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RESEARCH ARTICLE

Sex-specific microhabitat use is associated with sex-biased thermal physiology in *Anolis* lizards

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ABSTRACT

If fitness optima for a given trait differ between males and females in a population, sexual dimorphism may evolve. Sex-biased trait variation may affect patterns of habitat use, and if the microhabitats used by each sex have dissimilar microclimates, this can drive sex-specific selection on thermal physiology. Nevertheless, tests of differences between the sexes in thermal physiology are uncommon, and studies linking these differences to microhabitat use or behavior are even rarer. We examined microhabitat use and thermal physiology in two ectothermic congeners that are ecologically similar but differ in their degree of sexual size dimorphism. Brown anoles (*Anolis sagrei*) exhibit male-biased sexual size dimorphism and live in thermally heterogeneous habitats, whereas slender anoles (*Anolis apletophallus*) are sexually monomorphic in body size and live in thermally homogeneous habitats. We hypothesized that differences in habitat use between the sexes would drive sexual divergence in thermal physiology in brown anoles, but not slender anoles, because male and female brown anoles may be exposed to divergent microclimates. We found that male and female brown anoles, but not slender anoles, used perches with different thermal characteristics and were sexually dimorphic in thermal tolerance traits. However, field-active body temperatures and behavior in a laboratory thermal arena did not differ between females and males in either species. Our results suggest that sexual dimorphism in thermal physiology can arise from phenotypic plasticity or sex-specific selection on traits that are linked to thermal tolerance, rather than from direct effects of thermal environments experienced by males and females.

KEY WORDS: *Anolis*, Climate change, Habitat use, Sexual dimorphism, Thermal tolerance, Thermoregulation

INTRODUCTION

In many sexually reproducing animals, the phenotypes that give rise to high-fitness females are different from those that produce high-fitness males, even though the sexes share a genome. To resolve this 'intralocus sexual conflict', males and females may express different phenotypes via hormonal alteration of gene expression patterns or sex chromosome-specific genetic variation (Cox et al., 2015, 2017b; Cox and Calsbeek, 2009, 2010a,b; Ketterson et al., 2005; Mank, 2009; McGlothlin et al., 2019; McGlothlin and Ketterson, 2008). Divergence in male and female phenotypes is termed 'sexual dimorphism' and can arise from several processes. Sexual selection can lead to sexual dimorphism via female 'choosiness', whereby females select males that maximize their chances of producing high quality young (Bleu et al., 2012), or via male–male competition whereby males compete for access to females (Houde, 1988). Selection may also favor larger females because they are able to gestate larger clutches of eggs (Honěk, 1993), while larger males may have greater fitness because they are better able to defend and exploit high quality territories (Gabor, 1995). By contrast, sexual dimorphism may evolve for non-adaptive reasons. For example, pleiotropic effects of hormones can result in divergence between the sexes even in the absence of selection for alternative phenotypes (Flatt et al., 2005; Lichanska and Waters, 2008). These various mechanisms have generated differences between the sexes across many species and in a broad range of traits, including morphology (Butler et al., 2000; King, 2008), physiology (Cullum, 1998), life history (Lara-Ruiz and Chiarello, 2005) and behavior (Segovia and Guillamón, 1993).

Sexual dimorphism may also correspond to differences in the spatial and temporal niches occupied by males and females, regardless of whether adaptive or non-adaptive processes were responsible for generating divergent phenotypes (Butler, 2007). For instance, if males place a premium on defending territories and attracting potential mates, they may remain active for longer periods of the day (temporal niche divergence) and in the process be exposed to different abiotic conditions than females. The priorities of males may lead them to occupy more exposed areas of the habitat matrix to increase their chances of detecting competitors or attracting mates, while females remain hidden in sheltered microhabitats to reduce

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their exposure to predators (spatial niche divergence). Indeed, males of many lizard species are known to perch higher than females as they defend territories and try to remain visible to potential mates (Losos, 2009; Losos et al., 2004; Stuart et al., 2014; Zucker, 1986). In some habitats, these higher perches may have different thermal characteristics. Thus, it seems possible that sex-biased habitat usage in ectothermic species that live in thermally heterogeneous environments could expose the sexes to divergent selection.

To date, studies examining sexual dimorphism in thermal physiology are uncommon (Bodensteiner et al., 2020; Cecchetto and Naretto, 2015; Huey and Pianka, 2007) despite the potential for sex-biased variation in physiology to mediate the responses of species to climate change. If males and females differ in their thermal physiology in ways that affect survival probabilities, then rising temperatures might generate asymmetrical sex ratios through differential mortality. As one sex begins to outnumber the other, mating opportunities may be reduced, which could increase the risk of extinction (Schwanz and Janzen, 2008). In addition, niche overlap may increase as one sex is forced to track their preferred thermal environment, increasing resource competition between the sexes. While previous studies have reported sex-based differences in the thermal physiology of squamate reptiles (Beal et al., 2014; Brown and Weatherhead, 2000; Gilbert and Lattanzio, 2016; Lailvaux, 2007; Lailvaux et al., 2003; Lailvaux and Irschick, 2007), few have investigated the potential role of sex-biased habitat use and fine-scale thermal heterogeneity in driving these patterns.

