

Climate-induced patterns of activity and nest conditions select for genes associated with growth and maturation in a widespread lizard species

Christopher Parkinson

viper@clemson.edu

Clemson University <https://orcid.org/0000-0002-2020-6992>

Michael Sears

Arizona State University

Matthew McTernan

Clemson University

Article

Keywords: Climate, Biophysics, Genomics, Selection, Life History

Posted Date: June 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9941634/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations:

There is no duality of interest

Table 1 and 2 are available in the Supplementary Files section.

1 ABSTRACT:

2 How species adapt to local climates across their ranges remains a
3 central question in ecology and evolutionary biology. Though phenotypic
4 responses to climate are well documented, the genetic basis for these
5 adaptive responses remains largely unresolved. Here, we integrate
6 biophysical and genomic methods to identify genomic variants associated
7 with climate-induced patterns of activity and nest conditions across the
8 range of the Eastern fence lizard (*Sceloporus undulatus*). We first use a
9 biophysical model to estimate potential annual activity and incubation
10 temperatures for nests across the species' range. The biophysical
11 parameters were then used in gene-environmental association models to
12 identify candidate genes underlying adaptation to climate. The biological
13 functions of candidate genes were assessed through enrichment analyses.
14 Lastly, we use genome-wide linkage disequilibrium to identify evidence of
15 co-selection or epistatic interactions on candidate genes. In general, activity
16 time decreased with latitude, while the range of nest temperatures
17 increased with latitude. However, mean nest temperatures do not correlate
18 with latitude, longitude, or elevation. Genomic analyses identified 2040
19 candidate genes, of which 48 enriched for two KEGG pathways: the calcium
20 signaling pathway and the gonadotropin-releasing hormone pathway.

21 Calcium signaling dictates cell proliferation and migration through the
22 effects of growth factors (e.g., growth and development), while
23 gonadotropin-releasing hormone dictates the development of sexual traits
24 and the timing of sexual maturity. Phenotypic evidence from previous
25 studies indicates that selection on life history traits, such as body size and
26 age at maturation, underlie adaptation to climate in ectotherms, and our
27 results provide genetic support for this notion.

28

29 KEYWORDS:

30 Climate, Biophysics, Genomics, Selection, Life History

31 INTRODUCTION:

32 Understanding how species adapt to local climates throughout their
33 distribution remains a primary goal of ecological and evolutionary biology,
34 particularly as the global climate continues to shift (Franks and Hoffmann,
35 2012; Stein *et al.*, 2013; Sears *et al.*, 2019). Species occupy a realized
36 climatic niche, where their physiology has evolved to suit the environment
37 and enable population persistence (Vandermeer, 1972). However, many
38 species encounter a variety of climates, either due to expansion into new
39 areas or changes in local climate conditions. Studies investigating the
40 adaptive responses of animals to climate have documented putatively
41 adaptive responses in phenology, life history, behavior, and physiology
42 (Crozier and Hutchings, 2014; Urban *et al.*, 2014). However, while
43 phenotypic responses to climate have been well documented, the genetic
44 basis of these adaptive responses remains largely unresolved (Urban *et al.*,
45 2024). A better understanding of the genetics that facilitate adaptation to
46 climate may prove critical, as many species globally are experiencing
47 population declines due to rapid climate change (Urban, 2015, 2024) and
48 may rely on evolutionary rescue to avoid extinction (Hoffmann and Sgró,
49 2011; Carlson *et al.*, 2014). Further, genomic studies investigating climate
50 adaptation can reveal insights into less easily observed phenotypic

51 adaptations, providing a better understanding of how life adapts to climate
52 and improving predictions and conservational applications of adaptive
53 responses to climate change (Meek *et al.*, 2022; Urban *et al.*, 2024).

54 A key aspect of climate adaptation involves animals' ability to meet
55 their living costs (Wild *et al.*, 2025). These costs can be described as an
56 energy budget that must be allocated to growth, maintenance, reproduction,
57 activity, and storage (Sousa *et al.*, 2010). Within a given environment,
58 selection should dictate how energy is allocated across costs to maximize
59 fitness (Stearns, 1992). To fund the cost of living, animals must navigate
60 spatiotemporal heterogeneity in environmental temperatures to 1) avoid
61 dangerous temperature extremes (Sunday *et al.*, 2014), and 2) maintain
62 sufficient activity such that food acquisition and processing rates can fund
63 energetic demands (Congdon, 1989; Kearney, 2012). For ectotherms in
64 particular, this relationship is paramount, as they rely directly on
65 environmental heat sources to modulate body temperature (T_b), and the
66 capacity to be active and acquire food is temperature-dependent (Adolph
67 and Porter, 1993; Sinervo *et al.*, 2010). Consequently, species that span
68 climate gradients often show concordant variation in life history traits: cooler
69 climates that restrict activity produce slower-growing individuals that
70 mature at larger body sizes, whereas warmer climates produce faster-

71 growing individuals that mature at smaller body sizes (Morrison and Hero,
72 2003; Angilletta *et al.*, 2004). Larger body sizes often increase fitness by
73 increasing fecundity, while earlier maturation at smaller body sizes trades off
74 fecundity for a higher probability of survival to first reproduction (Healy *et al.*,
75 2019). If this “slow-fast continuum” of life history traits is adaptive, then
76 activity time provides a direct estimate of a major selective pressure
77 imposed by climate.

78 Selection by climate is further complicated in species with complex
79 life cycles (Kingsolver *et al.*, 2011; Levy *et al.*, 2015). While activity results
80 from mobile individuals selectively moving through their environment, early
81 life stages that are immobile (such as embryos in eggs) passively experience
82 environmental conditions. Developmental processes are temperature-
83 dependent in ectotherms, with higher temperatures increasing the rate of
84 development but reducing developmental efficiency, whereas cooler
85 temperatures either slow or arrest development entirely (Andrews *et al.*,
86 1997; Shine *et al.*, 1997; Carlo *et al.*, 2018). As a result, hotter incubation
87 conditions result in earlier hatching times but smaller individuals.
88 Conversely, cooler conditions result in delayed hatching but larger
89 hatchlings. Mothers can choose nest conditions and thus select nest sites
90 that optimize the development and survival of their offspring (Kolbe and

91 Janzen, 2002; Warner and Andrews, 2002a). However, nest conditions can
92 nonetheless vary across different climates despite maternal choice of nest
93 site (Angilletta *et al.*, 2009; Carlo *et al.*, 2018). As a result, selection may
94 adapt embryos across climates to optimize growth and development within
95 their specific nest conditions. Further, the date of oviposition and length of
96 incubation dictate the amount of potential activity hatchlings have to acquire
97 energy and grow prior to the onset of winter conditions (Sniegula *et al.*,
98 2024). Thus, incubation conditions and the knock-on effects on hatchling
99 and adult phenotypes (Jonsson *et al.*, 2022) likely pose a strong selection
100 pressure that can be used to understand adaptation to climate in species
101 with complex life cycles.

102 The Eastern fence lizard (*Sceloporus undulatus*) provides a model
103 system for studying genetic adaptation to climate. The species inhabits a
104 wide range of climates across the eastern United States (Fig. 1), and a wealth
105 of research has identified phenotypic variation that putatively adapts
106 populations to local climates. Adaptive responses in *S. undulatus* seem to
107 center on life-history traits, with individuals in cooler climates growing more
108 slowly, reaching sexual maturity later, and having larger body sizes than
109 those in warmer climates (Tinkle and Ballinger, 1972; Angilletta *et al.*, 2004;
110 Kearney, 2012). Nest conditions vary across climates (Angilletta *et al.*, 2009;

111 Carlo *et al.*, 2018), and survival rates are generally low (Tinkle and Ballinger,
112 1972; Andrews *et al.*, 2000). Thus, there is potential for strong selection on
113 developmental and growth processes during early life stages such that the
114 emergent pattern of life histories is adaptive across climates. Additionally,
115 life histories appear fixed across populations, indicating a genetic basis for
116 these traits (Niewiarowski and Roosenburg, 1993; Niewiarowski, 1995). The
117 recent publication of a *S. undulatus* reference genome (Westfall *et al.*, 2021)
118 now allows for a ‘genome up’ view through which we can identify adaptive
119 traits and/or support previous studies aimed at understanding phenotypic
120 adaptation to climate in this species.

121 In this study, we integrate biophysical and genomic methods to
122 identify genetic variants and associated genes under selection in climates
123 across the species distribution of the *S. undulatus*. We first use a biophysical
124 model to estimate potential annual activity and nest conditions for
125 populations across the full species’ range. We then use these estimates of
126 activity and nest conditions as predictors to identify genetic variants
127 putatively under selection across populations using two genome-
128 environment association (GEA) models. Genes harboring adaptive variants
129 are input into gene ontology and Kyoto Encyclopedia of Genes and Genomes
130 (KEGG) enrichment analyses to identify biological functions that may

131 underlie adaptation to climate. Lastly, we use genome-wide linkage
132 disequilibrium to identify evidence of co-selection or epistatic interactions
133 acting on candidate genes across the genome. Altogether, our study
134 leverages an integrative approach to identify the genetic basis of adaptation
135 to climate in a widespread ectothermic species.

136

137 METHODS:

138 *Sample Collection and Variant Calling*

139 We sampled 189 *S. undulatus* across 14 sites spanning the full range
140 of climates experienced across the species distribution (Fig. 1)(Table S1). Of
141 these, we sequenced 130 samples *de novo*, while we downloaded an
142 additional 59 whole-genome sequences from the National Center for
143 Biotechnology Information (accession code PRJNA656311)(Assis *et al.*,
144 2025). We extracted genomic DNA from tail tips for wild-caught individuals
145 and muscle from museum specimens using the DNeasy Blood and Tissue Kit
146 (#69506, Qiagen, Inc.). We prepared whole-genome DNA libraries using the
147 NEBNext Ultra II for DNA Library Prep kit (E6177L, NEBLabs, Inc.), with
148 indexes provided by the NEBNext Oligos for Illumina kit (E7600S, NEBLabs,
149 Inc.). We sequenced the 130 total *S. undulatus* genomic libraries on an

150 Illumina NovaSeq 6000 S4 flow cell and an Illumina NextSeq P3 flow cell,
151 with sequencing done to a target depth of 20x coverage.

152 We assessed read quality off the sequencers using FASTQC v0.12.1
153 (Andrews, 2010). Both adapter sequences and low-quality reads (Phred
154 score < 20) were trimmed using TRIM GALORE v0.6.10 (Krueger, 2015). We then
155 aligned trimmed reads to the *SceUnd_v1.1* reference genome for *S.*
156 *undulatus* (Westfall *et al.*, 2021) using BWA-MEM v0.7.17 (Li, 2013). We
157 called variants from the aligned reads using the GATK best-practices pipeline
158 (Van der Auwera *et al.*, 2013), which reduces potential bias due to batch
159 effects through joint variant calling. VCF files produced by the GATK pipeline
160 (GATK v4.6.1.0, PICARD v3.3.0) were then filtered using VCFTOOLS v0.1.17
161 (Danecek *et al.*, 2011). We removed single nucleotide polymorphisms (SNPs)
162 with minor allele frequency <5%, minimum sequencing depth <10x,
163 maximum sequencing depth >50x (to account for alignment errors or PCR
164 artifacts), and with missing data in more than 5% of individuals. We
165 additionally pruned the dataset for physically linked SNPs using the software
166 BCFtools v1.21 with the +prune plugin (Danecek *et al.*, 2021). Variants with
167 an $r^2 > 0.50$ were removed within a 10kb sliding window. The resulting filtered
168 VCF file contained 1,409,447 high-quality SNPs for use in downstream
169 analyses. We then annotated the VCF using SNPEff (Cingolani *et al.*, 2012).

170 *Nest Condition Estimates:*

171 To account for potential selection imposed by geographic variation in
172 nest incubation temperatures across the species' range, we estimated 20-
173 year averages of nest conditions for two time periods: 1941-1960 and 2001-
174 2020. Nest temperature was assumed to be equal to soil temperature, which
175 we estimated using the *NicheMapR* R package (Kearney and Porter, 2017).
176 We downloaded climate data from the ERA5 climate dataset (Hersbach et
177 al., 2020) and calculated soil temperatures using the "micro_era5()" function
178 in *NicheMapR*. We used temperatures at a soil depth of 6cm, which is the
179 typical nest depth for *S. undulatus* (Warner and Andrews, 2002a; Angilletta et
180 al., 2009; Carlo et al., 2018). To determine suitable nest conditions at each
181 site, we first calculated nest temperatures across a range of shade
182 conditions: 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 100%. For
183 each shade condition, we calculated the average temperature for each hour
184 of the year across the 20 years for each period.

185 To determine potential oviposition dates, we assumed mothers do
186 not oviposit until minimum nest temperatures are equal to or greater than
187 18°C, based on measurements of nest temperatures from the literature
188 (Warner and Andrews, 2002a; Angilletta et al., 2009; Carlo et al., 2018), and

189 knowledge that developmental rates slow down or cease at temperatures
190 less than 18°C (Christian *et al.*, 1986; Andrews *et al.*, 1997). We then
191 parameterized potential nest conditions using estimated developmental
192 time and expected survival rates across shade conditions, based on nest
193 temperatures. We used a previously published developmental time model to
194 estimate rates of development (Levy *et al.*, 2015):

195

$$196 \quad E = \left(\frac{0.00081 + 0.00067 \cdot t_{soil}}{24} \right)$$

197

198 where E is the hourly developmental rate, and t_{soil} refers to the soil
199 temperatures for a given shade value at 6 cm depth for each time point in the
200 nest temperature data. If development was not completed before the nest
201 conditions became too cold to continue, we categorized the nest as
202 unsuitable. Further, we binned embryo survivorship based on data from the
203 literature: nests that reached maximum temperatures of 42°C and above
204 resulted in 0% embryonic survival, nests that reached maximum
205 temperatures of 38-42°C resulted in 60% embryonic survival, and maximum
206 temperatures below 38°C resulted in 80% survival (Levy *et al.*, 2015; Carlo *et*
207 *al.*, 2018). Shade values that maximized embryonic survival and allowed for

complete development of embryos were considered optimal nests. We averaged optimal nest temperatures across shade conditions to obtain a single representative annual optimal nest temperature for each site within each time period. From these optimal nests, we calculated mean nest temperatures in the 1900s and 2000s, the change in mean nest temperatures between the 1900s and 2000s, the range of nest temperatures in the 1900s and 2000s, and the change in range of nest temperatures from the 1900s to 2000s.

Further, we used degree-day calculations to estimate changes in the total heat received by embryos in suitable nests between 1941-1960 and 2001-2020 (Zalom *et al.*, 1983). Degree days ($^{\circ}D$) for 2000s nests relative to 1900s nests were calculated relative to mean and maximum nest temperatures using the following equation:

$$D = \frac{(\sum_{day=1, hour=0}^{day=i, hour=j} (t_h - t_0)_{i,j})}{24}$$

where D = sum of $^{\circ}D$, t_h = mean or maximum nest temperatures ($^{\circ}C$) at time i on day j for nests in the 2000s, and t_0 = mean or maximum nest temperatures at time i on day j for nests in the 1900s.

