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Disciplines

Entomology | Evolution | Other Ecology and Evolutionary Biology | Population Biology | Terrestrial and Aquatic Ecology

Comments

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PHENOTYPIC PLASTICITY AND GEOGRAPHIC VARIATION IN THERMAL TOLERANCE AND WATER LOSS OF THE TSETSE *GLOSSINA PALLIDIPES* (DIPTERA: GLOSSINIDAE): IMPLICATIONS FOR DISTRIBUTION MODELLING

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Abstract. Using the tsetse, *Glossina pallidipes*, we show that physiologic plasticity (resulting from temperature acclimation) accounts for among-population variation in thermal tolerance and water loss rates. Critical thermal minimum (CT_{Min}) was highly variable among populations, seasons, and acclimation treatments, and the full range of variation was $9.3^{\circ}C$ (maximum value = $3.1 \times$ minimum). Water loss rate showed similar variation (max = $3.7 \times$ min). In contrast, critical thermal maxima (CT_{Max}) varied least among populations, seasons, and acclimation treatments, and the full range of variation was only approximately $1^{\circ}C$. Most of the variation among the four field populations could be accounted for by phenotypic plasticity, which in the case of CT_{Min} , develops within 5 days of temperature exposure and is lost rapidly on return to the original conditions. Limited variation in CT_{Max} supports bioclimatic models that suggest tsetse are likely to show range contraction with warming from climate change.

INTRODUCTION

Climate change and its interaction with human land use patterns are among the most significant threats facing biodiversity and society.¹ Therefore, forecasts of the interactive effects of climate change and land use are of considerable importance for predicting the future distribution and abundance of species and for enabling society to mitigate and cope with the effects of these changes.¹ Typically, predictions of climate change effects on species ranges are based on bioclimatic models,^{2,3} although there is substantial variety in the way in which this modeling is done. These models are often able to explain much of the variation in species distributions and abundances.^{4–7} They are therefore considered one of the most effective ways to obtain a rapid estimate of likely alterations in species' distributions.² Nonetheless, they have been criticized on a variety of grounds.⁸

From a physiologic perspective, the bioclimatic modeling approach raises concerns from three principal perspectives. Spatial variation in population responses to the environment is often not considered⁹; the rapid alterations to phenotypes that might take place through phenotypic plasticity in the form of developmental plasticity, acclimation, and hardening are typically ignored¹⁰; and the likely outcome of covariation among abiotic variables is often not adequately assessed.¹¹ In consequence, it has been proposed that physiologic studies and biophysical modeling should be used in concert with large scale bioclimatic studies of species responses to understand what the future might hold for various taxa under a changing climate.¹⁰

These concerns are equally, if not more, pertinent to understanding changes in the distributions of arthropod-borne diseases.¹² Nonetheless, it has been shown that climate change is likely to alter, or already has altered, the geographic distribution of at least some of these diseases,^{12,13} although in other cases, the effects are less straightforward to discern.^{11,14} Given that the distributions of vector species are important in

influencing changing patterns of disease transmission,^{12,13} considerable attention has been given to the likely effects of environmental change on vectors. For Africa, much of the attention has focused on malaria and its mosquito vectors.¹⁴ However, tsetse (*Glossina* spp. Diptera: Glossinidae), which are long-lived, hematophagous flies, with low reproductive rates, have also been the subject of consideration because they carry trypanosomes that have serious implications for human and animal health and are considered a major limitation to the socio-economic development of the continent.^{7,15}

Several studies have predicted that the ranges of African trypanosomiasis and their tsetse vectors are likely to change under forecast climate scenarios.^{16–18} These predictions arise from the fact that abiotic variables that will be affected by climate change, such as land surface temperature and saturation deficit, and other integrated environmental variables (e.g., the normalized difference vegetation index [NDVI]), are excellent correlates of tsetse distributions and abundances,^{4–7} explaining much of their variation. In some cases, the mechanisms underlying these relationships are likely to be indirect.^{7,4,19} However, the direct role of abiotic environmental variables in affecting tsetse life history parameters is well established.²⁰ Temperature has major effects on birth rates, development, and mortality, and especially strong relationships exist between adult mortality and ambient temperature, which in some species are markedly non-linear.^{20,21} Water availability is also known to affect mortality rates; puparia and teneral adults are most sensitive to dry conditions, whereas mature adults are most sensitive to temperature.²⁰

Despite the foregoing information on how responses to climate might vary among different tsetse species and subspecies, based both on remote sensing work^{5,6} and laboratory-based physiologic studies,²² inter-population physiologic variability and the rate of development and extent of phenotypic plasticity in tsetse are poorly understood (although some behavioral variation has been described²³). Nonetheless, it is clear that if the responses of these species to climate change are to be fully comprehended, such information, combined with estimates of gene flow^{24,25} and dispersal rates,^{20,25,26} is essential. We therefore undertook such a study by investigating between-population variability in seasonal acclimatization and the extent and time-course of phenotypic plasticity of

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thermal tolerance and desiccation resistance in *G. pallidipes* Austen.

