

Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests

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Summary

The preferred body temperature of ectotherms is typically inferred from the observed distribution of body temperatures in a laboratory thermal gradient. For very small organisms, however, that observed distribution might misrepresent true thermal preferences. Tiny ectotherms have limited thermal inertia, and so their body temperature and speed of movement will vary with their position along the gradient. In order to separate the direct effects of body temperature on movement from actual preference behaviour on a thermal gradient, we generate a null model (i.e. of non-thermoregulating individuals) of the spatial distribution of ectotherms on a thermal gradient and test the model using parameter values estimated from the movement of nematodes (*Caenorhabditis elegans*) at fixed temperatures and on a thermal gradient. We show that the standard lab strain N2, which is widely used in thermal gradient studies, avoids high temperature but

otherwise does not exhibit a clear thermal preference, whereas the Hawaiian natural isolate CB4856 shows a clear preference for cool temperatures (~17°C). These differences are not influenced substantially by changes in the starting position of worms in the gradient, the natal temperature of individuals or the presence and physiological state of bacterial food. These results demonstrate the value of an explicit null model of thermal effects and highlight problems in the standard model of *C. elegans* thermotaxis, showing the value of using natural isolates for tests of complex natural behaviours.

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Key words: *Caenorhabditis elegans*, diffusion model, natural variation, null model, thermal gradient, thermal preference.

Introduction

Temperature profoundly affects the physiological rates, ecology and fitness of ectotherms. Ectotherms that detect and respond to temperature can avoid extreme high and low temperatures that may be damaging or even lethal (Kingsolver and Watt, 1983; Grant and Dunham, 1988; Rosenzweig et al., 2005). Moreover, many ectotherms manipulate time of activity, habitat choice and posture in an attempt to achieve body temperatures, T_b , in narrow preferred (or 'selected') ranges (Cowles and Bogert, 1944; Heath, 1965; Huey et al., 1977; Christian et al., 1983; Stevenson, 1985; Kingsolver, 1987) and thereby increase the amount of time spent at physiologically optimal temperatures (Christian and Tracy, 1981; Huey, 1983; Kingsolver and Watt, 1983; Waldschmidt and Tracy, 1983; Huey et al., 1989; Hertz et al., 1993; Angilletta et al., 2002; Huey et al., 2003). Thermal preferences are usually measured by placing individuals in laboratory thermal gradients and then determining the distribution of their T_b (Licht et al., 1966; DeWitt and Friedman, 1979; Crawshaw, 1980). This distribution is assumed to represent the preferred or selected (Pough and Gans, 1982) temperature of the population.

However, if part of the gradient is cold enough to slow or immobilize the animal, the observed distribution of T_b may be biased by a 'cold trap' and thus not represent a true thermal preference. Similarly, if the time course of the experiment is too short for ectotherms to disperse to their preferred zone, observed T_b distributions can again be misleading.

These concerns are generally insignificant for gradient studies with vertebrate ectotherms such as lizards (Licht et al., 1966; Bennett and John-Alder, 1986; Angilletta et al., 2002), snakes (Huey et al., 1989; Blouin-Demers and Weatherhead, 2001) and fish (Beitinger and Fitzpatrick, 1979; Reynolds and Casterlin, 1979; Crawshaw, 1980). These ectotherms are large and thus have considerable thermal inertia (Spotila et al., 1973; Stevenson, 1985; Christian et al., 2006). Consequently, they can move around quickly in a laboratory thermal gradient, and thus their body temperature, and hence speed, will not change markedly with position in the gradient. Therefore, the distribution of their T_b should serve as a valid estimate of thermal preference, assuming temperatures in the cold zone do not trap these animals.

For microorganisms, however, these concerns are major.

Microorganisms have limited thermal inertia (Stevenson, 1985) and so their temperature and speed will vary with position on the gradient. Moreover, if they are small relative to the size of the gradient, they may take a long time to reach preferred zones. The direct interaction between temperature and locomotion has not been appreciated in prior thermal preference studies of microscopic ectotherms, and currently no empirical framework exists for evaluating whether the resulting temperature distribution is generated by preference or by kinetics or both. Resolving this issue is important because of widespread interest in thermal preferences of small and microscopic ectotherms such as *Caenorhabditis elegans* (Hedgecock and Russell, 1975; Mori and Ohshima, 1995; Ryu and Samuel, 2002; Yamada and Ohshima, 2003; Mohri et al., 2005; Biron et al., 2006; Luo et al., 2006; Ito et al., 2006), *Daphnia* (Kessler and Lampert, 2004), *Escherichia coli* (Salman et al., 2006) and *Dictyostelium discoideum* (Poff and Skokut, 1977).

A resolution to this dilemma can be achieved in several steps. First, one must develop a mathematical null model that predicts the expected (or null) distribution of microscopic ectotherms on a thermal gradient in the absence of thermal preference. In other words, the model is developed to predict the impact of temperature-mediated diffusion of non-regulating organisms. Second, to parameterize the model, one must quantify actual movement rates as a function of temperature. Then, deviations of the actual distribution of ectotherms from the null distribution provide evidence of active thermoregulation.

Null models have long been important to thermal biology. James Heath's classic beer-can experiment (Heath, 1964), which was probably the first null (experimental) model in physiological ecology, forced physiological ecologists to re-evaluate evidence of thermoregulation. Subsequent null models have extended this theme for field studies (Huey et al., 1979; Hertz et al., 1993; Huey et al., 2003). Surprisingly, however, null models have not been developed or applied for laboratory studies. In the present study, we integrate observations of temperature-dependent diffusion of microscopic ectotherms on a thermal gradient with a mathematical model of temperature-dependent dispersal. In doing so, we establish a framework for evaluating the potentially biasing role of temperature-dependent dispersal on thermal preference assays. To investigate the validity of this solution through simulations, we use empirical data obtained from the nematode *C. elegans* and apply our findings to empirical results for the standard *C. elegans* laboratory strain (N2) as well as to the most genetically divergent known strain of *C. elegans* (CB4856). We show that N2 displays an ambiguous thermal response whereas CB4856 is strongly cryophilic.