227 *Activity Time Estimates:*

228

229 We modeled annual activity time for both adult lizards and hatchlings
230 at each site using standard biophysical models applied to site-specific
231 climate data (Campbell and Norman, 1998; Sears *et al.*, 2011). We used
232 *NicheMapR* to calculate surface soil temperatures, 2-cm air temperatures,
233 and 2-m air temperatures from the ERA5 climate data downloaded for 1941-
234 1960 and 2001-2020. We calculated air and soil temperatures in full sun and
235 shade to capture the maximum and minimum temperature profiles at each
236 site. From these temperatures, we extracted monthly maximum and
237 minimum temperatures and averaged across the two 20-year time periods
238 for input into our biophysical model. We calculated radiation using standard
239 equations (Gates, 1980).

240 We estimated annual activity time using the approach outlined in
241 Sears *et al.* (2011). We calculated annual activity as the duration an
242 individual could remain active within its preferred T_b range on a
243 representative day of each month, and used spline interpolation to estimate
244 activity for all days of the year. We calculated body temperature for each
245 representative day by solving the following equation every minute over 24
246 hours:

247

248
$$T_{b2} = T_e + e^{\left(\frac{-t}{\tau}\right)}(T_{b1} - T_e)$$

249

250 where T_e is the operative temperature of the individual, t is the elapsed time
251 in minutes, τ is the time constant of the individual, T_{b1} is the initial body
252 temperature, and T_{b2} is the final body temperature. We calculated operative
253 temperatures as follows:

254

256
$$T_e = T_a + \frac{R_{abs} - \epsilon_s \sigma (T_a + 273.15)^4}{c_p (g_r + g_{Ha})}$$

255

257 where R_{abs} is the total radiation absorbed, ϵ_s is the surface emissivity, σ is the
258 Stefan-Boltzmann constant, c_p is the specific heat of air at constant
259 pressure, g_r is the radiative conductance, and g_{Ha} is the boundary layer
260 conductance for heat. We used 2 cm air temperature (i.e., the approximate
261 mid-body height of lizards off the substrate) for T_a . We calculated longwave
262 radiation from the ground and sky using ground surface temperatures and 2
263 m air temperatures, respectively. We modeled wind at 0.1 m/s.

264 For adult activity, we parameterized the model for a representative 10
265 g Eastern fence lizard with a snout-vent length (SVL) of 65 mm and venter

266 height of 1 cm above the substrate (Tinkle and Ballinger, 1972). We modeled
267 hatchlings with a body mass of 0.5 g, SVL of 25 mm, and a venter height of
268 0.5 cm (Tinkle and Ballinger, 1972; Warner and Andrews, 2002b; Carlo *et al.*,
269 2018). After 30 days of activity, we assumed that hatchlings had grown and
270 we modeled them as having a body mass of 2.5 g, an SVL of 41 mm, and a
271 venter height of 0.75 cm (Tinkle and Ballinger, 1972; Warner and Andrews,
272 2002b). We assumed lizards at each site to be active at a given time if the
273 calculated range of possible T_b values encompassed part of the T_b range of
274 active lizards (29-35°C)(Andrews, 1998). We estimated adult activity for the
275 entire year, whereas hatchling activity began only upon completion of
276 development, as determined by the developmental time model for the nest
277 conditions at each site. We summed potential activity time to calculate the
278 average potential annual activity for each site over the two 20-year periods.
279 As *S. undulatus* is heliothermic, we limited potential activity to only times
280 when the sun was above the horizon (e.g., cosine of the zenith angle > 1). We
281 estimated potential adult and hatchling activity in the 1900s and 2000s, and
282 changes in potential adult and hatchling activity from 1900s to 2000s.

283 Further, we calculated time above CT_{max} ($TACT_{max}$) as a metric of
284 potential heat stress at each site. Using T_e estimates for an individual in full
285 sun, we summed the minutes for which $T_e > CT_{max}$ for this species. We set

286 CT_{max} at 42°C (Crowley, 1985; Claunch *et al.*, 2021) and estimated TACT_{max}
287 for adults and hatchlings in the 1900s and 2000s, and the change in TACT_{max}
288 for adults and hatchlings between 1900s and 2000s.

289

290 *Assessment of Biophysical Variables*

291 We first looked for patterns of activity and nest variables across the
292 species' range using geographic predictors. We used linear mixed models to
293 test whether latitude, longitude, or elevation predicted patterns or changes
294 over time in activity or nest conditions. Interactive effects for latitude,
295 longitude, and elevation were included in the models. We then assessed the
296 degree of covariation among biophysical predictor variables using the
297 *corrplot* R package (Wei and Simko, 2024). Due to the high degree of
298 covariation between the activity and nest variables (Fig. S1), we performed a
299 principal component analysis (PCA) to generate PC axes for use in our
300 genomic analyses. The PCA was centered and scaled to account for unit
301 differences among variables. Further, we included latitude, longitude, and
302 elevation in the PCA to account for geographic variation among populations.
303 We determined the number of PC axes to include as predictors for our
304 genomic analyses by assessing a scree plot (Fig. S2). We then visualized

305 variable loadings on the PC axes using the *ggbiplot* R package (Vu and
306 Friendly, 2024).

307

308 *Gene-Environmental Association Modeling*

309 We used two gene-environmental association (GEA) approaches to
310 detect directional selection on SNPs in response to the biophysical
311 predictors: BayeScEnv (Villemereuil and Gaggiotti, 2015) and latent-factor
312 mixed models (LFMM2)(Caye *et al.*, 2019). We chose these models due to
313 their ability to account for deviations from neutrality caused by confounding
314 variables, specifically population structure. Following the approach
315 implemented by (Salloum *et al.*, 2022), we combined results across models
316 using the geometric mean of q-values to identify candidate SNPs.
317 Significance was assessed based on a mean q-value less than 0.05. Genes
318 that harbored candidate SNPs within 5kb were considered candidate genes
319 putatively adapting populations to differences in activity and nest conditions
320 across the species' range.

321 BayeScEnv is an F_{ST} -based genome scan program that leverages
322 genetic and environmental differences among populations to identify genetic
323 variants putatively under selection. The program uses a Markov Chain Monte

324 Carlo (MCMC) algorithm to compare three competing models: a neutral
325 model, and two locus-specific models. The neutral model acts as the null
326 against which the locus-specific models are tested. The two locus-specific
327 models then account for deviations from neutrality due to: 1) divergent
328 selection driven by the predictor variable, or 2) other process different from
329 local adaptation, such as allele surfing or background selection. We ran the
330 BayeScEnv program with the following parameters: 10 pilot runs with 2,000
331 iterations each; a thinning interval of 10; 5,000 output iterations; and a burn-
332 in length of 10,000. We used default priors for the MCMC chain. We used the
333 *coda* package in R to ensure the chain reached convergence with low
334 autocorrelation (Plummer *et al.*, 2006). Convergence was tested using the
335 Heidelberg and Welch’s convergence diagnostic procedure, and
336 autocorrelation was tested using the “autocorr.diag” function.

337 The LFMM2 model uses a latent variable regression to model allele
338 frequencies as a function of the environmental variable of interest across
339 populations, while accounting for a latent factor—in this case, population
340 structure. All analyses were implemented using the *LEA* package in R
341 (Frichot and François, 2015). We first assessed population structure in our
342 data using sparse non-negative matrix factorization, implemented by the
343 “snmf” function. We ran the “snmf” function for K values 1 through 20, with

344 10 repetitions each. Because the LFMM2 model does not tolerate missing
345 data, we used the resulting snmfProject to impute missing values in our
346 genotype matrix with the “impute” function. The imputed SNP dataset was
347 then regressed over the biophysical predictors using the “lfmm2” function,
348 and significant SNPs were identified using the “lfmm2_test” function.
349 Resulting p-values were converted to q-values to correct for multiple
350 comparisons using the R package *qvalue* (Storey *et al.*, 2023).

351

352 *Gene Ontology and KEGG Enrichment Analyses*

353 We used gene ontology (GO) and Kyoto Encyclopedia of Genes and
354 Genomes (KEGG) enrichment to determine whether biological processes or
355 pathways are enriched among candidate genes identified in the GEA
356 analysis. We input the full list of candidate genes into *ShinyGO* (Xijin Ge *et*
357 *al.*, 2020), an online resource for GO and KEGG enrichment analyses and
358 graphing. We used the *S. undulatus* genome as the background reference. A
359 false discovery rate (FDR) threshold of 0.05 was used to assess the
360 significance of assigned GO terms and KEGG pathways.

361 *Genome-wide Linkage Disequilibrium*

362 Selection on complex phenotypic traits or traits under pleiotropic
363 control may result in covariance in allele frequencies across the genome.
364 Therefore, we tested for patterns of interchromosomal linkage
365 disequilibrium (LD) among candidate SNPs identified by the GEA analyses.
366 Since linkage analyses increase exponentially with each additional SNP, we
367 downsampled to one SNP per candidate gene for this analysis. We used the
368 R package *GWLD* (Zhang *et al.*, 2023) to calculate LD among all pairs of
369 SNPs, using the standard r^2 metric. SNPs found in the top 99.5th percentile
370 in the resulting r^2 distribution were designated as in significant
371 disequilibrium. Further, we compared the r^2 distribution generated from the
372 candidate SNPs with 100 randomly generated SNP datasets to assess
373 whether rates of LD in the candidate SNPs differed from background levels.
374 We randomly sampled SNPs from the full SNP dataset using BCFtools within
375 a custom bash script, and then entered them into the *GWLD* pipeline. The
376 resulting r^2 distributions were compared with the candidate SNP distribution
377 using the Wilcoxon rank-sum test and the Fisher-Pitman permutation test,
378 implemented in base R and the R package *coin* (Hothorn *et al.*, 2008),
379 respectively.

380

381

382 RESULTS:

383 *Estimates of activity time and nest conditions*

384 Latitude strongly predicted activity time estimates across both adult
385 and hatchling life stages (Table 1). Within each time and life stage, our model
386 predicted that populations at lower latitudes had greater potential annual
387 activity than those at higher latitudes (Fig. 2a-b). For adults, our model
388 estimated activity times ranging from ~2280 hrs for the lowest potential
389 activity at the Indiana site to ~3170 hrs of potential activity at the eastern
390 Florida site. However, an interaction between longitude and elevation
391 predicted a change in potential activity for adults between the 1900s and
392 2000s (Table 1), with the largest increase in potential activity between
393 centuries occurring in the Tennessee site (149 hrs, a 5.8% increase from
394 1900s to 2000s) (Fig. 2e). Likewise, an interaction between latitude,
395 longitude, and elevation predicted a change in potential activity. Our model
396 predicted that hatchlings at mid-latitude sites west of the Appalachian
397 Mountains would experience the largest increase in potential activity time,
398 with increases of up to 313 hrs (a 25% increase) in western Kentucky (Fig. 2f).

399 Time above CT_{max} ($TACT_{max}$) also varied with latitude for both adult and
400 hatchling life stages and across both time periods. Populations at lower

401 latitudes had higher potential TACT_{max} than those at higher latitudes, with
402 TACT_{max} ranging from 1286-2091 hrs in adults and from 209-1107 hrs in
403 hatchlings. Change in TACT_{max} also varied with latitude for adults, with sites
404 at low latitudes showing an increase in potential TACT_{max} from the last
405 century to this century, the largest increase being 281 hrs in western Florida.
406 However, for hatchlings, an interaction between latitude, longitude, and
407 elevation predicted the change in TACT_{max}. In general, TACT_{max} increased
408 more at lower latitudes and elevations than at higher ones, with Arkansas
409 showing the largest increase (223 hrs).

410 The relationship between nest conditions and geographic predictors
411 was more complicated than with aspects of activity or TACT_{max}. Latitude and
412 elevation predicted degree days above mean nest temperatures between the
413 1900s and 2000s. While all nests were predicted to experience warming (*i.e.*,
414 more degree days)(Fig. 2g), the degree of warming was predicted to be higher
415 at lower latitudes and lower elevations than at higher latitudes or elevations
416 (e.g., ~52 degree days in western Florida versus 9.3 degree days in Indiana).
417 Concordantly, a change in mean nest temperatures between centuries was
418 predicted by interactions between both latitude and longitude with
419 elevation. Generally, sites at lower latitudes or lower elevations were
420 predicted to have warmed more than those at higher latitudes or elevations

421 (e.g., a 0.34°C increase in western Florida versus a 0.11°C increase in
422 Indiana). The range of nest temperatures also varied predictably with latitude
423 within both centuries, with higher ranges of nest temperatures experienced
424 at higher latitudes (e.g., 8.2°C range in Indiana versus 5.1°C range in western
425 Florida) (Fig. 2d). However, the change in the range of nest temperatures
426 between centuries was not predicted by any geographic predictor (Fig. 2h).
427 Mean nest temperatures (Fig. 2c) and degree days above maximum
428 temperatures also did not vary with any geographic predictor variable.

429 All biophysical model outputs are available in supplemental materials
430 as a table (Table S2). Maps of variables not shown in Fig. 2 are also available
431 (Fig. S3).

432

433 *PCA of activity time and nest conditions*

434 There was a high degree of correlation between latitude, longitude,
435 elevation, and our estimated variables for activity, $TACT_{max}$, and nest
436 conditions (Fig. S1). As such, we conducted a PCA to reduce collinearity in
437 our predictor variables. After assessing the scree plot (Fig. S2), we selected
438 the first two PC axes as predictors for genome-environmental association
439 models. The first PC axis (PC1) accounted for 58.5% of the variance and

440 primarily loaded latitude, elevation, and variables associated with activity
441 time, TACT_{max}, nest temperature range, and change in mean nest
442 temperature (Fig. 3). PC2 accounted for 14.2% of the variation and primarily
443 loaded longitude and variables associated with nests and hatchling activity,
444 including mean nest temperatures, change in mean nest temperatures, nest
445 temperature range, change in range nest temperatures, change in hatchling
446 activity, change in TACT_{max}, and degree days above maximum nest
447 temperatures.

448

449 *Genome-Environmental Association Analyses*

450 The number of candidate SNPs varied considerably between PC1 and
451 PC2. Using the geometric mean of the q-values from the BayeScEnv and
452 LFMM2 models, we identified 41 candidate SNPs associated with PC1 and
453 4,974 candidate SNPs associated with PC2 (Fig. 4). However, there was
454 overlap between SNPs identified by PC1 and PC2, as 40 of the 41 candidate
455 SNPs associated with PC1 were also identified by PC2. Of the 41 candidate
456 SNPs identified by PC1, 28 were associated with genes, hereafter referred to
457 as “candidate genes”. Likewise, of the 4,974 candidate SNPs identified by
458 PC2, 3,341 were associated with 2039 candidate genes. These candidate

459 gene sets represent 0.13% and 9.3% of the 21,891 genes found on the
460 chromosomes of the SceUnd_v1.1 *S. undulatus* reference genome,
461 respectively. The overlap in candidate SNPs between PC1 and PC2 led to a
462 concordant overlap in candidate genes, with 27 of the 28 candidate genes
463 identified by PC1 also identified by PC2 (Fig. 4). Genes identified by both PC
464 axes were dispersed relatively evenly across the genome, indicating the
465 potential for polygenic selection across multiple loci (Fig. S4), though
466 relatively few were found on chromosome 10, the putative X chromosome
467 for this species. Due to the high overlap between PC1 and PC2 candidate
468 genes, these datasets were combined for enrichment and genome-wide
469 linkage analyses, yielding 2040 candidate genes for the GO enrichment and
470 genome-wide linkage analysis.