MATERIALS AND METHODS

Field sampling and study populations. Adult flies were sampled from four locations representing different thermal habitats in Kenya, East Africa (Table 1). To determine seasonal variation in physiology, the Nguruman District (mid-altitude) and Lambwe District (high-altitude) populations were sampled twice within the same year in mid-July to mid-August 2003 (the end of the long rainy season) and in mid-November to mid-December 2003 (the end of the short rainy season). The Narok (highest altitude) and Kwale Districts (low-altitude) were sampled in November-December 2003. For each of the field experiments, flies were collected from odor-baited, biconical traps¹⁵ set out in the respective localities (key attractive components: 4-methyl-phenol, 3-*n*-propynol, and acetone). After removal from the traps, the flies were transported in an insulated container, lined with moist paper towel, to field laboratories. These were located a maximum of 2 hours away from each of the sampling sites of a given district. At the laboratories, the flies were separated into groups for each of the experimental procedures, after which experiments started immediately. Depending on the time of the experiment, flies were collected either in the morning or in the late afternoon, usually from 0800 to 1000 and from 1700 to 1800 hours. Thermal limit assessments were performed during the day using flies sampled in the morning, whereas desiccation experiments took place during the night using afternoon-collected flies.

Critical thermal limits. An insulated system of 11 double-jacketed isolation chambers was connected to a programmable water bath (LTD 20 with PZ1 programmer; Grant Instruments, Cambridge, UK) that regulated water flow around the chambers. A single fly was placed into each chamber, and a 40 SWG type-T thermocouple was inserted into the control chamber to measure chamber temperature. The flies were allowed to equilibrate for 10 minutes at either 16°C or 35°C before either minimum or maximum critical thermal limit assessments started, respectively.²⁷ After equilibration, chamber temperature was lowered or raised at 0.25°C/min. Preliminary experiments confirmed that body temperature did not lag behind or exceed chamber temperature at this rate of temperature change. Critical thermal minimum (CT_{Min}) was defined as the loss of coordinated muscle function, and critical thermal maximum (CT_{Max}) was defined as the onset of muscle spasms. The temperature at which either of these reactions occurred was recorded for each individual. These endpoints are readily identifiable for any species once an ob-

server is practiced,²⁷ variance about the endpoints is typically low, and here a single observer (C.J.K.) undertook all of this work. Moreover, this experimental procedure has been verified using thermolimit respirometry,²⁸ is widely used to assess thermal limits,²⁹ and the observer typically was not informed which acclimation treatment was being assessed. The procedure was repeated three times for both CT_{Min} and CT_{Max} to give a total $N = 30$ per population and trait. Preliminary, replicated experiments found no effect of sex, age, or feeding status on critical thermal limits (C. J. Klok and J. S. Terblanche, unpublished data). Critical thermal maxima were lethal, whereas minima were not. However, even in the case of CT_{Min}, all experimental animals were discarded after a trial.

Water loss rates. Male flies ($N = 16$), held individually in 5-mL cuvettes, were subjected to desiccation in flowing air (< 2.5% relative humidity [RH]) for 10 hours at $24.0 \pm 1.0^\circ\text{C}$ (PTC-1 Peltier-controlled temperature cabinet; Sable Systems, Las Vegas, NV).³⁰ Air was pumped through a scrubbing column containing silica gel and Drierite to remove residual water and into a mass flow controller (MFC). The MFC outlet was connected to a Sable Systems MF8 airflow manifold. Each outflow channel of the manifold was further split into two streams so that two cuvettes, each containing a fly, were attached to a single manifold channel. The air flow rate through each cuvette, tested with a second MFC, was regulated to produce a rate of 100 mL/min. Experiments took place during the night (2100–0700 hours), a period of minimal activity in tsetse, and were repeated twice per location ($N = 16$ per experiment), each time on individuals collected in the field on that particular day, to give a total $N = 32$. Each fly was weighed before and after a trial (Avery Berkel FA 304T, Fairmont, MN, electronic microbalance, 0.1-mg resolution), and water loss rate was expressed as milligrams of water per hour. Excretion usually takes place after a blood meal, and here, experiments took place well after such events. In addition, even in field-collected flies, where the time since feeding could not be accurately determined, no excretion in the cuvettes was observed. Therefore, mass loss was considered representative of water loss even though a small amount of mass change took place as a consequence of metabolism.²² After the trials, flies were dried to constant mass (~ 50 – 60°C for ~ 72 hours) and were re-weighed to determine water content. Solvent-based lipid extractions were used to determine lipid mass. Flies used in desiccation trials were dismembered and soaked in 2 mL chloroform:methanol (2:1) solution for approximately 48 hours to dissolve internal (body) lipids. Lipid content was estimated by determining dry body mass before and after lipid extraction.³¹

Activity levels. Several authors have suggested that differences in desiccation resistance among taxa might simply reflect divergence in activity levels.^{22,32} Because little is known about inter-population variation in tsetse activity levels, particularly under stressful conditions, the proportion of time individuals were active was estimated over a 30-min period for each population. Time active at 20°C, 24°C, 28°C, and 32°C was determined using a single electronic activity detector (AD-1; Sable Systems) that exported voltage data to a computer through Sable Systems DATACAN V software. These experiments were performed during the same field sampling periods reported above. Voltage data were sliced from the raw Datacan V (Sable Systems) data files, copied to MS-Excel, converted to absolute values, and sorted from low-

TABLE 1

Field sampling sites (coordinates in decimal degrees) and time of sampling of *G. pallidipes* for this study

Region	Coordinates (°)	Altitude (m)	Sampling season
Narok	1.12 S, 35.20 E	1,691	After short rains
Lambwe	0.64 S, 34.31 E	1,353	After long and short rains
Nguruman	1.85 S, 26.10 E	670	After long and short rains
Kwale	4.18 S, 39.46 E	388	After short rains

Both seasons were sampled during 2003

est to highest. Based on preliminary observations, we considered activity as any signal more than 0.01 V measured by the AD-1 (electronic spikes of 5 V were ignored and can easily be distinguished from true activity readings). The total time spent above this level of activity was expressed as a proportion of the total recording time. While it is well known that activity in tsetse is sensitive to feeding state,³³ the objective here was to determine whether differences in activity level were a likely cause of any differences in water loss rates between populations and not whether the flies activity overall differs between populations.

