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Disentangling thermal preference and the thermal dependence of movement in ectotherms

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ABSTRACT

Many ectotherms thermoregulate by choosing environmental temperatures that maximize diverse performance traits, including fitness. For this reason, physiological ecologists have measured preferred temperatures of diverse ectotherms for nearly a century. Thermal preference is usually measured by observing organism distributions on laboratory thermal gradients. This approach is appropriate for large ectotherms which have sufficient thermal inertia to decouple body temperatures from gradient temperatures. However, body temperatures and therefore speeds of movement of small ectotherms will closely track gradient temperature, making it difficult to distinguish between thermal preference and thermal dependence of movement. Here we develop and demonstrate the use of a patch model to derive the expected thermal gradient distribution given only the thermal dependence of movement. Comparison of this null distribution with the observed gradient distribution reveals thermal preference of small ectotherms.

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1. Introduction

Many ectotherms use behavior to regulate body temperature within a physiologically permissible range (Grant and Dunham, 1988) and often near a physiological optimum (Martin and Huey, 2008; Angilleta et al., 2002). An ectotherm's ability to avoid potentially lethal body temperatures and to increase the time it spends at optimal temperatures will have obvious and profound effects on its physiology and fitness. For this reason, physiologists have long been interested in measuring the preferred temperatures of organisms in relation to field body temperatures and the temperatures that maximize diverse performance traits and fitness (reviewed by Angilleta, 2009).

The preferred temperature, or thermal preference (T_{pref}) is difficult to measure in the field and so has been measured in the laboratory for ectotherms from lizards to fish and insects using a variety of techniques (Uvarov, 1931; Licht et al., 1966; Barbour and Racine, 1967). The most common approach is to establish a temperature gradient in a closed apparatus and monitor the position of a test animal or the distribution of a cohort of animals over time. The mean or median position of the single animal over time or of the cohort of animals at a set time is then taken as the thermal preference (Licht et al., 1966; DeWitt and Friedman, 1979).

This approach is likely appropriate for the measurement of T_{pref} of large ectotherms that have sufficient thermal inertia such that their speed of movement does not depend on their location on the gradient (Anderson et al., 2007; Licht et al., 1966). However, use of this technique for small ectotherms such as *Drosophila* and *Caenorhabditis elegans* is problematic. For small organisms, body temperature and also speed of movement closely track gradient temperature (Stevenson, 1985; Anderson et al., 2007; Dillon et al., 2009). Therefore, for small ectotherms, the implicit assumption that their location on the gradient depends only on T_{pref} is violated, making thermal gradient estimates of T_{pref} suspect.

Accurate measurement of thermal preferences of small ectotherms like *C. elegans* and *Drosophila* is becoming increasingly important as they are the de facto models for understanding the molecular, neuronal, and behavioral determinants of thermal sensation and thermal behavior (Garrity et al., 2010; Sayeed and Benzer, 1996; Hedgecock and Russell, 1975; Hong et al., 2008; Rosenzweig et al., 2005, 2008; Hamada et al., 2008; Luo et al., 2006; Ito et al., 2006). Unfortunately, analyses of thermal behavior of small ectotherms have largely ignored the thermal

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dependence of motion, neglecting to test the implicit assumption of a uniform distribution in the absence of thermal preference (but see Hong et al., 2006; Yamada and Ohshima, 2003; Anderson et al., 2007), and ignoring the null expectation could lead to serious misinterpretations of thermal preference data (Heath, 1964; Hertz et al., 1993).

Anderson et al. (2007) describe an attractive approach to circumventing this issue (see also Dillon et al., 2009). They derive a null model of *C. elegans* movement on a thermal gradient and estimate the effects of temperature on movement rates of worms to parametrize the model. Their diffusion-based model suggests, counter-intuitively, that worms will tend to approach a uniform distribution with some accumulation at the hot end of the gradient. Comparisons of worm distributions with null distributions generated by the model suggested that some worms did express a thermal preference independent of the thermal dependence of motion. Their diffusion-based model may not be the best approach for describing ectotherm movement on gradients because no-flux boundary conditions are assumed. In reality, the organisms likely turn back when they run into the boundary so the density of insects at the boundary can change with respect to time. Further, despite null distributions diverging strongly from uniform dispersion on the gradient, they estimated T_{pref} as the mean of the worm distribution. However, given some non-uniform null distribution, the actual distribution of animals on the gradient can only be considered to be the combination of thermal preference and the thermal dependence of motion.

Here we describe an approach to disentangling thermal preference from the thermal dependence of motion in small ectotherms. First, we develop a biologically inspired patch model to explicitly examine the (null) dynamics of animal distributions on thermal gradients in the absence of a thermal preference. We investigate how key experimental parameters determine the time to equilibrium and the resultant equilibrium distribution. We verify the model assumptions using data from fruit flies (*Drosophila melanogaster*) on a thermal gradient, and then demonstrate how to determine "true" thermal preference of small ectotherms on laboratory thermal gradients. We focus on *Drosophila* but emphasize that the approach is relevant for any small ectotherm.

2. A patch model for the null distribution

We used a two-dimensional patch model (Gustafson and Gardner, 1996; Hanski, 1998) to track the distribution of ectotherms on a thermal gradient. This model describes organism movement explicitly, allowing for direct incorporation of the thermal dependence of movement into predictions of null distributions. We divide the gradient of length L into patches of length equal to the body length of the animal, l (cm). So the total number of patches on the gradient is $n = L/l$. Let $P_i(t)$ denote the number of animals in patch i. It is mathematically equivalent to consider $P_i(t)$ to be the probability of finding a single animal in patch i at time t, but for the sake of brevity hereafter we describe the model in terms of distributions of multiple animals (but see Appendix for a demonstration of the model applied to individual animals tracked through time). If we assume that animals have no thermal preference, the movement from the ith to its two neighbor patches only depends on the animal's velocity, which depends on temperature.

