

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

Simon Baeckens^{1,2}, Jonathan B. Losos³, Duncan J. Irschick⁴, Jason J. Kolbe⁵,
Dan G. Bock^{1,6}

¹Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States

²Functional Morphology Lab, Department of Biology, University of Antwerp, Antwerp, Belgium

³Department of Biology, Washington University, St. Louis, MO, United States

⁴Department of Biology, University of Massachusetts, Amherst, MA, United States

⁵Department of Biological Sciences, University of Rhode Island, Kingston, RI, United States

⁶Centre for Biodiversity Genomics, University of Guelph, Guelph, Canada

Corresponding author: Evolution and Optics of Nanostructures Group, Department of Biology, Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium.

Email: simon.baeckens@uantwerp.be

S.B. and D.G.B. contributed equally to the work.

Abstract

As anthropogenic activities are increasing the frequency and severity of droughts, understanding whether and how fast populations can adapt to sudden changes in 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 the 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. Furthermore, 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 the water balance of invasive brown anoles is dictated by an 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, *Anolis sagrei*, rapid evolution, evaporative water loss, scalation

Desiccation poses a threat for all terrestrial organisms, but especially for those inhabiting xeric environments with limited or variable access to water resources (Alpert, 2005; Brown, 1968). The high demands of conserving water in arid habitats often lead to morphological and physiological adaptations that reduce an organism's water loss by evaporation (Alpert, 2005; Lundholm, 1976). 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 & Cox, 2015; Le Gaillard 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 (Chiang et al., 2021; Park et al., 2018), 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 & 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 &

Lee, 2020), allowing invasive populations to better cope with the novel environments they encounter (Bates & Bertelsmeier, 2021; Mooney & Cleland, 2001; Shine, 2012). Due to the abrupt nature of these human-assisted introduction events, selection can be strong and phenotypic change rapid (Hodgins et al., 2018; Prentis et al., 2008; Whitney & Gabler, 2008). The fruit fly *Drosophila subobscura*, for example, evolved an adaptive cline in wing size in only 20 years after the 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 (Huey et al., 2005; Moran & Alexander, 2014; Reznick et al., 2019; Stockwell et al., 2003).

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 introduction in the mid- to late-1800s (Williams, 1969), the species colonized the entire peninsula and expanded to the north and west (Bock et al., 2021; Kolbe et al., 2004). 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; see online supplementary material, 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., Dlugosch et al., 2015; Keller & Taylor, 2008; 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 & Schmidt-Nielsen, 1966; Dmi'el 1985, 2001; Kobayashi et al., 1983), we measured skin resistance to water loss and skin morphology, in addition to TEWL. 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 (Horton, 1972; Krakauer, 1970), 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., Kolbe et al., 2012; Losos et al., 1997), 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 the water balance physiology of invasive brown anoles is dictated by an 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.

Materials and methods

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 (Figure 1; see online supplementary material, Figure 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 revisited 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, US), 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 14 hr daylight/10 hr darkness scheme. Lizards were fed crickets (dusted with multivitamin powder) three times per week. We refer to De Meyer et al. (2019) for further details on the standardized housing conditions used in this study.

Water loss experiments

We measured both “total evaporative water loss” (TEWL, i.e., the combined effect of cutaneous water loss and water lost *via* the respiratory system), and skin resistance to water loss or “cutaneous water loss” (CWL, i.e., water lost through the skin epidermis) to assess patterns of water balance regulation in invasive *A. sagrei*. We used a subset of lizards ($N = 566$; see online supplementary material, Table S1) that survived transport from the field and an initial acclimation period of 20–30 days to comfortable hydric and thermic housing conditions (see above) for the TEWL experiments. We measured rates of TEWL following previous studies (e.g., Gunderson et al., 2011; Kolbe et al., 2014). Briefly, we quantified the change in body mass (as percentage mass lost) for lizards placed in an incubator (Percival Scientific, Inc., Perry, IA, USA) set at a constant temperature (30°C) and relative humidity (30%). These conditions aimed to replicate an arid environment with high evaporative potential (Greve et al., 2019). We chose not to expose the lizards to more extreme conditions to reduce

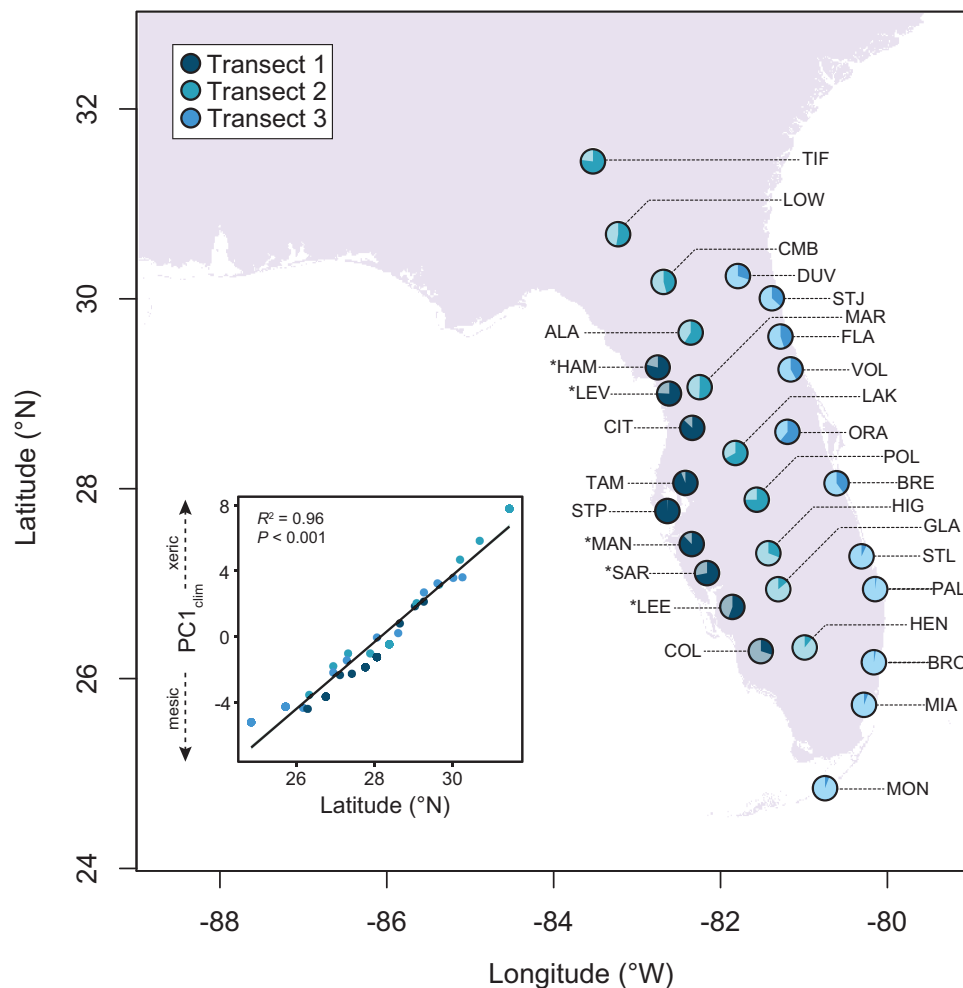


Figure 1. Geographical distribution of the *Anolis sagrei* populations used in this study. Shades of blue coloration illustrate the three different transects. For details on population IDs, see online supplementary material, [Table S1](#). Populations annotated with an asterisk were re-sampled in July to assess the role of phenology on the traits of interest. The opaque white pie slice overlaying the blue disks represents the population frequency of ancestry from Western Cuba (in %). The scatterplot shows the correlation between latitude and local climatic conditions (as $PC1_{clim}$; see methodology) of the study populations.

animal discomfort and the risk of fatalities. Experiments started in the morning before misting and one day after feeding. We weighed lizards twice, once before and once after a period of 5 hr in the incubator using an electronic balance (precision = 0.001 g). To facilitate airflow and to reduce animal activity during the experiments, lizards were placed individually in plastic mesh bags and suspended inside the dark incubator. Five lizards defecated during the experiments; hence, water loss data from these individuals were removed from further analyses. All experimental procedures described were approved by the Harvard University Institutional Animal Care and Use Committee (IACUC protocol # 26-11).

After completion of the TEWL rate experiments and following trait data collection for related projects, we euthanized all animals and excised from each individual a small section of skin, which we temporally stored in 70% ethanol until further processing. Liquid preservation has no significant effect on anole skin surface structure (Baeckens et al., 2019). This skin section consisted of the outer epidermis (circa 1 cm²) on the flank of the body (dorsolateral), posterior to the midpoint between the pectoral and pelvic girdle (see also Baeckens et al., 2019). Following scale morphology measurements, skin patches were used to estimate skin (trans-epidermal)

resistance to water loss following the protocols of Roberts and Lillywhite (1983) and Kattan and Lillywhite (1989). To do so, excised skin samples were first removed from the ethanol, lightly brushed with a fine paintbrush to remove any surface debris, dehydrated in a graded ethanol series, and air-dried. Next, we filled capless plastic PCR tubes with 200 μ l distilled water and covered the open top (which has an opening of 0.5 cm² diameter) of each tube with a single patch of skin (with the outer side of the skin facing upward). 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 CWL 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 hr (“CWL₉₀”) and, again, after 120 hr (“CWL₁₂₀”). Tubes that lost all their water after 90 or 120 hr were not weighted again; instead, they were classified as “empty” for an additional (binomial) variable, henceforth referred to as “cutaneous desiccation” (“CD₉₀” or “CD₁₂₀” depending on the time it took for the water to fully evaporate).