Within-generation physiologic plasticity. Logistic constraints precluded the use of field populations for investigations of phenotypic plasticity. Therefore, *G. pallidipes* from a laboratory colony maintained at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, International Atomic Energy Agency, Vienna, Austria, was used. The IAEA laboratory colony used in this study was established in 1975 from a natural population in Tororo, Uganda, close to the Kenyan border (and < 200 km from our Lambwe field site). Gene diversities over two mitochondrial loci were within the range of 18 field populations from Ethiopia and East and Southern Africa.³⁴ Therefore, the laboratory-reared flies may be considered genetically representative of flies from field populations. Puparia were immediately placed inside plastic containers and transferred to a climate chamber set to 24°C (24.6 ± 2.5°C, 12:12-hour L:D) and 76% RH as described previously.³⁵ Feeding of the newly emerged adults took place using a membrane-tray system³⁶ every alternative day, and at this time, container locations were randomized within the climate chamber. Care was taken to ensure that all treatment groups were handled for the same duration in transferal from the climate chamber to the feeding area and spent a similar amount of time outside the climate chamber while feeding (~25 minutes on each feeding day). Flies were held at 19°C, 24°C, and 29°C for 10 days in climate chambers with a synchronized photoperiod and constant 76% RH levels. The 24°C and 29°C groups were stored in temperature-regulated climate chambers (Labcon, Pretoria, South Africa; mean ± SD: 24.0 ± 2.3 [N = 1029]; 28.9 ± 1.7°C [N = 937]) and the 19°C group was stored in a Peltier-controlled temperature cabinet (mean ± SD: 19.4 ± 0.4°C [N = 905]). Acclimation temperatures were chosen to represent the mean annual temperatures of the respective geographic locations sampled (reported mean annual temperature—warm-temperate midlands [< 1,850 m]: 18–20°C; warm lowlands [900–1,200 m]: 22–24°C; hot lowlands [0–900 m]: 24–30°C).³⁷ All experimental and rearing facilities were contained within a quarantine approved, air-conditioned laboratory. The same system and methods as those used in the field experiments for determining geographic variation were used in the laboratory determination of critical thermal limits and desiccation rate.

A separate batch of colony-reared flies was used to determine whether the temperature acclimation effect observed in the CT_{Min} of *G. pallidipes* is time- and/or temperature-dependent and whether these changes in CT_{Min} are reversible. Flies were allowed to emerge in our laboratory as described above and were fed twice within 4 days after eclosion. However, a small number of flies seemed only to feed during the second feeding round. Thus, typically after two blood meals, flies were randomly assigned to four temperature ac-

climation groups (24°C, 21°C, 19°C, and 16°C; N = 180 ~ 220 per group, five to six cages per group). Humidity and photoperiod were regulated as described above, and randomization of cage locations within chambers followed the protocol adopted previously. Therefore, handling times were kept constant across all groups during feeding sessions and experimental treatments. The CT_{Min} of 10 randomly selected flies from each treatment group was determined on acclimation days 1, 3, 5, 7, and 9. After 9 days of acclimation, the remaining flies were all returned to 24°C for an additional 9-day period, after which another sample of flies was assessed on the tenth day. In this experiment, once a fly had been used for determination of CT_{Min}, it was discarded by bottling in 90% ethanol (i.e., no animals were re-used). Virgin females, separated from males into cages immediately after eclosion, were used to study effects of temperature acclimation on body mass. Feeding took place using the same methods described above only on days between experimental studies (i.e., flies were always in a fasted state during experiments).

Statistical analyses. Data were assessed for normality using a Shapiro-Wilk test before analyses. Because critical thermal limit data were normally distributed, analyses of variance (ANOVAs) using the raw data were used to compare critical thermal limits for both field and laboratory studies. Analyses of covariance (ANCOVAs) were used to test for differences in rates of water loss and lipid content between populations and seasons (i.e., mass-independent water loss rates), using mean experimental or dry mass as the covariate. Pearson product-moment correlations were used to assess the relationships between time spent active and temperature. Data are presented as means ± SE unless otherwise stated. Significance was set at P = 0.05. In no instances for any trait were experimental assessments performed on the same individual on more than one occasion (i.e., animals were not re-used).

RESULTS

Seasonal variation. In the only populations that were sampled on two occasions, season had a significant effect on the majority of the variables examined (Table 2). In the Lambwe and Nguruman populations, CT_{Min} increased and water loss rate declined after the short rains. Although lipid mass also declined in the Lambwe population, the change was not significant in flies from Nguruman. Seasonal changes in body mass and CT_{Max} were opposite in sign in the two populations, although the seasonal difference in CT_{Max} was only 0.4°C in both populations. Body mass increased in the Lambwe population but declined at Nguruman, leading to an overall difference in mass between the two populations after the short rains (Table 2; $F_{1,91} = 20.35$; $P < 0.0001$) but not after the long rains (Table 2; $F_{1,126} = 0.414$; $P = 0.52$).

Geographic variation. Using data collected within the same season after the short rains, CT_{Max} and CT_{Min} differed among the four populations (Table 3). The mid-altitude and low-altitude populations differed only marginally, whereas both CT_{Min} and CT_{Max} declined with a further increase in elevation. The slope of the two altitude-critical limit relationships also differed significantly (ANCOVA, $F_{1,234} = 59.42$; $P < 0.0001$), with CT_{Min} showing a steeper slope and stronger relationship with altitude than CT_{Max}. Overall, CT_{Min} varied by 3.3°C among the four populations, whereas CT_{Max} differed by only 0.4°C.