We compared morphology, habitat use, environmental temperature variation and thermal physiology between females and males in two congeners that are ecologically similar but differ in their habitat associations and degree of sexual size dimorphism. These data were originally collected as part of two large-scale transplant experiments in The Bahamas and Panamá, respectively, allowing us to perform robust tests of our hypotheses using sample sizes (N>700 for most traits) that are uniquely large for comparative physiology studies of vertebrates. The brown anole (*Anolis sagrei*) occupies habitats that are thermally heterogeneous in both space and time in The Bahamas, and displays male-biased sexual size dimorphism (Cox and Calsbeek, 2010a), whereas the slender anole (*Anolis apletophallus*) occupies habitats that are thermally homogeneous in both space and time in Panamá, and is sexually monomorphic in body size (Andrews, 1979; Ballinger et al., 1970). We expected that males of both species would perch higher than females in the vegetation, providing an opportunity to assess the morphological and ecological factors that favor sexual dimorphism in thermal physiology. Based on the physical structure of their habitats, we hypothesized that environmental temperature would vary with lizard perch height in the thermally heterogeneous habitat of brown anoles but not in the thermally homogeneous habitat of slender anoles. We further hypothesized that these patterns of microhabitat use would be associated with sexual dimorphism in thermal physiology in brown anoles, but not slender anoles.

MATERIALS AND METHODS

Study system

We examined sexual dimorphism in thermal physiology of the sexually size-dimorphic brown anole and sexually size-monomorphic slender anole. We studied adult brown anoles [male snout–vent length (SVL) >40 mm; female SVL >30 mm] on the island of Great Exuma in The Bahamas (23.5333°N, 75.8333°W), where they live along the sun-flecked edges of scrubby coppice forest (Logan et al., 2018). The mass of adult male brown anoles can be more than twice that of females (Cox and Calsbeek, 2010a). We also characterized a population of adult slender anoles (male and female SVL >38 mm) from Soberanía National Park, Panamá (9.1165°N, 79.6965°W), where they live in the dark, vine-tangled understory of lowland broad-leaf forest (Andrews and Sexton, 1981). Male and female slender anoles are nearly identical in both body length and mass. Note that the dewlaps of males are substantially larger in both species (Cox et al., 2017a; Rosso et al., 2020; Stapley et al., 2011). Despite these differences in habitat structure and sexual size dimorphism, brown anoles and slender anoles have broadly similar ecologies and life histories. Both species are territorial, generalist arthropod-predators that have nearly annual population turnover and breed during northern hemisphere summers (Andrews et al., 1989; Andrews and Nichols, 1990; Andrews and Stamps, 1994; Calsbeek, 2009; Cox et al., 2020a; Logan et al., 2014; Losos, 2009; Sexton, 1967; Sexton et al., 1972). The differences and similarities between these two species render them an ideal system for testing hypotheses regarding the ecological factors favoring sexual dimorphism in thermal physiology. All methods and procedures were carried out under Institutional Animal Care and Use protocols issued by Harvard University and the Smithsonian Tropical Research Institute.

Habitat use, field-active body temperatures and environmental temperatures

We captured brown anoles during June and July of 2017 and 2018. We captured slender anoles between June and November of 2017, 2018 and 2019. All lizards were caught by hand or lasso during their diurnal activity period, and their perch height when initially spotted by the researcher was measured in centimeters using a retractable tape measure. Internal (field-active) body temperatures were measured using a cloacal thermometer (Omega HH147U with type K thermocouple) within 20 s of capture. If a lizard was pursued for more than one minute prior to capture or handled for more than 20 s prior to thermometer insertion, body temperature was not recorded. We measured brown anole body temperatures in 2018 and slender anole body temperatures across all sampling years. We did not sample any individual lizard twice (all data are independent) because all lizards were subsequently transferred to experimental islands as part of separate studies.

We deployed iButton data-loggers (calibrated at factory: Embedded Data Systems, Lawrenceburg, KY, USA) that were set to record environmental temperatures (resolution: ±0.5°C) every 60–100 min, during the same general period of time that we captured wild individuals. For brown anole data-loggers, we suspended iButtons inside a short length of thin-walled copper piping that was painted to resemble the typical coloration of this species (Bakken, 1992; Dzialowski, 2005). Copper-based temperature data-loggers have been shown to generate accurate distributions of available temperatures for lizards that live in edge or open habitat (where solar radiation is the dominant mode of heat exchange), and we have used this method in the past to generate environmental temperature distributions for male brown anoles at our field site on Exuma (Logan et al., 2014, 2018, 2016). From April to June 2018, we deployed 20 data-loggers in forest-edge habitat by stopping at haphazardly chosen points along a narrow dirt road (near the town of George Town, Exuma) and then used a random distance into the forest (0–5 m in 1 m increments) and random height in the vegetation (0–2 m in 0.5 m increments). Data-loggers were fastened to branches with zip ties on either the top, bottom or side of the branch (position chosen randomly). Due to the size constraints associated with iButtons, it is possible that these data-loggers were too large to accurately estimate available temperatures for female brown anoles, which are much smaller than males. Thus, our temperature data-

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loggers for brown anoles probably under-estimated differences in environmental temperature between potential perch locations, at least from the perspective of females (see Discussion). From May to July 2018, we also deployed data-loggers across eight outlying islands (mean of 27 data-loggers per island) that were immediately adjacent to Exuma but still within the natural range of the brown anole. For these data-loggers, we chose haphazard points around each island (to cover as much of the island as possible), random cardinal directions and distances (0–5 m in 1 m intervals) from each of these points, and then random heights in the vegetation (0–2 m in 0.5 m intervals). In total, we sampled the temperatures of 239 potential perches of brown anoles.

