

# Thermal Fluctuation Correlates with Sex-Specific Effectiveness of Behavioral Thermoregulation in *Melanoplus differentialis*

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## Research Article

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## ABSTRACT

Environmental drivers of the evolution of behavioural thermoregulation are still being elucidated. Much attention has been given to mean temperatures in this respect, but less has been given to thermal heterogeneity. Here, we tested *in situ* effectiveness of thermoregulation of a generalist grasshopper at five sites that vary in temporal thermal heterogeneity to see if individuals from more heterogeneous sites thermo regulated more effectively. We sampled 1) operative temperatures, 2) preferred temperatures, 3) deviations between preferred and field body temperatures, 4) deviations between preferred and operative temperatures, and 5) effectiveness of thermoregulation (E). We found that: 1) operative temperatures did not differ among sites; 2) preferred temperatures did not differ among sites; 3) environmental thermal quality and accuracy of thermoregulation differed among sites, but there was not a clear correlation between these and temporal thermal heterogeneity; and 4) E differed among sites and partially followed our hypothesized pattern, but patterns differed between the sexes. We concluded that temporal thermal heterogeneity affects some organismal thermal properties, and may be a factor in differences in E among populations. However, spatial heterogeneity in thermal resources, which we did not measure, may be affecting the evolution of thermal properties in the populations we examined.

## INTRODUCTION

Insects face many thermal challenges and have developed a wide variety of mechanisms to overcome them [1]. One of these challenges is maximizing fitness in thermally fluctuating environments, which can dramatically impact the biology of many species [2]. Due to climate change, insects will be facing novel thermal stressors, and data are

needed that inform predictions about which populations are more at risk. Special attention has been paid to the vulnerability of tropical species to changes in mean temperature, but less has been paid to the vulnerability of temperate species to thermal variation, even though recent work concluded that temperate species will face levels of thermal stress similar to that of tropical species [3,4]. To maximize fitness, organisms should have preferred temperatures ( $T_{prefS}$ ) that maximize performance, and experiments with orthopterans [5]. Modeling efforts indicate that populations should evolve maximal performance near the body temperatures ( $T_{bS}$ ) which are most frequently experienced and empirical data support this. For example, in the field, Huey and Kingsolver correlated mean  $T_{bS}$  with thermal optimum for sprinting and upper thermal limits in lizards, and MacLean observed greater cold tolerance in *Drosophila* occurring in cooler climates [6-9]. Lab studies concur, as researchers have induced evolutionary shifts in organismal thermal limits by manipulating environmental temperatures; lower temperatures resulted in the evolution of cold tolerance, and higher temperatures resulted in the evolution of heat [10]. Furthermore, the thermal coadaptation hypothesis predicts that  $T_{prefS}$  should evolve to maximize performance at the most commonly encountered operative temperatures and some empirical studies support [11,12]. Thus, when individuals are able to maintain  $T_{prefS}$ , they should be able to maximize performance and, reciprocally,  $T_{prefS}$  should approximate temperatures at which performance is maximized [13,14]. Contrary to Blouin-Demers, Verheyen and Stoks found that daily thermal variation regimes, rather than mean temperatures, were more important in determining performance. However, the latter study examined intraspecific differences in dipterans, while the former examined interspecific differences in squamates. Thus, some climatic factors may be more important than others for the evolution of thermal limits in different taxa.

The temperature range for which performance lies above a given threshold relative to maximal performance is termed thermal performance breadth [15]. Thermal generalists have wide thermal performance breadths thus they should also have wider thermal preference ( $T_{pref}$ ) ranges than thermal specialists for the reasons stated above. Under certain conditions, environmental heterogeneity is expected to promote the evolution of generalists [7,16]. Intergenerational thermal heterogeneity is expected to be especially important in promoting the evolution of thermal generalists and experiments bear this out [6,7,17,18]. Intragenerational thermal heterogeneity, on the other hand, is not expected to drive thermal generalism. Rather, assuming there is a tradeoff between thermal breadth and optimal performance; thermal specialists are expected to evolve when intragenerational thermal heterogeneity is high. Gilchrist states that this occurs because, as long as there is even a short period of time during which an organism can grow and reproduce, natural selection favors increased efficiency over the evolution of environmental tolerance. Conversely, when intragenerational thermal heterogeneity is low and intergenerational thermal heterogeneity is high, a wide thermal breadth (thermal generalism) would make possible a period of time during which organisms could grow and reproduce and would therefore be favored. Conversely, spatial heterogeneity and behavioral thermoregulation can inhibit the evolution of wide performance breadths; when organisms can move among variable thermal patches in their environment to maintain desired  $T_{bS}$ , it relaxes the need for wide thermal breadths [19]. This is an example of the Bogert effect in which effective behavioral mechanisms obviate and slow the evolution of physiological mechanisms. Thus, thermal generalism is expected to evolve in a population when:

- intergenerational thermal heterogeneity is high,
- Spatial heterogeneity is low and 3) its individuals do not utilize behavioral thermoregulation to a great extent [20].

