4, 5 g l^{-1} NaCl, 1 mmol l^{-1} $MgSO_4$), rinsed them with M9 buffer, allowed them to settle and aspirated off excess M9 buffer in order to dilute out any bacteria washed off the plate. We transferred 40–410 worms *via* glass pipette onto the centre of gradient-equilibrated assay plates; most assays used between 100 and 200 worms per assay plate. We spread the worms and wicked away excess M9 buffer using a twisted KimWipe tissue (Kimberly-Clark 34120, Roswell, GA, USA). We let the worms explore the gradient for 1 h and then inverted the assay plate over chloroform to kill the worms. We marked the positions of the worms on the back of the assay plates with a felt pen and digitally scanned these images for analysis. We ran control assays using plates with no temperature gradient (at room temperature, ~23°C; reared at 17 or 23°C). This allowed us to control for any bias in the gradient apparatus and to use worms from the same timing and conditions as the experimental group.

Analysis

We adjusted the colour balance of the scanned images of the plates using ImageJ (version 1.44p, US National Institutes of Health,

Table 1. *Caenorhabditis briggsae* and *C. elegans* strains used for temperature-dependent behaviour assays

Species	Phylogeographic clade[a]	Strain	Geographic origin[a]
C. elegans	Bristol	N2	Bristol, UK
	Hawaiian	CB4856	Hawaii, USA
C. briggsae	Tropical	AF16	Ahmedabad, India
		ED3083	Johannesburg, South Africa
		VT847	Hawaii, USA
	Temperate	HK104	Okayama, Japan
		EG4181	Salt Lake City, USA
		JU439	Reykjavik, Iceland
		PB826	Ohio, USA
	Nairobi	ED3092	Nairobi, Kenya
		ED3098	Nairobi, Kenya
		ED3101	Nairobi, Kenya
	Kerala	JU1341	Kerala, India
		JU1345	Kerala, India
		JU1348	Kerala, India
	Montreal	QR24	Montreal, Canada
		QR25	Montreal, Canada

[a]Data are from Dolgin et al. (Dolgin et al., 2008) and Cutter et al. (Cutter et al., 2010).

Bethesda, MD, USA) in order to accentuate the coloured marks indicating worm position; we then set a threshold to leave a map of the worms' locations. We recorded the *x*- and *y*-positions of the centre of mass for each mark using the 'analyze particles' function in ImageJ. To quantify the trend in aggregation of worms on the plate, we calculated a thermotaxis index similar to that used by Ito et al. (Ito et al., 2006) by dividing each image into eight bins with equal weighting from –1 (cold) to +1 (warm). We calculate the thermotaxis index as:

$$\frac{\sum_{i=1}^{N} S_i}{N}, \quad (1)$$

where N is the number of worms and S_i is the weighted score for a given worm i. Each plate, comprised of 40–410 worms, was treated as one replicate. Within this range, we observed no significant correlation (Pearson's r, Bonferroni-adjusted $P>0.05$) between the thermotaxis index and the number of worms per plate in 39 of the 40 strain–condition combinations, and thus we conclude that there is no general influence of the abundance of worms in the assays.

Isothermal tracking on linear temperature gradients

Gradient apparatus

In this assay, we measured isothermal tracking behaviour by video recording paths of individual worms on a temperature gradient. We used the same aluminium gradient apparatus as in the accumulation assay above, but in a temperature-controlled room (22.5±1°C). To enhance image quality, we used two LED array lamps (Radionic ZX513, Chicago, IL, USA) angled from opposite sides of the plate on the gradient and placed thin black plastic on the aluminium block. We used standard 9 cm Petri plates with 6 ml of NGM left to dry for 20 min as our assay plates. To hold the plates in a repeatable position and to insulate the gradient, we used a fibreglass template with a circular hole bored through the centre. We recorded videos at 1 frame s^{-1} using a camera (A102f, Basler, Ahrensburg, Germany) controlled by a custom program written in LabVIEW (National Instruments, Austin, TX, USA). The gradient was set to 0.9°C cm^{-1}, similar to that used by Wasserman et al. (Wasserman et al., 2011), and centred around the respective rearing temperature for the condition tested.

Experimental setup

We picked young adult worms from well-fed, uncrowded plates that were incubated at 17, 23 or 29°C onto a transfer agar plate (free of bacteria). After 1 to 5 min, we picked 10 of these worms onto an assay plate, covered the plate with a lid and placed it on the gradient. To prevent condensation at higher temperatures from interfering with imaging, we spread a thin layer of detergent (Fisherbrand, Versa-clean 04-343, Waltham, MA, USA) across the underside of the lid. We recorded the worm behaviour for 30 min at 1 frame s^{-1}, but excluded the first 4 min of each recording in analysis because it takes at least 1 min for the gradient to equilibrate across the NGM assay plate.

Analysis

We processed images with a custom-written LabVIEW program to remove background artefacts and to visualize worm paths through slightly overlapping 2-min intervals. We identified isothermal tracks from these interval images by eye as we selected the start and end point of each path in ImageJ, which defined the 'bounding rectangle'. From this measure we calculated the start position of the track along the gradient, track angle and track length. We then defined an isothermal tracking event as any worm path that followed a path perpendicular (within 15 deg) to the gradient for at least five body lengths (45 pixels). We analyzed unitless track lengths, scaled by the plate diameter (900 pixels).

Statistical tests

To determine statistically significant differences among strains for thermotaxis and isothermal tracking assays, we used one-way ANOVA with Tukey–Kramer honestly significant difference (HSD) *post hoc* tests. To determine whether the strains' mean isothermal tracking temperatures were significantly different than a given rearing temperature, we subtracted the difference between the mean and the rearing temperature from each data point for each strain and ran a *t*-test and a Tukey–Kramer HSD on this adjusted distribution *versus* the original. We used JMP software (version 9, SAS Institute, Cary, NC, USA) to carry out all statistical tests.

