

EVOLUTION

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

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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, Hermon 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 (CT_{min}), 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 an extreme cold snap throughout the southern 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 CT_{min} compared to the previous winter. The southernmost population experienced the greatest increase in days below CT_{min} (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: 4.63%, 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 CT_{min} 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 CT_{min} . If the shifts observed in the spring were due to seasonal plasticity, we expected CT_{min} in BRO to return to prewinter 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

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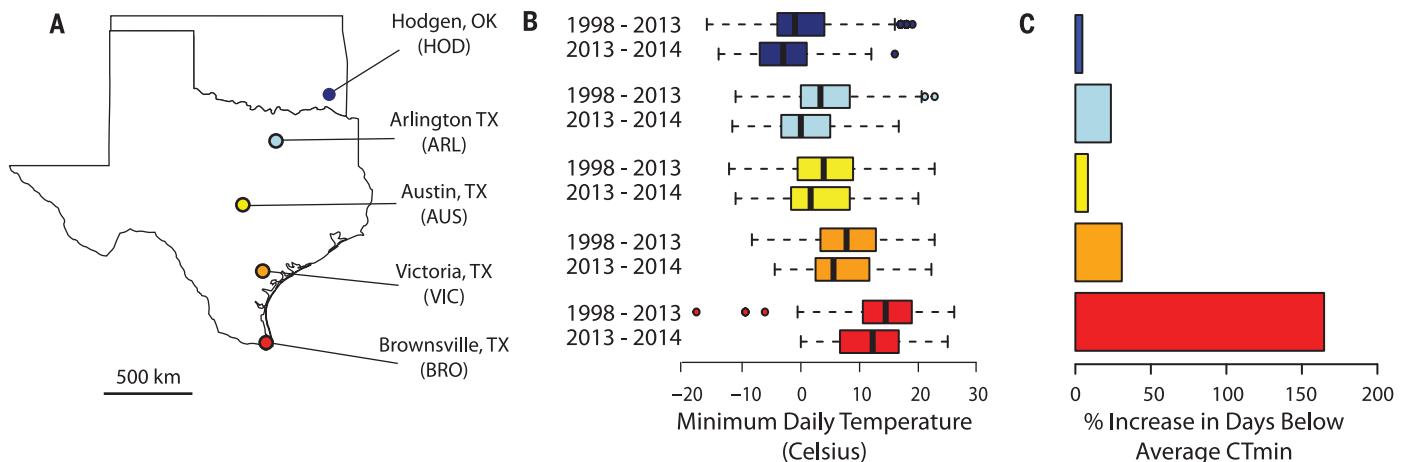


Fig. 1. Distribution of collection sites along a latitudinal cline. (A) Collection localities. (B) Minimum daily temperatures of 1998 to 2013 (November 1 to February 28). Daily values were averaged for the 15 years before the winter of 2013–2014. (C) Percentage increase in days below the minimum thermal limits of the population at each collection site. All data are ordered by latitude from top to bottom.

Fig. 2. Response of cold tolerance to the extreme winter of 2013–2014. (A)

Mean \pm SEM of critical thermal minimum (CT_{min}) in July and August of 2013 (summer 2013), April 2014 (spring 2014), and July 2014 (summer 2014). (B) Mean \pm SEM of (CT_{min}) during the summers of 2013 and 2014. Asterisks indicate that CT_{min} was significantly lower after the extreme cold event of 2013–2014.