We tested the above ideas using populations of the differential grasshopper (*Melanoplus differentialis*) that experience different degrees of thermal heterogeneity over time. We used environmental data from the field, as well as physiological and behavioral data from the lab, to attempt to answer the following questions:

1. Do commonly encountered  $T_{eS}$  determine temperature preferences? Drastic differences in intersite temperatures due to habitat type have been noted by other researchers [21]. Though we sampled from similar habitat across all sites for consistency, our (approximately equally spaced) sites spanned a 1,602 km range. Thus, the plant community and disturbance regimes likely varied at least slightly among our sites, resulting in different types and availability of microhabitats. For this reason and the arguments above, we hypothesized that differences in  $T_{eS}$  among sites would be present, and that these would correlate with  $T_{prefS}$  of individuals at those sites.

2. Do populations that experience more thermal heterogeneity over time behaviorally thermoregulate more effectively? As our sites differ in their thermal variability we concluded that it is more challenging for individuals to maintain  $T_{prefS}$  at some sites than at others due to differences in the degree of thermal fluctuation, and  $T_{eS}$  will more often deviate from individuals  $T_{prefS}$  at thermally heterogeneous sites. However, we also expected that individuals from these sites would have wider thermal preference ranges, and their environments would deviate more from their  $T_{prefS}$  [22]. Thus, we expected these individuals would thermo regulate more effectively, i.e. have high values of  $E$  (described in Methods). Generally, intergenerational thermal heterogeneity at the sites we sampled from declines as sites are considered from west to east. Westernmost sites tend to vary more in daily thermal maxima, daily thermal minima, and daily temperature range with the notable exception of the VA (easternmost) site having a daily temperature range second only to that of the KS (westernmost) site [22]. Thus, we hypothesized that there would be a general trend of decreasing effectiveness in behavioral thermoregulation from west to east.

This work will help to elucidate the role of temporal thermal regime on a population's ability to thermoregulate. By providing information on what climatic factors correlate with thermal limits and behavioral thermoregulation, this experiment informs whether or not conclusions about vulnerability to novel thermal stressors during the current situation of climate change can be drawn based on historical records of climatic variation.

## MATERIALS AND METHODS

### Measurement of $T_b$ and measurement and analysis of $T_e$

We conducted all field sampling from August 2, 2018 to August 10, 2018, spending 1-2 days at each site. We sampled field-active body temperatures ( $T_bS$ ) from sites in KS, MO, IL, KY, and VA (one site per state), the thermal regimes [22]. We captured individuals by hand and with a sweep net from 0900 to 1900 hours and inserted a thermocouple under the metasternum of the thorax 5-30 seconds after capture. As *M. differentialis* is a large-bodied species, and their thermal inertia is relatively high compared to smaller-bodied insects, e.g. *Drosophila*, we expect that differences among individuals in the latency between capture and measurement had minimal effects on sampled  $T_bS$ . We then put each individual sampled this way into a 46 × 46 × 46 cm stainless steel cage for transportation to the University of New Orleans, with the exception of those euthanized to obtain operative temperature ( $T_e$ ) values.

A  $T_e$  is an equilibrium temperature that a physical structure with the same color, size, and shape of a specific animal will attain in a given environment and describes an individual's internal  $T_b$  in the absence of thermoregulation. It has been measured in the past by sampling internal temperatures of objects such as copper tubes, agar models and carcasses of the animal of interest [23-27]. Operative temperatures can vary over time and space [28,29]. Spatially,  $T_{eS}$  can vary at both high and resolutions in an environment [30,31].

To measure  $T_{eS}$ , we adhered euthanized male and female *M. differentialis* at each field site ( $n=53$  among all sites) to a grass or forb we had observed live individuals on. We did not place these randomly because we were limited on both time and manpower. While a non-random placement may have biased  $T_e$  sampling, it was expected to yield more ecologically meaningful results than random placement would have, given our limitations. We affixed an approximately thorax-sized piece of double-sided masking tape to each individual's thorax and attached it to the stem of the grass or forb. We placed each specimen at the top of the stem and allowed the carcass to reach a steady-state (i.e.  $<0.1^\circ\text{C}$  change after five minutes) thoracic temperature, which we then recorded. For consistency and convenience, we used a yardstick to ensure that each grass or forb selected for taking these measurements was approximately 0.9 m in height. We repeated this for the middle (approximately 0.457 m) and bottom (0 m) of each stem, resulting in three samples per carcass. We took care to place each individual with its head facing upwards, as orientation can affect  $T_{eS}$  [26]. In addition, we ensured that each grasshopper was in the same posture, with its walking legs splayed out and its rear legs tucked underneath it. These positions on the stem, this orientation, and this posture accurately mimicked the state of individuals we observed in the field. We took these measurements between 900 and 1800 hours.