To measure environmental temperature distributions for slender anoles, we coated iButtons in Plasti Dip (Plasti Dip International, Blaine, MN, USA) for waterproofing, and then glued them to a short piece of pine wood. Copper models are unnecessary under dense forest canopy because heat transfer is dominated by convection rather than radiation in these environments (Bakken, 1992; Dzialowski, 2005). We deployed these data-loggers at our mainland site along two transects that penetrated the forest from the east and west of Pipeline Road in Soberanía National Park, Panamá, where they recorded environmental temperatures between July and November of 2017. We stopped at haphazardly chosen locations along each transect, placed each logger at a random side (left or right) and distance (0–5 m in 1 m increments) from the transect, a random height in the vegetation (0.5–2 m in 0.5 m increments), and a random position on the branch (top, bottom or side). We deployed 17 data-loggers along each transect for a total of 34 independently sampled locations on the mainland. We also deployed temperature data-loggers across 11 islands (mean of 23 per island) in Lake Gatún, which is adjacent to Soberanía National Park and falls within the natural range of slender anoles. To distribute these data-loggers, we used random cardinal directions and distances (0–5 m in 1 m intervals) from haphazardly chosen points covering as much of each island as possible, and then random heights (0.5–2 m in 0.5 m intervals) in the vegetation. In total, we sampled the temperatures of 282 potential perches of slender anoles. In Panamá, we did not deploy data-loggers at ground level because slender anoles are rarely on the ground and models would be lost in the leaf litter.

Morphology and thermal tolerance

Following capture, brown anoles were brought back to our makeshift laboratory in a rental house on the island of Exuma, The Bahamas, whereas slender anoles were taken to the Smithsonian laboratory facility in Gamboa, Panamá. We studied a broad suite of morphological and physiological traits (Table 1). After a minimum of 16 h acclimation to laboratory conditions (including one overnight period), we measured the full set of physiological traits in each lizard, although we randomized the order in which we measured these traits on a given batch of lizards to eliminate potential order effects. All physiological assays were conducted during the diurnal activity period of both species (between 07.00 and 18.00 h), and lizards were given a minimum of 90 min rest between physiological experiments. Mass was measured using a digital balance and SVL was measured using digital calipers. We measured the critical thermal minimum (CT_{min}), an index of cold tolerance (Angilletta, 2009; Campbell-Staton et al., 2017; Leal and Gunderson, 2012; Logan et al., 2020), in both species by placing lizards in small batches in an incubator set to 2°C and cooling them to a body temperature below the average CT_{min} of the population (determined via pilot trials). We then removed them from the incubator and observed them as they warmed to room temperature (*ca* 23°C), checking for a righting response every 5–10 s by gently flipping them onto their dorsal surface. CT_{min} was recorded as the body temperature (measured with an Omega HH147U type K cloacal thermometer) at which individuals regained their righting response. Our CT_{min} methodology differed from the typical approach that others have used. Most researchers slowly cool lizards until they lose their righting response rather than cooling them below CT_{min} and allowing them to warm towards room temperature. Nevertheless, our method generated realistic values that are similar to those produced by the traditional method (we directly compared the methods in Cox et al., 2020b) and permits the high-throughput processing of larger numbers of lizards because they can more easily be measured in batches (individuals are immobile at the start of the trial as opposed to the end). Despite cooling lizards below their CT_{min}, we observed no mortality from this assay and lizards recovered rapidly at the end of the trial.

Table 1. Trait comparisons between females and males of two lizard species, the brown anole (*Anolis sagrei*) and the slender anole (*Anolis apletophallus*)

	Brown anole		Slender anole	
Trait	Female	Male	Female	Male
Mass (g)	**1.44**±0.01 (386)	**3.54**±0.05 (410)	**1.70**±0.01 (471)	**1.57**±0.01 (553)
SVL (mm)	**37.98**±0.12 (381)	**50.20**±0.19 (409)	42.35±0.08 (473)	42.55±0.07 (555)
Perch height (cm)	**47.36**±3.97 (28)	**82.16**±5.23 (90)	**56.56**±1.73 (531)	**87.32**±1.84 (643)
Field-active T_b (°C)	32.20±0.30 (24)	32.89±0.20 (81)	27.74±0.05 (564)	27.83±0.05 (667)
Mean temperature chosen in arena (°C)	31.44±0.70 (23)	30.70±0.81 (41)	26.92±0.29 (49)	26.76±0.35 (43)
Minimum temperature chosen in arena (°C)	29.05±0.73 (23)	28.15±0.79 (41)	25.14±0.29 (49)	25.11±0.41 (43)
Maximum temperature chosen in arena (°C)	33.38±0.74 (23)	33.21±0.80 (41)	29.28±0.34 (49)	28.89±0.34 (43)
CT_{min} (°C)	**15.45**±0.13 (356)	**14.52**±0.14 (342)	13.76±0.09 (465)	13.98±0.10 (525)
VT_{max} (°C)	35.83±0.11 (359)	35.70±0.11 (341)	**29.54**±0.08 (483)	**29.82**±0.08 (551)
Panting threshold (°C)	**37.85**±0.11 (360)	**39.58**±0.11 (342)	–	–
RMR at 20°C (ml O_2 g^{-1} h^{-1})	–	–	0.27±0.03 (40)	0.25±0.02 (39)
RMR at 25°C (ml O_2 g^{-1} h^{-1})	0.75±0.04 (208)	0.40±0.02 (219)	–	–
RMR at 30°C (ml O_2 g^{-1} h^{-1})	–	–	**0.98**±0.09 (40)	**0.78**±0.06 (39)
RMR at 35°C (ml O_2 g^{-1} h^{-1})	1.14±0.06 (244)	0.59±0.02 (275)	–	–
Q_{10} of RMR	2.18±0.29 (152)	1.95±0.13 (183)	**5.23**±0.82 (40)	**3.63**±0.36 (39)

Values are raw means±s.e.m., with sample sizes in parentheses. The means for traits that significantly differed between the sexes are in bold. SVL, snout–vent length; T_b, body temperature. The critical thermal minimum, voluntary thermal maximum, and resting metabolic rate are denoted CT_{min}, VT_{max} and RMR, respectively. Significance was assessed with general linear models that included appropriate covariates such as 'year' for traits that were measured over multiple years, and 'mass' for traits that might be affected by body size or temperature ramping rate. Mass-specific values of RMR and Q_{10} are presented, although significance was assessed using general linear models with mass as a covariate.