The change in the number of animals in each gradient patch equals the number of animals that move in from neighboring patches (one on each side in this two-dimensional model) less the number of animals that move out of the patch. We derive the per capita influx rate from patch i to $i+1$ by dividing the animal velocity (body lengths per second) by the patch length, which we specify as one body length. Therefore, the influx rate from patch i to $i+1$ is $\nu(i)/1 = \nu(i)$ (units of body lengths/second).

We first describe the null expectation, i.e. the movement of individuals among patches P_1 to P_n on the thermal gradient when they have no thermal preference (with $n = L/l$).

$$\begin{cases} \dfrac{dP_1}{dt} = \nu_{21}P_2 - \nu_{12}P_1, \\ \dfrac{dP_i}{dt} = \nu_{i-1,i}P_{i-1} + \nu_{i+1,i}P_{i+1} - \nu_{i,i-1}P_i - \nu_{i,i+1}P_i, \quad i = 2, \ldots, n-1, \\ \dfrac{dP_n}{dt} = \nu_{n-1,n}P_{n-1} - \nu_{n,n-1}P_n, \end{cases} \tag{1}$$

Here the boundary patches are reflective, each only having one neighbor patch to go to or arrive from. We can find the equilibrium by setting the right hand sides of the model equal to zero, and rewriting in matrix form

$$\mathbf{V}\overrightarrow{P} = \overrightarrow{0}, \tag{2}$$

where

$$\mathbf{V} = \begin{pmatrix} -\nu_{12} & \nu_{21} & & & \\ \nu_{12} & -\nu_{21}-\nu_{23} & \nu_{32} & & \\ & \nu_{23} & -\nu_{32}-\nu_{34} & \nu_{43} & \\ & \ddots & \ddots & \ddots & \\ & & & \nu_{n-1,n} & -\nu_{n,n-1} \end{pmatrix}$$

and $\overrightarrow{P} = (P_1, P_2, \ldots, P_n)$.

For matrix $\mathbf{V}$, if we add the first row to the second row, and then add the new second column to the third row, and so on and so forth, we can prove that the rank of matrix $\mathbf{V}$ is $n-1$ and the equilibrium is given by

$$\overrightarrow{P}_s = \left(s, \frac{\nu_{12}}{\nu_{21}}s, \frac{\nu_{23}}{\nu_{32}}\frac{\nu_{12}}{\nu_{21}}s, \ldots, \frac{\nu_{n-1,n}}{\nu_{n,n-1}}\frac{\nu_{n-2,n-1}}{\nu_{n-1,n-2}} \cdots \frac{\nu_{12}}{\nu_{21}}s \right), \tag{3}$$

where s is decided by the equation and is the sum of all the elements of the equilibrium, which is equal to the initial number of animals placed on the gradient. The model (Eq. (1)) has a unique positive equilibrium which is decided by the movement velocity and the initial values.

If movement rates between patches were symmetric ($\nu_{ij} = \nu_{ji}$) the model equilibrium would be a uniform distribution on the gradient, with each patch having the same number of animals. Symmetric movement rates among patches would arise if the gradient apparatus was set at uniform temperature or if the velocity of ectotherm movement did not depend on temperature (i.e., a flat relationship in Fig. 1). However, if movement speed depends on temperature, there will be non-symmetric movement between patches, resulting in a non-uniform equilibrium distribution. Note that this non-uniform distribution is driven entirely by the thermal dependence of motion and is not an indicator of thermal preference.

3. Temperature dependence of walking speed

To evaluate the behavior of the model and estimate the effects of the thermal dependence of movement on null distributions, we used published estimates of *Drosophila* walking speed as a function of temperature (Fig. 1). These include measurements of maximum vertical climbing speed in a negative geotaxis assay (Strauss and Heisenberg, 1993; Crill et al., 1996; Gilchrist et al., 1997; Gibert et al., 2001; Roberts et al., 2003; Vaiserman et al., 2004; Gargano et al., 2005; Grotewiel et al., 2005; Dillon and Frazier, 2006), and of horizontal walking speeds of visually motivated (Strauss and Heisenberg, 1990, 1993; Martin and

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Ollo, 1996) and unmotivated (filmed, freely walking; Martin, 2004) flies (Table 1, Fig. 1). As with many thermal reaction norms (Huey and Kingsolver, 1989), overall the data are asymmetric, with a long tail at the cold end and a rapid drop-off at high temperatures. For purposes of illustration and use in the model simulations, we use the function $v(T)$ (Fig. 1, solid line) to describe the thermal dependence of walking speed. We use a fairly extreme case because it will have the largest effect on the null expectation, but we also evaluate effects of altered reaction norms on model predictions (Section 4.3). This function transforms temperature into fly walking velocity in any patch on the gradient. We denote $v_{i,j}$ as the velocity of individuals coming into patch i from patch j, where $j=i-1,i+1$ under the assumption that the experimental setup does not allow for flight between gradient patches.

4. Equilibrium of null distributions

Of considerable empirical interest is how long it takes for a set of animals to reach a stable distribution, and what factors affect this time to equilibrium (Table 2). We define the time to equilibrium as how long it takes (s) for the average per patch change in number of individuals from time t to time $t+1$ to be less than 1% of the total number of individuals on the gradient. Although this is an arbitrary definition, it is empirically appropriate–if 100 animals are placed on a gradient consisting of 10 patches, the distribution would be at equilibrium when the number of animals in each patch changed on average by less than 1 fly per time step. Time to equilibrium is largely insensitive to the initial placement of flies on the gradient, with all placements resulting in a stable equilibrium after 41–42 min (Fig. 2).

