

# When adaptation is slowed down: Genomic analysis of evolutionary stasis in thermal tolerance during biological invasion in a novel climate

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## Abstract

Research conducted during the past two decades has demonstrated that biological invasions are excellent models of rapid evolution. Even so, characteristics of invasive populations such as a short time for recombination to assemble optimal combinations of alleles may occasionally limit adaptation to new environments. Here, we investigated such genetic constraints to adaptation in the invasive brown anole (*Anolis sagrei*)—a tropical ectotherm that was introduced to the southeastern United States, a region with a much colder climate than in its native Caribbean range. We examined thermal physiology for 30 invasive populations and tested for a climatic cline in cold tolerance. Also, we used genomics to identify mechanisms that may limit adaptation. We found no support for a climatic cline, indicating that thermal tolerance did not shift adaptively. Concomitantly, population genomic results were consistent with the occurrence of recombination cold spots that comprise more than half of the genome and maintain long-range associations among alleles in invasive populations. These genomic regions overlap with both candidate thermal tolerance loci that we identified using a standard genome-wide association test. Moreover, we found that recombination cold spots do not have a large contribution to population differentiation in the invasive range, contrary to observations in the native range. We suggest that limited recombination is constraining the contribution of large swaths of the genome to adaptation in invasive brown anoles. Our study provides an example of evolutionary stasis during invasion and highlights the possibility that reduced recombination occasionally slows down adaptation in invasive populations.

## KEYWORDS

*Anolis sagrei*, genetic constraints, invasive species, rapid evolution, recombination suppression, thermal tolerance

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## 1 | INTRODUCTION

Human-mediated introductions of invasive species have provided some of the earliest indication that evolution can occur over contemporary time scales. In the mid-1900s, for instance, long before rapid evolution was considered ordinary (Reznick et al., 2019), studies of invasive plant and animal populations began reporting geographical clines in trait variation. These clines were inferred to have formed during the invasion, over just tens or hundreds of generations, implicating rapid adaptive evolution (e.g. Dobzhansky, 1965; Harper, 1965; Johnston & Selander, 1964). Since then, many other examples followed (e.g. Westley, 2011; reviewed in Hodgins et al., 2018), involving contemporary adaptation to variables such as temperature (e.g. Gilchrist et al., 2004; Huey et al., 2000) or seasonal variation (e.g. Colautti & Barrett, 2013; Dlugosch & Parker, 2008), as well as the rapid evolution of traits that facilitate invasive spread (Phillips et al., 2006; Whitney & Gabler, 2008). As a result, invasions are often recognized as natural experiments of rapid evolution. While these experiments are imperfect, given they were started at different timepoints and from different source populations, they offer rich opportunities for study because they span geographical and temporal scales that far exceed the scales of most planned laboratory or field experiments (Hodgins et al., 2018; Moran & Alexander, 2014; Sax et al., 2007).

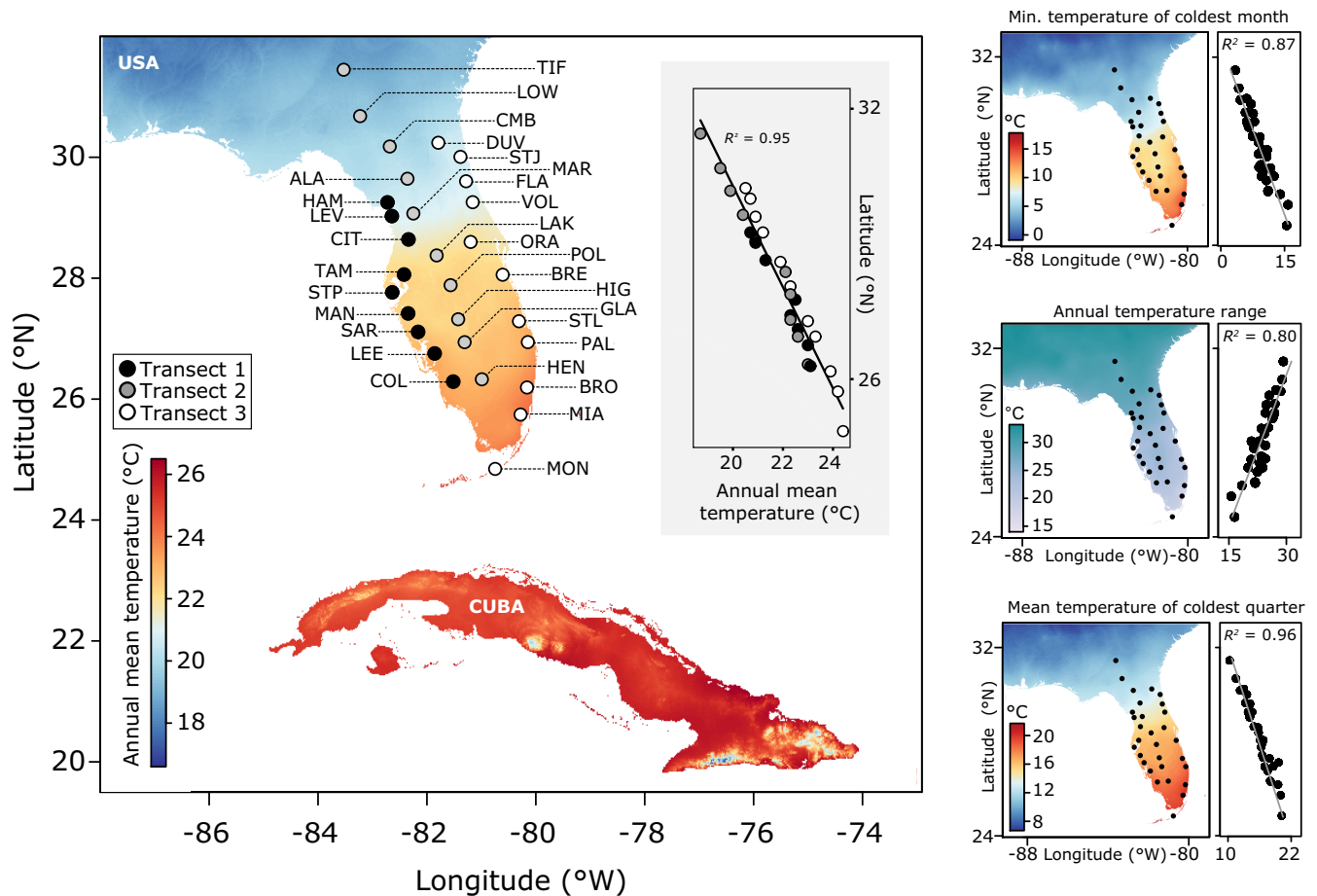
Aside from demonstrating that evolution can be rapid, research on invasive species has also helped to identify its underlying genetic mechanisms (reviewed in Bock et al., 2015). These studies have demonstrated that rapid evolution often involves standing genetic variants (e.g. Bock et al., 2018; Stern & Lee, 2020; Vandepitte et al., 2014) which, contrary to new mutations, are available as soon as the selective conditions change (Barrett & Schluter, 2008). Also, adaptive alleles that are already segregating in some populations can quickly be transferred via hybridization (e.g. DeVos et al., 2023; Valencia-Montoya et al., 2020). Lastly, research on invasive species has provided evidence that non-recombining regions of the genome like chromosomal inversions can drive rapid evolution (e.g. Battlay et al., 2023; Prevosti et al., 1988). These regions maintain combinations of alleles together and facilitate complex adaptation that is underpinned by multiple integrated phenotypes (Hoffmann et al., 2004; Lowry & Willis, 2010; Todesco et al., 2020).

While biological invasions have proven essential for understanding rapid evolution, their potential for revealing why adaptation may fail remains overlooked. This is notable for three reasons. First, our understanding of factors that constrain the rate and direction of adaptation is limited for invasive populations and for natural populations more broadly (Futuyma, 2010). Second, invasive species may be introduced to regions where rapid adaptation is challenging to achieve. This could occur, for example, if adaptation in an invasive species requires the evolution of trait values that are outside those displayed by the species across its ancestral range, necessitating new mutations or the rewiring of genetic and physiological pathways (Alexander & Edwards, 2010). Third, invasive populations often have a hybrid origin, and may contain chromosomal inversion

polymorphisms (Bock et al., 2015). While hybridization and chromosomal inversions can drive rapid adaptation during some invasions, they may also constrain adaptive responses in others. For example, hybridization may interfere with adaptation when it brings together divergent alleles that interact with one another, resulting in extrinsic outbreeding depression (Baeckens et al., 2023; Bock et al., 2021; Thompson et al., 2022). As well, due to the suppression of recombination, chromosomal inversions may slow down adaptation (Roesti et al., 2022) because when invasive species are introduced to new environments, recombination may be needed to stack together new advantageous arrangements of alleles and to break apart arrangements that are disadvantageous in the novel range (Roesti et al., 2022).