In brown anoles, we quantified two indices of heat tolerance, the voluntary thermal maximum (VT_{max}) and the panting threshold. In slender anoles, we only measured VT_{max} as very few individuals displayed a panting response during pilot trials. VT_{max} is the upper body temperature at which shade-seeking behavior is elicited and may manifest in nature as the seeking of cooler microhabitats when body temperature increases to a critical set-point (Camacho and Rusch, 2017; Virens and Cree, 2019; Weese, 1917). To measure VT_{max}, we moved lizards (contained in ventilated plastic containers) from room temperature (~23°C) to an incubator set to 50°C. We continuously monitored individuals until we observed escape behavior, which occurs abruptly after several minutes in the incubator and is easily distinguishable from exploratory movement. Once an individual engaged in escape behavior, we removed it from the incubator and recorded its body temperature (with a cloacal thermometer) as the VT_{max} for that individual. We followed the same procedure to measure panting thresholds in brown anoles, but body temperatures of lizards were measured when they displayed panting behavior (gaping for evaporative cooling; Loughran and Wolf, 2020) rather than when they exhibited escape behavior. For each batch of lizards, we randomized the order in which we measured thermal tolerance traits.

Behavior in thermal arenas

We tested for sexual dimorphism in thermoregulatory behavior by introducing males and females of both species to laboratory thermal arenas. Behavioral experiments were conducted after a minimum of 16 h such that lizards could acclimate to laboratory conditions and the chances that individuals were in peak digestive state was reduced. These arenas consisted of rectangular plastic bins (0.85×0.4×0.4 m; length×width×depth) with 250 W infrared heat lamps suspended over one end. The height of the heat lamps was adjusted to generate a thermal gradient between 20 and 45°C for brown anoles and between 22 and 38°C for slender anoles (verified with an infrared temperature gun). Note that we used thermal arenas with different temperature ranges because these species differ in the natural range of temperatures they encounter in the wild. Importantly, the gradients experienced by each species were equally broader than their respective thermal tolerance ranges, so the differences between the thermal arenas were unlikely to produced biased results. Indeed, the body temperatures achieved by each species in these gradients were close to what we predicted based on field-active body temperatures (see below). We replicated the natural relative humidity levels (~60–80%) in both slender anole and brown anole habitats by heating a pot of water over a hot plate to increase the humidity of the room. We only measured thermoregulatory behavior during the daytime activity period of each species (between 07:00 and 18:00 h). Prior to releasing lizards into arenas, we inserted a Type T cloacal thermocouple about 5 mm into each individual's cloaca and secured it with medical tape. Lizards were introduced individually to arenas and were given an acclimation period of 1 h before we began recording body temperatures. We then recorded body temperatures every 30 s for the ensuing hour. By design, laboratory thermal arenas are free of physical or ecological barriers to movement and therefore the body temperatures that individuals achieve are assumed to be optimal for physiological performance (Camacho and Rusch, 2017; Gilbert and Miles, 2017; Neel and McBrayer, 2018; Sannolo and Carretero, 2019; van Berkel and Clusella-Trullas, 2018). We included the mean (often referred to as 'preferred temperature'), minimum and maximum body temperatures achieved in the thermal arenas as traits to be included in subsequent analyses of sexual dimorphism in thermoregulatory behavior.

Thermal sensitivity of resting metabolic rate

We quantified resting metabolic rates (RMRs) in males and females of both species using fiber-optic closed system respirometry (PreSens Precision Sensing, Regensburg, Germany). We measured oxygen consumption ($\dot{V}_{O_2}$) at 25 and 35°C for brown anoles and at 20 and 30°C for slender anoles. As with our thermal arena experiments, we measured metabolic rates at different sets of temperatures between the species because of the different ranges of environmental temperatures experienced by these species in the wild (slender anoles would have perished if measured at 35°C, for example). Thus, we measured metabolic rates over the same magnitude of temperature change (10°C) for each species, but we chose each temperature range based on ecologically relevant thermal conditions. Individuals were placed in glass jars (355 ml volume) containing oxygen sensors which were themselves placed inside an incubator set to the experimental temperature. After 30 min for the system to come to thermal equilibrium, we used PreSens Measurement Studio 2 software (PreSens Precision Sensing) to record the O_2 concentration in each jar every 2 s for an additional 30 min. We used the slope of the relationship between O_2 concentration and time to calculate $\dot{V}_{O_2}$, which is the rate of oxygen consumption. We report this rate in mass-specific units when comparing means between the sexes in Table 1. We only included an estimate of $\dot{V}_{O_2}$ from an individual lizard at a given experimental temperature if we could extract a minimum of 5 min of reliable (linearly declining O_2 concentration) measurements from the 30 min trial. If we were able to extract reliable estimates of $\dot{V}_{O_2}$ at both experimental temperatures for an individual, we estimated the thermal sensitivity of RMR for that individual by calculating the temperature coefficient, or Q_{10}, using the standard formula (Logan et al., 2019; Watson and Burggren, 2016).