471 The analyses of the MCMC chain produced by the BayeScEnv model
472 showed both convergence (Cramer-von-Mises statistic, all $p > 0.05$) and low
473 autocorrelation (all $R < 0.05$). For the LFMM2 model, the sNMF analysis
474 identified $K=10$ as the most supported K value (Fig. S5). The run with the
475 lowest cross-entropy value for $K=10$ from the snmfProject was used to
476 impute missing data before input into the LFMM2 model.

477

478

479 *Gene Ontology and KEGG Enrichment Results*

480 Using the 2040 candidate genes identified across PC1 and PC2, we
481 identified 48 genes that significantly enriched for two KEGG pathways:
482 Gonadotropin-Releasing Hormone Signaling Pathway (GnRH pathway,
483 Path:sund04912), and Calcium Signaling Pathway (Ca²⁺ pathway,
484 Path:sund04020)(Table 2). For the GnRH pathway, 22 of the 106 pathway
485 genes were identified in our candidate gene dataset, whereas for the Ca²⁺
486 pathway, 39 out of 260 genes were identified. Of the 48 candidate genes, 13
487 were associated with both KEGG pathways, whereas 9 were associated with
488 the GnRH pathway and 26 were associated with only the Ca²⁺ pathway.
489 These genes include five calcium voltage-gated channel subunits (CACN),
490 four mitogen-activated protein kinases (MAPK), three calcium/calmodulin-
491 dependent protein kinases (CAMK), and three phospholipase C proteins
492 (PLC). No significantly enriched GO terms were found in our analysis.

493

494

495

496

497 *Genome-Wide Linkage Analysis*

498 One SNP per candidate gene was used to assess interchromosomal
499 linkage between candidate genes (see Methods). Of the 1,206,681 linkage
500 calculations across the 2040 candidate SNPs used for this analysis, an
501 overwhelming majority were found to not be in linkage disequilibrium ($r^2 <$
502 0.10)(Fig. 5A). The resulting r^2 distribution was heavily right-skewed, with a
503 mean of ~0.017 and a 99th percentile of 0.44. These values were consistently
504 greater than those obtained from linkage analyses using randomly selected
505 SNPs (Table S3). On average, the random SNP datasets had a mean r^2 of
506 ~0.008 and a 99th percentile of 0.17. Fisher-Pitman Permutation and
507 Wilcoxon rank sum tests supported the notion that the candidate SNPs were
508 in higher linkage than random, with 100/100 permutation tests and 97/100
509 Wilcox tests showing significant differences between the candidate SNP r^2
510 distribution and the r^2 distribution generated from random SNPs.

511 High interchromosomal linkage among candidate genes can indicate
512 co-selection in response to environmental selection or selection on epistatic
513 interactions among genes (Zhang, 2017). To identify candidate genes with
514 high linkage, we used a linkage threshold at the 99.5th percentile,
515 corresponding to an r^2 threshold of 0.48. Above this threshold, we found a

total of 639 SNPs in linkage disequilibrium with at least one other candidate gene, constituting 31.3% of the 2040 genes identified by our GEA analyses. Of the 639 candidate genes found to be in high linkage, 18 were previously identified to significantly enrich for KEGG pathways, while 6 were common genes identified across both PC1 and PC2 predictors in the GEA analyses. These represent 37.5% of the genes identified as enriching for KEGG pathways and 22.2% of the common genes identified by both PC1 and PC2 in the GEA analyses, respectively. In total there were 111 genes in significant linkage with either one of the KEGG enriching genes or one of the genes common to both PC1 and PC2 (Fig. 5b). Of the KEGG-enriching genes, 11 were involved in linkage groups with four or more candidate genes (Table S4): CACNA1C, CACNA1D, CACNA1H, LOC121936315 (CACNA1B-like protein), GNRH1, ITPR2, LOC121933610 (ADCY1-like protein), MAP3K3, MAPK13, NOS3, and PLCE1. Of these, the highest number of significant linkages to other candidate genes were found in CACNA1C (n=10), CACNA1H (n=10), MAP3K3 (n=13), MAPK13 (n=33), and PLCE1 (n=30). Further, MAPK13 and PLCE1 were in significant linkage with each other, and were linked to the same 29 candidate genes. A table of all the KEGG enriching genes and the genes to which they are linked is available in Supplemental Materials (Table S4).

536 DISCUSSION:

537

538 Selection imposed by climate induces local adaptation, but the
539 genetics underlying these adaptations remains poorly understood.
540 Knowledge of the adaptive traits and underlying genetics may prove critical
541 to conservation efforts, as many species will likely rely on adaptive evolution
542 to persist in rapidly changing climates (Hoffmann and Sgró, 2011; Urban *et*
543 *al.*, 2024). Here, we used biophysical estimates of activity time and nest
544 conditions across the Eastern fence lizard (*Sceloporus undulatus*) species'
545 range to identify genomic signatures of selection. Activity time dictates rates
546 of energy intake and expenditure and thus represents a fundamental control
547 over the ability of organisms to fund the cost of living (Congdon, 1989; Wild
548 *et al.*, 2025), whereas nest conditions affect rates of growth and
549 development, with downstream consequences to survival rates and life
550 histories (Andrews *et al.*, 2000; Carlo *et al.*, 2018; Jonsson *et al.*, 2022). Our
551 biophysical approaches identified a general pattern that potential activity
552 time decreases with latitude, while the range of nest temperatures increases
553 with latitude, and mean nest temperatures do not correlate with latitude,
554 longitude, or elevation. When input into GEA analyses, the suite of
555 biophysical variables identified a large number of candidate genes, with 48

556 that enriched for two KEGG pathways: the calcium signaling pathway and the
557 gonadotropin-releasing hormone pathway. Calcium signaling is associated
558 with cell proliferation and migration through the effects of growth factors
559 (e.g., growth and development)(Berridge, 1995; Whitaker, 2006), while
560 gonadotropin-releasing hormone dictates the development of sexual traits
561 and the timing of sexual maturity (Licht, 1979). Phenotypic evidence from
562 previous studies suggests that selection on life history traits such as body
563 size and age at maturation underlies adaptation to climate in ectotherms;
564 thus, our results provide genetic support for this notion (Crozier and
565 Hutchings, 2014; Urban *et al.*, 2014).

566 Life history theory focuses on the tradeoffs in resources allocated to
567 survival, growth, and reproduction (Stearns, 1992). When resources are
568 limited, selection should act to optimize the allocation of resources across
569 these costs, thereby maximizing fitness within a given environment (Stearns,
570 1992). Optimization of resource allocation appears to have led to the co-
571 evolution of life history traits, producing axes of variation in survival to
572 maturity, size at maturity, timing of reproduction, and reproductive
573 investment (Healy *et al.*, 2019). The results of our study suggest that
574 selection on growth processes and age at maturation underlie adaptation to
575 climates across the range of *S. undulatus*. Specifically, we identified 48

576 genes significantly enriched in two KEGG pathways: the calcium signaling
577 pathway and the gonadotropin-releasing hormone (GnRH) pathway. An
578 important role of calcium signaling is to regulate cell proliferation and
579 migration (Berridge, 1995). This may be particularly important during
580 embryogenesis and early development, as calcium signaling has been
581 implicated in regulating cell proliferation, migration, axis formation, and
582 organogenesis in animal embryos (Whitaker, 2006). Selection on aspects of
583 the calcium signaling pathway may therefore represent adaptations to nest
584 conditions across the range of *S. undulatus*. Our biophysical models
585 identified variation in both mean and range of nest temperatures
586 experienced across sites, with changes in nest temperatures occurring over
587 the last 60-80 years. As nest temperatures directly affect incubation time
588 and size at hatching (Andrews *et al.*, 1997; Shine *et al.*, 1997; Carlo *et al.*,
589 2018), selection may be acting on processes that control growth and
590 development pathways to optimize these processes within the range of
591 temperatures the embryo experiences. Indeed, a previous common garden
592 study using populations of *S. undulatus* from across the species' range
593 identified differences among populations in incubation time and embryonic
594 growth efficiency (Oufiero and Agilletta, Jr., 2006). The knock-on effects of
595 these early developmental processes may, at least in part, affect post-

596 hatching growth rates and adult body size patterns observed across the
597 species' range (Jonsson *et al.*, 2022).

598 Age at maturation post-hatching appears to be another adaptive life
599 history trait in *S. undulatus*. Warmer climates at lower latitudes facilitate
600 more activity time, which in turn increases opportunities for food acquisition
601 and reproductive output (Adolph and Porter, 1993). However, greater activity
602 comes with a trade-off: more active populations in southern latitudes
603 experience lower survival rates (likely due to greater exposure to
604 predators)(Adolph and Porter, 1993; Angilletta *et al.*, 2004). Survival to
605 maturation has been directly linked to age at maturation, such that
606 populations with lower survival rates mature earlier (Adolph and Porter,
607 1996), increasing the likelihood of reproducing before death (Stearns and
608 Crandall, 1981). As such, populations of *S. undulatus* in warmer climates
609 have evolved rapid post-hatching growth rates and earlier maturation at a
610 smaller body size relative to cooler-climate populations (Tinkle and
611 Ballinger, 1972; Adolph and Porter, 1996). Our study identified genes in the
612 GnRH pathway as under selection across our study populations, providing
613 genomic evidence supporting the view that age at maturation is an adaptive
614 trait selected by climate. The GnRH pathway regulates aspects of maturation
615 in animals by stimulating gonadotropins, specifically follicle-stimulating

616 hormone and luteinizing hormone (Counis *et al.*, 2005). These hormones
617 then affect sexual traits, including gonadal maturation, gamete formation,
618 and the production of sex-specific hormones such as testosterone and
619 progesterone (Licht, 1979). Therefore, selection on genes within the GnRH
620 pathway may act to control the timing of maturation across populations of *S.*
621 *undulatus*. Further, signal transmission of GnRH acts through calcium
622 signaling (Catt and Stojilković, 1989), linking the GnRH pathway to the
623 calcium-signaling pathway genes identified in our study. Indeed, many of the
624 genes we identified act in both the calcium signaling and GnRH pathways
625 (Table 2). Therefore, while we postulate that calcium signaling genes control
626 adaptive responses underlying embryonic growth and development under
627 different nest conditions, it is possible that the signatures of selection we
628 detect in calcium-signaling genes may also act in conjunction with the GnRH
629 pathway to control age at maturation.

630 Some gene families identified in this study have previously been
631 implicated in controlling aspects of growth, development, and sexual
632 maturation. The mitogen-activated protein kinase (MAPK) gene family
633 mediates intercellular signal transduction (Seger and Krebs, 1995).
634 Activation of a single kinase by an upstream mitogen or growth factor triggers
635 a cascade of downstream kinases that ultimately alter gene expression,

636 inducing multiple responses, including cell proliferation, differentiation,
637 migration, and cell death (Krens *et al.*, 2006). The MAPK signaling pathway
638 has been identified as controlling multiple aspects of vertebrate
639 development (Krens *et al.*, 2006; Craig *et al.*, 2008), as well as maturation of
640 oocytes in animals (Meinecke and Krischek, 2003; Ni *et al.*, 2015), due to the
641 interaction of MAPK activity and maturation-promoting factor (MPF)(Kotani
642 and Yamashita, 2002). Calcium/calmodulin-dependent protein kinases
643 (CAMK) has also been implicated in controlling aspects of organismal growth
644 (Braun and Schulman, 1995; Kahl and Means, 2003). This family of kinases
645 functions as a critical component of the calcium signaling pathway, acting
646 as effectors that translate calcium influx into changes in cellular function
647 through interactions with a wide range of substrates (Braun and Schulman,
648 1995). The action of calcium/calmodulin-dependent kinases has been
649 directly implicated in controlling aspects of muscle growth (Al-Shanti and
650 Stewart, 2009) and neurological development (Wayman *et al.*, 2008).
651 Further, CAMK genes have been implicated in control aspects of oocyte
652 maturation in yaks (Yang *et al.*, 2024) and pigs (Xu *et al.*, 2009), providing a
653 means by which they can potentially influence the age of maturation. Lastly,
654 phospholipase C (PLC) genes link the effects of extracellular messengers,
655 such as growth factors and hormones, to intracellular activities by

656 generating inositol 1,4,5-trisphosphate and diacylglycerol as secondary
657 messengers (Nakamura and Fukami, 2017). The phenotypic effects of PLC
658 activity putatively include effects on body size (Zhang *et al.*, 2024) and
659 embryonic development (Zhou and Hildebrandt, 2009). Due to the
660 fundamental roles the above gene families play as components of the
661 calcium signaling and GnRH pathways, as well as their influence on aspects
662 of embryonic development, organismal growth, and gamete maturation,
663 these genes are strong candidates for underpinning the adaptive phenotypic
664 responses of life histories to climate in *S. undulatus*.

665 The evolution of whole-organism phenotypes, such as those
666 described by life-history traits, can involve complex interactions among a
667 large number of genes (Whiting *et al.*, 2022). To that end, selection can act
668 on epistatic interactions between genes within networks or pathways,
669 resulting in patterns of interchromosomal linkage (Zhang, 2017). We
670 investigated this possibility and found elevated linkage in candidate genes,
671 exceeding background levels across the genome. Among the candidate
672 genes exhibiting elevated linkage were multiple MAPK genes and the PLCE1
673 gene, which were previously discussed as candidates underlying patterns of
674 life-history variation across populations of *S. undulatus*. In particular,
675 MAPK13 (found on chromosome four) and PLCE1 (found on chromosome

676 three) were found to form a linkage group with 29 other candidate genes,
677 including those critical for embryonic development (HOXB1, STAT3, DMXL1,
678 HDAC9, MED12)(Levy and Lee, 2002; McGraw *et al.*, 2003; Shin *et al.*, 2008;
679 Hubert and Wellik, 2023; Vontell *et al.*, 2025). Among these genes, a well-
680 known interaction has been described between KMT and HOX genes, in
681 which KMT (lysine N-methyltransferases) directly regulates HOX gene
682 expression during embryonic development (Milne *et al.*, 2002). While it may
683 be difficult to definitively link the effects of mutations across all candidate
684 genes, the overall evidence points to processes that control patterns of
685 embryonic development. Thus, it seems likely that selection via nest
686 conditions on processes of growth and development may underlie adaptive
687 patterns of growth and development as life histories become optimized
688 across climates.

689 Our study investigated the genetics that underlie adaptation to
690 climate in the *S. undulatus*, a well-studied model species in ecology and
691 evolution. Life history varies predictably across climate in *S. undulatus*
692 (Tinkle and Ballinger, 1972; Adolph and Porter, 1993), and other studies have
693 identified various life history traits as putatively under selection by climate in
694 other ectothermic species (Crozier and Hutchings, 2014; Urban *et al.*, 2014).
695 By integrating biophysical and genomic approaches, we identified a suite of

696 genes putatively under selection by climate-induced patterns in activity and
697 nest incubation conditions. Many of the candidate genes identified in this
698 study have known effects on processes of growth and development in
699 vertebrates. In particular, we identified candidate genes associated with the
700 calcium signaling and gonadotropin-releasing hormone pathways, which
701 have known effects on cell proliferation, embryonic development, and
702 sexual maturation. Our results ultimately suggest that selection acting on
703 early life stages (e.g., from embryonic development to sexual maturity) drives
704 patterns of life history in response to climate in a wide-spread lizard species.