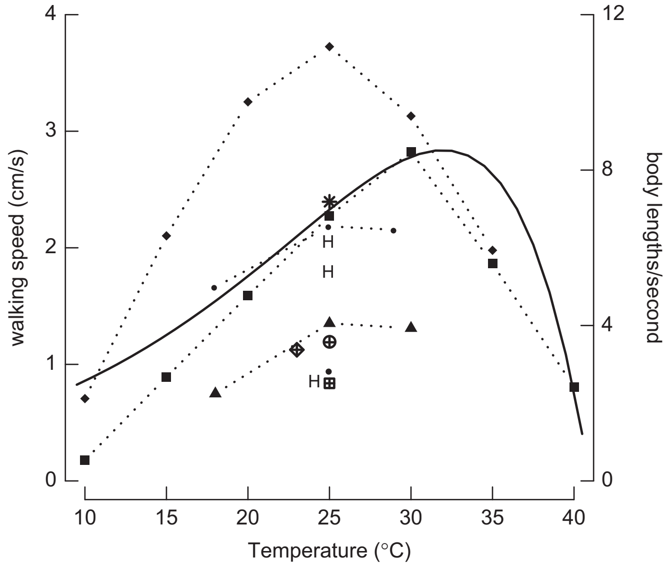

Fig. 1. Walking speed as a function of temperature from published studies (Table 1) using a negative geotaxis assay (black points and dotted lines), and from studies using a visually motivated horizontal walking speed assay ('H' points). The solid line depicts the relationship, $v(T)$, used in the model. The body lengths per second axis assumes fly body length of 1/3 cm.

4.1. Number of flies

Assuming no density-dependence, we can examine the effect of the initial number of flies placed on the gradient on the time to equilibrium and on equilibrium distributions. Experimentally, one might avoid density-dependence (i.e. flies impeding each other's movements), by keeping the gradient dimension perpendicular to the temperature axis sufficiently large (but this would not fix density-dependence arising from other factors, e.g. flies behaving as local attractors). Distributions took roughly 42 min to reach equilibrium, regardless of the initial number of flies placed on the gradient. Equilibrium distributions were qualitatively similar, but the resolution of the null distribution increased with increased initial number of flies (Fig. 3).

4.2. Steepness of the thermal gradient

We define the gradient steepness (ΔT) as the slope of temperature change across the length of the gradient (°C/cm, see Table 2). Note that changing the physical size of the gradient or changing the gradient temperature endpoints has the same effect on steepness, and therefore the same outcome (patch length is defined relative to gradient length). Changes in the steepness of the gradient strongly affected time to equilibrium (Fig. 4A). Temperature gradients shallower than 0.5 °C/cm greatly increased time to equilibrium whereas at steeper gradients, time to equilibrium did not change. Shallower gradients also resulted in slightly decreased accumulation at the cold end and increased accumulation at the hot end of the gradient (Fig. 4b).

4.3. Walking speed reaction norms

Variation in the shapes of thermal performance curves is well-documented (Huey and Stevenson, 1979; Gilchrist, 1995; Izem and Kingsolver, 2005) and can have important biological

Table 1
Drosophila walking speed data used to estimate the thermal dependence of motion.

Species	Notes	Reference
Maximum upward		
D. melanogaster	Four strains	Strauss and Heisenberg (1993)
D. melanogaster	$T_{dev}=18$, 25	Crill et al. (1996)
D. melanogaster	$T_{dev}=16.5$, 25, 29 °C	Gilchrist et al. (1997)
D. melanogaster	Age = 2,7,13 days, France or Congo	Gibert et al. (2001)
D. melanogaster	Heat-shock flies	Roberts et al. (2003)
D. melanogaster	Group geotactic measure	Vaiserman et al. (2004)
D. melanogaster	RING assay	Gargano et al. (2005)
D. melanogaster	RING, Tacc, aging	Grotewiel et al. (2005)
D. melanogaster	Sea-level	Dillon and Frazier (2006)
Spontaneous		
D. melanogaster	Buridan's paradigm	Strauss and Heisenberg (1990)
D. melanogaster	Buridan's, four strains	Strauss and Heisenberg (1993)
D. melanogaster	Buridan's paradigm	Martin and Ollo (1996)
Drosophila	Filmed in arena	Martin (2004)

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Table 2
Model parameters and properties of interest.

Parameter	Description	Values
L	Length of gradient (cm)	20–120
l	Fly body length (cm)	1/3
n	Number of patches$=L/l$	60–360
P_0	Number of flies on the gradient	200–1000
$T(0)$,$T(L)$	Minimum and maximum gradient temperatures (°C)	10, 40
$T(x)$	Temperature function $=T(L)-T(0)/Lx+T(0)$, $x\in[0,L]$	
$T(L)-T(0)/L$	Gradient steepness, ΔT (°C/cm)	0.25–1.5