Caenorhabditis elegans is a very small ectotherm that has been used extensively to study thermal preference behaviour (Hedgecock and Russell, 1975; Mori and Ohshima, 1995; Ryu and Samuel, 2002; Yamada and Ohshima, 2003; Mohri et al., 2005; Biron et al., 2006; Luo et al., 2006; Ito et al., 2006). This androdioecious (males and selfing hermaphrodites) soil nematode is approximately 1 mm in length and 0.6 µg as an adult (Ferris et al., 1995). At this size, the direct effects of environmental temperature on physiology can be substantial and wide ranging. Temperature impacts many evolutionarily and ecologically relevant aspects of *C. elegans* biology, including generation time, growth and reproduction rates (Byerly et al.,

1976), rate of population increase (Venette and Ferris, 1997) and swimming behaviour (Ryu and Samuel, 2002). Additionally, allele-specific sensitivity to temperature influences age at maturity, fertility and growth rate (Gutteling et al., 2007). *Caenorhabditis elegans*, therefore, provides an ideal system for investigating the underlying ecological and genetic basis of thermal preference.

A model of dispersion patterns under temperature-sensitive movement rates

To develop a null model of dispersion patterns under the sole and direct influence of temperature, we consider a random walk model of movement in which the rate of movement is temperature dependent. Such problems are often modelled using a diffusion approximation, which considers a population of identical individuals spreading out across a continuous spatial landscape (Berg, 1993; Murray, 1993). In general, diffusion models of this type assume that animals tend to move from more crowded areas to less crowded areas. Instead, we will assume that animals move in a random, Brownian fashion, but at a temperature-dependent rate. If rates of locomotion increase with temperature, then we might expect animals in cool sections of the gradients to tend to stay there, whereas many of those at warmer temperatures would tend to stream into cooler areas, resulting in an apparent cold preference even in a non-thermoregulator.

When movement rate depends on temperature, the equation for the change in density at a point in one-dimensional space is:

$$\frac{\partial f(t,x)}{\partial t} = \frac{\partial}{\partial x} \left(D(c(t,x)) \cdot \frac{\partial f(t,x)}{\partial x} \right), \quad (1)$$

where t is time, x is spatial position, c is temperature, f is the density of the animal, and D is the diffusion rate [p. 235 (Murray, 1993)]. For simplicity, we assume that temperature varies only with spatial position, not with time, as would be the case for a stable thermal gradient. This equation can be applied to a two-dimensional case as long as temperature is constant in the second dimension and we integrate density over the second dimension for each point on the first dimension.

For a specific function $c(x)$, we can solve Eqn 1 numerically to track the change in density with time. If the population is restricted to a finite spatial domain of $[0,L]$, as it often is experimentally, then we have no-flux boundary conditions where $\partial f(t,0)/\partial t = \partial f(t,L)/\partial t = 0$. In addition to tracking changes in density over time, we can also solve for the steady-state distribution that animals will eventually achieve. For a stable thermal gradient, we can write the steady-state condition as:

$$D'(c(x))c'(x) \frac{\partial f(x)}{\partial x} + D(c(x)) \frac{\partial^2 f(x)}{\partial x^2} = 0. \quad (2)$$

A constant-density function with the same mass as the initial population will satisfy Eqn 2 and is a steady state of the system. Hence, even when movement rates change with position (and therefore temperature), a uniform dispersion can result. Thus, the null model predicts that ectotherms lacking a thermal preference will be distributed uniformly along a gradient, at least at equilibrium.

We can evaluate this somewhat surprising result by using Eqn 2 to check other possible steady-state solutions, noting that it gives a differential equation for f in terms of the diffusion function whose solution is:

$$\frac{\partial f(x)}{\partial x} = \frac{A}{D(c(x))}, \quad (3)$$

where A is an arbitrary constant of integration. The boundary conditions require that the derivative of density approaches 0 as we move towards the boundary, so for an arbitrary diffusion function the only way for Eqn 3 to be satisfied is if $A=0$. Thus, again contrary to our intuitive expectation (above) that animals would collect at cool sections of the gradient, they should actually tend to spread out uniformly under a pure temperature-dependent diffusion model (although they might require some time to achieve that equilibrium). This is because at any given point the instantaneous rate of the number of individuals moving into that point matches the rate of those moving out of that point. If the boundaries are absorptive rather than reflective, as above, then we would expect animals to mostly collect nearest to the boundary at which they start. If there are no boundaries, then animals will not collect anywhere and will diffuse to cover the entire temperature spectrum.

Materials and methods

Individual-based simulation of the null model

Because the general differential equation is difficult to solve and because it is useful to test the underlying theory using numerical as well as analytical approaches, we constructed a simulation solution of the null model of temperature-based locomotion. Motivated by our empirical results (see below), we assumed that the rate of diffusion increases linearly with the temperature of an individual nematode over the range of temperatures considered here. The simulation used empirical estimates of this diffusion rate to describe the probability that an individual experiencing a particular temperature at time t would move to an adjacent position (or stay in the same place) at time $t+1$. The probability of movement to a particular location was assumed to be normally distributed, with a mean centred on the individual's current location and a variance determined by the estimated diffusion coefficient. The diffusion process was assumed to be time homogeneous, such that the diffusion coefficient was proportionally scaled for the timescale of locomotion being considered. For the results presented here, each individual's location was updated every 0.5 s.

After an individual's location was changed, its new temperature (and thus the probability distribution for future locomotion) was determined by its new location (assuming that T_b equilibrated instantaneously). Simulations were also conducted at fixed temperatures so that the simulated diffusion process could be checked against the actual diffusion process. In the simulation results presented below, null distributions were estimated by simulating the movement of individual nematodes on the gradient for 20 000 replicate experiments.