We analyzed  $T_{eS}$  with a repeated measures ANOVA using  $T_e$  ( $^\circ\text{C}$ ) as a response variable and site of origin, sex, stem position (three levels: top/middle/bottom), shade status as determined by visual observation at time of sampling (two levels:  $<$  or  $>$  50% shade), time of day (four levels: see below), and 'individual' as predictor variables. We coded 'individual' as a random variable in order to control for repeated measures on the same individuals. We recorded time of day as a categorical variable with four levels, with 901 to 1100 hours coded as "Morning," 1101 to 1300 coded as "Noon," 1301 to 1500 coded as "Afternoon," and 1501 to 1730 coded as "Late Afternoon." We performed this and all subsequent analyses in R [32].

### Measurement and analysis of $T_{prefS}$ and $T_{set}$

Preferred temperatures ( $T_{prefS}$ ) are specific  $T_{bS}$  that individuals attempt to attain to optimize performance, and they often vary within and among populations and individuals. Factors affecting  $T_{prefS}$  include acclimation effects feeding state sex and time of day [33-39]. Thus, an individual's  $T_{pref}$  is a moving target, and constant thermoregulation is necessary to maintain it, especially in a shifting environment. As such, if  $T_{prefS}$  are measured in the field, it is necessary to sample many individuals at different points in time. In the lab, however, one or more of these factors can be controlled for, releasing animals from ecological constraints and providing more meaningful results.

After field collection and measurement of  $T_{bS}$ , we transported field-collected individuals to the University of New Orleans. We determined  $T_{prefS}$  from August 14, 2018 to September 5, 2018 by placing each individual into a custom-made  $10.16 \times 91.44 \times 10.16$  cm wooden shuttle box. We covered the shuttle box with a clear acrylic sheet secured onto the main body of the shuttle box with adhesive hook and loop fasteners. We divided the shuttle box into seven  $10.16 \times 13.06 \times 10.16$  cm partitions ( $7.62 \times 9.8 \times 4.92$  cm of open space in each partition). We placed a space heater on one side and a window air conditioning unit on the other and connected each to the shuttle box with aluminum duct and detachable fittings. We cut a 2.54 cm square notch into the wooden dividers between each partition to allow individuals to freely pass between partitions. We alternated these notches from left to right in order to partially restrict air flow between partitions. The steady state temperatures of the partitions were, from the partition closest to the air conditioning unit to that closest to the space heater, 8.2, 9.2, 11.2, 15.4, 18.0, 44.3, and  $67.2^\circ\text{C}$ , respectively. For each  $T_{pref}$  trial, we allowed partitions to reach their steady state temperatures, then placed an individual into the middle partition and started the trial immediately. We did this (as opposed to allowing

for a behavioral adjustment period) in order to avoid bias that might arise from individuals wandering in the shuttle box before the trial started, potentially starting trials in hotter or colder partitions. After a 15-minute period, we obtained and recorded the  $T_b$  of each individual using the same protocol we used to obtain field  $T_b$ s as described above.

We used  $T_{prefS}$  to calculate each population's set-point range ( $T_{set}$ ). A  $T_{set}$  is a range within which individuals attempt to maintain their  $T_b$ s and can be calculated by taking a specified percentile of observed  $T_{prefS}$  (e.g. the central 50%) in a population [8]. Once a population's  $T_{set}$  is known, it can be used to calculate deviations between  $T_{prefS}$  and 1)  $T_b$ s ( $d_b$ s) and 2)  $T_e$ s ( $d_e$ s) in the population of interest. In this study, we used the central 50 percent of values of individuals from each site to determine  $T_{set}$ . Afterwards, we analyzed  $T_{prefS}$  with an ANOVA, using  $T_{pref}$  as a response variable, and sex, site of origin, weight, and their interactions as predictor variables. While weight can vary on a fine temporal scale due to feeding status and is not typically used for orthopterans, weight is a good predictor of pronotum length in this population [22]. Furthermore, we fasted individuals for 12 hours before testing. Thus, this variation was probably minimized.