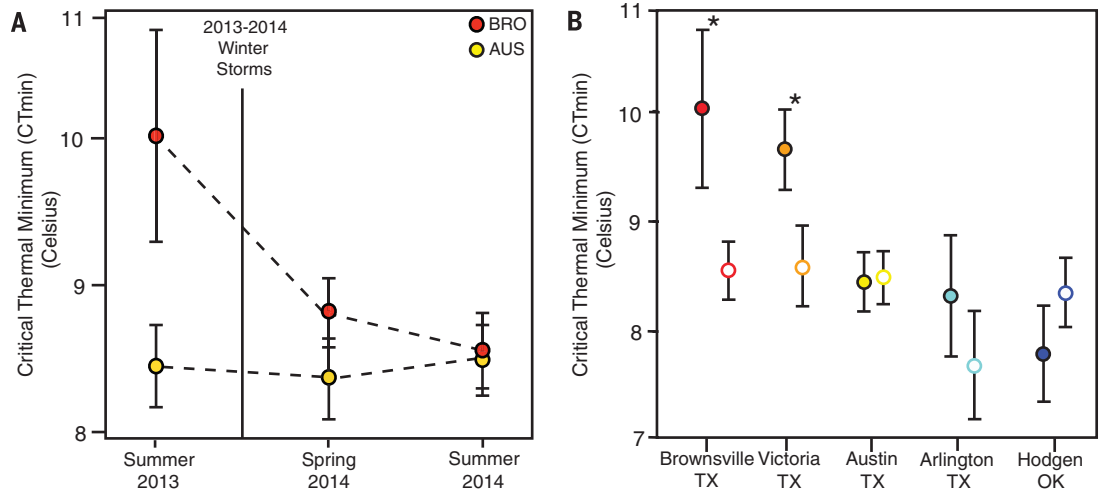
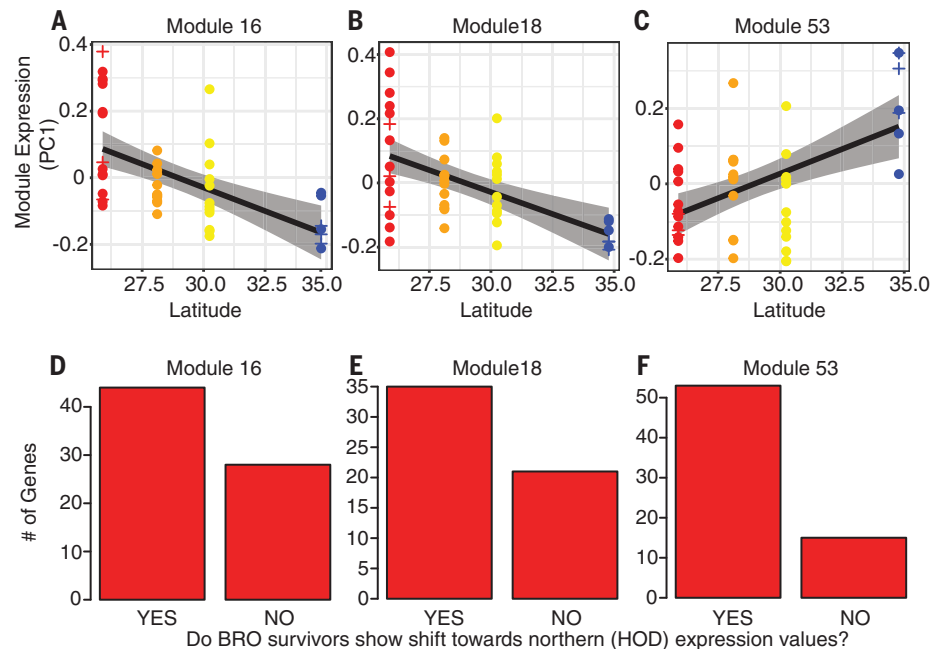


Fig. 3. Expression of regulatory modules associated with latitude. (A to C)

Associations between module expression and sampling latitude. Crosses and dots represent module expression scores from samples acclimated for 2 weeks at 20° and 30°C, respectively. (D to F) Bar plots representing the number of genes within each module that shift in expression in the southernmost population (BRO) after the storm in the same direction as gene expression differences between BRO and the northernmost site (HOD).



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 (11) 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 sig-

nificantly 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: $P = 0.041$; 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 (F_{ST}) 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 latitudinal cline. Absolute divergence, d_{XY} (12), was significantly elevated within southern versus northern F_{ST} outlier peaks (mean within-peak $d_{XY} = 0.00063$; mean outside-peak $d_{XY} = 0.00054$; Wilcoxon rank sum test: $P = 0.01$)