TABLE 2
Summary statistics for seasonal variation of physiological traits in *G. pallidipes* from the Lambwe and Nguruman populations

Population	Trait	Season		F ratio	df	P
		Long rains	Short rains			
Lambwe	CT _{Max} (°C)	45.0 ± 0.1	44.6 ± 0.1	11.18	58	< 0.01
	CT _{Min} (°C)	12.3 ± 0.2	13.4 ± 0.3	9.83	58	< 0.01
	Water loss rate (mg H ₂ O.h ⁻¹)*	0.620 ± 0.027	0.536 ± 0.032	6.95	51	< 0.05
	Body (dry) mass (mg)	11.74 ± 0.42	13.73 ± 0.73	6.38	59	< 0.05
	Body lipid content (mg)*	2.00 ± 0.07	1.89 ± 0.08	30.03	106	< 0.0001
Nguruman	CT _{Max} (°C)	44.6 ± 0.1	45.0 ± 0.1	12.00	56	< 0.01
	CT _{Min} (°C)	11.8 ± 0.2	13.8 ± 0.2	74.79	58	< 0.0001
	Water loss rate (mg H ₂ O.h ⁻¹)*	0.789 ± 0.042	0.474 ± 0.033	40.15	54	< 0.0001
	Body (dry) mass (mg)	11.62 ± 0.39	10.84 ± 0.41	7.13	47	< 0.05
	Body lipid content (mg)*	1.53 ± 0.07	0.99 ± 0.08	3.308	98	0.072

The results of either ANOVA or ANCOVA (*with dry mass as covariate) are shown.

Water loss rate also varied significantly among the populations (Figure 1). It seems unlikely that differences in activity levels among the populations could account for the variation in water loss rate. Total activity time was positively, although in one case weakly, correlated with temperature across the range of 20-32°C in all four populations (Narok: $r = 0.64$, $P < 0.01$; Lambwe: $r = 0.47$, $P = 0.068$; Nguruman: $r = 0.64$, $P < 0.01$; Kwale: $r = 0.50$, $P < 0.05$). Because there were no sex-related differences in activity time in any of the populations, sexes were pooled for the inter-population comparisons. There were no among-population differences for time spent active (ANCOVA [covariate: temperature]: $F_{3,59} = 1.38$; $P = 0.26$). Therefore, differences in activity probably did not account for differences in water loss rates.

Temperature acclimation of laboratory-bred flies. Ten days of laboratory acclimation at three different temperatures resulted in significant among-treatment variation in CT_{Max}, CT_{Min}, desiccation rate, and body water content (Table 4). CT_{Max} increased significantly with treatment temperature, but the change was less than 0.5°C. In contrast, at 19°C, CT_{Min} declined by approximately 3°C relative to the other treatments. Water loss rate showed similar threshold effects, declining significantly at the highest acclimation temperature, whereas body water content showed little change with acclimation temperature (Table 4).

In the time-course experiments, changes in CT_{Min} were induced in the 16°C and 19°C treatments only, and the magnitude of the induced change was quite similar (Figure 2). In contrast, the 21°C and 24°C treatments had no effect. In the former treatments, CT_{Min} had reached its nadir by the fifth

day of treatment and typically remained at a similar level for the remainder of the acclimation period (day 9). Ten days at 24°C were sufficient to completely reverse the effects of the acclimation treatments (i.e., at day 19, no groups differed from the 24°C group). Thus, significant day × treatment interactions were found for CT_{Min} ($F_{15,216} = 3.99$; $P < 0.0001$). The low treatment temperatures clearly came at some cost to normal adult development. Whereas flies gained mass in the 21°C and 24°C treatments and showed low and typically consistent among-individual variation, the flies at the lower temperatures did not show any steady progression in mass, and variation among individuals was considerable (Figure 3). The differential effect of treatment on change in mass over the 19 days is evidenced by a significant day × treatment interaction in a two-way ANOVA examining the effects of day and treatment on mass ($F_{15,120} = 2.7$, $P = 0.0013$).

Colony versus field flies. Upper and lower critical thermal limits differed among the laboratory-acclimated (colony) flies and field populations (Figure 4, A and B). However, the extent of this difference was only approximately 1°C for CT_{Max} but from 4°C to 10°C for CT_{Min}. Water loss rates also varied

TABLE 3
Geographic variation in mean population critical thermal maxima (CT_{Max}) and minima (CT_{Min}) for *G. pallidipes*

Trait	Mean ± SE (°C)	Min	Max	N
CT _{Max}				
Narok	44.4 ± 0.1	43.5	45.1	30
Lambwe	44.6 ± 0.1	43.7	45.2	30
Nguruman	45.0 ± 0.1	44.3	45.7	28
Kwale	45.0 ± 0.1	44.2	45.5	30
CT _{Min}				
Narok	10.5 ± 0.2	8.9	12.4	30
Lambwe	13.4 ± 0.3	11.8	16.0	30
Nguruman	13.8 ± 0.2	11.8	15.2	30
Kwale	13.8 ± 0.2	12.6	15.9	30

All flies were collected in the same season (Nov–Dec 2003). ANOVA: CT_{Max}: $F_{3,114} = 14.0$; $P < 0.0001$; CT_{Min}: $F_{3,116} = 70.35$; $P < 0.0001$