### Measurement and analysis of $d_b$ and $d_e$

We calculated the metric  $d_b$ , after Hertz et al [8]. The further an individual's  $T_b$  is from its  $T_{pref}$ , the higher  $d_b$  will be. This metric ( $d_b$ ) is inversely related to increasing accuracy and precision of thermoregulation, which depend on, among other things, the spatial configuration of thermal resources available to a population [40]. Sears, et al. constructed a model indicating that, when available  $T_e$ s are spread out, rather than clumped, it is easier for individuals to obtain  $T_{prefS}$  in the absence of competition. They subsequently validated their predictions with empirical data, further supporting the idea that spread-out  $T_e$ s allows individuals to maintain low  $d_b$ s. Another factor in determining  $d_b$  is the evolutionary strategy of the species in question; thermal specialists are expected to thermoregulate more carefully than generalists [40,41]. Thus, specialists should have smaller  $d_b$ s than generalists. However, a larger  $T_{set}$  in generalists may offset this effect.

To measure  $d_b$ , we took the absolute values of the differences between individual field  $T_b$ s and population  $T_{setS}$ . As these values fit a Poisson distribution, we analyzed them using a Generalized Linear Model (GLM), with  $d_b$  as a response variable and site of origin, sex, and pronotum length (body size) as predictor variables. We then summarized the GLM results with an analysis of deviance table. As there were significant effects of sex, and we were more interested in intersite differences, we subsequently performed sex-specific GLMs using the same variables, sans sex. As there was a significant effect of body size even when sexes were analyzed separately, we used the residuals from a GLM between pronotum length and  $d_b$  as a response variable in order to control for the effects of body size. As these residuals fit a Poisson distribution, but analyses utilizing this distribution are not applicable to negative values, we transformed all values by adding the absolute of the minimum value in the data set to each value. Finally, we used the emmeans package to obtain Estimated Marginal Means (EMMs) to perform a post-hoc analysis in order to determine which sites significantly differed in  $d_b$  [32,42].

We also calculated  $d_e$  after Hertz et al [8]. This measure is similar to  $d_b$ , except that it is a measure of how far an individual's environment, instead of its  $T_b$ , deviates from its  $T_{set}$ . To obtain  $d_e$ , we calculated the absolute deviations between  $T_e$ s and  $T_{set}$  at each site. As with  $d_b$ , we analyzed these data with a GLM, using  $d_e$  as a response variable and site of origin, sex, and body size as predictor variables. As there was a significant effect of body size, we performed a follow-up analysis as above with  $d_b$ , using the transformed residuals of a linear regression between body size and  $d_e$  as the response variable.

**Calculation of  $\bar{d}_b$ ,  $\bar{d}_e$ , and E**

We determined E in the field of each population after Hertz et al [8]. E gives a measure of the ability of individuals in a population to keep their Tbs near the Tset of the population, weighted by the amount of their environment's thermal deviation from the population's Tset. Values of E are useful for experimenters because they may be compared among multiple populations to determine interpopulation differences in the ability to thermoregulate effectively.

To calculate  $\bar{d}_b$  and  $\bar{d}_e$ , we used the mean  $d_b$  and  $d_e$  of each site, respectively. At each site, we calculated these for all individuals as well as male- and female-specific values for use in calculation of E. To obtain values of E, for each site, we used the formula  $1-(\bar{d}_b / \bar{d}_e)$ , as per Hertz et al [8]. Note that as individuals approach perfect thermoregulation ( $T_b=T_{set}$ ),  $\bar{d}_b$  will be low and E will be high. As environmental temperatures approach individuals'  $T_{prefS}$  ( $T_e=T_{set}$ ),  $\bar{d}_e$  will be low and E will also be low. More extreme and variable environments have higher values of  $\bar{d}_e$  and would consequently have higher values of E, all else being equal.

**RESULTS**

**Operative temperature, preferred temperature, and set-point range**

We had variable amounts of individuals for each analysis. Only position (ANOVA:  $F_{2,23} = 14.88$ ,  $p < 0.001$ ) and shade status (ANOVA:  $F_{2,23} = 7.918$ ,  $p = 0.01$ ) had a significant effect on  $T_{es}$ ; mean  $T_{es}$  were highest at mid-stem and in <50% shade. Neither sex nor population nor weight had a significant effect on  $T_{prefS}$  (ANOVA: All p values > 0.05).  $T_{set}$  lower and upper bounds ranged from 13.4 (IL) to 35.29°C MO (Tables 1 and 2).

**Table 1.** Sample sizes for experiments determining operative temperature ( $T_e$ ), preferred temperature ( $T_{pref}$ ), deviation of field body temperature from set-point temperature ( $d_b$ ), and deviation of  $T_e$  from set-point temperature ( $d_e$ ) of *Melanoplus differentialis* from field sites from Kansas (KS), Missouri (MO), Illinois (IL), Kentucky (KY), and Virginia (VA), United States.

