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Morphological and genomic responses to hurricanes arise and persist during a biological invasion

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Hurricanes can be a source of strong, episodic natural selection, especially for coastal and island populations. In *Anolis* lizards, selection favors morphological traits that enhance clinging performance under hurricane-force winds. However, we know little about the longer-term persistence of morphological and genomic responses to these pulse-like events. To address this limitation, we capitalized on the well-documented history of hurricanes and spread of the invasive brown anole lizard, *Anolis sagrei*, over the past 130 y in the southeastern United States. We used 30 sites with estimates of the number of hurricanes experienced since population establishment. We found that hurricane frequency is consistently related to morphological trait values that increase clinging performance—longer limbs and larger toepads. In contrast, traits with no known connection to clinging ability were not related to hurricane frequency. Our genomic results show that despite a complex genetic architecture for most traits, populations retain a signature of hurricane-mediated selection, with several loci being strongly associated with both hurricane frequency and longer limbs. Further, we found that hurricanes are a more persistent driver of among-population genomic differentiation than other environmental variables. These results solidify hurricanes as a major force shaping morphological and genomic variation in *Anolis* lizards. They also highlight how the evolutionary trajectories of populations will likely be altered as climate change modifies historical patterns of natural selection, such as those involving extreme weather events.

Anolis | episodic selection | extreme weather events | hurricanes | invasive species

One consequence of human-mediated climate change is an increased frequency of extreme weather events, such as heat waves, wildfires, and hurricanes (1–4). Recent analyses confirm connections between climate change and an increase in the strength and frequency of hurricanes in the North Atlantic (e.g., refs. 5–8). The high winds, waves, and storm surges associated with hurricanes can have devastating consequences for coastal environments, including direct mortality of organisms during a storm (e.g., refs. 9–14). If mortality is trait-dependent, then these extreme, pulse-like environmental perturbations can be a source of strong episodic natural selection on populations (15, 16). Indeed, recent studies document hurricane-induced selection (e.g., ref. 17) and morphological responses to hurricanes in wild populations, particularly in *Anolis* lizards (e.g., refs. 18–20). The predicted increase in hurricane activity and strength in the North Atlantic has the potential to alter patterns of selection and evolution for populations, especially in coastal areas and on islands.

Field studies and laboratory experiments show that high winds associated with hurricanes can result in natural selection on morphological traits related to clinging performance in *Anolis* lizards (17–24, but see refs. 25 and 26). For example, posthurricane populations of *Anolis scriptus* in the Turks and Caicos had larger toepads and longer forelimbs—traits that enhance clinging performance (21, 27)—compared to prehurricane populations (17). A key question related to the evolutionary potential of strong selection associated with hurricanes is whether the episodic nature of these events leads to long-term morphological evolution. Alternatively, routine (albeit weaker) selective pressures during the vastly longer time periods between hurricanes could counter those occurring during hurricanes to more consistently shape morphological variation. Examining the long-term effect of hurricane-induced selection on evolution of toepads and limbs in *Anolis* lizards, Donihue et al. (18) showed that toepad area had a positive relationship with hurricane activity across 12 island populations of *Anolis sagrei* and 188 *Anolis* species throughout the Neotropics over timeframes spanning thousands to millions of years (28, 29). Their analyses used the number of hurricanes in the previous 70 y as a surrogate for hurricane activity over the divergence times of these populations and species. Yet, analysis of the same dataset found no effects of hurricane activity on limb length variation. Thus, strong hurricane-induced

Significance

Climate change is increasing the frequency and severity of extreme weather events. Research shows hurricanes are a source of strong natural selection, favoring traits that enhance the clinging ability of lizards under strong winds. Investigating the invasion of the brown anole, we found that populations experiencing more hurricanes had longer limbs and larger toepads, trait values that increase clinging performance. This signature was mirrored at the genetic level, with several genomic regions showing strong associations with hurricane frequency and limb length, as well as extreme differentiation among populations. Our results suggest that increases in the frequency and strength of hurricane-induced selection will alter the balance of selective pressures on limbs and toepads, reshaping morphological and genomic variation in wild populations.

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selection (17) led to an evolutionary response for toepads and limbs (18, 20), but hurricane activity was associated with only toepad evolution over greater spatial and temporal scales (18).

Biological invasions have become useful scenarios for studying rapid evolution (30) including natural selection in response to shifting environmental conditions (e.g., ref. 31), genetic drift due to founder events (e.g., ref. 32), and admixture when introductions occur from multiple source populations (e.g., ref. 33). Investigations of adaptive evolutionary processes during invasions often feature novel spatial and temporal variation in environmental factors, such as latitudinal gradients or seasonal variation. One advantage of recent invasions is that the timing of introductions and spread within the non-native range can be ascertained in some cases, allowing researchers to estimate the time of exposure to novel conditions and to calculate the number of extreme events experienced. For example, capturing multiple incidents of extreme weather events, such as hurricanes, over the entire existence of a population can provide spatial variation needed to test hypotheses for their evolutionary effects. Despite the drawbacks of these quasi-experiments (e.g., no treatment controls or planned comparisons), biological invasions can be particularly useful if they capture natural variation in a putative explanatory variable that would otherwise be impossible to manipulate or infeasible to wait for future observations. The usefulness of invasions is bolstered when potential confounding factors of the invasion history, such as establishment times, native-range sources, patterns of admixture, and genetic ancestry, can be accounted for and when complementary insights can be derived from independent comparative and experimental evidence to support similar conclusions.

The *A. sagrei* invasion of the southeastern United States is well documented in many respects, making it an excellent system for understanding the effects of extreme weather events on morphological and genomic evolution. Dates of introduction and spread of *A. sagrei* are well known, and genetic analyses reveal introductions occurred from at least eight native-range source populations, mostly from Cuba (33, 34). Genetic ancestry of non-native populations is strongly influenced by admixture among genetically distinct sources and is associated with variation in skeletal morphology (34–36), water loss (37), and dewlap characteristics (38). Importantly for the current study, previous research found no evidence of adaptation of limb and toepad traits to local vegetation in the non-native range (34, 35), results that are counter to eco-morphological relationships that exist for *A. sagrei* in the Bahamas, part of its native range (39–41). However, these studies did not evaluate whether hurricane exposure was related to *A. sagrei* morphology. Fortunately for this study, hurricane activity in the southeastern United States is well documented. Indeed, detailed information on the paths and wind speeds is available for the 150+ hurricanes that have hit Florida since 1851 (https://coast.noaa.gov/hurricanes). By combining information on the date of establishment for *A. sagrei* populations with subsequent hurricane activity at these sites, we can evaluate whether these extreme weather events influence current patterns of limb, toepad, and genomic variation among non-native populations.

In this study, we capitalized on the well-documented invasion history of *A. sagrei* in Florida and Georgia to test whether morphological and genomic variation among non-native populations is consistent with an adaptive evolutionary response to hurricane activity over the period of invasion. We hypothesized that invasive populations frequently exposed to hurricanes will show an increase in trait values known to enhance clinging performance in this and other *Anolis* species, that is, toepad area and limb length (17, 18, 21, 24). In contrast, morphological traits with no known relationship to clinging ability should not be related to hurricane variation. We complement these analyses with genome-wide marker data to account for the influence of invasion history on trait variation (34, 35, 37).