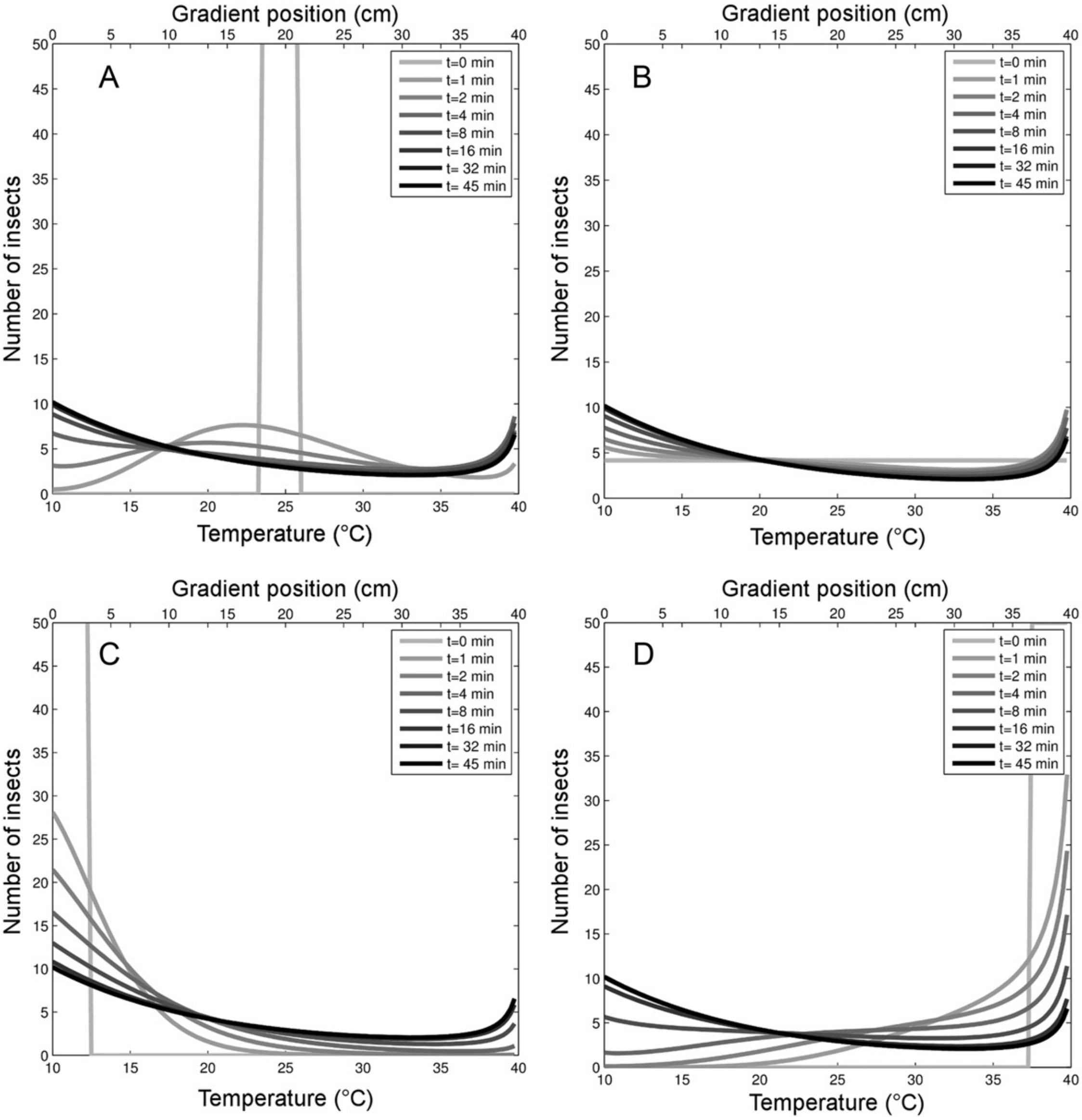

Fig. 2. Time course of fly distributions on the thermal gradient based solely on the thermal dependence of motion (Fig. 1, solid line). The number of flies in each gradient patch are tracked from their release either in the center (A), uniformly across the gradient (B), on the cold end (C), or on the hot end (D) of the gradient at time $t=0$ to equilibrium (41–42 min; see text for definition of equilibrium). Other model parameters (Table 2) were constant for all simulations: $L=45$ cm, $P_0=1000$ flies, $T(0)=10$ °C, $T(L)=40$ °C.

implications (DeWitt and Friedman, 1979; Izem and Kingsolver, 2005; Martin and Huey, 2008). In particular, thermal preferences are often compared among organisms from different environments (reviewed by Dillon et al., 2009). If these organisms differ in their walking speed reaction norms, interpretation of differences in preference curves is suspect. We compared the effects of changing characteristics of an idealized temperature–walking speed curve on the null distribution and time to equilibrium. Changes in maximum walking speed did not change time to equilibrium (41–42 min for all), but resulted in slightly

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different equilibrium distributions, with higher maximum speeds resulting in fewer flies in hot regions and more flies at the cold end of the gradient (Fig. 5). Further, the shape of the walking speed reaction norm strongly affects the null distribution curve as expected (Fig. 6), but not time to equilibrium (42 min).

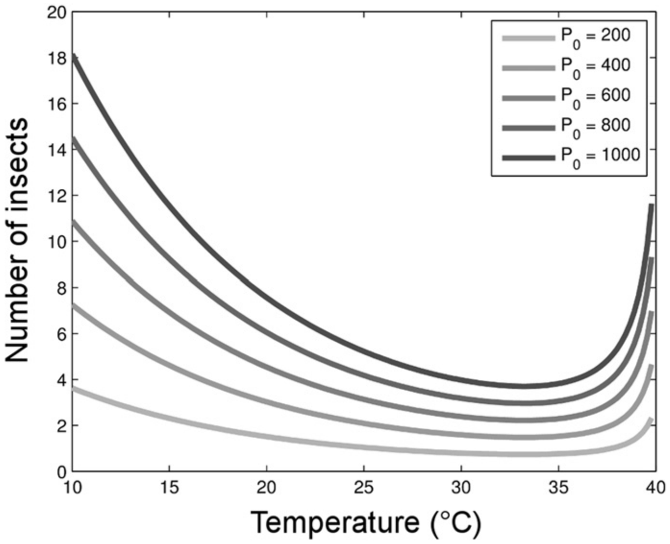

Fig. 3. Effect of initial number of organisms (P_0) on equilibrium distributions based on thermal dependence of motion. Time to equilibrium was 42 min for all distributions. All other model parameters (Table 2) were set as in Fig. 2.

5. Determining thermal preference: a cohort case study

Here we demonstrate application of this approach to data (Dillon and Huey, unpublished) from a laboratory population of *Drosophila melanogaster*. These flies were descended from wild flies collected from an apply orchard near Wenatchee, Washington, USA (47°37′N, 120°18 W) in June 2005 and kept in laboratory populations at constant densities of 1000–3000 flies. At the time of the preference and walking speed experiments, they had been in the lab for approximately 1 year.