The introduction of the Cuban brown anole (*Anolis sagrei*) in the southeastern United States (US) provides a promising opportunity to investigate factors that impede rapid adaptation during invasion. For one, the current invasive range of *A. sagrei*, which has expanded at an accelerated pace since the 1970s (Kolbe et al., 2004), covers a much wider latitudinal band than the species' native range (Angetter et al., 2011). As a result, invasive populations are exposed to a climate that is more variable, and considerably dryer and colder, than the one this species has experienced over much of its evolutionary history (Angetter et al., 2011; Baeckens et al., 2023). As such, pre-existing adaptive genetic variants could be of limited value. As well, invasive populations have a hybrid origin (Bock et al., 2021; Kolbe et al., 2004, 2008). The hybrid genome of invasive populations has previously been shown to contain divergent loci that interact epistatically, potentially interfering with adaptation (Baeckens et al., 2023; Bock et al., 2021). Moreover, recombination cold spots appear to be common along the genome of invasive populations, as inferred from strong genome-wide linkage disequilibrium (Bock et al., 2021). This characteristic may delay the reshuffling of alleles and the assembly of novel, highly fit genotypes. In line with these observations, a recent investigation of desiccation tolerance in invasive *A. sagrei* showed that a climatic cline in hydric physiology has not evolved, implying that invasive populations are not currently adapted physiologically to local climate conditions (Baeckens et al., 2023).

In this study, we build on previous findings on the ecology and evolution of the brown anole invasion in the southeastern US to investigate whether, indeed, climate adaptation is currently lacking in this system, and to identify genetic mechanisms that can explain this pattern. We focus on contemporary adaptation of thermal physiology to ambient temperature—the climatic variable that differs most strongly between the native and invasive ranges in this species (Figure 1). Because of the fitness benefits of a greater thermal tolerance for ectotherms in cold environments (Campbell-Staton et al., 2017; Campbell-Staton et al., 2018; Stroud et al., 2020), we hypothesize that in the northern, colder, extent of the invasive range of *A. sagrei*, natural selection will favour the survival and reproduction of individuals that tolerate relatively lower temperatures. To test this hypothesis, we combine climate data with measurements of thermal tolerance for invasive individuals obtained from 30 localities along a steep climatic gradient, seeking to determine whether a cline in



**FIGURE 1** Temperature trends and extremes across the brown anole invasive range, including our study sites. (left) Map of the localities for each of the 30 invasive populations sampled in this study; colour gradient shows annual mean temperature variation, ranging from red (warm) to blue (cold), across the native (Cuba) and invasive range (USA). Circle greyscale depict transect number (black=transect 1; grey=transect 2; white=transect 3). Locality IDs correspond to information given in Table S1. (right) Coloured maps visualizing geographical variation in the minimum temperature of the coldest month (top), annual temperature range (middle), and mean temperature of the coldest quarter (down). Scatterplots show the steep latitudinal cline in temperature that characterizes our study system.

thermal limits has evolved since the invasion started. Additionally, we rely on genotype-trait associations and on population genomic methods to establish whether thermal tolerance across the range of invasive brown anoles is shaped by limited genetic variation, epistatic interactions introduced by hybridization, and by reduced recombination. We specifically ask whether these genetic mechanisms can limit rapid adaptive responses in invasive populations of *A. sagrei*.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling and animal housing

During March–July 2018, we collected adult male lizards ( $n=589$ ) from 30 localities (average = 19.6 individuals per location; range = 13–28) distributed along three latitudinal transects from the southern tip of Florida to southern Georgia (Figure 1; Table S1). Previous studies on invasive *A. sagrei* indicate that thermal tolerance can change depending on ambient temperatures (Kolbe et al., 2014). Thus, to minimize the effect of conditions lizards experienced in the wild close to

the time of capture (i.e. acclimation), we followed previous studies (e.g. Kolbe et al., 2014; Leal & Gunderson, 2012) and kept all animals under uniform conditions in the lab before thermal limits measurements (detailed below). Thus, after capture, we transported all lizards to the animal care facility at Harvard University, where they were maintained in individual enclosures at the same temperature (~28°C) and relative humidity (80%; see Baeckens et al., 2023 for additional details on animal housing). This acclimation phase lasted for an average of 34 days (range 26–44 days; Table S2). During this time, we misted the lizards twice per day and fed them vitamin-dusted crickets three times per week. Animal husbandry and experimental protocols were approved by the Harvard University's Committee for Animal Care and Use (IACUC protocol #26–11).

### 2.2 | Phenotypic data collection: Critical thermal minimum

To assess the lower thermal limits of invasive individuals, we relied on the critical thermal minimum ( $CT_{min}$ ), which we measured

for a subset of 480 lizards distributed across all 30 localities (average = 16 individuals measured per location; range = 6–20; Table S1). Although not without limitations (Camacho & Rusch, 2017),  $CT_{min}$  is a widely-used metric to estimate lower thermal tolerances in ectotherms (Cowles & Bogert, 1944; Taylor et al., 2021), including *Anolis* lizards (Campbell-Staton et al., 2017; Huey & Webster, 1976; Leal & Gunderson, 2012; Muñoz et al., 2014). Briefly, it represents the body temperature at which an animal exposed to cold loses its locomotor function. Prior to  $CT_{min}$  measurements, to account for the possibility of experimenter bias, we anonymized the ID and population provenance of each lizard. We further processed the lizards in a random order, rather than sequentially by population.

At the beginning of  $CT_{min}$  trials, we took an initial measurement of body temperature for each lizard with a fine-gauge digital thermocouple (precision = 0.1°C) inserted into the cloaca. We then placed lizards in individual plastic containers in an incubator set at 17°C. After an average of 40 min (range 40–50 min; Table S2), at which point all lizards were still fully mobile, we again measured body temperature, and moved the plastic containers with the lizards to a large plastic tub with crushed ice, where we continued the cooling process. During this ice-cooling phase, we flipped each lizard on its back at one-minute intervals, and stimulated it to right itself by gently probing its thighs with a blunt forceps. If a lizard flipped over after 30 s, we continued to lower the body temperature by returning the lizard to the container with ice. When a lizard lost the ability to right itself in 30 s, we measured the body temperature, and recorded this value as the  $CT_{min}$ . Body temperatures were lowered at a rate of less than 1°C per min for all lizards except two, which experienced rates of 1.1 and 1.25°C per min during the ice cooling phase (Figure S1). Overall, rates of cooling averaged 0.28°C per min for the incubator phase, and 0.48°C per min for the ice phase (Figure S1; Table S2). We controlled the rate of cooling because the rate of temperature change during tolerance tests can alter an animal's performance (Terblanche et al., 2007).

### 2.3 | Environmental data collection: Temperature variation across the invasive range

We characterized the latitudinal climate space occupied by *A. sagrei* in the US based on a set of six bioclimatic variables (30 arcsec spatial resolution) retrieved from the WorldClim database (Fick & Hijmans, 2017). For this study, we were specifically interested in those bioclimatic variables that represent annual trends, seasonal variation, and extremes of temperature (Figures 1 and S2). For each sampling locality, we extracted data on (i) annual mean temperature (BIO1), (ii) minimum temperature of the coldest month (BIO6) and (iii) coldest quarter (BIO11), (iv) isothermality, which quantifies how large the day-to-night temperatures oscillate relative to the summer-to-winter oscillations—a low isothermal value indicates a low level of temperature variability within an average month relative to the year (BIO3), (v) temperature seasonality, which is a measure of temperature change over the course of the year (as standard deviation)—the

larger the value, the greater the variability of temperature (BIO4), and (vi) annual temperature range (BIO7). Each of the six variables correlates strongly with latitude (all  $p < .001$ ; Figure S2) indicating that the invasive range of brown anoles in the southeastern US exhibits a strong latitudinal thermal gradient. Northern populations experience relatively low annual mean temperatures (BIO1 of most northern locality = 18.7°C), substantial temperature fluctuations across the year (BIO4 = 62.9°C; BIO7 = 29.2°C), and extreme lows in winter (BIO6 = 3.4°C). In contrast, southern populations inhabit environments with high annual mean temperatures (BIO1 of most southern locality = 24.4°C), little temperature seasonality (BIO4 = 30.5°C; BIO7 = 15.6°C), and mild winters (BIO6 = 15.8°C).

### 2.4 | Genome-wide sequence data and genotyping

To investigate genetic factors that shape variation in  $CT_{min}$  across the invasive range of *A. sagrei*, we relied on genome-wide sequence data available from reduced-representation sequencing (i.e. ddRAD and quaddRAD; Table S3; see also Bock et al., 2021 for detailed information on sequencing library preparation and bioinformatic processing). This included sequence data for 560 (95%) of the 589 individuals we sampled in 2018 from the invasive range (see 'Sampling and animal housing' section above). We used these 560 sequencing libraries to investigate the genetic architecture of  $CT_{min}$  via genome-wide association mapping and to identify genomic regions of reduced recombination via population genomics (detailed below). We refer to the 560 samples as the 'Invasive range' data set. We also incorporated sequence data for an additional 120 individuals from the Cuba native range of *A. sagrei* (Table S3). These native-range individuals represent all major lineages known to have contributed ancestry to invasive populations (Kolbe et al., 2004). We used the 120 native-range samples to determine the ancestry of haplotype blocks identified during our screen for regions of reduced recombination (detailed below) and to contrast rates of linkage disequilibrium (LD) decay between ranges (see details in Supplementary Material). We refer to all 680 samples as the 'Native and invasive ranges' data set. We aligned quality-filtered sequence reads obtained as in Bock et al. (2021) to the most recent version of the *A. sagrei* genome (v2.1; Geneva et al., 2022). We then called single nucleotide polymorphisms (SNPs) using the dDocent pipeline (v2.2.20; Puritz et al., 2014) and filtered them using stringent criteria (see details in Supplementary Material).

