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HYBRIDIZATION BETWEEN A NATIVE LIZARD (ANOLIS CAROLINENSIS) AND A CRYPTIC INVADER (A. PORCATUS) ACROSS AN URBAN LANDSCAPE

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BY

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ABSTRACT

Invasive species impact native biota through competition, predation, and habitat alteration but can also transform native populations through hybridization. Outcomes of such hybridization events are variable and may result in the formation of a stable hybrid tension zone, extinction of one parental species via genetic swamping, hybrid speciation, or adaptive introgression. Hybrid population dynamics are further complicated by anthropogenic habitat disturbance, which has been shown to influence patterns of admixture and introgression between native and invasive species. Hybridization between the native lizard *Anolis carolinensis* and a morphologically similar invader (*A. porcatus*) in South Miami, Florida provides an ideal opportunity to study outcomes of admixture across a heterogeneous landscape composed of both urban and forested habitat patches. We used single nucleotide polymorphism (SNP) data to describe patterns of introgression occurring in the *A. carolinensis* x *A. porcatus* hybrid system, as well as to test for a relationship between urbanization and non-native ancestry. Our findings indicate that hybridization between *A. carolinensis* and *A. porcatus* was likely a limited, historic event, and that contemporary immigration of *A. porcatus* is negligible. We identified two distinct genetic clusters within the hybrid population and related these to differences in recent patterns of hybrid backcrossing with *A. carolinensis*. Investigation of genomic clines revealed rapid introgression and disproportionate representation of *A. porcatus* alleles at many loci, as well as a total lack of evidence for reproductive isolation between parental species. We also found evidence for a positive relationship between

urbanization and *A. porcatus* ancestry, though the mechanism driving this association remains unclear. Ultimately, our findings demonstrate the persistence of non-native genetic material even in the absence of ongoing immigration, indicating that hybridization management strategies should focus on preserving native alleles (rather than simply removing invasive individuals) in populations where admixture has already occurred. However, we also note that not all outcomes of interspecific admixture should be considered intrinsically negative. Hybridization of native species with ecologically robust invaders can lead to adaptive introgression, which in turn may facilitate the long-term survival of populations or species otherwise unable to adapt to global, anthropogenically-mediated change.

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PREFACE

This thesis is submitted in manuscript format. Chapter 1 is in preparation for submission to the journal of Molecular Ecology. References and section headings have been formatted in accordance with guidelines set forth by the target journal.

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CHAPTER 1

HYBRIDIZATION BETWEEN A NATIVE LIZARD (*ANOLIS CAROLINENSIS*) AND A CRYPTIC INVADER (*A. PORCATUS*) ACROSS AN URBAN LANDSCAPE

This chapter is in preparation for submission to the journal of Molecular Ecology.

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INTRODUCTION

Invasive species are well-known and frequently maligned for their negative ecological impacts on native organisms, which often involve predation, competition, and habitat alteration (Case and Bolger 1991; Gibbons et al. 2000; Gordon 1998; Kraus 2015). However, in some species introductions, invaders also interact with native species through hybridization (e.g., Ellstrand and Schierenbeck 2000; Fitzpatrick and Shaffer 2007; Huxel 1998; Vuillaume et al. 2015). Hybridization between native and invasive species has been observed across a wide range of plant and animal taxa, though the majority of research concerning this topic has centered around birds, mammals, fishes, and plants (Largiader 2007). Such events threaten the genetic integrity of native populations, and in some cases may even result in the extinction of rare native species (Rhymer and Simberloff 1996; Riley et al. 2003). The dangers of genetic swamping and reduction (or loss) of native genotypes are exacerbated by invasion scenarios in which the invader is morphologically indistinguishable from the native species with which it is hybridizing (Morais and Reichard 2018; Riley et al. 2003; Wegener et al. 2019; Wielstra et al. 2016). These cryptic invaders cannot be visually identified, and as a result, can spread, persist, and impact native species, all while remaining entirely undetected.

Possible outcomes of interspecific admixture are diverse, and will vary on a situational basis. When hybridizing species are parapatric, a stable tension zone (a stationary band of hybrid individuals resulting from parental dispersal and held in place by negative selective pressure) may form and persist (Barton and

Hewitt 1989; Gay et al. 2008; Pinto et al. 2019). Though the majority of hybridization research has been conducted in or designed to model tension zone systems (Gompert et al. 2017), prolonged admixture may also lead to adaptive introgression (Hedrick 2013; Whitney et al. 2010), the formation of a new, hybrid species (Mallet 2007; Salazar et al. 2010; Schumer et al. 2014), or the extinction of one parental species (Todesco et al. 2016; Wolf et al. 2001). These less commonly studied outcomes may be more likely in invasion scenarios, since invaders usually spread from an introduction point within the range of a native species rather than into the native population from a range boundary. The outcome ultimately realized is determined by ecological and genomic factors not yet fully understood. Gompert et al. (2017) list hybrid zone size, parental range size, asymmetry in range overlap, environmental characteristics, population size, and hybrid fitness as elements that likely contribute to evolutionary outcomes of hybridization. Immigration rate is also an influential factor (Vinogradova and Galkina 2020), and propagule pressure (a variable summarizing immigration in an invasion context) has been shown to be a strong predictor of introgression between introduced and native species (Bennett et al. 2010; Fitzpatrick and Shaffer 2007). When assessing observed outcomes (extinction vs. speciation vs. stability), species boundaries are often blurry and difficult to delineate beyond the broad categories of early versus late-stage speciation (Gompert et al. 2014, 2017; Mallet 2007). Hybridization occurring during the earliest stages of speciation is characterized by high levels of gene flow, large numbers of hybrid individuals, and a lack of fixed loci indicative of reproductive isolation, while

hybridization occurring during the latter stages will display opposite patterns (Gompert et al. 2017; Payseur 2010).