To estimate thermal preference, 922 flies were placed into the middle of a lane (40 × 7.6 cm) on a thermal gradient (see Appendix A for application of the approach to an individual over time). Water flowing through the two ends of an aluminum block and maintained at low and high temperatures by water bath circulators (Haake, Paramus, NJ, USA) created a linear temperature gradient ranging from 16.6 to 32.9 °C across the surface of the block. After 57 min on the gradient, which was covered by black felt to remove phototactic cues (Markow, 1979), flies were anesthetized with CO_2. A high resolution digital photograph (Nikon Coolpix 4500, Nikon, Melville, NY, USA) taken from directly above the gradient recorded the position of each fly. Because the change in temperature across the gradient was linear

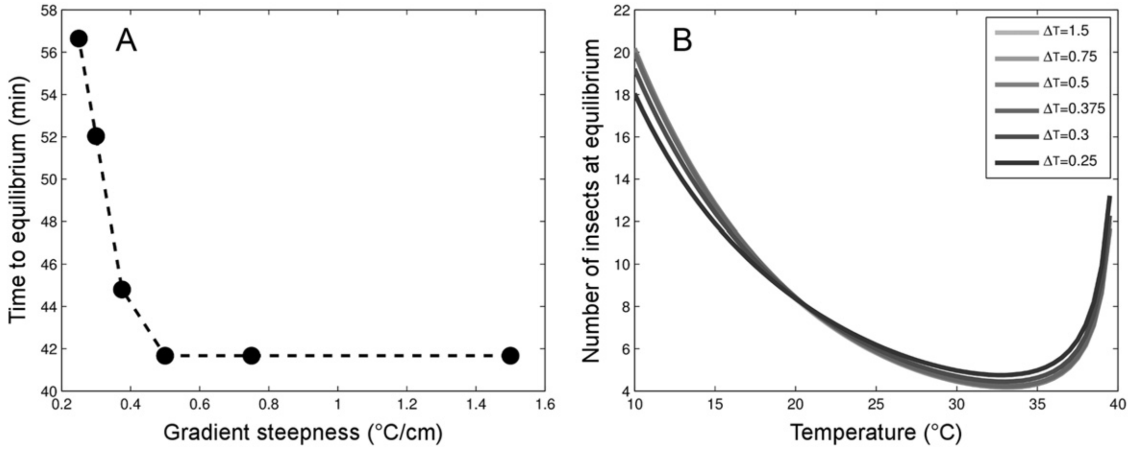

Fig. 4. Effects of gradient steepness, ΔT (°C/cm) on time to equilibrium (A) and equilibrium distributions (B). All other model parameters (Table 2) were set as in Fig. 2.

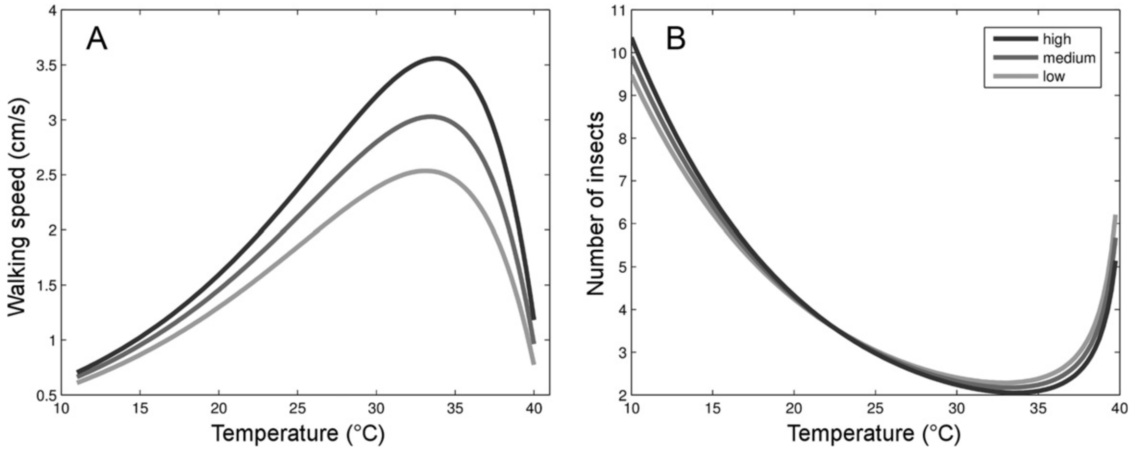

Fig. 5. Effects of changes in maximum walking speed (A) on equilibrium distributions (B). All other model parameters (Table 2) were set as in Fig. 2, and time to equilibrium was 42 min for all curves.

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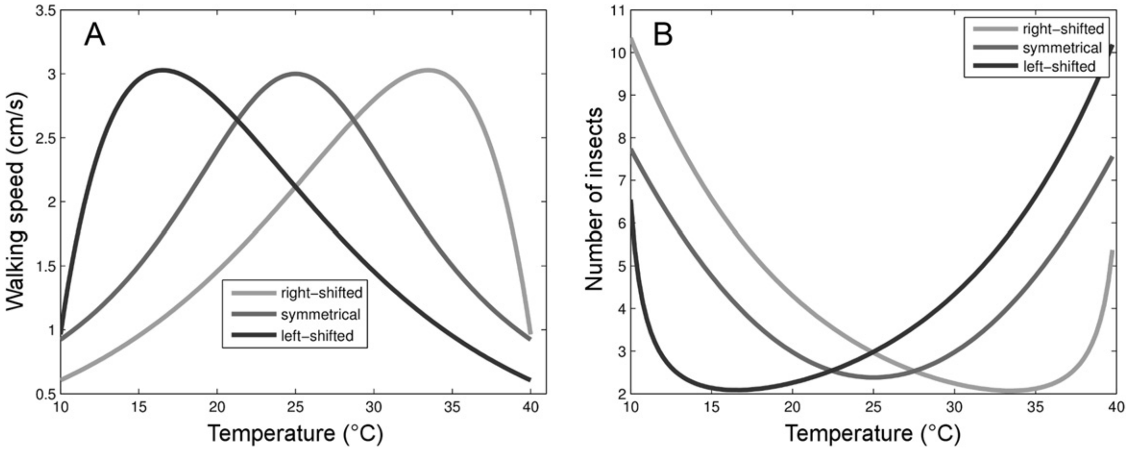

Fig. 6. Effects of changes in the shape of walking speed reaction norms (A on equilibrium distributions (B). All other model parameters (Table 2) were set as in Fig. 2, and time to equilibrium was 42 min for all curves.