Statistical analyses

In both brown and slender anoles, we tested for a relationship between environmental temperature and potential perch height by averaging all temperatures recorded by each data-logger within the daily activity cycle of both species (07.00–18.00 h). We then used general linear models (GLMs) with Tukey's post hoc comparisons to compare data-loggers that were deployed at different heights. 'Locality' was included as a covariate in these models to account for site-level variation. We plotted the average temperatures recorded by all data-loggers at a given height as a function of time of day to examine temporal variation in environmental temperature at each height.

To test for sexual dimorphism in morphology, thermal physiology and behavior in each species, we ran separate GLMs with sex as the independent variable and the trait or habitat use variable as the dependent variable. 'Year' was included as a covariate in models testing for differences in variables measured across multiple years (e.g. field-active body temperature in slender anoles or RMR in both species). We first tested for differences in CT_{min}, VT_{max} or panting threshold between the sexes using models with mass as a covariate, as thermal tolerance measurements can be affected by ramping rate which is dependent on body size (larger lizards will heat or cool more slowly than smaller lizards). In brown anoles, because male and female body size distributions are largely non-overlapping, we conducted an additional set of trait comparisons after standardizing mass (converting to a mean of zero and unit variance) within each sex. However, because our main effects remained significant in mass-standardized analyses, we only report results from our analyses that included raw values of mass. RMRs at each experimental temperature and Q_{10} values were compared between sexes (separately for each species) using GLMs

with mass as a covariate. Finally, we tested for a relationship between lizard perch height and field-active body temperature in both study species using GLMs with 'body temperature' as the dependent variable and 'perch height' and 'sex' as independent variables. Interaction terms were insignificant for both species and were therefore removed from the final models. In all models, we used a significance threshold of α=0.05. Probability plots and residual distributions were inspected prior to running GLMs to ensure that the data conformed to model assumptions. All traits included as dependent variables in statistical models are listed in Table 1. Statistical analyses were conducted in SYSTAT (Systat Software, Inc.).

RESULTS

Environmental temperatures

In brown anole habitat, the mean ($F_{4,226}$=9.001, P<0.001) and maximum ($F_{4,226}$=10.094, P<0.001) environmental temperatures recorded by data-loggers declined with potential perch height in the vegetation (Fig. 1B,C). Post hoc comparisons revealed that this pattern was driven largely by significant differences between ground-level data-loggers and those at other heights. The minimum environmental temperature also declined with potential perch height in brown anole habitat (Fig. 1A), but this result was only marginally significant ($F_{4,226}$=2.330, P=0.057). The daily amplitude of mean environmental temperature increased with potential perch height in brown anole habitat (Fig. 1D). In slender anole habitat, there was no significant relationship between potential perch height and minimum ($F_{3,267}$=0.434, P=0.729), mean ($F_{3,267}$=1.225, P=0.301) or maximum ($F_{3,267}$=1.887, P=0.132) data-logger temperature (Fig. 1E–G), and the daily amplitude of environmental temperature did not vary with data-logger height (Fig. 1H).

Morphology, habitat use and field-active body temperature

Male brown anoles had greater mass ($F_{1,788}$=1682.578, P<0.001) and SVL ($F_{1,786}$=2784.869, P<0.001) compared with female brown anoles (Table 1). Female slender anoles had statistically greater mass ($F_{1,1020}$=106.108, P<0.001) compared with males, but the mean difference between sexes was much greater in brown anoles than in slender anoles (Table 1). Male and female slender anoles did not significantly differ in SVL ($F_{1,1024}$=3.530, P=0.061).

Males of both species perched about 60% higher in the vegetation than females (brown: $F_{1,114}$=13.500, P<0.001; slender: $F_{1,1170}$=142.690, P<0.001; Fig. 2). Field-active body temperatures did not differ between males and females of either species (brown: $F_{1,100}$=2.176, P=0.143; slender: $F_{1,1150}$=0.470, P=0.493; Table 1) and did not depend on perch height in either species (brown: $F_{1,100}$<0.001, P=0.983; slender: $F_{1,1150}$=0.233, P=0.630).

Behavior in thermal arenas, thermal tolerance and resting metabolic rates

The mean ($F_{1,62}$=0.383, P=0.538), minimum ($F_{1,62}$=0.581, P=0.449) and maximum ($F_{1,62}$=0.020, P=0.888) body temperatures achieved in laboratory thermal arenas did not differ between male and female brown anoles. The mean ($F_{1,89}$=0.126, P=0.724), minimum ($F_{1,89}$=0.008, P=0.930) and maximum ($F_{1,89}$=0.672, P=0.415) body temperatures achieved in thermal arenas also did not differ between male and female slender anoles.

In brown anoles, males had lower CT_{min} ($F_{1,693}$=11.893, P=0.001) and higher panting thresholds ($F_{1,695}$=62.359, P<0.001), whereas VT_{max} ($F_{1,695}$=0.236, P=0.627) did not differ between the sexes. In slender anoles, CT_{min} did not differ between males and females ($F_{1,948}$=1.376, P=0.241), whereas VT_{max} was higher in males (although the difference in means was small and probably ecologically irrelevant: $F_{1,984}$=5.959, P=0.015; Table 1).

While RMR was greater in male brown anoles compared with females at both 25 and 35°C (Table 1; Fig. 3A), this effect was entirely explained by differences in mass between the sexes. The Q_{10} of RMR did not differ between the sexes in brown anoles ($F_{1,332}$=0.007, P=0.935; Fig. 3A) irrespective of whether mass was included as a covariate. Although RMR did not differ between male and female slender anoles at 20°C ($F_{1,74}$=0.443, P=0.508), females had higher RMRs at 30°C ($F_{1,74}$=4.145, P=0.045; Fig. 3B). The Q_{10} of RMR was also greater in females ($F_{1,74}$=4.540, P=0.036; Fig. 3B).