Issues of species invasion and hybridization can be further complicated when occurring across landscapes disrupted by anthropogenic activity (Grabenstein and Taylor 2018; Riley et al. 2003). A study by Riley et al. (2003) found that hybridization between a native tiger salamander and a cryptic invader occurred at much higher levels in artificial water bodies than in natural breeding pools, and that these differences were better explained by non-random patterns of mating and survival than by the relative proportions of hybrid individuals in each pool. These observations suggest that invaders may be more successful (and thus hybridize more frequently) in breeding habitats which have been disturbed from their natural state. For organisms which live and breed in habitats occupied by humans, urbanization can be a major source of disturbance. Urban habitats are characterized by environmental alterations, including reduced canopy cover and an increase in impervious surface area; together, these features facilitate rapid surface heating and lead to diurnal temperatures up to 10°C warmer than those observed in corresponding natural habitats (Kim 1992). This “urban heat island” effect requires organisms living in cities to adapt to warmer conditions, and is especially critical for ectothermic species, which rely on external conditions and thermoregulatory behaviors to maintain an operational body temperature (Ackley et al. 2015; Battles and Kolbe 2019; Hall and Warner 2018). Furthermore, urban heat island conditions have also been linked to an increase in pollutant concentration (Sarrat et al. 2006), as well as changes in

patterns of wind, humidity, and precipitation (Taha 1997). Since anthropogenic habitat disturbance has been shown to play a role in reducing reproductive barriers and promoting hybridization between both naturally co-occurring species (Grabenstein and Taylor 2018) and native-invasive species pairs (Beninde et al. 2018; Riley et al. 2003; Walters et al. 2008), it is essential that studies of hybrid populations in urban areas consider the possible implications of habitat heterogeneity.

A recent study of *Anolis* lizard populations in south Florida determined that invasive Cuban green anoles (*Anolis porcatus*) have hybridized with native green anoles (*Anolis carolinensis*) in the city of South Miami, resulting in a population of hybrid origin with a genetic makeup distinct from that of either parent species (Wegener et al. 2019). While the study confirmed that *A. carolinensis* collected from a natural wildlife management area 135 km north of South Miami displayed no evidence of hybridization, the current geographic range of the hybrid population remains unknown since the two species involved are not morphologically distinct (Camposano 2011; Wegener et al. 2019). A few previous instances of hybridization among anole species have been recorded (Gabot-Rodriguez et al. 2020; Gorman and Atkins 1968; Jenssen 1977; Jezkova et al. 2013; Kohler et al. 2010; MacGuigan et al. 2016), though such events are generally quite rare within the nearly 400-species *Anolis* clade (Losos 2009). Approximately 33% of the nuclear DNA in the hybrid green anole population (based on analysis of 18 microsatellite loci) is estimated to have resulted from the introduction of *A. porcatus*, and 35% of the hybrid anoles carry mitochondrial

DNA sequences indicative of maternal *A. porcatus* ancestry. Despite the fact that the two parental species have been geographically isolated since before the Pliocene (separation is thought to have occurred at least 6 mya; Campbell-Staton et al. 2012), rapid interspecific admixture following reintroduction of the two lineages was not entirely unexpected due to limited morphological divergence between *A. carolinensis* and *A. porcatus* (Camposano 2011; Tollis and Boissinot 2014; Wegener et al. 2019).

Though neither species invasions nor hybrid zones are uncommon, this particular hybridization event is notable for multiple reasons. First, the genetically distinct nature of the hybrid population is indicative of historic admixture followed by population differentiation, implying that, though hybrid anoles are continuing to reproduce with one another (and potentially with pure *A. carolinensis* at range boundaries and/or regions of sympatry), ongoing hybridization with *A. porcatus* is unlikely (Lavretsky et al. 2015; Wegener et al. 2019). Together, the hypothesized low (likely zero) rate of ongoing *A. porcatus* immigration and the likely restricted location of the original invaders within the broad native range of *A. carolinensis* constitute a unique scenario in which the impact of unusually low propagule pressure on hybridization outcomes can be assessed. Second, the occurrence of *A. porcatus* x *A. carolinensis* hybridization in a region characterized by a patchwork of urban and remnant natural forest habitats presents a valuable opportunity to study interactions between anthropogenic habitat disturbance and invasive-native hybridization, while also shedding light on an otherwise cryptic invasion. Very few natural systems have been described in which hybridization,

invasion, urbanization, and species of cryptic nature coincide to permit simultaneous study of all four factors.

In accounting for the role that anthropogenic habitat disturbance may play in facilitating hybridization in the South Miami *Anolis* system, it is necessary to consider both the niche typically filled by the hybridizing species, as well as how the habitat associated with this niche differs between urban and natural forest settings. Both *A. carolinensis* and *A. porcatus* are arboreal species typically found on tree trunks or in the forest canopy (i.e., trunk-crown habitat specialists; Losos 2009), but will also utilize artificial perches in urban areas. From a lizard's perspective, urban habitats will be characterized by smoother substrates, greater amounts of impervious surface area, sparser canopy cover, and higher temperatures relative to corresponding natural habitats (Battles and Kolbe 2019; Winchell et al. 2016). Multiple studies have also found that urban anoles tend to have larger body sizes than those living in natural forest habitats; while the cause of this phenomenon is uncertain, differences in food availability and predation pressure across habitat types have been postulated as potential explanations (Hall and Warner 2017; Thawley et al. 2019). Among these differences in habitat characteristics, the relative increase in average temperature in anthropogenically disturbed areas suggests a mechanism by which urban habitats could promote increased hybridization. Studies of anoles and other ectotherms have found that thermal tolerance limits are correlated with the natural thermal conditions existing across a species' geographic range (Grigg and Buckley 2013; Gunderson et al. 2018; Hertz et al. 1979; Sunday et al. 2010). If tropical *A. porcatus* is better