705

706 **Acknowledgments:**

707 We thank Dr. Donald Miles, Dr. Lance McBrayer, Dr. Sharon Bewick, Dr.
708 Henry John-Alder, Robert Chamberlin, and Tara Cronin for assistance with
709 field collections. Funding for this work was provided to MRM by the *Theodore*
710 *Roosevelt Memorial Grant* from by The American Museum of Natural History,
711 the *EE Williams Grant* from by the Herpetologists' League, the Biological
712 Sciences Professional Development Course-Grant in Aid of Research, a "R"
713 initiative seed grant to MWS & CLP from Clemson University and the US
714 National Science Foundation to CLP (DEB 1822417). We thank the Clemson
715 University Genomics and Bioinformatics Facility for support and help with

716 data generation, which receives support from the College of Science and two
717 Institutional Development Awards (IDeA) from the National Institute of
718 General Medical Sciences of the National Institute of Health under grant
719 numbers P20GM146584 and P20GM139769. Scientific specimens were
720 collected with permissions from the Alabama Department of Conservation
721 and Natural Resources, the Florida Fish and Wildlife Conservation
722 Commission and Florida Department of Agriculture and Consumer Services,
723 the Indiana Department of Natural Resources, the Kentucky Department of
724 Fish and Wildlife Resources, the New Jersey Division of Fish and Wildlife, the
725 North Carolina Wildlife Resources Commission, the Ohio Department of
726 Natural Resources, the South Carolina Division of Wildlife and Freshwater
727 Fisheries, the Virginia Department of Wildlife Resources, and the United
728 States Department of Agriculture. Additionally, Greg Colwell provided
729 *Sceloporus undulatus* samples from the KY population via the Yale-Peabody
730 Museum.

731

732 **Author Contributions:**

733 MRM and MWS conceived and designed the experiments. CLP and MWS
734 provided funding and resources for the experiments, while MRM performed
735 the experiments. MRM, MWS, and CLP analyzed the data. MRM wrote the

736 initial draft of the manuscript; and all authors edited and finalized the
737 manuscript.

738

739 **Competing Interests:**

740 The authors have no conflicts of interest to report.

741

742 **Data Archiving:**

743 The raw genomic sequences used in this study have been uploaded to NCBI
744 under BioProject PRJNA1433456 and is set to be released within the next
745 year.

746

747 **Ethics Approval Statement:**

748 All work conducted herein was approved by the Clemson University
749 Institutional Animal Care and Use Committee under Animal Use Protocol
750 #2022-0264.

751 LITERATURE CITED:

- 752 Adolph SC, Porter WP (1993). Temperature, Activity, and Lizard Life
753 Histories. *The American Naturalist* **142**: 273–295.
- 754 Adolph SC, Porter WP (1996). Growth, Seasonality, and Lizard Life Histories:
755 Age and Size at Maturity. *Oikos* **77**: 267–278.
- 756 Al-Shanti N, Stewart CE (2009). Ca²⁺/calmodulin-dependent transcriptional
757 pathways: potential mediators of skeletal muscle growth and
758 development. *Biological Reviews* **84**: 637–652.
- 759 Andrews RM (1998). Geographic variation in field body temperature of
760 *Sceloporus* lizards. *Journal of Thermal Biology* **23**: 329–334.
- 761 Andrews S (2010). FastQC: A Quality Control Tool for High Throughput
762 Sequence Data. Available online at:
763 <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- 764 Andrews RM, Mathies T, Warner D (2000). Effect of Incubation Temperature
765 on Morphology, Growth, and Survival of Juvenile *Sceloporus*
766 *undulatus*. *Herpetological Monographs* **14**: 420–431.
- 767 Andrews RM, Qualls CP, Rose BR (1997). *Effects of Low Temperature on*
768 *Embryonic Development of Sceloporus Lizards*.
- 769 Angilletta MJ, Niewiarowski PH, Dunham AE, Leaché AD, Porter WP (2004).
770 Bergmann's Clines in Ectotherms: Illustrating a Life-History
771 Perspective with Sceloporine Lizards. *The American Naturalist* **164**:
772 168–183.
- 773 Angilletta MJ, Sears MW, Pringle RM (2009). Spatial Dynamics of Nesting
774 Behavior: Lizards Shift Microhabitats to Construct Nests with
775 Beneficial Thermal Properties. *Ecology* **90**: 2933–2939.
- 776 Assis BA, Sullivan AP, Marciniak S, Bergey CM, Garcia V, Szpiech ZA, et al.
777 (2025). Genomic signatures of adaptation in native lizards exposed to
778 human-introduced fire ants. *Nature Communications* **16**: 89.
- 779 Berridge MJ (1995). Calcium signalling and cell proliferation. *BioEssays* **17**:
780 491–500.

- 781 Braun AP, Schulman H (1995). The Multifunctional Calcium/Calmodulin-
782 Dependent Protein Kinase: From Form to Function. *Annual Review of*
783 *Physiology*: 417–445.
- 784 Campbell GS, Norman JM (1998). *An introduction to environmental*
785 *biophysics*. Springer-Verlag: New York.
- 786 Carlo MA, Riddell EA, Levy O, Sears MW (2018). Recurrent sublethal warming
787 reduces embryonic survival, inhibits juvenile growth, and alters
788 species distribution projections under climate change. *Ecology*
789 *Letters* **22**: 104–116.
- 790 Carlson SM, Cunningham CJ, Westley PAH (2014). Evolutionary rescue in a
791 changing world. *Trends in Ecology and Evolution* **29**: 521–530.
- 792 Catt KJ, Stojilković SS (1989). Calcium Signaling and Gonadotropin
793 Secretion. *Trends in Endocrinology and Metabolism*: 15–20.
- 794 Caye K, Jumentier B, Lepeule J, François O (2019). LFMM 2: Fast and
795 accurate inference of gene-environment associations in genome-
796 wide studies. *Molecular Biology and Evolution* **36**: 852–860.
- 797 Christian KA, Tracy CR, Porter WP (1986). The Effect of Cold Exposure during
798 Incubation of *Sceloporus undulatus* Eggs. *Copeia* **1986**: 1012–1014.
- 799 Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, *et al.* (2012). A
800 program for annotating and predicting the effects of single nucleotide
801 polymorphisms, SnpEff. *Fly* **6**: 80–92.
- 802 Claunch NM, Nix E, Royal AE, Burgos LP, Corn M, DuBois PM, *et al.* (2021).
803 Body size impacts critical thermal maximum measurements in
804 lizards. *Journal of Experimental Zoology Part A: Ecological and*
805 *Integrative Physiology* **335**: 96–107.
- 806 Congdon JD (1989). Proximate and Evolutionary Constraints on Energy
807 Relations of Reptiles. *Physiological Zoology* **62**: 356–373.
- 808 Counis R, Laverrière J-N, Garrel G, Bleux C, Cohen-Tannoudji J, Lerrant Y, *et*
809 *al.* (2005). Gonadotropin-releasing hormone and the control of
810 gonadotrope function. *Reprod Nutr Dev* **45**: 243–254.

811 Craig EA, Stevens MV, Vaillancourt RR, Camenisch TD (2008). MAP3Ks as
812 central regulators of cell fate during development. *Developmental*
813 *Dynamics* **237**: 3102–3114.

814 Crowley SR (1985). Thermal sensitivity of sprint-running in the lizard
815 *Sceloporus undulatus*: support for a conservative view of thermal
816 physiology. *Oecologia* **66**: 219–225.

817 Crozier LG, Hutchings JA (2014). Plastic and evolutionary responses to
818 climate change in fish. *Evolutionary Applications* **7**: 68–87.

819 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, *et al.*
820 (2011). The variant call format and VCFtools. *Bioinformatics* **27**: 2156–
821 2158.

822 Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, *et al.* (2021).
823 Twelve years of SAMtools and BCFtools. *GigaScience* **10**.

824 Fick SE, Hijmans RJ (2017). WorldClim 2: new 1-km spatial resolution
825 climate surfaces for global land areas. *International Journal of*
826 *Climatology* **37**: 4302–4315.

827 Franks SJ, Hoffmann AA (2012). Genetics of Climate Change Adaptation.
828 *Annual Review of Genetics* **46**: 185–208.

829 Frichot E, François O (2015). LEA: An R package for landscape and ecological
830 association studies. *Methods in Ecology and Evolution* **6**: 925–929.

831 Gates DM (1980). *Biophysical Ecology*. Springer-Verlag: New York.

832 Healy K, Ezard THG, Jones OR, Salguero-Gómez R, Buckley YM (2019).
833 Animal life history is shaped by the pace of life and the distribution of
834 age-specific mortality and reproduction. *Nature Ecology and*
835 *Evolution* **3**: 1217–1224.

836 Hersbach H, Bell B, Berrisford P, Hirahara S, Horányi A, Muñoz-Sabater J, *et*
837 *al.* (2020). The ERA5 global reanalysis. *Quarterly Journal of the Royal*
838 *Meteorological Society*: 1999–2049.

839 Hoffmann AA, Sgró CM (2011). Climate change and evolutionary adaptation.
840 *Nature* **470**: 479–485.

841 Hothorn T, Hornik K, Wiel MA van de, Zeileis A (2008). Implementing a Class
842 of Permutation Tests: The coin Package. *Journal of Statistical*
843 *Software* **28**: 1–23.

844 Hubert KA, Wellik DM (2023). Hox genes in development and beyond.
845 *Development* **150**: dev192476.

846 Jonsson B, Jonsson N, Hansen MM (2022). Knock-on effects of
847 environmental influences during embryonic development of
848 ectothermic vertebrates. *The Quarterly Review of Biology* **97**: 95–139.

849 Kahl CR, Means AR (2003). Regulation of Cell Cycle Progression by
850 Calcium/Calmodulin-Dependent Pathways. *Endocr Rev* **24**: 719–736.

851 Kearney M (2012). Metabolic theory, life history and the distribution of a
852 terrestrial ectotherm. *Functional Ecology* **36**: 167–179.

853 Kearney MR, Porter WP (2017). NicheMapR – an R package for biophysical
854 modelling: the microclimate model. *Ecography*: 664–674.

855 Kingsolver JG, Woods HA, Buckley LB, Potter KA, Maclean HJ, Higgins JK
856 (2011). Complex Life Cycles and the Responses of Insects to Climate
857 Change. *Integrative and Comparative Biology* **51**: 719–732.

858 Kolbe JJ, Janzen FJ (2002). Impact of Nest-Site Selection on Nest Success
859 and Nest Temperature in Natural and Disturbed Habitats. *Ecology* **83**:
860 269–281.

861 Kotani T, Yamashita M (2002). Discrimination of the Roles of MPF and MAP
862 Kinase in Morphological Changes That Occur during Oocyte
863 Maturation. *Developmental Biology* **252**: 271–286.

864 Krens SFG, Spaink HP, Snaar-Jagalska BE (2006). Functions of the MAPK
865 family in vertebrate-development. *FEBS Letters* **580**: 4984–4990.

866 Krueger F (2015). Trim Galore!: wrapper script for automated quality and
867 adapter trimming and quality control. Available online at:
868 <https://github.com/FelixKrueger/TrimGalore>.

869 Levy O, Buckley LB, Keitt TH, Smith CD, Boateng KO, Kumar DS, *et al.* (2015).
870 Resolving the life cycle alters expected impacts of climate change.
871 *Proceeding of the Royal Society B: Biological Sciences* **282**: 1–9.

872 Levy DE, Lee C (2002). What does Stat3 do? *J Clin Invest* **109**: 1143–1148.

873 Li H (2013). Aligning sequence reads, clone sequences and assembly contigs
874 with BWA-MEM. *arXiv:13033997[q-bioGN]*.

875 Licht P (1979). Reproductive Endocrinology of Reptiles and Amphibians:
876 Gonadotropins. *Annu Rev Physiol* **41**: 337–351.

877 McGraw S, Robert C, Massicotte L, Sirard M-A (2003). Quantification of
878 Histone Acetyltransferase and Histone Deacetylase Transcripts
879 During Early Bovine Embryo Development. *Biol Reprod* **68**: 383–389.

880 Meek MH, Beever EA, Barbosa S, Fitzpatrick SW, Fletcher NK, Hellmann JJ
881 (2022). Understanding Local Adaptation to Prepare Populations for
882 Climate Change. *BioScience*: 1–12.

883 Meinecke B, Krischek C (2003). MAPK/ERK kinase (MEK) signalling is required
884 for resumption of meiosis in cultured cumulus-enclosed pig oocytes.
885 *Zygote* **11**: 7–16.

886 Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, *et al.* (2002).
887 MLL Targets SET Domain Methyltransferase Activity to Hox Gene
888 Promoters. *Molecular Cell* **10**: 1107–1117.

889 Morrison C, Hero J-M (2003). Geographic variation in life-history
890 characteristics of amphibians: a review. *Journal of Animal Ecology* **72**:
891 270–279.

892 Nakamura Y, Fukami K (2017). Regulation and physiological functions of
893 mammalian phospholipase C. *J Biochem* **161**: 315–321.

894 Ni H, Sheng X, Cui X, Gu M, Liu Y, Qi X, *et al.* (2015). Epidermal Growth
895 Factor-Mediated Mitogen-Activated Protein Kinase3/1 Pathway Is
896 Conducive to In Vitro Maturation of Sheep Oocytes. *PLOS ONE* **10**:
897 e0120418.

- 898 Niewiarowski PH (1995). Effects of Supplemental Feeding and Thermal
899 Environment on Growth Rates of Eastern Fence Lizards, *Sceloporus*
900 *undulatus*. *Herpetologica* **51**: 487–496.
- 901 Niewiarowski PH, Roosenburg W (1993). Reciprocal Transplant Reveals
902 Sources of Variation in Growth Rates of the Lizard *Sceloporus*
903 *Undulatus*. *Ecology* **74**: 1992–2002.
- 904 Oufiero CE, Agilletta, Jr. MJ (2006). Convergent evolution of embryonic
905 growth and development in the eastern fence lizard (*Sceloporus*
906 *undulatus*). *Evolution* **60**: 1066–1075.
- 907 Plummer M, Best N, Cowles K, Vines K (2006). CODA: Convergence
908 Diagnosis and Output Analysis for MCMC. *R News* **6**: 7–11.
- 909 Salloum PM, Santure AW, Lavery SD, de Villemereuil P (2022). Finding the
910 adaptive needles in a population-structured haystack: A case study in
911 a New Zealand mollusc. *Journal of Animal Ecology* **91**: 1209–1221.
- 912 Sears MW, Raskin E, Angilletta Jr. M (2011). The World Is not Flat: Defining
913 Relevant Thermal Landscapes in the Context of Climate Change.
914 *Integrative and Comparative Biology* **51**: 666–675.
- 915 Sears MW, Riddell EA, Rusch TW, Angilletta MJ (2019). The World Still Is Not
916 Flat: Lessons Learned from Organismal Interactions with
917 Environmental Heterogeneity in Terrestrial Environments. *Integrative*
918 *and Comparative Biology* **59**: 1049–1058.
- 919 Seger R, Krebs EG (1995). The MAPK signaling cascade. *The FASEB Journal* **9**:
920 726–735.
- 921 Shin CH, Chung W-S, Hong S-K, Ober EA, Verkade H, Field HA, *et al.* (2008).
922 Multiple roles for Med12 in vertebrate endoderm development.
923 *Developmental Biology* **317**: 467–479.
- 924 Shine R, Elphick MJ, Harlow PS (1997). The Influence of Natural Incubation
925 Environments on the Phenotypic Traits of Hatchling Lizards. *Ecology*
926 **78**: 2559–2568.