DISCUSSION

Our study revealed that several thermal tolerance traits differed between the sexes in the sexually size-dimorphic brown anole, whereas this pattern was largely absent from the sexually size-monomorphic slender anole. We found that males of both species perched higher than females, although environmental temperature varied with potential perch height only in the patchy habitat occupied by brown anoles. Neither species displayed sexual dimorphism in field-active body temperature or thermoregulatory behavior in a laboratory thermal arena. This suggests that differences between sexes in thermal physiology did not result from selection acting directly on performance in different thermal environments, but rather from interactions between thermal physiology and other traits that differ between males and females. Our results indicate that species displaying sexual size dimorphism may also be dimorphic in thermal physiology, even when the sexes do not differ in field-active body temperature or microhabitat use. Furthermore, our results imply that sex-specific selection on non-thermal traits may indirectly drive divergence in thermal physiology between the sexes, with implications for the responses of species to climate change.

Brown and slender anoles live in different structural habitats (Andrews and Sexton, 1981; Ballinger et al., 1970; Logan et al., 2014, 2018; Stapley et al., 2015). Brown anoles thrive along the edges of scrubby coppice forest in The Bahamas, whereas slender anoles live under the canopy of dense lowland forest in Panamá. We found that environmental temperature varied with potential perch height in brown anole habitat, but not in the homogenous forest understory habitat of slender anoles (Fig. 2). Indeed, maximum environmental temperatures in brown anole habitat differed between ground level and 2 m height by 6°C, and the daily amplitude of environmental temperature was fivefold less at 2 m height relative to ground level (Fig. 1). These estimates of environmental temperature variation are likely to be conservative, as our temperature data-loggers were larger than female brown anoles and probably underestimated differences in temperature between potential perch locations from the perspective of females (because larger data-loggers have higher thermal inertia). It is possible that higher perches are cooler, on average, because they are exposed to greater levels of convection (wind).

Even though males of both species perched substantially higher in the vegetation than females, we predicted that only brown anoles would be sexually dimorphic in thermal physiology due to the thermal heterogeneity of their habitat. Consistent with this hypothesis, brown anoles exhibited sexual dimorphism in two of the three thermal tolerance traits we measured (Table 1). Both cold tolerance and heat tolerance were greater in males, and this pattern was not simply an indirect effect of their greater body size relative to