adapted to the warmer temperatures that dominate urban settings than *A. carolinensis*, the original invaders would have thrived in the city, ultimately reproducing through hybridization with the more abundant native green anole. If a competitive advantage over native genotypes is passed on to hybrid offspring in the form of an enhanced thermal tolerance, the resulting hybrids should outcompete pure *A. carolinensis*—but only in the warmer urban habitats where non-native genotypes are adaptive. An ability to withstand higher temperatures may become increasingly advantageous as current climate change and urbanization trends contribute to an increase in environmental temperatures (Battles and Kolbe 2019; Frishkoff et al. 2019; Huey et al. 2009), and could favor the persistence of hybrid genotypes in a region with little to no ongoing *A. porcatus* immigration.

In this study, we generated and used SNP data from strategically sampled hybrid individuals—as well as from the two parental species (*A. carolinensis* and *A. porcatus*)—to thoroughly describe the genetic nature and extent of hybridization occurring across the green anole genome in South Miami, FL. In doing so, we shed light on an otherwise cryptic invasion and filled existing knowledge gaps regarding hybridization outcomes in a unique hybrid zone characterized by an uncommonly low parental immigration rate. Furthermore, we tested for a relationship between hybrid prevalence and anthropogenic habitat disturbance by comparing green anole genotypes to corresponding measurements of canopy cover and impervious surface area (quantitative indicators of urbanization), hypothesizing that non-native alleles are associated

primarily with urban habitats due to a breakdown of reproductive barriers facilitated by the thermal similarity of urban heat island conditions to those favored by *A. porcatus* in its tropical native range in Cuba. Ultimately, our research provides valuable insight into the topics of genetic swamping resulting from cryptic invasion, the relationship between urbanization and invasion success, and human impacts on reproductive barriers, and contributes meaningfully to a deeper scientific understanding of the genetic and ecological factors influencing hybridization outcomes.

MATERIALS AND METHODS

Study species

Anolis carolinensis is an arboreal (trunk-crown) lizard found throughout much of the southeastern USA, and is the only *Anolis* species native to the United States (Campbell-Staton et al. 2012; Losos 2009). It is a popular model organism for which an abundance of genetic and natural history data are available (Losos and Schneider 2009) but belongs to a taxonomic group currently underrepresented in studies of hybridization (Largiader 2007). *Anolis porcatus* is invasive in Florida and is morphologically indistinct from *A. carolinensis* (Camposano 2011; Kolbe et al. 2007; Wegener et al. 2019). Though *A. porcatus* is native to Cuba and the two species have had allopatric distributions since before the Pliocene, they are known to be capable of hybridizing. Hybrid green anoles have been identified in the South Miami area, which is thought to be the original location of the *A. porcatus* invasion (Wegener et al. 2019).

Sample Selection and Processing

Samples of muscle and liver tissue were obtained from the Kolbe Lab tissue library at the University of Rhode Island and were strategically selected to construct a sample set with balanced sex ratios, diverse temporal sampling, and hybrid representatives from a variety of locations near South Miami, FL. In addition to the 63 hybrid samples selected in this manner, 15 *A. porcatus* from Western Cuba, 2 green anoles of unknown ancestry from Parkland, FL (included to assess whether hybrid genotypes had spread to this previously unsampled location), and 2 *A. smaragdinus* from Abaco Island, The Bahamas (included as an outgroup) were also selected from the same collection. Tissue samples from 14 *A. carolinensis* from Hobe Sound, FL—a location 185 kilometers north of the known, hybrid population in South Miami—were obtained from the cryogenic collection of the Harvard Museum of Comparative Zoology. Genomic DNA was extracted from all 96 samples using a commercially available Bioline DNA extraction kit, and extract concentration and purity were assessed via nanodrop. The DNA samples were then sent to Admera Health in New Jersey, USA for preparation of a double digest restriction-site associated DNA (ddRAD) library using the restriction enzymes *SphI* and *EcoRI*, size selection with AMPure XP beads (ligated DNA was recovered at the 80% bead volume), and 150-bp paired-end sequencing on the Illumina HiSeq 4000 platform. To increase hybrid sample size, preexisting sequence data from an additional fourteen individuals (including five replicate individuals sequenced in both libraries) were also included in the

final sequence dataset. Prior to sequencing, these samples were digested with the same restriction enzymes as all other samples in our study, but underwent a slightly different size selection procedure retaining fragments between 550 and 710 base pairs in length (Bock et al. 2021).

Data Trimming and Filtering

A quality control check for all raw sequence data was conducted using FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Read trimming was then performed using Trimmomatic v0.39 (Bolger et al. 2014) to exclude Illumina-specific sequences from the dataset, remove bases below a quality score of 20 from the start and end of each read, cut any windows (width = 3 base pairs) within which the average quality score fell below 15, and remove individual reads with a total length of less than 36 base pairs. Trimmed reads were mapped against the *Anolis carolinensis* reference genome (NCBI RefSeq database (O'Leary et al. 2016), accession GCF_000090745.1) using dDocent v2.7.8 (Puritz et al. 2014) with a mismatch penalty of 4 and a gap opening penalty of 6. SNPs were then called from this assembly and filtered in dDocent under both 70% and 95% call rate specifications to create versions of the dataset suitable for both genome-wide and individual-based analyses. For both call rates, only biallelic markers were retained, complex variants (non-SNPs) were excluded, markers with quality scores less than or equal to 20 were removed, and genotypes with fewer than 4 reads were marked as missing data. Additional filters to exclude markers with minor allele frequencies less than or equal to 3%

(present in 3 or fewer samples) and to remove individual samples with more than 30% missing data were applied prior to downstream analyses as appropriate. To reduce potential effects of linkage disequilibrium (LD), loci were also pruned using the snp_autoSVD function of the R package bigsnpr (Prive 2018) prior to use in genetic clustering analyses. This function identifies and iteratively removes regions of long-range LD by using PCA and Mahalanobis distance to detect outlier SNPs.

