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Introduction history and hybridization determine the hydric balance of an invasive lizard facing a recent climate niche shift

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S.B., J.B.L., D.J.I., J.J.K., and D.G.B. designed research; S.B. and D.G.B. performed research, analyzed data, and wrote the original draft of the manuscript. All authors contributed to revisions of the manuscript.

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The authors have no conflict of interest to declare.

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Abstract: — As anthropogenic activities are increasing the frequency and severity of droughts, understanding whether and how fast populations can adapt to sudden changes to their hydric environment is critically important. Here, we capitalize on the introduction of the Cuban brown anole lizard (*Anolis sagrei*) in North America to assess contemporary evolution of a widespread terrestrial vertebrate to an abrupt climatic niche shift. We characterized hydric balance in 30 populations along a large climatic gradient. We found that while evaporative and cutaneous water loss varied widely, there was no climatic cline, as would be expected under adaptation. Further, the skin of lizards from more arid environments was covered with smaller scales, a condition thought to limit water conservation and thus be maladaptive. In contrast to environmental conditions, genome-averaged ancestry was a significant predictor of water loss. This was reinforced by our genome-wide association analyses, which indicated a significant ancestry-specific effect for water loss at one locus. Thus, our study indicates that water balance of invasive brown anoles is dictated by environment-independent introduction and hybridization history and highlights genetic interactions or genetic correlations as factors that might forestall adaptation. Alternative water conservation strategies, including behavioral mitigation, may influence the brown anole invasion success and require future examination.

Keywords: — invasive species, hybridization, natural selection, rapid evolution, evaporative water loss, scalation, *Anolis sagrei.*

1. Introduction

Desiccation poses a threat for all terrestrial organisms, but especially for those inhabiting xeric environments with limited or variable access to water resources (Brown 1968; Alpert 2005). The high demands of conserving water in arid habitats often leads to morphological and physiological adaptations that reduce an organism's water loss by evaporation (Lundholm 1976; Alpert 2005). Macroevolutionary studies spanning a wide range of animal groups have, indeed, established a strong relationship between species' total evaporative water loss (TEWL) and the hydric conditions of their local habitat: species from arid environments typically lose water at a lower rate than their mesic counterparts (squamates: Cox and Cox 2015, Le Gailliard et al. 2021; mammals: Van Sant et al. 2012; birds: Albright et al. 2017; amphibians: Lertzman-Lepofsky et al. 2020; insects: Addo-Bediako et al. 2001). Convergence in reduced water loss for lineages that have colonized arid habitats demonstrates that species can adjust their water loss levels to match the local hydric conditions on a macro-evolutionary timescale. However, whether and how different populations respond to changes to their hydric environment over micro-evolutionary timescales is less understood. Anthropogenic activities are changing the Earth's climate in unprecedented ways, including by increasing the frequency, duration, and intensity of droughts (Park et al. 2018; Chiang et al. 2021), which makes understanding the scope of rapid adaptive responses to hydric environments critical for predicting the future persistence of populations.

Species introduced by humans to areas beyond their native range can experience rapid and drastic environmental change, as a direct result of the translocation event or following the spread of these species outward from the site of introduction (Moran and Alexander 2014). Such environmental change can involve a novel suite of interacting species or different climates (Lodge 1993; Sakai et al. 2001). If the optimal phenotype to survive and reproduce under novel conditions differs from the phenotype favored under ancestral conditions, phenotypic change may occur via plasticity, genetic adaptation, or both (e.g., Bock et al. 2018; Corl et al. 2018; Stern and Lee 2020), allowing invasive populations to better cope with the novel environments they encounter (Mooney and Cleland 2001; Shine 2012; Bates and Bertelsmeier 2021). Due to the abrupt nature of these human-assisted introduction events, selection can be strong and phenotypic change rapid (Prentis et al. 2008; Whitney and Gabler 2008; Hodgins et al. 2018). The fruit fly *Drosophila subobscura*, for example, evolved an adaptive cline in wing size in only 20 years after initial introduction to the Americas from their native range in Afro-Eurasia (Huey et al. 2000). Therefore, biological invasions offer excellent

opportunities to study population responses to environmental change over contemporary timescales (Stockwell et al. 2003; Huey et al. 2005; Moran and Alexander 2014; Reznick et al. 2019).

The invasion of the brown anole lizard (*Anolis sagrei*) in North America offers an ideal opportunity to examine the micro-evolutionary response of a widespread terrestrial vertebrate to an abrupt climatic niche shift. Native to the Caribbean, *A. sagrei* arrived in the United States (US) from Cuba by means of repeated anthropogenic introductions at various locations across Florida (Kolbe et al. 2004). Since the first introductions in the mid- to late-1800's (Williams 1969), the species colonized the entire peninsula and expanded to the north and west (Kolbe et al. 2004; Bock et al. 2021). Its invasive range currently spans a broad latitudinal extent (~24°N to 33°N), which is much greater and well beyond that of its native range in Cuba (~20°N to 23°N; Angetter et al. 2011). Consequently, the thermic and hydric conditions experienced by invasive *A. sagrei* differ in both breadth and magnitude from those experienced by conspecifics in the native range (Angetter et al. 2011; Kolbe et al. 2014; Table S5).

Previous studies have shown that invasive populations in the south-eastern US were seeded by at least eight different introductions from genetically divergent native-range populations (Kolbe et al. 2004). Since their introduction, these lineages have been interbreeding in Florida (Kolbe et al. 2008), creating a mosaic of admixed ancestry across the peninsula that appears to have stabilized, at least over the past 15 years (Bock et al. 2021). Thus, aside from the abrupt climate niche shift, the brown anole invasion presents an opportunity to study how invasion history (i.e., the sequence and source of introduction events) and post-introduction hybridization jointly contribute to trait variation and contemporary local adaptation (e.g., Keller and Taylor 2008; Dlugosch et al. 2015; Querns et al. 2022).

In this study, we examined the hydric balance of *A. sagrei* from populations across a large part of its non-native range in the US. Because reptiles lose much of their water passively through the skin (e.g., Bentley and Schmidt-Nielsen 1966; Kobayashi et al. 1983; Dmi'el 1985, 2001), we measured skin resistance to water loss and skin morphology, in addition to total evaporative water loss. Because of this species' success in its non-native range in the US, we hypothesize that lizards have rapidly adapted to the local climatic conditions. Therefore, we expect a relationship between habitat aridity and evaporative water loss. Furthermore, with

the assumption that water mostly evaporates through the spaces between the skin scales of anoles (Krakauer 1970; Horton 1972), we predict that lizards from drier habitats have evolved larger scales, thereby reducing the area of exposed interscalar skin. Anoles are model systems of rapid adaptation (e.g., Losos et al. 1997; Kolbe et al. 2012), with recent studies documenting rapid adaptive responses to extreme climate events (e.g., Campbell-Staton et al. 2017, 2020; Donihue et al. 2018, 2020). Therefore, we predict that the hydric balance of invasive populations will be aligned to their respective local climate, independent of the identity of native-range lineages that contributed to their ancestry. Alternatively, a lack of a climatic cline in trait variation might indicate that water balance physiology of invasive brown anoles is dictated by environment-independent introduction and hybridization history. To test this alternative hypothesis, we integrate genome-averaged estimates of ancestry obtained from reduced-representation sequencing and methods for association mapping of traits that have been optimized for admixed populations.

2. Materials and methods

2. 1. Animal sampling and housing

In 2018, we captured 589 adult male brown anoles by noose from 30 populations across Florida and southern Georgia (US). Populations were distributed along three latitudinal transects (Fig. 1; Fig. S1; Table S1). Transect 1 (west) was sampled in March (n = 151; 9 populations), transect 2 (central) was sampled in May (n = 195; 10 populations), and transect 3 (east) was sampled in July (n = 200; 11 populations). To assess the consistency of our traits of interest over time (i.e., phenological effect), we re-visited five populations from transect 1 in July, and caught an additional 43 individuals (hereafter referred to as temporal replicates). After capture, lizards were transported to the animal facility at Harvard University (Cambridge, MA, USA), where they were housed individually in custom-built acrylic terraria of 36 cm high, 30 cm deep, and 14 cm wide. Each terrarium contained a layer of autoclaved organic potting soil as substrate, plastic foliage, and a wooden dowel (1 cm diameter) for perching. Room temperature was maintained at 28°C and terraria were misted at least twice a day with reverse osmosis water to sustain around 80% relative humidity in the lizard enclosures. Water-vapor resistant (F32T8) fluorescent bulbs provided proper lighting that followed a 14h daylight/10h darkness scheme. Lizards were fed crickets (dusted with multivitamin powder) three times per week. We refer to Meyer et al. (2019) for further details on the standardized housing conditions used in this study.

