

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

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1 **Title:**

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

4

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18

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21

22 **Author contributions:**

23 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,
24 analyzed data, and wrote the original draft of the manuscript. All authors contributed to
25 revisions of the manuscript.

26

27 **Conflict of interest:**

28 The authors have no conflict of interest to declare.

29

30 **Data archiving:**

31 Raw sequence data are stored in the Sequence Read Archive (SRA) under BioProject accession
32 number PRJNA737437. Additional files related to the reference genome are archived on
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54 **Abstract:** — As anthropogenic activities are increasing the frequency and severity of droughts,
55 understanding whether and how fast populations can adapt to sudden changes to their hydric
56 environment is critically important. Here, we capitalize on the introduction of the Cuban
57 brown anole lizard (*Anolis sagrei*) in North America to assess contemporary evolution of a
58 widespread terrestrial vertebrate to an abrupt climatic niche shift. We characterized hydric
59 balance in 30 populations along a large climatic gradient. We found that while evaporative
60 and cutaneous water loss varied widely, there was no climatic cline, as would be expected
61 under adaptation. Further, the skin of lizards from more arid environments was covered with
62 smaller scales, a condition thought to limit water conservation and thus be maladaptive. In
63 contrast to environmental conditions, genome-averaged ancestry was a significant predictor
64 of water loss. This was reinforced by our genome-wide association analyses, which indicated
65 a significant ancestry-specific effect for water loss at one locus. Thus, our study indicates that
66 water balance of invasive brown anoles is dictated by environment-independent introduction
67 and hybridization history and highlights genetic interactions or genetic correlations as factors
68 that might forestall adaptation. Alternative water conservation strategies, including
69 behavioral mitigation, may influence the brown anole invasion success and require future
70 examination.

71

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

74 **1. Introduction**

75 Desiccation poses a threat for all terrestrial organisms, but especially for those inhabiting xeric
76 environments with limited or variable access to water resources (Brown 1968; Alpert 2005).
77 The high demands of conserving water in arid habitats often leads to morphological and
78 physiological adaptations that reduce an organism's water loss by evaporation (Lundholm
79 1976; Alpert 2005). Macroevolutionary studies spanning a wide range of animal groups have,
80 indeed, established a strong relationship between species' total evaporative water loss
81 (TEWL) and the hydric conditions of their local habitat: species from arid environments
82 typically lose water at a lower rate than their mesic counterparts (squamates: Cox and Cox
83 2015, Le Gailliard et al. 2021; mammals: Van Sant et al. 2012; birds: Albright et al. 2017;
84 amphibians: Lertzman-Lepofsky et al. 2020; insects: Addo-Bediako et al. 2001). Convergence
85 in reduced water loss for lineages that have colonized arid habitats demonstrates that species
86 can adjust their water loss levels to match the local hydric conditions on a macro-evolutionary
87 timescale. However, whether and how different populations respond to changes to their
88 hydric environment over micro-evolutionary timescales is less understood. Anthropogenic
89 activities are changing the Earth's climate in unprecedented ways, including by increasing the
90 frequency, duration, and intensity of droughts (Park et al. 2018; Chiang et al. 2021), which
91 makes understanding the scope of rapid adaptive responses to hydric environments critical
92 for predicting the future persistence of populations.

93

94 Species introduced by humans to areas beyond their native range can experience rapid and
95 drastic environmental change, as a direct result of the translocation event or following the
96 spread of these species outward from the site of introduction (Moran and Alexander 2014).
97 Such environmental change can involve a novel suite of interacting species or different
98 climates (Lodge 1993; Sakai et al. 2001). If the optimal phenotype to survive and reproduce
99 under novel conditions differs from the phenotype favored under ancestral conditions,
100 phenotypic change may occur via plasticity, genetic adaptation, or both (e.g., Bock et al. 2018;
101 Corl et al. 2018; Stern and Lee 2020), allowing invasive populations to better cope with the
102 novel environments they encounter (Mooney and Cleland 2001; Shine 2012; Bates and
103 Bertelsmeier 2021). Due to the abrupt nature of these human-assisted introduction events,
104 selection can be strong and phenotypic change rapid (Prentis et al. 2008; Whitney and Gabler
105 2008; Hodgins et al. 2018). The fruit fly *Drosophila subobscura*, for example, evolved an
106 adaptive cline in wing size in only 20 years after initial introduction to the Americas from their
107 native range in Afro-Eurasia (Huey et al. 2000). Therefore, biological invasions offer excellent