To check for anomalies indicative of sample contamination, vcflib (Garrison et al. 2021) was used to calculate allele balance ratios for all heterozygote calls supported by 15 or more reads within the fully filtered, 70% call rate dataset. Any samples for which more than 20% of calls displayed ratios less than 0.2 or greater than 0.8 were removed from all versions of the dataset (O'Leary et al. 2018). An identity by state (IBS) analysis was performed using the package SNPRelate (Zheng et al. 2012) in R version 3.5.1 (R Core Team 2018) to check for library effects, comparing pairwise IBS values of replicate samples sequenced in both libraries to pairwise values for all other sample combinations. Per-sample heterozygosity rates were also calculated with vcflib and compared among replicate sample pairs, after which the version of each pair with the lower number of reads was removed from all versions of the dataset.

Individual-based Analyses

To investigate patterns of genetic clustering and admixture at the individual level, a subset of 10,000 SNPs was selected randomly from the fully-

filtered, LD-pruned, 95% call rate dataset. Principal components and their corresponding eigenvalues were calculated using the R package adegenet v.2.1.1 (Jombart and Ahmed 2011), and visualized using the package ggplot2 (Wickham 2016). Discriminant analysis of principal components (DAPC) was also performed using the adegenet package, which employs the *k*-means algorithm to identify the number of theoretical genetic clusters for which BIC is minimized and between-group variation is maximized. Cluster membership probabilities were determined for each individual based on an optimized number of principal components, and used to assign each individual to one of the clusters identified by the *k*-means algorithm.

Individual admixture proportions were estimated using the admixture ancestry model of STRUCTURE v2.3.4 (Pritchard et al. 2000) with a maximum of 8 possible genetic groups (K = 8). Twenty independent runs were performed for each possible value of K = 1-8. Each STRUCTURE simulation was run for a total of 150,000 Markov Chain Monte Carlo (MCMC) steps preceded by a burn-in phase of equal length. The most likely value of K was inferred via the Evanno method (Evanno et al. 2005), and the associated individual admixture proportions were plotted using the R package pophelper v2.3.1 (Francis 2017).

Ancestry informative markers (AIMs) displaying fixed differences between *A. carolinensis* and *A. porcatus* were identified and isolated using custom script in conjunction with BEDTools v2.27.1 (Quinlan and Hall 2010). AIM genotypes for each individual were then converted to allele counts and used to calculate hybrid index values with the R package introgress v1.2.3 (Gompert and Buerkle

2010). These values were visualized in a triangle plot displaying individual hybrid class membership as a function of the relationship between hybrid index and interspecific heterozygosity, as well as an ancestry plot displaying the genotype of each individual at each ancestry informative marker.

Population-based Analyses

To describe the extent of genetic differentiation between the hybrid anoles and parental species, weighted fixation index (F_{ST}) values were calculated in windows (width = 50,000 base pairs) across the genome for all pairwise combinations of the hybrid, *A. carolinensis*, and *A. porcatus* sample groups. To accomplish this, missing data in the partially filtered version of the 70% call rate dataset (not filtered for percent missing data per individual) were imputed with BEAGLE v5.2 (Browning et al. 2018), and F_{ST} calculations were performed using the weir-fst-pop function of vcftools (Danecek et al. 2011).

Patterns of introgression at individual loci were examined via Bayesian estimation of genomic clines using bgc v1.03 (Gompert and Buerkle 2012). For this analysis, only fixed AIMs were tested, and any AIMs missing allele depth values (an effect of the decomposition of multiple nucleotide polymorphisms during the AIM identification process) were excluded from the dataset. The genomic cline model was run for 100,000 MCMC steps (the first 50,000 of which were discarded as burn-in), and included both a genotype-uncertainty component with a sequence error probability of 0.001 and an ICARrho component accounting for linked loci with a maximum free recombination distance of 0.5.

Loci categorized as alpha and/or beta outliers were identified and plotted using the R package ClineHelpR v0.0.0.9000 (Martin and Chafin 2021) and mapped to an ideogram of the *A. carolinensis* reference genome with custom script.

Environmental Association Analysis

To investigate the relationship between hybrid genotypes and anthropogenic habitat disturbance, canopy cover and impervious surface area were selected as quantitative proxies of urbanization—specifically, the urban heat island environment. These variables are commonly employed metrics of urbanization (Borden et al. 2021) and are expected to be negatively correlated (i.e., canopy cover will be low and impervious surface area will be high in distinctly urban habitats). Using GPS coordinates for the site of capture associated with each hybrid individual sampled, values for both variables were extracted from the 2016 US Forest Service Tree Canopy Cover and 2016 Percent Developed Imperviousness GIS layers produced by the National Land Cover Database (Yang et al. 2018). These layers provide habitat data at a spatial resolution of 30 x 30 m, which should be interpreted as representing the average condition across the home range of an individual anole, rather than the specific microhabitat in which that individual was observed. Arboreal anoles (*A. carolinensis* included) have been found to utilize larger quantities of two-dimensional space than their more terrestrial counterparts (Schoener and Schoener 1982), with a recent study of *A. carolinensis* habitat use in an urban setting (Weber et al. 2021) reporting individual home ranges as varying from a

minimum of 16 m^2 to a maximum of 1,538 m^2 in size (average = 260 m^2 for males and 410 m^2 for females).