Further, we hypothesized that repeated exposure to hurricanes will leave a genomic signature consistent with natural selection for increased size of traits that enhance clinging ability. To this end, we investigated the genetic architecture of morphological traits and combined this information with genomic differentiation analyses and genome-wide scans for associations with environmental variables.

Results

Invasion Timeline and Hurricane History. Dates of establishment for the *A. sagrei* introduction in Florida and Georgia ranged from 1887 for Monroe County (i.e., the Florida Keys) to 2002 for Hernando County on the Gulf coast in northern Florida (Dataset S1 and Fig. 1). As of 2017, the 30 invasive *A. sagrei* populations in this study were established for an average of 43 y, ranging from 15 to 130 y. When excluding Monroe County, all other observations of *A. sagrei* populations date from 1941 or later, a 76-y period of invasion. Hurricane activity since the establishment of each non-native population ranged from 1 to 19 Category 1 or greater hurricanes passing within 100 km of each site (Fig. 1), potentially impacting *A. sagrei* populations across Florida and southern Georgia.

Associations between Morphological Traits, Hurricane Frequency, and Genetic Ancestry. We found significant relationships with hurricane activity for traits known to be functionally related to clinging ability and therefore predicted to be under selection during hurricane-force winds. All limb and toepad measures were positively related to the number of hurricanes with most being statistically significant or nearly so (Table 1 and Fig. 2). Specifically,
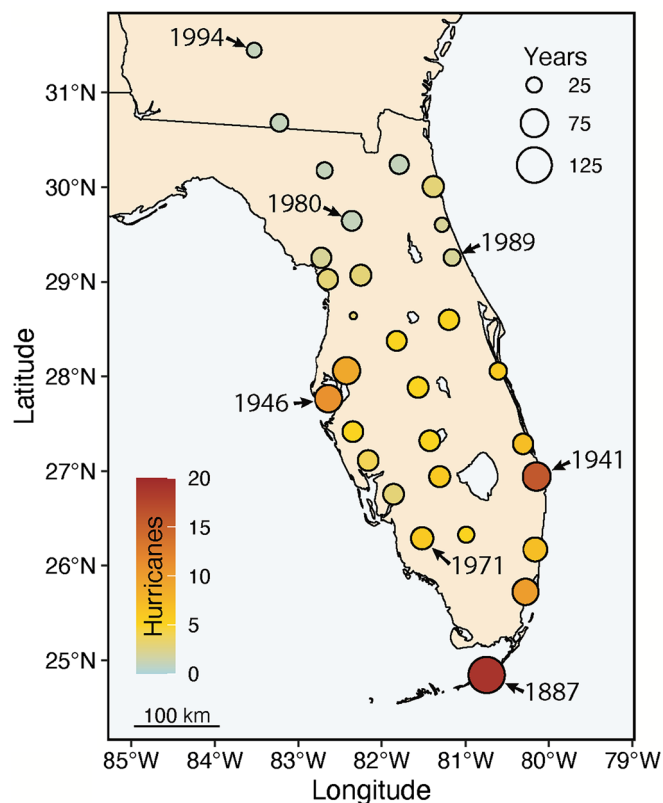
Fig. 1. History of hurricanes and invasion of *A. sagrei* across Florida and southern Georgia. Circles on the map represent each of the 30 localities sampled for this study. The color gradient shows the number of hurricanes experienced at each site since that population was established, and the size of the circle represents the number of years each introduced population has been established. Localities labeled with a year indicate dates of establishment for some populations.

Table 1. Results of linear mixed models assessing effects of variation in hurricane frequency and genetic ancestry on trait variation among non-native *A. sagrei* populations

Trait category	Trait	Term	Est.	*SE*	CI$_l$	CI$_u$	*P*	R^2_m	R^2_c
Toe	Lamellae hind	Intercept	−0.34	0.16			0.04	0.09	0.12
		Hurricane frequency	0.03	0.02	−0.01	0.06	0.12		
		Genetic ancestry	0.31	0.23	−0.13	0.75	0.20		
		Spatial covariate	**0.24**	**0.07**	**0.10**	**0.37**	**0.00**		
Toe	Lamellae fore	Intercept	−0.49	0.15			0.00	0.08	0.10
		Hurricane frequency	**0.04**	**0.02**	**0.01**	**0.08**	**0.02**		
		Genetic ancestry	0.43	0.22	0.02	0.85	0.06		
		Spatial covariate	**0.22**	**0.07**	**0.09**	**0.35**	**0.00**		
Toe	Toepad hind	Intercept	0.26	0.19			0.19	0.04	0.12
		Hurricane frequency	0.03	0.02	−0.01	0.07	0.16		
		Genetic ancestry	**−0.76**	**0.28**	**−1.29**	**−0.23**	**0.01**		
		Spatial covariate	0.12	0.09	-0.05	0.29	0.19		
Toe	Toepad fore	Intercept	0.16	0.20			0.41	0.03	0.12
		Hurricane frequency	0.02	0.02	−0.02	0.06	0.40		
		Genetic ancestry	−0.50	0.29	−1.05	0.07	0.09		
		Spatial covariate	0.02	0.09	−0.16	0.19	0.84		
Limb	Hind digit length	Intercept	−0.14	0.17			0.42	0.04	0.09
		Hurricane frequency	**0.06**	**0.02**	**0.02**	**0.10**	**0.01**		
		Genetic ancestry	−0.27	0.25	−0.75	0.22	0.29		
		Spatial covariate	0.09	0.08	−0.06	0.24	0.25		
Limb	Fore digit length	Intercept	−0.13	0.19			0.49	0.01	0.08
		Hurricane frequency	0.04	0.02	0.00	0.08	0.10		
		Genetic ancestry	−0.12	0.28	−0.66	0.45	0.67		
		Spatial covariate	0.12	0.09	−0.05	0.29	0.18		
Limb	Humerus length	Intercept	−0.29	0.16			0.08	0.01	0.05
		Hurricane frequency	0.04	0.02	0.00	0.07	0.07		
		Genetic ancestry	0.24	0.24	−0.22	0.70	0.32		
		Spatial covariate	0.04	0.08	−0.11	0.18	0.61		
Limb	Ulna length	Intercept	−0.35	0.20			0.09	0.02	0.11
		Hurricane frequency	**0.05**	**0.02**	**0.00**	**0.09**	**0.05**		
		Genetic ancestry	0.20	0.29	−0.37	0.79	0.50		
		Spatial covariate	0.06	0.10	−0.13	0.24	0.54		
Limb	Tibia length	Intercept	−0.18	0.18			0.32	0.05	0.11
		Hurricane frequency	**0.05**	**0.02**	**0.01**	**0.09**	**0.02**		
		Genetic ancestry	−0.08	0.27	−0.59	0.43	0.77		
		Spatial covariate	0.01	0.09	−0.15	0.17	0.90		
Limb	Femur length	Intercept	−0.24	0.17			0.17	0.05	0.09
		Hurricane frequency	**0.05**	**0.02**	**0.02**	**0.09**	**0.01**		
		Genetic ancestry	0.01	0.25	−0.46	0.49	0.96		
		Spatial covariate	−0.01	0.08	−0.16	0.14	0.91		
Limb	Hindlimb length	Intercept	−0.23	0.17			0.20	0.04	0.09
		Hurricane frequency	**0.06**	**0.02**	**0.02**	**0.10**	**0.01**		
		Genetic ancestry	−0.08	0.26	−0.57	0.41	0.76		
		Spatial covariate	0.05	0.08	−0.11	0.20	0.56		
Limb	Forelimb length	Intercept	−0.35	0.18			0.06	0.02	0.07
		Hurricane frequency	**0.05**	**0.02**	**0.01**	**0.09**	**0.02**		
		Genetic ancestry	0.20	0.26	−0.30	0.71	0.45		
		Spatial covariate	0.08	0.08	−0.08	0.24	0.33		