108 opportunities to study population responses to environmental change over contemporary
109 timescales (Stockwell et al. 2003; Huey et al. 2005; Moran and Alexander 2014; Reznick et al.
110 2019).

111

112 The invasion of the brown anole lizard (*Anolis sagrei*) in North America offers an ideal
113 opportunity to examine the micro-evolutionary response of a widespread terrestrial
114 vertebrate to an abrupt climatic niche shift. Native to the Caribbean, *A. sagrei* arrived in the
115 United States (US) from Cuba by means of repeated anthropogenic introductions at various
116 locations across Florida (Kolbe et al. 2004). Since the first introductions in the mid- to late-
117 1800's (Williams 1969), the species colonized the entire peninsula and expanded to the north
118 and west (Kolbe et al. 2004; Bock et al. 2021). Its invasive range currently spans a broad
119 latitudinal extent (~24°N to 33°N), which is much greater and well beyond that of its native
120 range in Cuba (~20°N to 23°N; Angetter et al. 2011). Consequently, the thermic and hydric
121 conditions experienced by invasive *A. sagrei* differ in both breadth and magnitude from those
122 experienced by conspecifics in the native range (Angetter et al. 2011; Kolbe et al. 2014; Table
123 S5).

124

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

134

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

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

155

156 **2. Materials and methods**

157 **2. 1. Animal sampling and housing**

158 In 2018, we captured 589 adult male brown anoles by noose from 30 populations across
159 Florida and southern Georgia (US). Populations were distributed along three latitudinal
160 transects (Fig. 1; Fig. S1; Table S1). Transect 1 (west) was sampled in March (n = 151; 9
161 populations), transect 2 (central) was sampled in May (n = 195; 10 populations), and transect
162 3 (east) was sampled in July (n = 200; 11 populations). To assess the consistency of our traits
163 of interest over time (i.e., phenological effect), we re-visited five populations from transect 1
164 in July, and caught an additional 43 individuals (hereafter referred to as temporal replicates).
165 After capture, lizards were transported to the animal facility at Harvard University
166 (Cambridge, MA, USA), where they were housed individually in custom-built acrylic terraria of
167 36 cm high, 30 cm deep, and 14 cm wide. Each terrarium contained a layer of autoclaved
168 organic potting soil as substrate, plastic foliage, and a wooden dowel (1 cm diameter) for
169 perching. Room temperature was maintained at 28°C and terraria were misted at least twice
170 a day with reverse osmosis water to sustain around 80% relative humidity in the lizard
171 enclosures. Water-vapor resistant (F32T8) fluorescent bulbs provided proper lighting that
172 followed a 14h daylight/10h darkness scheme. Lizards were fed crickets (dusted with
173 multivitamin powder) three times per week. We refer to Meyer et al. (2019) for further details
174 on the standardized housing conditions used in this study.

175

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 cm²) 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 cm²

210 diameter) of each tube with a single patch of skin (with the outer side of the skin facing
211 upwards). We used thermoplastic stretch film to tightly seal the patch edges to the outside of
212 the tube. In this way, the tube could only lose water as vapor through the excised skin
213 epidermis. Rates of cutaneous water loss were measured by calculating the change in mass
214 (as percentage lost) of the skin-wrapped tubes held at a constant temperature (30°C) and
215 relative humidity (30%) in the incubator. Each test tube was weighted to the nearest 0.0001
216 g before and after a period in the incubator of 90 hours (“CWL₉₀”) and, again, after 120 hours
217 (“CWL₁₂₀”). Tubes that lost all their water after 90 or 120 hours were not weighted again;
218 instead, they were classified as “empty” for an additional (binomial) variable, henceforth
219 referred to as “cutaneous desiccation” (“CD₉₀” or “CD₁₂₀” depending on the time it took for
220 the water to fully evaporate).