### 2.5 | Genome-wide association mapping

Of the 458 individuals with both  $CT_{min}$  measurements and genome-wide SNP data available, we excluded 37 individuals from 5 populations on transect 1 that were obtained at a different time of the year as compared to the rest of the samples on that transect (Table S1). This was done to account for the possibility that thermal tolerance measurements are influenced by ontogeny or phenology (e.g. Baeckens et al., 2023; Pottier et al., 2022; Ruthsatz

et al., 2022). Also, we focused on SNPs that passed quality filtering and that were positioned on the 14 largest scaffolds of the reference genome, which correspond to the expected number of chromosomes for *A. sagrei* and comprise over 99% of the full assembly length (Geneva et al., 2022). Association analyses were performed based on a set of SNPs that excluded markers in strong LD (i.e. 37,355 SNPs; see Supplementary Material for details on LD filtering).

We used two different association mapping approaches. First, we relied on the commonly used linear mixed model implemented in GEMMA (v0.94; Zhou & Stephens, 2012), which assumes that a given genetic variant has the same effect across individuals. Second, we relied on the ancestry-specific model implemented in asaMap (Skotte et al., 2019). Designed for admixed populations, the asaMap model allows for the identification of loci with ancestry-specific effects. Such ancestry-specific effects may occur because of interactions among divergent alleles that evolved in isolation, but that now segregate within a mosaic genome because of hybridization (e.g. Baeckens et al., 2023; Bock et al., 2021). Based on simulations, Skotte et al. (2019) indicate these models can identify different loci. Specifically, the standard model provides increased power when associations are not ancestry specific, whereas the ancestry-specific model is superior when the effect of loci depends on ancestry (Skotte et al., 2019). Both the GEMMA and asaMap models accounted for potential confounding effects of genetic relationships among samples and the membership of samples to each of the three transects (see additional details provided as Supplementary Material).

## 2.6 | Identifying genomic regions of reduced recombination

Recent studies have illustrated how short-read sequence data, including data obtained via reduced-representation sequencing, can be used to detect regions of the genome with low recombination, such as chromosomal inversions (e.g. Battlay et al., 2023; Harringmeyer & Hoekstra, 2022; Huang et al., 2020; Todesco et al., 2020). These methods rely on the fact that reduced recombination creates a distinct signature in the genome in terms of population structure and genetic diversity. Because reduced recombination can delay adaptation when the environment colonized by an invasive species is novel (Roesti et al., 2022), we sought to identify such recombination cold spots in the mosaic genome of invasive *A. sagrei*. We followed recent studies (e.g. Huang et al., 2020) and used the Lostruct R package (Li & Ralph, 2019) to perform windowed principal component analyses (PCAs; see additional details provided as Supplementary Material). We restricted the Lostruct scan to the 'Invasive range' data set for two reasons. First, our goal was to detect outliers of population structure that are segregating in the invasive range. Second, these methods rely on contrasting signals of population structure along the genome and are therefore well-suited for settings where hybridization and gene flow are occurring. In *A. sagrei*, these conditions are met for invasive populations but not for native

populations, which are highly structured genome-wide and contain only rare hybrids (Bock et al., 2021; Kolbe et al., 2004).

While Lostruct outliers have previously been shown to represent genomic regions of reduced recombination (e.g. Battlay et al., 2023; Harringmeyer & Hoekstra, 2022; Huang et al., 2020; Todesco et al., 2020), other non-mutually exclusive processes including natural selection or introgression can generate similar patterns (Li & Ralph, 2019). A common approach to bolster the conclusion that Lostruct outliers correspond to recombination cold spots relies on PCA and estimates of heterozygosity. Specifically, in a PCA performed using SNPs from regions of reduced recombination, samples are expected to form distinct clusters, corresponding to homozygotes for different haplotypes, as well as heterozygotes. In PCA space, heterozygote genotypes should be intermediate relative to homozygous genotypes. As well, these intermediate genotypes should have extreme levels of heterozygosity (Battlay et al., 2023; Harringmeyer & Hoekstra, 2022; Huang et al., 2020; Todesco et al., 2020).

To investigate whether these predictions are met for the Lostruct outliers that we identified, we further analysed patterns of population structure and heterozygosity at these genomic regions. By including native-range samples in these analyses, we were able to infer the ancestry of chromosomal blocks segregating in invasive populations. For each Lostruct outlier window, we conducted a PCA using the adegenet v2.1.8 R package (Jombart & Ahmed, 2011). For invasive *A. sagrei*, because of extensive divergence among native-range lineages that are hybridizing in Florida (Kolbe et al., 2004), we expect a minimum of six genotype clusters for genomic recombination cold spots. These correspond to 1: Western Cuba (WC) homozygous genotypes, 2: Central Cuba (CC) homozygous genotypes, 3: West-Central Cuba homozygous genotypes and WC/CC heterozygotes, 4: Eastern/East-Central Cuba (EC) homozygous genotypes, 5: CC/EC heterozygotes, and 6: WC/EC heterozygotes. Note that, while Eastern Cuba and East-Central Cuba are different *A. sagrei* lineages (Kolbe et al., 2004), they are closely related and harder to separate using PCA (e.g. Bock et al., 2021). As well, West-Central Cuba *A. sagrei* are intermediate in PCA space relative to Western Cuba and Central Cuba *A. sagrei*, and therefore difficult to distinguish from WC/CC heterozygotes. With this information, we used kmeans clustering in R3.6.0 (R Core Team, 2019) to categorize samples in six clusters. Further, we quantified heterozygosity rate for each sample and at each MDS outlier region as the proportion of heterozygous sites out of all sequenced sites for each genotype. We then used linear models to compare the heterozygosity rate of invasive-range samples assigned to intermediate PCA clusters (i.e. WC/EC and CC/EC), relative to heterozygosity of all other invasive-range samples. To further verify that Lostruct outlier regions correspond to sections of the genome with reduced recombination, we used PLINK (v1.90b6.24; Purcell, 2007) to estimate rates of LD decay (see additional details provided as Supplementary Material).

Lostruct outlier regions may also contribute to  $F_{ST}$  outliers, when haplotypes from regions of reduced recombination occur at contrasting frequencies between populations, potentially because they



are involved in adaptive divergence. To compare the contribution of Lostruct outlier and non-outlier regions to genetic differentiation in the native and invasive ranges of *A. sagrei*, we used the window-based  $F_{ST}$  estimates reported by Bock et al. (2021) for the same dataset. For each of the two ranges, these estimates consist of windowed  $F_{ST}$  (50 kb), averaged across independent pairwise population comparisons. We classified each window as 'outlier' or 'non-outlier' depending on whether they overlapped with regions of reduced recombination highlighted by our Lostruct scan. We then tested for differences between window categories for each range, using linear models in R. These had average  $F_{ST}$  as the response variable, and window category as the predictor variable.

## 2.7 | Genome-wide ancestry estimates

Previous studies have highlighted an important contribution of introduction and hybridization history in shaping trait variation across the invasive range of *A. sagrei* (e.g. Baeckens et al., 2023; Bock et al., 2021; Kolbe et al., 2007; Pita-Aquino et al., 2022). Thus, prior to investigating the relationship between  $CT_{min}$  and climate variables (described below), we sought to incorporate information on genome-wide ancestry so that population genetic structure can be properly accounted for. To do so, we used Western Cuba ancestry estimates (hereafter referred to as 'ancestry') reported in Baeckens et al. (2023) for the same individuals as those used here. These estimates were obtained based on a set of 10,000 random genome-wide SNPs, which were used to estimate admixture proportions from a  $K=2$  analysis performed in STRUCTURE (v.2.3.4; Pritchard et al., 2000).

## 2.8 | Trait-environment associations

To assess whether variation in environmental thermal conditions explained variation in lizard thermal tolerance, we performed linear mixed effects analyses of the relationship between  $CT_{min}$  (response variable) and each of the six bioclimatic (predictor) variables, which were run in R using the package *lme4* (Bates et al., 2015). Aside from the bioclimatic variable in question, models also contained "body mass," "cooling rate," "acclimation time," and "ancestry" as fixed effects to control for the potential scaling effect of thermal tolerance with size (Leiva et al., 2019), thermal assay conditions (Chown et al., 2009; Terblanche et al., 2007), thermal acclimation (Pintor et al., 2016), and lizard introduction history and degree of hybridization (Bock et al., 2021). As random effects, we included "population" nested within "transect" to avoid pseudo-replication. With six bioclimatic variables of interest, we built six models and compared each of these six "full models" against the "null model" (i.e. the full model without the bioclimatic effect in question) to test for the effect of the focal bioclimatic variable on  $CT_{min}$ . We also ran an additional analysis testing whether lizard body mass was ancestry-determined. Body mass was log-transformed prior to analysis to meet model

assumptions. Visual inspection of model residual plots and Shapiro-Wilk's tests did not reveal any obvious deviations from homoscedasticity or normality.