176 **2. 2. Water loss experiments**

177 We measured both "total evaporative water loss" (TEWL, i.e., the combined effect of
178 cutaneous water loss and water lost *via* the respiratory system), and skin resistance to water
179 loss or "cutaneous water loss" (CWL, i.e., water lost through the skin epidermis) to assess
180 patterns of water balance regulation in invasive *A. sagrei*. We used a subset of lizards (N =
181 566; Table S1) that survived transport from the field and an initial acclimation period of 20-30
182 days to comfortable hydric and thermic housing conditions (see §2.1) for the TEWL
183 experiments. We measured rates of TEWL following previous studies (e.g., Gunderson et al.
184 2011, Kolbe et al. 2014). Briefly, we quantified the change in body mass (as percentage mass
185 lost) for lizards placed in an incubator (Percival Scientific, Inc., Perry, IA, USA) set at a constant
186 temperature (30 °C) and relative humidity (30%). These conditions aimed to replicate an arid
187 environment with high evaporative potential (Greve et al. 2019). We chose not to expose the
188 lizards to more extreme conditions to reduce animal discomfort and the risk of fatalities.
189 Experiments started in the morning before misting and one day after feeding. We weighed
190 lizards twice, once before and once after a period of five hours in the incubator using an
191 electronic balance (precision = 0.001 g). To facilitate airflow and to reduce animal activity
192 during the experiments, lizards were placed individually in plastic mesh bags and suspended
193 inside the dark incubator. Five lizards defecated during the experiments; hence, water loss
194 data from these individuals were removed from further analyses. All experimental procedures
195 described were approved by the Harvard University Institutional Animal Care and Use
196 Committee (IACUC protocol # 26-11).
197
198 After completion of the TEWL rate experiments and following trait data collection for related
199 projects, we euthanized all animals and excised from each individual a small section of skin,
200 which we temporally stored in 70% ethanol until further processing. Liquid-preservation has
201 no significant effect on anole skin surface structure (Baeckens et al. 2019). This skin section
202 consisted of the outer epidermis (circa 1 cm^2) on the flank of the body (dorsolateral), posterior
203 to the midpoint between the pectoral and pelvic girdle (see also Baeckens et al. 2019).
204 Following scale morphology measurements (see §2.3), skin patches were used to estimate
205 skin (trans-epidermal) resistance to water loss following the protocols of Roberts and
206 Lillywhite (1983) and Kattan and Lillywhite (1989). To do so, excised skin samples were first
207 removed from the ethanol, lightly brushed with a fine paintbrush to remove any surface
208 debris, dehydrated in a graded ethanol series, and air-dried. Next, we filled capless plastic PCR
209 tubes with 200 µL distilled water and covered the open top (which has an opening of 0.5 cm^2

diameter) of each tube with a single patch of skin (with the outer side of the skin facing upwards). We used thermoplastic stretch film to tightly seal the patch edges to the outside of the tube. In this way, the tube could only lose water as vapor through the excised skin epidermis. Rates of cutaneous water loss were measured by calculating the change in mass (as percentage lost) of the skin-wrapped tubes held at a constant temperature (30°C) and relative humidity (30%) in the incubator. Each test tube was weighted to the nearest 0.0001 g before and after a period in the incubator of 90 hours ("CWL_{90}") and, again, after 120 hours ("CWL_{120}"). Tubes that lost all their water after 90 or 120 hours were not weighted again; instead, they were classified as "empty" for an additional (binomial) variable, henceforth referred to as "cutaneous desiccation" ("CD_{90}" or "CD_{120}" depending on the time it took for the water to fully evaporate).

2. 3. Skin morphology

We measured average scale size from each individual skin sample by digitizing (using ImageJ; Schneider et al. 2012) the surface area of nine different scales (following Baeckens et al. 2019, 2021) on images obtained with a stereomicroscope (Leica M165 C). We then calculated mean scale surface area per individual. Additionally, we obtained data on body length (as snout-to-vent length, SVL) for all lizards using digital calipers. Relative scale size was then calculated by regressing log-transformed scale area against log-transformed SVL and extracting the residual values for each individual.

2. 4. Climate space

We extracted climate data from the WorldClim database (Fick and Hijmans 2017), using the geographical location of each study population. WorldClim provides long-term (monthly) average climate conditions on a spatial resolution of 1 km^2 (Fick and Hijmans 2017). We retrieved data on 19 bioclimatic variables (BIO1-BIO19) representing different measures for annual trends, seasonality, and extremes of temperature and precipitation (Fick and Hijmans 2017). In addition, we gathered data on the average annual precipitation (P), the highest monthly mean temperature (T_{max}), and the lowest monthly mean temperature (T_{min}) to calculate a single measure for aridity, the $\log_{10}(Q)$ index, where $Q = P / ((T_{max} + T_{min}) \times (T_{max} - T_{min})) \times 1000$. A lower Q indicates arid environments, whereas a higher Q indicates mesic environments (e.g., Tieleman et al. 2003, Oufiero et al. 2011; Wegener et al. 2014; Baeckens et al. 2018; Hlubeň et al. 2021; Muñoz-Garcia et al. 2022). To reduce the number of climatic variables for subsequent analyses, we performed a principal component analysis (PCA) with

all 20 climatic variables as input (*prcomp* function; variables scaled to unit variance). Because many of the bioclimatic variables are strongly intercorrelated (Fick and Hijmans 2017), a PCA is particularly useful as it uses orthogonal transformation to convert a set of correlated variables into a set of orthogonal, uncorrelated axes (Bolker 2008). The number of non-trivial components to be retained was determined based on the Kaiser-Guttman criterion (Peres-Neto et al. 2004). The PCA yielded three component axes with eigenvalues larger than 1 that jointly explained a total of 87.2% of the variation (PC1: 55.4%; PC2: 22.0%; PC3: 9.8%; Fig. S2; Table S2).

The latitudinal climate space occupied by *A. sagrei* largely could be largely explained by PC1 (linear model: $R^2 = 0.96$, t = 26.59, $P < 0.001$; Fig. 1), and not by PC2 ($R^2 < 0.01$, t = -0.48, $P = 0.657$) or PC3 ($R^2 < 0.01$, t = 0.01, $P = 0.994$). Hence, PC1 was used in all further climate-related analyses as the main climate variable (hereafter coined "$PC1_{clim}$") describing the latitudinal climate extent of the brown anole invasion. Relative to northern populations, southern populations are characterized by high negative values for $PC1_{clim}$ (Fig. 1). Based on the loadings of $PC1_{clim}$ (Table S2), southern populations inhabit a tropical climate with high mean temperatures, little annual temperature change, and rainfall all year round with summer peaks. By contrast, northern populations experience lower annual temperatures, especially during winters, and little precipitation seasonality. The invasive range of *A. sagrei* in the south-eastern US thus shows a strong latitudinal climate gradient, with a relatively warmer and more humid climate towards the south, and a relatively cooler and more xeric climate towards the north. $PC2_{clim}$ can be interpreted as temperature seasonality and $PC3_{clim}$ as temperature seasonality mixed with precipitation (Table S2).

2. 5. Ancestry inference

To characterize ancestry, we relied on reduced-representation sequencing (i.e., ddRADseq) data, which were available for most (544/566; 96.1%) of our experimental animals (see Bock et al. 2021 for detailed molecular and bioinformatics methods). Briefly, single nucleotide polymorphisms (SNPs) were obtained based on alignments of sequence reads to version 2.1 of the *A. sagrei* reference genome (Geneva et al. 2021). From a final filtered set of 120,387 SNPs, we randomly selected 10,000 markers, which we used for ancestry inference in STRUCTURE v.2.3.4 (Pritchard et al. 2000). The best-supported number of genetic clusters in this dataset was two ($K = 2$; Bock et al. 2021). Therefore, we used 20 independent STRUCTURE runs, all of which considered a K of 2. Replicate runs consisted of 150,000 MCMC repetitions,

with a burn-in of the same length. We then identified the run with the highest Ln probability of the data from which we extracted ancestry proportions. This approach partitions the ancestry of each individual as either Western Cuba ancestry or admixed ancestry (Bock et al. 2021). We used this information to extract percentage Western Cuba ancestry for each individual. For simplicity, we refer to this metric as "ancestry" hereafter.

2. 6. Statistics

To assess whether variation in climate conditions can explain variation in morphology and physiology in *A. sagrei*, we regressed each response variable separately (i.e., TEWL, CWL_{90}, CWL_{120}, CD_{90}, CD_{120}, relative scale size, body mass, SVL) against each of the three PC_{clim} separately. In each linear mixed-effect model, we included "transect" (three-level factor) as a fixed effect (interacting with PC_{clim}) and also "ancestry" as fixed effect. Here, and in all other models, the factor "population" was included as a random effect to avoid pseudo-replication. We used the function *lme* (*nlme* package, Pinheiro et al. 2021) for all continuous dependent variables, and *glmer* (binomial distribution; *lme4* package, Bates et al. 2015) for the binomial variables CD_{90} and CD_{120} (scored as either "empty" or "not empty"). To examine the relationship between the rate of total water loss and the rate of cutaneous water loss, we regressed each CWL measure against TEWL (interacting with transect), with ancestry incorporated as random variable. Lastly, to examine the relationship between the two water loss measurements (CWL and TEWL) and skin morphology, we regressed each water loss measurement against relative scale size (interacting with transect), also with ancestry as random variable. In all models, the interaction effect with "transect" was eliminated when non-significant. Also, the factor "population" was included as a random effect to avoid pseudo-replication. Additionally, all the above models included only individuals obtained during our first visit of each population (i.e., we excluded the 43 individuals considered as temporal replicates).

To test for an effect of phenology on our traits of interest, we compared lizards from the five populations on transect 1 sampled at two timepoints, in March (n = 84) and July (n = 43). We regressed each physiological and morphological variable (except for CD_{90} and CD_{120}) separately against $PC1_{clim}$ interacting with "time of sampling" (two-level factor, with "March" corresponding to the March trip, and "July" corresponding to the July trip). The interaction was eliminated from the models when non-significant. The variables CD_{90} and CD_{120} were excluded from these models, as the percentages of fully evaporated tubes were too low for

the models to converge. Similar to models described above for the complete dataset, "population" was included as a random effect.

All analyses were performed in R 3.6.0 (R Core Team, 2019). Diagnostic plots were checked for appropriate residual distributions for all fitted models. Significance of fixed effects is reported based on F-tests calculated using Kenward-Roger degrees of freedom approximation or Wald χ^2-tests for LMMS and GLMMs respectively. We corrected for multiple testing by applying a false discovery rate correction (Benjamini and Hochberg 1995).