(Continued)

Table 1. (Continued)

Trait category	Trait	Term	Est.	SE	CI$_l$	CI$_u$	P	R^2_m	R^2_c
Torso	Pelvis width	Intercept	−0.27	0.15			0.08	0.05	0.07
		Hurricane frequency	−0.03	0.02	−0.07	0.00	0.06		
		Genetic ancestry	**0.80**	**0.22**	**0.40**	**1.21**	**0.00**		
		Spatial covariate	−0.11	0.07	−0.24	0.01	0.11		
Torso	Pectoral width	Intercept	−0.26	0.18			0.16	0.12	0.18
		Hurricane frequency	−0.01	0.02	−0.05	0.03	0.57		
		Genetic ancestry	**0.56**	**0.26**	**0.06**	**1.06**	**0.04**		
		Spatial covariate	0.14	0.08	−0.02	0.29	0.11		
Head size	Head length	Intercept	0.01	0.18			0.94	0.00	0.05
		Hurricane frequency	0.01	0.02	−0.03	0.05	0.57		
		Genetic ancestry	−0.11	0.26	−0.63	0.39	0.67		
		Spatial covariate	0.06	0.08	−0.10	0.22	0.49		
Head size	Head width	Intercept	−0.28	0.15			0.07	0.04	0.05
		Hurricane frequency	0.00	0.02	−0.03	0.03	0.98		
		Genetic ancestry	**0.50**	**0.22**	**0.09**	**0.90**	**0.03**		
		Spatial covariate	0.04	0.07	-0.08	0.17	0.52		
Body size	Body size	Intercept	−0.12	0.26			0.65	0.01	0.24
		Hurricane frequency	0.00	0.03	−0.07	0.06	0.91		
		Genetic ancestry	0.37	0.36	−0.32	1.06	0.31		
		Spatial covariate	−0.03	0.13	−0.28	0.21	0.80		

The number of hurricanes is the number of Category 1 or greater storms passing within 100 km of a site from the year of establishment to 2017 for each non-native population. Genetic ancestry is the proportion of Western Cuba ancestry as estimated by a STRUCTURE analysis of K = 2 groups (see ref. 34). The spatial covariate describes a spatial axis from NNW to SSE across Florida and southern Georgia based on a PCA using latitude and longitude. Morphological trait categories include toepad, limb, torso, head, and body size. Estimates were derived from scaled and centered size-adjusted morphology measures. CI are calculated from Profile Likelihood in confint(). Statistically significant (P < 0.05) factors are in bold. Marginal and conditional R^2 values labeled as R^2_m and R^2_c.

lizards in populations experiencing more hurricanes during the course of their invasion had longer limbs and larger toepads. In contrast, traits thought to be unrelated to clinging performance (i.e., head length, head width, pectoral width, and pelvic width) were not significantly related to the number of hurricanes (Table 1 and Fig. 2). Some of these traits (i.e., head width, pectoral width,
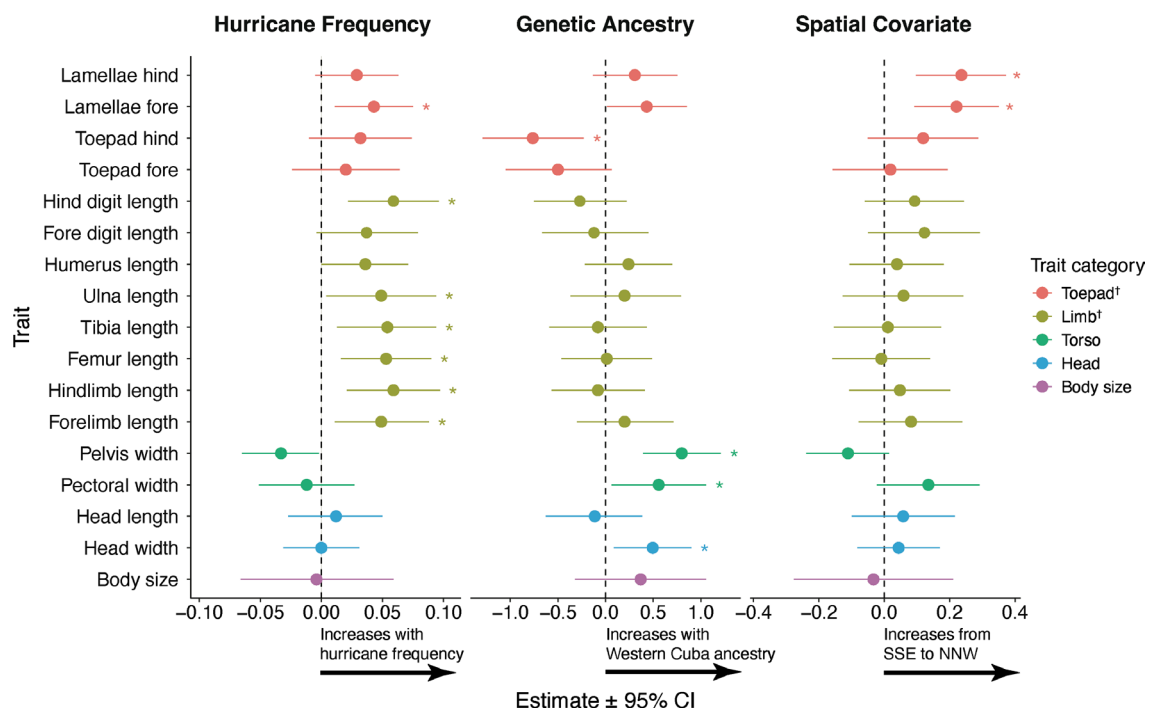

Fig. 2. Estimates and 95% CI for effects of hurricane frequency, genetic ancestry, and the spatial covariate on morphological variation among 30 introduced populations of *A. sagrei*. Colors reflect trait categories: toepad, limb, torso, head, and body size. Toepad and limb traits are associated with clinging performance in *Anolis* lizards and are indicated with the †. Asterisks indicate significant effects from linear mixed models (see Table 1 for statistical results).

and pelvic width) were related to genetic ancestry with increasing trait size being positively correlated with the proportion of Western Cuba ancestry, whereas among limb and toepad traits, only hindlimb toepad area was related to Western Cuba ancestry. The spatial covariate had a significant effect on only the number of lamellae, with an increase in both forelimb and hindlimb lamellae in populations when moving from the SSE to the NNW (Table 1 and Fig. 2).