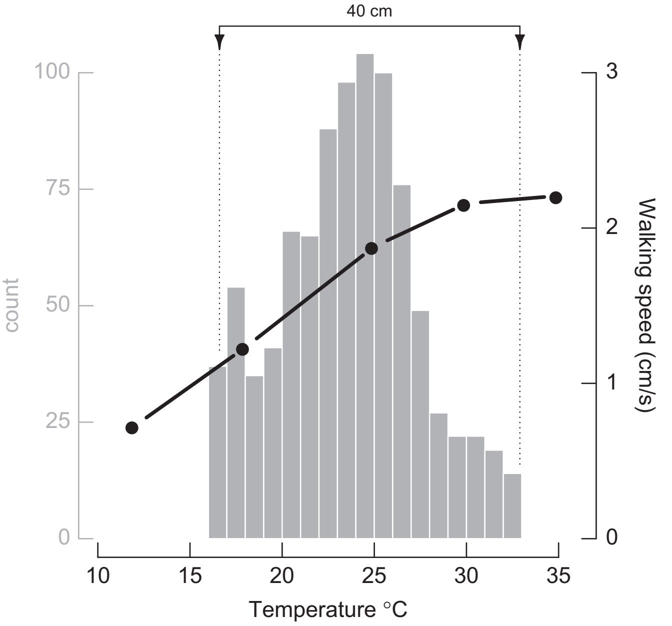

Fig. 7. Distribution of 922 *Drosophila melanogaster* (gray bars, left axis) on a laboratory thermal gradient (linear from 16.6 to 32.9 °C across 40 cm, endpoints indicated by arrows above histogram) 57 min after being placed in the center of the gradient. Walking speed as a function of temperature for flies from the same laboratory population (black points and line, right axis). See text for details.

and known, we could determine the temperature of each fly on the gradient to create a thermal gradient distribution (Fig. 7, gray bars).

We measured maximum walking speed (cf. Crill et al., 1996) of flies from the same population. Twenty flies were placed individually into test tubes (1.6×12.5 cm W × H), which were then submerged in a water bath at one of five temperatures (12, 18, 25, 30, and 35 °C). After 10 minutes of acclimation, each fly was knocked to the bottom of the tube and the time it took to run 6 cm up the side of the tube was estimated with a stopwatch (to 0.01 s). Each fly was tested three times in every temperature and we took the fastest time as the estimate of maximum walking speed. The mean of maximum walking speed across the 20 flies in each temperature treatment is shown in Fig. 7 (points and line).

Using these walking speed data, the characteristics of the laboratory experiment (Fig. 7), and the patch model (Eq. (1)), we generated an expected null distribution for these flies. The null distribution reached equilibrium after 30 minutes (well below the 57 minute run time of the experiment) and revealed an expected accumulation of flies at the cold end of the gradient (points and line in Fig. 8A). We distinguished between preferred temperatures and avoided temperatures by subtracting the null

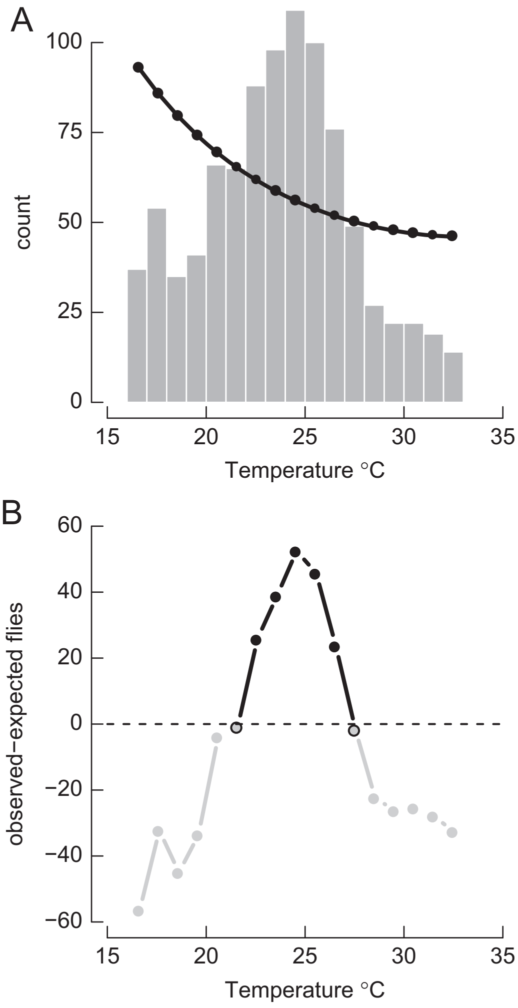

Fig. 8. (A) Comparison of null distribution of 922 *Drosophila melanogaster* (black points and lines) and measured distribution (gray bars). (B) Subtracting the expected from the observed fly numbers reveals temperatures actively preferred (black) and avoided (gray). Thermal preference can be taken as the mean of preferred temperatures (24.6 °C).

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expectation from the experimental data (Fig. 8B). The mean of the preferred temperatures (black points in Fig. 8B) was 24.6 °C, substantially higher than either the mean (23.6 °C) or median (23.8 °C) of the experimental data, suggesting that, in this case, ignoring the null expectation (i.e., the thermal dependence of motion) resulted in an underestimate of preferred temperature.