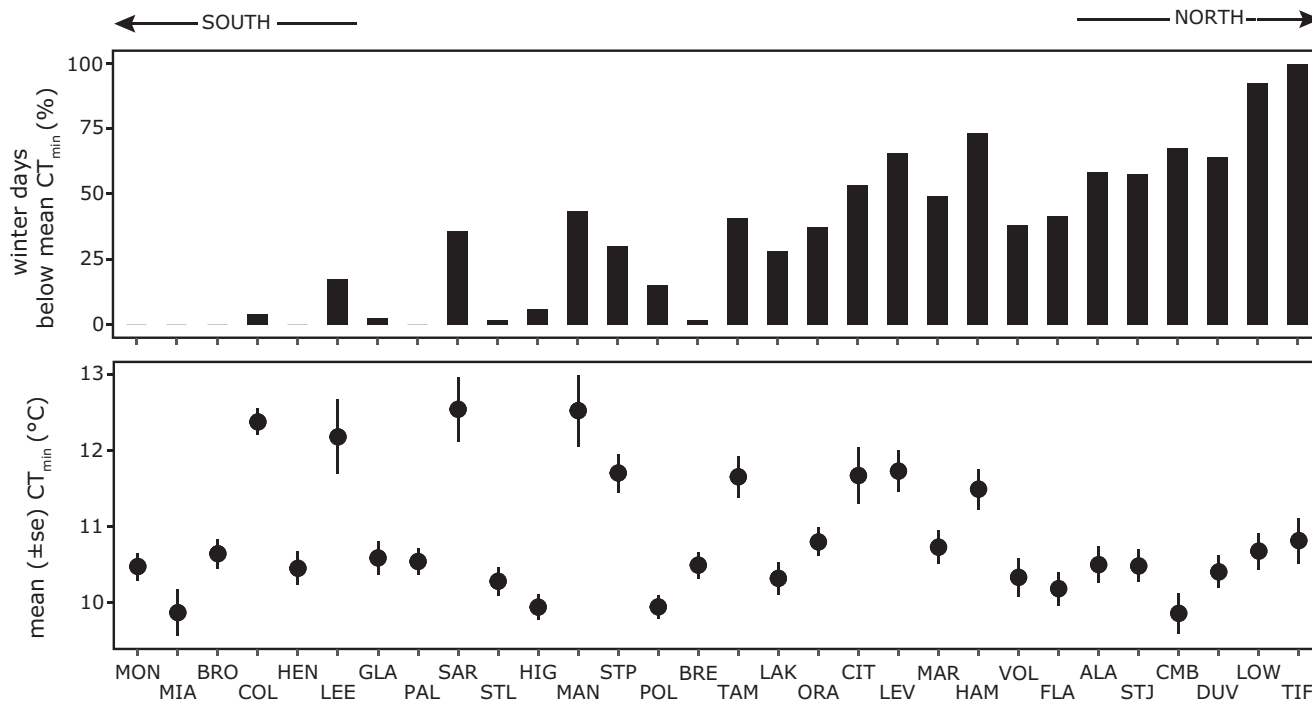
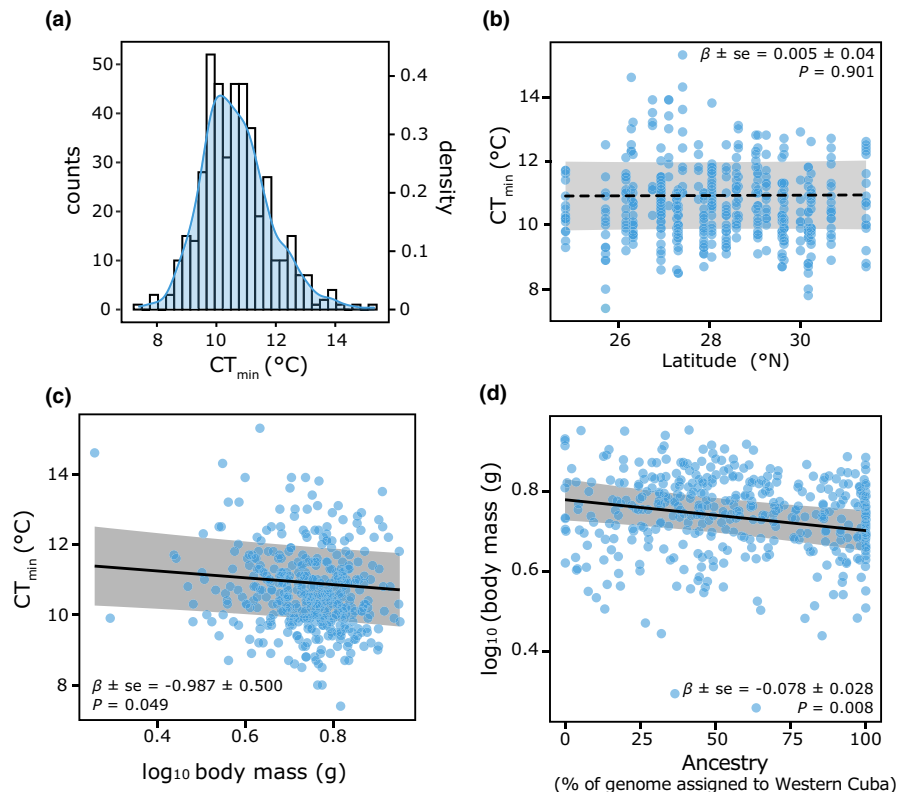
## 3 | RESULTS

The thermal tolerance of invasive brown anoles varied substantially among the 30 populations that were sampled across southeastern US. Population means ( $\pm SE$ ) of  $CT_{min}$  ranged from  $9.8 \pm 0.3^\circ C$  in Colombia County to  $12.5 \pm 0.4^\circ C$  in Sarasota County. The lowest observed  $CT_{min}$  value among all individuals was  $7.4^\circ C$ , whereas the highest was  $15.3^\circ C$  (mean =  $10.6^\circ C$ ; 25th percentile =  $9.9^\circ C$ ; 75th percentile =  $11.3^\circ C$ ) (Figures 2 and 3). Climate was an overall poor predictor of  $CT_{min}$  (Figure S3): model comparisons revealed that none of the bioclimatic variables, which describe temperature trends, fluctuations, and extremes across the invasion range of the brown anole, were significantly correlated with  $CT_{min}$  (Table 1). The null model showed that  $CT_{min}$  was significantly, albeit marginally, affected by lizard body mass—with bigger lizards tolerating colder temperatures—but not by cooling rate or ancestry (Table 1). Additional analyses, however, exposed an indirect effect of ancestry on  $CT_{min}$  via body size (Figure 2). Specifically, lizards with low percentage of Western Cuba ancestry were, on average, larger ( $\beta \pm SE = -0.078 \pm 0.028$ ,  $t = -2.75$ ,  $p = .008$ ), and those larger lizards also withstood lower temperatures ( $\beta \pm SE = -0.987 \pm 0.500$ ,  $t = -1.97$ ,  $p = .049$ ).

The standard GWAS performed using GEMMA identified two loci that were associated with  $CT_{min}$  at the suggestive significance level (Figure 4a). These loci were located on chromosome 2 (i.e. locus 1; coordinate of lead association SNP: 27,630,520;  $p = 1.4 \times 10^{-5}$ ; Figure 4a,b) and on chromosome 3 (i.e. locus 2; coordinate of lead association SNP: 235,762,309;  $p = 1.76 \times 10^{-5}$ ; Figure 4a,c). For both loci, differences in average  $CT_{min}$  between the two homozygous genotype classes were large (locus 1:  $1.54^\circ C$ ; locus 2:  $1.11^\circ C$ ; Figure 4b,c), and on par with differences in average  $CT_{min}$  among populations (Table S1). Nonetheless, alleles at these loci occur at low-to-moderate frequencies (locus 1: 4.1%; locus 2: 8.7%) across invasive populations. Consequently, the percentage variance explained (PVE) by these loci was low (i.e. locus 1 PVE: 5.2%; locus 2 PVE: 4.8%). As well, allele frequencies at these loci are either not correlated with latitude (e.g. locus 1;  $p = .13$ ; Figure S4) or are marginally correlated with latitude, but in a direction opposite to expectation had these loci been under selection (i.e. alleles associated with reduced cold tolerance are more common at higher latitudes; locus 2;  $p = .04$ ; Figure S4).