Skin morphology

We measured the 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 the 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 the log-transformed scale area against the log-transformed SVL and extracting the residual values for each individual.

Climate space

We extracted climate data from the WorldClim database ([Fick & Hijmans, 2017](#)), using the geographical location of each study population. WorldClim provides long-term (monthly) average climate conditions on a spatial resolution of 1 km² ([Fick & 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 & 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 $Q = P / ((T_{\max} + T_{\min}) \times (T_{\max} - T_{\min})) \times 1,000$. A lower Q indicates arid environments, whereas a higher Q indicates mesic environments (e.g., [Baeckens et al., 2018](#); [Hlubeň et al., 2021](#); [Muñoz-García et al., 2022](#); [Oufiero et al., 2011](#); [Tieleman et al., 2003](#); [Wegener et al., 2014](#)). 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 & Hijmans, 2017](#)), a PCA is particularly useful as it uses an orthogonal transformation to convert a set of correlated variables into a set of orthogonal, uncorrelated axes ([Bolker, 2008](#)). The number of nontrivial components to be retained was determined based on the Kaiser-Guttman criterion ([Peres-Neto et al., 2005](#)). 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%; see online supplementary material, [Figure S2](#); [Table S2](#)).

The latitudinal climate space occupied by *A. sagrei* could be largely explained by PC1 (linear model: $R^2 = 0.96$, $t = 26.59$, $p < .001$; [Figure 1](#)), and not by PC2 ($R^2 < 0.01$, $t = -0.48$, $p = .657$) or PC3 ($R^2 < 0.01$, $t = 0.01$, $p = .994$). 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} ([Figure 1](#)). Based on the loadings of PC1_{clim} (see online supplementary material, [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 toward the south, and a relatively

cooler and more xeric climate toward the north. PC2_{clim} can be interpreted as temperature seasonality and PC3_{clim} as temperature seasonality mixed with precipitation (see online supplementary material, [Table S2](#)).

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., 2022](#)). 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 the percentage of Western Cuba ancestry for each individual. For simplicity, we refer to this metric as “ancestry” hereafter.

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, CWL₉₀, CWL₁₂₀, CD₉₀, CD₁₂₀, 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 a 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 CD₉₀ and CD₁₂₀ (scored as either “empty” or “not empty”). To examine the relationship between the rate of total water loss and the rate of CWL, we regressed each CWL measure against TEWL (interacting with transect), with ancestry incorporated as a 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 a random variable. In all models, the interaction effect with “transect” was eliminated when nonsignificant. Also here 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 time points, in March ($n = 84$) and July ($n = 43$). We regressed each physiological and morphological variable (except for CD₉₀ and CD₁₂₀) 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 nonsignificant. 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 the 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. The 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 & Hochberg, 1995).

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 the genomic background (i.e., ancestry-specific GWAS; Rio et al., 2020; Skotte et al., 2019). 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 quantitative trait loci (QTLs) and divergent loci elsewhere in the genome (Bock et al., 2021; Mackay, 2014; Rio et al., 2020). 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 regard 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 by 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., 2022). 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 & 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 was 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 nonreference alleles) as the predictor variable. Lastly, we estimated the effect sizes of QTL alleles for each group as linear model R^2 values.

Results

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 the south-eastern US. The TEWL 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 ($p < .001$). While there was no clear trend toward a lower TEWL in lizards from the north that inhabit more arid environments ($PC1_{\text{clim}}$, $F = 3.22$, $p = .084$; Figure 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 ($F = 4.70$, $p = .031$; Figure 2C). We also found a significant effect of sampling transect on TEWL (Figure 2A and C). The average TEWL of lizards from transect 1 (0.96%) was significantly, albeit marginally, higher than lizards from transect 2 (0.77%; $t = 3.20$, $p = .008$) and transect 3 (0.86%; $t = 2.71$, $p = .018$); TEWL did not significantly differ among lizards from transects 2 and 3 ($t = 0.47$, $p = .639$). Also, $PC2_{\text{clim}}$ and $PC3_{\text{clim}}$ did not significantly correlate with TEWL (all $p > .4$; see online supplementary material, Table S4).

Rates of CWL 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 a within-population variance. Neither of the two measures were significantly related to $PC1_{\text{clim}}$ (CWL_{90} , $F = 0.18$, $p = .674$; CWL_{120} , $F = 0.10$, $p = .758$) or to $PC2_{\text{clim}}$ and $PC3_{\text{clim}}$ (all $p > .3$; see online supplementary material, Table S4). Similar to TEWL, CWL_{90} was dependent on ancestry ($F = 10.83$, $p = .001$) and transect, with CWL_{90} values being higher in transect 1 than in transects 2 ($t = 2.61$, $p = .023$) and 3 ($t = 3.17$, $p = .012$); no significant CWL_{90} difference was found between transects 2 and 3 ($t = 1.01$, $p = .321$). In contrast, we found no effect of the transect ($F = 1.05$, $p = .363$) or ancestry ($F = 2.77$, $p = .097$) on variation in CWL_{120} . Analyses on our second proxy for skin resistance against water loss—“cutaneous desiccation”—did show an effect of $PC1_{\text{clim}}$ (CD_{90} , $\chi^2 = 7.29$, $p = .007$; CD_{120} , $\chi^2 = 3.58$, $p = .058$) but not of $PC2_{\text{clim}}$ or $PC3_{\text{clim}}$ (all $p > .3$; see online supplementary material, 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 (Figure 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} , $z = 2.70$, $p = .021$; CD_{120} , $z = 1.89$, $p = .059$) and not for transect 2 (CD_{90} , $z = 0.28$, $p = .781$; CD_{120} ,

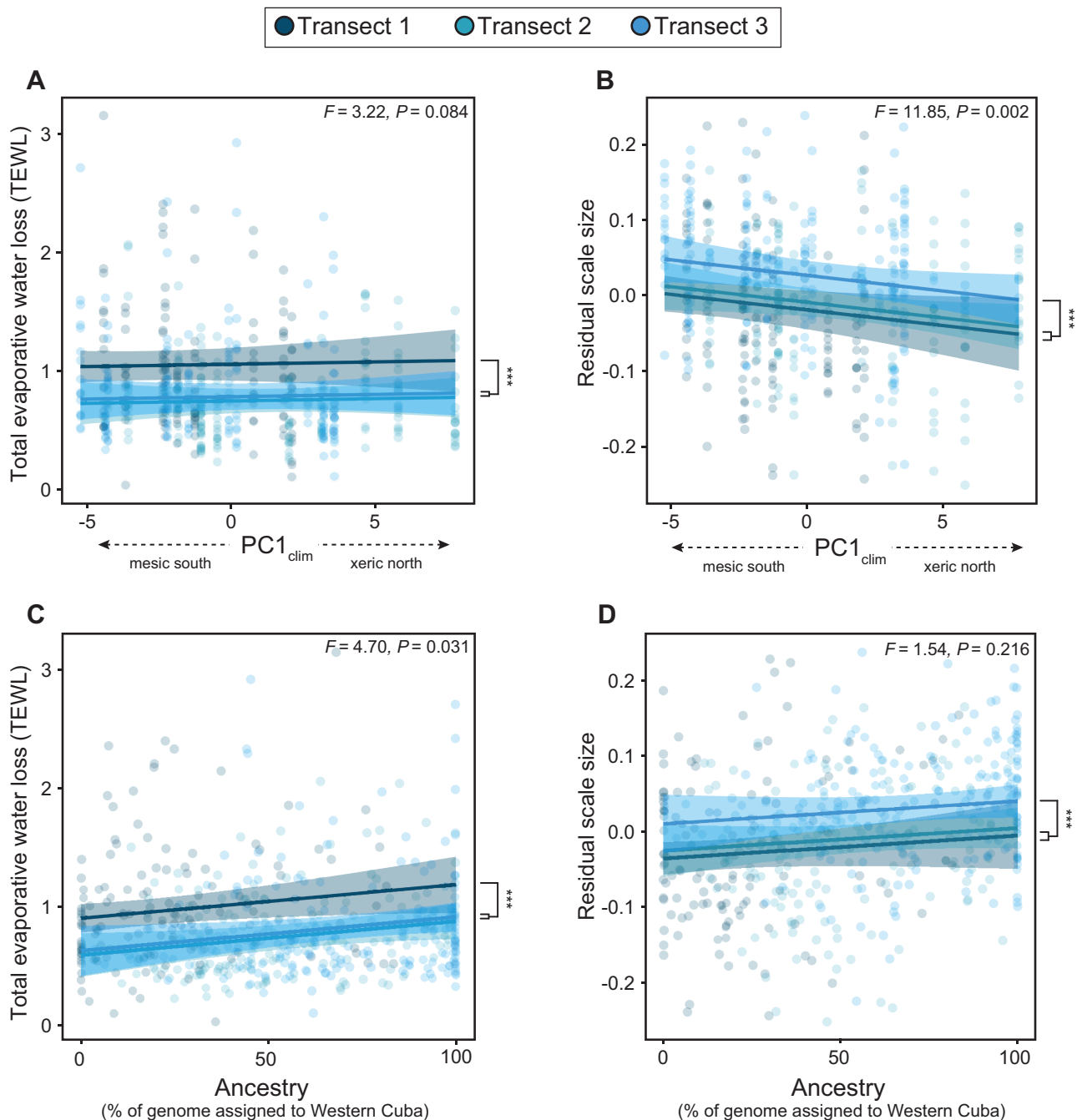


Figure 2. Variation in water balance physiology and skin morphology in invasive brown anoles. Scatterplots showing total evaporative water loss and residual (i.e., body size-corrected) scale size against $PC1_{clim}$ (A and B), and ancestry (C and D), respectively. Colors denote transect numbers. Asterisks indicate statistical significance among transect intercepts.

