EVOLUTION

Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard

Shane C. Campbell-Staton,1,2 Zachary A. Cheviron,2 Nicholas Rochette,1 Julian Catchen,1 Jonathan B. Losos,3 Scott V. Edwards3

Extreme environmental perturbations offer opportunities to observe the effects of natural selection in wild populations. During the winter of 2013–2014, the southeastern United States endured an extreme cold event. We used thermal performance, transcriptomics, and genome scans to measure responses of lizard populations to storm-induced selection. We found significant increases in cold tolerance at the species’ southern limit. Gene expression in southern survivors shifted toward patterns characteristic of northern populations. Comparing samples before and after the extreme winter, 14 genomic regions were differentiated in the surviving southern population; four also exhibited signatures of local adaptation across the latitudinal gradient and implicate genes involved in nervous system function. Together, our results suggest that extreme winter events can rapidly produce strong selection on natural populations at multiple biological levels that recapitulate geographic patterns of local adaptation.

In 1898, Hermann Bumpus provided the first measurement of the effects of natural selection operating on a wild population (1). By comparing house sparrows that survived a severe snowstorm to those that perished, he was able to quantify selection on body size and shape. Events like the 1898 snowstorm can result in intense episodes of rapid demographic and evolutionary change (1–4) and, despite their brevity, may account for a large portion of total selection experienced by a population (5). However, despite advances in technology and statistical inference, there are still surprisingly few empirical examples of natural selection imposed by intense weather events (1, 3, 4) and fewer still have investigated the regulatory and genetic mechanisms targeted by such events (6, 7).

Here, we investigate the effects of natural selection in response to an extreme cold event at the phenotypic, regulatory, and genetic level in wild populations of the green anole lizard, Anolis carolinensis. We measured critical thermal minimum (CTmin), the temperature at which lizards lose coordination under cold challenge (8), at five sites along a latitudinal transect in August 2013 (Fig. 1A). During winter 2013–2014, weakening of the arctic low-pressure zone (the “polar vortex”) led to extreme cold snap throughout the southeastern United States (Fig. 1), resulting in minimum temperatures that were significantly colder than those in the previous 15 years at all sites (Welch’s two sample t test; Brownsville, Texas (BRO): P < 0.01; Victoria, Texas (VIC): P = 0.047; Austin, Texas (AUS): P = 0.021; Arlington, Texas (ARL): P < 0.01; Hodgen, Oklahoma (HOD): P < 0.01). Because cold tolerance in this species naturally varies with latitude (9), we estimated the local intensity of storm-induced cold stress at each site as the number of days each population experienced temperatures below its mean CTmin compared to the previous winter. The southernmost population experienced the greatest increase in days below CTmin (BRO: 164.71%, 28 days). Populations farther north also experienced increased cold stress, although to a lesser degree (VIC: 30.77%, 20 days; AUS: 8.33%, 7 days; ARL: 26.9%, 20 days; HOD: 463%, 5 days).

We hypothesized that the extreme cold may have exerted natural selection on these populations, eliminating less cold-hardy individuals. Therefore, we investigated whether survivors of the 2013–2014 winter storms displayed greater cold tolerance than individuals sampled the previous year. We revisited BRO and AUS in April 2014 to measure CTmin of the survivors. The southernmost population (BRO) showed a significant increase in cold tolerance (linear mixed effects model, t = −2.09, P = 0.043), whereas AUS showed no change (t = −0.182, P = 0.856) (Fig. 2). To rule out potential effects of seasonal plasticity, we returned to these localities during late July 2014 to remeasure CTmin. If the shifts observed in the spring were due to seasonal plasticity, we expected CTmin in BRO to return to prevwinter estimates the following summer. Instead, the increase in cold tolerance in BRO was maintained through the summer (t = −2.72, P = 0.009). We revisited the remaining sites during late July 2014 to measure the geographic extent of this effect. VIC also displayed a significant increase in cold tolerance (t = −2.057, P < 0.05), whereas more northern sites did not (AUS: t = 0.116, P = 0.908; ARL: t = −0.818, P = 0.429; HOD: t = 1.064, P = 0.299). Because cold tolerance is heritable (9) and locally adapted (9–10) in the green anole, strong selection may lead to rapid evolutionary response of this phenotype.

Next, we tested the hypothesis that the cold snap selected for individuals in southern populations with regulatory phenotypes more similar to their northern counterparts. We sequenced

1University of Illinois, Urbana-Champaign, IL, USA. 2University of Montana, Missoula, MT, USA. 3Harvard University, Cambridge, MA, USA.