The *asaMap* GWAS, which can detect loci with ancestry-specific effects, pointed to one locus as significant at the suggestive significance level, based on the LD-pruned marker set (Figure 4a). This locus was located on chromosome 6 (i.e. locus 3; coordinate of lead association SNP: 51,011,483;  $p = 1.05 \times 10^{-5}$ ; Figure 4a,d), and had a small effect size (PVE: 1.7%; hybridization-limited samples only; Figure 4d). The model considered by *asaMap* (i.e. the M2 vs. M5 model; Skotte et al., 2019) tested whether there is an effect only for

**FIGURE 2** Observed variation in, and correlates of, lizard critical thermal minimum. (a) Histogram (left-side y-axis) overlaid by a density plot (right-side y-axis) illustrating the variation in critical thermal minimum ( $CT_{min}$ ) detected in this study. (b) Scatterplot of  $CT_{min}$  against latitude. Relationship between (c)  $CT_{min}$  and body mass, and between (d) body mass and ancestry showing the indirect effect of ancestry on  $CT_{min}$  through body mass.

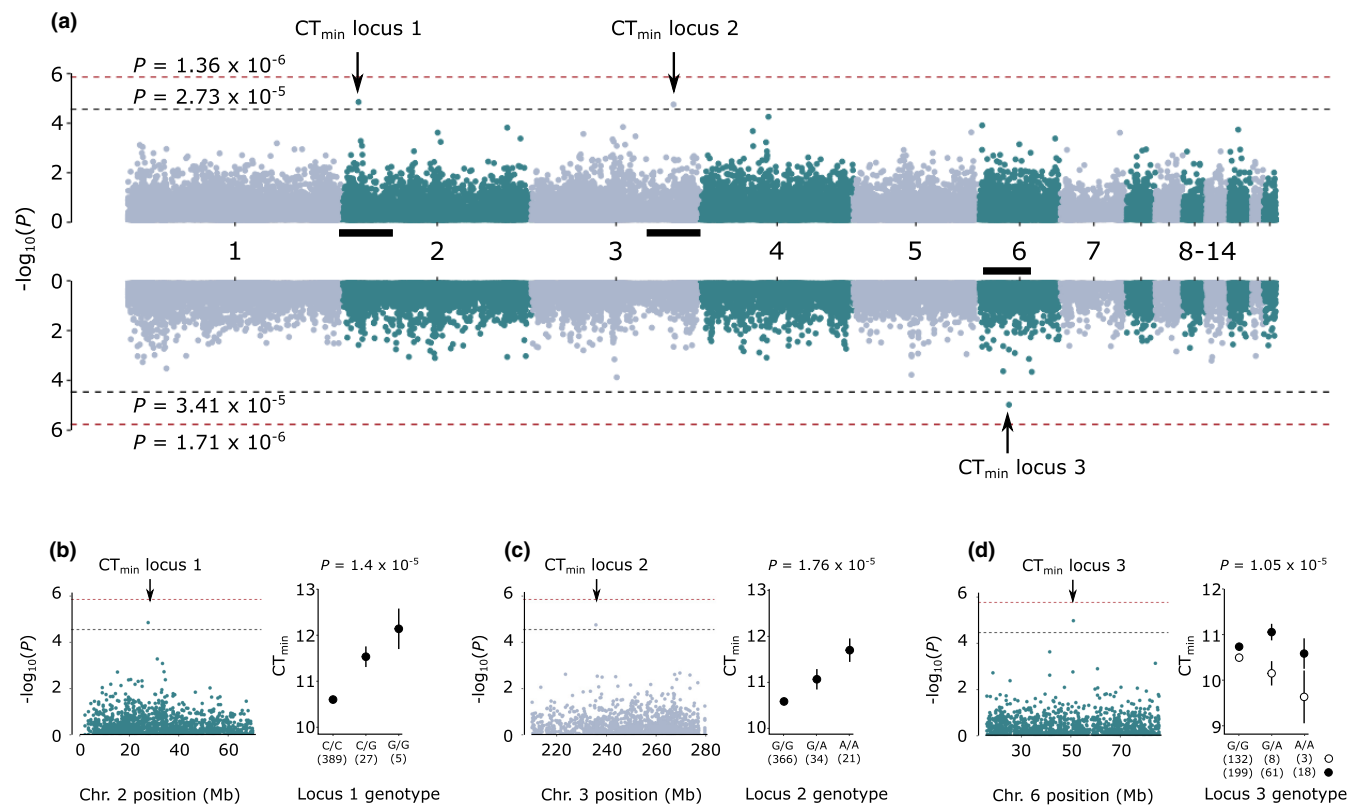


**FIGURE 3** Estimation of the cold stress experienced by lizards at each locality as the relative number of days each population experienced temperatures below its average critical thermal limit during winter. Mean ( $\pm SE$ ) critical thermal minima per population are visualized in the bottom graph; the upper barplot shows the average number of winter days per year (in %) for which minimum environmental temperatures are lower than the mean population critical thermal minimum. Daily minimum temperature (obtained from the CHELSA-W5E5 dataset; Krager et al., 2022) were averaged for eleven winters from 2005 to 2016 (November 1 to February 28; total of 120 days). Populations are ordered by latitude from left (south) to right (north).

TABLE 1 Climatic predictors of critical thermal minimum ( $CT_{min}$ ).

Model	Model output						Model comparisons	
	Fixed effect	$\beta$	SE	df	t-value	p-value	$\chi^2$	p-value
Null model	(Intercept)	10.977	0.825	31.15	13.308	<.001		
	Body mass	-0.987	0.500	387.39	-1.974	.049		
	Cooling rate	0.140	0.449	397.57	0.312	.755		
	Acclimation time	0.019	0.014	394.41	1.31	.190		
	Ancestry	-0.066	0.239	46.29	-0.277	.783		
Full model (bio1)	BIO1	-0.003	0.005	27.19	-0.512	.613	0.251	.616
Full model (bio3)	BIO3	0.006	0.021	27.15	0.287	.776	0.080	.778
Full model (bio4)	BIO4	<0.001	<0.001	27.35	0.512	.613	0.205	.617
Full model (bio6)	BIO6	-0.002	0.002	27.93	-0.628	.535	0.379	.538
Full model (bio7)	BIO7	0.002	0.002	27.15	0.724	.475	0.505	.478
Full model (bio11)	BIO11	-0.001	0.003	27.62	-0.506	.617	0.246	.620

Note: Summary of the null model output and its comparison with six different full models, each testing for the effect of a different bioclimate variable on  $CT_{min}$  (response variable). Estimates ( $\beta$ ), standard errors (SE), degrees of freedom (df), t- and p-values of the fixed effects are provided, in addition to the outcome ( $\chi^2$  and p-value) of the likelihood ratio tests comparing modes.



**FIGURE 4** Genome-wide association mapping of  $CT_{min}$ . (a) Associations on chromosomes 1–14, using a standard model (top) or an ancestry-specific model (bottom). Blue and teal are used to differentiate between markers on separate chromosomes. The dashed lines indicate the two significance thresholds, while black bars show the approximate location of chromosomal regions highlighted in panels b–d. For panels b–d, the figure on the right shows mean ( $\pm SE$ )  $CT_{min}$  values for each genotype at the lead GWAS SNP, with sample sizes given in parentheses under each genotype, and p values as obtained from each association analysis. For the ancestry-specific locus (panel d), empty circles denote samples with limited hybrid ancestry, while black circles denote samples with common hybrid ancestry.

individuals of one lineage. Based on the ADMIXTURE scores used by asaMap, we infer that individuals with an effect for locus 3 are characterized by limited hybridization and have predominantly Western Cuba ancestry (Figure 4d).

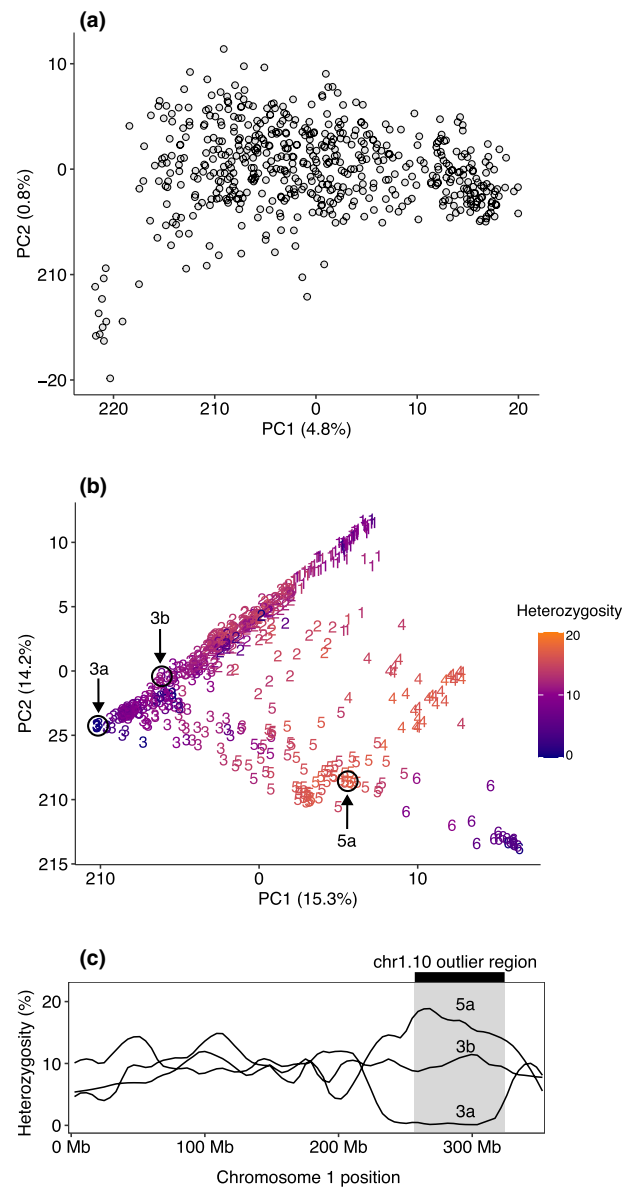
The Lostruct scan identified 1378 windows of 50 SNPs each, that had a different population structure signal than the rest of the genome (Figures 5 and S5 and S6; Table S4). These outlier windows were distributed along all seven macrochromosomes