221

222 **2. 3. Skin morphology**

223 We measured average scale size from each individual skin sample by digitizing (using ImageJ;
224 Schneider et al. 2012) the surface area of nine different scales (following Baeckens et al. 2019,
225 2021) on images obtained with a stereomicroscope (Leica M165 C). We then calculated mean
226 scale surface area per individual. Additionally, we obtained data on body length (as snout-to-
227 vent length, SVL) for all lizards using digital calipers. Relative scale size was then calculated by
228 regressing log-transformed scale area against log-transformed SVL and extracting the residual
229 values for each individual.

230

231 **2. 4. Climate space**

232 We extracted climate data from the WorldClim database (Fick and Hijmans 2017), using the
233 geographical location of each study population. WorldClim provides long-term (monthly)
234 average climate conditions on a spatial resolution of 1 km² (Fick and Hijmans 2017). We
235 retrieved data on 19 bioclimatic variables (BIO1-BIO19) representing different measures for
236 annual trends, seasonality, and extremes of temperature and precipitation (Fick and Hijmans
237 2017). In addition, we gathered data on the average annual precipitation (P), the highest
238 monthly mean temperature (T_{max}), and the lowest monthly mean temperature (T_{min}) to
239 calculate a single measure for aridity, the log₁₀(Q) index, where $Q = P / ((T_{max} + T_{min}) \times (T_{max} -$
240 $T_{min})) \times 1000$. A lower Q indicates arid environments, whereas a higher Q indicates mesic
241 environments (e.g., Tieleman et al. 2003, Oufiero et al. 2011; Wegener et al. 2014; Baeckens
242 et al. 2018; Hlubeň et al. 2021; Muñoz-Garcia et al. 2022). To reduce the number of climatic
243 variables for subsequent analyses, we performed a principal component analysis (PCA) with

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

252

253 The latitudinal climate space occupied by *A. sagrei* largely could be largely explained by PC1
254 (linear model: $R^2 = 0.96$, $t = 26.59$, $P < 0.001$; Fig. 1), and not by PC2 ($R^2 < 0.01$, $t = -0.48$, $P =$
255 0.657) or PC3 ($R^2 < 0.01$, $t = 0.01$, $P = 0.994$). Hence, PC1 was used in all further climate-related
256 analyses as the main climate variable (hereafter coined “PC1_{clim}”) describing the latitudinal
257 climate extent of the brown anole invasion. Relative to northern populations, southern
258 populations are characterized by high negative values for PC1_{clim} (Fig. 1). Based on the loadings
259 of PC1_{clim} (Table S2), southern populations inhabit a tropical climate with high mean
260 temperatures, little annual temperature change, and rainfall all year round with summer
261 peaks. By contrast, northern populations experience lower annual temperatures, especially
262 during winters, and little precipitation seasonality. The invasive range of *A. sagrei* in the south-
263 eastern US thus shows a strong latitudinal climate gradient, with a relatively warmer and more
264 humid climate towards the south, and a relatively cooler and more xeric climate towards the
265 north. PC2_{clim} can be interpreted as temperature seasonality and PC3_{clim} as temperature
266 seasonality mixed with precipitation (Table S2).

267

268 **2. 5. Ancestry inference**

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

278 with a burn-in of the same length. We then identified the run with the highest Ln probability
279 of the data from which we extracted ancestry proportions. This approach partitions the
280 ancestry of each individual as either Western Cuba ancestry or admixed ancestry (Bock et al.
281 2021). We used this information to extract percentage Western Cuba ancestry for each
282 individual. For simplicity, we refer to this metric as “ancestry” hereafter.