*Corresponding author. Email: shane.campbellstaton@gmail.com

Fig. 1. Distribution of collection sites along a latitudinal cline. (A) Collection localities. (B) Minimum daily temperatures of 1998 to 2013 (November 1 to February 28). Daily values were averaged for the 15 years before the winter of 2013–2014. (C) Percentage increase in days below the minimum thermal limits of the population at each collection site. All data are ordered by latitude from top to bottom.
48 liver transcriptomes of lizards collected before and after the focal winter. Before the winter storms, eight individuals were sampled from each end of the latitudinal transect following a 14-day common-garden acclimation to either 30°C (BRO: N = 4; HOD: N = 4) or 20°C (BRO: N = 4; HOD: N = 4). An additional 10 animals were also sampled from the mid-latitude sites before the winter storms and acclimated to 30°C for 14 days (VIC: N = 6; AUS: N = 4). After the winter storms, 22 animals were sampled from four sites (BRO: N = 8; VIC: N = 6; AUS: N = 8) after a 14-day, 30°C acclimation period. We used the program WGCNA (17) to identify modules of coexpressed genes across all 48 liver transcriptomes. Scores from the first principal components analysis axis of variation were used as a measure of module expression and regressed against latitude of origin (Fig. 3). We identified 57 coexpression modules, three of which were significantly associated with latitude after controlling for variation in mass, sex, and acclimation condition (table S1).

Next, we used individuals from the 30°C acclimation to test for storm-mediated shifts in gene expression. We found that winter survivors in BRO displayed shifts in gene expression predominantly toward mean expression levels of the northernmost population (HOD) (exact binomial test; Module 16: P = 0.038; Module 18: P = 0.044; Module 53: P < 0.001) (Fig. 3). These shifts support the hypothesis that the extreme winter at the southernmost site selected for survivors with regulatory phenotypes more similar to lizards that frequently endure harsher winters farther north. This trend was not apparent in survivors at the other collection sites (see the supplementary materials), likely due to the lesser effect of the winter storms at these sites.

Finally, we identified putative genomic targets of storm-induced selection in the southernmost site (BRO) by mapping RNA sequencing reads from individuals collected before (N = 8) and after (N = 8) the winter storms. Fourteen genomic regions displayed significantly elevated genetic divergence (fixation index (Fst) outlier peaks) between individuals collected before and after the storm (bootstrap resampling, P < 0.05) (Fig. 4A). Four of these regions also show signatures of genetic divergence between BRO and the northernmost site (HOD) (Fig. 4A), suggesting that the extreme winter event targeted regions of the genome that may also be involved in local adaptation across the latitudinalcline. Absolute divergence, d_{ST} (12), was significantly elevated within southern versus northern Fst outlier peaks (mean within-peak d_{ST} = 0.00063; mean outside-peak d_{ST} = 0.00054; Wilcoxon rank sum test: P = 0.01).
GABA is an inhibitory neurotransmitter crucial to nerve function. The acetylcholinesterase orthologs (SLC6A1 and SLC6A8) within two gamma-aminobutyric acid (GABA) transporters play an important role in neurotransmission (14, 15). These events may have drastic effects on natural populations by inducing intense episodes of natural selection and driving evolution on contemporary time scales (16). Understanding the biological effects of these events has important implications for the continued survival of species around the globe. This study demonstrates that such events can rapidly induce natural selection at the phenotypic, regulatory, and genetic levels resulting in patterns of divergence similar to those driven by local adaptation along natural climatic gradients.

**Fig. 4. Genomic scan for targets of storm-mediated selection.** (A) Scan for genetic differentiation between lizards collected before and after the winter of 2013–2014 from BRO. Gray points represent differentially expressed genes under FST outlier peaks (bootstrap resampling, P \(\geq 0.05\)) in north (HOD) versus south (BRO) comparisons. Red and green dots indicate regions of significantly elevated FST between samples (bootstrap resampling, P \(\leq 0.01\) and P \(\leq 0.05\), respectively). Black lines indicate differentially expressed genes within FST outlier peaks. (B to F) Expression differences between pre- and post-storm BRO samples at gene expression outliers. Genes shown are associated with cholinesterase activity and sodium symporter activity.