- 927 Sinervo B, Méndez-de-la-Cruz F, Miles DB, Heulin B, Bastiaans E, Villagrán-
 928 Santa Cruz M, *et al.* (2010). Erosion of lizard diversity by climate
 929 change and altered thermal niches. *Science* **328**: 894–899.
- 930 Sniegula S, Stoks R, Golab MJ (2024). Insect responses to seasonal time
 931 constraints under global change are facilitated by warming and
 932 counteracted by invasive alien predators. *Scientific Reports* **14**:
 933 24565.
- 934 Sousa T, Domingos T, Poggiale JC, Kooijman SALM (2010). Dynamic energy
 935 budget theory restores coherence in biology. *Philosophical*
 936 *Transactions of the Royal Society B: Biological Sciences* **365**: 3413–
 937 3428.
- 938 Stearns SC (1992). *The Evolution of Life Histories*. Oxford University Press.
- 939 Stearns SC, Crandall RE (1981). Quantitative predictions of delayed maturity.
 940 *Evolution* **35**: 455–463.
- 941 Stein BA, Staudt A, Cross MS, Dubois NS, Enquist C, Griffis R, *et al.* (2013).
 942 Preparing for and managing change: Climate adaptation for
 943 biodiversity and ecosystems. *Frontiers in Ecology and the*
 944 *Environment* **11**: 502–510.
- 945 Storey J, Bass A, Dabney A, Robinson D (2023). qvalue: Q-value estimation
 946 for false discovery rate control.
- 947 Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, *et al.*
 948 (2014). Thermal-safety margins and the necessity of thermoregulatory
 949 behavior across latitude and elevation. *Proceedings of the National*
 950 *Academy of Sciences* **111**: 5610–5615.
- 951 Tinkle DW, Ballinger RE (1972). *Sceloporus Undulatus*: A Study of the
 952 Intraspecific Comparative Demography of a Lizard. *Ecology* **53**: 570–
 953 584.
- 954 Urban MC (2015). Accelerating extinction risk from climate change. *Science*
 955 **348**: 571–573.
- 956 Urban MC (2024). Climate change extinctions. *Science*: 1123–1128.

957 Urban MC, Richardson JL, Freidenfelds NA (2014). Plasticity and genetic
 958 adaptation mediate amphibian and reptile responses to climate
 959 change. *Evolutionary Applications* **7**: 88–103.

960 Urban MC, Swaegers J, Stoks R, Snook RR, Otto SP, Noble DWA, *et al.* (2024).
 961 When and how can we predict adaptive responses to climate change?
 962 *Evolution Letters* **8**: 172–187.

963 Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, del Angel G, Levy-
 964 Moonshine A, *et al.* (2013). From fastQ data to high-confidence
 965 variant calls: The genome analysis toolkit best practices pipeline.
 966 *Current Protocols in Bioinformatics*: 1–33.

967 Vandermeer JH (1972). Niche theory. *Ann Rev Ecol Syst* **3**: 107–132.

968 Villemereuil P, Gaggiotti OE (2015). A new FST-based method to uncover
 969 local adaptation using environmental variables (RB O’Hara, Ed.).
 970 *Methods in Ecology and Evolution* **6**: 1248–1258.

971 Vontell AM, Willeke L, Iyyanar PPR, Stottmann RW (2025). *Dmx1* is required
 972 for survival in the mouse to organogenesis stages of development.
 973 *Differentiation* **146**: 100917.

974 Vu V, Friendly M (2024). *_ggbiplot*: A Grammar of Graphics Implementation of
 975 Biplots_.

976 Warner D, Andrews RM (2002a). Nest-site selection in relation to
 977 temperature and moisture by the lizard. *Herpetologica* **58**: 399–407.

978 Warner D, Andrews RM (2002b). Laboratory and field experiments identify
 979 sources of variation in phenotypes and survival of hatchling lizards.
 980 *Biological Journal of the Linnean Society* **76**: 105–124.

981 Wayman GA, Lee Y-S, Tokumitsu H, Silva A, Soderling TR (2008). Calmodulin-
 982 Kinases: Modulators of Neuronal Development and Plasticity. *Neuron*
 983 **59**: 914–931.

984 Wei T, Simko V (2024). ‘corrplot’: Visualization of a Correlation Matrix.

985 Westfall AK, Telemeco RS, Grizante MB, Waits DS, Clark AD, Simpson DY, *et*
 986 *al.* (2021). A chromosome-level genome assembly for the eastern

987 fence lizard (*Sceloporus undulatus*), a reptile model for physiological
988 and evolutionary ecology. *GigaScience* **10**: 1–17.

989 Whitaker M (2006). Calcium at Fertilization and in Early Development.
990 *Physiological Reviews* **86**: 25–88.

991 Whiting JR, Paris JR, Parsons PJ, Matthews S, Reynoso Y, Hughes KA, *et al.*
992 (2022). On the genetic architecture of rapidly adapting and
993 convergent life history traits in guppies. *Heredity* **128**: 250–260.

994 Wild KH, Huey RB, Pianka ER, Clusella-Trullas S, Gilbert AL, Miles DB, *et al.*
995 (2025). Climate change and the cost-of-living squeeze in desert
996 lizards. *Science* **387**: 303–309.

997 Xijin Ge S, Jung D, Yao R (2020). ShinyGO: a graphical gene-set enrichment
998 tool for animals and plants. *Bioinformatics* **36**: 2628–2629.

999 Xu B-Z, Li M, Xiong B, Lin S-L, Zhu J-Q, Hou Y, *et al.* (2009). Involvement of
1000 calcium/calmodulin-dependent protein kinase kinase in meiotic
1001 maturation of pig oocytes. *Animal Reproduction Science* **111**: 17–30.

1002 Yang Y, Chen Z, Yang C, Chen H, Song X, Li Y, *et al.* (2024). Calcium
1003 promoting maturity of oocytes through calcium/calmodulin-
1004 dependent protein kinase II of yak (*Bos grunniens*). *Italian Journal of*
1005 *Animal Science* **23**: 895–906.

1006 Zalom FG, Goodell P, Wilson LT, Bentley WJ (1983). *Degree-days: the*
1007 *calculation and use of heat units in pest management*. Univeresity of
1008 California Division of Agriculture and Natural Resources: Davis,
1009 California.

1010 Zhang J (2017). Epistasis Analysis Goes Genome-Wide. *PLOS Genetics* **13**:
1011 e1006558.

1012 Zhang R, Wu H, Li Y, Huang Z, Yin Z, Yang CX, *et al.* (2023). GWLD: an R
1013 package for genome-wide linkage disequilibrium analysis. *G3: Genes,*
1014 *Genomes, Genetics* **13**.

1015 Zhang J, Zha X, Yang G, Ma X, La Y, Wu X, *et al.* (2024). Polymorphisms of TXK
1016 and PLCE1 Genes and Their Correlation Analysis with Growth Traits in
1017 Ashidan Yaks. *Animals* **14**: 3506.

1018 Zhou W, Hildebrandt F (2009). Molecular cloning and expression of
1019 phospholipase C epsilon 1 in zebrafish. *Gene Expression Patterns* **9**:
1020 282–288.

1021

1022 **Figure Legends:**

1023

1024 Figure 1. A) Map of the locations for the fourteen populations of *Sceloporus*
1025 *undulatus* used in this study. a) Mean annual temperatures and b) annual
1026 temperature range were plotted to illustrate climatic patterns across the
1027 species' range. Names and locations of each site are shown as points on the
1028 map. The data used for these maps were obtained from the WorldClim2 (Fick
1029 and Hijmans, 2017) database for illustrative purposes. Photo credit for the *S.*
1030 *undulatus* specimen goes to Diego R. Quirola.

1031

1032 Figure 2. Multiple biophysical variables describing activity patterns and nest
1033 conditions were estimated to use as predictors for gene-environmental
1034 association analyses. The maps show patterns of these variables across
1035 populations, with some exemplars shown here: a) patterns of annual adult
1036 activity averaged from 2001-2020, b) patterns of annual hatchling activity
1037 averaged from 2001-2020, c) mean nest temperatures averaged from 2001-
1038 2020, d) range of nest temperatures averaged from 2001-2020, e) change in
1039 patterns of adult activity from 1941-1960 to 2001-2020, f) change in patterns
1040 of hatchling activity from 1941-1960 to 2001-2020, g) degree days (C°/day)

1041 above mean nest temperature from 1941-1960 to 2001-2020, and h) change
1042 in range of nest temperatures from 1941-1960 to 2001-2020.

1043

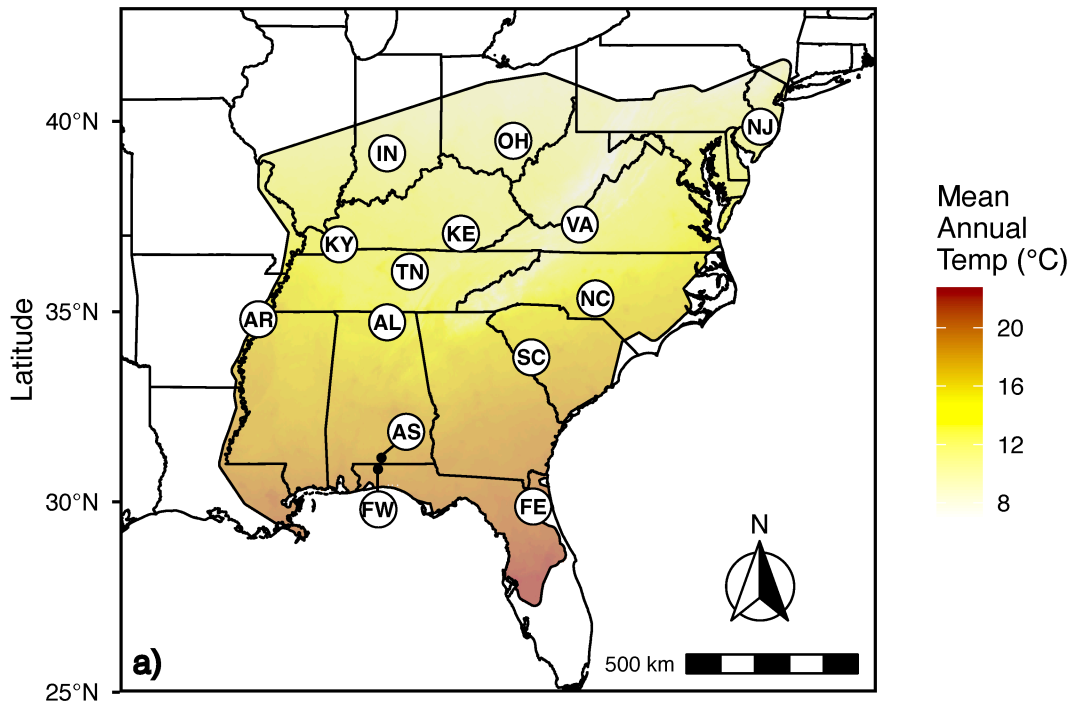
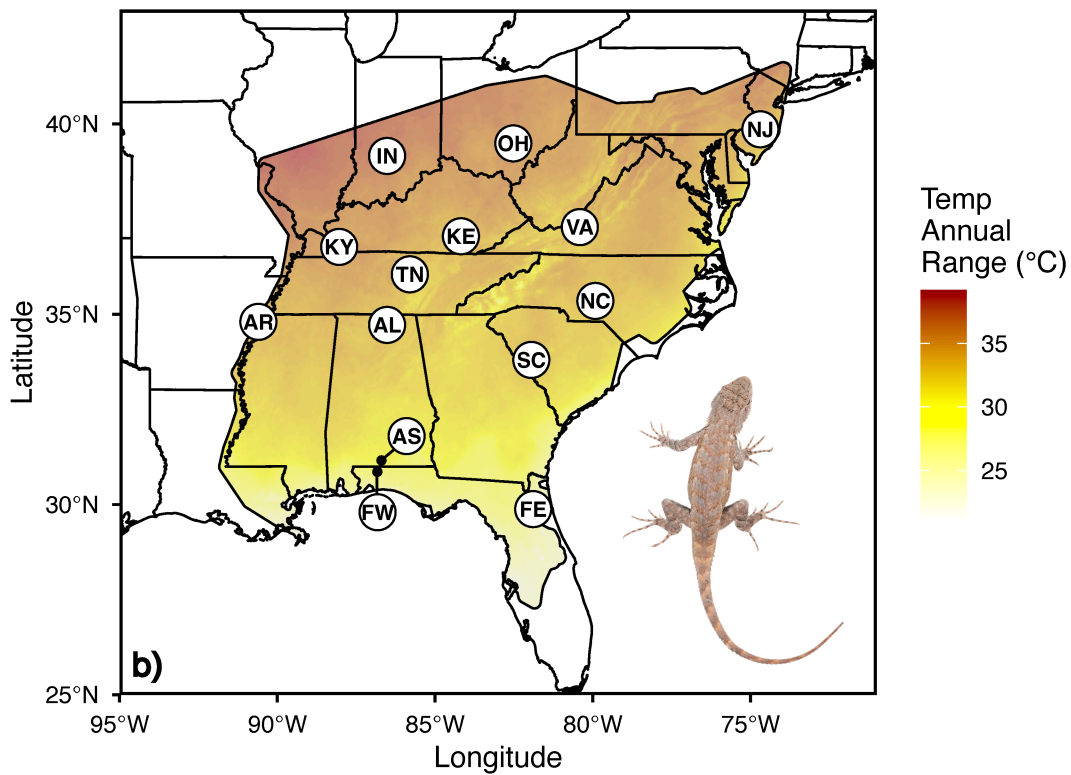
1044 Figure 3. A principal component analysis (PCA) was used to reduce
1045 multidimensionality among the correlated biophysical predictor variables.
1046 The locations of each site in PC spaces are shown in the biplot on the left.
1047 The loadings for each variable onto the PC axes are shown by the bar plots
1048 on the right. Variables that correlate with latitude mostly loaded onto PC1,
1049 while multiple variables associated with nest conditions and hatchling
1050 activity loaded onto PC2.