2. 7. Genome-wide association of hydric balance traits

Recent methodological developments in genome-wide association study (GWAS) allow the identification of alleles with effects that vary depending on genomic background (i.e., ancestry-specific GWAS; Skotte et al. 2019; Rio et al. 2020). In systems where admixture is frequent, as is the case for invasive *A. sagrei*, such genomic background effects are likely to arise due to epistatic interactions between focal QTLs and divergent loci elsewhere in the genome (Mackay 2014; Rio et al. 2020; Bock et al. 2021). Thus, while ancestry-specific GWAS studies based on ddRADseq do not provide a complete view of the genetic architecture of traits due to the sparser coverage of the genome, they can be informative with regards to the effect of ancestry on trait variation in hybrids when ancestry-specific associations are identified. Moreover, these GWAS approaches include steps for mitigating the effects of population structure and are optimized for recently admixed populations (e.g., Skotte et al. 2019).

To conduct an ancestry-specific GWAS, we followed the methods described in Bock et al. (2021). We included the TEWL and relative scale size traits, which we analyzed in conjunction with 120,232 quality-filtered SNPs from the 50 largest scaffolds in the *A. sagrei* reference genome (Geneva et al. 2021). We then relied on the GWAS model implemented in asaMap (Skotte et al. 2019). To correct for population structure, we included as covariates the first 10 principal components from a genetic PCA, following Skotte et al. (2019). The genetic PCA was calculated in the *adegenet* R package (v. 2.1.1; Jombart and Ahmed, 2011), and used 10,000 random genome-wide SNPs. Lastly, to obtain additional information on SNPs spanning any significant GWAS association peaks, we annotated all markers using snpEff v. 5.0 (Cingolani et al. 2012). SNPs predicted to lead to an amino acid change that also overlapped an association peak were further tested for linkage disequilibrium versus the lead GWAS SNP using PLINK v1.90b6.24 (Purcell 2007).

To further verify asaMap results, we partitioned the samples in one group with mostly Western Cuba ancestry (n = 151; hereafter referred to as the "hybridization limited" group), and a second group with mostly admixed ancestry (n = 393; hereafter referred to as the "hybridization common" group; see Bock et al. 2021 for details on delineating these groups). We used this grouping because it corresponds to the major population genetic differences among *A. sagrei* in Florida (Bock et al. 2021). We reasoned that spurious GWAS associations that are driven by unaccounted population structure (e.g., Platt et al. 2010; Shen et al. 2013) should disappear when considering these groups separately. Alternatively, if most population genetic subdivisions have been properly accounted for, we expected to find significant effects of QTL alleles on trait values, in one or both sample groups. For each of the "hybridization limited" and "hybridization common" sample groups, we then built linear models, with log-transformed trait values as the response variable, and QTL genotype (i.e., number of non-reference alleles) as the predictor variable. Lastly, we estimated effect sizes of QTL alleles for each group as linear model R^2 values.

3. Results

3. 1. Variation in hydric balance traits across a climatic gradient

We found considerable variation in rates of water loss and skin morphology in *A. sagrei* across its invasive range in south-eastern US. The total evaporative water loss of the lizards in our study ranged from 0.04% to 3.15% (of body mass lost) with among-population variance in TEWL being 3.4 times greater than the within-population variance ($P < 0.001$). While there was no clear trend towards a lower TEWL in lizards from the north that inhabit more arid environments ($PC1_{clim}$, $F = 3.22$, $P = 0.084$; Fig. 2A), we found that rates of water loss were significantly affected by anole ancestry: lizards with high percentages of Western Cuba ancestry showed high TEWL rates ($F = 4.70$, $P = 0.031$; Fig. 2C). We also found a significant effect of sampling transect on TEWL (Fig. 2A,C). Average TEWL of lizards from transect 1 (0.96%) was significantly, albeit marginally, higher than lizards from transect 2 (0.77%; $t = 3.20$, $P = 0.008$) and transect 3 (0.86%; $t = 2.71$, $P = 0.018$); TEWL did not significantly differ among lizards from transect 2 and 3 ($t = 0.47$, $P = 0.639$). Also, $PC2_{clim}$ and $PC3_{clim}$ did not significantly correlate with TEWL (all $P > 0.4$; Table S4).

Rates of cutaneous water loss ranged from 7.67% to 62.20% for CWL_{90}, and from 9.40% to 62.49% for CWL_{120}; among-population variance was respectively, 2.8 and 2.2 times greater

than within-population variance. Neither of the two measures were significantly related to $PC1_{clim}$ (CWL_{90}, $F = 0.18$, $P = 0.674$; CWL_{120}, $F = 0.10$, $P = 0.758$) or to $PC2_{clim}$ and $PC3_{clim}$ (all $P > 0.3$; Table S4). Similar to TEWL, CWL_{90} was dependent on ancestry ($F = 10.83$, $P = 0.001$) and transect, with CWL_{90} values being higher in transect 1 than in transect 2 ($t = 2.61$, $P = 0.023$) and 3 ($t = 3.17$, $P = 0.012$); no significant CWL_{90} difference was found between transects 2 and 3 ($t = 1.01$, $P = 0.321$). In contrast, we found no effect of transect ($F = 1.05$, $P = 0.363$) or ancestry ($F = 2.77$, $P = 0.097$) on variation in CWL_{120}. Analyses on our second proxy for skin resistance against water loss —"cutaneous desiccation"— did show an effect of $PC1_{clim}$ (CD_{90}, $\chi^2 = 7.29$, $P = 0.007$; CD_{120}, $\chi^2 = 3.58$, $P = 0.058$) and of $PC2_{clim}$ or $PC3_{clim}$ (all $P > 0.3$; Table S4). More specifically, water-filled tubes covered with skin from lizards that inhabit mesic environments dried up significantly faster than did tubes covered with skin from lizards that inhabit arid environments (Fig. 3). In other words, cutaneous desiccation probability was higher for lizards from mesic areas than for lizards from arid areas. This was, however, only true for transect 1 (CD_{90}, $z = 2.70$, $P = 0.021$; CD_{120}, $z = 1.89$, $P = 0.059$) and not for transect 2 (CD_{90}, $z = 0.28$, $P = 0.781$; CD_{120}, $z = 0.34$, $P = 0.973$) or transact 3 (CD_{90}, $z = 0.54$, $P = 0.781$; CD_{120}, $z = 0.49$, $P = 0.934$). Approximately 7.3% of the skin-wrapped tubes were completely evaporated after 90 hours (transect 1: 8.7%; transect 2: 9.4%; transect 3: 3.9%), which increased to circa 13.6% after 120 hours (transect 1: 17.4%; transect 2: 18.2%; transect 3: 5.1%). Ancestry did not affect CD_{90} ($\chi^2 = 0.27$, $P = 0.607$) and CD_{120} ($\chi^2 = 2.21$, $P = 0.137$).

Neither body mass nor size significantly differed among lizards that inhabit different climate conditions (mass, $F = 2.53$, $P = 0.124$; size, $F = 1.14$, $P = 0.295$) or that were sampled on different transects (mass, $F = 0.69$, $P = 0.508$; size, $F = 1.59$, $P = 0.224$); only lizards from transect 3 were marginally, but significantly, bigger (13% in mass and 5% in size) than lizards from transect 1 (mass, $t = 2.30$, $P = 0.030$; size, $t = 2.29$, $P = 0.030$). Ancestry, however, determined both mass ($F = 5.26$, $P = 0.022$) and size ($F = 3.39$, $P = 0.066$) with lizards with low proportions of Western Cuba ancestry being larger and heavier than those with more Western Cuba ancestry. Relative scale size was unaffected by ancestry ($F = 1.54$, $P = 0.216$; Fig. 2D), but did significantly vary across areas of different aridity and among transects (Fig. 4). The skin of lizards originating from more arid areas was covered with smaller scales (relative to their body size) than the skin of lizards inhabiting more mesic environments ($F = 11.85$, $P = 0.002$; Fig. 2B). Moreover, the average relative scale size of lizards sampled on transect 3 was larger than that of lizards from transect 1 ($t = 2.47$, $P = 0.031$) and 2 ($t = 2.67$, $P = 0.031$); no significant difference in relative scale size was found between transect 1 and 2 lizards ($t = 0.59$, $P = 0.561$).

The relationship (slope) of relative scale size over aridity did not significantly differ among transects (interaction effect, $F = 1.75$, $P = 0.196$).

3. 2. Interrelationships among measures of water loss rates and morphology

We found no significant relationship between TEWL and any of the CWL measures (CWL_{90}, $F = 0.38$, $P = 0.537$; CWL_{120}, $t = 1.95$, $P = 0.164$; CD_{90}, $\chi^2 = 0.08$, $P = 0.782$; CD_{120}, $\chi^2 = 0.03$, $P = 0.872$). Similarly, no significant relationship was found between relative scale size and any of the water loss measures (TEWL, $F = 0.05$, $P = 0.826$; CWL_{90}, $F < 0.01$, $P = 0.943$; CWL_{120}, $F = 0.03$, $P = 0.872$; CD_{90}, $\chi^2 = 0.38$, $P = 0.538$; CD_{120}, $\chi^2 = 0.05$, $P = 0.837$). Large and heavy lizards had lower rates of TEWL than small lizards of low mass (mass, $F = 39.31$, $P < 0.001$; size, $F = 12.88$, $P < 0.001$).

3. 3. Phenology

On transect 1, lizards sampled in March did not significantly differ from lizards sampled in July in relative scale size ($F = 0.31$, $P = 0.583$) and cutaneous water loss (CWL_{90}, $F = 1.03$, $P = 0.0314$; CWL_{120}, $F = 0.20$, $P = 0.659$). However, in July, lizards were on average 4% larger and 12% heavier, and lost 42% less water than lizards sampled in March (mass, $F = 11.62$, $P < 0.001$; size, $F = 9.63$, $P = 0.002$; TEWL, $F = 17.70$, $P = 0.001$; Fig. 5).