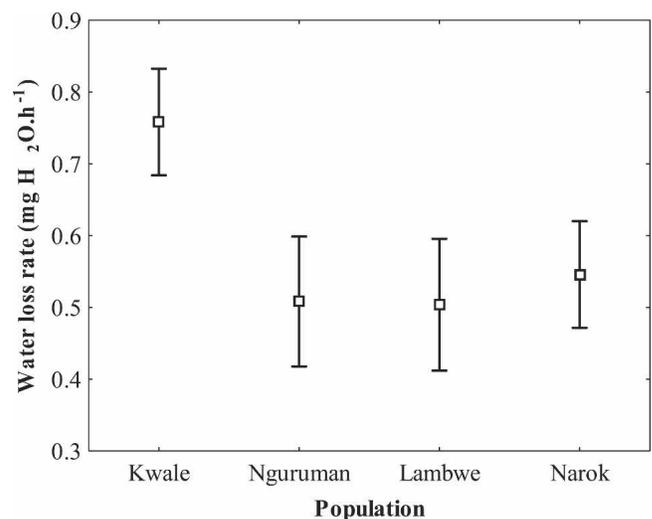


FIGURE 1. Geographic variation of water loss rate in *G. pallidipes*. Note that desiccation rate is highest in the low altitude population from Kwale (388 m). All flies were sampled in the same season (November to December 2003; Narok: 1,691 m; Lambwe: 1,353 m; Nguruman: 670 m).

TABLE 4

Upper (CT_{Max}) and lower (CT_{Min}) critical thermal limits, water loss rate at 24°C and initial body water content after 10 days of temperature acclimation in laboratory-reared *G. pallidipes* adults

Acclimation temperature	CT_{Max} (°C)	CT_{Min} (°C)	Water loss rate* (mg H ₂ O.h ⁻¹)	Body water content (mg H ₂ O)
19°C (19.5 ± 0.4)	43.9 ± 0.1 ^A (20)	4.5 ± 0.1 ^A (20)	0.464 ± 0.061 ^A (8)	29.9 ± 0.7 ^A (8)
24°C (23.4 ± 0.9)	44.4 ± 0.1 ^B (10)	7.8 ± 0.3 ^B (12)	0.393 ± 0.017 ^A (9)	25.2 ± 2.8 ^B (9)
29°C (28.8 ± 1.7)	44.2 ± 0.1 ^{A,B} (20)	7.2 ± 0.2 ^B (20)	0.213 ± 0.047 ^B (8)	23.4 ± 1.5 ^B (8)
<i>F</i>	5.10	97.34	7.89	2.77
<i>P</i>	< 0.01	< 0.001	< 0.003	0.085

* ANCOVA with dry body mass as covariate.

Recorded climate chamber temperatures presented in parentheses (mean ± SD) after treatment temperature.

Similar superscript letters denote statistically homogeneous groups (post hoc unequal sample HSD).

All analyses by means of ANOVA unless otherwise specified.

among the field and laboratory populations, and here, the maximum difference among groups (Kwale and the 29°C treatment group) was substantial, although none of the other groups differed from each other (Figure 4C). In contrast, dry mass varied little among the populations and groups (Figure 4D).

DISCUSSION

Physiologic implications. The temperature and water balance traits differed substantially from each other in the extent of their variability. Of the three traits, CT_{Max} varied least among populations (0.6°C), seasons (0.4°C), and acclimation treatments (0.5°C), and the full range of variation among all of these samples was only 1.1°C (maximum value, 1.02 times larger than the minimum). In contrast, CT_{Min} was more variable, differing by 3.3°C, 1–2°C, and 3.3°C among the populations, seasons, and acclimation treatments, respectively, and

the full range of variation was 9.3°C (maximum value = 3.1 × minimum). Water loss rate showed similar, substantial variation, with the overall maximum value, across laboratory and field populations, being 3.7 times the minimum. These results are consistent with work across a range of insect taxa. In insects, upper critical temperatures show much less variation than lower critical temperatures among populations and acclimation treatments,³⁸ between selection regimens,³⁹ and among species at a variety of spatial scales.⁴⁰ Substantial differences in water loss rates among populations, among seasons within a population, and between humidity acclimation treatments are also common.^{38,41,42} However, only a few studies have examined temperature acclimation or acclimatization effects on desiccation resistance, with some finding notable effects of temperature,^{38,43} and others finding no influence.³⁰ Thus, upper thermal critical limits that are not especially variable, and water loss rates and lower thermal limits that are more flexible are typical of insects in general. In consequence, their occurrence in *G. pallidipes* is not entirely unexpected.⁴⁴

A further significant finding is that the magnitude of the among-population differences was identical to or smaller than the magnitude of the acclimation effects in the laboratory-reared flies. Phenotypic plasticity might therefore account for all of the among-population variation in *G. pallidipes*. However, because of logistic constraints, each population was not examined to determine the extent of its phenotypic plasticity. Therefore, the extent to which among-population variation really is accounted for by phenotypic plasticity could not be fully determined. Nonetheless, seasonal acclimatization of CT_{Min} , CT_{Max} , and water loss rates in the Lambwe and Ngoruman populations encompassed 50–100% of the among-population variation in these traits. Together with the results from the laboratory acclimation trials, these findings provide substantive evidence for the idea that most of the variation among populations could be accounted for by phenotypic rather than genetic differences. Similar results have been obtained for two independent assessments of *Drosophila melanogaster*.^{38,45} Thus, it seems likely that much of the among-population variation in these traits examined in *G. pallidipes* is a consequence of phenotypic plasticity.