RESULTS

Thermotaxis and isothermal tracking in *C. briggsae*

When assayed for classic temperature-dependent behaviours on linear thermal gradients, we found that *C. briggsae* performs

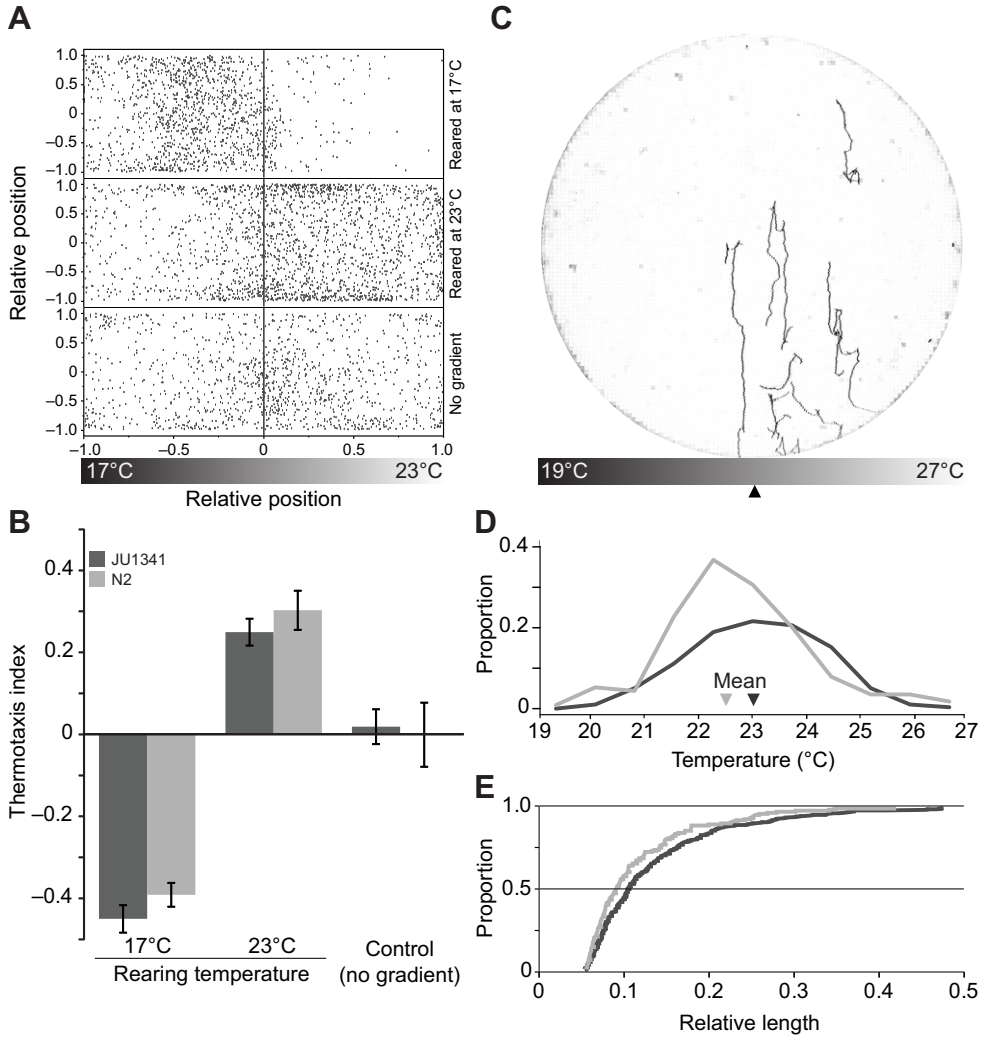

Fig. 1. *Caenorhabditis briggsae* performs temperature-dependent behaviours similar to those of *C. elegans*. (A) *C. briggsae* strain JU1341 migrates towards its rearing temperature on a linear thermal gradient ranging from 17°C (left) to 23°C (right). This panel displays the *x*- and *y*-positions of all worms relative to the centre of the plate (axes not to scale) after 1 h. The top plot shows the response to a rearing temperature of 17°C (11 assays, 1293 worms), the middle plot a rearing temperature of 23°C (14 assays, 1920 worms) and the bottom plot no temperature gradient (control; 13 assays, 1123 worms). (B) Comparison of mean ± s.e.m. thermotaxis index across assay plates between *C. briggsae* strain JU1341 (dark) and *C. elegans* strain N2 (light) under the same conditions as in A. (C) An example of several worms of strain JU1341 performing isothermal tracking over a period of 200 s on a 0.9°C cm^{-1} gradient ranging from 19 to 27°C. (D) Distribution of isothermal tracks along a thermal gradient for *C. briggsae* strain JU1341 and *C. elegans* strain N2; the gradient is the same as in C. Data include 296 isothermal tracking events for JU1341 with a mean of 23.1°C, and 157 events for N2 with a mean of 22.7°C; arrowheads indicate mean tracking temperature. (E) Cumulative distribution plot of lengths of isothermal tracking events from D; track length is a unitless measure scaled relative to the plate diameter.

thermotaxis and isothermal tracking similarly to *C. elegans* (Fig. 1). For example, both *C. briggsae* strain JU1341 and *C. elegans* strain N2 execute thermophilic and cryophilic thermotaxis towards their rearing temperature when reared at 17 or 23°C and tested on a gradient centred at 20°C (Fig. 1A). We quantified thermotaxis aggregation measurements by calculating a thermotaxis index, a weighting of the position of the worms along the gradient, where a negative and positive index correspond to cryophilic and thermophilic behaviour, respectively (Fig. 1B). Like *C. elegans*, *C. briggsae* also performs isothermal tracking near its rearing temperature, with isothermal tracks identified as long straight paths perpendicular to a thermal gradient (Fig. 1C). When we compare the distribution of isothermal tracking events across the temperature gradient, both *C. briggsae* strain JU1341 and *C. elegans* strain N2 are most likely to track near their rearing temperature (Fig. 1D). In addition, both strains have a similar pattern of isothermal track lengths (Fig. 1E; for all strains, see supplementary material Fig. S1). However, not all *C. briggsae* strains that we tested are similar to *C. elegans*, but rather show interspecific and intraspecific differences in both thermotaxis and isothermal tracking behaviour.