3.4. Genetic architecture of hydric balance traits

The GWAS did not identify any region as significantly associated with relative scale size. By contrast, for TEWL, we identified one locus (hereafter "TEWL QTL"; Fig. 6A) on the proximal end of chromosome 3, which was associated with trait values at the suggestive genome-wide significance threshold ($P < 1.4 \times 10^{-5}$; Fig. 6A). The GWAS model in this case considered a different effect in each of the two genetic clusters (corresponding to Western Cuba ancestry and to admixed ancestry). In line with this result, and as expected if population structure was properly accounted for, we found that the genotype at this QTL is significantly associated with TEWL values within one of our sample groups, the "hybridization limited" group ($F = 11.77$, $P = 8 \times 10^{-4}$; Fig. 6B). For these *A. sagrei* samples, the TEWL QTL behaves as a medium-effect locus, explaining 6.8% of trait variance. By contrast, the same two alleles do not explain any trait variance in the "hybridization common" sample group ($F = 0.13$, $P = 0.722$; Fig. 6B).

The TEWL QTL spans ~35 Mb on chromosome 3 (start coordinate: ~30 Mb; end coordinate: ~65 Mb) and includes 355 genes. Overall, there were 3,053 SNPs that overlap this region, of

447 which 1,399 (45.8%) were inferred to be genic, and 1,654 (54.2%) were inferred to be
448 intergenic. Further, while we emphasize that identifying candidate genes is beyond the scope
449 of this study and likely not possible using ddRADseq, there were 21 genic SNPs, distributed
450 across 11 genes (Table S6), predicted to lead to an amino acid change. None of these SNPs
451 were, however, in strong linkage disequilibrium with the lead GWAS SNP (R^2 values ranged
452 from 0.002 to 0.341; Table S6).
453
454 **4. Discussion**
455 **4. 1. Lack of a climate cline in evaporative water loss**
456 The relationship between total evaporative water loss (TEWL) and habitat aridity, where
457 species and populations living in arid habitats are characterized by lower TEWL, has been
458 established in several reptile taxa (reviewed by Mautz 1982a and Le Galliard et al. 2021),
459 including in *Anolis* lizards in their native range in Central America and the Caribbean (e.g.,
460 Sexton and Heatwole 1968; Hillman and Gorman 1977; Hillman et al. 1979; Hertz 1980; Dmi'el
461 et al. 1997). In invasive *A. sagrei*, this pattern has previously been documented as well, albeit
462 based on a survey of three geographically diverse populations (Kolbe et al. 2014). In contrast
463 to these previous studies, we find little evidence for an aridity cline in TEWL among recently
464 introduced populations of *A. sagrei* in south-eastern US. Rather, patterns of TEWL variation
465 among the 30 invasive populations studied here are shaped primarily by ancestry and
466 phenology. Below, we discuss reasons that can underlie the discrepancies between our results
467 and those of previous studies.
468
469 First, the lack of a climatic cline in TEWL can reflect constraints imposed on rapid adaptation
470 that result from the invasion process itself. For example, hybridization among divergent
471 lineages in invasive brown anole populations may have resulted in novel antagonistic genetic
472 interactions among alleles at loci controlling TEWL and loci elsewhere in the genome. Indeed,
473 the only TEWL locus that we identified using ancestry-specific GWAS appears to be exposed
474 to such genetic interactions (Fig. 6). We emphasize, however, that ddRADseq data only
475 sparsely covers the genome, and therefore our results should not be viewed as a
476 comprehensive interrogation of the genetic architecture of TEWL or scale size. Nonetheless,
477 we note that while genetic interactions have typically been considered as contributing to
478 intrinsic (i.e., environment-independent) genetic incompatibilities, studies in yeast (Dettman
479 et al. 2007) and stickleback fish (Thompson et al. 2022) have provided evidence of similar
480 environment-dependent incompatibilities. In invasive brown anoles as well, Bock et al. (2021)

identified an adaptive locus that controls limb length, and that is subject to similar deleterious genetic interactions in hybrids. As well, invasive brown anoles are characterized by large-scale linkage disequilibrium and limited contemporary gene flow among populations (Bock et al. 2021). Linkage disequilibrium among alleles under selection can restrict adaptation *via* a process known as Hill-Robertson interference (Hill and Robertson 1966). Likewise, while large-scale gene flow can swamp local adaptation, intermediate levels of gene flow are often beneficial for adaptation in variable environments and at range margins (Tigano and Friesen 2016; Bontrager and Angert 2018). Thus, limited contemporary genetic exchange among invasive brown anole populations, as has been documented among the populations under study here (Bock et al. 2021), may be forestalling the spread of adaptive alleles.

Second, lack of adaptation in rates of water loss might be due to the limited time that has passed since the establishment of invasive *A. sagrei* populations. We consider this possibility less likely, however. This is because strong selection and rapid evolutionary change in morphology and physiology has been documented repeatedly for *Anolis* species*,* over much shorter timescales. Green anole lizards (*A. carolinensis*) in southern US, for instance, showed greater cold tolerance and a corresponding shift in putative genomic targets of selection a year after experiencing a cold snap (Campbell-Staton et al. 2017). In response to hurricanes, survivors in two island populations of Southern Bahamas anoles (*A. scriptus*) had larger, stronger-gripping, toepads that likely aided in their ability to cling to vegetation during high-speed winds (Donihue et al. 2018, 2020). Note, however, that these examples involved rapid strong directional selection imposed by acute, extreme climate events (see also Grant et al. 2017). In the case of brown anole populations surveyed here, selection pressure for decreased TEWL in arid regions might not be strong enough. Yet, the climatic conditions experienced by invasive brown anoles differs substantially in both breadth and magnitude from those experienced by their native island counterparts in Cuba (Angetter et al. 2011; Kolbe et al. 2014; Table S5). Also, the range of climatic variation experienced by brown anoles in the US is comparable to that observed in other *Anolis* species for which adaptive changes in TEWL have been documented. For instance, Gunderson et al. (2011) observed significant differences in water loss rates between two populations of Puerto Rican crested anoles (*A. cristatellus*) from habitats that differed on average by 12% in relative humidity. This value is comparable to the circa 10% difference recorded between the most northern sampled population (Tifton, GA) and Miami (FL), one of our more southern populations (Kolbe et al. 2014). Future studies could capitalize on extreme drought events to better understand rapid evolutionary change in *Anolis*

water balance physiology. Although previous demonstrations of extreme climate-induced evolutionary change in wild anoles began with a serendipitous baseline from which to measure selection (e.g., Campbell-Staton et al. 2017, 2018; Donihue et al. 2018, 2020), strategic sampling at intervals in sites where extreme events are likely to occur should be feasible (discussed in Grant et al. 2017).

Third, the lack of a climatic cline in TEWL may imply that invasive brown anoles employ alternative strategies for coping with limited or variable access to water resources. For instance, behavioral adjustments of hydroregulation, thermoregulation, and water uptake might enable lizards from the relatively arid north to counter desiccation risks. By selecting humid and low temperature microenvironments and by remaining relatively inactive in the field, lizards can reduce water loss. Also, in xeric environments, lizards may opportunistically maximize water intake (e.g., during thundershowers; Bradshaw and Shoemaker 1967; Minnich and Schoemaker 1970), transition to a more fluid-rich diet (Warburg 1964; Nagy et al. 1991; Znari and Nagy 1997) or close their eyes for longer periods of time whilst basking hence minimizing ocular water loss (Waldschmidt and Porter 1987). In this context, additional field data on microhabitat selection, body temperatures, diel activity patterns, food intake, and ocular behavior would provide valuable insights into the contribution of hydroregulatory behavior (Pirtle et al. 2019).

Other explanations for the absence of a climate cline in TEWL in our study might have a methodological basis. First, we used online climate databases with a spatial scale of 1 km to retrieve abiotic data of our study populations, rather than measuring environmental conditions at the lizards' perch sites in the field (e.g., Gunderson et al. 2011). Environmental data on a microgeographical scale is preferred because water loss rates are dictated by the conditions of the immediate surrounding (Hertz 1980). Collecting such data is, however, extremely labor intensive as it is only of value when obtained repeatedly and periodically (i.e., hourly, daily, and across all seasons). Second, there are several techniques to assess evaporative water loss, which may all yield slightly variable TEWL estimates (Mautz 1982a). Selecting a single method to measure TEWL in squamate reptiles is not straightforward because the exact protocol depends on research questions and context (Le Galliard et al. 2021). We used the most widely used protocol for squamate reptiles (i.e., measurements of body mass loss in the laboratory) because of its simplicity and suitability for high-throughput TEWL quantification of large numbers of specimens in a non-destructive manner.

549

550 **4. 2. Effects of body size and phenology on evaporative water loss**

551 Water is lost by evaporation through several routes, including the respiratory passages, ocular
552 membranes, excretory expenditures, and, most importantly, the skin surface—the leading
553 avenue of water loss in squamate reptiles (Chew and Dammann 1961; Bentley and Schmidt-
554 Nielsen 1966; Standaert and Johansen 1974; Lillywhite and Maderson 1982; Feder and
555 Burggren 1985; Lillywhite 2006). Because evaporation rates are direct functions of the surface
556 area over which flux of water occurs, laws in allometric scaling (Gould 1971) dictate that large
557 animals (1) lose more water and (2) have lower mass specific water loss strictly due to
558 decreasing ratios of surface to mass compared to small animals (Mautz 1982a). In
559 concordance, we find water loss to increase with strong negative allometry to body mass in
560 brown anoles (natural log of mass lost over natural log of body mass; slope = 0.46 with CI =
561 0.27 - 0.55): large anoles lose more water than small anoles in absolute, but not relative,
562 terms. Increasing body size in dry habitats could thus be an alternative adaptive strategy to
563 improving physiological capacities to resist water loss in lizards (Oufiero et al. 2011; Hlubeň et
564 al. 2021). Our results, however, do not support this possibility. We find that while body size
565 explains much of the among-population variation in brown anole water loss, size does not
566 follow a latitudinal or climatic cline; rather, body size is ancestry-dependent: anoles with high
567 proportions of Western Cuba ancestry are generally smaller and, due to a high surface-to-
568 volume ratio, have a relatively large TEWL. The body size distribution of brown anoles across
569 their invasion range is thus a result of introduction and hybridization history, which occurs
570 independent of the local environment (Kolbe et al. 2004, 2007, 2008; Bock et al. 2021).