(Figure 6) and were aggregated in 50 large regions (8.77 Mb–125.46 Mb in length; average 53.34 Mb; Table S4; Figure S5). While Lostruct outlier regions for any given MDS coordinate only spanned 0.7%–8.9% of the genome, across all 40 MDS coordinates they covered 1.09 Gb, or 57% of the total length of the reference genome, and 55% of named genes in the reference genome (Geneva et al., 2022).

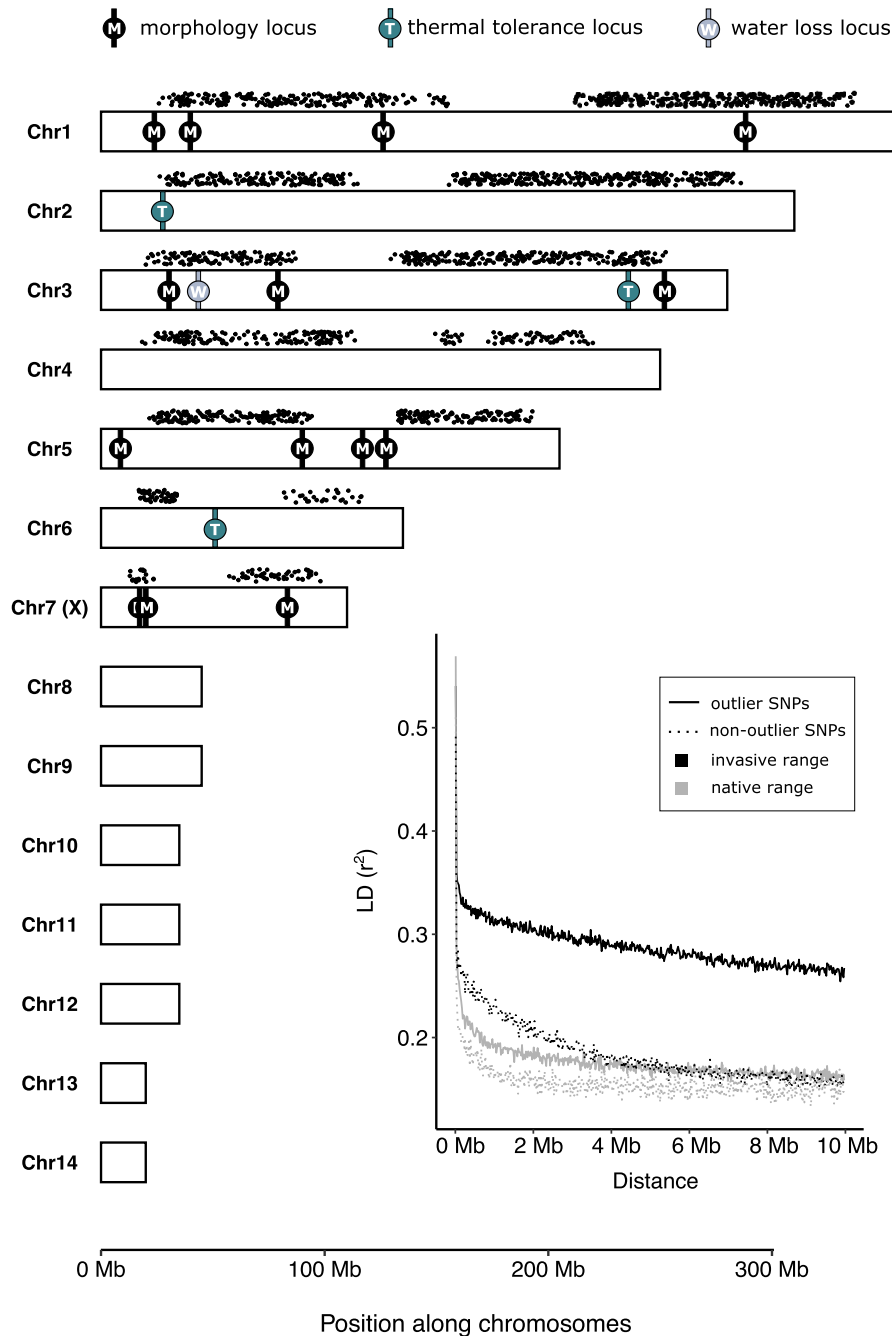
Lostruct outlier regions displayed a pattern of population structure and genetic diversity that is consistent with genomic recombination cold spots. Specifically, outlier regions are characterized by strong population structure as inferred by PCA (Figures 5 and S6), with PC1 and PC2 explaining a sizeable proportion of overall variation (range 19.41%–49.74%; average 28.97%; Table S4). This is substantially higher than the proportion of variation explained by PC1 and PC2 when using a set of genome-wide SNPs that have been filtered for LD (i.e. 5.6%; Figure 5a; see also Bock et al., 2021 for a detailed analysis of genome-wide population structure and diversity in this system). Samples could be assigned to distinct clusters in PCA space for most of the outlier regions (e.g. 95.7%, or 45/47 of autosomal outlier regions; Table S4; Figure S6). Moreover, in all instances for which the ancestry of PCA clusters could be inferred using native-range data (Figure S6), samples that were part of intermediate clusters (i.e. clusters 4 and 5; Figures 5 and S6) were considerably more diverse (Table S4; Figures 5b and S6), as expected if intermediate clusters consist of heterozygotes for long-range haplotypes that originated in different lineages. By contrast, samples assigned to the rest of PCA clusters had either intermediate or null heterozygosity (Figures 5b,c and S6). This is in accord with expectations if these samples contain different haplotypes that originated from the same lineage (resulting in intermediate heterozygosity), or the same long-range haplotype that has not recombined within or across lineages (resulting in null heterozygosity). Lastly, we found that populations established at different times during the *A. sagrei* invasion (e.g. 1932 vs. 2002) maintain polymorphism at Lostruct outlier regions (e.g. 30.1–66.9 Mb; Figure S7). Thus, multiple long-range haplotypes that originated from different lineages are maintained at these sites, and have not been significantly eroded by recombination within the past 70 years (Figure S7).

The rate of LD decay differed between outlier and non-outlier regions of the genome for both the native and the invasive ranges (Figure 6). For example, for SNPs separated by 2 Mb, outlier genomic regions are characterized by stronger LD ( $r^2$ ) than non-outlier genomic regions in the invasive range (outlier regions: mean  $r^2=0.30 \pm 0.04$ ; non-outlier regions: mean  $r^2=0.20 \pm 0.03$ ) and in the native range (outlier regions: mean  $r^2=0.19 \pm 0.01$ ; non-outlier regions: mean  $r^2=0.16 \pm 0.01$ ; Table S5). However, in the invasive range, LD persists over much longer stretches of the genome in outlier regions relative to non-outlier regions. For example, at 10 Mb, LD remains high in outlier regions of the genome in invasive populations (mean  $r^2=0.26 \pm 0.03$ ) but decreases to levels characteristic of the native range for SNPs in non-outlier regions (mean  $r^2=0.16 \pm 0.03$ ; Figure 6; Table S5).



**FIGURE 5** Characterization of the chr1.10 Lostruct outlier region. (a) PCA of invasive-range samples performed using 37,355 LD-filtered genome-wide SNPs. (b) PCA of invasive-range samples performed using 5150 SNPs within the chr1.10 outlier region. Numbers indicate the cluster that each individual was assigned to, using the kmeans method. Colour-coding is used to represent the heterozygosity rate of each individual, at the chr1.10 region. Arrows point to the individuals represented in panel c. A PCA of the same outlier region that includes native-range data is shown in Figure S6. (c) Heterozygosity (%) calculated in 5 Mb non-overlapping windows along the length of chromosome 1, for three samples highlighted in panel b. Grey shading indicates the position of the chr1.10 outlier region.