Variation in thermotaxis among strains of *C. briggsae*

We tested five natural strains of *C. briggsae* and two strains of *C. elegans* under several rearing and assay conditions to discover significant variation in thermotaxis among them (Fig. 2). The only condition that produced no variation of the mean thermotaxis index among strains was the control, which has no temperature gradient across the plate, and on average has no bias towards either end of the assay plate ($F_{6,68}$=0.23, P=0.97; Fig. 2A). We first tested animals on a gradient that ranged from 17 to 23°C, and found that all strains reared at 17°C taxed in the cryophilic direction towards their rearing temperature, as indicated by negative thermotaxis indices (Fig. 2B). Despite the consistently negative thermotaxis index values, strains still differed significantly from each other ($F_{6,89}$=6.45, P<0.0001). When we reared worms at 23°C and tested them on this same gradient, so that their rearing temperature was at the warm end, we observed both cryophilic and thermophilic behavioural responses, depending on the strain ($F_{6,114}$=37.42, P<0.0001; Fig. 2C). *Caenorhabditis briggsae* strains JU1341 and QR25, as well as *C. elegans* strains N2 and CB4856, taxed towards their rearing temperature in the thermophilic direction, as anticipated from previous work in *C. elegans*. However, *C. briggsae* strains AF16, ED3092 and HK104 all behaved in a cryophilic manner under these conditions. We again assayed the strains reared at 23°C, but assayed them with a warmer gradient centred at 23°C, and found that the mean thermotaxis indices were closer to a neutral score, except for AF16 ($F_{6,60}$=5.61, P=0.0001; Tukey's HSD, P<0.05), which continued to tax in a strongly cryophilic manner; strains ED3092 and N2 also were cryophilic under these conditions (Fig. 2D). Under this condition, the distributions of worms along

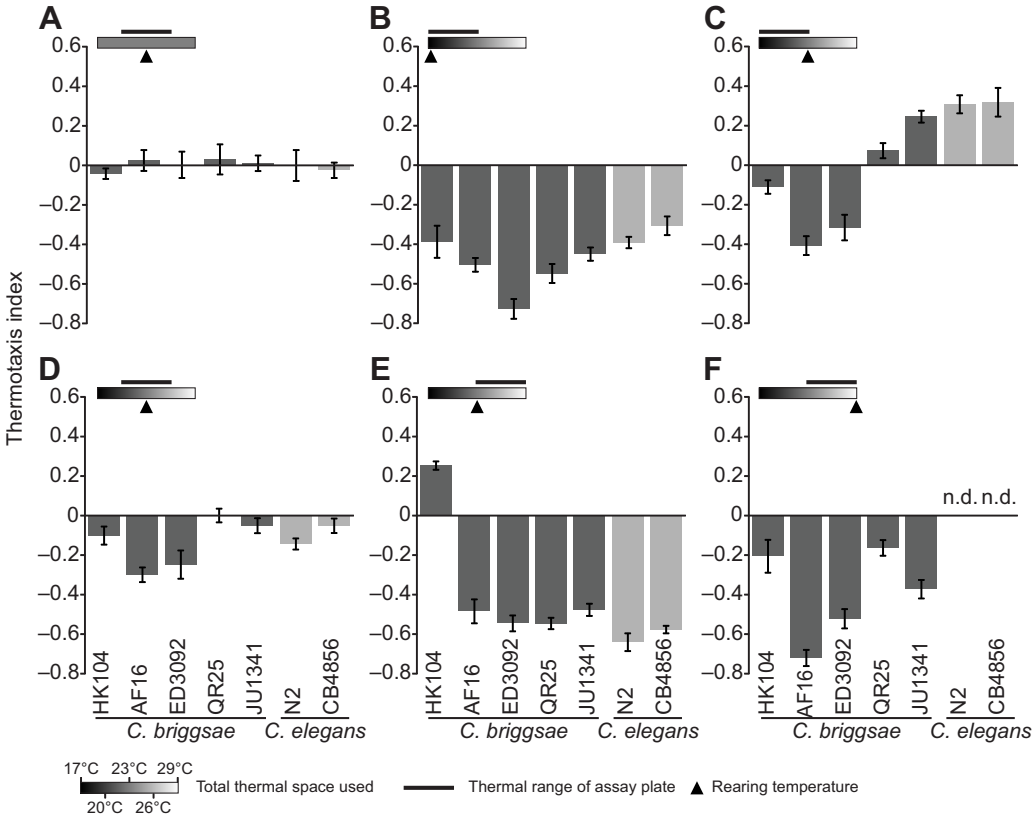

Fig. 2. Variation in thermotaxis across strains and assay conditions for *C. briggsae* and *C. elegans*. These plots show the mean thermotaxis index for each of five wild isolate strains of *C. briggsae* and two *C. elegans* strains, in response to six conditions that manipulate rearing temperature and the assay gradient. The symbols in the top left of each plot aid with comparison of plot conditions (see key, bottom): the total thermal range used across these experiments is represented by the black (17°C) to white (29°C) gradient and assay plate position in this range is represented by the bar above the gradient with the rearing temperature indicated by the arrowhead (at 17, 23 or 29°C). A thermotaxis index of –1 indicates all worms accumulate on the coldest eighth of the gradient, and +1 corresponds to accumulation at the warmest eighth. We placed worms at the centre of the plate to start the assays and left them for 1 h on a 0.5°C cm^{-1} thermal gradient (except control). Error bars indicate ±s.e.m. (N=7–23). (A) Control condition for all strains; combines data from strains that were reared at 17 and 23°C and assayed on plates left at room temperature with no temperature gradient. (B) Strains reared at 17°C and assayed on a gradient from 17 to 23°C. (C) Strains reared at 23°C and assayed on the same gradient as in B. (D) Strains reared at 23°C and assayed on a gradient from 20 to 26°C. (E) Strains reared at 23°C and assayed on a gradient from 23 to 29°C. (F) Strains reared at 29°C and assayed on same gradient as in E. Distributions of individual worms for these data can be seen in supplementary material Fig. S2.

the gradient were qualitatively closer together (cf. supplementary material Fig. S2A,D).