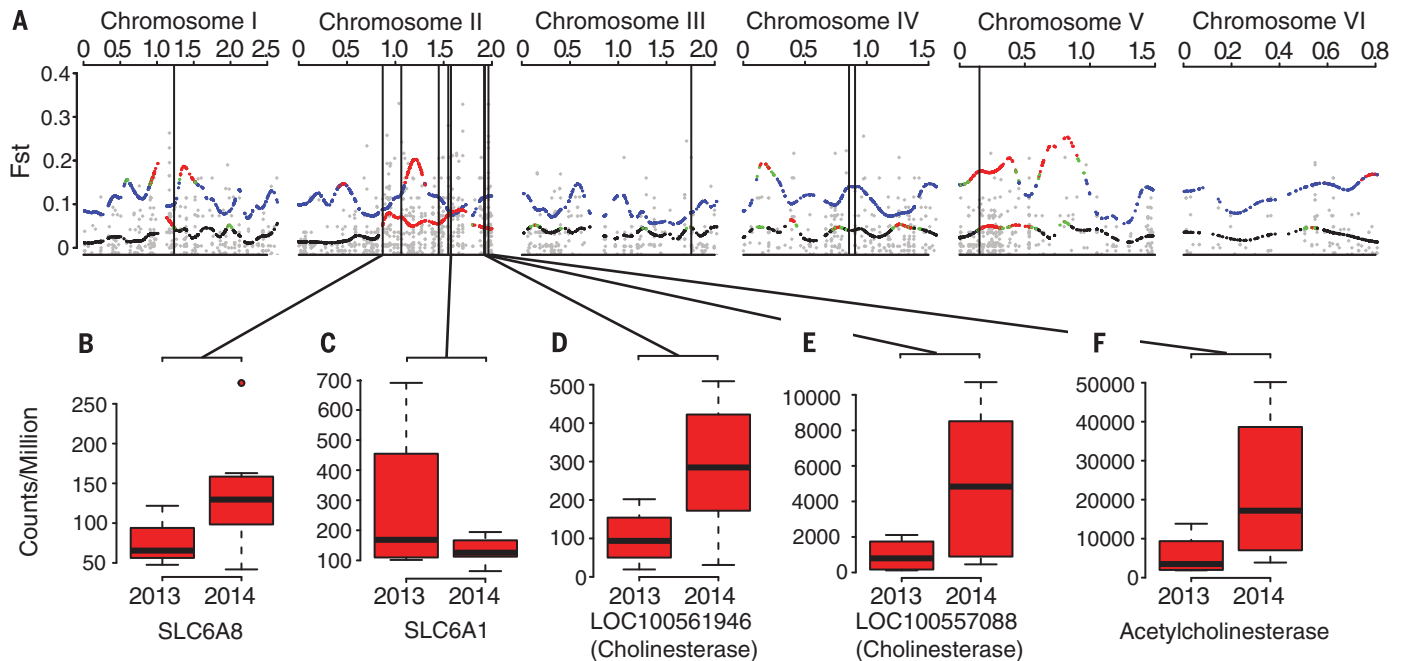


Fig. 4. Genomic scan for targets of storm-mediated selection. (A)

Scan for genetic differentiation between lizards collected before and after the winter of 2013–2014 from BRO. Gray points represent individual values of F_{ST} for each single-nucleotide polymorphism. Black dots indicate nonsignificant F_{ST} values within 5-Mb windows (bootstrap resampling, $P \geq 0.05$) in pre- versus post-storm comparisons. Blue dots indicate nonsignificant F_{ST} values within 5-Mb windows (bootstrap resampling,

$P \geq 0.05$) in north (HOD) versus south (BRO) comparisons. Red and green dots indicate regions of significantly elevated F_{ST} between samples (bootstrap resampling, $P < 0.01$ and $P < 0.05$, respectively). Black lines indicate differentially expressed genes within F_{ST} 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.

(figs. S5 and S6), suggesting that the observed patterns of differentiation are not an artifact of low-diversity regions across the species' genome (12).

Differentially expressed genes under F_{ST} peaks ($N = 12$) were enriched for carboxylic ester hydrolase activity (GO:00052689, $P = 0.012$) and sodium symporter activity (GO:0005328, $P = 0.014$; GO:000345, $P = 0.016$), which participate in maintenance of synaptic function, a proposed contributor to temperature-induced loss of function and death in ectothermic species (13). Three of these genes [acetylcholinesterase, LOC100557088 (cholinesterase), and LOC100561946 (cholinesterase)] are acetylcholinesterase orthologs that play an important role in neurotransmission (14). Additionally, two gamma-aminobutyric acid (GABA) transporters (SLC6A1 and SLC6A8) within F_{ST} outlier peaks are associated with sodium symporter activity. GABA is an inhibitory neurotransmitter crucial to the maintenance of muscle tone. All five genes are located on chromosome 2 of the green anole genome, under a single F_{ST} outlier peak (Fig. 4, B to F). Differential expression of these genes in the liver may reflect functional differences throughout the autonomic nervous system. Further study is needed to investigate the potential role of these genes in cold-dependent organismal function.

Extreme weather events are expected to increase in frequency and magnitude due to human-mediated

climate change (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.

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SUPPLEMENTARY MATERIALS

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.

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