Associations between the selected environmental variables and hybrid SNPs were assessed using two separate latent factor mixed models (LFMMs) with K values determined by results from STRUCTURE, DAPC, and the broken stick stopping rule. To maximize the number of loci tested, the 70% call rate dataset was used as input for these analyses. LFMM was selected over other environmental association modeling methods because it accounts for neutral and population genetic structure as a random factor and has been shown to be a suitable modelling choice for scattered, individual-based sampling designs as in this study (Rellstab et al. 2015). Both models used a least-squares estimation method with ridge penalties and were run in R with the package LFMM v2 (Caye et al. 2019). Following initial model estimation, the genomic inflation factor (GIF) for each model was manually adjusted to achieve an appropriate p-value distribution. Correction for multiple comparisons was implemented by converting p-values to q-values with the package qvalue v2.12.0 (Storey et al. 2015) and assessing significance at a false discovery rate (FDR) threshold of 0.05. The NCBI *Anolis carolinensis* Annotation Release 102 feature table (O'Leary et al. 2016) was then used to determine the location of significant loci relative to known genomic features. To further investigate the relationship between urbanization and hybrid genotypes, Wilcoxon rank-sum, Mantel, and partial Mantel tests were also used to compare the distributions of habitat characteristics associated with

groups of hybrid individuals displaying differing proportions of *A. porcatus* ancestry (these groups were assigned based on results of the DAPC analysis).

RESULTS

Following trimming, mapping, and filtering of the raw sequence data, 147,594 SNPs from 103 individuals (70 hybrid anoles, 16 *A. carolinensis*, 15 *A. porcatus*, and 2 *A. smaragdinus*; Tables S1, S2) were retained in the 95% call rate dataset, while 222,567 SNPs from 100 individuals were retained in the 70% call rate dataset (these were the same individuals retained under the 95% call rate filtering scheme, minus the two outgroup *A. smaragdinus* and hybrid sample MIA_670). Assessment of per-sample allele balance ratios revealed abnormal values indicative of sample contamination for hybrid samples AC_35 and JJK_1950, which were excluded from all versions of the dataset. IBS analysis confirmed that differences between the five technical replicates were minimal (Figure S1), allowing data from the nine hybrid samples sequenced in a separate library to be included in all analyses.

Principal component analysis produced well-defined clusters for both parental species, while hybrid individuals were distributed along a diagonal line (Figure 1). This line spanned the intermediate space between the *A. carolinensis* and *A. porcatus* clusters along the first principal component (PC1) but was extreme relative to both parental clusters along PC2. Collectively, PC1 and PC2 described 25.6% of the observed genetic variation, with no other PC axis describing more than 2.2% of the variation individually. The two samples of

unknown ancestry from Parkland, FL fell within the *A. carolinensis* cluster rather than along the hybrid continuum and so were classified as pure *A. carolinensis* for all downstream analyses.

DAPC indicated a K value of 4 (Figure S2), correctly identifying *A. carolinensis* and *A. porcatus* as distinct genetic groups and subdividing the 70 hybrid individuals into two separate clusters of n = 31 (hereafter referred to as hybrid group 1) and n = 39 (hybrid group 2) (Figures 2A, 2B). While discriminant function 1 placed both hybrid groups close to *A. carolinensis* and far from *A. porcatus*, discriminant function 2 situated the hybrid clusters closest to *A. porcatus*, with group 2 located further from the *A. carolinensis* cluster than group 1 (Figures 2C, 2D). Geographically, individuals assigned to hybrid group 1 were associated primarily with the northernmost, southernmost, and westernmost sampling sites, whereas group 2 individuals were associated almost exclusively with the central and easternmost sites (i.e., primarily in and around South Miami; Figure 3). When DAPC was repeated with a dataset containing only the hybrid individuals, K = 2 was the most likely number of groups; individual group assignments remained consistent with previous results.

Unlike DAPC, STRUCTURE supported a K value of 3 (Figure S3), assigning the *A. carolinensis* and *A. porcatus* samples to their own distinct groups while representing the hybrid samples as an admixed population composed of a mixture of genetic material from *A. carolinensis, A. porcatus,* and a third genetic group (Figure 4). Among the hybrid samples, ancestry for 3 individuals was inferred to be 100% consistent with this third genetic group, while

no individual shared more than 67% inferred ancestry with *A. carolinensis* or more than 2% inferred ancestry with *A. porcatus*. When forced to divide all individuals among K = 2 populations, STRUCTURE combined *A. carolinensis* and *A. porcatus* into a single group, while still displaying hybrids as admixed individuals with ancestry from this combined parental group and a second, distinct group. An identical version of the analysis run with only the hybrid samples supported a K value of 2, assigning admixture proportions generally consistent with the hybrid groups identified by DAPC.