As *C. briggsae* is capable of growing and reproducing at temperatures a few degrees warmer than *C. elegans*, we tested the same strains on a gradient that ranged from 23 to 29°C. When we tested worms reared at 23°C on this gradient, we found that most strains showed cryophilic thermotaxis towards their rearing temperature, with the exception of strain HK104 (Fig. 2E). The cryophilic behaviour of most strains is largely similar to that in the previous condition where we tested worms reared at a temperature that corresponded to the cold end of the gradient (compare Fig. 2B and 2E). HK104 is a distinct exception. While HK104 shows cryophilic thermotaxis towards a rearing temperature of 17°C on the 17–23°C gradient, when the assay conditions are shifted higher by 6°C, they unexpectedly performed thermophilic thermotaxis away from their rearing temperature ($F_{6,75}$=87.07, P<0.0001; all pairs Tukey's HSD, P<0.05). When we tested the *C. briggsae* strains reared at 29°C on this same 23–29°C gradient, none of the strains exhibited a thermophilic response towards their rearing temperature; instead we observed differing degrees of cryophilic movement (Fig. 2F). Under this condition, strains AF16 and ED3092 are more cryophilic than most other strains ($F_{4,57}$=23.25, P<0.001; all pairs Tukey's HSD, P<0.05, except for ED3092–JU1341). We were unable to assay *C. elegans* under these conditions, as the warmer rearing temperature resulted in a high proportion of dauer and 'sick' worms.

In summary, we identified three basic patterns of thermotaxis across the distinct wild genetic backgrounds of *C. briggsae*. First, strains AF16 and ED3092 display a consistently strong cryophilic preference. Second, a pattern of behaviour similar to that of *C. elegans* is demonstrated by strains JU1341 and QR25. Finally, strain HK104 tends to have weaker cryophilic tendencies and a peculiar thermophilic response on a warmer gradient.

Variation in isothermal tracking among strains of *C. briggsae*

In addition to the striking heritable variation in thermotaxis behaviours, we also observed differences among strains of *C. briggsae* and *C. elegans* in assays for isothermal tracking. For these assays, we reared worms at either 17 or 23°C and recorded isothermal tracks on steeper temperature gradients (0.9°C cm^{-1}) than we had used for thermotaxis assays (0.5°C cm^{-1}), to promote strong isothermal tracking behaviour. We found that, overall, most isothermal tracking behaviour occurred within a few degrees of the

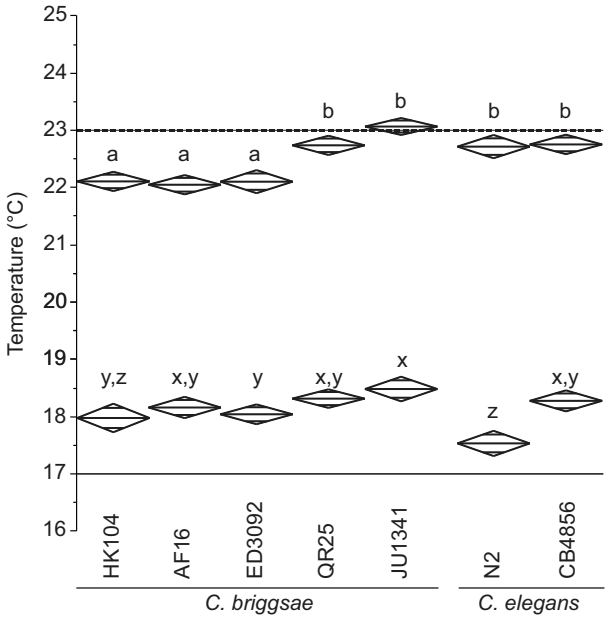

Fig. 3. Variation in mean isothermal tracking temperatures among strains of *C. briggsae* and *C. elegans*. Worms reared at 17°C were tested on a gradient from 13 to 21°C (bottom) and worms reared at 23°C were tested on a gradient from 19 to 27°C (top) so that rearing temperature was at the centre of the assay plates. Solid and dashed horizontal lines indicate rearing temperatures for 17 and 23°C rearing conditions, respectively. Centre line of diamond indicates the mean of the isothermal tracking temperatures and diamond tips represent the 95% confidence intervals (see also supplementary material Fig. S3). Mean isothermal tracking temperatures not sharing the same letter are significantly different (Tukey–Kramer HSD, $P<0.05$). Number of recorded tracks per condition varied from 93 to 296. For the 17°C raised condition at the bottom, the mean isothermal tracking temperatures for all strains are statistically significant from the same distributions centred at the rearing temperature, $P<0.0001$ for each strain (see Materials and methods).