571

572 While genetically determined body size may partly explain the geographical variation in water
573 loss, phenologically determined body size explains much of the variation observed among
574 transects. Anoles gradually change in size over time with intervals of growth rate spurs during
575 the wet season when food availability increases (Andrews 1976; Stamps 1977; Schoener and
576 Schoener 1978; Dunham et al. 1988). Since populations surveyed here were sampled at
577 different timepoints (field work spanned 81 days, from late March to early July; Table S1), we
578 likely sampled populations at different growth stages, which may explain the smaller body
579 size and relatively high rates of water loss of lizards sampled during the first transect, in
580 March, earlier in the season (Fig. 2). We validated this phenology effect by re-visiting five
581 populations from transect one (sampled in March) in July and, indeed, observed a substantial
582 population-level increase in body size and mass, and decrease in percentage water loss rate

583 (Fig. 5). While the effect of phenology may complicate searches for climatic patterns in animal
584 water loss, it is an inevitable factor in extensive field studies at large geographic scales that
585 should be taken into account.
586
587 **4. 3. Scalation and skin resistance to water loss**
588 The scaled integument is a significant avenue of water loss in squamates (Bentley and
589 Schmidt-Nielsen 1966; Lillywhite and Maderson 1982). Specifically, the hinge regions between
590 the scales are often considered the dominant routes for cutaneous water movement because
591 interscalar tissue (as opposed to scale tissue) contains a thinner layer of keratin and hence, a
592 lower diffusion distance for passive water exchange (Horton 1972; Maderson 1972; Minnich
593 1982). Following this premise, one expects (1) squamates with larger scales and lower
594 interscalar tissue surface area to have a higher skin resistance to water loss than those with
595 smaller scales and (2) arid dwelling squamates to have larger scales than those inhabiting
596 mesic environments (Warburg 1966; Horton 1972; Lillywhite and Maderson 1982). Indeed, in
597 a range of different lizard groups, scale size was found to be inversely correlated with water
598 loss rates (*Sphaerodactylus*: MacLean and Hold 1979; MacLean 1985; *Sceloporus:* Acevedo
599 2009) and positively correlated with habitat aridity (e.g. *Sceloporus*: Oufiero et al. 2011,
600 Wishingrad and Thomson 2020; *Liolaemus*: Tully and Cruz 2019; *Uta*: Soulé 1966; *Gallotia*:
601 Thorpe and Baez 1987), including *Anolis* (Wegener et al. 2014) and even *A. sagrei* in their
602 native range (Lister, 1976; Calsbeek et al. 2006). Contrary to expectations based on these prior
603 studies, in invasive populations of *A. sagrei*, we find no evidence for a relationship between
604 scale size and evaporative water loss, and an inverse relationship between scale size and
605 habitat aridity.
606 First, the lack of a relationship between scalation and water loss in invasive brown
607 anoles might be due to a low contribution of cutaneous water loss relative to other avenues
608 of evaporative water loss. This seems unlikely however, because rates of cutaneous water loss
609 typically exceed respiratory water loss in various species of squamates (Standaert and
610 Johansen 1974; Mautz 1982b; Le Galliard et al. 2021), including the congener *A. cristatellus*
611 for which cutaneous water loss comprises roughly three-quarters of the total evaporative
612 water loss (Dmi'il et al. 1997). Second, water loss might occur primarily through the scale
613 tissue rather than through the interstitial skin at scale edges, which is contrary to as what
614 originally hypothesized by Krakauer (1970) and Horton (1972). If true, one would expect the
615 relationship between scale size and habitat aridity to run in opposite direction, with smaller
616 scales in more arid environments, as has been observed in *Anolis oculatus (*Malhotra and

617 Thorpe 1997) for instance. This would clarify the negative relationship between habitat aridity
618 and scale size in our dataset, yet the absence of a link between scale size and water loss
619 prevents us from interpreting the climatic cline in scale size as an adaptive response for
620 efficient water conservation. Third, the lipids in the epidermis may play a prime role in
621 regulating integument permeability, rather than the scales *per se*. An increase in skin
622 permeability following lipid extraction has been reported for *Anolis carolinensis*, which
623 suggests that lipids may be an important component of the water barrier in anoles (Kattan
624 and Lillywhite 1989). Histochemical studies of the epidermis of brown anoles across their
625 invasive range would provide valuable information on how lipid quality and quantity are
626 involved in evolutionary or physiological adjustments to habitat. Fourth, functional trade-offs
627 may constrain scale size evolution. The skin plays a crucial role in many functions other than
628 protecting against extreme hydric and thermic conditions, such as contributing to structural
629 coloration (e.g., Saenko et al. 2013; Nicolaï et al. 2021) and taking part in locomotion (e.g.,
630 Spinner et al. 2013; Martinez et al. 2021) and body cleansing (e.g., Hiller 2009; Watson et al.
631 2015). If two or more functions pose conflicting demands on the same scale design, then
632 simultaneous "optimization" becomes impossible, and trade-offs will result in a compromise
633 phenotype (reviewed by Garland et al. 2022). Future studies that integrate skin biomechanics,
634 functional morphology, and phylogenetic comparative methods are encouraged as they may
635 reveal the existence of trade-off with other relevant functions or may indicate other
636 constraints to biological "optimization".
637
638 **5. Outlook**
639 The brown anole is an emerging model organism that has served as a workhorse of
640 evolutionary and ecological research for more than six decades (Losos 2009; Geneva et al.
641 2021). Over this timespan, numerous experimental and observational studies have
642 documented natural selection and local adaptation in populations in the native range
643 (reviewed in Losos 2009). Following the human-mediated introduction of this species to the
644 south-eastern US, *A. sagrei* spread rapidly such that it now represents the most abundant
645 terrestrial vertebrate in peninsular Florida (Campbell 2000). The invasive range of this species
646 in Florida is also characterized by a novel climate, with relatively drier conditions than those
647 typical of its Caribbean ancestral range (Angetter et al. 2011). Given these considerations, we
648 set out expecting to find a climatic cline in total evaporative water loss and in skin scale size,
649 in a direction that is consistent with adaptive divergence of brown anole populations across
650 Florida.

Our results show that trait variation in the invasive range of this species is unlikely to be the result of rapid local adaptation. Instead, we find that among-population differences in water loss traits are shaped primarily by phenology and ancestry. These results echo those obtained for limb length, a trait known to be involved in local adaptation of native *A. sagrei* populations, but for which invasive populations show limited evidence of adaptation (Kolbe et al. 2007; Bock et al. 2021). Our findings highlight the possibility that characteristics of invasive populations, such as high linkage disequilibrium or detrimental genetic variation introduced by hybridization, might in some cases forestall adaptive responses, even in invasive species that, at face value, would seem to be primed for a rapid adaptive response. Even more broadly, our study illustrates the importance of using a multi-pronged research strategy that combines large-scale geographical sampling with temporal data, and with information from physiology, functional morphology, and genetics. Such an integrative approach is likely to give us the best chance of teasing apart factors that shape the evolution of invasive populations (Kueffer et al. 2013).

References

Acevedo, G.A. 2009. Ecomorphology of the mexican fence lizards of the *Sceloporus formosus* group (Squamata: Phyrnosomatidae). PhD Dissertation. The University of Texas at Arlington, TX, USA.

Addo-Bediako, A. Chown, S.L. & Gaston, K.J. 2001. Revisiting water loss in insects: a large scale view. J. Insect Physiol. 47:1377-1388.

Albright, T.P., Mutiibwa, D., Gerson, A.R, Smith E.K., Talbot, W.A., O'Neill, J.J, McKechnie, A.E., & Wolf, B.O. 2017. Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. Proc. Natl. Acad. Sci. U. S. A. 114:2283-2288.

Alpert, P. 2005. The limits and frontiers of desiccation-tolerant life. Int. Comp. Biol. 45:685-695.

Andrews, R.M. 1976. Growth rate in island and mainland anoline lizards. Copeia 1976:477-482.

Angetter, L.S., Lötters, S. & Rödder, D. 2011. Climate niche shift in invasive species: the case of the brown anole. Biol. J. Linn. Soc. 104:943-954.

684 Baeckens, S. Martín, J., García-Roa, R., Pafilis, P., Huyghe, K. & Van Damme, R. 2018.
685 Environmental conditions shape the chemical signal design of lizards. Funct. Ecol.
686 32:566-580.

687 Baeckens, S., Wainwright, D.K., Weaver, J.C., Irschick, D.J., & Losos, J.B. 2019. Ontogenetic
688 scaling patterns of lizard skin surface structure as revealed by gel-based stereo-
689 profilometry. J. Anat. 235:346-56.

690 Baeckens, S. Temmerman, M., Gorb, S.N., Neto, C., Whiting, M.J. & Van Damme, R. 2021.
691 Convergent evolution of skin surface microarchitecture and increased skin
692 hydrophobicity in semi-aquatic anole lizards. J. Exp. Biol. 224:jeb242939.

693 Bates D., Mächler M., Bolker B. & Walker S. 2015. Fitting linear mixed-effects models using
694 lme4. J. Stat. Soft. 67:1-48.

695 Bates, O. & Bertelsmeier, C. 2021. Climatic niche shifts in introduced species. Curr. Biol.
696 31:1252-1266.

697 Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate: a practical and
698 powerful approach to multiple testing. J. R. Stat. Soc. B 57:289-300.