Nematode strain maintenance

The *C. elegans* natural isolate CB4856 and wild-type Bristol strain, N2, were obtained from the *Caenorhabditis* Genetics

Center (University of Minnesota, Minneapolis, MN, USA) and inbred by selfing for 10 generations to generate isogenic lines that were immediately frozen. Independent trails were run on thawed stocks in order to minimize any genetic changes that might accumulate over time. Stocks were allowed to acclimate for two generations out of the freezer before use to minimize any direct effects of freezing *per se*. Nematodes were maintained at 20°C on nematode growth medium-lite (NGM-lite; US Biological, Marblehead, MD, USA) seeded with *E. coli* strain OP50 unless otherwise indicated (Brenner, 1974; Sulston and Hodgkin, 1988).

Dispersal at fixed temperatures

We needed estimates of temperature-dependent locomotion at fixed temperatures to simulate the dispersal of *C. elegans* on a thermal gradient. Therefore, we measured the rate at which worms dispersed from a common release site at uniform temperatures (14°C, 20°C and 24°C). Age-synchronized populations (Stiernagle, 1999) of nematodes in the final larval stage (L4) were washed from uncrowded NGM-lite plates with S-basal (0.1 mol l⁻¹ NaCl, 0.006 mol l⁻¹ K₂HPO₄, 0.044 mol l⁻¹ KH₂PO₄, 1 ml of 5 mg ml⁻¹ cholesterol in ethanol), allowed to settle in microcentrifuge tubes, and transferred to 1 cm² Whatman 1.0 Qualitative filter paper (Florham Park, NJ, USA). By briefly inverting the filter paper onto the bacterial lawn that covered the thermal gels (NGM-lite prepared in 10.2×17.5×0.5 cm plastic frames backed with transparency film, seeded with OP50 and incubated overnight at room temperature), we were able to efficiently transfer approximately 300 readily motile worms to each thermal gel.

Assays were performed in temperature-controlled incubators at 14°C, 20°C and 24°C. Thermal gels (covered to prevent evaporation) were equilibrated to the appropriate temperature prior to each experiment. Nematode distribution was recorded *via* pen onto a transparency film with the aid of a stereomicroscope after 10, 60, 160, and 260 min. The data were then scanned to a computer, converted to digital format and analyzed with Image Pro Plus 5.1 (MediaCybernetics Inc., Silver Spring, MD, USA) to determine the location (x,y) of individual worms relative to a central origin.

Thermal gradient

Three thermal gradients, similar in design to that of Yamada and Ohshima (Yamada and Ohshima, 2003), were placed in a climate-controlled room with an ambient temperature of 15°C. Each gradient consisted of an aluminium plate (25×45×0.2 cm) secured with clamps to 2 cm²-thick aluminium bases. A 1-cm channel was drilled through the base (side to side), allowing the continuous circulation of water or ethylene glycol from external baths controlled at 50°C and 2°C. To facilitate temperature measurements and repeatable gel placement on the gradient, a thin plastic sheet containing a 1 cm² grid was secured with clamps to the aluminium plate; a thin layer of glycerol was placed between the plastic and aluminium, thereby holding the plastic flat against the plate. Thermal gels, in plastic frames with the transparency backing intact, were placed directly on the 1 cm² grid. This setup produced a stable and robust linear gradient averaging 1.15 deg. cm⁻¹ (s.e.m. 0.013 deg. cm⁻¹) on each gradient from

10.5 to 29°C (Fig. S1 in supplementary material). A second frame (14×22×5 cm) of heavy acetate with detachable lid was used to reduce airflow over the gels.

We mapped the surface temperature of thermal gels on each gradient apparatus to 1 cm² resolution using a thermistor probe (Omega 408; Stamford, CT, USA) attached to a handheld thermometer (Omega HH41; accuracy ±0.015°C, resolution ±0.01°C). The time course and stability of these readings was verified using temperature data loggers (iButton®, DS1921G; accuracy ±1°C, resolution ±0.5°C; Maxim Integrated Products Inc., Dallas, TX, USA). These data were used to generate a model of the surface temperature of the gels on each gradient using JMP 4.0 (SAS Institute, Cary, NC, USA). Temperature change across this portion of the gradient was highly linear, so a linear model accurately predicted temperature. Using this linear model, we then estimated the temperature experienced by an individual nematode at any given (x,y) coordinate. Relative to past studies, which assign worms to coarsely resolved temperature or location categories, our design allows more accurate measurement of the temperature experienced by each worm.

Thermal preference assay

Age-synchronized populations of naive nematodes (L4) were applied to thermal gels *via* filter-paper transfer at a specific location on each thermal gradient corresponding to 10°C, 20°C or 24°C depending on the experiment. Thermal gels were equilibrated on the thermal gradients for 1 h prior to the assays. Nematodes were allowed to experience a thermal gradient for up to 8 h before location of individual worms was recorded onto a transparency film with the aid of a lighted 5× magnifier. These data were converted to digital format and analyzed as previously described. Given the size of an individual nematode (<1 mm), the thermal time constant (Christian et al., 2006) for acclimation to the temperature of the agar surface is much less than one second, thus justifying the assumption that location and temperature of an individual are equivalent.

Food quality

If nematodes were tested on gradients with live bacteria for food, their position might be influenced by the effects of temperature on bacterial metabolism and reproduction and hence density. To ascertain if nematode distribution was influenced by these effects we assayed thermal preference using dead bacteria (heat- or UV-killed bacteria). Heat-killed bacteria were prepared by exposing liquid cultures of *E. coli* OP50 to 75°C for 1 h (Couillault and Ewbank, 2002). Dead bacterial cells were concentrated and spread onto thermal gels to approximate a live *E. coli* lawn and allowed to dry overnight at room temperature. To prepare UV-killed bacterial lawns, thermal gels were seeded with OP50 and grown overnight at room temperature, then irradiated for 4 min at 100 mJ cm⁻² in a UV Crosslinker FB-UVXL-1000 (FisherBiotech, Pittsburgh, PA, USA) and incubated at 37°C overnight. Thermal gels resulting from these treatments were tested for the presence of live bacteria by inoculating sterile NGM-lite plates with streaks from the treated lawns and incubating for 24 h at 37°C. Plates that tested positive for bacterial growth were eliminated (Gems and Riddle, 2000).