Among the 222,567 SNPs contained in the 70% call rate dataset, 10,269 were identified as fixed AIMs. These markers were used to produce a triangle plot (Figure 5) for which the bottom two vertices represent the ratio of interspecific heterozygosity (IH) to hybrid index (HI) associated with each parental species, the top vertex represents the IH:HI ratio expected for an F1 hybrid, and the midpoints of the upper sides represent IH:HI ratios expected for backcrosses between F1 hybrids and parental individuals. The location of the majority of the hybrid individuals within the center of the triangle suggests that the hybrid population is the result of many generations of backcrossing (i.e., composed of F2+ generation hybrids) while the spread of the points toward the right side of the triangle reveals that group 1 hybrid individuals are the result of more recent backcrosses with *A. carolinensis* than individuals assigned to group 2. A marker ancestry plot produced with the fixed AIM data (Figure 6) also illustrates the high level of introgression occurring across the hybrid genome, although a few fixed loci (vertical bands) and genomic regions of low admixture

(horizontal fragments) can be visually identified as regions of consistent color occurring within the ancestry plot.

For hybrid group 1, weighted F_{ST} values averaged for windows across the genome (width = 50,000 base pairs) were lower when compared to *A. carolinensis* (mean = 0.207) than to *A. porcatus* (mean = 0.349), indicating that group 1 hybrids are less genetically differentiated from native *A. carolinensis* (Figure 7A). Alternately, hybrid group 2 displayed slightly lower weighted F_{ST} values when compared to *A. porcatus* (mean = 0.325, vs. 0.385 for comparison with *A. carolinensis*) across all but a portion of the second chromosome, where F_{ST} values converged (Figure 7B). The same genomic region also displayed a pattern shift for group 2, with pairwise F_{ST} with respect to *A. porcatus* increasing near the center of chromosome 2, while simultaneously decreasing with respect to *A. carolinensis*. For all genomic windows within both groups, hybrid comparisons with parental species always yielded F_{ST} values lower than those corresponding to genetic differentiation between the parental species themselves (mean = 0.601).

A total of 8,551 fixed AIMs were examined via Bayesian estimation of genomic clines. Of these loci, 53.5% were identified as alpha outliers, beta outliers, or both (Table 1; Figures 8A, 8B). The alpha parameter relates observed ancestry proportions to hybrid index scores, with positive alpha values indicating higher-than-expected contributions of genetic material from *A. carolinensis* and negative alpha values indicating excess *A. porcatus* ancestry. The beta parameter describes the rate of introgression at each locus, with positive beta

values signifying slow rates of introgression, while negative beta values signify relatively faster rates of introgression (Gompert and Buerkle 2011, 2012). All loci identified as beta outliers displayed negative beta scores, suggesting that no ancestry-informative allele from either parental species is being selected against. Of the loci identified as alpha outliers, 91.8% were negative, revealing that alleles indicative of *A. porcatus* ancestry are more than nine times more likely to be overrepresented in the hybrid genome than those unique to *A. carolinensis*. Finally, among loci identified as both alpha and beta outliers, 98.7% displayed negative values for both alpha and beta—a combination representing rapid introgression of *A. porcatus* alleles. When mapped to the *A. carolinensis* genome, loci identified as alpha outliers were distributed across all six macrochromosomes (Figure 9A), while those identified as beta outliers were concentrated nearly exclusively on chromosome 3 (Figures 9B, 9C).

A wide range of both canopy cover (0-80%) and impervious surface area (0-70%) conditions were represented among the 68 hybrid individuals for which location data were available. As expected, values for these variables were negatively correlated ($r = -0.759$, $p < 0.0001$; Figure S4). The LFMM testing the association between canopy cover and hybrid genotype (manually adjusted GIF = 0.96; Figure S5A) identified a single, significant locus at position 19,549,307 on chromosome 1 ($q = 0.0176$). A Manhattan plot of the model (Figure 10A) also revealed a series of closely-positioned, near-significant loci on an unassigned scaffold (NW_003338792.1), the clustering of which suggest that a more liberal FDR threshold would also have identified the locus at position 1,663,486 as

significantly correlated with canopy cover. The LFMM testing the association between impervious surface area and hybrid genotype (manually adjusted GIF = 1.00; Figure S5B) identified two loci in close proximity on chromosome 2 (positions 135,319,503 and 135,321,966, Figure 10B) as significant (q = 0.0037 for both loci). Of the three significant loci identified by the two models, all are located in currently unannotated regions of the *A. carolinensis* reference genome. All of these loci also display the same consistently homozygous genotype across both parental species, while an alternate allele occurs at low frequencies (7.5 – 8.0%) among hybrids. The near-significant locus identified by the canopy cover LFMM falls within the protein coding sequence for subunit epsilon of the eukaryotic initiation factor (eIF) 2B. Subunit epsilon contains the catalytic domain of eIF2B, which regulates mRNA translation by increasing the rate of guanine nucleotide exchange on eIF2. This in turn accelerates the rate at which the 40S ribosomal subunit is supplied with the initiator methionyl tRNA required for translation initiation (Stelzer et al. 2016; Wang et al. 2001). All *A. carolinensis* individuals in our study are missing data for this locus; both alleles occur among *A. porcatus* (at frequencies of 71.4 and 28.6%), while all hybrid individuals with sequence data at this locus are homozygous for the more common allele.

We found that both canopy cover ($mean_1$ = 37.4%, $mean_2$ = 17.8%, p = 0.0021) and impervious surface area ($mean_1$ = 14.9%, $mean_2$ = 24.8%, p = 0.0132) differed significantly between hybrid groups, with group 1 individuals occurring in more natural habitats (higher canopy cover and lower impervious

surface area) than group 2 individuals. However, we also documented significant spatial autocorrelation among observations for both canopy cover (Mantel r = 0.1378, p = 0.0050) and impervious surface area (Mantel r = 0.1384, p = 0.0190), suggesting spatial non-independence for these variables. With the confounding effect of geographic location held constant, we confirmed a significant correlation of hybrid group membership with canopy cover (Mantel r = 0.1044, p = 0.0050), but not with impervious surface area (Mantel r = 0.0159, p = 0.1748).