rearing temperature (Fig. 3, supplementary material Fig. S3). We detected significant differences in the mean temperature of isothermal tracking for the different strains (reared at 17°C, $F_{6,1081}=8.09$, $P<0.0001$; reared at 23°C, $F_{6,1519}=23.09$, $P<0.0001$). When reared at 23°C and tested on a gradient from 19 to 27°C, isothermal tracks on average occurred at, or slightly below, the rearing temperature (Fig. 3, top). For this condition, the strains can be divided into two phenotypic clusters according to mean isothermal tracking temperature: AF16, ED3092 and HK104 produce isothermal tracks on average 1°C cooler than the rearing temperature, whereas isothermal tracks for JU1341 and QR25 (and the two *C. elegans* strains) occur at or just below the rearing temperature (Fig. 3). The strains with the lower mean temperature of tracking on this assay (AF16 and ED3092) are also the strains that typically exhibited strong cryophilic thermotaxis (compare these strains throughout Fig. 2 and Fig. 3). We also observed significant differences in the mean isothermal track temperature among strains when reared at 17°C and tested on a gradient ranging from 13 to 21°C. For this condition the mean isothermal tracking temperatures were higher than the rearing temperature (Fig. 3, bottom). Although *C. briggsae* strains AF16, ED3092 and HK104 tend to perform isothermal tracking at a lower mean temperature than QR25 and JU1341, they do not clearly separate into statistically distinguishable groups as described above for the warmer rearing condition. *Caenorhabditis elegans* shows greater disparity in isothermal tracking between strains than *C. briggsae* under these colder conditions (reared at 17°C).

Thermotaxis within and among clades of *C. briggsae*

We tested thermotaxis behaviour in an additional 10 strains of *C. briggsae* to provide multiple genetic backgrounds for each of the phylogeographic groups that have been reported in *C. briggsae* (Table 1). We reared all worms at 23°C and tested them on the gradient from 17 to 23°C, because this condition showed high variation among strains in our initial assays (Fig. 2C) and is similar to conditions used in other studies (Ito et al., 2006; Jurado et al., 2010). All strains from the 'Tropical' and 'Nairobi' phylogeographic groups were consistently cryophilic (Fig. 4). By contrast, distinct genetic backgrounds from within the 'Temperate', 'Montreal' and 'Kerala' phylogeographic groups exhibited heterogeneous thermal responses (Fig. 4), ranging from weakly cryophilic (HK104, JU1348) to strongly thermophilic (JU439).

DISCUSSION

These experiments quantify heritable differences for temperature-dependent behaviour in *C. briggsae*. As an emerging model for determining the genetic basis of phenotypic variation (Baird et al., 2005; Hillier et al., 2007; Ross et al., 2011; Koboldt et al., 2010), these data further position *C. briggsae* as a model system for the genetic analysis of complex behavioural traits. We compared genetic backgrounds for the ability to perform thermotaxis and isothermal tracking within and between species by assaying 15 natural isolate strains of *C. briggsae* alongside two *C. elegans* strains, and demonstrated that *C. briggsae* can sense and navigate a thermal landscape in a manner similar to *C. elegans*. The direction of thermotaxis and the temperature at which isothermal tracking occurs in *C. briggsae* are both affected by the rearing temperature of the worms, just as in *C. elegans*. However, we also discovered striking heritable variation among the *C. briggsae* wild genetic backgrounds that often exceeded differences between species or between clades within *C. briggsae*. Moreover, a preference for taxis towards rearing temperature is not evident in some *C. briggsae* strains under some assay conditions, which we discuss below.

Variation within *C. elegans* for temperature-dependent behaviour

For *C. elegans* strain N2, our experiments largely replicate the behavioural patterns reported previously. However, *C. elegans* strain CB4856 differs behaviourally in our hands from some previous reports: both Jurado et al. (Jurado et al., 2010) and Anderson et al. (Anderson et al., 2007) found CB4856 to respond cryophilically when reared at 23 or 24°C, whereas we observed a thermophilic response similar to N2. Anderson et al. (Anderson et al., 2007) assayed thermotaxis on a steeper gradient than we did, and both prior studies (Anderson et al., 2007; Jurado et al., 2010) used assay plates comprised of different media than the NGM agar plates on which the worms were reared, which might have led to a context-specific association of food, temperature and medium. Such circumstances could potentially change the association of food and temperature, and block thermophilic behaviour in some assay conditions (Law et al., 2004).

Heritable variation within *C. briggsae* for temperature-dependent behaviour

We observed consistent differences among strains of *C. briggsae* across distinct assays and rearing conditions, which is indicative of heritable differences among the strains. There are three broad

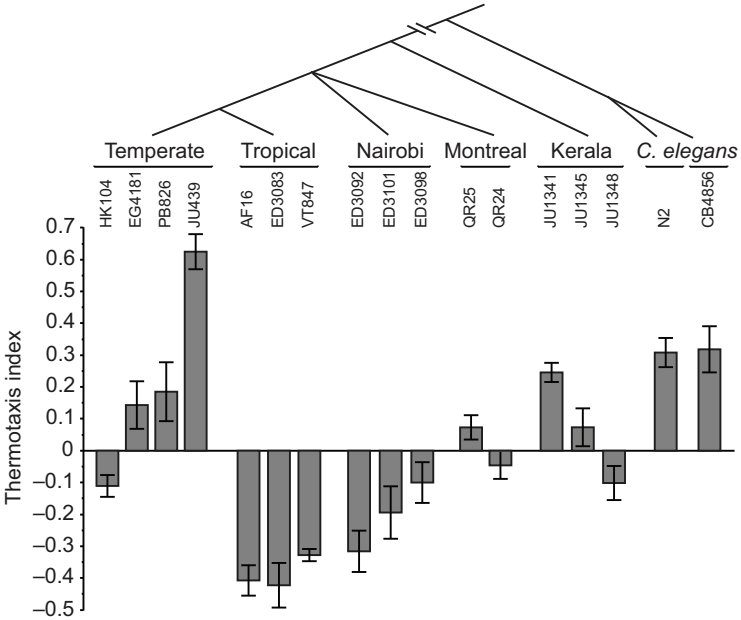

Fig. 4. *Caenorhabditis briggsae* genealogical topology overlaying patterns in thermotaxis behaviour. Genealogy based on Cutter and Choi (Cutter and Choi, 2010) and Jovelin and Cutter (Jovelin and Cutter, 2011). Behaviour patterns consist of thermotaxis data from the 23°C reared treatment on a 17–23°C gradient, similar to that shown in Fig. 2C, ordered according to strain relationships. For both *C. elegans* and strains HK104, AF16, ED3092, QR25 and JU1341, data are identical to those in Fig. 2C and from a minimum *N*=12 plates for each strain; all other strains tested had either four or five plates tested.