Cultivation temperature and fed vs unfed trials

In addition to testing worms with access to food (above), we also ran tests for worms without food so that we could compare our results with those of prior studies, which usually tested unfed worms. We raised replicate N2 and CB4856 populations at 14°C, 20°C or 24°C for two generations before assaying thermal preference in fed and in unfed trials. Populations – age synchronized by transferring eggs in non-crowded conditions to 10 cm NGM plates seeded with *E. coli* OP50 and grown to the final larval stage at the appropriate temperature – were assayed as above with the following exceptions. In unfed trials, thermal gels were not seeded, and assay duration was reduced to 1 h due to rapid migration of worms off the assay surfaces.

Statistical analyses

Results were analyzed using a factorial nested analysis of variance with main effects of strain, cultivation temperature and starting temperature, and with replicate as a nested effect. Replicate was treated as a random effect, whereas all other factors were considered fixed. Standard errors were calculated from the overall model *via* a least-squares means approach, with the whole-plate replicate (rather than the individual nematode) as the unit of sampling variation.

Results

Dispersal at fixed temperatures

We focus first on the CB4856 natural isolate and use the variance in individual locations at a particular time as our measure of dispersion. Rate of dispersal increased almost linearly with temperature (Figs 1, 2). Individuals at 10°C dispersed very little, even after 60 min, while those at higher temperatures, such as 20°C, moved rapidly across the 9 cm plates in roughly an hour (Fig. 2). The temperature-dependant rate of dispersal (Fig. 1) was used as the diffusion coefficient in simulations outlined above. The simulation accurately reconstructed the dynamics of dispersal at each temperature at multiple time points (Fig. 2) except for very cold temperatures, in which the diffusion approximation slightly overestimated the rate of movement. These patterns suggest that both model assumptions (linearity in the diffusion coefficient, time-homogeneous nature of diffusion) are not strongly violated in

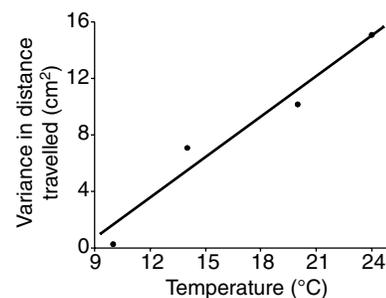


Fig. 1. Rate of dispersal/diffusion at fixed temperatures for CB4856 measured as the variance in individual straight-line distance travelled after 60 min (see Fig. 2 for the actual distributions). Increase in the rate of diffusion (D) with temperature (T) is fairly linear over this range ($R^2=0.92$), as fit by the equation $D=-0.41+0.046T$.