$z = 0.34$, $p = .973$) or transect 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 hr (transect 1: 8.7%; transect 2: 9.4%; and transect 3: 3.9%), which increased to circa 13.6% after 120 hr (transect 1: 17.4%; transect 2: 18.2%; and transect 3: 5.1%). Ancestry did not affect CD_{90} ($\chi^2 = 0.27$, $p = .607$) and CD_{120} ($\chi^2 = 2.21$, $p = .137$).

Neither body mass nor size significantly differed among lizards that inhabit different climate conditions (mass, $F = 2.53$, $p = .124$; size, $F = 1.14$, $p = .295$) or that were sampled on different transects (mass, $F = 0.69$, $p = .508$; size, $F = 1.59$, $p = .224$); only lizards from transect 3 were marginally, but

significantly, bigger (13% in mass and 5% in size) than lizards from transect 1 (mass, $t = 2.30$, $p = .030$; size, $t = 2.29$, $p = .030$). Ancestry, however, determined both mass ($F = 5.26$, $p = .022$) and size ($F = 3.39$, $p = .066$) 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 ($F = 1.54$, $p = .216$; Figure 2D), but did significantly vary across areas of different aridity and among transects (Figure 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 ($F = 11.85$, $p = .002$; Figure 2B). Moreover, the average relative scale size of lizards sampled

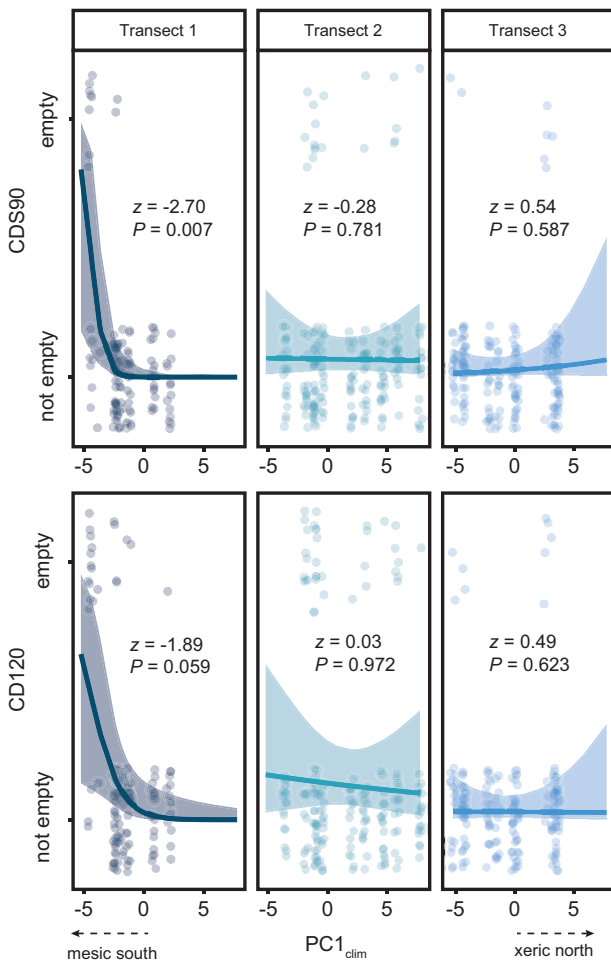


Figure 3. Variation in skin resistance against water loss in invasive brown anoles. Scatterplots of cutaneous desiccation (i.e., fully evaporated “empty,” or “not empty”) after (top) 90 hr (CD_{90}) and (bottom) 120 hr (CD_{120}) for all three transects.

on transect 3 was larger than that of lizards from transects 1 ($t = 2.47, p = .031$) and 2 ($t = 2.67, p = .031$); no significant difference in relative scale size was found between transects 1 and 2 lizards ($t = 0.59, p = .561$). The relationship (slope) of relative scale size over aridity did not significantly differ among transects (interaction effect, $F = 1.75, p = .196$).

Interrelationships among measures of water loss rates and morphology

We found no significant relationship between TEWL and any of the CWL measures ($CWL_{90}, F = 0.38, p = .537$; $CWL_{120}, t = 1.95, p = .164$; $CD_{90}, \chi^2 = 0.08, p = .782$; $CD_{120}, \chi^2 = 0.03, p = .872$). Similarly, no significant relationship was found between relative scale size and any of the water loss measures (TEWL, $F = 0.05, p = .826$; $CWL_{90}, F < 0.01, p = .943$; $CWL_{120}, F = 0.03, p = .872$; $CD_{90}, \chi^2 = 0.38, p = .538$; $CD_{120}, \chi^2 = 0.05, p = .837$). Large and heavy lizards had lower rates of TEWL than small lizards of low mass (mass, $F = 39.31, p < .001$; size, $F = 12.88, p < .001$).

Phenology

On transect 1, lizards sampled in March did not significantly differ from lizards sampled in July in relative scale size

($F = 0.31, p = .583$) and CWL ($CWL_{90}, F = 1.03, p = .0314$; $CWL_{120}, F = 0.20, p = .659$). However, in July, lizards were on average 4% larger and 12% heavier, and lost 42% less water than lizards sampled in March (mass, $F = 11.62, p < .001$; size, $F = 9.63, p = .002$; TEWL, $F = 17.70, p = .001$; Figure 5).

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”; Figure 6A) on the proximal end of chromosome 3, which was associated with trait values at the suggestive genome-wide significance threshold ($p < 1.4 \times 10^{-5}$; Figure 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 ($F = 11.77, p = 8 \times 10^{-4}$; Figure 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 ($F = 0.13, p = .722$; Figure 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 which 1,399 (45.8%) were inferred to be genic, and 1,654 (54.2%) were inferred to be intergenic. Furthermore, while we emphasize that identifying candidate genes is beyond the scope of this study and likely not possible using ddRAD-seq, there were 21 genic SNPs, distributed across 11 genes (see online supplementary material, Table S6), predicted to lead to an amino acid change. None of these SNPs were, however, in strong linkage disequilibrium with the lead GWAS SNP (R^2 values ranged from 0.002 to 0.341; see online supplementary material, Table S6).

Discussion

Lack of a climate cline in evaporative water loss

The relationship between TEWL and habitat aridity, where species and populations living in arid habitats are characterized by lower TEWL, has been established in several reptile taxa (reviewed by Mautz, 1982a and Le Galliard et al., 2021), including in *Anolis* lizards in their native range in Central America and the Caribbean (e.g., Dmi'el et al., 1997; Hertz, 1980; Hillman & Gorman, 1977; Hillman et al., 1979; Sexton & Heatwole, 1968). In invasive *A. sagrei*, this pattern has previously been documented as well, albeit based on a survey of three geographically diverse populations (Kolbe et al., 2014). In contrast to these previous studies, we find little evidence for an aridity cline in TEWL among recently introduced populations of *A. sagrei* in the south-eastern US. Rather, patterns of TEWL variation among the 30 invasive populations studied here are shaped primarily by ancestry and phenology. Below, we discuss reasons that can underlie the discrepancies between our results and those of previous studies.

First, the lack of a climatic cline in TEWL can reflect constraints imposed on rapid adaptation that result from the invasion process itself. For example, hybridization among divergent lineages in invasive brown anole populations may have resulted in novel antagonistic genetic interactions among alleles at loci controlling TEWL and loci elsewhere in the genome. Indeed, the only TEWL locus that we identified using ancestry-specific GWAS appears to be exposed to such genetic interactions (Figure 6). We emphasize, however, that ddRADseq data only sparsely cover the genome, and therefore our results should not be viewed as a comprehensive

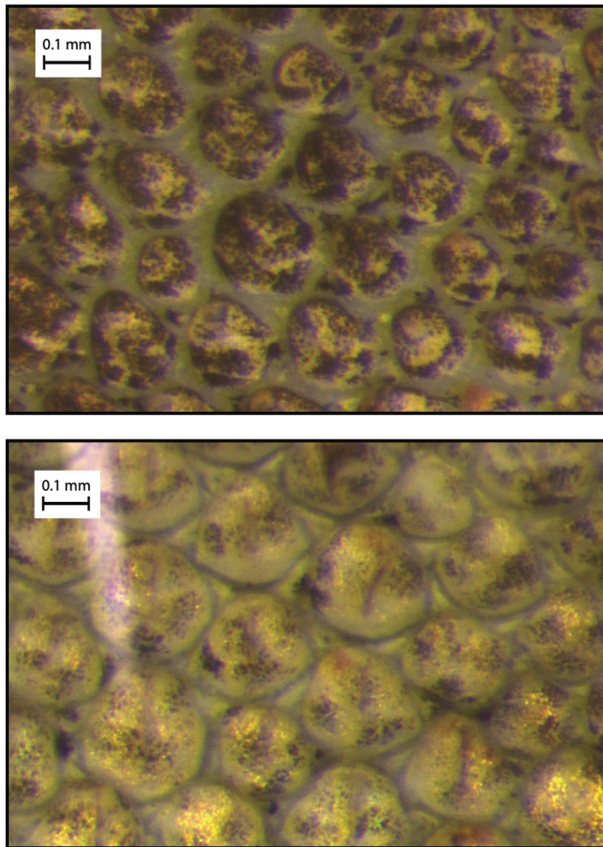


Figure 4. Size variation in anole skin scales. Images of the skin surface of two similar-sized anoles from Florida populations in the north (top) and south (bottom) illustrate the relatively larger scales of anoles from the south.