In the case of CT_{Min} these plastic changes take place rapidly. Within 5 days of exposure to a different temperature, flies held at the lower temperatures had substantially altered their CT_{Min} , and by 9 days after their return to a common, higher temperature environment, CT_{Min} had returned to its original value. Whether the rapid phenotypic change found in *G. pallidipes* is typical of CT_{Min} or similar traits in other insect species is not clear, largely because the time-course of thermal

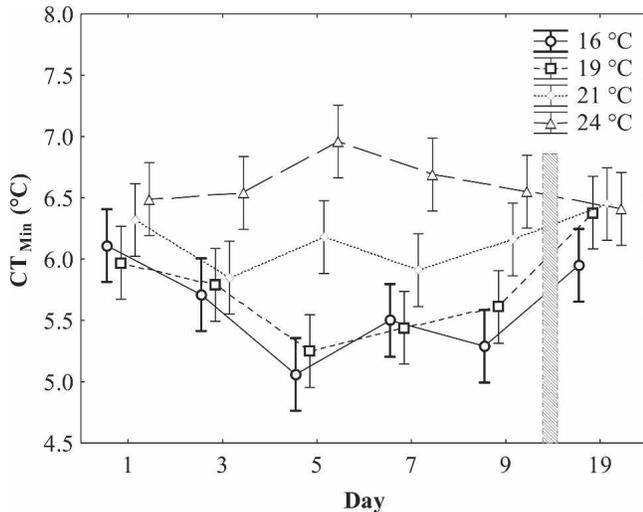


FIGURE 2. The time-course of acclimation for CT_{Min} in *G. pallidipes* adults. After 9 days of acclimation to 16°C, 19°C, 21°C, and 24°C, all acclimation groups were returned to 24°C for 10 days of recovery (tested on day 19). (Recorded climate chamber temperatures [mean ± SD]: 16: 15.7 ± 0.37°C; 19: 18.6 ± 0.22°C; 21: 20.6 ± 0.50°C; 24: 24.4 ± 1.38°C). *N* = 10 flies for all days and all acclimation temperature groups. There was no effect of acclimation on 21°C and 24°C acclimation groups, but both 16°C and 19°C had significantly reduced CT_{Min} by day 5 (ANOVA: 16°C: $F_{5,54} = 8.70$; $P < 0.0001$; 19°C: $F_{5,54} = 9.31$; $P < 0.0001$; 21°C: $F_{5,54} = 1.99$; $P = 0.095$; 24°C: $F_{5,54} = 1.38$; $P = 0.25$). By day 19, all acclimation groups were similar ($F_{3,36} = 2.64$; $P = 0.064$).