In addition to the contrasting patterns of LD highlighted above, outlier and non-outlier genomic regions make different contributions to population differentiation in the native and invasive ranges, as estimated by  $F_{ST}$ . In the native range, outlier genomic regions are characterized by stronger differentiation (average  $F_{ST}=0.121$ ) than non-outlier genomic regions (average  $F_{ST}=0.094$ ;  $p < 2 \times 10^{-16}$ ;



**FIGURE 6** Lostruct outlier regions in the *A. sagrei* genome. Dots above chromosomes indicate the location of outlier windows (50 SNPs each) identified by Lostruct. These outliers display a pattern of population structure and genetic diversity consistent with reduced recombination (Figures 5 and S6). Also indicated is the location of candidate loci (identified via GWAS) for body size and shape (i.e. morphology; Bock et al., 2021), water loss (Baeckens et al., 2023), and thermal tolerance (this study). The inset shows the decay of LD with increased distance among SNPs from autosomes 1–6. Figure S4 shows where outlier and non-outlier SNPs were selected from, along these chromosomes. For both categories of SNPs, LD is averaged across populations in the invasive range (black) and the native range (grey). Table S5 includes detailed information on LD values observed, including the range and standard deviation.

Figure S8a). In the invasive range, by contrast, average range-wide  $F_{ST}$  was 2.8–3.5 times smaller, with outlier genomic regions only moderately more differentiated (average  $F_{ST}=0.033$ ), than non-outlier genomic regions (0.032;  $p=.02$ ; Figure S8b).

## 4 | DISCUSSION

Phenotypic and genomic investigations of invasive species conducted over the past two decades have identified multiple instances of rapid evolution, some of which occurred over tens of generations or less (e.g. Bock et al., 2018; Colautti & Barrett, 2013; Phillips et al., 2006). Examples such as these have reshaped our understanding of the

speed of evolution (Hodgins et al., 2018; Westley, 2011), while also revealing how invasive species can become dominant members of communities to which they are ecological and evolutionary newcomers (Bock et al., 2015; Handley et al., 2011; Lee, 2002). Given frequent evidence of rapid evolution during invasions and considering the success of the *A. sagrei* invasion in the southeastern US, we expected to find that invasive populations of this species have sustained evolutionary shifts in cold temperature tolerance in a manner that is consistent with local adaptation to climate. Instead, our results do not provide any indication that invasive populations are physiologically adapted to the new and more challenging thermal habitats that they occupy in the southeastern US. Our population genomic analyses complemented these results by revealing that

many regions of the genome bear a signature that is expected of recombinationally inert segments. Therefore, our results are consistent with the possibility that reduced recombination is forestalling adaptation that is dependent on novel combinations of existing genetic variation. Below, we contextualize these findings considering current information on the ecology and evolution of the brown anole invasion, as well as the genetic mechanisms that can interfere with adaptation.

#### 4.1 | Lack of a climatic cline in cold tolerance and alternative strategies to mitigate cold stress

The lack of a relationship between cold tolerance and the thermal habitat occupied by invasive populations is surprising because tropical ectotherms have narrow thermal tolerance ranges, such that even small changes in temperature can incur substantial fitness costs (Janzen, 1967; Kingsolver et al., 2013; Tewksbury et al., 2008). One possibility is that, because the colonization of the invasive range generally progressed in a northward direction (Kolbe et al., 2004), populations at the northern range edge are too recent and have not yet experienced intense selection episodes imposed by cold temperatures. We consider this possibility unlikely, considering that even the most northern populations that we surveyed here have been established for over 20 years, based on the earliest reports of *A. sagrei* at those sites (e.g. TIF: 23 years, Campbell & Hammontree, 1995; LOW: 30 years, Echternacht et al., 1995; CMB: 26 years; Campbell, 1996). During this time, these populations have repeatedly been exposed to winter temperatures below their critical thermal limit (Figure 3). Given limited evidence for physiological adaptation to new thermal extremes and considering the success of this species throughout its climatically novel invasive range, alternative strategies for coping with cold must be at play.

Thermal acclimation via phenotypic plasticity, in which thermal sensitivities are labile and can shift depending on individuals' recent or anticipated environmental experience (Somero, 2010), is one alternative strategy that can help buffer physiological impacts of temperature change (Chown et al., 2009). While tropical ectotherms such as *A. sagrei* are generally thought to have limited acclimation abilities (Gunderson et al., 2017; Gunderson & Stillman, 2015; Huey et al., 2009), a prior study in invasive *A. sagrei* from two populations in Florida does report acclimation in response to high-temperature as well as low-temperature treatments (Kolbe et al., 2012). Whether this is more generally true for populations across the invasive range is currently unknown. Evidence from another non-native anole in Florida, *A. cristatellus*, indicates that one introduced population has acquired low-temperature acclimation ability relative to its native-range source population, whereas another introduced population has not (Kolbe et al., 2014). Thus, it is possible that genetic polymorphisms for thermal plasticity are maintained within *Anolis* species (Campbell-Staton et al., 2020). If this is the case for invasive *A. sagrei* as well, thermal acclimation ability could be a target of selection. Indeed, previous studies indicate that invasive populations can

evolve 'jack-of-all-trades' phenotypic plasticity (Richards et al., 2006), which allows them to cope with a wider range of environmental conditions (e.g. Hufbauer et al., 2012). Note, however, that it remains unclear whether acclimation ability is advantageous in cases when populations are exposed to sudden and drastic dips to low temperatures. More likely, such plasticity will facilitate survival of individuals in cases when temperatures drop gradually. Regardless, a promising direction for future study could be to determine if *A. sagrei* populations established at different latitudes vary in their capacity for acclimation to cold. Because our study included thermal tolerance measurements for individuals maintained in uniform, benign conditions, we could not investigate this possibility.

Another strategy that ectotherms can use to mitigate unfavourable thermal conditions is behavioural adjustment (Huey et al., 2012; Kearney et al., 2009), such as shuttling between sun and shade, modifying body posture, and regulating activity times (Cowles & Bogert, 1944; Huey & Slatkin, 1976). For invasive brown anoles, however, none of these strategies would be effective at night, when individuals are inactive and perched on exposed vegetation (Muñoz et al., 2014; Stroud et al., 2020). Indeed, at high latitudes and during the night, invasive *A. sagrei* would experience temperatures below their critical thermal limits for most days of the winter (Figure 3). Alternatively, invasive brown anoles may be able to escape the challenges of cold stress if they preferentially inhabit urban areas at higher latitudes, and if they make use of thermally advantageous structures at these sites (Battles & Kolbe, 2019; Kolbe et al., 2016). Consistent with this possibility, the two most northern populations that we sampled occurred on hotel grounds, which were the only locations where we could find *A. sagrei* at those latitudes. Even north of the continuous invasive range of the species, in Alabama, previous studies have tracked an isolated population consisting of hundreds of individuals that survived for 10 years in a greenhouse and its immediate surroundings (Hulbert et al., 2020; Warner et al., 2021). Individuals in this population did not differ in terms of thermal tolerance from invasive individuals obtained at lower latitudes in Georgia and Florida (Hulbert et al., 2020) but appeared to behaviorally adjust activity times to limit temperature stress at the cost of reduced foraging time (Hulbert et al., 2020). Only after the roof of this greenhouse was removed due to wind damage after a storm did the *A. sagrei* population go extinct, likely because of exposure to below-freezing temperatures the following winter (Warner et al., 2021). Whether nearby urban areas, which are typically warmer and more thermally heterogeneous (Battles & Kolbe, 2019; Rizwan et al., 2008), are operating as thermal safe havens for brown anoles at high latitudes requires further investigation.

A limitation of our study is that we relied on field-collected animals to obtain thermal tolerance data. As such, we cannot confidently exclude the occurrence of maternal effects or effects of the environment experienced prior to capture which last longer than our acclimation phase. Such confounding variables could have impacted thermal tolerance measurements, as well as downstream analyses that relied on these trait data, such as climatic cline analyses and genotype-trait associations. A previous study in *A. sagrei*

with a longer acclimation time indicated, however, that the largest shifts in thermal tolerance occur during the first two weeks upon exposure to a new ambient temperature (Kolbe et al., 2014). Thus, these results suggest that our acclimation phase, which averaged 34 days (range 26–44 days; Table S2), should have removed large  $CT_{\min}$  differences related to field conditions. Moreover, long-term environmental effects on thermal tolerance should have resulted in a positive relationship between cold tolerance and environmental conditions, which we did not detect. Lastly, we note that our climatic cline analyses and genotype-trait associations accounted for the potential of transect-specific effects, which could occur if thermal tolerances vary depending on the time of year when lizards are captured and measured.