N	KS	MO	IL	KY	VA	Total
Te	14	15	12	6	6	53
Tpref	21	31	19	23	20	114
db	38	40	43	29	40	190
de	14	15	12	6	6	53

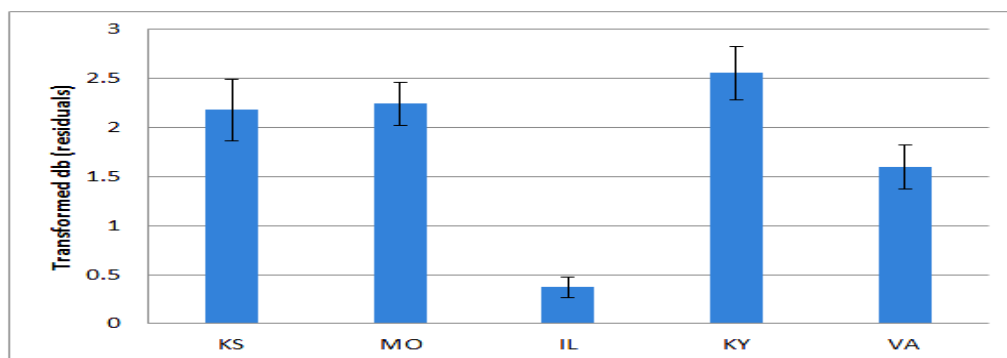
**Table 2.** Lower and upper bounds of set-point temperatures ( $T_{set}$ ) of *Melanoplus differentialis* populations from field sites from Kansas (KS), Missouri (MO), Illinois (IL), Kentucky (KY), and Virginia (VA), United States.

States	$T_{set}$ Lower Bound (°C)	$T_{set}$ Upper Bound (°C)
KS	15.27	32.66
MO	16.665	35.29
IL	13.4	34.62
KY	15.89	32.745
VA	15.935	35.055

**Deviation of body temperatures and operative temperatures from preferred temperatures**

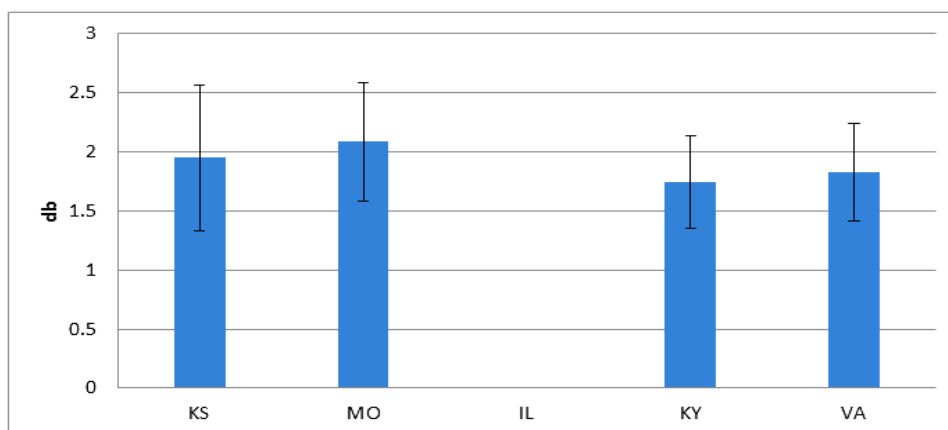
Site (Analysis of deviance table:  $X^2_{4,191} = 130.392$ ,  $p < 0.001$ ), sex (analysis of deviance table:  $X^2_{1, 191} = 9.107$ ,  $p = 0.003$ ), and body size (analysis of deviance table:  $X^2_{1, 191} = 8.684$ ,  $p = 0.003$ ) affected  $d_b$ . When we analyzed males separately, body size (analysis of deviance table:  $X^2_{1,128} = 4.993$ ,  $p = 0.026$ ) and site of origin still affected  $d_b$  (analysis of deviance table:  $X^2_{1,128} = 4.993$ ,  $p = 0.026$ ). When we controlled for the effect of body size, site still affected male  $d_b$  (analysis of deviance table:  $X^2_{5,128} = 118.67$ ,  $p < 0.001$ ). IL was significantly lower in  $d_b$  than all other sites (EMMs: all  $p$  values  $< 0.001$ ). No other sites significantly differed from one another in male  $d_b$  (EMMs: all  $p$  values  $> 0.05$ ) (Figure 1).