### REFERENCES AND NOTES


### ACKNOWLEDGMENTS

M. Fujita, Y. Stuart, and A. Jaffe provided assistance in collecting samples. National Science Foundation, Harvard University, and University of Illinois provided funding. Sequence data are available from the National Center for Biotechnology Information: SAMN06042490 to SAMN06042537. Cold tolerance data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.g500m. Samples are accessioned in the Harvard Museum of Comparative Zoology (MCZ Cryogenic 4448-4586).

### SUPPLEMENTARY MATERIALS

[www.sciencemag.org/content/357/6350/495/suppl/DC1](www.sciencemag.org/content/357/6350/495/suppl/DC1)

Materials and Methods Figs. S1 to S8
Table S1
References (17–27)
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Extreme events bring rapid change

Environmental adaptation is often considered a slow process. However, extreme events, such as heat waves or cold snaps, can produce rapid changes, both morphologically and genetically. Campbell-Staton et al. studied a population of green anole lizards during an extreme cold snap in the southern United States (see the Perspective by Grant). After the cold snap, the lizards showed greater cold resistance and displayed changes in six genomic regions that are important for regulation of function in the cold. Understanding how extreme climatic events influence adaptive potential will become increasingly important as the climate becomes more volatile.

Science, this issue p. 495; see also p. 451
Supplementary Materials for

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Shane C. Campbell-Staton,* Zachary A. Cheviron, Nicholas Rochette, Julian Catchen, Jonathan B. Losos, Scott V. Edwards

*Corresponding author. Email: shane.campbellstaton@gmail.com

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This PDF file includes:

Materials and Methods
Figs. S1 to S8
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References
Supplementary Materials

Animal Care Approval:

All animal husbandry and experimental protocols were approved by the University of Illinois IACUC protocol 14049 and Harvard University IACUC protocol 26-11.

Supplemental Methods and Results:

Study sites, temperature analyses and lizard collection:

We collected climatic, phenotypic and genetic data from five collection sites distributed across a latitudinal gradient (Brownsville, TX (BRO): 25.894346, -97.489169; Victoria, TX (VIC): 28.818269, -97.024316; Austin, TX (AUS): 30.244070, -97.720786, Arlington, TX (ARL): 32.768686, -97.083690 and Hodgen, OK (HOD): 34.784382, -94.694722). During the summer of 2013 we measured cold tolerance of 108 lizards from these five sites after two-week exposures to 20°C (BRO: N = 10, VIC: N = 10, AUS: N = 12, ARL: N = 9, HOD: N = 10) and 30°C (BRO: N = 9, VIC: N = 15, AUS: N = 14, ARL: N = 9, HOD: N = 10). To estimate the intensity of winter storms at each locality, we obtained daily minimum temperature data from weather stations using the National Oceanic and Atmospheric Association (NOAA) online data tools. Minimum temperature values were collected for sixteen winters (November 1 – February 28) from 1998-2014. We used Welch two sample t-tests to quantify differences in minimum temperature distributions between the focal winter (2013-2014) and the fifteen years previous.

The winter of 2013-2014 was significantly colder at all five sites studied (difference of -2.35°C, averaged across all five sites). BRO, the coldest temperature experienced during the winter of 2013-2014 was -1.1°C, compared to an average low of 7.96°C across the previous 15 years. There were 45 total days below average CT_min (sub-critical days). Sub-
critical bouts were 1-7 days in duration, with between 1 and 11 days in between. In VIC, min temp was -4.4°C compared to a 15-year average low of 3.48°C. There were 85 total days below $CT_{\text{min}}$, with sub-critical bout lasting 1-20 days separated by 2-7 days in between. In AUS, the minimum winter temperature was -11.1°C compared to a 15-year average low of 0.37°C. There were 91 sub-critical days broken into 3-20 day bouts with between 1-3 days in between. In ARL, he minimum temperature during the winter of 2013-2014 was -11.7°C compared to a 15-year average low of -0.59°C. There were 110 total sub-critical days in 3-20 day bouts with 1-3 days in between. In HOD, the minimum winter temperature of 2013-2014 was -14°C compared to a 15-year average low of -4.73°C. Lizards at this site experienced 111 sub-critical days, clustered in 3 – 61 day intervals with 1-3 days in between.

Figure S1 displays daily minimum winter temperatures for 2013-2014 and the 15 years previous.