Journal of Experimental Biology (2021) 224, jeb235697. doi:10.1242/jeb.235697

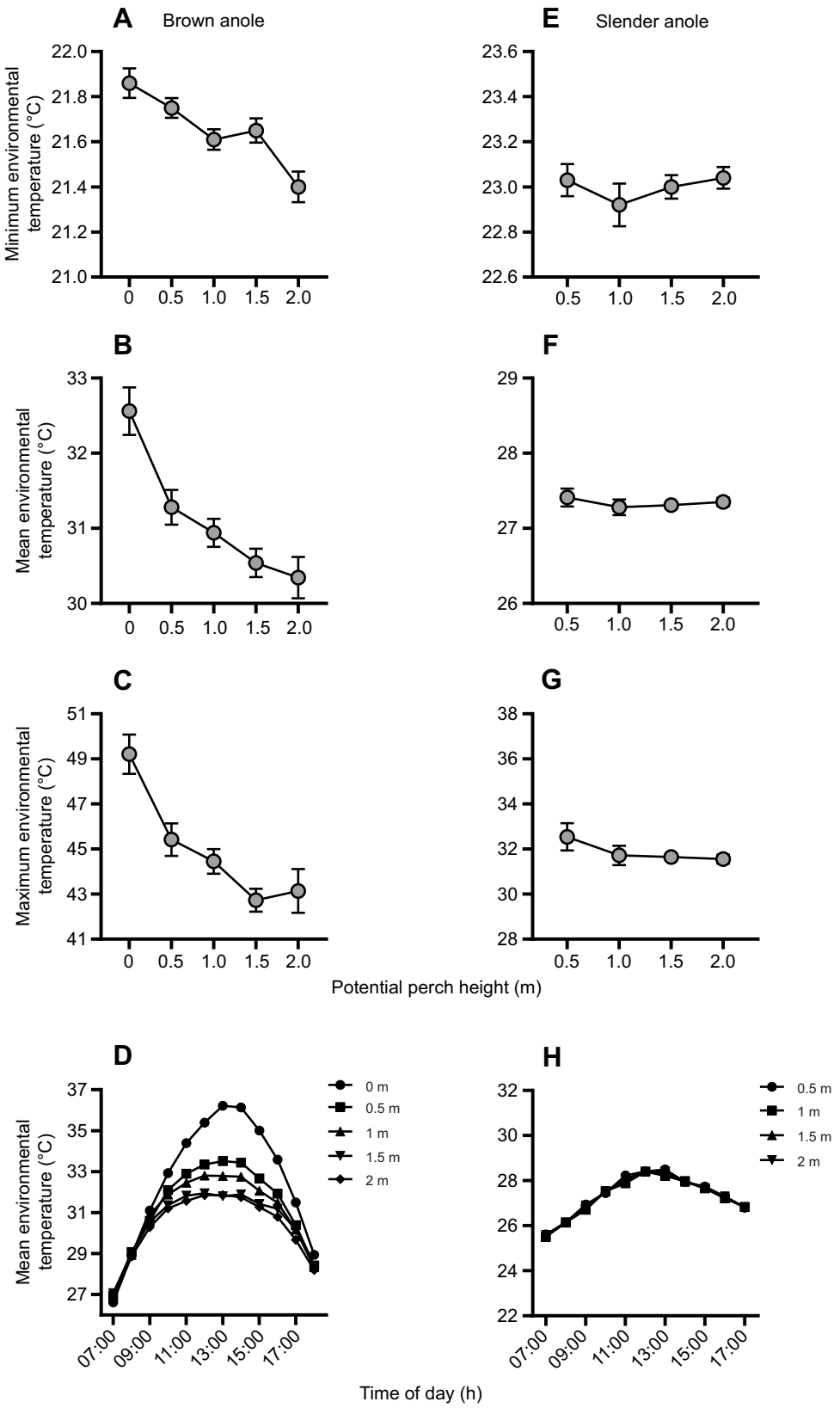

Fig. 1. The relationship between potential perch height and environmental temperature in brown anole and slender anole habitat. Minimum (A), mean (B) and maximum (C) environmental temperature declined with increasing potential perch height, and the daily amplitude of environmental temperature was much greater at lower heights (D) in brown anole habitat. By contrast, the minimum (E), mean (F), maximum (G) and daily amplitude (H) of environmental temperature did not vary substantially with potential perch height in slender anole habitat (lines almost completely overlap in panel H). Data from brown anole habitat are from temperature loggers deployed randomly at 239 potential perches. Data from slender anole habitat are from temperature loggers deployed randomly at 282 potential perches.

females (the main effect of 'sex' remained significant in all models after accounting for body size). By contrast, slender anoles exhibited little sexual dimorphism in the physiological variables measured (note that while male slender anoles did have slightly higher VT_{max} values than females, this difference was small and probably not ecologically irrelevant). Taken together, our results indicate that brown anoles are dimorphic in thermal physiology while slender anoles are monomorphic, and this pattern is not solely explained by

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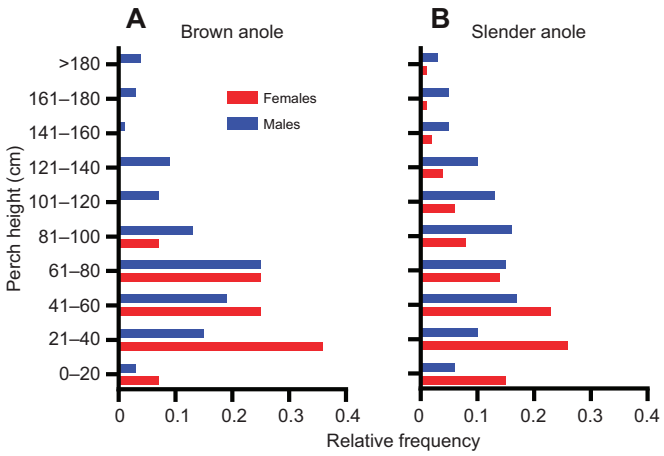

Fig. 2. Frequency distributions of perch height for male and female brown and slender anoles. Males perched higher in the vegetation than females (P<0.05) in both species.

differences in sexual size dimorphism (e.g. sex-biased differences in mass) between the species.

Observed patterns were somewhat different for the case of resting metabolic rates. The differences in RMRs of male and female brown anoles at both experimental temperatures were entirely explained by body size differences between the sexes. Similarly, male and female brown anoles did not differ in the thermal sensitivity (Q_{10}) of RMR (Fig. 3). In contrast, female slender anoles had higher RMRs than males at warmer temperatures (30°C), leading to a higher thermal sensitivity of RMR in females of that species. This was a surprising result given the similar body sizes and thermal environments experienced by male and female slender anoles. We studied both species during their respective breeding seasons, so one explanation for this pattern is that egg-bound female slender anoles have higher metabolic rates at warm temperatures. However, many of the female brown anoles we captured should have been gravid as well, yet we did not observe the same pattern in

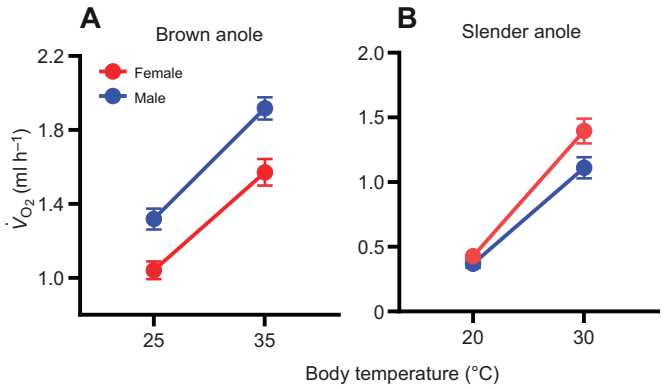

Fig. 3. Resting metabolic rates ($\dot{V}_{O_2}$) of male and female brown and slender anoles. Brown anoles, N=641; slender anoles, N=79. (A) Male brown anoles had higher resting metabolic rates (RMR) than females at both experimental temperatures, although these differences were entirely explained by body size differences between the sexes. The thermal sensitivity (Q_{10}) of RMR did not differ between the sexes in brown anoles (P>0.05). (B) Female slender anoles had greater RMRs at warm (30°C) but not cold (20°C) temperatures, leading to greater thermal sensitivity of RMR in female slender anoles (P<0.05 for Q_{10}). Error bars represent standard error of the mean. Note that raw RMR values are presented here, whereas mass-specific values are presented in Table 1.

brown anoles. The higher thermal sensitivity of RMR in female slender anoles requires further study.