Genetic Architecture of Morphological Traits. To understand which genomic regions underlie variation in our 17 focal morphological traits, we used 120,135 quality-filtered single-nucleotide polymorphisms (SNPs) and two genome-wide association study (GWAS) models (*Methods*). We identified 109 quantitative trait loci (QTLs) across all traits (range 2 to 13 loci per trait; Dataset S2). Most QTLs (86.2%; 94 loci) only passed the suggestive significance threshold. Also, most QTLs (76.2%) had small effect sizes (PVE 0.7 to 4.8%; Dataset S2 and Fig. 3). Only 17.4% and 6.4% of QTLs were classified as moderate (PVE 5.0 to 9.9%) and large effect size loci (PVE 10.7 to 13.3%), respectively (Dataset S2). The sex determining region (SDR) on the sex chromosome had the most QTLs (35 loci across 12 traits). Of these, two loci were of large effect (PVE 11.4 to 13.1%), including a locus that was associated with ulna length, humerus length, forelimb length, tibia length, and hindlimb length (Dataset S2), located within a region of the sex chromosome previously linked to limb length variation in this species (34). For toepad traits, the strongest association was found for hindlimb number of lamellae on chromosome 2 (Fig. 4). This QTL was identified by both GWAS models (Fig. 4 *A* and *B*) and produced moderate effect sizes (PVE 7.28 to 7.36%; Fig. 4 *B* and *C*). Nonetheless, hurricane frequency was not a significant predictor of allele frequency at this locus (Fig. 4D).

Genomic Signatures of Hurricane Adaptation. The intersection of GWAS, genomic differentiation, and hurricane frequency genotype–environment analyses (GEA) revealed 15 shared outlier SNPs on the sex chromosome, grouped into four loci based on linkage disequilibrium (LD; Dataset S3 and *SI Appendix*, Fig. S1). Of these, eight SNPs (grouped into two loci) were associated with femur length (Fig. 5A), and seven SNPs (grouped into two loci) were associated with pelvis width (Dataset S3 and *SI Appendix*, Fig. S1). While pelvis width has not previously been linked to morphological evolution in response to hurricanes in *Anolis* lizards, the pelvis is a critical component of limb functioning that serves

as an attachment point for hindlimb muscles. Also, our linear mixed models identified a marginally nonsignificant association between pelvis width variation and hurricane frequency (P = 0.06; Table 1). Permutation tests confirmed that the number of loci that are outliers across all three methods is significantly greater than expected by chance for both traits (P = 9.99 × 10^{-5}). Most of the shared outliers were located within or near a sex chromosome region previously reported to be under selection in the native range of *A. sagrei*, and to be associated with multiple limb length traits (*SI Appendix*, Fig. S1; ref. 34). Given the link between limb length and hurricane frequency, we focused on the femur length outlier SNP showing the strongest selection signal across all three metrics (i.e., GWAS, differentiation, and GEAs; Fig. 5). After controlling for genome-wide ancestry, we found that hurricane frequency is a significant predictor of allele frequency at this candidate adaptive locus (Fig. 5B).

The intersection of GEA and genomic differentiation analyses revealed 12 candidate hurricane adaptation loci, of which four were located within the SDR of the sex chromosome, while the rest were classified as autosomal/pseudoautosomal regions (PAR) loci (Fig. 6). All loci inferred to be under selection by other environmental variables were autosomal/PAR loci (Fig. 6). Finally, candidate hurricane adaptation loci exhibited stronger among-population differentiation than those linked to other environmental factors (Fig. 6).

Discussion

By combining information on the chronology of the *A. sagrei* invasion and history of hurricane exposure for each population in the southeastern United States with patterns of morphological, environmental, and genomic variation, we found strong evidence that hurricanes shape morphological traits related to clinging performance as well as genomic differentiation among invasive populations. Limb length and aspects of toepad size were positively related to hurricane frequency among populations, a pattern that likely developed over 130 y of the invasion. Even though most traits analyzed here appeared to have a polygenic basis, with many small-effect loci affecting phenotypic variation, we identified several loci with a signature consistent with adaptive divergence in response to hurricane exposure. Moreover, loci inferred to contribute to hurricane adaptation showed the strongest genomic differentiation among populations, consistent with hurricane activity being a particularly important driver of adaptive divergence.
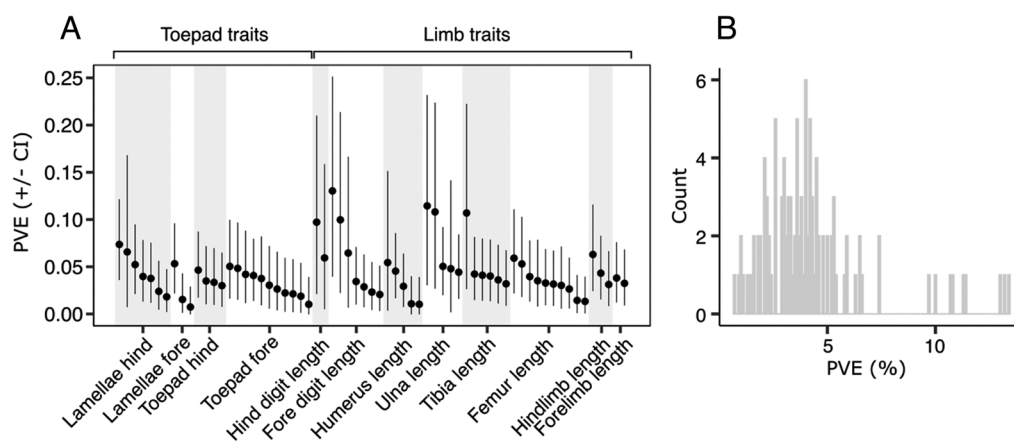