699 Bentley, P. & Schmidt-Nielsen, K. 1966. Cutaneous water loss in reptiles. Science 152:1523.

700 Bock, D.G., Kantar, M.B., Caseys, C., Matthey-Doret, R. & Rieseberg, L.H. 2018. Evolution of
701 invasiveness by genetic accommodation. Nat. Ecol. Evol. 2:991-999.

702 Bock, D.G., Baeckens, S., Pita-Aquino, J.N., Chejanovski, Z.A., Michaelides, S.N., Muralidhar,
703 P., Lapiedra, O., Park, S., Menke, D.B., Geneva, A.J., Losos, J.B. & Kolbe, J.J. 2021.
704 Changes in selection pressure can facilitate hybridization during biological invasion in a
705 Cuban lizard. - Proc. Natl. Acad. Sci. U. S. A. 118:e2108638118.

706 Bolker, B.M. 2008. Ecological models and data in R. Princeton University Press, Princeton.

707 Bontrager, M. & Angert, A.L. 2019. Gene flow improves fitness at a range edge under climate
708 change. Evol. Lett. 3:55-68.

709 Bradshaw, S.D. & Shoemaker, V.H. 1967. Aspects of water and electrolyte changes in a field
710 population of *Amphibolurus* lizards. Comp. Biochem. Physiol. 20:855-865.

711 Brown, G. 1968. Desert Biology: Special Topics on the Physical and Biological Aspects of Arid
712 Regions, Volume I. Academic Press, New York.

713 Calsbeek, R. Knouft, J.H. & Smith, T.B. 2006. Variation in scale numbers is consistent with
714 ecologically based natural selection acting within and between lizard species. Evol.
715 Ecol. 20:377-394.

716 Campbell, T.S. 2000. Analysis of the effects of an exotic lizard (*Anolis sagrei*) on a native
717 lizard (*Anolis carolinensis*) in Florida, using islands as experimental units. PhD
718 Dissertation. University of Tennessee at Knoxville, TN, USA.

719 Campbell-Staton S.C., Cheviron, Z.A., Rochette, N.C., Catchen, J., Losos, J.B. & Edwards, S.E.
720 2017. Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the
721 green anole lizard. Science 357:495-498.

722 Campbell-Staton, S.C., Bare, A., Losos, J.B., Edwards, S.E., & Cheviron, Z.A. 2018.
723 Physiological and regulatory underpinnings of geographic variation in reptilian cold
724 tolerance across a latitudinal cline. Mol. Ecol. 27:2243-2255.

725 Campbell-Staton, S.C., Winchell, K.M., Rochette, N.C., Fredette, J., Maayen, I., Schweizer,
726 R.M. & Catchen, J. 2020. Parallel selection on thermal physiology facilitates repeated
727 adaptation of city lizards to urban heat islands. Nat. Ecol. Evol. 4:652-658.

728 Chew, R.M. & Dammann, A.E. 1961. Evaporative water loss of small vertebrates, as
729 measured with an infrared analyzer. Science 133: 384-385.

730 Chiang, F., Mazdiyasni, O. & AghaKouchak, A. 2021. Evidence of anthropogenic impacts on
731 global drought frequency, duration, and intensity. Nat. Commun. 12:2754.

732 Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X. & Ruden,
733 D.M. 2012. A program for annotating and predicting the effects of single nucleotide
734 polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118;
735 iso-2; iso-3. Fly. 6:80-92.

736 Corl, A., Bi, K., Luke, C., Challa, A.S., Stern, A.J., Sinervo, B. & Nielsen, R. 2018. The genetic
737 basis of adaptation following plastic changes in coloration in a novel environment.
738 Curr. Biol. 28:2970-2977.

739 Cox, C.L. & Cox, R.M. 2015. Evolutionary shifts in habitat aridity predict evaporative water
740 loss across squamate reptiles. Evolution 69:2507-2516.

741 De Meyer, J., Donihue, C.M., Scantlebury, D., Ng, J., Glor, R.E., Losos, J.B. & Geneva, A.J.
742 2019. Protocol for setting up and rearing a successful lizard room. Pp. 37-62 *in* J.T.
743 Stroud, A.J. Geneva & J.B. Losos, eds. Anolis Newsletter VII. Washington University,
744 MO.

745 Dettman, J.R., Sirjusingh, C., Kohn, L.M. & Anderson, J.B. 2007. Incipient speciation by
746 divergent adaptation and antagonistic epistasis in yeast. Nature 447:585-588.

747 Dlugosch, K.M., Anderson, S.R., Braasch, J., Cang, F.A. & Gillette, H.D. 2015. The devil is in
748 the details: genetic variation in introduced populations and its contributions to
749 invasion. Mol. Ecol. 24:2095-2111.

750 Donihue, C.M., Herrel, A., Fabre, A.C., Kamath, A., Geneva, A.J., Schoener, T.W., Kolbe, J.J. &
751 Losos, J.B. 2018. Hurricane-induced selection on the morphology of an island lizard.
752 Nature 560: 88-91.

753 Donihue, C.M. Kowaleski, A.M., Losos, J.B., Algar, A.C., Baeckens, S., Buchkowski, R.W.,
754 Fabre, A.C., Frank, H.K., Geneva, A.J., Reynolds, R.G., Stroud, J.T., Velasco, J.A., Kolbe,
755 J.J., Mahler, D.L. & Herrel, A. 2020. Hurricane effects on neotropical lizards span
756 geographic and phylogentic scales. Proc. Natl. Acad. Sci. U. S. A. 117:10429-10434.

757 Dmi'el F., Perry, G. & Lazell, J. 1997. Evaporative water loss in nine insular populations of the
758 lizard *Anolis cristatellus* group in the British Virgin Islands. Biotropica. 29:111-116.

759 Dmi'el, R. 1985. Skin resistance to water loss in a desert snake. J. Therm. Biol. 10:145-149.

760 Dmi'el, R. 2001. Skin resistance to evaporative water loss in reptiles: a physiological adaptive
761 mechanism to environmental stress or a phyletically dictated trait? Isr. J. Zool. 47:56-
762 67.

763 Dunham, A.E., Miles, D.B. & Reznick, D.N. 1988. Life history patterns in squamate reptiles.
764 Pp. 443–320 *in* C.C. Gans and R.B. Huey, eds. Biology of the Reptilia, Volume 16,
765 Ecology B Defense and Life History. Academic Press, New York.

766 Feder, M.E. & Burggren, W.W. 1985. Cutaneous gas exchange in vertebrates: design,
767 patterns, control and implications. Biol. Rev. 60:1-45.

768 Fick, S.E. & Hijmans, R.J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for
769 global land areas. Int. J. Climatol. 37:4302-4315.

770 Garland, T., Downs, C.J. & Ives, A.R. 2022. Trade-offs (and constraints) in organismal biology.
771 Physiol. Biochem. Zool. 95:82-112.

772 Geneva, A.J., Park, S., Bock, D.G., de Mello, P., Sarigol, F., Tollis, M., Donihue, C.M., Reynolds,
773 G., Feiner, N., Rasys, A.M., Lauderdale, J.D., Minchey, S.G., Alcala, A.J., Infante, C.R.,
774 Kolbe, J.J., Schluter, D., Menke, D.B. & Losos, J.B. 2021. Chromosome-scale genome
775 assembly of the brown anole (*Anolis sagrei),* a model species for evolution and ecology.
776 bioRxiv, 2021.09.28.462146. https://doi.org/10.1101/2021.09.28.462146.

777 Gould, S.J. 1971. Geometric similarity in allometric growth: a contribution to the problem of
778 scaling in the evolution of size. Am. Nat. 105:113-136.

779 Grant, P.R., Grant, R.B., Huey, R.B., Johnson, M.T.J., Knoll, A.H. & Schmitt, J. 2017. Evolution
780 caused by extreme events. Proc. R. Soc. B. 372:20160146.

781 Greve, P., Roderick, M.L., Ukkola, A.M. & Wada, Y. 2019. The aridity Index under global
782 warming. Environ. Res. Lett. 14:124006.

783 Gunderson, A.R., Siegel, J. & Leal, M. 2011. Tests of the contribution of acclimation to
784 geographic variation in water loss rates of the West Indian lizard *Anolis cristatellus*. J.
785 Comp. Physiol. B 181:965-972.

786 Hellmich, W.C. 1951. On ecotypic and autotypic characters, a contribution to the knowledge
787 of the evolution of the genus *Liolaemus* (Iguanidae). Evolution 5:359-369.

788 Hertz, P.E. 1980. Responses to dehydration in *Anolis* lizards sampled along altitudinal
789 transects. Copeia 1980:440-446.

790 Hill, W.G. & Robertson, A. 1966. The effect of linkage on limits to artificial selection. Genet.
791 Res. 8:269-294.

792 Hiller, U. 2009. Water repellence in gecko skin: how do geckos keep clean? Pp. 47-53 *in* S.N.
793 Gorb, ed. Functional Surfaces in Biology. Springer, New York.

794 Hillman, S.S. & Gorman, G.C. 1977. Water loss, desiccation tolerance, and survival under
795 desiccating conditions in 11 species of Caribbean *Anolis*. Oecologia 29:105-116.

796 Hillman, S., Gorman, G.C. & Thomas, R. 1979. Water loss in *Anolis* lizards: evidence for
797 acclimation and intraspecific differences along a habitat gradient. Comp. Biochem.
798 Physiol. A 62:491-494.

799 Hodgins, K.A., Bock, D.G. & Rieseberg, L.H. 2018. Trait evolution in invasive species. Annu.
800 Plant Rev. Online 1:1-37.