patterns of behaviour. First, *C. briggsae* strains AF16 and ED3092 tend toward cryophilic thermotaxis, even under conditions where one expects to see thermophilic behaviour towards the rearing temperature. These two strains also perform isothermal tracking at a lower average temperature when reared at 23°C. Second, strain HK104 also showed taxis away from its rearing temperature, but this strain seems unique as it was the only strain to move away thermophilically (Fig. 2E). Third, strains JU1341 and QR25 performed similarly to the two *C. elegans* strains. This included a clear preference for the rearing temperature in thermotaxis assays and isothermal tracking at higher temperatures when reared at 23°C. However, these between-species consistencies in behaviour patterns do not hold for isothermal tracking behaviour of strains reared at 17°C. For instance, JU1341 and QR25 differ from N2 in average isothermal tracking temperature ($F_{6,1081}$=8.09, P<0.0001; Tukey's HSD, P<0.0001). Under this condition, however, the rank order of the average tracking temperatures is relatively consistent with the clusters of behavioural patterns: AF16, ED3092 and HK104 have the lowest average tracking temperatures while JU1341 and QR25 are nominally higher (but not statistically significant). We hypothesize that common underlying pathways affect behaviour on both assays, which would yield, for example, the cryophilic tendencies of AF16 and ED3092 in both thermotaxis and isothermal tracking experiments. However, there would still need to be distinct components causing differences between assays. Further dissection of these behaviours, quantified separately, would help uncover the mechanisms that generate the disparate thermotactic responses.

Natural phenotypic variation has been explored in *C. briggsae* for several traits, including male tail morphology (Baird, 2001; Baird et al., 2005), vulval cell fate (Delattre and Félix, 2001; Dolgin et al., 2008) and fecundity (Fodor et al., 1983; Prasad et al., 2011). Our findings of heritable behaviour differences among wild genetic backgrounds that can exceed interspecific differences underscores the importance of quantifying intraspecific variability as a reference for contrasts between species.

Behaviour patterns and *C. briggsae* genealogical relationships

A sequential origin for broad patterns of behaviour seems plausible when mapped onto a genealogical network relating *C. briggsae* genetic backgrounds (Fig. 4). The 'Kerala' clade corresponds to the most basal genetic group within *C. briggsae* (Cutter and Choi, 2010; Cutter et al., 2010; Jovelin and Cutter, 2011), from which strain JU1341 derives, and is the most similar to *C. elegans* in thermotaxis behaviour. Strains from the 'Nairobi' and 'Tropical' phylogeographic groups behave similarly with strong cryophilic tendencies, suggesting that some change in behaviour arose in their common ancestor. The 'Montreal' clade strains, including strain QR25, exhibit relatively weak thermal responses; the genealogical position of these strains remains unresolved (Jovelin and Cutter, 2011). The most recent split in the *C. briggsae* lineage separates the 'Tropical' and 'Temperate' clades. And yet, strains of 'Tropical' origin appear consistently cryophilic in contrast to the heterogeneous responses among 'Temperate' clade strains, with HK104 yielding some distinctive behaviours (Figs 2, 4). Curiously, population genetic data indicate that 'Temperate' clade strains differ from each other less in DNA sequence than do strains within the 'Tropical' clade (Cutter et al., 2006; Cutter and Choi, 2010), suggesting a mismatch between genome-wide levels of polymorphism and phenotypic disparity. Prasad et al. (Prasad et al., 2011) reported that fecundity also varied among strains within the same genetic group, but that the largest differences distinguished strains from different genetic groups, in contrast to the patterns we observe here for thermal behaviours.

Strains AF16 and HK104 have been cultivated in laboratories for much longer than other *C. briggsae* strains, and might have accumulated adaptations to laboratory conditions and thus may not be archetypal strains for natural behaviour from their respective clades (especially HK104, see Fig. 2C,E, Fig. 4). Laboratory adaptations in *C. elegans* strain N2 were found to pleiotropically affect multiple behaviours, as strains were selected unintentionally for those animals that did not burrow into agar (McGrath et al., 2009). However, *C. elegans* N2 behaves differently from AF16 and HK104 in our thermotaxis assays, suggesting that any putative lab adaptations are not convergent for these behaviours (McGrath et al., 2011).

Tendency toward cryophilic behaviour

We have not been able to elicit thermophilic behaviour in *C. briggsae* strains AF16 or ED3092. Gradient steepness (°C cm^{-1}) could be a

factor: *C. elegans* tends to show cryophilic preferences on steeper gradients (Jurado et al., 2010; Nakazato and Mochizuki, 2009). Although we did not exhaustively test alternative gradients, we did not observe thermophilic behaviour for AF16 on shallower gradients in pilot experiments (data not shown). Albeit opposite to *C. elegans*, it is conceivable that a steeper gradient could be required for taxis towards a relatively warmer thermal memory. Strains AF16 and ED3092 show a preference to their rearing temperature on our isothermal tracking assay, which was performed on a steeper gradient than the thermotaxis assays (0.9 *versus* 0.5°C cm^{-1}). However, the assay timing or the absolute temperature of the gradient could also contribute (Ito et al., 2006; Jurado et al., 2010). We did not assay thermotaxis with a time series or test thermotaxis at the extreme low end of the comfortable temperature range for this species [near 14°C for fecundity (Prasad et al., 2011)]. Similarly ectothermic, but parasitic, worms have been shown to navigate up thermal gradients to the point of death (McCue and Thorson, 1964; Hitcho and Thorson, 1972), and yet it seems similarly maladaptive for these *C. briggsae* strains to do the opposite and navigate down a gradient to the point at which they can no longer move. One possibility is that high temperatures impose a strong selective force in nature that has resulted in the evolution of a robust anti-thermophilic response.