## 4.2 | Genetic mechanisms of evolutionary stasis of cold tolerance

Because the impact of low environmental temperatures can likely only be partially buffered by phenotypic plasticity and thermoregulatory behaviour in invasive *A. sagrei*, and because these mitigation strategies likely impose costs (e.g. Hulbert et al., 2020; Warner et al., 2021), the absence of physiological divergence in cold tolerance in invasive *A. sagrei* is puzzling. One possibility is that adaptation in thermal tolerance has been limited by genetic constraints. Our genomic analyses allowed us to evaluate three such potential constraints. The first constraint that we considered is that genetic variation for cold tolerance is limited, preventing an adaptive response in invasive populations. We consider this possibility unlikely for two reasons. First, we observed substantial differences in average  $CT_{\min}$  among populations across the invasive range (Figure 3). If most of these differences are genetically based rather than a result of plasticity (see discussion above regarding the acclimation of field-collected animals prior to  $CT_{\min}$  measurements), there should be ample variation for natural selection to act on. Second, the standard GWAS analysis, even though it was based on relatively sparse coverage of the genome, did identify two loci that were associated with  $CT_{\min}$  (Figure 4). Allele frequencies at these loci did not, however, co-vary with latitude in the direction predicted by local adaptation (Figure S4), indicating it is unlikely that they have been under selection during the colonization of the invasive range. We emphasize, however, that these associations should be viewed as candidate  $CT_{\min}$  loci and require further validation. For example, alleles at both loci were not associated with  $CT_{\min}$  when considering a more stringent Bonferroni-adjusted significance threshold (Figure 4a). As well, the minor genotype class for both loci was characterized by low sample sizes (locus 1:  $N=5$ ; locus 2:  $N=21$ ; Figure 4b,c), a condition that can lead to false-positive associations (Shen & Carlborg, 2013).

The second constraint that we considered is that negative genetic interactions introduced by hybridization are interfering with adaptation. Our results do not support this scenario, given that we did not identify ancestry as a significant predictor of  $CT_{\min}$  (Table 1).

Also, the ancestry specific GWAS, which we used to pinpoint loci that may be affected by epistasis in a hybrid genomic background, identified only one small-effect  $CT_{\min}$  locus (Figure 4d). This contrasts with results from other phenotypic traits in invasive brown anoles (e.g. Baeckens et al., 2023; Bock et al., 2021; Kolbe et al., 2007; Pita-Aquino et al., 2022) which have reported a stronger effect of ancestry and identified several loci with ancestry-specific effects (e.g. Baeckens et al., 2023; Bock et al., 2021). In this context, it is possible that loci involved in the control of  $CT_{\min}$  are less exposed to epistatic genetic interactions. Note, however, that an additional confounding factor is the smaller sample size available for our  $CT_{\min}$  analyses compared to these other studies, which could have limited our ability to detect genetic interactions.

The third constraint we considered, that reduced recombination is delaying adaptation of invasive populations, is consistent with results from our population genomic scan. Specifically, we found that extensive stretches of the genome in invasive populations display patterns of population structure and heterozygosity that are consistent with large haploblocks within which recombination has not had the opportunity to reshuffle genetic diversity between haplotypes (Figures 6 and S5 and S6). These regions cover 57% of the genome and 55% of the genes. As well, they overlap with both  $CT_{\min}$  loci that we identified using the standard GWAS approach (i.e.  $CT_{\min}$  locus 1 on chromosome 2;  $CT_{\min}$  locus 2 on chromosome 3; Figures 4a and 6), and with several candidate loci identified in previous studies of morphology (Bock et al., 2021) and physiology (Baeckens et al., 2023; Figure 6). Thus, it is likely that these regions contain genes that are part of numerous genetic and physiological pathways and could be involved in forestalling adaptation in other traits in invasive brown anoles (e.g. Baeckens et al., 2023; Bock et al., 2021; Kolbe et al., 2007).

In the native range, the same regions of the genome exhibit limited recombination, but to a much smaller degree (Figure 6). Thus, the pattern we report here is likely a result of genome biology, which we expect to be manifested across the native and invasive ranges, and hybridization of divergent lineages, which we expect to be manifested mainly in the invasive range where hybridization is common (Bock et al., 2021; Kolbe et al., 2004, 2008). For example, invasive populations may contain different arrangements of chromosomal inversions because of repeated introductions from the native range and subsequent hybridization. If these inversions are only rarely polymorphic within native range populations, they would prevent recombination to a much smaller degree than in the invasive range. Indeed, our population genomic scan did not identify any heterozygotes at these genomic regions in the native range (i.e. no native-range individuals were assigned to PCA clusters 4 or 5; Figure S6), consistent with limited within-population polymorphism. A corollary of this argument is that recombination cold spots identified here make a much smaller contribution to adaptive divergence in the invasive range, where linkage extends over long genomic regions, than in the native range, where linkage decreases over much shorter distances (Figure 6). Consistent with this expectation, we find that  $F_{ST}$  values calculated from recombination cold spots are less extreme in the invasive range

than they are in the native range (Figure S8). At the single-locus level as well, three of the recombination cold spots that we identified (i.e. regions chr7.1, chr7.2, and chr7.3; Figure S6; Table S4) overlap with a limb-length locus that retains the signature of repeated adaptive divergence, but only in the native range (Bock et al., 2021).

We note, however, that strong reduction in recombination need not involve inversions and may also be a result of factors such as high sequence divergence, or the assembly of heterochromatin (e.g. Hunter et al., 1996; Sun et al., 2017; Termolino et al., 2016). Indeed, the genomic regions we detected using the Lostruct scan did not form sharp peaks in MDS scores along chromosomes (Figure S6), as expected of inversions (e.g. Battlay et al., 2023; Harringmeyer & Hoekstra, 2022; Huang et al., 2020; Todesco et al., 2020). As well, outlier regions were overlapping across segments of each chromosome, with different start and end coordinates, consistent with infrequent recombination events within these regions (Figure S5). Thus, it is possible that Lostruct outlier regions represent collinear stretches of the genome, that have reduced recombination. Further supporting this possibility, a study of the congener *A. carolinensis* revealed valleys of recombination that were similarly distributed along the middle of chromosome arms (Bourgeois et al., 2019), indicating that the recombination landscape may be conserved across anoles. In this context, an important direction of future study should be to obtain genetic maps for *A. sagrei*, which can provide direct confirmation of our population genomic inferences of reduced recombination. As well, future studies could rely on long-read sequencing to establish whether recombinationally inert chromosomal segments that we report here consist of inversions that have different orientations among *A. sagrei* lineages.

In addition to mechanisms discussed above, LD can be elevated in invasive populations due to demographic changes that occur during the invasion, such as population bottlenecks (e.g. Flanagan et al., 2021). In the case of invasive *A. sagrei*, two lines of evidence suggest that demographic changes are unlikely to be the leading driver of elevated levels of LD. Firstly, population bottlenecks should lead to elevated LD across the genome, rather than in distinct chromosomal segments as observed here. Secondly, we found that large blocks of high LD segregate within some of the earliest invasive populations that have been reported for *A. sagrei* in Florida (e.g. MON; Figure S7). This is contrary to expectations if demography were a leading driver of LD, in which case large haploblocks should be present mostly in populations that have been established very recently, for which recombination has not had enough time to break down LD.

Recombination cold spots such as inversions are known to be important for adaptation and speciation, particularly when divergence is occurring with gene flow. Indeed, many previous studies have mapped multiple adaptive traits within inversions (e.g. Battlay et al., 2023; Harringmeyer & Hoekstra, 2022; Huang et al., 2020; Todesco et al., 2020), highlighting the important role of such genomic regions for maintaining the cohesion of genomic cassettes involved in adaptation. Recent studies have also pointed to scenarios in which reduced recombination could be detrimental. This includes, for example, cases when several loci need to be decoupled as part of

adaptation to new environments (Roesti et al., 2022). In the context of the brown anole invasion, alleles that confer cold tolerance could be linked to alleles that control different, maladaptive traits, leading to trait correlations that slow down adaptation. Alternatively, recombination cold spots may contain higher loads of deleterious mutations that decrease the adaptive value of alleles contained in these regions of the genome (e.g. Berdan et al., 2021; Jay et al., 2021; but see Huang et al., 2022; Harringmeyer & Hoekstra, 2022). In the context of the brown anole invasion, these deleterious mutations could involve epistatic interactions, as have been reported for several traits in this system (e.g. Baeckens et al., 2023; Bock et al., 2021). Importantly, a genetic architecture such as the one described here, characterized by strong linkage among alleles, can impact local adaptation even when there is ample variation in a quantitative trait, as we observed for cold tolerance across the invasive range of *A. sagrei* (Figure 3). We note, however, that definitively demonstrating these effects will be challenging and will require identifying the adaptive as well as detrimental alleles that co-localize within regions of reduced recombination such as inversions, and unlocking them, potentially using gene editing approaches (Roesti et al., 2022; Schmidt et al., 2020).

Beyond the contribution of recombination cold spots to adaptation, results from our study illustrate the utility of biological invasions for advancing our understanding of why rapid adaptation may sometimes fail. With the accelerating implementation of genomics in the study of invasive species, this contribution is likely to become increasingly important in coming years, expanding the value of invasive species as fortuitous experiments in nature.

#### AUTHOR CONTRIBUTIONS

All authors designed the study; Dan G. Bock and Simon Baeckens collected and analysed the data and drafted an initial version of the manuscript. All authors contributed to revisions of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

All sequence data are publicly available on the NCBI Sequence Read Archive, as BioProject PRJNA737437. All trait data is included in the Supplementary Material, as Table S2 (provided as a separate data file). Scripts used in the analyses as well as additional data files used in the analyses are available from FigShare (DOI: [10.6084/m9.figshare.23576973](https://doi.org/10.6084/m9.figshare.23576973)).

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