To estimate the intensity of cold stress on green anole populations, we measured the difference in the number of days in which each population experienced temperatures below their mean functional limit ($CT_{\text{min}}$). $CT_{\text{min}}$ was measured during the July and August of 2013 (see the following section for details of regarding measurement of $CT_{\text{min}}$). Lizards from the 30°C acclimation group were used for this analysis. The percent of total days with minimum temperatures below this mean was estimated for the winters before and after the summer of 2013 at each site. The percent increase in days between the two winters was used as a measure of cold stress severity caused by the extreme weather event of 2013-2014.

In the April of 2014 we collected lizards from two of our previous collections sites (BRO and AUS) and August of 2014 we revisited all five localities to test for significant shifts in cold tolerance in the survivors of the storm and their offspring.
Analyses of phenotypic selection:

To test for significant shifts in cold tolerance in the survivors of this extreme winter event, we measured functional performance during cold exposure. Animals collected during the spring and summer of 2014 (BRO: N = 32, VIC: N = 17, AUS: N = 33, ARL: N = 6, HOD: N = 14) were brought into common laboratory conditions and acclimated for 14 days at 30°C. We inserted a 20-gauge digital thermocouple approximately 5mm inside the cloaca of each lizard to continuously measure internal body temperature (Tb). Beginning from room temperature, we cooled the Tb of each lizard 1°C per minute. We then flipped the lizard onto its back and stimulated it with forceps, allowing it 30 seconds to right itself, repeating this protocol periodically until it was unable to right itself within the given window of time. The body temperature at which an animal could no longer right itself after 30 seconds was recorded as its CTmin. Cooling rate during each trial was recorded for statistical analyses.

We then compared cold tolerance of lizards collected in the spring and summer of 2014 to measurements taken from lizards collected in the summer of 2013 at the same sites after acclimation to similar laboratory conditions (2-weeks at 30°C). For each site, we tested the association between CTmin and collection date, using cooling rate during each trial as a random variable. To confirm that lizards captured in the summer after the storm were survivors from the previous year, we used a nonlinear growth equation (17) to estimate birth date based on snout-vent length.

Transcriptome sequencing:

Lizards from all acclimation and experimental groups were anesthetized immediately upon the conclusion of experimentation using 5% vaporized isoflurane and euthanized via cervical dislocation. Liver tissue was taken immediately following euthanasia, flash frozen in liquid nitrogen and stored at -80°C. We sequenced the full liver transcriptomes of 48
individuals collected before and after the winter storm event. 26 individuals were sequenced from before the storm event. Eight individuals were sequenced from each end of the latitudinal transect (Brownsville, TX and Hodgen, OK). For both of these sites, four animals were exposed one of two, two-week acclimation treatments (30°C or 20°C). Ten animals were selected from mid-latitude sites (Victoria, TX N=6; Austin, TX N=4) after 30°C two-week acclimation treatment. Additionally, we sequenced 22 individuals collected after the winter storm (Brownsville, TX N = 8, Victoria, TX N = 6, Austin, Texas N = 8). Each of these individuals was acclimated to 30°C for two weeks prior to sampling. Total RNA was extracted from each sample using Trizol RNA isolation reagents. Transcriptome libraries were constructed using a Wafergen mRNA directional library preparation kit. Each library was sequenced for 75bp reads using the Illumina Nextseq 500 platform. We used Trimmomatic (18) to filter the resultant sequences based on quality. Sequence quality was assessed for each read in a 4bp sliding window. Sequences were trimmed once Phred33 quality scores fell below an average of 15 within the window. Adapter sequences, if detected, were removed. Quality controlled sequences were then mapped to the Anolis carolinensis genome (19) using Tophat2 (20). Raw reads counts were normalized by library size using the cpm function in edgeR (21). These values were quantile normalized and log transformed for the gene co-expression network analyses below.

**Identification of regulatory modules associated with cold tolerance:**

Pair-wise Pearson correlations between each pair of genes were used to create signed regulatory networks in WGCNA (11). We first used Pearson correlations to identify and remove outlier samples (Figure S2). We then computed an adjacency matrix and used a soft thresholding approach to approximate a scale free topological network (22) (Figure S3). Topological overlap was used to create cluster dendrograms based on hierarchical clustering
(Figure S4). We identified regulatory modules as branches of the resulting cluster tree using the dynamic tree-cutting method (11) and correlated modules \( R^2 = 0.75 \) were merged for downstream analyses.