interrogation of the genetic architecture of TEWL (or scale size). Nonetheless, we note that while genetic interactions have typically been considered as contributing to intrinsic (i.e., environment-independent) genetic incompatibilities, studies in yeast (Dettman et al., 2007) and stickleback fish (Thompson et al., 2022) have provided evidence of similar 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 & Robertson, 2007). 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 (Bontrager & Angert, 2018; Tigano & Friesen, 2016). 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, the 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 the strong selection and rapid evolutionary change in morphology and physiology have been documented repeatedly for *Anolis* species, over much shorter timescales. Green anole lizards (*A. carolinensis*) in the 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 differ substantially in both breadth and magnitude from those experienced by their native island counterparts in Cuba (Angetter et al.,

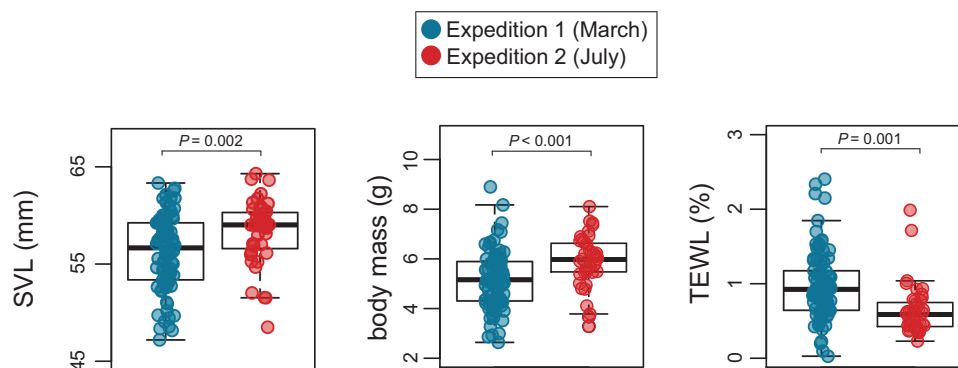


Figure 5. The effect of phenology on hydric balance. (A) Body size, (B) mass, and (C) TEWL of anoles from five populations (transect 1) sampled during a first expedition, early in the season (in March; blue color) and re-sampled during a second expedition, late in the season (in July; red color).

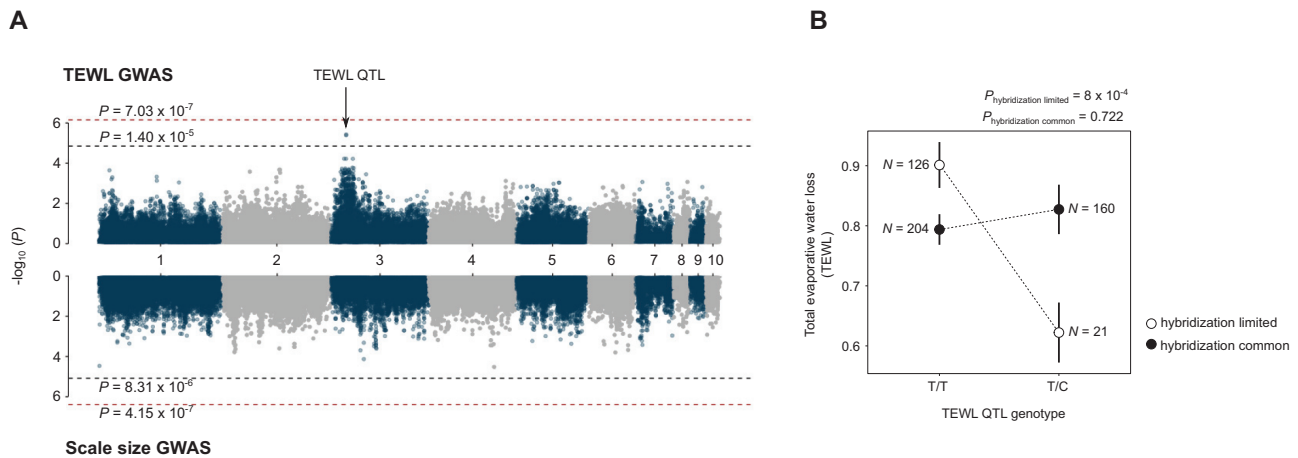


Figure 6. Genetic architecture of hydric balance. (A) Genome-wide association for TEWL (top) and relative scale size (bottom). While SNPs from 50 scaffolds were considered for these analyses, only markers on the 10 largest scaffolds are shown here, to improve readability. For TEWL, the arrow points to one ancestry-specific QTL on chromosome 3 that passed the suggestive association threshold. No significant associations were identified for relative scale size. (B) Mean (\pm 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.

2011; Kolbe et al., 2014; see online supplementary material, 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 the 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 hydoregulation, 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 & Shoemaker, 1967; Minnich & Schoemaker, 1970), transition to a more fluid-rich diet (Nagy et al., 1991; Warburg, 1964; Znari & Nagy, 1997) or close their eyes for longer periods of time while basking hence minimizing ocular water loss (Waldschmidt & 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 hydoregulatory 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 nondestructive manner.

Effects of body size and phenology on evaporative water loss

Water is lost by evaporation through several routes, including the respiratory passages, ocular membranes, excretory expenditures, and, most importantly, the skin surface—the leading avenue of water loss in squamate reptiles (Bentley & Schmidt-Nielsen, 1966; Chew & Dammann, 1961; Feder & Burggren, 1985; Lillywhite, 2006; Lillywhite & Maderson, 1982; Standaert & Johansen, 1974). Because evaporation rates are direct functions of the surface area over which flux of water occurs, laws in allometric scaling (Gould, 1971) dictate that large animals (a) lose more water and (b) have lower mass-specific water loss strictly due to decreasing ratios of surface to mass compared to small animals (Mautz, 1982a). In concordance, we find water loss to increase with strong negative allometry to body mass in brown anoles (natural log of mass lost over natural log of body mass; slope = 0.46 with CI = 0.27–0.55): large anoles lose more water than small

anoles in absolute, but not relative, terms. Increasing body size in dry habitats could thus be an alternative adaptive strategy to improving physiological capacities to resist water loss in lizards (Hlubeň et al., 2021; Oufiero et al., 2011). Our results, however, do not support this possibility. We find that while body size explains much of the among-population variation in brown anole water loss, size does not follow a latitudinal or climatic cline; rather, body size is ancestry-dependent: anoles with high proportions of Western Cuba ancestry are generally smaller and, due to a high surface-to-volume ratio, have a relatively large TEWL. The body size distribution of brown anoles across their invasion range is thus a result of introduction and hybridization history, which occurs independent of the local environment (Bock et al., 2021; Kolbe et al., 2004, 2007, 2008).

While genetically determined body size may partly explain the geographical variation in water loss, phenologically determined body size explains much of the variation observed among transects. Anoles gradually change in size over time with intervals of growth rate spurs during the wet season when food availability increases (Andrews, 1976; Dunham et al., 1988; Schoener & Schoener, 1978; Stamps, 1977). Since populations surveyed here were sampled at different time points (fieldwork spanned 81 days, from late March to early July; see online supplementary material, Table S1), we likely sampled populations at different growth stages, which may explain the smaller body size and relatively high rates of water loss of lizards sampled during the first transect, in March, earlier in the season (Figure 2). We validated this phenology effect by revisiting five populations from transect 1 (sampled in March) in July and, indeed, observed a substantial population-level increase in body size and mass, and a decrease in percentage water loss rate (Figure 5). While the effect of phenology may complicate searches for climatic patterns in animal water loss, it is an inevitable factor in extensive field studies at large geographic scales that should be taken into account.

Scalation and skin resistance to water loss

The scaled integument is a significant avenue of water loss in squamates (Bentley & Schmidt-Nielsen, 1966; Lillywhite & Maderson, 1982). Specifically, the hinge regions between the scales are often considered the dominant routes for cutaneous water movement because interscalar tissue (as opposed to scale tissue) contains a thinner layer of keratin and hence, a lower diffusion distance for passive water exchange (Horton, 1972; Maderson, 1972; Minnich, 1982). Following this premise, one expects (a) squamates with larger scales and lower interscalar tissue surface area to have a higher skin resistance to water loss than those with smaller scales and (b) arid dwelling squamates to have larger scales than those inhabiting mesic environments (Horton, 1972; Lillywhite & Maderson, 1982; Warburg, 1966). Indeed, in a range of different lizard groups, scale size was found to be inversely correlated with water loss rates (*Sphaerodactylus*: MacLean & Holt, 1979; MacLean, 1985; *Sceloporus*: Acevedo, 2009) and positively correlated with habitat aridity (e.g., *Sceloporus*: Oufiero et al., 2011, Wishingrad & Thomson, 2020; *Liolaemus*: Tulli & Cruz, 2018; *Uta*: Soule, 1966; *Gallotia*: Thorpe & Baez, 1987), including *Anolis* (Wegener et al., 2014) and even *A. sagrei* in their native range (Calsbeek et al., 2006; Lister, 1976). Contrary to expectations based on these prior studies, in invasive populations of *A. sagrei*, we find no evidence for a

relationship between scale size and evaporative water loss and an inverse relationship between scale size and habitat aridity.