801 Horton, D.R. 1972. Lizard scale size and adaptation. Syst. Zool. 21:441-443.

802 Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra L. 2000. Rapid evolution of a
803 geographic cline in size in an introduced fly. Science 287:308-309.

804 Huey, R.B., Gilchrist, G.W. & Hendry A.P. 2005. Using invasive species to study evolution:
805 case studies with *Drosophila* and salmon. Pp. 139-164 *in* D.F. Sax, J.J. Stachowicz & S.D.
806 Gaines, eds. Species Invasions: Insights into Ecology, Evolution and Biogeography.
807 Sinauer Associates, Sunderland.

808 Hlubeň, M., Kratochvíl, L. & Gvoždík, L. & Strarostová, Z. 2021. Ontogeny, phylogeny and
809 mechanisms of adaptive changes in evaporative water loss in geckos. J. Evol. Biol.
810 34:1290-1301.

811 Jombart, T. & Ahmed, I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide
812 SNP data. Bioinformatics 27:3070-3071.

813 Kattan, G.H. & Lillywhite, H.B. 1989. Humidity acclimation and skin permeability in the lizard
814 *Anolis carolinensis*. Physiol. Zool. 62:593-606.

815 Keller, S.R. & Taylor, D.R. 2008. History, chance and adaptation during biological invasion:
816 Separating stochastic phenotypic evolution from response to selection. Ecol. Lett.
817 11:852-866.

818 Kolbe, J.J., Glor, R.E., Schettino, L.R., Lara, A.C., Larson, A. & Losos, J.B. 2004. Genetic
819 variation increases during biological invasion by a Cuban lizard. Nature 431:177-181.

820 Kolbe, J.J., Larson, A. & Losos, J.B. 2007. Differential admixture shapes morphological
821 variation among invasive populations of the lizard *Anolis sagrei*. Mol. Ecol. 16:1579-
822 1591.

823 Kolbe, J.J., Larson, A., Losos, J B. & de Queiroz, K. 2008. Admixture determines genetic
824 diversity and population differentiation in the biological invasion of a lizard species.
825 Biol. Lett. 4:434-437.

826 Kolbe, J.J., Leal, M., Schoener, T.W., Spiller, D.A. & Losos, J.B. 2012. Founder effects persist
827 despite adaptive differentiation: A field experiment with lizards. Science 335:1086-
828 1089.

829 Kolbe, J.J., Ehrenberger, J.C., Moniz, H.A. & Angilletta, M.J.J. 2014. Physiological variation
830 among invasive populations of the brown anole (*Anolis sagrei*). Physiol. Biochem. Zool.
831 87:92-104.

832 Kobayashi, D., Mautz, W.J. & Nagy, K.A. 1983. Evaporative water loss: humidity acclimation
833 in *Anolis carolinensis* lizards. Copeia 1983:701-704.

834 Krakauer, T. 1970. The ecological and physiological control of water loss in snakes. PhD
835 Dissertation. University of Florida at Gainesville, FL, USA.

836 Kueffer, C., Pysek, P. & Richardson, D.M. 2014. Integrative invasion science: model systems,
837 multi-site studies, focused meta-analysis and invasion syndromes. New Phytol.
838 200:615-633.

839 Le Galliard, J.F., Chabaud, C., de Andrade, D.O.V., Brischoux, F., Carretero, M.A., Dupoué, A.,
840 Gavira, R.S.B., Lourdais, O., Sannolo, M. & Van Dooren T.J.M. 2021. A worldwide and
841 annotated database of evaporative water loss rates in squamate reptiles. Glob. Ecol.
842 Biogeogr. 30:1938-1950.

843 Lertzman-Lepofsky, G.F., Kissel, A.M., Sinervo, B. & Palen, W.J. 2020. Water loss and
844 temperature interact to compound amphibian vulnerability to climate change. Global
845 Change Biol. 26:4868-4879.

846 Lillywhite, H.B. 2006. Water relations of tetrapod integument. J. Exp. Biol. 209:202-226.

847 Lillywhite, H. B. & Maderson, P.F.A. 1982. Skin structure and permeability. Pp. 397-422 *in*
848 C.C. Gans, F.H. Pough, eds. Biology of the Reptilia, Volume 12: Physiology. Academic
849 Press, New York.

850 Lister, B.C. 1976. The nature of niche expansion in West Indian *Anolis* lizards I: Ecological
851 consequences of reduced competition. Evolution 30:659-676.

852 Lodge, D. 1993. Biological invasions: lessons for Ecology. Trends Ecol. Evol. 8:133-137.

853 Losos, J.B., Warheitt, K.I. & Schoener, T.W. 1997. Adaptive differentiation following
854 experimental island colonization in Anolis lizards. Nature 387:70-73.

855 Losos, J.B. 2009. Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles.
856 University of California Press, California.

857 Lundholm, B. 1976. Adaptations in arid ecosystems. Ecol. Bull. 24:19-27.

858 Mackay, T.F. 2014. Epistasis and quantitative traits: using model organisms to study gene-
859 gene interactions. Nat. Rev. Genet. 15:22-33.

860 MacLean, W.P. 1985. Water-loss rates of *Sphaerodactylus parthenopion* (Reptilia:
861 Gekkonidae), the smallest amniote vertebrate. J. Comp. Biochem. Physiol. Part A
862 82:759-761.

863 Maderson, P.F.A. 1972. When? Why? and How? Some speculations on the evolution of the
864 vertebrate integument. Am. Zool. 12:159-171.

865 Malhotra, A. & Thorpe, R.S. 1997. Size and shape variation in a Lesser Antillean anole, *Anolis*
866 *oculatus* (Sauria: Iguanidae) in relation to habitat. Biol. J. Linn. Soc. 60:53-72.

867 Martinez, A., Nguyen, D., Basson, M.S., Medina, J., Irschick, D.J. & Baeckens, S. 2021.
868 Quantifying surface topography of biological systems from 3D scans. Methods Ecol.
869 Evol. 12:1265-1276.

870 Mautz, W.J. 1982a. Correlation of respiratory and cutaneous water losses of lizards with
871 habitat aridity. J. Comp. Physiol. 149:25-30.

872 Mautz, W.J. 1982b. Patterns of evaporative water loss. Pp. 443-481 *in* F.H. Pough & C.C.
873 Gans, eds. Biology of the Reptilia, Volume 12: Physiological Ecology. Academic Press,
874 New York.

875 Minnich, J.E. & Shoemaker, V.H. 1970. Diet, behaviour and water turnover in the desert
876 iguana, *Dipsosaurus dorsalis*. Amer. Midl. Natur. 84:496-509.

877 Minnich, J.E. 1982. The use of water. Pp. 325-396 *in* C.C. Gans & F.H. Pough, eds. Biology of
878 the Reptilia, Physiology C. Academic Press, New York.

879 Mooney, H.A. & Cleland, E.E. 2001. The evolutionary impact of invasive species. Proc. Natl.
880 Acad. Sci. U. S. A. 98:5446-5451.

881 Moran, E.V. & Alexander, J.M. 2014. Evolutionary responses to global change: lessons from
882 invasive species. Ecol. Lett. 17:637-649.

883 Muñoz-Garcia, A., Ben-Hamo, M., Pilosof, S., Williams, J.B. & Korine C. 2022. Habitat aridity
884 as a determinant of the trade-off between water conservation and evaporative heat
885 loss in bats. J. Comp. Physiol. B 192:325-333.

886 Nagy, K.A., Clarke, B.C., Seely, M.K., Mitchell, D. & Lighton, J.R. 1991. Water and energy
887 balance in Namibian Desert sand-dune lizards, *Angolosaurus skoogi* (Andersson, 1916).
888 Funct. Ecol. 5:731-739.

889 Nicolai, M. P. J., D'Abla, L., Goldenberg, J., Gansemans, Y., Van Nieuwburgh, F., Clusella-
890 Trullas, S. & Shawkey, M.D. 2021. Untangling the structural and molecular mechanisms
891 underlying colour and rapid colour change in a lizard, *Agama atra*. Mol. Ecol. 30:2262-
892 2284.

893 Oufiero, C.E., Adolph, S.C., Gartner, G.E.A. & Garland, Jr. T. 2011. Latitudinal and climatic
894 variation in body size and dorsal scale rows in *Sceloporus* lizards: a phylogenetic
895 perspective. Evolution 65:3590-3607.

896 Oufiero, C.E. & Van Sant, M.J. 2018. Variation and repeatability of cutaneous water loss and
897 skin resistance in relation to temperature and diel variation in the lizard *Sceloporus*
898 *consobrinus*. J. Comp. Physiol. B 188:671-681.

899 Park, C.E., Jeong, S.J., Joshi, M., Osborn, T.J., Ho, C.H., Piao, S., Chen, D., Liu, J., Yang, H.,
900 Park, H., Kim, B.M. & Feng, S. 2018. Keeping global warming within 1.5 °C constrains
901 emergence of aridification. Nat. Clim. Chang. 8:70-74.

902 Peres-Neto, P.R., Jackson, D.A. & Somers, K.M. 2004. How many principal components?
903 Stopping rules for determining the number of non-trivial axes revisited. Comput Stat
904 Data Anal. 49:974-997.

905 Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. 2021. nlme: linear and nonlinear mixed effects
906 models. R package version 3.1-152. Retrieved on 12 October 2022 from https://cran.r-
907 project.org/web/packages/nlme/.

908 Pirtle, E.I., Tracy, C.R. & Kearney, M.R. 2019. Hydroregulation. A neglected behavioral
909 response of lizards to climate change? Pp. 343-374 *in* V. Bels & A. Russell, eds.
910 Behavior of Lizards: Evolutionary and mechanistic perspectives. CRC Press, Boca Raton.

911 Platt, A., Vilhjálmsson. B.J. & Nordborg, M. 2010. Conditions under which genome-wide
912 association studies will be positively misleading. Genetics. 186:1045-1052.