Fig. 3. Effect sizes of QTLs associated with morphological traits in *A. sagrei*. (A) Effect sizes (with 95% CI) for QTLs associated with 12 toepad and limb traits known to enhance clinging performance and that are likely under selection during hurricanes. (B) Histogram of effect sizes for 109 QTLs identified across all 17 traits, including torso, head, and body size in addition to toepad and limb traits.
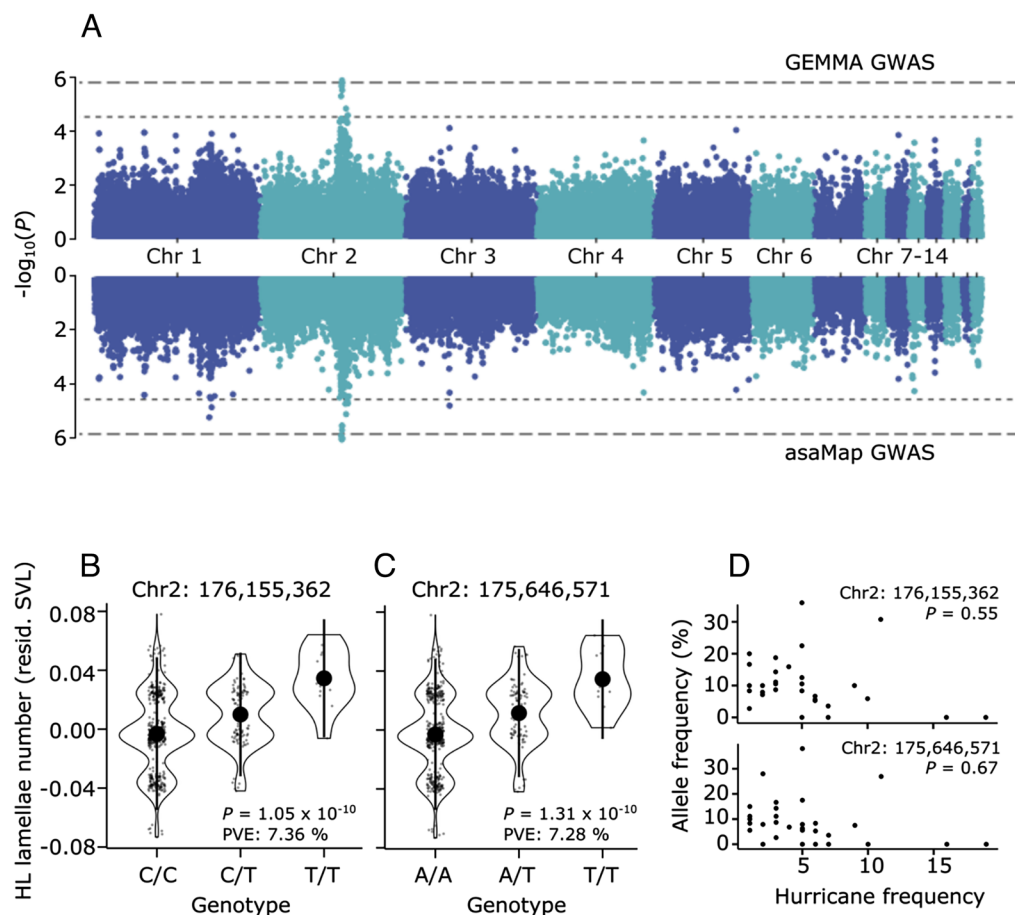
Fig. 4. GWAS results for the hindlimb number of lamellae. (*A*) Manhattan plot of the GEMMA association model (*Top*) and the asaMap association model (*Bottom*). The short-dashed lines show the suggestive genome-wide significance threshold, whereas the long-dashed lines show the stringent genome-wide significance threshold. Panels (*B*) and (*C*) illustrate the relationship between genotypes at the lead GEMMA SNP (Chr2: 176,155,362) or the lead asaMap SNP (Chr2: 175,646,571) and phenotype. Panel (*D*) shows the relationship between population-averaged allele frequency and hurricane frequency at the two SNPs. Only the larger-effect variant (Chr2: 175,646,571) was considered for downstream analyses, given that the two SNPs are in linkage disequilibrium.

Previous studies of *A. sagrei* populations in its invasive range failed to support hypotheses for local adaptation of morphology related to locomotor performance (34, 35). These studies found no relationship between limb length and perch diameter, or between toepad size and perch height, both well-established morphology-performance-habitat use relationships in anoles (41–43). One key assumption for the current study was that trait values when each introduced population was established were
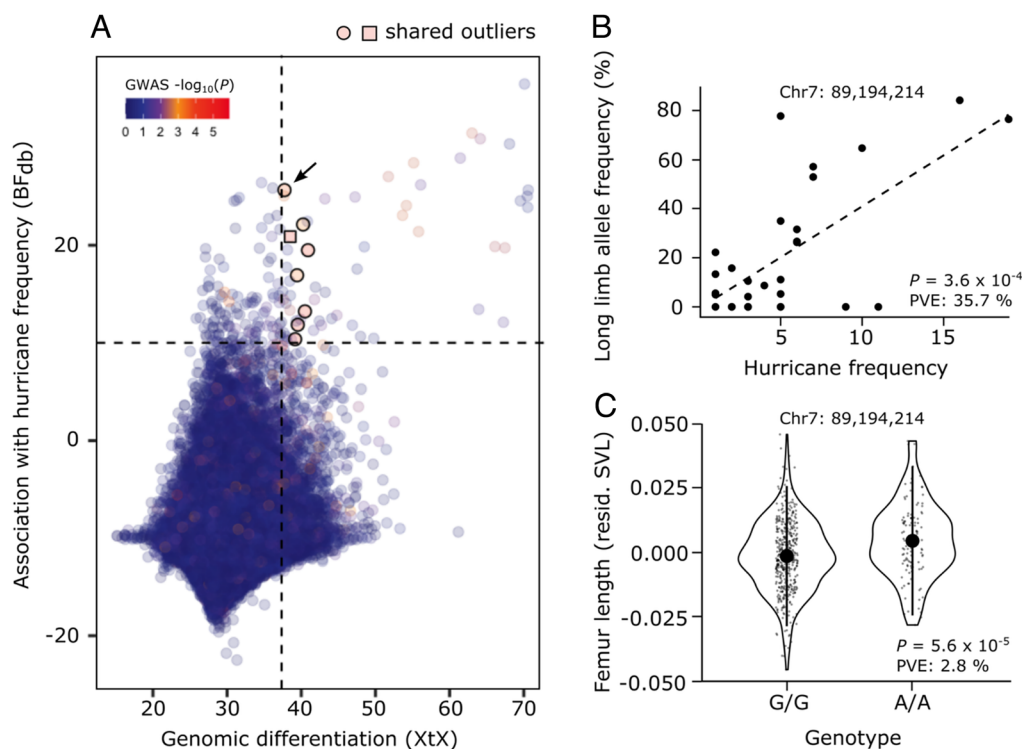

Fig. 5. Identification of candidate adaptive loci. (*A*) Outliers across genomic differentiation, associations with hurricane frequency, and GWAS for femur length. The dashed lines indicate the genome-wide significance thresholds. The eight shared outlier SNPs are highlighted and grouped into two loci, indicated by circles and squares, with the arrow pointing to the SNP used for panels *B* and *C*. (*B*) Association between hurricane frequency and population-averaged allele frequency. The PVE is estimated as partial R^2 from a linear model that additionally includes genome-wide ancestry as a covariate (see *SI Appendix, Appendix C* for additional details). (*C*) Association between genotype and femur length at the same shared outlier SNP.
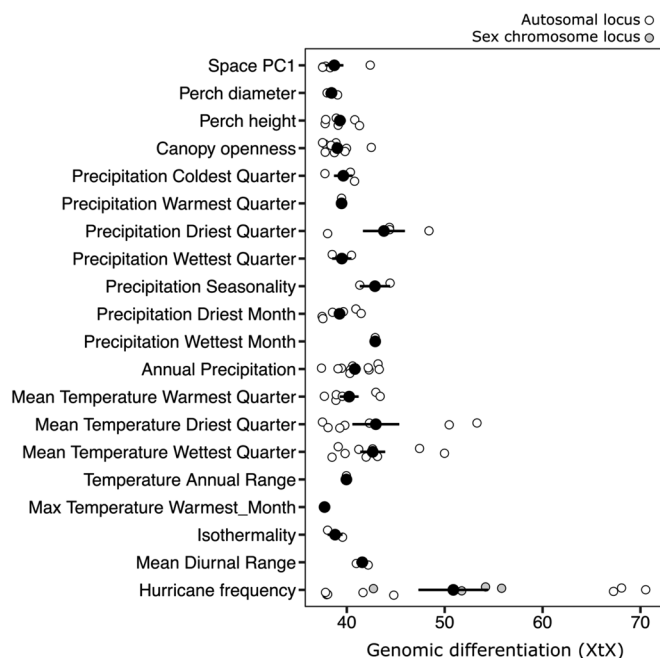
Fig. 6. Genomic differentiation at candidate adaptive loci across all environmental variables. Loci located within the autosomes or PARs are shown in white, whereas loci located within the SDR of the sex chromosome are shown in light gray. For each environmental variable, black circles indicate mean differentiation of all outliers (±SEM). Four environmental variables are not shown, given that no candidate adaptive loci were identified in those cases.