First, the lack of a relationship between scalation and water loss in invasive brown anoles might be due to a low contribution of CWL relative to other avenues of evaporative water loss. This seems unlikely, however, because rates of CWL typically exceed respiratory water loss in various species of squamates (Le Galliard et al., 2021; Mautz, 1982b; Standaert & Johansen, 1974), including the congener *A. cristatellus* for which CWL comprises roughly three-quarters of the TEWL (Dmi'il et al., 1997). Second, water loss might occur primarily through the scale tissue rather than through the interstitial skin at scale edges, which is contrary to what was originally hypothesized by Krakauer (1970) and Horton (1972). If true, one would expect the relationship between scale size and habitat aridity to run in the opposite direction, with smaller scales in more arid environments, as has been observed in *Anolis oculatus* (Malhotra & Thorpe, 1997), for instance. This would clarify the negative relationship between habitat aridity and scale size in our dataset, yet the absence of a link between scale size and water loss prevents us from interpreting the climatic cline in scale size as an adaptive response for efficient water conservation. Third, the lipids in the epidermis may play a prime role in regulating integument permeability, rather than the scales *per se*. An increase in skin permeability following lipid extraction has been reported for *Anolis carolinensis*, which suggests that lipids may be an important component of the water barrier in anoles (Kattan & Lillywhite, 1989). Histochemical studies of the epidermis of brown anoles across their invasive range would provide valuable information on how lipid quality and quantity are involved in evolutionary or physiological adjustments to habitat. Fourth, functional trade-offs may constrain scale size evolution. The skin plays a crucial role in many functions other than protecting against extreme hydric and thermic conditions, such as contributing to structural coloration (e.g., Nicolai et al., 2021; Saenko et al., 2013) and taking part in locomotion (e.g., Martinez et al., 2021; Spinner et al., 2013) and body cleansing (e.g., Hiller, 2009; Watson et al., 2015). If two or more functions pose conflicting demands on the same scale design, then simultaneous “optimization” becomes impossible, and trade-offs will result in a compromise phenotype (reviewed by Garland et al., 2022). Future studies that integrate skin biomechanics, functional morphology, and phylogenetic comparative methods are encouraged as they may reveal the existence of trade-offs with other relevant functions or may indicate other constraints to biological “optimization.”

Conclusion

The brown anole is an emerging model organism that has served as a workhorse of evolutionary and ecological research for more than six decades (Geneva et al., 2022; Losos, 2009). Over this timespan, numerous experimental and observational studies have documented natural selection and local adaptation in populations in the native range (reviewed in Losos, 2009). Following the human-mediated introduction of this species to the south-eastern US, *A. sagrei* spread rapidly such that it now represents the most abundant terrestrial vertebrate in peninsular Florida (Campbell, 2000). The invasive range of this species in Florida is also characterized by a novel climate, with relatively drier conditions than those typical of its Caribbean ancestral range (Angetter et al., 2011). Given these considerations, we set out expecting to find a climatic cline in TEWL

and in skin scale size, in a direction that is consistent with the adaptive divergence of brown anole populations across 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 the local adaptation of native *A. sagrei* populations, but for which invasive populations show limited evidence of adaptation (Bock et al., 2021; Kolbe et al., 2007). 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 multipronged 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., 2014).

Data availability

Raw sequence data are stored in the Sequence Read Archive (SRA) under BioProject accession number PRJNA737437. Additional files related to the reference genome are archived on Harvard Dataverse, <https://doi.org/10.7910/DVN/TTKBFU>. Other data and codes can be downloaded from FigShare (10.6084/m9.figshare.21303798).

Author contributions

S.B., J.B.L., D.J.I., J.J.K., and D.G.B. designed the 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.

Funding statement

This work was made possible by funding from the Fonds Wetenschappelijk Onderzoek (FWO 12I8819N, 12I8822N) and the Belgian American Educational Foundation (BAEF) (to S.B.); National Science Foundation Grant DEB-1927194 (to J.B.L.); National Science Foundation Grant DEB-1354897 and funds from the University of Rhode Island (to J.J.K.); and a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship, a Banting Postdoctoral Fellowship, and a Barbour award from the Harvard Museum of Comparative Zoology (to D.G.B.). Additionally, this project was made possible through the support of a grant from the John Templeton Foundation. The opinions expressed in this publication are those of the authors and do not necessarily reflect the views of the John Templeton Foundation.

Conflict of interest: The authors have no conflict of interest to declare.

Acknowledgments

We thank J.N. Pita-Aquino and Z.A. Chejanovski for support in the field, M. Baeckens for help with scalation imag-

ing, C. Hahn and M. Gage for assistance with lizard care, and R. Stelkens and two anonymous reviewers for providing constructive comments on an earlier version of the manuscript. We are also most grateful to the late J. Breeze for his help with setting up and managing the lizard common garden, as well as for intellectual input on Bostonian music and ice-cream parlors in New England.

References

- Acevedo, G.A. 2009. *Ecomorphology of the Mexican fence lizards of the Sceloporus formosus group (Squamata: Phrynosomatidae)*. PhD Dissertation. The University of Texas at Arlington.
- Addo-Bediako, A., Chown, S. L., Gaston, K. J. (2001). Revisiting water loss in insects: A large scale view. *Journal of Insect Physiology*, 47(12), 1377–1388. [https://doi.org/10.1016/S0022-1910\(01\)00128-7](https://doi.org/10.1016/S0022-1910(01)00128-7)
- 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. *Proceedings of the National Academy of Sciences USA*, 114(9), 2283–2288. <https://doi.org/10.1073/pnas.1613625114>
- Alpert, P. (2005). The limits and frontiers of desiccation-tolerant life. *Integrative and Comparative Biology*, 45(5), 685–695. <https://doi.org/10.1093/icb/45.5.685>
- Andrews, R. M. (1976). Growth rate in island and mainland anoline lizards. *Copeia*, 477(3), 482.
- Angetter, L. S., Lötters, S., & Rödder, D. (2011). Climate niche shift in invasive species: The case of the brown anole. *Biological Journal of the Linnean Society*, 104(4), 943–954. <https://doi.org/10.1111/j.1095-8312.2011.01780.x>
- Baeckens, S., Martín, J., García-Roa, R., Pafilis, P., Huyghe, K., & Van Damme, R. (2018). Environmental conditions shape the chemical signal design of lizards. *Functional Ecology*, 32, 566–580. <https://doi.org/10.1111/1365-2435.12984>
- Baeckens, S., Temmerman, M., Gorb, S. N., Neto, C., Whiting, M. J., & Van Damme, R. (2021). Convergent evolution of skin surface microarchitecture and increased skin hydrophobicity in semi-aquatic anole lizards. *Journal of Experimental Biology*, 224(19), jeb.242939. <https://doi.org/10.1242/jeb.242939>
- Baeckens, S., Wainwright, D. K., Weaver, J. C., Irschick, D. J., & Losos, J. B. (2019). Ontogenetic scaling patterns of lizard skin surface structure as revealed by gel-based stereo-profilometry. *Journal of Anatomy*, 235(2), 346–356. <https://doi.org/10.1111/joa.13003>
- Bates, O. K., & Bertelsmeier, C. (2021). Climatic niche shifts in introduced species. *Current Biology*, 31(19), R1252–R1266. <https://doi.org/10.1016/j.cub.2021.08.035>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bentley, P. J., & Schmidt-Nielsen, K. (1966). Cutaneous water loss in reptiles. *Science*, 152(3717), 1547–1549. <https://doi.org/10.1126/science.151.3717.1547>
- Bock, D. G., Baeckens, S., Pita-Aquino, J. N., Chejanovski, Z. A., Michaelides, S. N., Muralidhar, P., Lapiedra, O., Park, S., Menke, D. B., Geneva, A. J., Losos, J. B., & Kolbe, J. J. (2021). Changes in selection pressure can facilitate hybridization during biological invasion in a Cuban lizard. *Proceedings of the National Academy of Sciences USA*, 118(42), e2108638118. <https://doi.org/10.1073/pnas.2108638118>
- Bock, D. G., Kantar, M. B., Caseys, C., Matthey-Doret, R., & Riesberg, L. H. (2018). Evolution of invasiveness by genetic accommodation. *Nature Ecology and Evolution*, 2(6), 991–999. <https://doi.org/10.1038/s41559-018-0553-z>