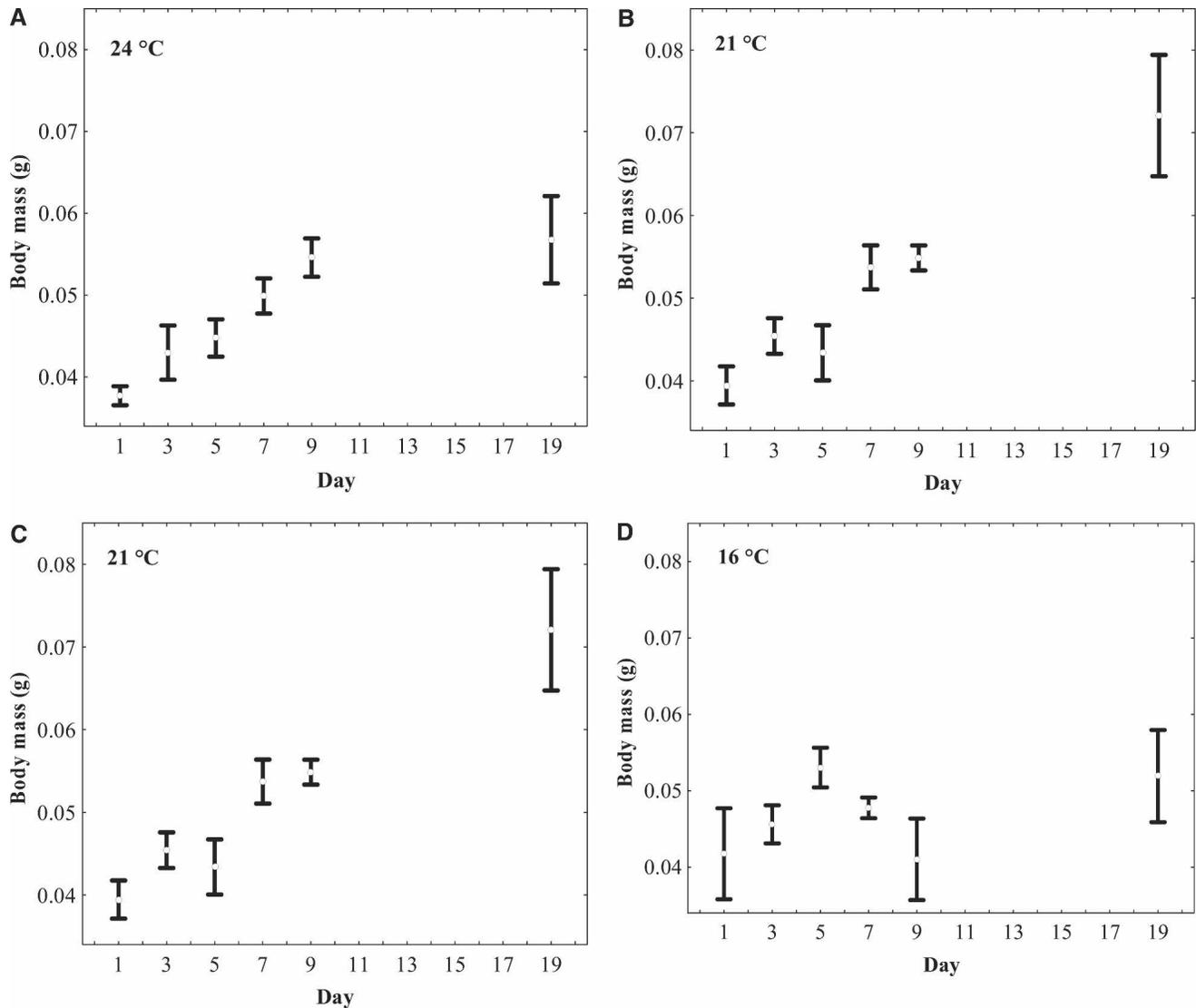


FIGURE 3. Body mass (grams; mean \pm SE) of virgin female *G. pallidipes* during the course of acclimation to (A) 24°C, (B) 21°C, (C) 19°C, and (D) 16°C. All flies were returned to 25°C after day 9 of acclimation. All mass measurements were obtained on non-feeding days (i.e., 24 h after feeding). Although no differences were found in body mass on completion of the acclimation treatments, there was a significant day \times treatment interaction (see text for statistics).

acclimation (rather than developmental plasticity) has not been thoroughly studied in the group. Nonetheless, several studies have documented similar rapid changes in physiologic traits.^{46–48} Therefore, reasonably rapid acclimation effects are probably not unusual in insects.

Finally, whereas CT_{Max} , water loss rate, and dry mass were quite similar across all of the populations and acclimation treatments, CT_{Min} differed substantially between the field-collected and laboratory-reared flies (Figure 4). The latter had CT_{Min} s that were 3–9°C lower than those of the field-collected flies. In consequence, it seems likely that differences in the phenotypic plasticity of the upper and lower critical thermal limits extend to the ease with which these traits can evolve. Higher acclimation temperatures in laboratory flies, or lower acclimation temperatures in field flies, might have further altered CT_{Min} . However, this seems unlikely given that more extreme temperatures are debilitating to flies over the longer term.^{7,20} Therefore, the differences between the

field and laboratory flies might have evolved as a consequence of conditions in the laboratory. The IAEA laboratory colony used in this study was established in 1975. Assuming linear evolutionary change, approximately 175 generations in the laboratory, and CT_{Min} values of the original Ugandan field population similar to those found at Lambwe, this would equate to an approximately 1°C change every 29 generations (i.e., 6°C/175 generations). While the reasons for laboratory evolution are not always clear, substantial changes in animals held in colonies have been documented in several studies.^{49,50} Whatever the reason for the evolved difference, the most noteworthy feature thereof is that this evolution has been restricted to CT_{Min} and has not taken place in the other traits. In consequence, it seems likely that most of the variation in the other traits is a consequence of phenotypic plasticity, whereas the variation found in CT_{Min} is a mixture of plasticity and evolved differences between laboratory and field-collected *G. pallidipes*. In addition, it raises questions about