independent of the number of hurricanes they would experience such that any patterns detected must have arisen during the invasion. Although past studies found some associations between morphology and genetic ancestry, resulting from the widespread admixture of genetic variation from multiple source populations in the native range (33–35), we found little support for the role of genetic ancestry in explaining limb and toepad variation when hurricane frequency was included in models as an explanatory variable. Rather, results from this study suggest that episodic selection associated with hurricanes is relatively more important for shaping morphological variation than routine selection on the same traits associated with locomotor performance between hurricanes, at least on the time scale of a century.

Hurricane-Induced Episodic Selection and the Persistence of Trait Adaptation. Over 100 hurricanes have hit Florida since the start of the *A. sagrei* invasion. Our results are consistent with hurricane-induced selection and its associated evolutionary response shaping limb and toepad traits in non-native *A. sagrei* populations during this time. These results solidify the conclusions that hurricanes are a source of episodic selection (17) with lasting evolutionary effects on both limb and toepad variation. Yet, at longer temporal scales, patterns among populations and species of anoles are consistent with adaptation to hurricanes for toepads, but not limbs (18). To reconcile this discrepancy between evolutionary patterns at different temporal scales, we propose that routine selection between hurricanes on limbs for locomotor performance commonly opposes the direction of selection on limbs during hurricanes; however, selection on toepads for clinging, albeit stronger during hurricanes, is in the same direction as routine selection between hurricanes. That is, hurricanes favor longer limbs and larger toepads for clinging, yet selection during intervals between hurricanes favors shorter limbs for locomotion but larger toepads for clinging in the Bahamas (18), but not Florida. Evidence exists that selection and adaptive differentiation in the limb length

of *A. sagrei* is related to the diameter of perches used by lizards in the Bahamas (40, 44, 45), and this generates a positive relationship between limb length and perch diameter among populations in this part of the native range (39, 40).

Even if correct, this hypothesis does not explain why patterns in the non-native range (Florida and Georgia) align with hurricane adaptation, whereas those in the native range (Bahamas) indicate local adaptation to vegetation. Both regions experience active hurricane seasons; however, without quantifying hurricane activity throughout the Bahamas it is difficult to compare the relative influence of hurricane-induced selection in each region. Additionally, perch-diameter use differs between regions with native Bahamian populations using a narrower range of mean perch diameters (range = 2.5 to 10.0 cm, N = 12 populations; ref. 39) compared to non-native populations (range = 2.1 to 61.8 cm, N = 30 populations; ref. 34). Yet, how this discrepancy might translate to differences in selection pressures associated with locomotion is difficult to discern.

Certainly, other sources of selection may influence limb-length variation (e.g., ref. 46), including other ecological differences between the invasive and native-range populations and variation in responses to hurricanes among *Anolis* species. For example, in contrast to our findings for *A. sagrei*, shorter hindlimbs were favored during hurricanes for *A. scriptus* in the Turks and Caicos (17), perhaps reducing drag forces and increasing clinging ability (23). We are unaware of support for any toepad function other than clinging in arboreal habitats. Thus, limb variation over longer temporal scales may be influenced more than toepads by the weaker, sustained routine selection during interhurricane periods compared to the stronger, virtually instantaneous episodic selection during a hurricane.

Genomic Signatures of Adaptation to Hurricane Disturbance. Detecting the genomic signature of natural selection is especially challenging when polygenic adaptation is driven by pulse-like events such as hurricanes. On one hand, historical selection signals—those driven by recurring hurricane events that accumulate over many generations—can be obscured at some adaptive loci by processes acting between hurricanes, such as genetic drift or selection in the opposite direction (e.g., due to negative pleiotropic effects; ref. 47). These forces can erase the signature of episodic selection, especially when adaptation involves only slight changes in allele frequencies across many loci. On the other hand, if interhurricane forces have only a minimal impact on adaptive allele frequencies after hurricanes subside, analyses that target historical selection signals can still identify small-effect adaptive loci if they capture subtle allele frequency changes that accumulate over time (47).

Our results are consistent with a polygenic architecture for the morphological traits analyzed here, with 2 to 13 QTLs identified per trait, and most loci exhibiting only small effect sizes. However, reduced-representation sequencing examines only a fraction of the genome (48). Indeed, we estimate that our sequencing approach effectively sampled 31.2% of the genome at high LD ($r^2 > 0.5$) and 98.7% of the genome at moderate LD ($r^2 > 0.3$). Therefore, we expect that many more QTLs affecting variation of these traits await detection. Despite these challenges, our analyses revealed several loci bearing signatures of hurricane-driven selection. These include two sex-chromosome loci associated with increased femur length. These loci showed strong genetic differentiation among populations and had alleles linked to longer femurs that increased in frequency in populations exposed to more hurricanes.

The analyses performed without considering genotype-trait associations revealed 12 candidate hurricane adaptation loci. These included four sex-chromosome loci and eight autosomal loci. By comparison, loci inferred to have been under selection in response

to other environmental variables were exclusively autosomal. Therefore, our results point to a relatively greater contribution of the sex chromosome during hurricane adaptation. One possible interpretation is that the sex chromosome is a frequent target of hurricane-mediated selection due to its role in shaping limb length variation in *A. sagrei* (34). Connecting the signatures of selection that we report here with variation at candidate adaptive traits will, however, require larger association mapping populations and denser marker coverage along the genome. These conditions will facilitate the identification of additional small-effect loci that affect trait variation, potentially revealing more details of the genetic architecture of limbs as well as toepads.