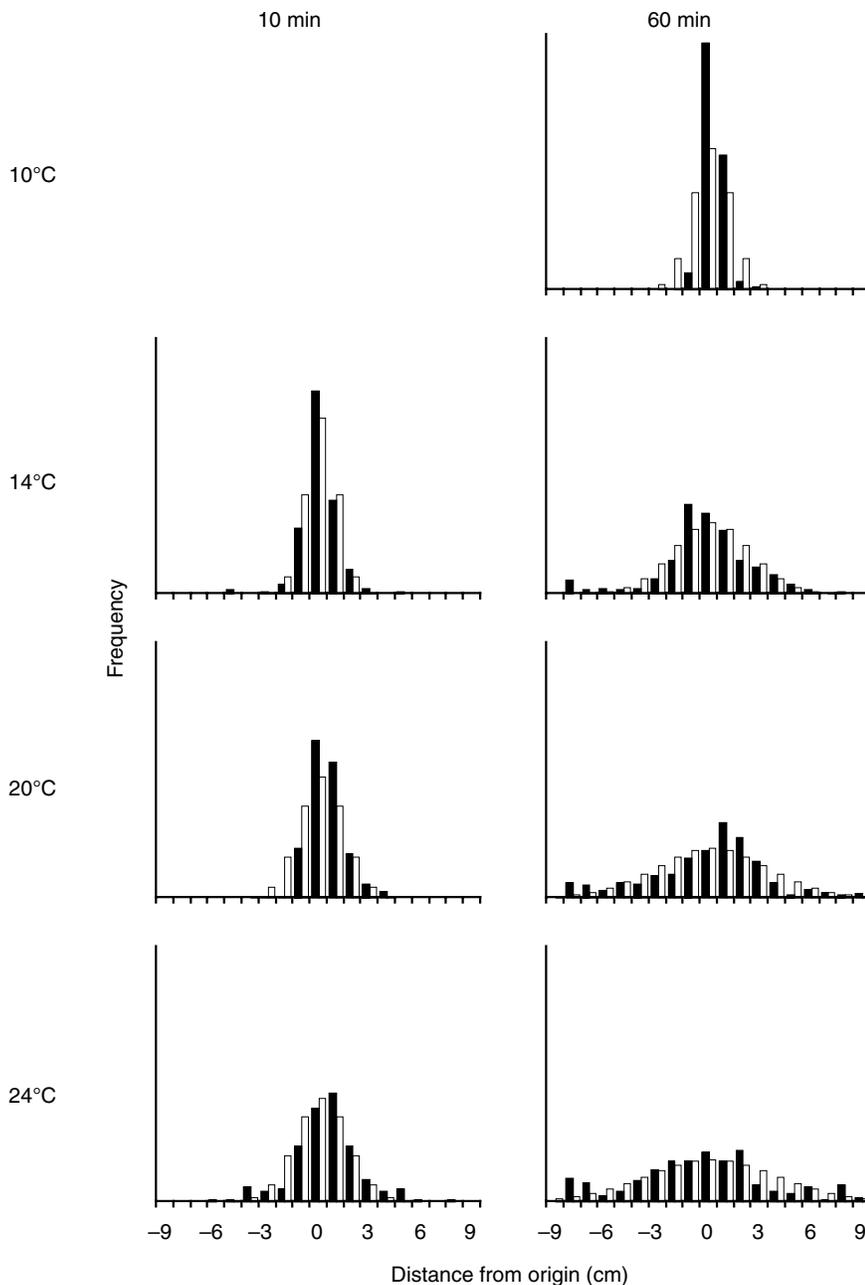


Fig. 2. Dispersal at fixed temperatures for the CB4856 natural isolate. The filled bars show the actual distributions of individual nematodes allowed to freely disperse for 10 or 60 min at a given temperature (mean number of individuals assayed per temperature=452). Individuals did not disperse after 10 min at 10°C. Open bars show the predicted distribution of individuals derived from the diffusion simulation based on the temperature-dependent linear change in diffusion coefficient estimated in Fig. 1. The diffusion process closely approximates the actual dispersal distribution at both time scales.

Dispersal and preference on a thermal gradient

Temperature-dependent (null) diffusion on a thermal gradient should be similar to diffusion at a fixed temperature, except that the rate of diffusion changes differentially with the location (and thus temperature) of an individual. Using the temperature–diffusion relationship measured above, we estimated how the distribution of individual nematodes (non-thermoregulating) would be expected to change during a 24 h period after a fixed start at 24°C (Fig. 3). Even after 15–30 min, individuals should diffuse over much of the upper part of the gradient but should take substantially longer to diffuse throughout the colder regions. Nevertheless, as predicted by the analytical model, the overall distribution tends to flatten out over time, although it is still slightly biased towards warmer temperatures even after 24 h (Fig. 3).

Comparing the actual movements of the CB4856 natural isolate to the null temperature-dependent diffusion model clearly shows that CB4856 displays thermal preference for colder temperatures (Fig. 4). This is most clearly seen early in the response: individuals not exhibiting thermal preference would be expected to still be relatively close to the starting position,

this system. Therefore, we used coefficients estimated at these fixed temperatures to generate a null model of temperature-dependent locomotion against which the thermal preference assays could be compared. The diffusion rate will obviously not remain linear across all temperatures, and there is evidence that the rate of diffusion declines at higher temperatures in other strains (results not shown), as must be the case as the upper thermal limit is approached. Because there is some expectation that physiological processes might increase exponentially with temperature, we also fit an exponential model to the data (which produced a poor fit relative to the linear model, $R^2=0.75$). Using the exponential model for the simulations produced results that are quantitatively very similar and qualitatively identical to those of the linear model and so are not presented here.

whereas real worms actually moved quickly into the colder region. At 8 h, the predicted null distribution has flattened out substantially, but the actual distribution of worms is still concentrated in the cold region, but making them less qualitatively different than earlier in the process (although they are still distinct; Kolmogorov-Smirnov test, $P<0.05$).

Whereas CB4856 exhibits a clear thermal preference, the standard lab strain, N2, responds qualitatively differently. We therefore constructed a null model for N2, using the approach outlined above, and used it to analyze the behaviour of N2 on a thermal gradient. As will be explored more fully below, N2 is largely immobile on the gradient, especially in the presence of food (Fig. 5). N2 apparently avoids extremely hot temperatures on the gradient, but its behaviour in the other parts of the thermal spectrum is quite consistent with the null dispersal model. As

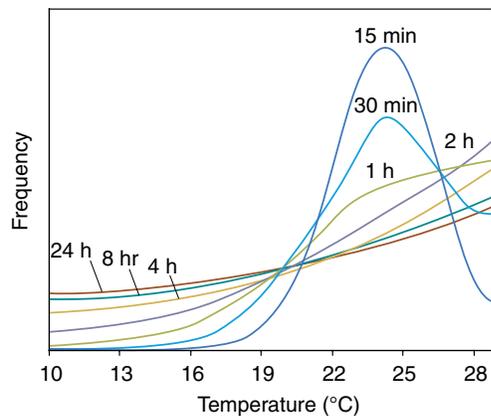


Fig. 3. Expected distribution of individuals from the CB4856 natural isolate across a thermal gradient starting at 24°C, assuming that locomotion is determined only by the temperature-dependent rate of diffusion. Results are from the diffusion simulation parameterized by the estimated diffusion coefficients at fixed temperatures (Fig. 1).

can be seen by comparing Fig. 4 and Fig. 5, N2 moves substantially less than does CB4856 over the same time period.

Initial starting conditions

The mean thermal preferences of N2 and CB4856 differ by more than 4°C [Figs 4 and 5; N2, 21.66 ± 0.213 (mean \pm s.e.m.); CB4856, 16.97 ± 0.298] under standard assay conditions (8 h, fed, 24°C start temperature, 20°C cultivation temperature). Note, however, that mean thermal preferences of N2 were always within a few degrees of each start temperature that we evaluated (Fig. 6; 14°C, 14.98 ± 0.239 °C; 20°C, 18.68 ± 0.0453), again suggesting that N2 lack a thermal preference beyond an avoidance of extreme temperatures. By contrast, CB4856 always migrated towards a preferred temperature range cooler than the cultivation temperature, regardless of start position (Fig. 6; 14°C, 14.55 ± 0.341 ; 20°C, 16.25 ± 0.348). Although the final position of both strains correlated with start temperature (N2, $F_{2,6.06} = 217.22$, $P < 0.0001$; CB4856, $F_{2,12.99} = 4.98$, $P = 0.025$), the effect of start temperature was much larger in N2 (Fig. 6).