1051

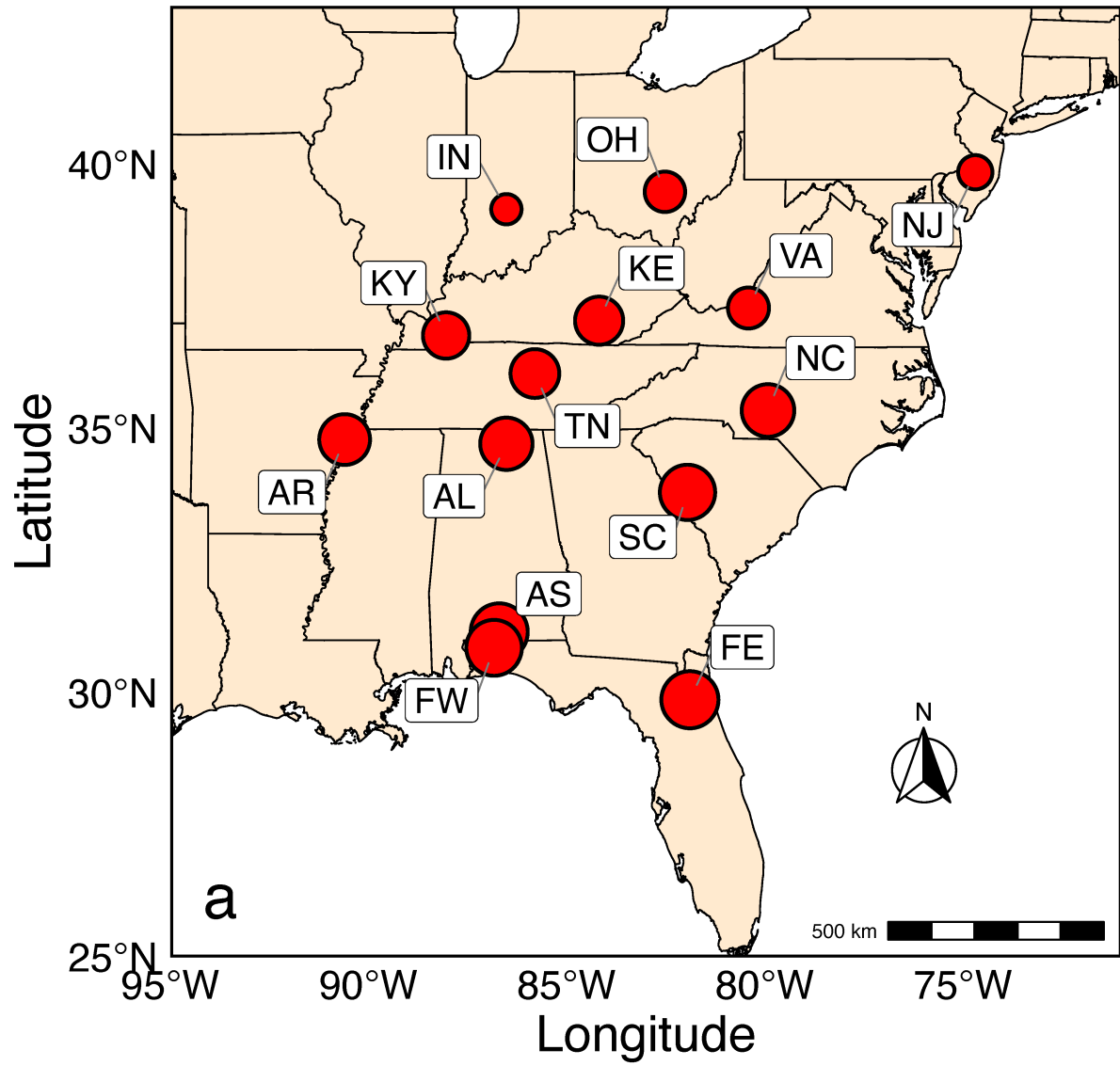
1052 Figure 4. The output of the GEA analyses identified 41 candidate SNPs
1053 associated with PC1 and 4,974 candidate SNPs associated with PC2. There
1054 were 27 candidate genes identified across both PC1 and PC2, and their
1055 locations and names are shown. Q-values were calculated using the
1056 geometric mean of the outputs from both LFMM2 and BayeScEnv, and a
1057 significance threshold of 0.05 was employed to identify candidate SNPs
1058 significantly associated with PC1 or PC2 (candidates shown in red).

1059

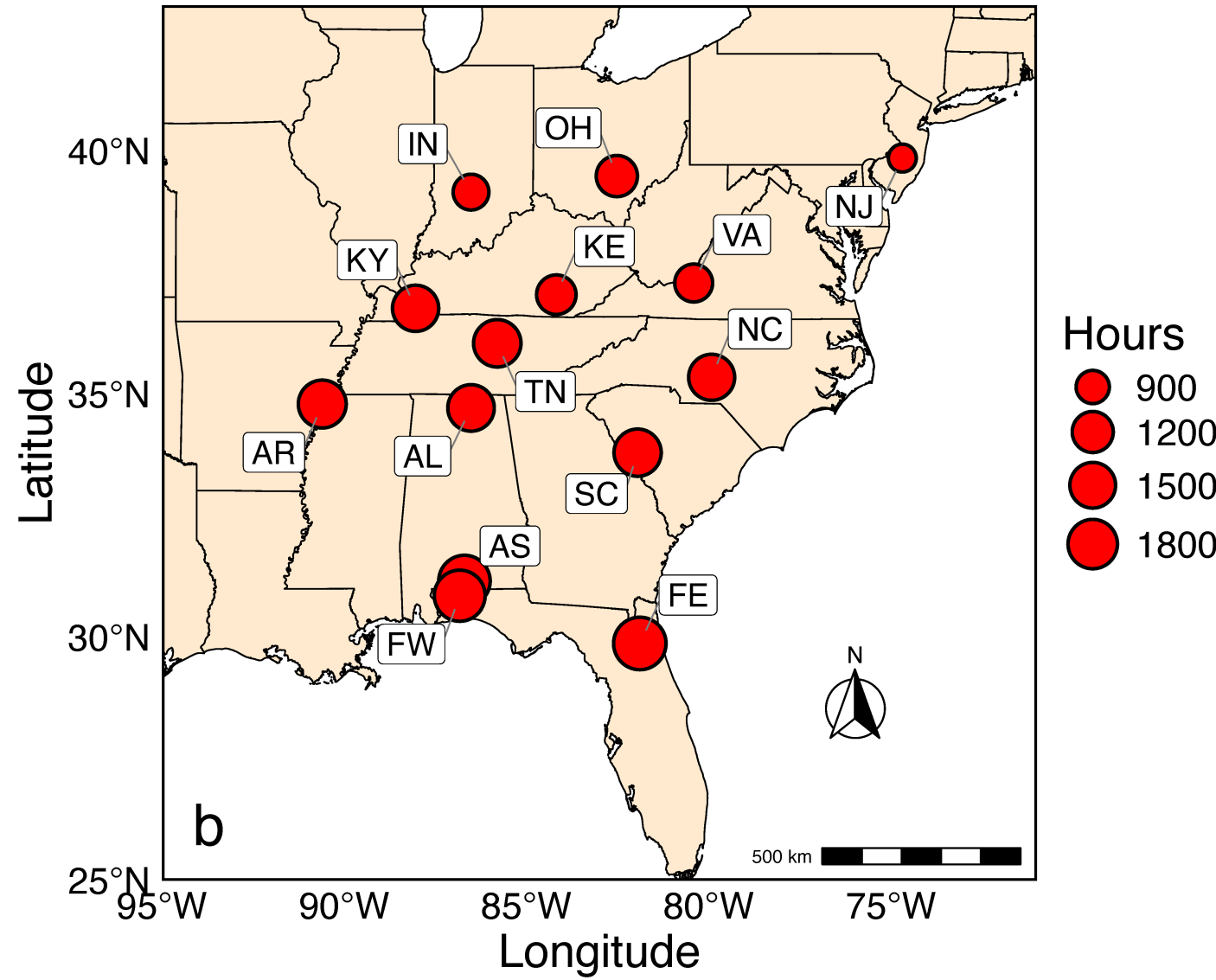
1060 Figure 5. We used genome-wide linkage disequilibrium to identify
1061 correlations between candidate genes identified using LFMM2 and
1062 BayeScEnv. A) Histogram of the distribution of linkage values (r^2) for the
1063 1,206,681 comparisons between the 2040 candidate SNPs. A large majority
1064 of the SNPs showed no evidence of linkage disequilibrium (mean r^2 =
1065 ~ 0.017). B) Candidate genes that harbor SNPs in significant linkage ($r^2 > 0.48$,
1066 the 99.5th percentile) are plotted with linkage values shown by the color-
1067 coded connections. The locations of 18 genes that significantly enrich for
1068 calcium signaling and gonadotropin-releasing hormone pathways are shown
1069 (LOC121933610 “ADCY1-like”, LOC121936315 “CACNA1B-like”, CACNA1C,
1070 CACNA1D, CACNA1H, GDNF, GNRH1, ITPR2, MAP2K3, MAP3K3, MAPK13,
1071 NOS3, PHKB, LOC121926791 “PLA2G4D-like”, LOC121926840 “PLA2G4E-
1072 like”, PLCE1, PTK2B, and RYR2). Additionally, the location of six candidate
1073 genes identified across both PC1 and PC2 are plotted (CDH5, CR1, AQP11,
1074 ST6GAL2, WNT2B, ASXL3).