913 Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. & Lowe, A.J. 2008. Adaptive
914 evolution in invasive species. Trends Plant Sci. 13:288-294.

915 Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using
916 multilocus genotype data. Genetics 155:945-959.

917 Purcell, S. 2007. PLINK: a tool set for whole-genome association and population-based
918 linkage analyses. Am. J. Hum. Genet. 81:559-575.

919 Putman, B.J., Gasca, M., Blumstein, D.T. & Pauly, G.B. 2019. Downsizing for downtown: limb
920 lengths, toe lengths, and scale counts decrease with urbanization in western fence
921 lizards (*Sceloporus occidentalis*). Urban Ecosyst. 22:1071-1081.

922 Querns, A., Wooliver, R., Vallejo-Marín, M. & Sheth, S.N. 2022. The evolution of thermal
923 performance in native and invasive populations of *Mimulus guttatus*. Evol. Lett. 6:136-
924 148.

925 R Development Core Team, R: A Language and Environment for Statistical Computing
926 (www.R-project.org/) (R Foundation for Statistical Computing, 2019).

927 Reznick, D.N., Losos, J.B. & Travis, J. 2019. From low to high gear: there has been a paradigm
928 shift in our understanding of evolution. Ecol. Lett. 22:233-244.

929 Rio, S., Mary-Huard, T., Moreau, C., Bauland, C., Palaffre, C., Madur, D., Combes, V. &
930 Charcosset, A. 2020. Disentangling group specific QTL allele effects from genetic
931 background epistasis using admixed individuals in GWAS: An application to maize
932 flowering. PLoS Genet. 16:e1008241.

933 Roberts, J.B. & Lillywhite, H.B. 1983. Lipids and the permeability of the epidermis from
934 snakes. J. Exp. Zool. 228:1-9.

935 Saenko, S.V., Teyssier, J., Marel, D.V. & Milinkovitch, M.C. 2013. Precise colocalization of
936 interacting structural and pigmentary elements generates extensive color pattern
937 variation in *Phelsuma* lizards. BMC Biol. 11:105.

938 Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S.,
939 Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M.,
940 Thompson, J.N. & Weller, S.G. 2001. The population biology of invasive species. Annu.
941 Rev. Ecol. Syst. 32:305-332.

942 Schneider, C.A., Rasband, W.S. & Eliceiri, W. S. 2012. NIH Image to ImageJ: 25 years of image
943 analysis. Nat. Methods 9:671-675.

944 Schoener, T.W. & Schoener, A. 1978. Estimating and interpreting body-size growth in some
945 *Anolis* lizards. Copeia 1978:390-405.

946 Sexton, O.J. & Heatwole, H. 1968. An experimental investigation of habitat selection and
947 water loss in some anoline lizards. Ecology 49:762-767.

948 Shen, X. & Carlborg, O. 2013. Beware of risk for increased false positive rates in genome-
949 wide association studies for phenotypic variability. Front. Genet. 4:93.

950 Shine, R. 2012. Invasive species as drivers of evolutionary change: cane toads in tropical
951 Australia. Evol. Appl. 5:107-116.

952 Skotte, L., Jorsboe, E., Korneliussen, T.S., Moltke, I. & Albrechtsen, A. 2019. Ancestry-specific
953 association mapping in admixed populations. Genet. Epidemiol. 43:506-521.

954 Soule, M. 1966. Trends in the insular radiation of a lizard. Am. Nat. 100:47-64.

955 Spinner, M., Gorb, S.N. & Westhoff, G. 2013. Diversity of functional microornamentation in
956 slithering geckos *Lialis* (Pygopodidae). Proc. R. Soc. B. 280:20132160.

957 Stamps, J.A. 1977. Rainfall, moisture and dry season growth rates in *Anolis aeneus*. Copeia
958 1977:415-419.

959 Standaert, T. & Johansen, K. 1974. Cutaneous gas exchange in snakes. J. Comp. Physiol.
960 89:313-320.

961 Stern, D.B. & Lee, C.E. 2020. Evolutionary origins of genomic adaptations in an invasive
962 copepod. Nat. Ecol. Evol. 4:1084-1094.

963 Stockwell, C.A., Hendry, A.P. & Kinnison, M.T. 2003. Contemporary evolution meets
964 conservation biology. Trends Ecol. Evol. 18:94-101.

965 Thompson, K.A., Peichel, C.L., Rennison, D.J., McGee, M.D., Albert, A.Y.K., Vines, T.H.,
966 Greenwood, A.K., Wark, A.R., Brandvain, Y., Schumer, M. & Schluter D. 2022. Analysis
967 of ancestry heterozygosity suggests that hybrid incompatibilities in threespine
968 stickleback are environment dependent. PLoS Biol. 20:e3001469.

969 Thorpe, R.S. & Baez, M. 1987. Geographic variation within an island: univariate and
970 multivariate contouring of scalantion, size, and shape of the lizard *Gallotia galloti*.
971 Evolution 41:256-268.

972 Tieleman, B.I., Williams, J.B. & Blommer, P. 2003. Adaptation of metabolism and evaporative
973 water loss along an aridity gradient. Proc. R. Soc. B 270:207-214.

974 Tigano, A. & Friesen V.L. 2016. Genomics of local adaptation with gene flow. Mol. Ecol.
975 25:2144-2164.

976 Van Sant, M.J., Oufiero, C.E., Muñoz-Garcia, A., Hammond, K.A. & Williams, J.B. 2012. A
977 Phylogenetic approach to total evaporative water loss in mammals. Physiol. Biochem.
978 Zool. 85:526-532.

979 Waldschmidt, S.R. & Porter, W.P. 1987. A model and experimental test of the effect of body
980 temperature and wind speed on ocular water loss in the lizard *Uta stansburiana*.
981 Physiol. Zool. 60:678-686.

982 Warburg, M.R. 1964. The influence of ambient temperature and humidity on the body
983 temperature and water loss from two Australian lizards, *Tiliqua rugosa* (Gray)
984 (Scincidae) and *Amphibolurus barbatus cuvier* (Agamidae). Aust. J. Zool. 13:331-350.

985 Warburg, M.R. 1966. On the water economy of several Australian geckos, agamids, and
986 skinks. Copeia 2:230-235.

987 Watson, G.S., Green, D.W., Schwarzkopf, L., Li, X., Cribb, B.W., Myhra, S. & Watson, J.A.
988 2015. A gecko skin micro/nano structure - A low adhesion, superhydrophobic, anti-
989 wetting, self-cleaning, biocompatible, antibacterial surface. Acta Biomater. 21:109-122.

990 Williams, E.E. 1969. The ecology of colonization as seen in the zoogeography of anoline
991 lizards on small islands. Q. Rev. Biol. 44:345-389.

992 Wegener, J., Gartner, G.E.A. & Losos, J.B. 2014. Lizard scales in an adaptive radiation:
993 variation in scale number follows climatic and structural habitat diversity in *Anolis*
994 lizards. Biol. J. Linn. Soc. 112:570-579.

995 Whitney, K.D. & Gabler, C.A. 2008. Rapid evolution in introduced species, “invasive traits”
996 and recipient communities: Challenges for predicting invasive potential. Divers. Distrib.
997 14:569-580.

998 Wishingrad, V. & Thomson, R.C. 2020. Ecological variability is associated with functional trait
999 diversity in the western fence lizard (*Sceloporus occidentalis*). Biol. J. Linn. Soc.
1000 129:414–424.

1001 Znari, M. & Nagy, K.A. 1997. Field metabolic rate and water flux in free-living Bribon’s agama
1002 (*Agama impalearis*) in Morocco. Herpetologica 53:81-88.

Figure captions:

Fig. 1: — Geographical distribution of the *Anolis sagrei* populations used in this study. Shades of blue coloration illustrate the three different transects. Population IDs correspond to Table S1. Populations annotated with an asterisk were re-sampled in July to assess the role of phenology on the traits of interest. The opaque white pie slice overlaying the blue disks represent the population frequency of haplotypes from Western Cuba (in %). The scatterplot shows the correlation between latitude and local climatic conditions (as $PC1_{clim;}$ see methodology) of the study populations.

Fig. 2: — Water balance physiology and skin morphology. Scatterplots showing total evaporative water loss and residual (i.e., body size-corrected) scale size against $PC1_{clim}$ **(A-B)**, and ancestry **(C-D)**, respectively. Colors denote transect number. Asterisks indicate statistical significance among transect intercepts.

Fig. 3: — Skin resistance against water loss. Scatterplots of cutaneous desiccation (i.e., fully evaporated "empty", or "not empty") after (top) 90h (CD_{90}) and (bottom) 120h (CD_{120}) for all three transects.

Fig. 4: — Size variation in anole skin scales. Images of the skin surface of two similar-sized anoles from Florida populations in the north (top) and south (bottom) illustrating the relative larger scales of anoles from the south.

Fig. 5: — The effect of phenology on hydric balance. (A) Body size, **(B)** mass, and **(C)** TEWL of anoles from five populations (transect 1) sampled during a first expedition, early in the season (in March; blue color) and re-sampled during a second expedition, late in the season (in July; red color).

Fig. 6: — Genetic architecture of hydric balance. (A) Genome-wide association for TEWL (top) and relative scale size (bottom). While SNPs from 50 scaffolds were considered for these analyses, only markers on the 10 largest scaffolds are shown here, to improve readability. For TEWL, the arrow points to one ancestry-specific QTL on chromosome 3 that passed the suggestive association threshold. No significant associations were identified for relative scale size. **(B)** Mean (+/- SE) TEWL for each genotype class at the TEWL chromosome 3 QTL, shown separately for *A. sagrei* with limited and common hybrid ancestry. Note that the C/C genotype class was excluded given that only one individual from the hybridization limited group had this genotype.

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