Comparisons of XtX values for loci inferred to be adaptive across different environmental contexts revealed that hurricane frequency is a stronger driver of among-population genetic differentiation than 23 other microhabitat- and climate-related variables. This finding aligns with our broader results pointing to hurricane disturbance as a major force shaping morphological and genomic variation in invasive *A. sagrei*, and helps explain results from previous studies in this system, which found limited evidence of adaptive divergence in response to the local vegetation used for perching (34, 35, 41), canopy openness (38), water availability (37), or temperature (49). Even so, we emphasize that hurricane adaptation loci identified here should be viewed as candidates until further supporting evidence becomes available. Many of the environmental variables that we considered are correlated and follow a strong north-to-south progression in our study region, as does genome-wide ancestry (34–36). Although hurricane frequency also follows this north–south gradient of genome-wide ancestry (33–35), hurricane effects on allele frequency remained significant after controlling for ancestry. Even so, it remains possible that some of these loci represent false positives. Additionally, hurricane frequency and time since colonization are strongly correlated among populations in our study region. But aside from greater hurricane exposure, we are unaware of any reason why longer-established populations would have larger values of clinging-related traits and increased frequencies of alleles associated with such traits, but no such relationships with other traits. Ideally, follow-up analyses should compare morphological and genomic variation in these populations before and immediately after a hurricane strike, as in previous studies (e.g., ref. 17). While such analyses may favor the detection of large-effect loci that undergo substantial shifts in allele frequency between generations, they are robust to many of the confounding factors inherent in historical approaches.

Conclusions

The invasion of *A. sagrei* to the southeastern United States provides a rare opportunity to put a timestamp on the start of adaptive evolution for populations in this region. Coupled with the chronology of spread in the non-native range and history of hurricanes over a 130-y period, we can estimate the relative strength of selection pressure due to hurricane exposure. The consistency of morphological responses for traits related to clinging ability and genomic responses underlying some of these traits strongly supports adaptive evolution to hurricane-associated selective pressures. We now have evidence for the adaptive evolution of morphology in response to hurricanes at multiple temporal and spatial scales, including estimates of selection differentials from measurements before and after hurricanes (17), trait change associated with hurricanes (e.g., refs. 19 and 22), evolutionary responses to selection in the next generation (18, 20), population-level divergence over more than a century of a biological invasion (this study),

differentiation among *A. sagrei* populations (toepads only; ref. 18), and divergence among species in the *Anolis* clade (toepads only; ref. 18). These results solidify hurricanes as an important force shaping morphological evolution in *Anolis* lizards.

Lizards are surely not the only species potentially experiencing selection during hurricanes. Understanding of episodic selection may be enhanced by studies on the evolutionary effects of hurricanes on other species, not only to understand the generality of hurricane effects on adaptive evolution but also to predict how climate-change-induced increases in hurricane strength and frequency will affect the balance of selective pressures and patterns of morphological diversity.

Methods

Sampling and Housing of Lizards. In March-July 2018, we collected the 561 adult male *A. sagrei* used in this study. After capturing lizards at sites in Florida (N = 28) and Georgia (N = 2) along three transects, we shipped them to the animal facility at Harvard University (Cambridge, MA). Lizards were housed individually in terraria with soil substrate, plastic foliage, and a wooden dowel for perching. Room temperature was 28 °C and terraria were misted at least twice per day to maintain 80% relative humidity. Fluorescent bulbs provided lighting on a 14 h light/10 h dark daily cycle. Lizards were fed crickets dusted with multivitamin powder three times per week. All procedures were approved by the Harvard University Institutional Animal Care and Use Committee (IACUC protocol #26-11). For more details on collecting and housing conditions, see Bock et al. (34) and Baeckens et al. (37).

Chronology of the Brown Anole Invasion. The first published record in Florida of what is now considered *A. sagrei* indicated its presence in the Florida Keys in the late 1800s (50). *Anolis sagrei* was not confirmed in peninsular Florida until over a half-century later along the southeast coast (i.e., Jupiter in 1941 and Coral Gables in 1951) and on the west-central coast (i.e., St. Petersburg in 1946 and Tampa in 1947) (51, 52). We reconstructed the chronology of the *A. sagrei* invasion in Florida and Georgia using dates from published observations and museum specimen records from the Museum of Comparative Zoology at Harvard University, the Natural History Museum at the University of Kansas, and the Florida Museum of Natural History at the University of Florida (Dataset S1). We assigned dates to our 30 sites based on when *A. sagrei* was first documented in the corresponding county. Given most dates are based on the year of publication, they may underestimate the time non-native populations have been established at a particular site.

Hurricane Data Collection. In a previous study investigating the relationship between hurricanes and morphology in *Anolis* lizards, Donihue et al. (18) counted a hurricane strike when a storm passed within 30 km of a lizard locality with a maximum wind speed at or exceeding 148 km/h (92 mph or a strong Category 1 hurricane). Their statistical models were somewhat sensitive to distance from the hurricane and windspeed thresholds. Another study of hurricane effects on anoles, Rabe et al. (19), detected limb-length changes in *Anolis carolinensis* after Hurricane Irma, which was a Category 1 storm with wind speeds of 139 km/h (86 mph) when it passed ~150 km to the west of their study site. They reported sustained, near hurricane-force winds of 115 km/h (71 mph) close to their study site during Hurricane Irma. Variation in hurricane direction, distance from a site, storm size, and speed, among other factors, will influence wind speeds experienced by organisms at a particular site. Given the potential for hurricane effects on lizards at greater distances from sites or from lower wind speeds than accounted for by Donihue et al. (18), we increased the distance from the path of a hurricane in our study. We used data from the NOAA Historical Hurricane Track website (https://coast.noaa.gov/hurricanes) to calculate the number of hurricanes (i.e., wind speed of at least 119 km/h or 74 mph) within 100 km of each site. In some instances, we needed to interpolate wind speeds between data points, which were generally at 6-h intervals along the path of a hurricane. This allowed us to determine whether hurricane-force winds were present when a storm passed closest to each study site. For each site, we only calculated the number of hurricanes in the period from the estimated date of population establishment to 2017, the year prior to our March-July 2018 collection of lizards. Using these methods, we were able to estimate the

number of hurricanes potentially impacting non-native *A. sagrei* populations during the time since their establishment in Florida and Georgia.

Morphological Data Collection. Lizards were euthanized approved by the Harvard University Institutional Animal Care and Use Committee (IACUC protocol #26-11) and images taken with a flatbed scanner and an X-ray machine. We measured morphological traits from these scaled scanner and X-ray images using the ObjectJ plug-in (https://sils.fnwi.uva.nl/bcb/objectj) for the program ImageJ (53). We measured toepad area for the longest toes on the forelimb (3rd toe) and hindlimb (4th toe). The repeatability of this method was confirmed by measuring the toepad area of the longest forelimb and hindlimb toes on both the left and right sides of a lizard three times each for the first 15 specimens. Repeated measurements were highly consistent with intraclass correlation coefficients of 98.99% for left forelimb toepad area, 99.02% for right forelimb toepad area, 98.97% for left hindlimb toepad area, and 99.18% for right hindlimb toepad area. Therefore, a single measurement of the forelimb and hindlimb toepads was considered reliable for each specimen. We preferentially measured right limb toepads; however, if the right digit was missing or damaged, then we measured the left toepad instead. In cases where the right toepad was not sufficiently flat against the scanning surface, we measured both the left and right toepads and the larger of the two areas was used in subsequent analyses. We also counted the number of lamellae (i.e., enlarged, subdigital scales) visible within the polygon created to measure toepad area. Toepad area and lamella number were successfully measured for the forelimbs and hindlimbs of all lizards (N = 561) except for one hindlimb lamella count because the lizard was shedding its skin. All toepad trait measurements were carried out by A.S.P. The following linear body and limb measurements for the same lizards were taken from Bock et al. (34): snout-vent length (SVL), head length, head width, pectoral width, pelvic width, humerus length, ulna length, 3rd toe of the forefoot length, forelimb length, femur length, tibia length, 4th toe of the hindfoot, and hindlimb length. Repeatability was high for these traits (overall mean 98.5%, ranging from 93.6 to 99.6% among traits). All body and limb measurements were done by D.G.B. The influence of body size (SVL) on all non-SVL traits was accounted for by extracting residuals from linear regressions of each log-transformed focal trait against log-transformed SVL in R.