6. Recommendations and discussion

Implicit ignorance of the null expectation of gradient distributions in thermal preference studies of small ectotherms is ubiquitous (reviewed by Dillon et al., 2009, but see Anderson et al., 2007). Here we have outlined a straightforward approach that explicitly incorporates the effects of the thermal dependence of motion on estimates of thermal preference:

1. Choose endpoint temperatures that are not deleterious to your organisms as the resultant null distribution (no simulations necessary) is a pile of dead animals at the hot end and/or immobile animals at the cold end.
2. Measure the reaction norm of walking speed as a function of temperature using a technique appropriate for your animal. The most appropriate method is to track movement of organisms on the gradient set to uniform temperatures.
3. Use the patch distribution model (Eq. (1)) to estimate times to equilibrium and equilibrium distributions for a range of parameters (Table 2) that are experimentally feasible.
4. Design the experimental setup such that gradient length, steepness, and number of animals result in an experimentally attractive time to equilibrium.
5. Estimate animal position as a function of temperature using the chosen parameters. Starting animals in the center of the gradient or distributing them evenly will result in the fastest time to equilibrium for the most common shapes of thermal dependence of motion curves. Animals should be left on the gradient for at least as long as the model-determined time to equilibrium.
6. Subtract model expectations from observed distributions to estimate the range and mean of preferred temperatures.

It has long been argued that thermal preference should closely match temperatures that optimize performance (e.g. sprint speed, fecundity, fitness; need citations). However, empirically, thermal optima often exceed preferred body temperatures for diverse ectotherms (e.g. Huey and Bennett, 1987). At least two hypotheses have been proposed to explain this observation. The mismatch between preference and performance may actually be expected if organisms are imperfect thermoregulators (Martin and Huey, 2008) with asymmetrical performance curves. Asymmetrical performance costs of thermoregulatory errors predict that T_{pref} should be lower than T_{opt}; how much lower depends on the breadth and degree of asymmetry of the performance curves (Martin and Huey, 2008). Even without asymmetrical performance curves, the positive correlation between performance and thermal optima for performance (Frazier et al., 2006; Angilleta et al., 2010) suggests that organisms will maximize fitness when their mean (preferred) body temperatures are below the thermal optimum (Angilleta, 2009).

For small ectotherms with low thermal inertia, the null approach outlined here suggests a third hypothesis for the commonly observed mismatch between preference and performance: traditional estimates of thermal preference tend to be low because the excess time spent at low temperatures (Fig. 7) is not due to preference but is driven by the thermal dependence of motion (accumulation at the cold end is predicted by the null distribution; Fig. 8A). In both the cohort (Fig. 8) and individual-based (Fig. 10) examples, we estimated substantially higher T_{pref} than would be estimated by traditional means or medians of gradient distributions. More generally, incorporating the null expectation as outlined here will always yield higher estimates of T_{pref}, given similar shapes of movement curves and of gradient distributions. Reanalysis of previous data using the approach outlined here may reveal closer correspondence of T_{pref} and T_{opt} than has previously been found (Fig. 10).

One assumption of the approach outlined here is that organisms are always moving on the gradient. However, they may stop periodically, temporarily decoupling their speed of movement from the temperature of the gradient. If organisms do stop on the gradient, the patch model described here would tend to underestimate times to equilibrium. This suggests an attractive enhancement of the model: thermal preference could be modeled as probability of stopping such that organisms choose to stop or to move, in which case their movement is determined by the temperature of the gradient. Increased probability of stopping would then be associated with preferred regions of the gradient (see also Barber and Crawford, 1977).

Appendix A. Determining thermal preference for individuals over time

For convenience or to avoid group effects (Regal, 1971), one may prefer to determine thermal preference of small ectotherms by tracking the position of an individual over time, rather than using the cohort approach described above. This is easily done with a minor reinterpretation of the model (Eq. (1)). For the individual case, $P_i(t)$ denotes the probability of finding the animal in patch i (rather than the number of animals in patch i). The final distribution is given by

$$\int_0^{t_e} P_i(t)\,dt, \tag{4}$$

where t_e is the time to equilibrium, as defined for the cohort case.

To demonstrate application of this approach, we analyzed data (Dillon and Huey, unpublished) on movement of individual flies from the same laboratory population (see above) in narrow (40 × 0.5 × 0.3 cm L × W × H) lanes on the same laboratory thermal gradient (see Section 5). These experiments were used to measure heat avoidance so the gradient temperature settings were not ideal for measuring T_{pref}; however we analyze the data here to demonstrate application of the approach to the case of following individuals over time. Fly movement was digitally recorded from above (Sony HandyCam DCR-TRV480, 30 fps) for 28 min. We subsequently estimated fly location and therefore temperature every 1 s by digitizing (Rasband, 2012) the gradient position of the fly (Fig. 9, black hash marks) for every 30th frame from exported image sequences. The cumulative distribution of the fly over time (Fig. 9, gray bars) is analogous to the population distribution at the end of the experiment in the cohort example (Section 5).

We used walking speed data (Fig. 7, black fitted line), characteristics of the laboratory experiment (Fig. 9), and the patch model (Eq. (1)) to generate an expected null distribution for these flies with starting positions equal to their experimental starting positions (Fig. 9, location of black hash mark at time 0). For direct comparison with experimental data, we took the cumulative probabilities for each patch over the course of the experiment (Eq. (4) with t_e=28), normalized across all 120 patches, and multiplied the normalized probability (sum across patches equal to 1) by the total number of seconds in the experiment to determine expected number of seconds spent in a given patch.