283

284 **2. 6. Statistics**

285 To assess whether variation in climate conditions can explain variation in morphology and
286 physiology in *A. sagrei*, we regressed each response variable separately (i.e., TEWL, CWL₉₀,
287 CWL₁₂₀, CD₉₀, CD₁₂₀, relative scale size, body mass, SVL) against each of the three PC_{clim}
288 separately. In each linear mixed-effect model, we included “transect” (three-level factor) as a
289 fixed effect (interacting with PC_{clim}) and also “ancestry” as fixed effect. Here, and in all other
290 models, the factor “population” was included as a random effect to avoid pseudo-replication.
291 We used the function *lme* (*nlme* package, Pinheiro et al. 2021) for all continuous dependent
292 variables, and *glmer* (binomial distribution; *lme4* package, Bates et al. 2015) for the binomial
293 variables CD₉₀ and CD₁₂₀ (scored as either “empty” or “not empty”). To examine the
294 relationship between the rate of total water loss and the rate of cutaneous water loss, we
295 regressed each CWL measure against TEWL (interacting with transect), with ancestry
296 incorporated as random variable. Lastly, to examine the relationship between the two water
297 loss measurements (CWL and TEWL) and skin morphology, we regressed each water loss
298 measurement against relative scale size (interacting with transect), also with ancestry as
299 random variable. In all models, the interaction effect with “transect” was eliminated when
300 non-significant. Also, the factor “population” was included as a random effect to avoid
301 pseudo-replication. Additionally, all the above models included only individuals obtained
302 during our first visit of each population (i.e., we excluded the 43 individuals considered as
303 temporal replicates).

304 To test for an effect of phenology on our traits of interest, we compared lizards from
305 the five populations on transect 1 sampled at two timepoints, in March (n = 84) and July (n =
306 43). We regressed each physiological and morphological variable (except for CD₉₀ and CD₁₂₀)
307 separately against PC1_{clim} interacting with “time of sampling” (two-level factor, with “March”
308 corresponding to the March trip, and “July” corresponding to the July trip). The interaction
309 was eliminated from the models when non-significant. The variables CD₉₀ and CD₁₂₀ were
310 excluded from these models, as the percentages of fully evaporated tubes were too low for

311 the models to converge. Similar to models described above for the complete dataset,
312 “population” was included as a random effect.

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

318

319 **2. 7. Genome-wide association of hydric balance traits**

320 Recent methodological developments in genome-wide association study (GWAS) allow the
321 identification of alleles with effects that vary depending on genomic background (i.e.,
322 ancestry-specific GWAS; Skotte et al. 2019; Rio et al. 2020). In systems where admixture is
323 frequent, as is the case for invasive *A. sagrei*, such genomic background effects are likely to
324 arise due to epistatic interactions between focal QTLs and divergent loci elsewhere in the
325 genome (Mackay 2014; Rio et al. 2020; Bock et al. 2021). Thus, while ancestry-specific GWAS
326 studies based on ddRADseq do not provide a complete view of the genetic architecture of
327 traits due to the sparser coverage of the genome, they can be informative with regards to the
328 effect of ancestry on trait variation in hybrids when ancestry-specific associations are
329 identified. Moreover, these GWAS approaches include steps for mitigating the effects of
330 population structure and are optimized for recently admixed populations (e.g., Skotte et al.
331 2019).

332

333 To conduct an ancestry-specific GWAS, we followed the methods described in Bock et al.
334 (2021). We included the TEWL and relative scale size traits, which we analyzed in conjunction
335 with 120,232 quality-filtered SNPs from the 50 largest scaffolds in the *A. sagrei* reference
336 genome (Geneva et al. 2021). We then relied on the GWAS model implemented in asaMap
337 (Skotte et al. 2019). To correct for population structure, we included as covariates the first 10
338 principal components from a genetic PCA, following Skotte et al. (2019). The genetic PCA was
339 calculated in the *adegenet* R package (v. 2.1.1; Jombart and Ahmed, 2011), and used 10,000
340 random genome-wide SNPs. Lastly, to obtain additional information on SNPs spanning any
341 significant GWAS association peaks, we annotated all markers using snpEff v. 5.0 (Cingolani et
342 al. 2012). SNPs predicted to lead to an amino acid change that also overlapped an association
343 peak were further tested for linkage disequilibrium versus the lead GWAS SNP using PLINK
344 v1.90b6.24 (Purcell 2007).