DISCUSSION

In this study, we used high-resolution SNP data to describe the nature and extent of hybridization that has occurred between native *A. carolinensis* and non-native *A. porcatus* in South Miami, FL, and investigated the influence of urbanization on hybridization between these species. Our results support a description of the hybrid population as genetically unique relative to both parental species (Wegener et al. 2019) and also reveal the presence of two distinct genetic subsets within the hybrid population. We report rapid introgression of *A. porcatus* alleles at a large number of individual loci, demonstrating that hybrids have retained non-native alleles even in the absence of ongoing immigration of this non-native species. We also provide evidence for a positive association of urban habitats with increased *A. porcatus* ancestry, though our hypothesis regarding the role of thermal conditions in facilitating hybridization is neither supported nor disproved. Ultimately, our findings reveal an unusual case of adaptive introgression resulting from a limited (possibly isolated) introduction

event, demonstrating the combined ability of anthropogenic disturbance and selective pressure to influence hybridization outcomes.

Hybrid population structure

We documented a genetically diverse hybrid population, with individuals spanning nearly the full distance between *A. carolinensis* and *A. porcatus* along the first principal component axis. However, all individuals were extreme relative to both parental species along the second PC axis. This genetic divergence agrees with the microsatellite-based results of Wegener et al. (2019) and supports the hypothesis of an historic hybridization event followed by discontinuation of *A. porcatus* immigration. Differentiation of the hybrid population along the second axis likely corresponds to mutations and/or changes in allele frequencies that accumulated independently within the hybrid population during the period following the original hybridization event. Such allele frequency change may occur randomly as an outcome of genetic drift, or can result—directly or indirectly—from selective pressure (Barton 2000; Buffalo and Coop 2020).

The diagonal angling of the PCA hybrid line toward the *A. carolinensis* cluster can also be explained by the historic admixture hypothesis. If immigration of *A. porcatus* was restricted and is no longer occurring, contemporary hybrid anoles have only the options of breeding with hybrids or backcrossing with native *A. carolinensis*. This is because the limited pool of introduced *A. porcatus* available for mating would quickly have disappeared. Backcrossing of hybrid

individuals with the native parent should reduce the degree of hybrid differentiation displayed by offspring (resulting in lower PC2 scores for individuals with greater proportions of recent *A. carolinensis* ancestry), while perpetual crossing of hybrid individuals should maintain or increase the divergence of offspring from their historic parents (resulting in higher PC2 scores for individuals with little to no recent *A. carolinensis* ancestry). A competing (though seemingly less likely) explanation for the observed pattern is that the hybrid line angles directly toward a theoretical cluster representing the original parental *A. porcatus* propagule, which was in some way genetically distinct from the *A. porcatus* sampled in our study. While our samples were collected from the western *A. porcatus* clade in Cuba, which displays mitochondrial haplotypes most similar to those observed in the South Miami hybrid population (Kolbe et al. 2007; Wegener et al. 2019), the possibilities of substructure within the western clade or founder effects resulting from a small immigrant propagule remain.

Substructure of the hybrid population resulted in the assignment of hybrid individuals to two groups, with discriminant function 2 placing hybrid group 1 in closer proximity to *A. carolinensis* than group 2. These group assignments were further supported by genetic clustering analyses performed with STRUCTURE, which estimated higher *A. carolinensis* ancestry proportions for hybrids belonging to group 1. Though the hybrid range boundary is currently unknown, the spatial distribution of group 1 individuals, which occurred primarily along the outermost sites sampled in our study, suggests that these individuals are closer to the range boundary and thus more likely to encounter and backcross with pure *A.*

carolinensis than group 2 individuals located closer to the (putative) center of the hybrid swarm. Indeed, patterns of hybrid ancestry and heterozygosity at loci displaying fixed differences between parental species confirm that the difference between hybrid groups is related to patterns of backcrossing with *A. carolinensis.* These results also display a notable lack of evidence for any recent backcrossing of hybrid individuals with *A. porcatus*, providing additional support for the hypothesis that hybridization was a limited, historic event, and that contemporary *A. porcatus* immigration is low or nonexistent.

It is necessary to note that although both plant and animal hybrids resulting from crosses among three parental species have been reported (Bi and Bogart 2006; Floate et al. 2016; McDonald et al. 2008), we consider contribution of genetic material from a third species to be an unlikely explanation for the patterns observed in our study system. Since our analyses clearly identified substructure within the hybrid population in addition to differentiating between *A. carolinensis* and *A. porcatus*, any higher-tier genetic structure resulting from a third parental species would also have been revealed had it been present. Furthermore, hybridization among *Anolis* species is rare to begin with (Jezkova et al. 2013; Losos 2009), and—beyond *A. porcatus*—no anole species known to occur in South Miami is thought to be capable of producing fertile hybrids with *A. carolinensis* (Morris et al. 2021). The divergent features of the hybrid population are thus best interpreted as resulting from genetic differentiation of *A. carolinensis* x *A. porcatus* hybrids following historic admixture, rather than evidence for tri-specific hybridization with an unknown third parent.