Effect of food quality on thermal preference

Because growth and reproduction of their food (*E. coli*) will vary with temperature, nematodes on a thermal gradient seeded with live bacteria might be responding to differential growth and density of bacterial food with temperature rather than to temperature *per se*. Therefore, we compared the behaviour of nematodes with live *versus* dead food. Thermal preference was unaffected by food source (Fig. 7; $F_{2,18.8} = 3.13$, $P = 0.07$). However, CB4856 had a significantly lower thermal preference than did N2 ($F_{1,18.8} = 80.07$, $P < 0.0001$; Tukey's HSD, $P < 0.05$), consistent with the results presented above.

Food availability and cultivation temperature

When food availability and cultivation temperature were manipulated, CB4856 and N2 again differed significantly in observed thermal preferences (Fig. 8; $F_{1,27.5} = 247.17$, $P < 0.001$). We used shorter (1 h) assays for these tests, which resulted in

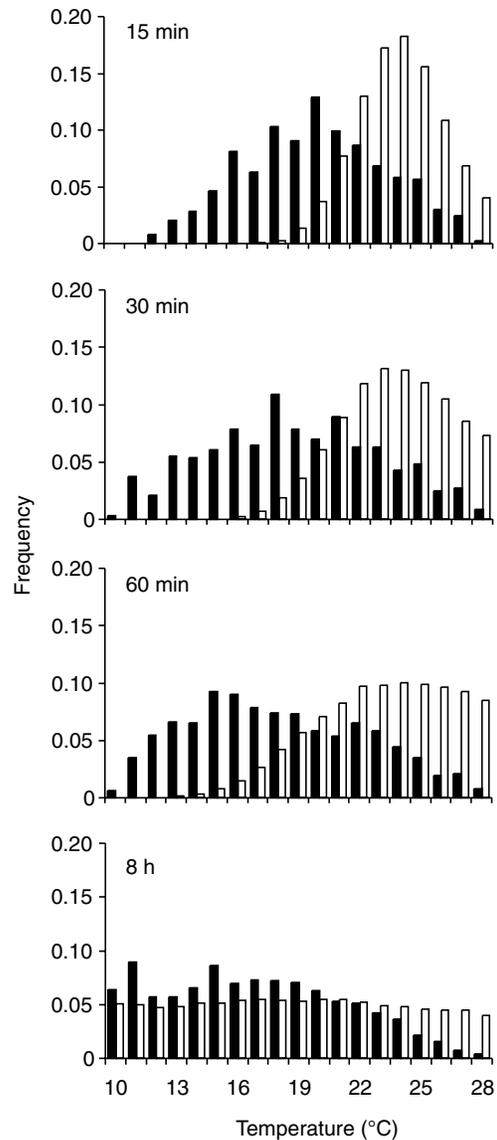


Fig. 4. Comparison of the actual distribution of individuals of CB4856 on a thermal gradient at different time points (filled bars), with the null temperature-dependent-only diffusion prediction from the diffusion simulation (open bars). Individuals starting at 24°C clearly move towards the colder region of the thermal gradient at a much quicker rate than would be expected under the null model. Sample sizes for the empirical distributions: 15 min, $N = 495$; 30 min, $N = 556$; 60 min, $N = 1077$; 8 h, $N = 886$.

slightly higher estimates of thermal preference than observed in our standard (8-h) assay, because of the reduced response time (e.g. see Fig. 4). Location after one hour shows the strongest signal of preference, however (Fig. 4), and the overall responses at these two time scales are very similar. Here, food and cultivation temperature had significant effects ($F_{1,27.5} = 13.51$, $P = 0.001$ and $F_{2,27.5} = 8.78$, $P = 0.001$, respectively), although this effect is driven almost entirely by the response of CB raised at 14°C and assayed in the absence of food, as this group is the only treatment within that strain that differs significantly from the other treatments (Tukey's HSD, $P < 0.05$). Cultivation of

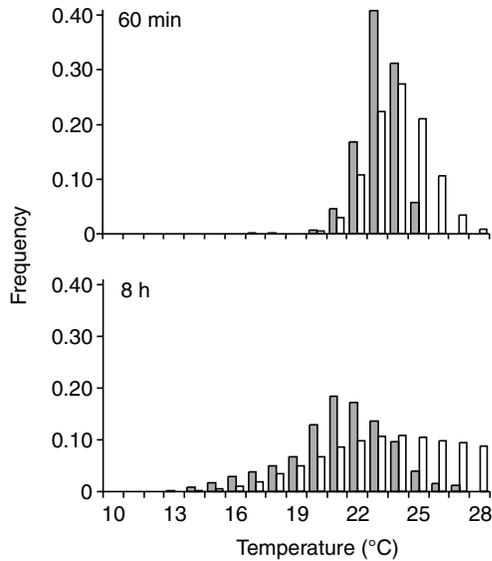


Fig. 5. Comparison of the actual distribution of individuals of N2 on a thermal gradient at different time points (filled bars), with the null temperature-dependent-only diffusion prediction from the diffusion simulation (open bars). Individuals starting at 24°C avoid high temperatures, but otherwise do not move. Sample sizes for the empirical distributions: 60 min, $N=432$; 8 h, $N=649$.

CB4856 at 14°C resulted in clumping behaviour not witnessed at other cultivation temperatures and may explain this shift in thermal behaviour.

Discussion

Temperature-dependent locomotion in microscopic ectotherms

Laboratory measurements of thermal preferences of ectotherms are often used as an index of physiologically optimal

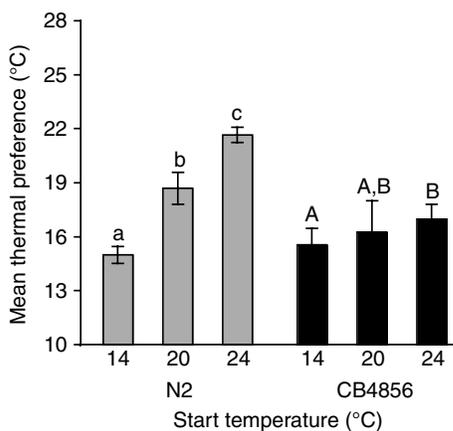


Fig. 6. Effect of start temperature on thermal preference in N2 (grey bars) and in CB4856 (black bars) cultivated at 20°C. CB4856 individuals consistently preferred cool temperatures, whereas N2 tended to disperse around their start temperature. Mean thermal preferences are based on a mean of four replicates, with 168 individuals per replicate (4038 total individuals). Values are least-square means \pm 2 s.e.m. Within each strain, treatments not connected by the same letter are significantly different ($P<0.05$; Tukey HSD).