- Bolker, B.M. 2008. *Ecological models and data in R*. Princeton University Press.
- Bontrager, M., & Angert, A. L. (2018). Gene flow improves fitness at a range edge under climate change. *Evolution Letters*, 3(1), 55–68. <https://doi.org/10.1002/evl3.91>
- Bradshaw, S. D., & Shoemaker, V. H. (1967). Aspects of water and electrolyte changes in a field population of *Amphibolurus* lizards. *Comparative Biochemistry and Physiology*, 20(3), 855–865. ISSN 0010-406X. [https://doi.org/10.1016/0010-406X\(67\)90058-8](https://doi.org/10.1016/0010-406X(67)90058-8)
- Brown, G. 1968. *Desert biology: Special topics on the physical and biological aspects of arid regions*, Vol. I. Academic Press.
- Calsbeek, R. Knouft, J. H., & Smith, T. B. (2006). Variation in scale numbers is consistent with ecologically based natural selection acting within and between lizard species. *Evolutionary Ecology*, 20, 377–394. <https://doi.org/10.1007/s10682-006-0007-y>
- Campbell, T.S. 2000. *Analysis of the effects of an exotic lizard (Anolis sagrei) on a native lizard (Anolis carolinensis) in Florida, using islands as experimental units*. PhD Dissertation. University of Tennessee at Knoxville.
- Campbell-Staton, S. C., Bare, A., Losos, J. B., Edwards, S. E., & Cheviron, Z. A. (2018). Physiological and regulatory underpinnings of geographic variation in reptilian cold tolerance across a latitudinal cline. *Molecular Ecology*, 27(9), 2243–2255. <https://doi.org/10.1111/mec.14580>
- Campbell-Staton, S. C., Cheviron, Z. A., Rochette, N., Catchen, J., Losos, J. B., & Edwards, S. V. (2017). Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science*, 357(6350), 495–498. <https://doi.org/10.1126/science.aam5512>
- Campbell-Staton, S. C., Winchell, K. M., Rochette, N. C., Fredette, J., Maayen, I., Schweizer, R. M., & Catchen, J. (2020). Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. *Nature Ecology and Evolution*, 4(4), 652–658. <https://doi.org/10.1038/s41559-020-1131-8>
- Chew, R. M., & Dammann, A. E. (1961). Evaporative water loss of small vertebrates, as measured with an infrared analyzer. *Science*, 133(3450), 384–385. <https://doi.org/10.1126/science.133.3450.384>
- Chiang, F., Mazdiyasni, O., & AghaKouchak, A. (2021). Evidence of anthropogenic impacts on global drought frequency, duration, and intensity. *Nature Communications*, 12(1), 2754. <https://doi.org/10.1038/s41467-021-22314-w>
- Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)*, 6(2), 80–92. <https://doi.org/10.4161/fly.19695>
- Corl, A., Bi, K., Luke, C., Challa, A. S., Stern, A. J., Sinervo, B., & Nielsen, R. (2018). The genetic basis of adaptation following plastic changes in coloration in a novel environment. *Current Biology*, 28(18), 2970–2977.e7. <https://doi.org/10.1016/j.cub.2018.06.075>
- Cox, C. L., & Cox, R. M. (2015). Evolutionary shifts in habitat aridity predict evaporative water loss across squamate reptiles. *Evolution*, 69(9), 2507–2516. <https://doi.org/10.1111/evo.12742>
- De Meyer, J., Donihue, C.M., Scantlebury, D., Ng, J., Glor, R.E., Losos, J.B. & Geneva, A.J. 2019. Protocol for setting up and rearing a successful lizard room. In J. T. Stroud, A. J. Geneva, & J. B. Losos (Eds.), *Anolis newsletter VII* (pp. 37–62). Washington University.
- Dettman, J. R., Sirjusingh, C., Kohn, L. M., & Anderson, J. B. (2007). Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature*, 447(7144), 585–588. <https://doi.org/10.1038/nature05856>
- DLugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., & Gillette, H. D. (2015). The devil is in the details: Genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology*, 24(9), 2095–2111. <https://doi.org/10.1111/mec.13183>
- Dmi'el, R. (1985). Effect of body size and temperature on skin resistance to water loss in a desert snake. *Journal of Thermal Biology*, 10(3), 145–149. ISSN 0306-4565. [https://doi.org/10.1016/0306-4565\(85\)90018-X](https://doi.org/10.1016/0306-4565(85)90018-X)
- Dmi'el, R. (2001). Skin resistance to evaporative water loss in reptiles: A physiological adaptive mechanism to environmental stress or a phylogenetically dictated trait? *Israel Journal of Zoology*, 47(1), 56–67. Taylor & Francis. <https://doi.org/10.1560/ENQ9-KD7R-WFGW-KUQW>
- Dmi'el, R., Perry, G., & Lazell, J. (1997). Evaporative water loss in nine insular populations of the lizard *Anolis cristatellus* group in the British Virgin Islands. *Biotropica*, 29, 111–116. <https://doi.org/10.1111/j.1744-7429.1997.tb00012.x>
- Donihue, C. M., Herrel, A., Fabre, A. C., Kamath, A., Geneva, A. J., Schoener, T. W., Kolbe, J. J., & Losos, J. B. (2018). Hurricane-induced selection on the morphology of an island lizard. *Nature*, 560(7716), 88–91. <https://doi.org/10.1038/s41586-018-0352-3>
- Donihue, C. M., Kowaleski, A. M., Losos, J. B., Algar, A. C., Baeckens, S., Buchkowski, R. W., Fabre, A. C., Frank, H. K., Geneva, A. J., Reynolds, R. G., Stroud, J. T., Velasco, J. A., Kolbe, J. J., Mahler, D. L., & Herrel, A. (2020). Hurricane effects on neotropical lizards span geographic and phylogenetic scales. *Proceedings of the National Academy of Sciences USA*, 117(19), 10429–10434. <https://doi.org/10.1073/pnas.2000801117>
- Dunham, A. E., Miles, D. B., & Reznick, D. N. 1988. Life history patterns in squamate reptiles. In C. C. Gans, & R. B. Huey (Eds.), *Biology of the reptilia*, Vol. 16, *Ecology B defense and life history* (pp. 443–320). Academic Press.
- Feder, M. E., & Burggren, W. W. (1985). Cutaneous gas exchange in vertebrates: Design, patterns, control and implications. *Biological Reviews of the Cambridge Philosophical Society*, 60(1), 1–45. <https://doi.org/10.1111/j.1469-185x.1985.tb00416.x>
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302–4315. <https://doi.org/10.1002/joc.5086>
- Garland, T. Jr, Downs, C. J., & Ives, A. R. (2022). Trade-offs (and constraints) in organismal biology. *Physiological and Biochemical Zoology*, 95(1), 82–112. <https://doi.org/10.1086/717897>
- Geneva, A. J., Park, S., Bock, D. G., de Mello, P. L. H., Sarigol, F., Tollis, M., Donihue, C. M., Reynolds, R. G., Feiner, N., Rasys, A. M., Lauderdale, J. D., Minchey, S. G., Alcalá, A. J., Infante, C. R., Kolbe, J. J., Schluter, D., Menke, D. B., & Losos, J. B. (2022). Chromosome-scale genome assembly of the brown anole (*Anolis sagrei*), an emerging model species. *Communications Biology*, 5(1), 1126. <https://doi.org/10.1038/s42003-022-04074-5>
- Gould, S. J. (1971). Geometric similarity in allometric growth: A contribution to the problem of scaling in the evolution of size. *American Naturalist*, 105(942), 113–136.
- Grant, P. R., Grant, B. R., Huey, R. B., Johnson, M. T. J., Knoll, A. H., & Schmitt, J. (2017). Evolution caused by extreme events. *Philosophical Transactions of the Royal Society London B: Biological Sciences*, 372(1723), 20160146. <https://doi.org/10.1098/rstb.2016.0146>
- Greve, P., Roderick, M. L., Ukkola, A. M., & Wada, Y. (2019). The aridity Index under global warming. *Environmental Research Letters*, 14(12), 124006. IOP Publishing. <https://doi.org/10.1088/1748-9326/ab5046>
- Gunderson, A. R., Siegel, J., & Leal, M. (2011). Tests of the contribution of acclimation to geographic variation in water loss rates of the West Indian lizard *Anolis cristatellus*. *Journal of Comparative Physiology B*, 181(7), 965–972. <https://doi.org/10.1007/s00360-011-0576-0>
- Hertz, P. E. (1980). Responses to dehydration in *Anolis* lizards sampled along altitudinal transects. *Copeia*, 440(3), 446. <https://doi.org/10.2307/1444519>
- Hill, W. G., & Robertson, A. (2007). The effect of linkage on limits to artificial selection. *Genetical Research*, 89(5-6), 311–336. <https://doi.org/10.1017/S001667230800949X>
- Hiller, U. 2009. Water repellence in gecko skin: How do geckos keep clean? In S. N. Gorb (Ed.), *Functional surfaces in biology* (pp. 47–53). Springer.