Conclusions

Heritable variation in temperature-dependent behaviour suggests the possibility of underlying adaptation to different temperature regimes in nature. Given that fecundity varies with temperature for *C. briggsae* (Prasad et al., 2011), we expected that variation in behaviour might vary in a concordant manner, as behaviour is the main way for these animals to regulate body temperature. Although we demonstrated heritable variation in behaviour, it remains ambiguous whether this variation represents differential adaptation to distinct environmental conditions. *Caenorhabditis briggsae* as a model system for temperature-dependent behaviour has the potential to reveal the molecular details of these behaviours, and future work should endeavour to further characterize *C. briggsae* thermotaxis, including temporal dynamics and locomotory properties of individual animals. A quantitative genetics approach taking advantage of this natural variation will help uncover the causative nucleotide differences between strains, and help us to understand the molecular basis and evolutionary processes that drive behavioural responses to temperature stimuli in these organisms (Bendesky and Bargmann, 2011; Ross et al., 2011).

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REFERENCES

Anderson, J. L., Albergotti, L., Proulx, S., Peden, C., Huey, R. B. and Phillips, P. C. (2007). Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests. *J. Exp. Biol.* **210**, 3107-3116.

Anderson, J. L., Albergotti, L., Ellebracht, B., Huey, R. B. and Phillips, P. C. (2011). Does thermoregulatory behavior maximize reproductive fitness of natural isolates of *Caenorhabditis elegans*? *BMC Evol. Biol.* **11**, 157.

Baird, S. E. (2001). Strain-specific variation in the pattern of caudal papillae in *Caenorhabditis briggsae* (Nematoda : Rhabditidae); implications for species identification. *Nematology* **3**, 373-376.

Baird, S. E., Davidson, C. R. and Bohrer, J. C. (2005). The genetics of ray pattern variation in *Caenorhabditis briggsae*. *BMC Evol. Biol.* **5**, 3.

Bendesky, A. and Bargmann, C. I. (2011). Genetic contributions to behavioural diversity at the gene–environment interface. *Nat. Rev. Genet.* **12**, 809-820.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Cassata, G., Kagoshima, H., Andachi, Y., Kohara, Y., Dürrenberger, M. B., Hall, D. H. and Bürglin, T. R. (2000). The LIM homeobox gene ceh-14 confers thermosensory function to the AFD neurons in *Caenorhabditis elegans*. *Neuron* **25**, 587-597.

Colosimo, M. E., Brown, A., Mukhopadhyay, S., Gabel, C. V., Lanjuin, A. E., Samuel, A. D. T. and Sengupta, P. (2004). Identification of thermosensory and olfactory neuron-specific genes via expression profiling of single neuron types. *Curr. Biol.* **14**, 2245-2251.

Cutter, A. D. and Choi, J. Y. (2010). Natural selection shapes nucleotide polymorphism across the genome of the nematode *Caenorhabditis briggsae*. *Genome Res.* **20**, 1103-1111.

Cutter, A. D., Félix, M.-A., Barrière, A. and Charlesworth, D. (2006). Patterns of nucleotide polymorphism distinguish temperate and tropical wild isolates of *Caenorhabditis briggsae*. *Genetics* **173**, 2021-2031.

Cutter, A. D., Yan, W., Tsvetkov, N., Sunil, S. and Félix, M.-A. (2010). Molecular population genetics and phenotypic sensitivity to ethanol for a globally diverse sample of the nematode *Caenorhabditis briggsae*. *Mol. Ecol.* **19**, 798-809.

Delattre, M. and Félix, M.-A. (2001). Polymorphism and evolution of vulval precursor cell lineages within two nematode genera, *Caenorhabditis* and *Oscheius*. *Curr. Biol.* **11**, 631-643.

Dolgin, E. S., Félix, M.-A. and Cutter, A. D. (2008). Hakuna Nematoda: genetic and phenotypic diversity in African isolates of *Caenorhabditis elegans* and *C. briggsae*. *Heredity* **100**, 304-315.

Fodor, A., Riddle, D. L., Nelson, F. K. and Golden, J. W. (1983). Comparison of a new wild-type *Caenorhabditis briggsae* with laboratory strains of *C. briggsae* and *C. elegans*. *Nematologica* **29**, 203-216.

Hedgecock, E. M. and Russell, R. L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **72**, 4061-4065.

Hillier, L. W., Miller, R. D., Baird, S. E., Chinwalla, A., Fulton, L. A., Koboldt, D. C. and Waterston, R. H. (2007). Comparison of *C. elegans* and *C. briggsae* genome sequences reveals extensive conservation of chromosome organization and synteny. *PLoS Biol.* **5**, e167.

Hitcho, P. J. and Thorson, R. E. (1972). Behavior of free-living and plant-parasitic nematodes in a thermal gradient. *J. Parasitol.* **58**, 599.

Hodgkin, J. and Doniach, T. (1997). Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* **146**, 149-164.

Inada, H., Ito, H., Satterlee, J. S., Sengupta, P., Matsumoto, K. and Mori, I. (2006). Identification of guanylyl cyclases that function in thermosensory neurons of *Caenorhabditis elegans*. *Genetics* **172**, 2239-2252.

Ito, H., Inada, H. and Mori, I. (2006). Quantitative analysis of thermotaxis in the nematode *Caenorhabditis elegans*. *J. Neurosci. Methods* **154**, 45-52.