345

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

360

361 **3. Results**

362 **3. 1. Variation in hydric balance traits across a climatic gradient**

363 We found considerable variation in rates of water loss and skin morphology in *A. sagrei* across
364 its invasive range in south-eastern US. The total evaporative water loss of the lizards in our
365 study ranged from 0.04% to 3.15% (of body mass lost) with among-population variance in
366 TEWL being 3.4 times greater than the within-population variance ($P < 0.001$). While there
367 was no clear trend towards a lower TEWL in lizards from the north that inhabit more arid
368 environments ($PC1_{\text{clim}}$, $F = 3.22$, $P = 0.084$; Fig. 2A), we found that rates of water loss were
369 significantly affected by anole ancestry: lizards with high percentages of Western Cuba
370 ancestry showed high TEWL rates ($F = 4.70$, $P = 0.031$; Fig. 2C). We also found a significant
371 effect of sampling transect on TEWL (Fig. 2A,C). Average TEWL of lizards from transect 1
372 (0.96%) was significantly, albeit marginally, higher than lizards from transect 2 (0.77%; $t = 3.20$,
373 $P = 0.008$) and transect 3 (0.86%; $t = 2.71$, $P = 0.018$); TEWL did not significantly differ among
374 lizards from transect 2 and 3 ($t = 0.47$, $P = 0.639$). Also, $PC2_{\text{clim}}$ and $PC3_{\text{clim}}$ did not significantly
375 correlate with TEWL (all $P > 0.4$; Table S4).

376

377 Rates of cutaneous water loss ranged from 7.67% to 62.20% for CWL_{90} , and from 9.40% to
378 62.49% for CWL_{120} ; among-population variance was respectively, 2.8 and 2.2 times greater

379 than within-population variance. Neither of the two measures were significantly related to
380 PC1_{clim} (CWL₉₀, $F = 0.18$, $P = 0.674$; CWL₁₂₀, $F = 0.10$, $P = 0.758$) or to PC2_{clim} and PC3_{clim} (all $P >$
381 0.3 ; Table S4). Similar to TEWL, CWL₉₀ was dependent on ancestry ($F = 10.83$, $P = 0.001$) and
382 transect, with CWL₉₀ values being higher in transect 1 than in transect 2 ($t = 2.61$, $P = 0.023$)
383 and 3 ($t = 3.17$, $P = 0.012$); no significant CWL₉₀ difference was found between transects 2 and
384 3 ($t = 1.01$, $P = 0.321$). In contrast, we found no effect of transect ($F = 1.05$, $P = 0.363$) or
385 ancestry ($F = 2.77$, $P = 0.097$) on variation in CWL₁₂₀. Analyses on our second proxy for skin
386 resistance against water loss —“cutaneous desiccation”— did show an effect of PC1_{clim} (CD₉₀,
387 $\chi^2 = 7.29$, $P = 0.007$; CD₁₂₀, $\chi^2 = 3.58$, $P = 0.058$) and of PC2_{clim} or PC3_{clim} (all $P > 0.3$; Table S4).
388 More specifically, water-filled tubes covered with skin from lizards that inhabit mesic
389 environments dried up significantly faster than did tubes covered with skin from lizards that
390 inhabit arid environments (Fig. 3). In other words, cutaneous desiccation probability was
391 higher for lizards from mesic areas than for lizards from arid areas. This was, however, only
392 true for transect 1 (CD₉₀, $z = 2.70$, $P = 0.021$; CD₁₂₀, $z = 1.89$, $P = 0.059$) and not for transect 2
393 (CD₉₀, $z = 0.28$, $P = 0.781$; CD₁₂₀, $z = 0.34$, $P = 0.973$) or transect 3 (CD₉₀, $z = 0.54$, $P = 0.781$;
394 CD₁₂₀, $z = 0.49$, $P = 0.934$). Approximately 7.3% of the skin-wrapped tubes were completely
395 evaporated after 90 hours (transect 1: 8.7%; transect 2: 9.4%; transect 3: 3.9%), which
396 increased to circa 13.6% after 120 hours (transect 1: 17.4%; transect 2: 18.2%; transect 3:
397 5.1%). Ancestry did not affect CD₉₀ ($\chi^2 = 0.27$, $P = 0.607$) and CD₁₂₀ ($\chi^2 = 2.21$, $P = 0.137$).