To identify regulatory modules associated with latitude, we summarized module expression using a principal components analysis (PCA) of gene expression for each module with the `blockwiseModules` function in WGCNA. Eigengenes were calculated as the first principal component (PC1) of regulatory variation for each module (11). We used module eigengene values to test for associations between module expression and latitude by Pearson correlation with the `cor` function of WGCNA. P-values for the correlation were determined by a Student’s asymptotic test using the function `corPvalueStudent` (Table S1). We corrected for multiple testing by applying a Bonferroni correction. Once candidate modules were identified (corrected p < 0.05), we corroborated associations with latitude via additional statistical analyses. Linear mixed effects models were built for each module using sex and body weight as random variables and acclimation condition as a covariate. We then compared subordinate models, removing latitude as an effect. We then compared these models via likelihood ratio test. Modules showing significant improvement of model fit with inclusion of latitude as a variable were retained as strong candidates for further analysis.

We used gProfileR, implemented in R, to identify gene ontology categories enriched in latitude-associated modules. We performed an unordered query, filtering for significant results only and excluding electronic annotations with a minimum functional category size of three. FDR was implemented to correct for multiple testing and a strong hierarchical filtering threshold was applied. We combined all genes within latitude-associated modules and compared this list against all genes expressed in the liver transcriptome. Latitude-
associated modules were enriched for genes that participate in antigen processing and presentation (GO:0019882, p << 0.001) and antigen binding (GO:0003823, p << 0.001)

**Analysis of regulatory response to selection:**

We used regulatory modules showing significant correlations with latitude to test for evidence of regulatory response to the extreme winter event. For each candidate module identified in the WGCNA analysis we calculated the direction of gene expression shift for each gene at a given site after the storm. Additionally, we calculated the direction of gene expression shift between the site of interest and the northernmost site in the latitudinal transect (HOD). Lastly, we used an exact binomial test to estimate the significance of directional bias of gene expression in the module. This test was performed on each module for each site separately. Significant bias towards northern gene expression was found at the southernmost site (BRO, See Main Text), but this trend was not apparent in survivors at the other collection sites (VIC: Module 16: p = 0.722, Module 18: p = 0.571, Module 53: p = 0.529; AUS: Module 16: p = 0.205, Module 18: p = 0.252, Module 53: p = 0.909), likely due to the lesser impact of the storm at these sites.

**Identification of candidate genomic regions under natural selection**

To identify genomic regions showing significant differentiation after the extreme winter event, we aligned the cleaned reads from transcriptome samples to the *A. carolinensis* genome with STAR v. 2.5.1b (23) using a two-pass approach. The first pass used the Ensembl v. 74 (24) splice-sites annotations (via the --sjdbGTFfile option), and the parameters for de novo splice-junction discovery (--outSJfilterCountTotalMin and --outSJfilterCountUniqueMin) were set to 6-5-5-5. Uniquely mapped reads with less than 3 mismatches were retained (--outFilterMultimapNmax and --outFilterMultimapNmax options). Alignment files were processed with Picard v. 2.1.1 and SNPs were called with the
GATK HaplotypeCaller and GenotypeGVCF v. 3.5 (25) using the recommended RNAseq parameters (-stand_call_conf 20 -stand_emit_conf 20 --dontUseSoftClippedBases). The resulting SNPs were then filtered based on strand bias and coverage-corrected quality as recommended (FS>30 and QD<2), as well as for depth ≥5, missing data <20%, heterozygosity <70% and minor allele frequency >3% (i.e. 3 observations). Population genetics statistics were obtained using the Populations program from Stacks v. 1.42 (26). $F_{ST}$ values were calculated using the AMOVA method (27), and smoothed using a sigma of 5 Mb for the weight distribution. The significance of the deviation of the smoothed values to the genomic average was estimated using 10,000 bootstrap resamplings.

To ensure that the results of the BRO selection scans were not artifacts of limited sample sizes, we conducted an additional pre- vs post-storm selection scan, pooling samples from both populations showing a significant shift in cold tolerance (BRO + VIC, N= 16 before, N = 16 after). The results of this analysis were very similar overall (Figure S5). Additionally, absolute genetic divergence ($d_{XY}$, (12)) was calculated in non-overlapping 25kb windows across each chromosome. Windows that contained no single nucleotide polymorphisms were removed from the analysis. We tested for elevated $d_{XY}$ values within $F_{ST}$ peaks identified in north (HOD) vs. south (BRO+VIC) comparisons with respect to non-differentiated regions across the six macrochromosomes of the species’ genome using a Wilcoxon rank sum test (Figure S6, S7). Plots were drawn in R using custom scripts.