- Hillman, S. S., & Gorman, G. C. (1977). Water loss, desiccation tolerance, and survival under desiccating conditions in 11 species of Caribbean *Anolis*: Evolutionary and ecological implications. *Oecologia*, 29(2), 105–116. <https://doi.org/10.1007/BF00345791>
- Hillman, S., Gorman, G. C., & Thomas, R. (1979). Water loss in *Anolis* lizards: Evidence for acclimation and intraspecific differences along a habitat gradient. *Comparative Biochemistry and Physiology Part A: Physiology*, 62(2), 491–493. ISSN 0300-9629. [https://doi.org/10.1016/0300-9629\(79\)90091-4](https://doi.org/10.1016/0300-9629(79)90091-4)
- Hlubeň, M., Kratochvíl, L., Gvoždík, L., & Strarostová, Z. (2021). Ontogeny, phylogeny and mechanisms of adaptive changes in evaporative water loss in geckos. *Journal of Evolutionary Biology*, 34(8), 1290–1301. <https://doi.org/10.1111/jeb.13891>
- Hodgins, K. A., Bock, D. G., & Rieseberg, L. H. (2018). Trait evolution in invasive species. In J. A. Roberts (Ed.), *Annual Plant Reviews Online*. <https://doi.org/10.1002/9781119312994.apr0643>
- Horton, D. R. (1972). Lizard scale size and adaptation. *Systematic Zoology*, 21(4), 441–443. <https://doi.org/10.1093/sysbio/21.4.441>
- Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D., & Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science*, 287(5451), 308–309. <https://doi.org/10.1126/science.287.5451.308>
- Huey, R.B., Gilchrist, G.W. & Hendry A.P. 2005. Using invasive species to study evolution: case studies with *Drosophila* and salmon. In D. F. Sax, J. J. Stachowicz, & S. D. Gaines (Eds.), *Species invasions: Insights into ecology, evolution and biogeography* (pp. 139–164). Sinauer Associates.
- Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27, 3070–3071.
- Kattan, G. H., & Lillywhite, H. B. (1989). Humidity acclimation and skin permeability in the lizard *Anolis carolinensis*. *Physiological Zoology*, 62(2), 593–606.
- Keller, S. R., & Taylor, D. R. (2008). History, chance and adaptation during biological invasion: Separating stochastic phenotypic evolution from response to selection. *Ecology Letters*, 11(8), 852–866. <https://doi.org/10.1111/j.1461-0248.2008.01188.x>
- Kobayashi, D., Mautz, W. J., & Nagy, K. A. (1983). Evaporative water loss: Humidity acclimation in *Anolis carolinensis* lizards. *Copeia*, 1983(3), 701–704. <https://doi.org/10.2307/1444335>
- Kolbe, J. J., Ehrenberger, J. C., Moniz, H. A., & Angilletta, M. J. Jr. (2014). Physiological variation among invasive populations of the brown anole (*Anolis sagrei*). *Physiological and Biochemical Zoology*, 87(1), 92–104. <https://doi.org/10.1086/672157>
- Kolbe, J. J., Glor, R. E., Schettino, L. R., Lara, A. C., Larson, A., & Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, 431(7005), 177–181. <https://doi.org/10.1038/nature02807>
- Kolbe, J. J., Larson, A., & Losos, J. B. (2007). Differential admixture shapes morphological variation among invasive populations of the lizard *Anolis sagrei*. *Molecular Ecology*, 16(8), 1579–1591. <https://doi.org/10.1111/j.1365-294X.2006.03135.x>
- Kolbe, J. J., Larson, A., Losos, J. B., & de Queiroz, K. (2008). Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. *Biology Letters*, 4(4), 434–437. <https://doi.org/10.1098/rsbl.2008.0205>
- Kolbe, J. J., Leal, M., Schoener, T. W., Spiller, D. A., & Losos, J. B. (2012). Founder effects persist despite adaptive differentiation: A field experiment with lizards. *Science*, 335(6072), 1086–1089. <https://doi.org/10.1126/science.1209566>
- Krakauer, T. 1970. *The ecological and physiological control of water loss in snakes*. PhD Dissertation. University of Florida at Gainesville.
- Kueffer, C., Pysek, P., & Richardson, D. M. (2014). Integrative invasion science: Model systems, multi-site studies, focused meta-analysis and invasion syndromes. *New Phytologist*, 200(3), 615–633. <https://doi.org/10.1111/nph.12415>
- Le Galliard, J.-F., Chabaud, C., de Andrade, D. O. V., Brischoux, F., Carretero, M. A., Dupoué, A., Gavira, R. S. B., Lourdais, O., Sannolo, M., & Van Dooren, T. J. M. (2021). A worldwide and annotated database of evaporative water loss rates in squamate reptiles. *Global Ecology and Biogeography*, 30, 1938–1950. <https://doi.org/10.1111/geb.13355>
- Lertzman-Lepofsky, G. F., Kissel, A. M., Sinervo, B., & Palen, W. J. (2020). Water loss and temperature interact to compound amphibian vulnerability to climate change. *Global Change Biology*, 26(9), 4868–4879. <https://doi.org/10.1111/gcb.15231>
- Lillywhite, H. B. (2006). Water relations of tetrapod integument. *Journal of Experimental Biology*, 209(Pt 2), 202–226. <https://doi.org/10.1242/jeb.02007>
- Lillywhite, H. B., & Maderson, P. F. A. (1982). Skin structure and permeability. In C. C. Gans, & F. H. Pough (Eds.), *Biology of the reptilia*, Vol. 12: *Physiology* (pp. 397–422). Academic Press.
- Lister, B. C. (1976). The nature of niche expansion in West Indian *Anolis* lizards I: Ecological consequences of reduced competition. *Evolution*, 30(4), 659–676. <https://doi.org/10.1111/j.1558-5646.1976.tb00947.x>
- Lodge, D. (1993). Biological invasions: Lessons for ecology. *Trends in Ecology and Evolution*, 8(4), 133–137. [https://doi.org/10.1016/0169-5347\(93\)90025-K](https://doi.org/10.1016/0169-5347(93)90025-K)
- Losos, J. B. (2009). *Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles*. University of California Press.
- Losos, J. B., Warheitt, K. I., & Schoener, T. W. (1997). Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature*, 387, 70–73. <https://doi.org/10.1038/387070a0>
- Lundholm, B. (1976). Adaptations in arid ecosystems. *Ecological Bulletins*, 24, 19–27. <https://www.jstor.org/stable/20112537>
- Mackay, T. F. (2014). Epistasis and quantitative traits: using model organisms to study gene-gene interactions. *Nature Reviews Genetics*, 15(1), 22–33. <https://doi.org/10.1038/nrg3627>
- MacLean, W. P. (1985). Water-loss rates of *Sphaerodactylus parthenopion* (Reptilia: Gekkonidae), the smallest amniote vertebrate. *Comparative Biochemistry and Physiology Part A: Physiology*, 82, 759–761.
- MacLean, W. P., & Holt, R. D. (1979). Distributional patterns in St. Croix *Sphaerodactylus* lizards: The taxon cycle in action. *Biotropica*, 11, 189–195.
- Maderson, P. F. A. (1972). When? Why? and How? Some speculations on the evolution of the vertebrate integument. *American Zoologist*, 12(1), 159–171. <https://doi.org/10.1093/icb/12.1.159>
- Malhotra, A., & Thorpe, R. S. (1997). Size and shape variation in a Lesser Antillean anole, *Anolis oculatus* (Sauria: Iguanidae) in relation to habitat. *Biological Journal of the Linnean Society*, 60(1), 53–72. <https://doi.org/10.1111/j.1095-8312.1997.tb01483.x>
- Martinez, A., Nguyen, D., Basson, M. S., Medina, J., Irschick, D. J., & Baeckens, S. (2021). Quantifying surface topography of biological systems from 3D scans. *Methods in Ecology and Evolution*, 12, 1265–1276. <https://doi.org/10.1111/2041-210X.13603>
- Mautz, W. J. (1982a). Correlation of respiratory and cutaneous water losses of lizards with habitat aridity. *Journal of Comparative Physiology*, 149, 25–30. <https://doi.org/10.1007/BF00735711>
- Mautz, W. J. (1982b). Patterns of evaporative water loss. In F. H. Pough, & C. C. Gans (Eds.), *Biology of the reptilia*, Vol. 12: *Physiological ecology* (pp. 443–481). Academic Press.
- Minnich, J.E. 1982. The use of water. In C. C. Gans, & F. H. Pough (Eds.), *Biology of the Reptilia*, *Physiology C* (pp. 325–396). Academic Press.
- Minnich, J. E., & Shoemaker, V. H. (1970). Diet, behaviour and water turnover in the desert iguana, *Dipsosaurus dorsalis*. *American Midland Naturalist*, 84(2), 496–509. <https://doi.org/10.2307/2423863>
- Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences USA*, 98(10), 5446–5451. <https://doi.org/10.1073/pnas.091093398>
- Moran, E. V., & Alexander, J. M. (2014). Evolutionary responses to global change: Lessons from invasive species. *Ecology Letters*, 17(5), 637–649. <https://doi.org/10.1111/ele.12262>
- Muñoz-García, A., Ben-Hamo, M., Pilosof, S., Williams, J. B., & Korine, C. (2022). Habitat aridity as a determinant of the trade-off between water conservation and evaporative heat loss in bats.