398

399 Neither body mass nor size significantly differed among lizards that inhabit different climate
400 conditions (mass, $F = 2.53$, $P = 0.124$; size, $F = 1.14$, $P = 0.295$) or that were sampled on
401 different transects (mass, $F = 0.69$, $P = 0.508$; size, $F = 1.59$, $P = 0.224$); only lizards from
402 transect 3 were marginally, but significantly, bigger (13% in mass and 5% in size) than lizards
403 from transect 1 (mass, $t = 2.30$, $P = 0.030$; size, $t = 2.29$, $P = 0.030$). Ancestry, however,
404 determined both mass ($F = 5.26$, $P = 0.022$) and size ($F = 3.39$, $P = 0.066$) with lizards with low
405 proportions of Western Cuba ancestry being larger and heavier than those with more Western
406 Cuba ancestry. Relative scale size was unaffected by ancestry ($F = 1.54$, $P = 0.216$; Fig. 2D), but
407 did significantly vary across areas of different aridity and among transects (Fig. 4). The skin of
408 lizards originating from more arid areas was covered with smaller scales (relative to their body
409 size) than the skin of lizards inhabiting more mesic environments ($F = 11.85$, $P = 0.002$; Fig.
410 2B). Moreover, the average relative scale size of lizards sampled on transect 3 was larger than
411 that of lizards from transect 1 ($t = 2.47$, $P = 0.031$) and 2 ($t = 2.67$, $P = 0.031$); no significant
412 difference in relative scale size was found between transect 1 and 2 lizards ($t = 0.59$, $P = 0.561$).

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

415

416 **3. 2. Interrelationships among measures of water loss rates and morphology**

417 We found no significant relationship between TEWL and any of the CWL measures (CWL₉₀, F
418 $= 0.38$, $P = 0.537$; CWL₁₂₀, $t = 1.95$, $P = 0.164$; CD₉₀, $\chi^2 = 0.08$, $P = 0.782$; CD₁₂₀, $\chi^2 = 0.03$, $P =$
419 0.872). Similarly, no significant relationship was found between relative scale size and any of
420 the water loss measures (TEWL, $F = 0.05$, $P = 0.826$; CWL₉₀, $F < 0.01$, $P = 0.943$; CWL₁₂₀, $F =$
421 0.03 , $P = 0.872$; CD₉₀, $\chi^2 = 0.38$, $P = 0.538$; CD₁₂₀, $\chi^2 = 0.05$, $P = 0.837$). Large and heavy lizards
422 had lower rates of TEWL than small lizards of low mass (mass, $F = 39.31$, $P < 0.001$; size, $F =$
423 12.88 , $P < 0.001$).

424

425 **3. 3. Phenology**

426 On transect 1, lizards sampled in March did not significantly differ from lizards sampled in July
427 in relative scale size ($F = 0.31$, $P = 0.583$) and cutaneous water loss (CWL₉₀, $F = 1.03$, $P = 0.0314$;
428 CWL₁₂₀, $F = 0.20$, $P = 0.659$). However, in July, lizards were on average 4% larger and 12%
429 heavier, and lost 42% less water than lizards sampled in March (mass, $F = 11.62$, $P < 0.001$;
430 size, $F = 9.63$, $P = 0.002$; TEWL, $F = 17.70$, $P = 0.001$; Fig. 5).