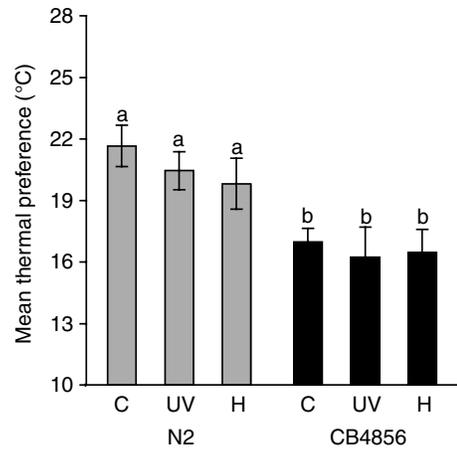


Fig. 7. Thermal preference in N2 (grey bars) and CB4856 (black bars) is not influenced by food quality. Control (C): thermal gels seeded with *E. coli* strain OP50 were incubated at room temperature overnight. Ultra-violet (UV): prepared as for control then subjected to UV radiation to kill the bacteria. Heat (H): bacteria grown in liquid culture overnight, killed *via* exposure to high temperature, concentrated, and applied to thermal gels to approximate a live lawn. Mean thermal preferences are based on 3–8 replicates with an average of 144 individuals per replicate for a total of 3446 observations. Values are least square means \pm 2 s.e.m. Treatments not sharing the same letter are significantly different ($P<0.05$; Tukey HSD).

temperatures (Huey and Bennett, 1987; Angilletta et al., 2002) and of thermoregulatory ‘targets’ for field-active animals (Hertz et al., 1993). Thermal preferences are typically estimated from the distribution of body temperatures of animals in a laboratory thermal gradient (Licht et al., 1966). Prior studies have implicitly assumed that the distribution of body temperatures reflects only the organism’s thermal preference and is thus not

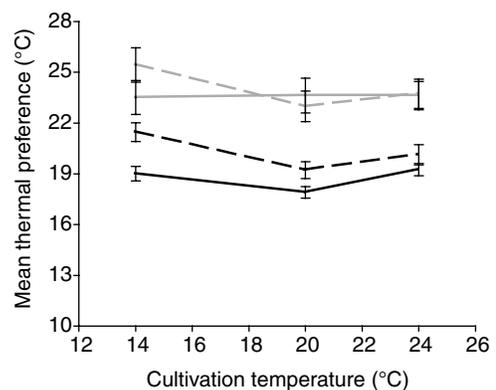


Fig. 8. Neither N2 (grey lines) nor CB4856 (black lines) migrates to their cultivation temperature regardless of food availability. Because starved worms rapidly migrate off unseeded thermal preference gels, the duration of these assays was reduced to 1 h, resulting in the observation of slightly higher estimates of mean thermal preference (see text). Worms raised for two generations at 14°C, 20°C or 24°C were assayed for thermal preference with OP50 present on the thermal gel (solid line) or without food (broken line). Mean thermal preferences are based on 3–5 replicates with a mean of 181 individuals per replicate for a total of 7059 individuals. Values are least square means \pm 2 s.e.m.

biased by the direct physiological effects of temperature on its directed movement in the gradient. This may be reasonable for large organisms that have thermal inertia and thus can move quickly around in the gradient without a major change in T_b but is unreasonable for microscopic organisms such as bacteria, protists and nematodes. Speed of movement of these small ectotherms will vary with their position in the gradient, potentially leading to temperature distributions that reflect the thermal dependence of movement as well as inherent thermal preference. To be able to evaluate the potential bias, we developed a null model for temperature-dependent diffusion and explicitly parameterized that model with empirical estimates of temperature-dependent rates of locomotion. This null model predicts the temperature distribution of a hypothetical ectotherm with temperature-dependent movement but without a thermal preference, and it allows us to rigorously evaluate the hypothesis that body temperatures in a gradient reflect only thermal preference.

Part of the motivation for generating this null model was our intuition that organisms that ventured into the cold sections of a gradient might simply be becoming 'trapped' there because their speed would be reduced. Obviously, if locomotion were to decline to zero at some temperature on the gradient, then individuals that experienced that temperature would indeed be trapped: in this case, low temperature would be an absorbing boundary for a random walk along the thermal gradient. However, as long as coldest temperature in the gradient is above this minimum temperature threshold, our theoretical analysis suggests – contrary to our simple intuition – that individuals moving randomly with respect to temperature (but at a temperature-dependent speed) will eventually spread uniformly over the gradient (Eqn 3). Certainly, for the time scales and diffusion rates of the strains studied here, the slowing of diffusion at low temperatures (Fig. 1) will not cause nematodes to accumulate at low temperatures (Fig. 3). Thus, rapid movement of the Hawaiian natural isolate CB4856 into the low-temperature portion of the gradient indicates true cryophilic behaviour, not low-temperature trapping (Fig. 4). By contrast, the standard lab strain N2 exhibits some tendency to avoid high temperatures but does not move substantially from where it is placed; therefore, N2 does not exhibit a true thermal preference, at least in this environment (Figs 5, 6). These between-isolate results are robust to differences in rearing temperature, food quality and overall food availability. In the absence of an explicit null model, these results would be much more difficult to interpret.