**Figure 1.** Mean transformed values ( $\pm 1$ SE) of residuals from a linear model using the deviations between field body temperatures and set-point temperatures ( $d_b$ ) of *Melanoplus differentialis* males from field sites from Kansas (KS), Missouri (MO), Illinois (IL), Kentucky (KY), and Virginia (VA), United States, as a response variable and pronotum length as a predictor variable. We transformed residual values by adding the absolute minimum value in the data set to all values. IL was significantly lower than all other sites (expected marginal means: all  $p$  values  $< 0.001$ ), but no other sites significantly differed from one another (expected marginal means: all  $p$  values  $> 0.05$ ).



When we analyzed females by themselves, site was the only factor significantly affecting  $d_b$  (analysis of deviance table:  $X^2_{5,63} = 52.339$ ,  $p < 0.001$ ). IL had a mean value of zero. As in males, female  $d_b$  in the IL population was significantly lower than that of all other populations (Figure 2).

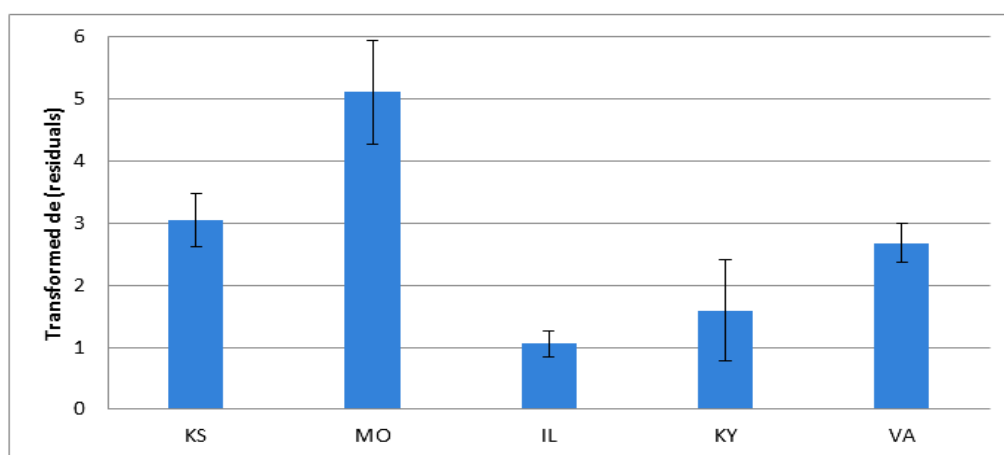
**Figure 2.** Mean values ( $\pm 1$ SE) of deviations between field body temperatures and set-point temperatures ( $d_b$ ) of *Melanoplus differentialis* females from field sites from Kansas (KS), Missouri (MO), Illinois (IL), Kentucky (KY), and Virginia (VA), United States. IL was significantly lower than all other sites (expected marginal means: all  $p$  values  $< 0.001$ ), but no other sites significantly differed from one another (expected marginal means: all  $p$  values  $> 0.05$ ).



Body size significantly affected  $d_e$  (Analysis of deviance table:  $X^2_{1,53}=7.319$ ,  $p=0.007$ ), as did site (Analysis of deviance table:  $X^2_{4,53} =171.59$ ,  $p<0.001$ ), but not sex (Analysis of deviance table:  $X^2_{1,53} = 0.77$ ,  $p>0.05$ ). When we controlled for body size, site (Analysis of deviance table:  $X^2_{5,53} = 115.591$ ,  $p<0.001$ ), but not sex (Analysis of deviance table:  $X^2_{1,53} = 2.594$ ,  $p>0.05$ ), significantly affected  $d_e$ .

KS  $d_e$  and MO  $d_e$ , was significantly higher than that of IL, and MO  $d_e$  was significantly higher than that of KY (EMMs: all  $p$  values $<0.009$ ). No other sites differed significantly from each other in  $d_e$  (EMMs: all  $p$  values  $> 0.05$ ) (Figure 3).

**Figure 3.** Mean transformed values ( $\pm 1SE$ ) of residuals from a linear model using the deviations between environmental temperatures and set-point temperatures ( $d_e$ ) of *Melanoplus differentialis* individuals from field sites from Kansas (KS), Missouri (MO), Illinois (IL), Kentucky (KY), and Virginia (VA), United States, as a response variable and pronotum length as a predictor variable. We transformed residual values by adding the absolute minimum value in the data set to all values.



### Effectiveness of thermoregulation

In males, and when both sexes were considered together, E was highest at the MO site and, while mathematically undefined, was lowest at the KY site. When females were considered by themselves, E was highest at the IL site and lowest at the VA site (Table 3).

**Table 3.** Values of E, a measure of a population's ability to thermoregulate, of males and females in populations of *M. differentialis* from field sites from Kansas (KS), Missouri (MO), Illinois (IL), Kentucky (KY), and Virginia (VA), United States. E for KY males is mathematically undefined, as  $\bar{d}_e$  was zero for that population.