431

432 **3.4. Genetic architecture of hydric balance traits**

433 The GWAS did not identify any region as significantly associated with relative scale size. By
434 contrast, for TEWL, we identified one locus (hereafter “TEWL QTL”; Fig. 6A) on the proximal
435 end of chromosome 3, which was associated with trait values at the suggestive genome-wide
436 significance threshold ($P < 1.4 \times 10^{-5}$; Fig. 6A). The GWAS model in this case considered a
437 different effect in each of the two genetic clusters (corresponding to Western Cuba ancestry
438 and to admixed ancestry). In line with this result, and as expected if population structure was
439 properly accounted for, we found that the genotype at this QTL is significantly associated with
440 TEWL values within one of our sample groups, the “hybridization limited” group ($F = 11.77$, P
441 $= 8 \times 10^{-4}$; Fig. 6B). For these *A. sagrei* samples, the TEWL QTL behaves as a medium-effect
442 locus, explaining 6.8% of trait variance. By contrast, the same two alleles do not explain any
443 trait variance in the “hybridization common” sample group ($F = 0.13$, $P = 0.722$; Fig. 6B).

444

445 The TEWL QTL spans ~35 Mb on chromosome 3 (start coordinate: ~30 Mb; end coordinate:
446 ~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)

481 identified an adaptive locus that controls limb length, and that is subject to similar deleterious
482 genetic interactions in hybrids. As well, invasive brown anoles are characterized by large-scale
483 linkage disequilibrium and limited contemporary gene flow among populations (Bock et al.
484 2021). Linkage disequilibrium among alleles under selection can restrict adaptation *via* a
485 process known as Hill-Robertson interference (Hill and Robertson 1966). Likewise, while large-
486 scale gene flow can swamp local adaptation, intermediate levels of gene flow are often
487 beneficial for adaptation in variable environments and at range margins (Tigano and Friesen
488 2016; Bontrager and Angert 2018). Thus, limited contemporary genetic exchange among
489 invasive brown anole populations, as has been documented among the populations under
490 study here (Bock et al. 2021), may be forestalling the spread of adaptive alleles.

491

492 Second, lack of adaptation in rates of water loss might be due to the limited time that has
493 passed since the establishment of invasive *A. sagrei* populations. We consider this possibility
494 less likely, however. This is because strong selection and rapid evolutionary change in
495 morphology and physiology has been documented repeatedly for *Anolis* species, over much
496 shorter timescales. Green anole lizards (*A. carolinensis*) in southern US, for instance, showed
497 greater cold tolerance and a corresponding shift in putative genomic targets of selection a
498 year after experiencing a cold snap (Campbell-Staton et al. 2017). In response to hurricanes,
499 survivors in two island populations of Southern Bahamas anoles (*A. scriptus*) had larger,
500 stronger-gripping, toepads that likely aided in their ability to cling to vegetation during high-
501 speed winds (Donihue et al. 2018, 2020). Note, however, that these examples involved rapid
502 strong directional selection imposed by acute, extreme climate events (see also Grant et al.
503 2017). In the case of brown anole populations surveyed here, selection pressure for decreased
504 TEWL in arid regions might not be strong enough. Yet, the climatic conditions experienced by
505 invasive brown anoles differs substantially in both breadth and magnitude from those
506 experienced by their native island counterparts in Cuba (Angetter et al. 2011; Kolbe et al.
507 2014; Table S5). Also, the range of climatic variation experienced by brown anoles in the US is
508 comparable to that observed in other *Anolis* species for which adaptive changes in TEWL have
509 been documented. For instance, Gunderson et al. (2011) observed significant differences in
510 water loss rates between two populations of Puerto Rican crested anoles (*A. cristatellus*) from
511 habitats that differed on average by 12% in relative humidity. This value is comparable to the
512 circa 10% difference recorded between the most northern sampled population (Tifton, GA)
513 and Miami (FL), one of our more southern populations (Kolbe et al. 2014). Future studies could
514 capitalize on extreme drought events to better understand rapid evolutionary change in *Anolis*