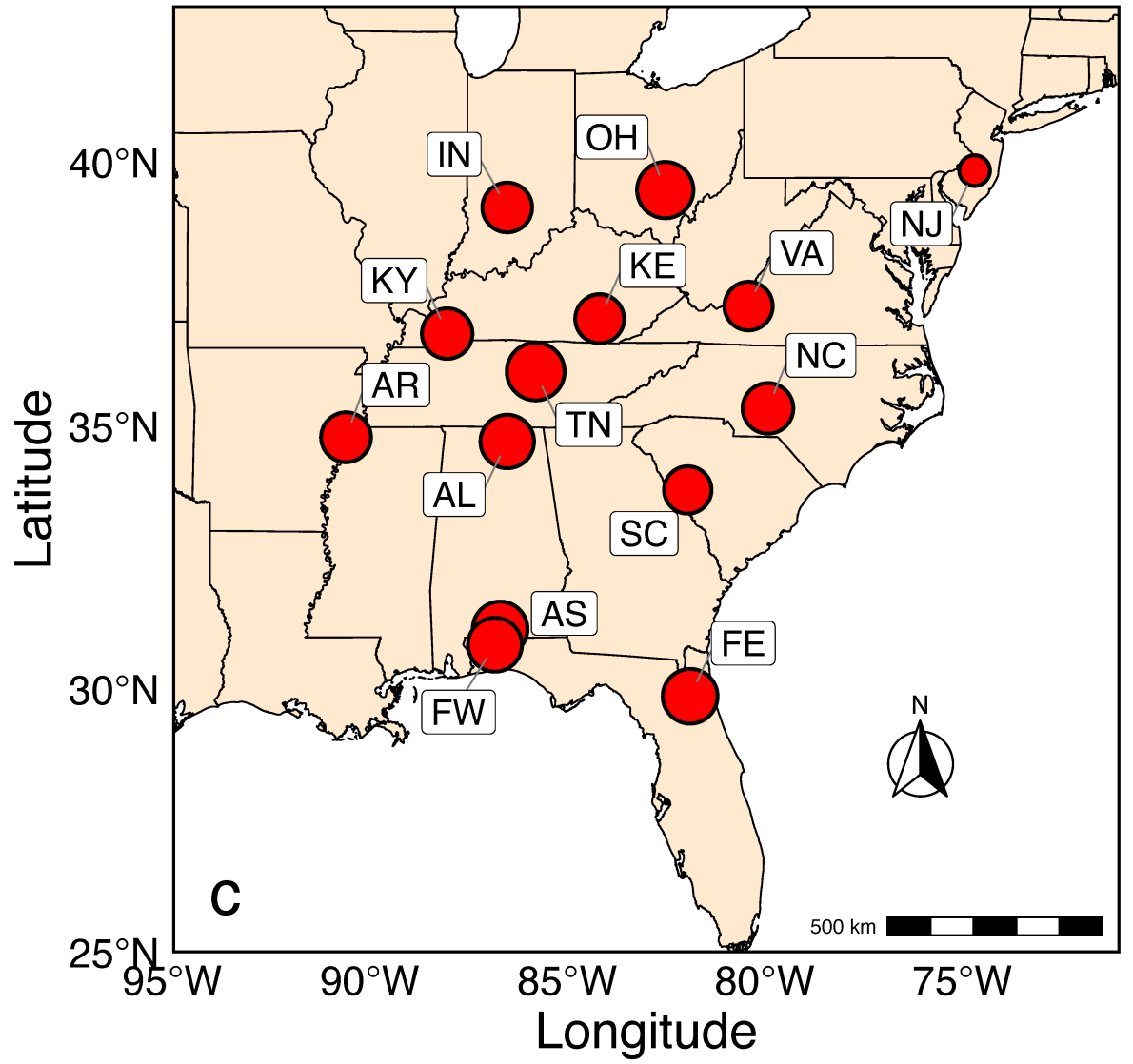
Mean Adult Activity 2001–2020



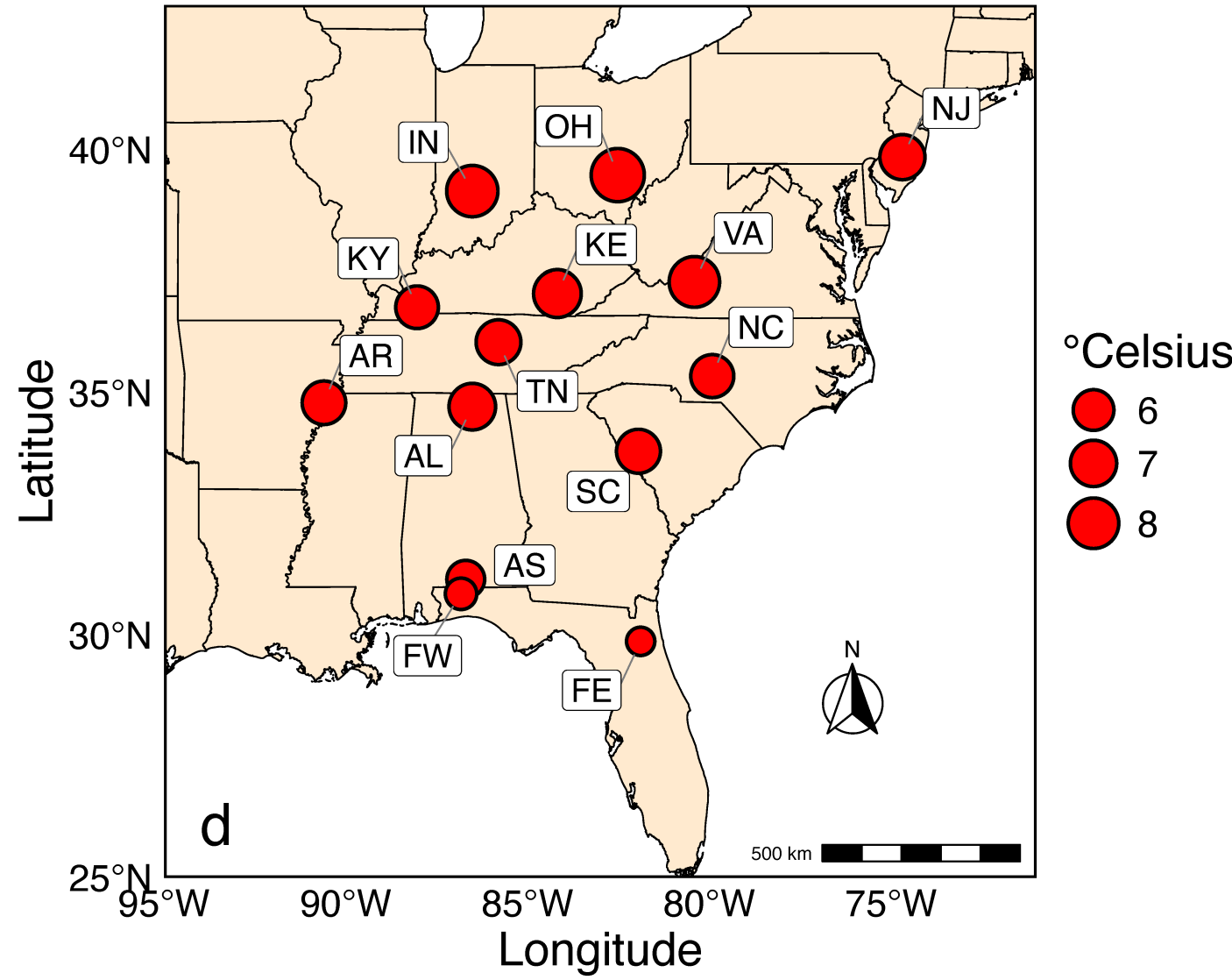
Mean Hatchling Activity 2001–2020



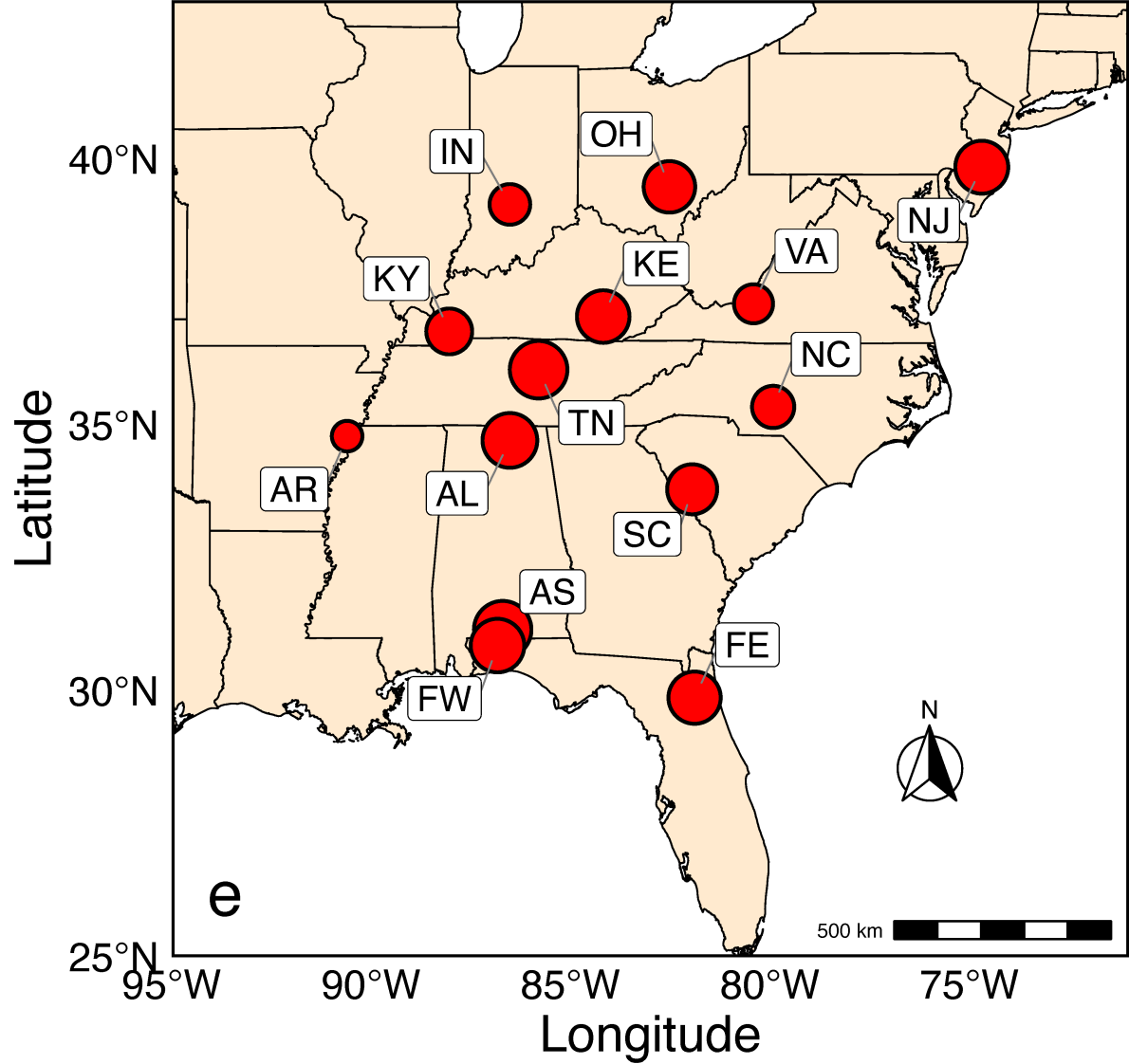
Mean Nest Temperatures 2001–2020



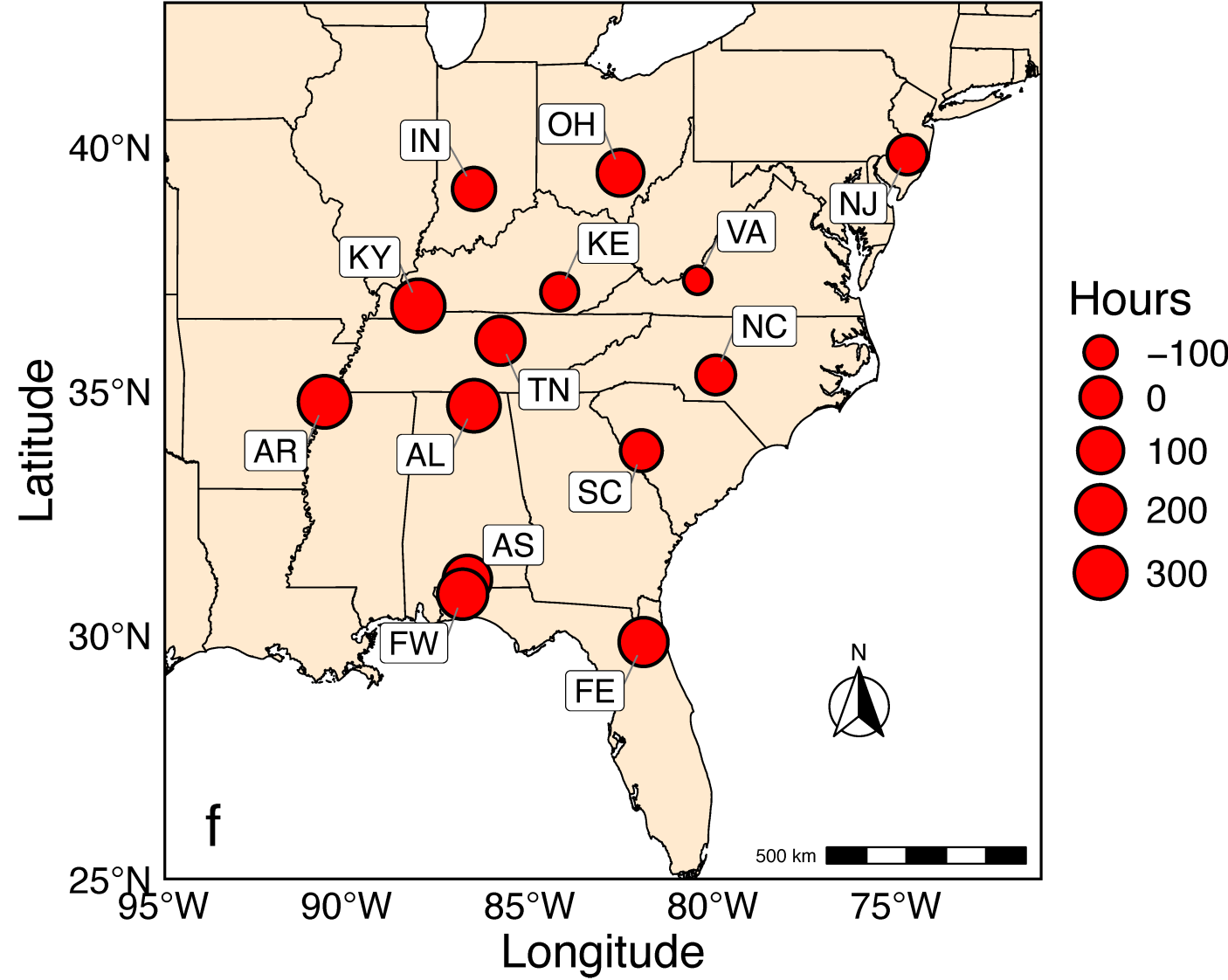
Range of Nest Temperatures 2001–2020



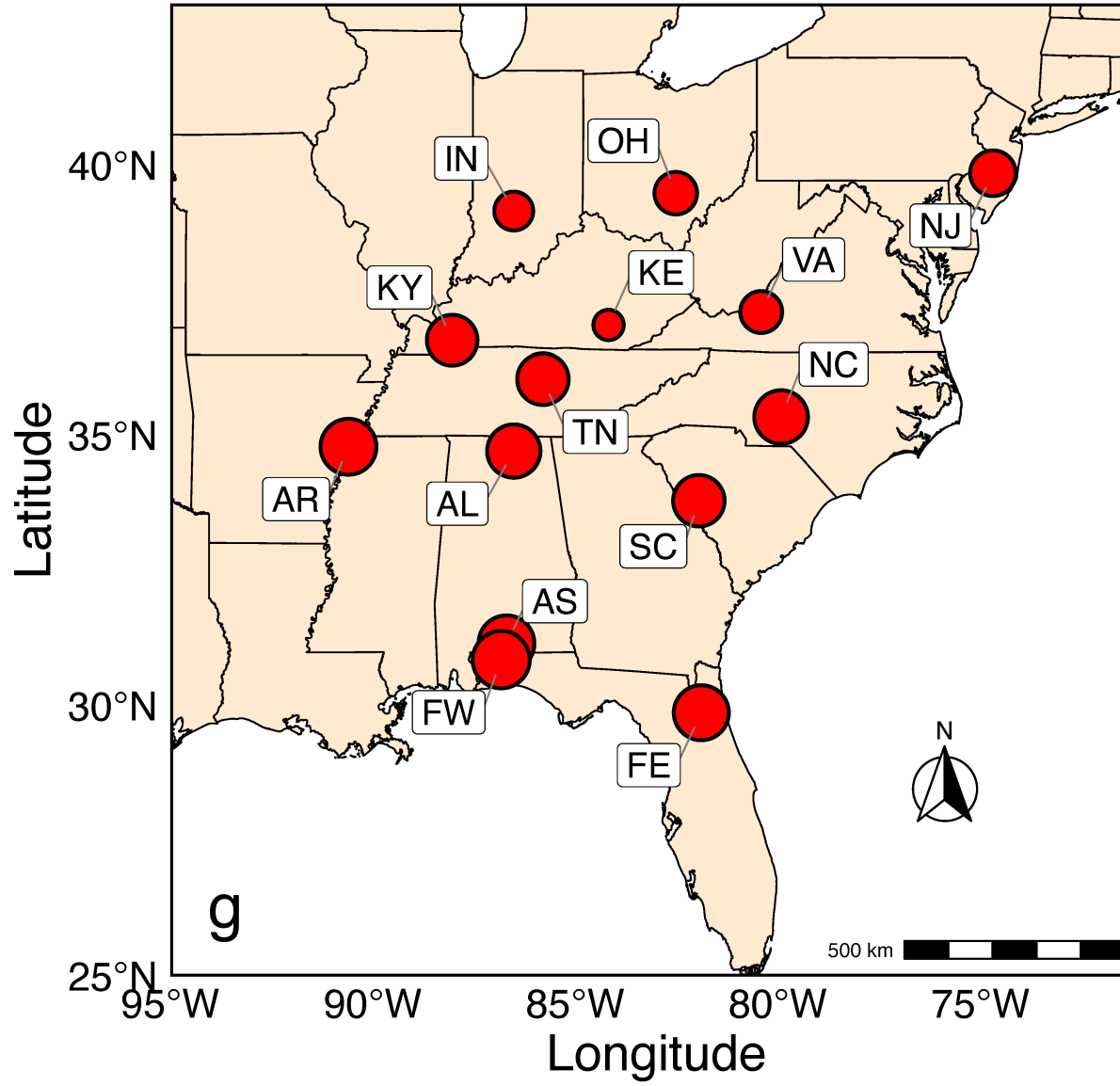
Change in Adult Activity 1900s–2000s



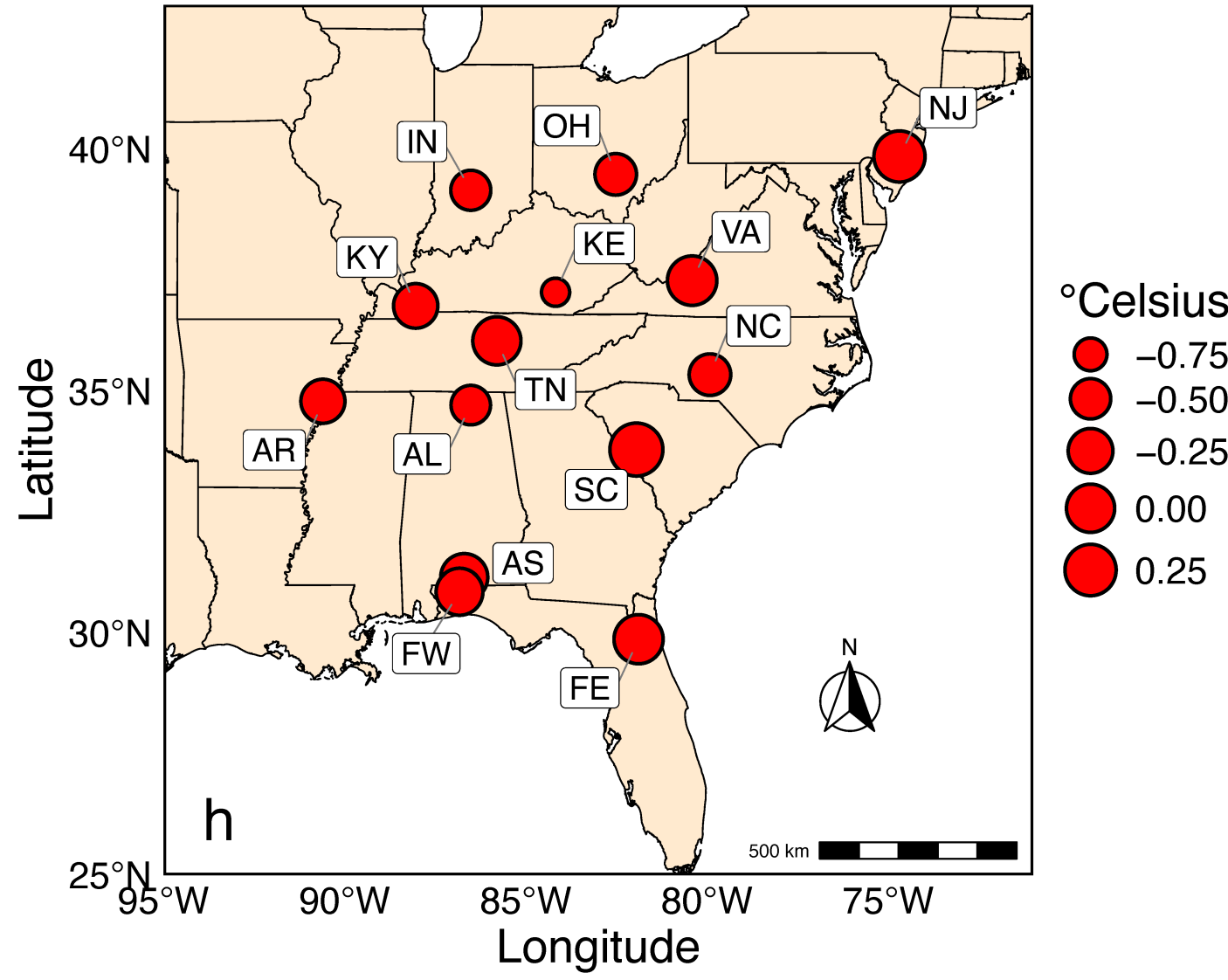
Change in Hatchling Activity 1900s–2000s