Jovelin, R. and Cutter, A. D. (2011). MicroRNA sequence variation potentially contributes to within-species functional divergence in the nematode *Caenorhabditis briggsae*. *Genetics* **189**, 967-976.

Jurado, P., Kodama, E., Tanizawa, Y. and Mori, I. (2010). Distinct thermal migration behaviors in response to different thermal gradients in *Caenorhabditis elegans*. *Genes Brain Behav.* **9**, 120-127.

Koboldt, D. C., Staisch, J., Thillainathan, B., Haines, K., Baird, S. E., Chamberlin, H. M., Haag, E. S., Miller, R. D. and Gupta, B. P. (2010). A toolkit for rapid gene mapping in the nematode *Caenorhabditis briggsae*. *BMC Genomics* **11**, 236.

Law, E., Nuttley, W. M. and van der Kooy, D. (2004). Contextual taste cues modulate olfactory learning in *C. elegans* by an occasion-setting mechanism. *Curr. Biol.* **14**, 1303-1308.

Luo, L., Clark, D. A., Biron, D., Mahadevan, L. and Samuel, A. D. T. (2006). Sensorimotor control during isothermal tracking in *Caenorhabditis elegans*. *J. Exp. Biol.* **209**, 4652-4662.

McCue, J. F. and Thorson, R. E. (1964). Behavior of parasitic stages of helminths in a thermal gradient. *J. Parasitol.* **50**, 67-71.

McGrath, P. T., Rockman, M. V., Zimmer, M., Jang, H., Macosko, E. Z., Kruglyak, L. and Bargmann, C. I. (2009). Quantitative mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors. *Neuron* **61**, 692-699.

McGrath, P. T., Xu, Y., Ailion, M., Garrison, J. L., Butcher, R. A. and Bargmann, C. I. (2011). Parallel evolution of domesticated Caenorhabditis species targets pheromone receptor genes. *Nature* **477**, 321-325.

Mori, I. and Ohshima, Y. (1995). Neural regulation of thermotaxis in *Caenorhabditis elegans*. *Nature* **376**, 344-348.

Murray, R. L. and Cutter, A. D. (2011). Experimental evolution of sperm count in protandrous self-fertilizing hermaphrodites. *J. Exp. Biol.* **214**, 1740-1747.

Nakazato, K. and Mochizuki, A. (2009). Steepness of thermal gradient is essential to obtain a unified view of thermotaxis in *C. elegans*. *J. Theor. Biol.* **260**, 56-65.

Nigon, V. and Dougherty, E. C. (1949). Reproductive patterns and attempts at reciprocal crossing of *Rhabditis elegans* Maupas, 1900, and *Rhabditis briggsae* Dougherty and Nigon, 1949 (Nematoda: Rhabditidae). *J. Exp. Zool.* **112**, 485-503.

Ohnishi, N., Kuhara, A., Nakamura, F., Okochi, Y. and Mori, I. (2011). Bidirectional regulation of thermotaxis by glutamate transmissions in *Caenorhabditis elegans*. *EMBO J.* **30**, 1376-1388.

Prasad, A., Croydon-Sugarman, M. J. F., Murray, R. L. and Cutter, A. D. (2011). Temperature-dependent fecundity associates with latitude in *Caenorhabditis briggsae*. *Evolution* **65**, 52-63.

Ramot, D., MacInnis, B. L., Lee, H.-C. and Goodman, M. B. (2008). Thermotaxis is a robust mechanism for thermoregulation in *Caenorhabditis elegans* nematodes. *J. Neurosci.* **28**, 12546-12557.

Rockman, M. V. and Kruglyak, L. (2009). Recombinational landscape and population genomics of *Caenorhabditis elegans*. *PLoS Genet.* **5**, e1000419.

Ross, J. A., Koboldt, D. C., Staisch, J. E., Chamberlin, H. M., Gupta, B. P., Miller, R. D., Baird, S. E. and Haag, E. S. (2011). *Caenorhabditis briggsae* recombinant

inbred line genotypes reveal inter-strain incompatibility and the evolution of recombination. *PLoS Genet.* **7**, e1002174.
Ryu, W. S. and Samuel, A. D. T. (2002). Thermotaxis in *Caenorhabditis elegans* analyzed by measuring responses to defined thermal stimuli. *J. Neurosci.* **22**, 5727-5733.
Samuel, A. D. T., Silva, R. A. and Murthy, V. N. (2003). Synaptic activity of the AFD neuron in *Caenorhabditis elegans* correlates with thermotactic memory. *J. Neurosci.* **23**, 373-376.
Satterlee, J. S., Ryu, W. S. and Sengupta, P. (2004). The CMK-1 CaMKI and the TAX-4 Cyclic nucleotide-gated channel regulate thermosensory neuron gene expression and function in *C. elegans. Curr. Biol.* **14**, 62-68.
Stiernagle, T. (2006). Maintenance of *C. elegans*. In *WormBook: The Online Review of C. elegans Biology* (ed. The *C. elegans* Research Community), doi/10.1895/wormbook.1.101.1.
Tanizawa, Y., Kuhara, A., Inada, H., Kodama, E., Mizuno, T. and Mori, I. (2006). Inositol monophosphatase regulates localization of synaptic components and behavior in the mature nervous system of *C. elegans. Genes Dev.* **20**, 3296-3310.
Wasserman, S. M., Beverly, M., Bell, H. W. and Sengupta, P. (2011). Regulation of response properties and operating range of the AFD thermosensory neurons by cGMP signaling. *Curr. Biol.* **21**, 353-362.
Zariwala, H. A., Miller, A. C., Faumont, S. and Lockery, S. R. (2003). Step response analysis of thermotaxis in *Caenorhabditis elegans. J. Neurosci.* **23**, 4369-4377.