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

520

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

534

535 Other explanations for the absence of a climate cline in TEWL in our study might have a
536 methodological basis. First, we used online climate databases with a spatial scale of 1 km to
537 retrieve abiotic data of our study populations, rather than measuring environmental
538 conditions at the lizards' perch sites in the field (e.g., Gunderson et al. 2011). Environmental
539 data on a microgeographical scale is preferred because water loss rates are dictated by the
540 conditions of the immediate surrounding (Hertz 1980). Collecting such data is, however,
541 extremely labor intensive as it is only of value when obtained repeatedly and periodically (i.e.,
542 hourly, daily, and across all seasons). Second, there are several techniques to assess
543 evaporative water loss, which may all yield slightly variable TEWL estimates (Mautz 1982a).
544 Selecting a single method to measure TEWL in squamate reptiles is not straightforward
545 because the exact protocol depends on research questions and context (Le Galliard et al.
546 2021). We used the most widely used protocol for squamate reptiles (i.e., measurements of
547 body mass loss in the laboratory) because of its simplicity and suitability for high-throughput
548 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; Nicolai 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.

651

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

666

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1003 **Figure captions:**

1004 **Fig. 1: — Geographical distribution of the *Anolis sagrei* populations used in this study.**
1005 Shades of blue coloration illustrate the three different transects. Population IDs correspond
1006 to Table S1. Populations annotated with an asterisk were re-sampled in July to assess the role
1007 of phenology on the traits of interest. The opaque white pie slice overlaying the blue disks
1008 represent the population frequency of haplotypes from Western Cuba (in %). The scatterplot
1009 shows the correlation between latitude and local climatic conditions (as $PC1_{\text{clim}}$; see
1010 methodology) of the study populations.

1011
1012 **Fig. 2: — Water balance physiology and skin morphology.** Scatterplots showing total
1013 evaporative water loss and residual (i.e., body size-corrected) scale size against $PC1_{\text{clim}}$ **(A-B)**,
1014 and ancestry **(C-D)**, respectively. Colors denote transect number. Asterisks indicate statistical
1015 significance among transect intercepts.

1016
1017 **Fig. 3: — Skin resistance against water loss.** Scatterplots of cutaneous desiccation (i.e., fully
1018 evaporated “empty”, or “not empty”) after (top) 90h (CD_{90}) and (bottom) 120h (CD_{120}) for all
1019 three transects.

1020
1021 **Fig. 4: — Size variation in anole skin scales.** Images of the skin surface of two similar-sized
1022 anoles from Florida populations in the north (top) and south (bottom) illustrating the relative
1023 larger scales of anoles from the south.

1024
1025 **Fig. 5: — The effect of phenology on hydric balance. (A)** Body size, **(B)** mass, and **(C)** TEWL of
1026 anoles from five populations (transect 1) sampled during a first expedition, early in the season
1027 (in March; blue color) and re-sampled during a second expedition, late in the season (in July;
1028 red color).

1029
1030 **Fig. 6: — Genetic architecture of hydric balance. (A)** Genome-wide association for TEWL (top)
1031 and relative scale size (bottom). While SNPs from 50 scaffolds were considered for these
1032 analyses, only markers on the 10 largest scaffolds are shown here, to improve readability. For
1033 TEWL, the arrow points to one ancestry-specific QTL on chromosome 3 that passed the
1034 suggestive association threshold. No significant associations were identified for relative scale
1035 size. **(B)** Mean (+/- SE) TEWL for each genotype class at the TEWL chromosome 3 QTL, shown
1036 separately for *A. sagrei* with limited and common hybrid ancestry. Note that the C/C genotype
1037 class was excluded given that only one individual from the hybridization limited group had this
1038 genotype.

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