States	Both sexes	Male	Female
KS	0.22	-0.42	0.67
MO	0.75	0.78	0.69
IL	0.63	-22.23	1
KY	-5.96	Undefined	-1.31
VA	-3.9	-10.7	-2.48



## DISCUSSION

### Operative and preferred temperatures

Our hypothesis that  $T_{es}$  will differ among sites was not supported. These results concur with some other studies with ectotherms. Mueller and Gienger found that mean  $T_e$  for two snakes (*Agkistrodon spp.*) species differed among months, but not sites positioned 100 km apart and differing by 50 m in elevation [28]. Conversely, only the microclimatic conditions we measured, position on stem and shade status, affected  $T_{es}$ , indicating that microclimatic factors may be more important than site or diurnal fluctuation in temperature in determining  $T_{es}$  at our sites. Similarly, Pincebourde and Suppo found that spatial temperature distributions of tropical ectotherms vary drastically on a fine scale, and concluded that this variation regulates the ability of insects to attain  $T_{prefS}$  [30]. Further, modeling efforts indicate that fine-scale spatial heterogeneity plays a role in determining organismal performance and thermal adaptation [12,43]. This is likely to be the case at our sites, as behavioral thermoregulation would be most appropriate in an environment that varies spatially in thermal resources, and there appears to be more intrasite than intersite variability in  $T_{es}$  among our populations. Thus, there is likely ample opportunity for our experimental individuals to behaviorally thermoregulate, and selection for effective behavioral thermoregulation is likely present due to intrasite structural complexity. This result, paired with the lack of observed correlation between  $T_{es}$  and  $T_{set}$  at our sites, agrees with the models referenced above. Two caveats to our conclusion are that thermal preferences for insects 1: can differ from the lab to the field 2: can have low heritability and 3: thermal properties can be plastic in *Melanoplus* species [44-47].

Our hypothesis that individuals from sites with higher  $T_{es}$  will have higher  $T_{prefS}$  could not be tested, as we detected no differences among sites in  $T_{es}$ . Furthermore, we detected no intersite differences in  $T_{prefS}$ , consistent with results from some other researchers [48]. Taucare-Ríos et al. have noted differences in  $T_{prefS}$  among some, but not all, populations of spiders from six sites [49]. Three of these sites were coastal and had relatively low mean daily temperature, while the other three were inland and had relatively high mean daily temperature ranges.  $T_{prefS}$  were lower at the coastal sites, indicating that differences in daily thermal fluctuations may have played a role in the selection of  $T_{prefS}$  by individuals in that study. While most of the sites we collected from differed in daily range these differences may not have been great enough to create the same pattern in intersite  $T_{prefS}$  in the populations we sampled, or intrasite spatial heterogeneity may have offset these effects [22].

A major limitation of our study is that we had a very small resolution of each site's  $T_{es}$ . While we eliminated seasonal effects by sampling all sites within eight days of each other, and similar atmospheric conditions prevailed at each site during the sampling period (personal observation), we had a low sample size for  $T_e$  (Table 1). If we had a larger sample size and duration for  $T_{es}$ , we would have been able to better characterize the thermal resources at each site. Furthermore, we placed all carcasses used to obtain  $T_{es}$  in the same posture. Though this served to control for the effect of posture, it also resulted in sampling a more restricted range of  $T_{es}$  than are actually available to our populations.

Another reason we may not have detected differences in  $T_{prefS}$  could be gene flow among populations, even if the ideal thermal optima were different among sites. *Melanoplus differentialis* is a strong flier, and in the model developed by Day describing gene flow, thermal heterogeneity, and the coevolution of thermal optima among intraspecific competitors, thermal heterogeneity coupled with gene flow inhibited the divergence of thermal optima among competitors. If this holds true for our populations, then there is likely some degree of gene flow among

them. Lastly,  $T_{prefS}$  can vary within populations for a variety of reasons as described above, and have an intrinsically large standard error as compared to other thermal properties, e.g. thermal tolerance. For this reason, larger sample sizes than ours may be required to detect differences in  $T_{prefS}$  [50].