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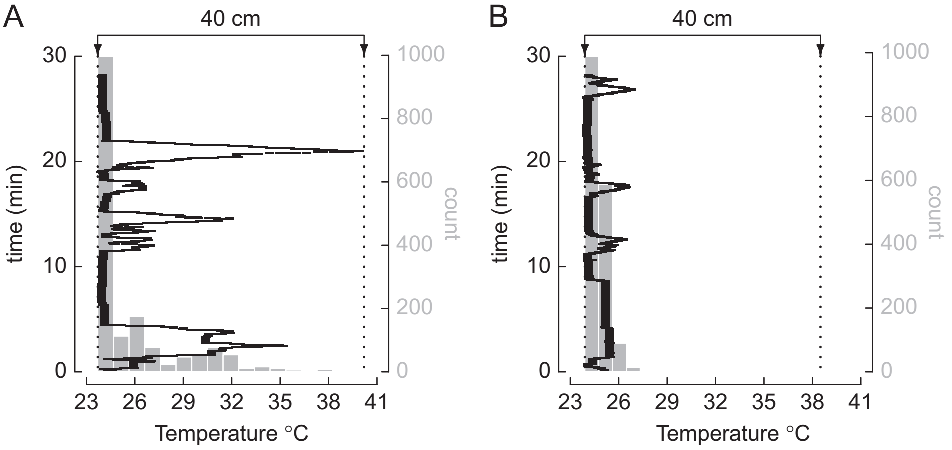

Fig. 9. Position over time of individual flies (one shown in A, the other in B) on a laboratory thermal gradient. Fly positions (indicated by black hash marks and left *y* axes) were recorded every second for 28 min by digitization of video, with the start of the experiment shown at the bottom of the figure and time increasing upwards. The cumulative distribution of these flies over the 28 min period is indicated by the gray bars and right-hand *y*-axis (number of seconds spent in each temperature bin). The edges of the 40 cm gradient are indicated by the vertical dotted lines.

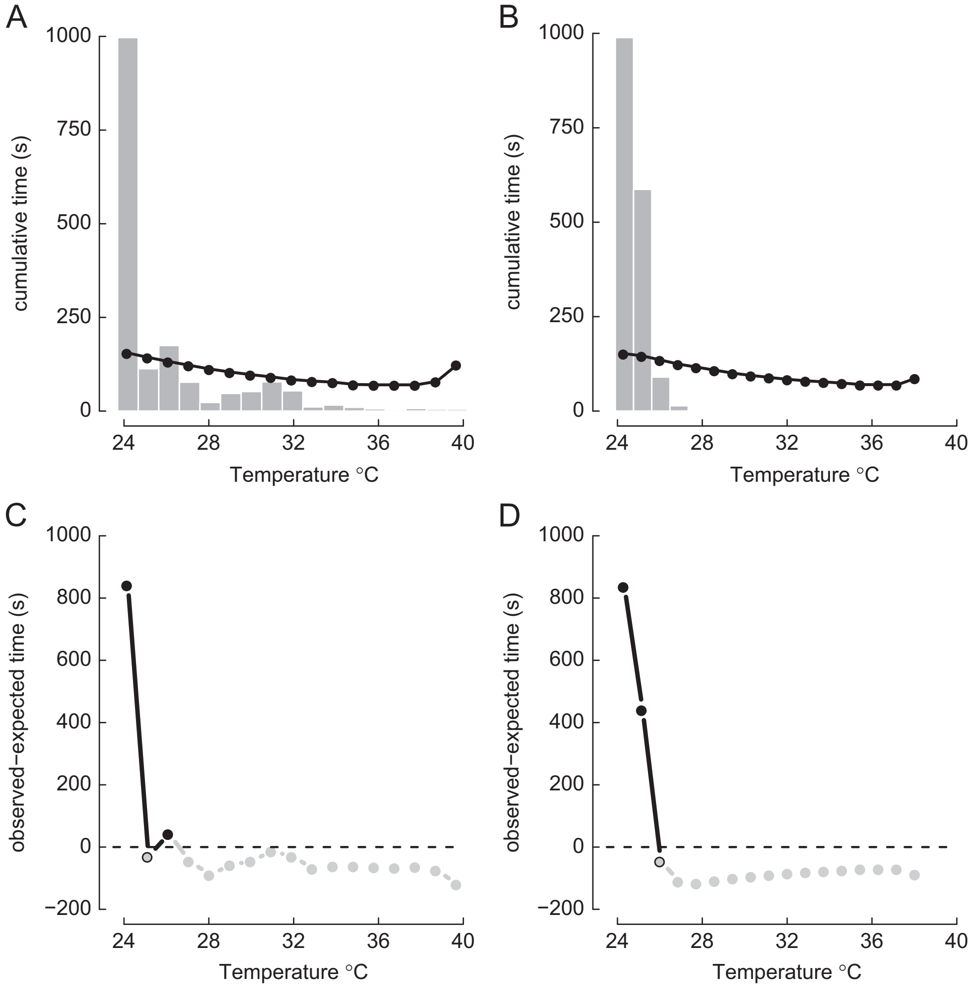

Fig. 10. (A,B) Comparison of null (black points and lines) and empirical (gray bars) distributions of two individual flies over time (see Fig. 9). (C, D) Subtracting the expected (null) from the observed (empirical) times reveals temperatures actively preferred (black) and avoided (gray).

The null expectation for both flies was a slight accumulation in the two ends of the gradient with the minimum number of flies predicted near ~36 °C (Fig. 10A, B, black points and lines). Both flies actively preferred temperatures below ~26 °C (Fig. 10C, D, black points) and avoided higher temperatures (Fig. 10C, D, gray points). Because the experiments from which these data come were originally set up to measure avoidance of high temperatures and not T_{pref}, they give us no information about preference or

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avoidance of temperatures below 24 °C. However, the estimated thermal preferences (weighted means of preferred temperatures) for fly 1 and fly 2 were 24.3 and 24.6, respectively, remarkably consistent with the population estimate of 24.6 (Fig. 8).

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