Statistical Analyses. We focused our analyses on explaining variation in toepad size and limb length based on the number of hurricanes experienced by a population. Previous analyses found no relationship between limb length and perch diameter, or between number of lamellae and perch height, among non-native *A. sagrei* populations in Florida and Georgia (34, 35). However, these studies did identify an effect of genetic ancestry on some aspects of morphological and physiological variation among non-native *A. sagrei* populations (34, 35, 37, 49). Therefore, we assessed the effect of hurricane history and genetic ancestry on *A. sagrei* morphology with a series of linear mixed models using the *lmer()* function in the *lme4* package in R (54). Response variables were residual trait values and body size (i.e., SVL) with fixed effects including the number of hurricanes experienced by a population and genetic ancestry (see below for details on Western Cuba ancestry estimates). The covariate structure included population as a random effect to account for variation among sites not related to our main hypotheses, and a spatial effect based on the latitude and longitude of each sampling site. This spatial covariate was included because several phenotypic traits of *A. sagrei* exhibit clines with latitude and longitude in Florida (e.g., refs. 34, 35, and 37). Moreover, our hurricane number dataset indicates the existence of latitudinal and longitudinal trends due to the historical range expansion of *A. sagrei* in Florida and the tendency for hurricanes to make landfall in southeastern Florida. We combined latitude and longitude in a principal components analysis to reduce this spatial covariate to a single variable describing a spatial axis from NNW to SSE. We also detected a positive correlation ($r = 0.88$) between the number of hurricanes per population and time since population establishment. Because our goal was to test a series of a priori predictions derived from theory and existing evidence for hurricane-mediated evolution of toepad and limb traits, we did not include time in our analysis. However, we recognize the necessity of additional sampling and studies to better account for elapsed time. We address the relevance of time since establishment in the *Discussion*.

We generated individual models for each morphological trait. We also explored more complex model structure including two-way interactions between hurricane frequency and the other covariates (i.e., genetic ancestry and the spatial

covariate). Model comparison showed that the additive models had lowest AIC values for all traits, so we proceeded with and report results of additive models only. Based on previous studies, we expected traits related to clinging performance to mediate survival during high winds and thus to evolve in ways that reflect hurricane history. Traits related to clinging performance include forelimb length, humerus length, ulna length, forelimb digit length, hindlimb length, femur length, tibia length, hindlimb digit length, forelimb toepad area, hindlimb toepad area, forelimb toepad lamellae, and hindlimb toepad lamellae. In contrast, other traits with no known relationship to clinging ability would not be expected to contribute to differential survival during high winds. These include head width, head length, pectoral width, and pelvic width. We report *P*-values for two-tailed tests.

Genome-Wide SNP Data and Ancestry Estimates. We used a set of 120,135 quality-filtered SNPs available for our lizards (see *SI Appendix, Appendix A* for additional details on SNP filtering). These markers were scored based on the most recent version of the *A. sagrei* genome v. 2.1 (55) and were derived from reduced-representation sequencing (see refs. 34, 35, and 49 for information on the molecular and bioinformatics methods used). To estimate the fraction of the genome that has been sampled by these SNPs, we used LD decay data for these populations (34). Specifically, we applied windows of ±20 kb (average $r^2 = 0.525$) and ±1.2 Mb (average $r^2 = 0.304$) around quality-filtered SNPs for chromosomes 1 to 14, merging overlapping regions to calculate the sampled proportions (31.2% and 98.7%, respectively). Finally, we used estimates of ancestry that we previously reported for these populations and individuals (38), representing the inferred proportion of the genome derived from the Western Cuba lineage in the native range of *A. sagrei*.

Genome-Wide Association Mapping. We relied on the four toepad descriptors reported here, as well as the 13 skeletal traits from Bock et al. (34), to understand the genetic architecture of all traits using GWAS. The analysis pipeline followed Bock et al. (34) with modifications (see *SI Appendix, Appendix B* for detailed methodology). Briefly, we relied on a standard linear-mixed model implemented in GEMMA v. 0.94 (56), as well as the ancestry-specific association model implemented in asaMap (57). The association analyses were conducted separately for SNPs located on the autosomes and the PARs (118,295 SNPs) and for those within the SDR of the X chromosome (chromosome 7; 1,840 SNPs). These separate analyses were conducted to ensure that the confounding effect of population structure is properly accounted for, particularly for SNPs in the SDR, which exhibit strong differentiation among populations in this dataset (34). We then summarized the number of independent associations detected for each trait and estimated the effect size of each locus as percent variance explained (PVE) using linear models in R (*SI Appendix, Appendix B*).

Genetic Differentiation and Genotype–Environment Associations. To identify genomic regions with the signature of hurricane-associated natural selection, we combined genomic differentiation analyses and GEAs. These analyses were performed separately for SNPs on the autosomes/PARs and the SDR. The differentiation analyses used the XtX statistic, as implemented in BayPass v. 2.3 (58). To control for population structure, we ran the BayPass core model using SNPs pruned for LD (see *SI Appendix, Appendix A* for details on LD pruning). From this analysis, we extracted the population allele frequency covariance matrices, which were used as input for subsequent runs that included all SNPs. To determine a threshold for designating highly differentiated SNPs, we simulated pseudo-observed datasets (PODs) of the same number of SNPs, using the *simulate.baypass* R function. After running the BayPass core model using the PODs, we used the distribution of XtX to select a cutoff for highly differentiated SNPs at a 5% false discovery rate threshold.

For the GEAs, we used the standard covariate BayPass model, built separately for autosomal/PAR SNPs and SDR SNPs. To account for population structure, we used the same covariance matrices as in the differentiation analyses. We tested for associations between SNPs and hurricane frequency, as well as 23 other microhabitat and environmental variables. These included 19 descriptors of temperature and precipitation (BIO1-BIO19) obtained from WorldClim (59), canopy openness measurements (reported in ref. 38), perch height and perch diameter measurements (reported in ref. 34), and the spatial covariate used in the morphology analyses, which summarizes latitude and longitude for our sites. These additional

variables were used to compare the genomic signature of hurricane adaptation with that of adaptation to other environmental factors. We used the BayPass *scalecov* option to standardize covariates. To designate "strong" associations, we used Jeffreys' rule (Bayes Factor BF$_{is}$ > 10 deciban units; ref. 60). We then intersected the GWAS, genomic differentiation, and GEAs, as well as only the differentiation and GEAs, to find SNP outliers across multiple methods (*SI Appendix, Appendix C*).

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