### Deviation of body temperature and operative temperature from preferred temperature

Males and females from all sites except IL had comparable  $d_{bS}$ . As we found no intersite differences in  $T_{prefS}$  (one of the major factors determining  $d_b$ ), the most likely cause for the relatively low  $d_b$  at the IL site is low  $\bar{d}_e$ ; individuals from the IL site would not have to thermoregulate as carefully to keep their  $T_{bS}$  within the population's  $T_{set}$  due to the relatively stable temperatures there. However, KY had values of  $d_e$  comparable to those of IL without a concomitant decrease in values of  $d_b$ . Thus, differences in  $d_b$  between IL and KY could also be due to differences in the spatial configuration of those two sites. Differences in spatial heterogeneity are important to the energetic costs of thermoregulation and these affect a population's evolutionary trajectory towards either specialism or generalism. While there is no argument that orthopterans can behaviorally thermoregulate to great effect some ectotherms thermoregulate more carefully than others [43,51,52]. If this is occurring at our sites, this could be due to a tradeoff between optimal performance and performance breadth; in a spatially heterogeneous environment, selection would favor higher maximal performance (specialists), whereas selection would favor wide performance breadths (generalists) in a spatially homogeneous environment. However, we did not formally measure or analyze differences in spatial heterogeneity among our sites, so we cannot draw any conclusions regarding this.

While we did not test for the Bogert effect across our populations, demonstrated that spatial variation in available environmental temperatures can offset the Bogert effect in some traits. As we detected significant effects of microclimatic factors (shade coverage, stem position) on  $T_{eS}$  (which affect the calculation of  $d_e$ ), this may be happening at the IL site [53].

Other reasons insects might actively vary  $T_{bS}$  include thermal fine-tuning to optimize tradeoffs between resource acquisition and energy expenditure optimization of flight energetics and behavioral fever to minimize or eliminate mycosis [54,55]. Thus, there are a variety of incidental reasons that  $d_{bS}$  may have differed between IL and KY.

The KS and MO sites had higher values of  $d_e$  than some of the more thermally homogeneous sites, indicating that the quality of the thermal environment was lower at those sites. While spatial heterogeneity has been indicated as a contributor to the thermal quality of an environment temporal heterogeneity appears to detract from it in the sites considered in this study [22,43]. If this holds true for other sites and taxa, this has implications for the survival of populations inhabiting environments with differing degrees of thermal fluctuation in a warming world.

### Effectiveness of thermoregulation

Our hypothesis that populations from temporally thermally heterogeneous environments will have higher values of  $E$  was partially supported; when males were considered exclusively or collectively with females, there was a general trend of decreasing  $E$  from west to east, with the exception of populations from the most geographically extreme sites (KS and VA; Table 3). Lynch and Gabriel modeled the evolution of performance breadth (the temperature range at which performance is above a specified level) and concluded that performance breadth is directly related to intergenerational heterogeneity. Gilchrist's model is similar in that it also indicates that intergenerational heterogeneity plays a role in the evolution of performance breadth, but it differs from Lynch and Gabriel's in that it suggests that the optimal performance breadth tightly follows the ratio of intra- to intergenerational variation; narrow performance breadths should evolve when the variation within generations equals or exceeds the variation

among generations. This difference is due to the way in which performance contributes to fitness in each model (geometric mean and additive performance over an individual's lifetime in Lynch and Gabriel's and Gilchrist's models, respectively). While we did not measure the ratio of intra- to intergenerational variation, the pattern of  $E$  roughly follows the predictions made by Lynch and Gabriel, assuming that wider performance breadths result in wider ranges of  $T_{set}$ , and therefore larger values of  $E$ .

However, when females were considered exclusively, IL had the highest value of  $E$  and VA had the lowest. *M. differentialis* males are smaller than females, and body size affected  $d_b$  (in males) and  $d_e$  (in both sexes), critical components of  $E$ . Thus, it is no surprise that  $E$  differs between the sexes. Other causes of sexual differences in thermoregulation in insects have been noted, such as longer diurnal activity periods in males' microsite selection by male orthopterans to optimize  $T_{bs}$  for calling and the modulation of  $T_{bs}$  to increase ovarian development rate in females. Pivnick and McNeil observed that, despite having lower masses, male butterflies consistently had higher thoracic temperatures and hypothesized that this may be due to sexual selection favoring the ability of males, but not females, to fly in suboptimal conditions [56-59].

## CONCLUSION

Thus, in the future, it may be useful to investigate sex-specific selection for differing  $T_{bs}$  as a driving factor in the difference in sex-specific patterns of  $E$  among the populations we sampled. We conclude that 1: variability in thermal resources for *M. differentialis* are due more to microsite than among-site differences, 2: the populations we sampled from have differences in  $d_b$  which are most likely driven by differences in thermal variability, and 3: temporal thermal variability may be associated with effective thermoregulation in the populations of *M. differentialis* we sampled from, but this trend does not hold for both sexes.

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## TREATMENT OF ANIMAL SUBJECTS

All treatment of experimental individuals complied with ARRIVE and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

## DECLARATION OF INTEREST

None

## CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

**Devin B Preston:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft, writing - review and editing.

**Steven G Johnson:** Funding acquisition, Investigation, Project administration, Supervision, Writing - review and editing.

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