As we hypothesized, brown anoles displayed marked sexual size dimorphism, divergence in habitat use between the sexes, and a correlation between perch height and environmental temperature. Furthermore, brown anole sexual size-dimorphism was accompanied by sexual dimorphism in thermal tolerance and metabolic rates. Because male brown anoles use higher and cooler perches, it follows that they might have greater cold tolerance if males are not perfect thermoregulators and selection acts primarily to increase cold tolerance during the diurnal activity period. Although we observed greater cold tolerance in males, we also found that they had greater heat tolerance, which runs counter to the relationship between perch height and environmental temperature. Indeed, mean and maximum environmental temperatures in brown anole habitat were substantially lower at the higher locations where males perch (Fig. 1). It seems unlikely that the relationship between environmental temperature and perch height is the direct cause of sexual dimorphism in thermal physiology in brown anoles because field-active body temperatures did not differ between the sexes and perch height did not correlate with body temperature in either sex (we observed the same pattern in slender anoles). Moreover, we did not detect sexual dimorphism in the thermoregulatory behavior of either species in laboratory thermal arenas, suggesting that the sexes prefer similar body temperatures for optimizing physiological performance.

The likely explanation for the observed lack of difference in field-active body temperatures between the sexes in brown anoles is that males and females behaviorally thermoregulate within their respective strata in the vegetation (i.e. body temperature is invariant with respect to mean environmental temperature at any given perch height; Cox et al., 2018; Fey et al., 2019; Huey et al., 2003; Muñoz and Bodensteiner, 2019). Numerous studies have shown that terrestrial ectotherms are remarkably efficient behavioral thermoregulators when they occur in spatially heterogeneous habitats (Fey et al., 2019; Gunderson and Leal, 2012; Hertz et al., 1993; Huey, 1974; Huey et al., 2003; Kearney et al., 2009; Logan, 2019; Logan et al., 2013, 2019; Muñoz and Losos, 2018; Muñoz et al., 2014; Neel and McBrayer, 2018). Huey and Pianka (2007) surveyed 56 lizard species and found that males and females rarely differed in their field-active body temperatures, and when they did, differences were typically small (<1°C). Thus, many habitats occupied by terrestrial ectotherms may permit both males and females to shuttle between sunny and shady patches to maintain a narrow range of body temperatures even when average thermal conditions differ between microhabitats. Of course, not all habitats are thermally heterogenous and therefore not all species are effective thermoregulators. In our study, slender anoles were thermoconformers when active (Ballinger et al., 1970), but environmental temperatures were similar at all heights in the vegetation so the sexes did not differ in body temperature despite differing in their perching behavior.

Although Huey and Pianka (2007) reported a lack of sexual dimorphism in field-active body temperatures in lizards, other authors have reported sexual dimorphism in thermal tolerance and thermal ecology in a range of terrestrial ectotherms (Beal et al., 2014; Brown and Weatherhead, 2000; Lailvaux, 2007; Lailvaux et al., 2003; Lailvaux and Irschick, 2007). For example, several studies have demonstrated sexual dimorphism in response to laboratory thermal tolerance or thermoregulation assays (Beal et al., 2014; Lailvaux and Irschick, 2007; Mathies and Andrews, 1997), while others have shown that field-active body temperatures do differ between males and females of some species (especially when females are gravid;

Beal et al., 2014; Brown and Weatherhead, 2000; Lailvaux et al., 2003; Woolrich-Piña et al., 2015). These differences often persist even after accounting for sexual size dimorphism (Beal et al., 2014). Thus, sexual dimorphism in at least some aspects of thermal physiology appears to be relatively common in ectotherms, yet few studies have successfully revealed the mechanisms that drive these differences. Whether males and females live in different thermal environments that select for divergent thermal physiologies, or sexual dimorphism in thermal physiology is a by-product of other differences between the sexes that are unrelated to body temperature, remains an open question. The reproductive status of females is a prime candidate for a driver of sex differences in thermal physiology, as gravidity has been shown to affect thermoregulatory behavior in several species of reptiles (Charland, 1995; Charland and Gregory, 1990; Isaac and Gregory, 2004; Mathies and Andrews, 1997; Woolrich-Piña et al., 2015).

Male and female brown anoles differed in thermal tolerance despite a lack of divergence in field-active body temperatures or behavior in thermal arenas. This suggests that sexual dimorphism in thermal physiology can arise as a result of sex-specific selection on (or plasticity in) other traits that are linked to thermal tolerance but do not themselves correspond to body temperature variation. Potential candidates for these lurking variables that may be linked to thermal physiology include circulating hormone (e.g. testosterone) concentrations or immune system components, both of which are known to differ between the sexes of many vertebrate species (Cox et al., 2015, 2017b; Pap et al., 2010; but see Kelly et al., 2018). Although our approach to exploring the causes of sex-based differences in thermal physiology was integrative and data-rich, our inference was somewhat limited because we only compared two species. Further research is needed to understand the causes of physiological dimorphism in ectotherms. We recommend that future studies include measurements of selection on thermal physiology in males and females while accounting for selection on potentially correlated traits such as body size, hormone concentrations, and components of immune function. It might also be fruitful to pair ethological observations with physiological experiments to understand why males and females of some species prefer similar body temperatures despite differing in thermal tolerance.

Our observation that male brown anoles occupy cooler microclimates and have greater heat tolerance than females has implications for understanding how climate warming might impact sexually dimorphic species. Our work suggests that climate warming may disproportionately affect sexually dimorphic ectotherms, possibly reducing reproductive output in females or generating asymmetrical sex ratios. These effects could cause population crashes or extinction if ectotherms are not able to compensate for environmental change with behavioral buffering, acclimatization or genetic adaptation.

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The authors declare no competing or financial interests.

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