Thermal preference in C. elegans

In pioneering research on thermal preference in *C. elegans*, Hedgecock and Russell (Hedgecock and Russell, 1975) observed that well-fed worms move towards – but starved worms disperse away from – their cultivation temperature when assayed on a laboratory thermal gradient. These observations have served as the backbone of thermotaxis research in this important model organism and have facilitated advances in the identification of thermotaxis-related genes (Cassata et al., 2000; Colosimo et al., 2004; Satterlee et al., 2004; Mohri et al., 2005; Tanizawa et al., 2006; Inada et al., 2006), the study of learning and memory (Samuel et al., 2003; Mohri et al., 2005; Kodama

et al., 2006) and characterization of the neural network for thermotaxis (Mori and Ohshima, 1995; Zariwala et al., 2003; Samuel et al., 2003; Clark et al., 2006). However, the definitive roles of cultivation temperature and feeding state (i.e. fed *versus* starved) in *C. elegans* thermal preference, although supported in several recent studies (Mori and Ohshima, 1995; Mohri et al., 2005; Ito et al., 2006), are controversial (Yamada and Ohshima, 2003; Luo et al., 2006). Neither of the strains we evaluated (N2 and CB4856) conform to the standard expectation. Rather, N2 demonstrates thermal preference only in the sense that it avoids extreme high temperatures (Fig. 5), but otherwise it conforms closely to the null model and thus shows thermal neutral behaviour in a gradient. This outcome is consistent with the results of Yamada and Ohshima, who propose that avoidance of extreme temperatures is more important than cultivation temperature in dictating N2 dispersal on a thermal gradient (Yamada and Ohshima, 2003). This is also qualitatively consistent with the data of Ryu and Stewart (Ryu and Stewart, 2002) and Clark et al. (Clark et al., 2007a), who find that N2 exhibits an initial cryophilic response followed by isothermal tracking, although we do not find a strong role for cultivation temperature. Ryu and Stewart do find that N2 will exhibit isothermal tracking at a wide variety of temperatures, however (Ryu and Stewart, 2002).

N2 appears to lack preference for a specific temperature or range and thus differs from CB4856 as well as other organisms. Thus, the behaviour of N2 on a thermal gradient deviates from a canonical notion (Reynolds and Casterline, 1979) of thermal preference as representing single and narrow preferred temperature range. The behaviour of N2 in our assays is not easily explained by the three factors predicted to determine ectotherm distribution on thermal gradients: thermal preference behaviour, temperature-dependent physiological rates (Fig. 1) and time (Fig. 5). N2 appears to be 'unmotivated' to disperse from any moderate temperature on a thermal gradient when food is present, even though it is fully capable of locomotion at all temperatures found in our gradient and can cover substantial distances in the time allotted on the gradient (Fig. 1). Importantly, the behaviour of N2 is consistent over a range of cultivation temperatures, start temperatures, food quantities and food quality: thus, the results obtained here are unlikely to be artefacts of different assay designs between this and previous studies.

Historically, most thermal preference assays for *C. elegans* have been conducted without food. However, when well-fed nematodes are moved to food-free environments they swim up to 10-fold faster than when on food (de Bono and Bargmann, 1998; Sawin et al., 2000) and exhibit dispersal behaviour within approximately 40 min of starvation (Gray et al., 2005; Wakabayashi et al., 2004). This increased dispersal probably represents food seeking with temperature serving as a cue and likely confounds traditional thermal preference assays performed without food. Part of our motivation for testing for thermal preference in the presence of food, which is a new paradigm in *C. elegans*, is that we hypothesized that thermal preference in the presence of food might be different (and more ecologically relevant in terms of the long-term physiological response of the nematodes) than the apparent food cue or food-seeking behaviour traditionally observed for thermal preference

in the absence of food. To our surprise, we did not see a strong influence of the presence or absence of food on thermal preference (Fig. 8).

Striking differences in the thermal behaviour of CB4856 versus N2 (Figs 4, 5) indicate that thermal preference differs between strains of *C. elegans*. Prior research on thermotaxis in *C. elegans* focused almost exclusively on N2 or on mutants in an N2 background. However, the behaviour of N2 in a gradient (Fig. 6) is clearly atypical of other ectotherms and even of 'wild' strains of *C. elegans* such as CB4856 and other strains (L.A., J.L.A., R.B.H. and P.C.P., unpublished). We suspect that the atypical behaviour of N2 evolved as a consequence of its unusual and extensive cultivation history. N2 was collected prior to 1956 near Bristol, UK (Nicholas et al., 1959) and was maintained in unusual axenic laboratory conditions for nearly a decade (Dougherty et al., 1959; Nicholas et al., 1959) before being received by S. Brenner in 1964 (Brenner, 1974). During its long history of laboratory cultivation, N2 has experienced thousands of generations of evolution at room temperature: during this time, N2's normal thermoregulatory behaviours might well have decayed *via* mutation accumulation (Ajie et al., 2005), or even have become maladaptive in a homogeneous and constant thermal environment.

Given the strong and mounting evidence regarding the genetic and neurological basis of thermotaxis in *C. elegans* (e.g. Mori and Ohshima, 1995; Zariwala et al., 2003; Mohri et al., 2005; Clark et al., 2007b), it is clear that N2 does in fact respond to temperature. However, specific results have varied from laboratory to laboratory. This could be a reflection of differences in laboratory apparatus, laboratory-specific environmental conditions, and/or slight genetic differences between particular laboratory N2 strains [such differences have been observed in longevity studies (e.g. Gems and Riddle, 1996)]. Whatever the causes of these differences, they suggest that defining thermosensory behaviour within the standard lab strain may be noisy and that the field could benefit from a more robust model. Certainly in our assays, the magnitude of the response in thermal preference is much stronger in CB4856 than in N2. In any case, our results suggest that long-established laboratory stocks such as N2 may be inappropriate subjects as experimental models of normative behaviours. Natural isolates appear better suited for studies of complex behaviours such as thermotaxis.

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