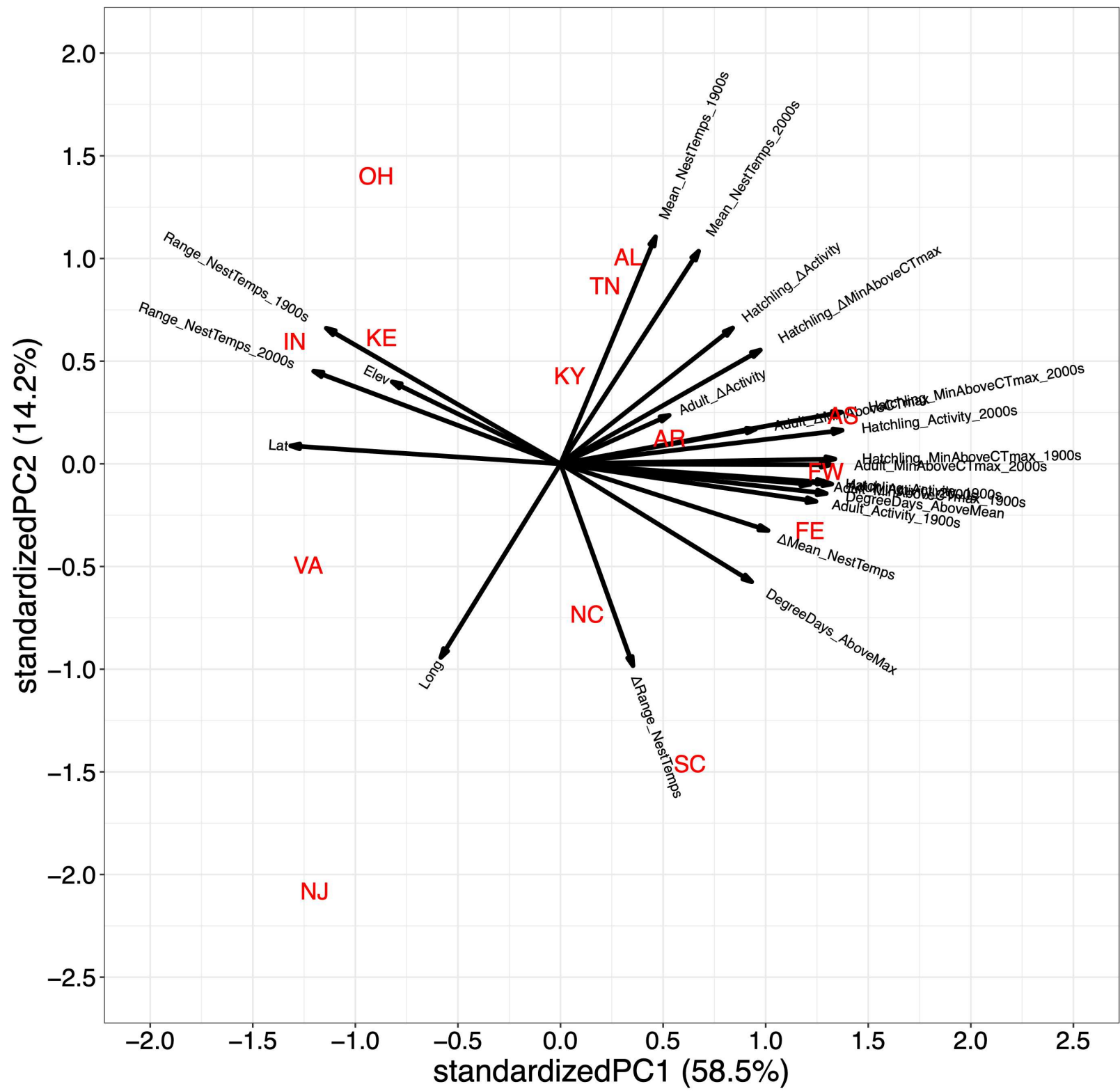
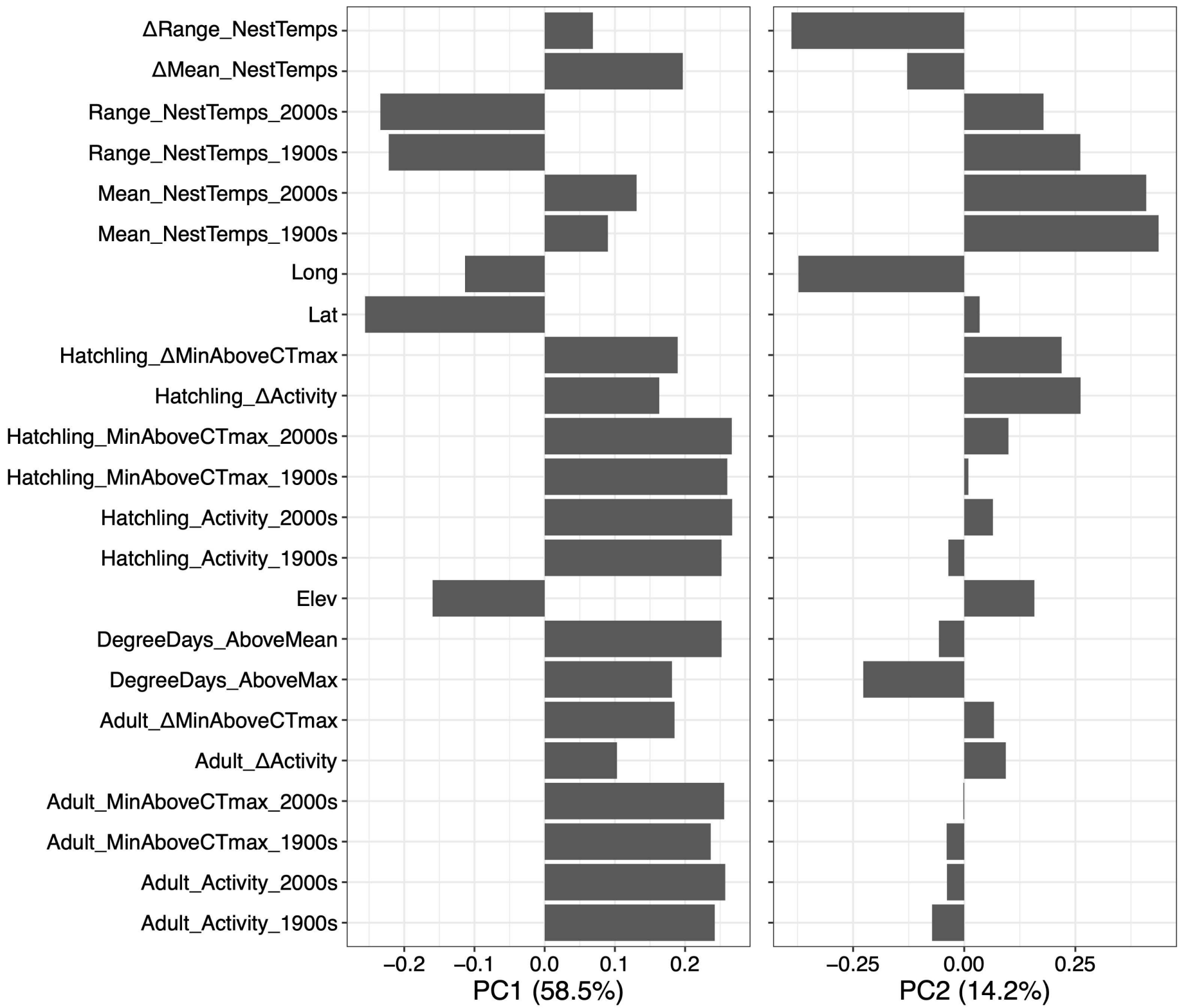


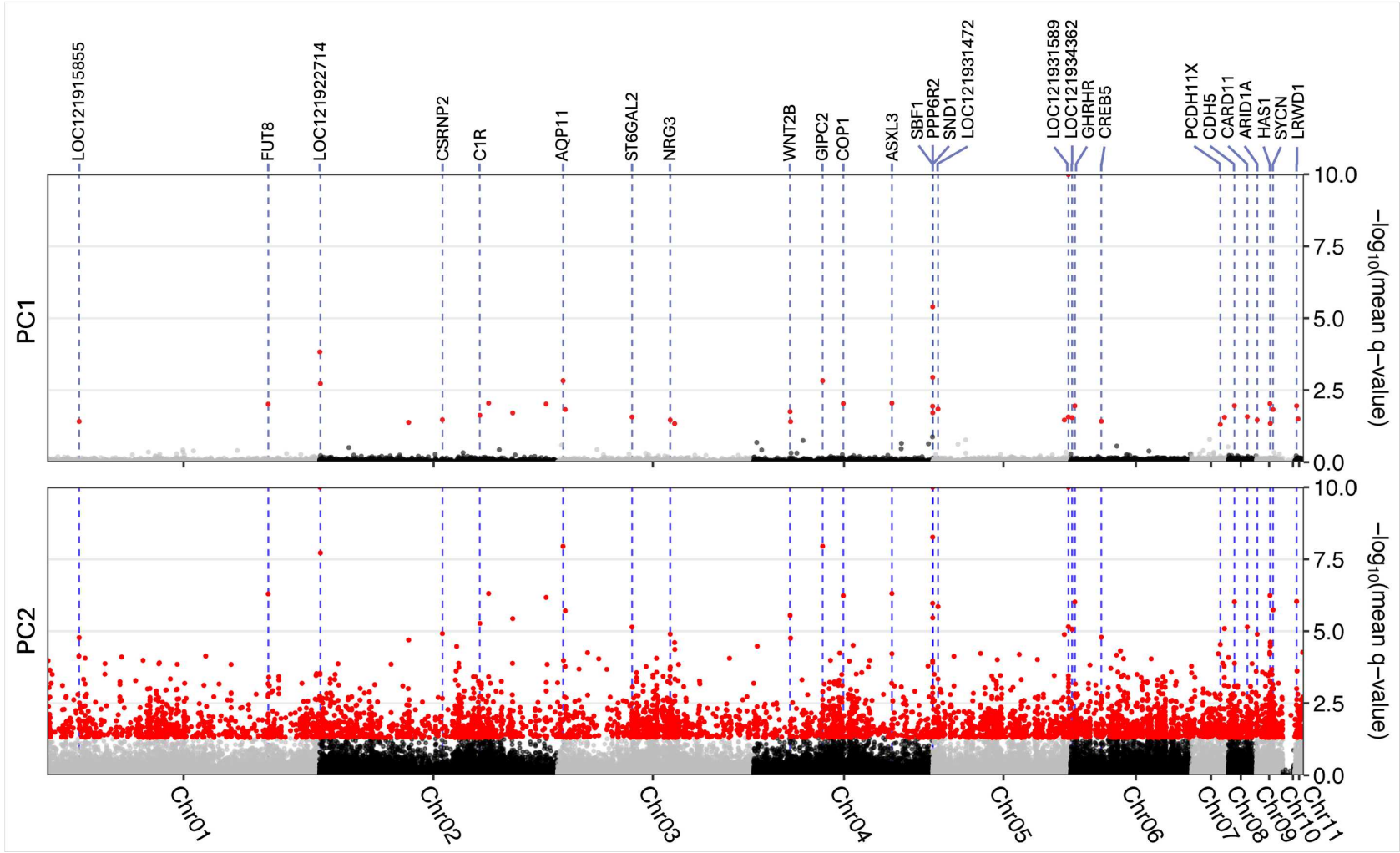
Degree Days Above Mean Nest Temperature 1900s vs. 2000s



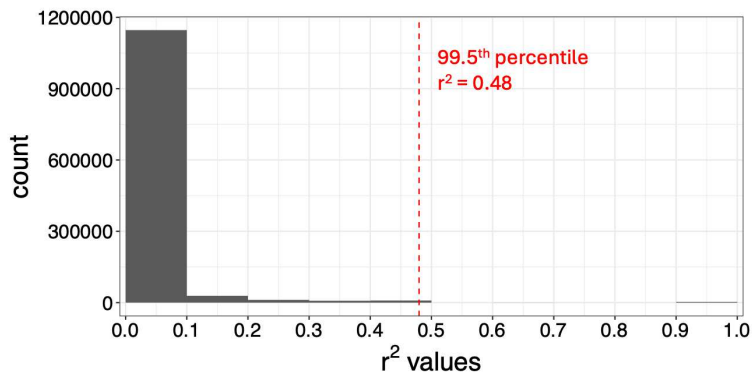
Change in Range of Nest Temperatures 1900s–2000s



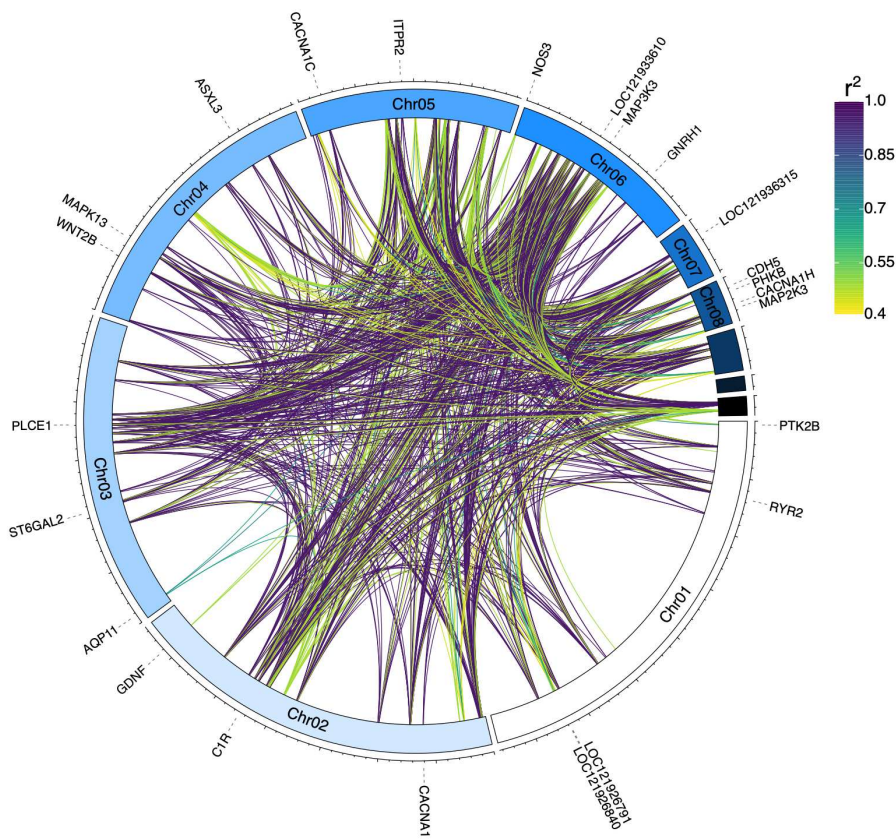




a)



b)



Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.zip](#)
- [Table1PredictorsLatLongElevLinearModelsResultsTable.xlsx](#)
- [Table2KEGGENrichmentGenes.xlsx](#)