To find putative targets of storm-mediated selection under $F_{ST}$ peaks, we searched the liver transcriptome for individual loci that both displayed significant differences in gene expression and lay within regions of the genome with significant differentiation ($F_{ST} < 0.05$) in survivors of the winter storms. We used DESeq2 to identify differentially expressed genes in the liver, focusing on the two collection sites showing significant shifts in cold tolerance.
(BRO + VIC). We estimated size factors (the geometric mean of all samples) for each gene, and then estimated beta prior variance, matching a normal distribution using the upper quantile of the finite maximum likelihood estimate betas. Lastly, we tested for significance of coefficients in a negative binomial generalized linear model, using previously calculated size factors and dispersion estimates. Genes showing a significance level of $p < 0.05$ after correction for false discovery rate, were identified as differentially expressed (Figure S8). To further corroborate differences in gene expression, each built a linear mixed model for each strong candidate gene, using sex, mass, and population-of-origin as random effects. Results of these analyses corroborate a significant effect of collection time (before vs after the extreme winter) on candidate gene expression (SLC6A1: $p = 0.049$, SLC6A8: $p << 0.01$, LOC100561946 (Cholinesterase): $p << 0.01$, LOC100557088 (Cholinesterase): $p << 0.01$, Acetylcholinesterase: $p << 0.01$).

**Supplemental Figure Legends:**

Figure S1: Climatic variation during extreme winter of 2013-2014 and fifteen years previous. Dotted lines indicate average CTmin estimated during July of 2013 for each collection site. 1998-2013 plots are reported as daily means (colored lines) and standard errors (black outlines).

Figure S2: Sample dendrogram indicating mean pairwise correlations between transcriptome samples. Those individuals marked as outliers (showing low mean correlation with other samples) were removed from further analysis.

Figure S3: Graph of scale free topology in relation to soft threshold power. Asterisk indicates the soft threshold power chosen for module detection.
Figure S4: **Cluster dendrogram displaying patterns of correlated gene expression within the green anole liver transcriptome.** Each gene is represented by an individual branch. Module colors are used to identify groups of highly correlated genes.

Figure S5: **Pooled Fst selection scan of BRO + VIC.** A) Genome-wide scan for differentiation between lizards collected before and after the winter of 2013-2014 from sites that showed a significant shift in cold tolerance (BRO and VIC). Grey points represent individual values of F_{ST} for each SNP. Black and dots indicate non-significant F_{ST} values within 5Mb windows (bootstrap resampling, p \geq 0.01) in pre- vs post- storm comparisons. Blue dots indicate non-significant F_{ST} values within 5Mb windows (bootstrap resampling, p \geq 0.01) in north (HOD) vs. south (BRO+VIC) comparisons. Red dots indicate regions of significant F_{ST} between samples (bootstrap resampling, p < 0.01). Grey columns represent genomic regions that are outliers in both F_{ST} scans. Black lines indicate differentially expressed genes within F_{ST} outlier peaks B) Regulatory differences (normalized by library size) between pre- and post-storm samples at gene expression outliers in the southern two sites. Black dots represent mean expression in southern two sites combined, black lines represent standard error of the mean. Red and orange dots represent individual expression of individuals from BRO and VIC respectively.

Figure S6: **Plot of smoothed Fst values (blue dots: p \geq 0.01, red dots p < 0.01) and dxy (black dots) across each macrochromosome of the green anole genome.**

Figure S7: **Dxy values compared within Fst peaks vs. outside of Fst peaks, averaged across all 6 macrochromosomes.** Dots indicate mean values error bars indicates standard error of the mean.

Figure S8: **Analysis of differential expression between southern populations (BRO + VIC) collected before (August 2013) vs after (August 2014) the extreme winter storms.**
Dots indicate individual genes. Red dots are gene expression outliers (FDR adjusted p < 0.05).

Table S1: **Strength of correlation ($R^2$) and significance (p) between PC1 of regulatory module expression and Latitude, based on Student’s asymptotic T-test conducted in WGCNA (11).** P values in bold are significant after Bonferroni correction for multiple testing (q < 0.05).
Sample dendrogram and trait heatmap

outlierC
Scale independence

SFT, signed $R^2$

Soft Threshold (power)
Fst Peaks

Outside of Fst Peaks

dxy
DESeq2

log fold change

mean of normalized counts
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