- Journal of Comparative Physiology B*, 192(2), 325–333. <https://doi.org/10.1007/s00360-021-01425-2>
- Nagy, K. A., Clarke, B. C., Seely, M. K., Mitchell, D., & Lighton, J. R. (1991). Water and energy balance in Namibian Desert sand-dune lizards, *Angolosaurus skoogi* (Andersson, 1916). *Functional Ecology*, 5(6), 731–739. <https://doi.org/10.2307/2389535>
- Nicolai, M. P. J., D'Abla, L., Goldenberg, J., Gansemans, Y., Van Nieuwburgh, F., Clusella-Trullas, S., & Shawkey, M. D. (2021). Untangling the structural and molecular mechanisms underlying colour and rapid colour change in a lizard, *Agama atra*. *Molecular Ecology*, 30(10), 2262–2284. <https://doi.org/10.1111/mec.15901>
- Oufiero, C. E., Gartner, G. E., Adolph, S. C., & Garland, T. Jr. (2011). Latitudinal and climatic variation in body size and dorsal scale rows in *Sceloporus* lizards: A phylogenetic perspective. *Evolution*, 65(12), 3590–3607. <https://doi.org/10.1111/j.1558-5646.2011.01405.x>
- Park, C. E., Jeong, S. J., Joshi, M., Osborn, T. J., Ho, C. H., Piao, S., Chen, D., Liu, J., Yang, H., Park, H., Kim, B. M., & Feng, S. (2018). Keeping global warming within 1.5 °C constrains emergence of aridification. *Nature Climate Change*, 8, 70–74. <https://doi.org/10.1038/s41558-017-0034-4>
- Peres-Neto, P. R., Jackson, D. A., & Somers, K. M. (2005). How many principal components? Stopping rules for determining the number of non-trivial axes revisited. *Computational Statistics and Data Analysis*, 49(4), 974–997. ISSN 0167-9473. <https://doi.org/10.1016/j.csda.2004.06.015>
- Pineiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2021). nlme: linear and nonlinear mixed effects models. R package version 3.1-152. Retrieved on 12 October 2022 from <https://cran.r-project.org/web/packages/nlme/>.
- Pirtle, E. I., Tracy, C. R. & Kearney, M. R. 2019. Hydroregulation. A neglected behavioral response of lizards to climate change? In V. Bels, & A. Russell (Eds.), *Behavior of lizards: Evolutionary and mechanistic perspectives* (pp. 343–374). CRC Press.
- Platt, A., Vilhjálmsson, B. J., & Nordborg, M. (2010). Conditions under which genome-wide association studies will be positively misleading. *Genetics*, 186(3), 1045–1052. <https://doi.org/10.1534/genetics.110.121665>
- Prentis, P. J., Wilson, J. R. U., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Plant Science*, 13(6), 288–294. <https://doi.org/10.1016/j.tplants.2008.03.004>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945–959. <https://doi.org/10.1093/genetics/155.2.945>
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Sklar, P., de Bakker, P. I., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81(3), 559–575. <https://doi.org/10.1086/519795>
- Querns, A., Wooliver, R., Vallejo-Marín, M., & Sheth, S. N. (2022). The evolution of thermal performance in native and invasive populations of *Mimulus guttatus*. *Evolution Letters*, 6(2), 136–148. <https://doi.org/10.1002/evl3.275>
- R Development Core Team. R: A Language and Environment for Statistical Computing (www.R-project.org/) (R Foundation for Statistical Computing, 2019).
- Reznick, D. N., Losos, J. B., & Travis, J. (2019). From low to high gear: There has been a paradigm shift in our understanding of evolution. *Ecology Letters*, 22(2), 233–244. <https://doi.org/10.1111/ele.13189>
- Rio, S., Mary-Huard, T., Moreau, C., Bauland, C., Palaffre, C., Madur, D., Combes, V., & Charcosset, A. (2020). Disentangling group specific QTL allele effects from genetic background epistasis using admixed individuals in GWAS: An application to maize flowering. *PLoS Genetics*, 16(3), e1008241. <https://doi.org/10.1371/journal.pgen.1008241>
- Roberts, J. B., & Lillywhite, H. B. (1983). Lipids and the permeability of the epidermis from snakes. *Journal of Experimental Zoology*, 228, 1–9. <https://doi.org/10.1002/jez.1402280102>
- Saenko, S. V., Teyssier, J., Marel, D. V., & Milinkovitch, M. C. (2013). Precise colocalization of interacting structural and pigmentary elements generates extensive color pattern variation in *Phelsuma* lizards. *BMC Biology*, 11, 105. <https://doi.org/10.1186/1741-7007-11-105>
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N., & Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics*, 32(1), 305–332. <https://doi.org/10.1146/annurev.ecolsys.32.081501.114037>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- Schoener, T. W., & Schoener, A. (1978). Estimating and interpreting body-size growth in some *Anolis* lizards. *Copeia*, 1978(3), 390–405.
- Sexton, O. J., & Heatwole, H. (1968). An experimental investigation of habitat selection and water loss in some anoline lizards. *Ecology*, 49(4), 762–767. <https://doi.org/10.2307/1935543>
- Shen, X., & Carlborg, O. (2013). Beware of risk for increased false positive rates in genome-wide association studies for phenotypic variability. *Frontiers in Genetics*, 4, 93. <https://doi.org/10.3389/fgene.2013.00093>
- Shine, R. (2012). Invasive species as drivers of evolutionary change: Cane toads in tropical Australia. *Evolutionary Applications*, 5(2), 107–116. <https://doi.org/10.1111/j.1752-4571.2011.00201.x>
- Skotte, L., Jorsboe, E., Korneliusson, T. S., Moltke, I., & Albrechtsen, A. (2019). Ancestry-specific association mapping in admixed populations. *Genetic Epidemiology*, 43(5), 506–521. <https://doi.org/10.1002/gepi.22200>
- Soule, M. (1966). Trends in the insular radiation of a lizard. *American Naturalist*, 100(910), 47–64.
- Spinner, M., Gorb, S. N., & Westhoff, G. (2013). Diversity of functional microornamentation in slithering geckos *Lialis* (Pygopodidae). *Proceedings of the Royal Society of London. Series B*, 280(1772), 20132160. <https://doi.org/10.1098/rspb.2013.2160>
- Stamps, J. A. (1977). Rainfall, moisture and dry season growth rates in *Anolis aeneus*. *Copeia*, 1977(3), 415–419.
- Standaert, T., & Johansen, K. (1974). Cutaneous gas exchange in snakes. *Journal of Comparative Physiology*, 89, 313–320. <https://doi.org/10.1007/BF00695349>
- Stern, D. B., & Lee, C. E. (2020). Evolutionary origins of genomic adaptations in an invasive copepod. *Nature Ecology and Evolution*, 4(8), 1084–1094. <https://doi.org/10.1038/s41559-020-1201-y>
- Stockwell, C. A., Hendry, A. P., & Kinnison, M. T. (2003). Contemporary evolution meets conservation biology. *Trends in Ecology and Evolution*, 18(2), 94–101. ISSN 0169-5347. [https://doi.org/10.1016/S0169-5347\(02\)00044-7](https://doi.org/10.1016/S0169-5347(02)00044-7)
- Thompson, K. A., Peichel, C. L., Rennison, D. J., McGee, M. D., Albert, A. Y. K., Vines, T. H., Greenwood, A. K., Wark, A. R., Brandvain, Y., Schumer, M., & Schluter, D. (2022). Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. *PLoS Biology*, 20(1), e3001469. ISSN 0169-5347. <https://doi.org/10.1371/journal.pbio.3001469>
- Thorpe, R. S., & Baez, M. (1987). Geographic variation within an island: Univariate and multivariate contouring of scalation, size, and shape of the lizard *Gallotia galloti*. *Evolution*, 41(2), 256–268. <https://doi.org/10.1111/j.1558-5646.1987.tb05795.x>
- Tieleman, B. I., Williams, J. B., & Blommer, P. (2003). Adaptation of metabolism and evaporative water loss along an aridity gradient. *Proceedings of the Royal Society of London. Series B*, 270(1511), 207–214. <https://doi.org/10.1098/rspb.2002.2205>
- Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, 25(10), 2144–2164. <https://doi.org/10.1111/mec.13606>
- Tulli, M. J., & Cruz, F. B. (2018). Are the number and size of scales in *Liolaemus* lizards driven by climate? *Integrative Zoology*, 13, 579–594.

- Van Sant, M. J., Oufiero, C. E., Muñoz-Garcia, A., Hammond, K. A., & Williams, J. B. (2012). A phylogenetic approach to total evaporative water loss in mammals. *Physiological and Biochemical Zoology*, 85(5), 526–532. <https://doi.org/10.1086/667579>
- Waldschmidt, S. R., & Porter, W. P. (1987). A model and experimental test of the effect of body temperature and wind speed on ocular water loss in the lizard *Uta stansburiana*. *Physiological Zoology*, 60(6), 678–686. <https://doi.org/10.1086/physzool.60.6.30159982>
- Warburg, M. R. (1964). The influence of ambient temperature and humidity on the body temperature and water loss from two Australian lizards, *Tiliqua rugosa* (Gray) (Scincidae) and *Amphibolurus barbatus cuvier* (Agamidae). *Australian Journal of Zoology*, 13(2), 331–350. <https://doi.org/10.1071/ZO9650331>
- Warburg, M. R. (1966). On the water economy of several Australian geckos, agamids, and skinks. *Copeia*, 1966(2), 230–235. <https://doi.org/10.2307/1441129>
- Watson, G. S., Green, D. W., Schwarzkopf, L., Li, X., Cribb, B. W., Myhra, S., & Watson, J. A. (2015). A gecko skin micro/nano structure—A low adhesion, superhydrophobic, anti-wetting, self-cleaning, biocompatible, antibacterial surface. *Acta Biomaterialia*, 21, 109–122. <https://doi.org/10.1016/j.actbio.2015.03.007>
- Wegener, J. E., Gartner, G. E. A., & Losos, J. B. (2014). Lizard scales in an adaptive radiation: Variation in scale number follows climatic and structural habitat diversity in *Anolis* lizards. *Biological Journal of the Linnean Society*, 113(2), 570–579. <https://doi.org/10.1111/bij.12380>
- Whitney, K. D., & Gabler, C. A. (2008). Rapid evolution in introduced species, “invasive traits” and recipient communities: Challenges for predicting invasive potential. *Diversity and Distributions*, 14, 569–580. <https://doi.org/10.1111/j.1472-4642.2008.00473.x>
- Williams, E. E. (1969). The ecology of colonization as seen in the zoogeography of anoline lizards on small islands. *Quarterly Review of Biology*, 44(4), 345–389. <https://doi.org/10.1086/406245>
- Wishegrad, V., & Thomson, R. C. (2020). Ecological variability is associated with functional trait diversity in the western fence lizard (*Sceloporus occidentalis*). *Biological Journal of the Linnean Society*, 129(2), 414–424. <https://doi.org/10.1093/biolinnean/blz179>
- Znari, M., & Nagy, K. A. (1997). Field metabolic rate and water flux in free-living Bribon’s agama (*Agama impalearis*) in Morocco. *Herpetologica*, 53(1), 81–88.