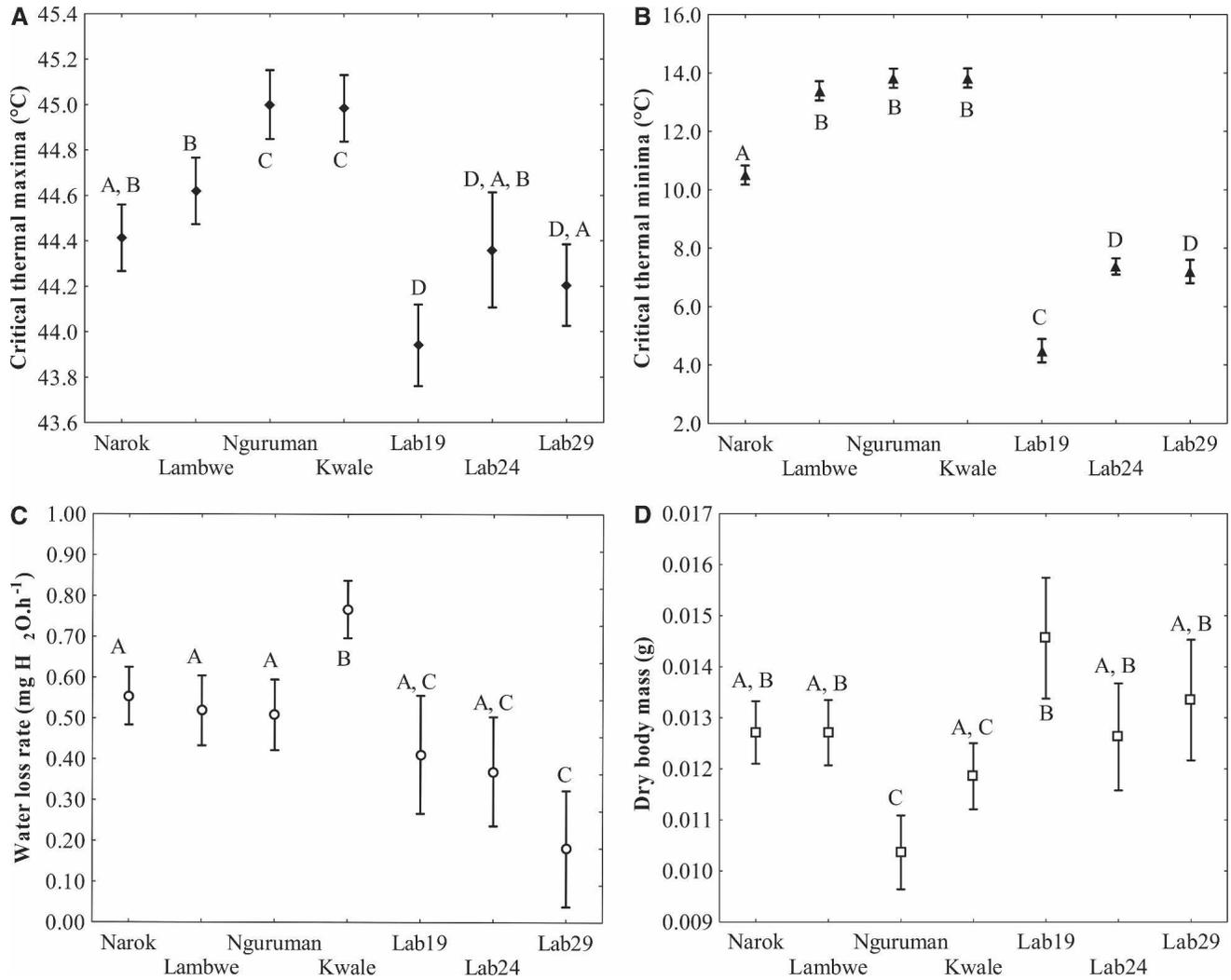


FIGURE 4. Traits means (\pm SE) in field and laboratory populations of *G. pallidipes* of (A) critical thermal maxima (ANOVA, $F_{6,161} = 22.38$; $P < 0.0001$); (B) critical thermal minima (ANOVA, $F_{6,195} = 453.20$; $P < 0.0001$); (C) water loss rate (ANCOVA, covariate: dry body mass; $F_{6,126} = 12.378$; $P < 0.0001$; least squares adjusted means \pm 95% confidence limits); and (D) body size as dry body mass from all desiccation experiment flies (ANOVA; $F_{6,239} = 8.43$; $P < 0.0001$; means \pm 95% confidence limits). The numerals in Lab19, Lab24, and Lab29 refer to the temperature of acclimation in laboratory-reared flies for periods of 9–10 days.

the extent to which the laboratory flies are genetically representative of field-collected flies, at least in the case of this trait. Future work exploring the underlying genetic basis of CT_{Min} would be required to resolve this question.⁵¹

Implications for distributional modeling. Although the critical thermal maxima of adult *G. pallidipes* documented here are higher than the temperatures that induce mortality in the field,²⁰ these findings nonetheless substantiate correlative work on the determinants of the distribution of this species and provide several insights into the likely responses of tsetse to climate change. In the first instance, univariate and multivariate analysis of climate and productivity (NDVI) variables showed that temperature variables are much better predictors of *G. pallidipes* presence and absence than NDVI variables.^{5,6} These results are in keeping with population level analyses that show a strong relationship between increasing temperature and elevated mortality in *G. pallidipes* adults.²⁰ Our results suggest a causal basis for the determination of *G. pallidipes* distributions by temperature. It seems that, in this spe-

cies, tolerance to high temperatures cannot change much either by phenotypic plasticity or by evolution. Such change in tolerance would result in a weaker relationship between temperature and population dynamics, and in doing so, would reduce the strength of the relationships between temperature and the presence/absence of the fly species.⁵² The limited variation in CT_{Max} also provides a causal explanation for why, in statistical models of tsetse distribution, the mean temperature difference between areas of fly presence and absence may be less than 1°C.¹⁹

Limited scope for change in upper critical temperatures might also explain the narrow variation in this trait among populations despite the fact that gene flow between *G. pallidipes* populations is typically low.^{25,34} From a theoretical perspective, limited gene flow between populations should promote diversification as long as gene flow is not so low that novel variants that have successfully survived similar conditions elsewhere are seldom introduced into a population.^{53,54} However, if the trait itself cannot evolve because it is globally

constrained,⁵⁵ changes in gene flow are unlikely to affect trait diversification.

From these data, it might seem difficult to discern why flies are not able to colonize lower temperature areas. However, lower temperature thresholds are likely set by development of the larvae rather than by adult capabilities.⁵⁶ Moreover, in our acclimation experiments, we showed that, although flies are able to survive longer-term maintenance at low temperatures, their muscle growth and development (which accounts for the largest change in mass in adult tsetse⁵⁷) is compromised, given that mass declines substantially by days 7–9, and variance in mass between individuals was larger in the lower than in the high temperature treatments (Figure 3). Therefore, low temperatures are likely to compromise adults also.

To date, most climate change predictions suggest that tsetse species would show a reduction in the range of the flies owing to increases in temperature.¹⁷ Our data support these findings because they show limited plasticity in upper critical temperatures. If upper thermal tolerances are inflexible, increases in temperature are likely to lead to a reduction in range size as suggested by modeling approaches.⁷ What altered rainfall patterns are likely to mean is more difficult to discern because water loss rates in adult flies respond to altered ambient conditions,⁴⁴ water balance effects are likely to be most significant for puparia,^{20,56} which were not investigated here, and fine-scale predictions of rainfall change are typically not available.¹ Therefore, it is difficult to be able to predict what the outcome of the interaction between fly desiccation resistance and changing water availability might be. Nonetheless, our results provide further evidence in support of the idea that, owing to changes in land use and climate, at least some tsetse may be brought under autonomous control in parts of their natural ranges.⁵⁸

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