Introgressive hybridization

Patterns of introgression within the hybrid population were revealed both through the calculation of pairwise windowed F_{ST} values, as well as Bayesian estimation of genomic clines. We demonstrated that group 2 individuals are characterized by near-equal contributions of genetic material from both parental species, while group 1 individuals (as expected due to patterns of backcrossing) are more similar to *A. carolinensis* across the genome—especially on chromosome 2. However, group 1 displayed similar patterns of divergence from *A. carolinensis* and *A. porcatus* along most of chromosome 3, revealing that both hybrid groups have retained relatively equal proportions of alleles from each parental species at this location. These patterns were more thoroughly investigated via genomic cline analysis, which compared allele frequencies for individual loci to the genome-wide average (note that this analysis was not conducted separately for hybrid groups 1 and 2 because it requires a wide range of hybrid index values for accurate identification of outlying loci—since hybrid group was found to be directly linked to hybrid index, subdivision of groups would have reduced statistical power). Although hybrid ancestry averages for AIM loci showed a much higher genetic contribution from *A. carolinensis* overall, a large proportion (40.3%) of individual markers displayed higher than expected *A. porcatus* ancestry in comparison to the average across all AIM loci. Furthermore, a quarter of these loci (10% of all AIMs) displayed both excess *A. porcatus* ancestry and unusually rapid rates of introgression (i.e., negative alpha +

negative beta outliers), while only 0.1% of AIMs departed from null expectations regarding increased introgression of *A. carolinensis* alleles (i.e., positive alpha + negative beta outliers). Fixed AIM loci can show drastically larger proportions of significant alpha and beta outliers than markers that do not display fixed differences between parental species (McFarlane et al. 2021), so the percentage values reported here should not be extrapolated as representative of introgression rates across the entire hybrid genome. However, the notable inequality in counts of positive and negative outliers for both the alpha and beta parameters demonstrates a clear trend of rapid introgression, which has resulted in the accumulation and retention of *A. porcatus* alleles rather than those of the more abundant, native *A. carolinensis*. While genomic cline analyses conducted in several other hybrid systems have identified similar imbalances in positive and negative alpha outliers, few report equally large differences in beta outliers associated with a particular parental species (Haines et al. 2019; Oswald et al. 2019; Souissi et al. 2018; Sung et al. 2018). McFarlane et al. (2021) report AIM-based genomic cline patterns for hybrids of native red deer (*Cervus elaphus*) and introduced sika (*C. nippon*) in Scotland that do meet both of these criteria, though their findings are ultimately opposite to ours in that alleles from the native deer were shown to be rapidly introgressing into the population of the invader, rather than vice versa.

Caution must be applied when interpreting the evolutionary cause of observed genomic cline patterns, as significant outliers may indicate the work of directional selective pressure within a population, but can also result from genetic

drift (Fitzpatrick 2013; Gompert and Buerkle 2011). Indeed, considering the large number of significant loci identified by most genomic cline analyses, it is inevitable that at least some outliers will be linked to drift rather than selection (Gompert and Buerkle 2012; McFarlane et al. 2021). Because genetic drift is a random process and natural selection is not, comparison of clines measured in replicate populations can be used to distinguish between these causes when fitness values of individual alleles are unknown (Jeffery et al. 2017; Teeter et al. 2009). However, additional *A. carolinensis* x *A. porcatus* hybrid populations resulting from replicate introductions are not known to exist, and for this reason selection and drift remain confounded in our study system. Nonetheless, the lopsided distributions of positive versus negative alpha and beta outliers observed in the existing hybrid population—coupled with the strong association between negative beta outliers and loci displaying excess *A. porcatus* ancestry—comprise a non-random pattern. Whether the result of selective pressure or genetic drift, our results provide clear evidence that non-native *A. porcatus* alleles are disproportionately represented in the hybrid genome, and that many of these alleles display unusually rapid rates of introgression. These trends defy neutral expectations, especially when the large differences in population size and immigration rate between the two parental species are taken into account.

In addition to the patterns described above, the total lack of positive beta outliers in our dataset also warrants discussion. Positive beta values correspond to reduced rates of introgression, and loci involved in reproductive isolation between species will deviate from genome-wide averages in this way (Gompert

and Buerkle 2010; McFarlane et al. 2021; Taylor et al. 2014). Wegener et al. (2019) have suggested revision of the currently paraphyletic *A. porcatus* group such that the western clade of *A. porcatus* is subsumed into *A. carolinensis*, citing morphological similarity (Camposano 2011) and violation of the biological species concept (Mayr 1982) as justifications for this taxonomic alternation. Hybridization scenarios often blur species boundaries, and an increased understanding of the genetic details of admixture and introgression has led many to critique the biological species concept as insufficient for delineating species status (Gompert et al. 2014, 2017; Mallet 2007, 2020). To ameliorate the difficulties of assigning hybridizing organisms to the discreet "different species" and "same species" categories set forth by the biological species concept, hybrid systems should be viewed as representing various intermediate stages of the speciation process, with early stages characterized by abundant gene flow, many hybrid individuals, and little to no reproductive isolation, while later stages display opposite characteristics (Gompert et al. 2017; Payseur 2010). The total absence of positive beta outliers among the fixed AIM loci in our study demonstrates that, despite an estimated 6–12 million-year period of geographic isolation (Campbell-Staton et al. 2012; Wegener et al. 2019), none of the loci we sequenced and analyzed are contributing to reproductive isolation between *A. carolinensis* and *A. porcatus*. This lack of evidence for reproductive isolation indicates that speciation differentiating the two anoles is still in its earliest stages, and considerably strengthens the argument that *A. carolinensis* and western *A. porcatus* have not yet diverged enough to constitute unique species. Future

studies of anole diversification and speciation should assess locus-specific cline width for other known hybrid populations produced by putatively distinct parental species. If no evidence of reproductive isolation between parental species (i.e., positive beta outliers) is present, species status may need to be reassessed.

Relationship between urbanization and hybridization

Only three loci were found to be significantly associated with environmental variables indicative of urbanization (i.e., canopy cover or impervious surface area)—a surprisingly small proportion of the 222,567 loci tested. While most studies with similar methods identify hundreds to thousands of significant SNPs (Bekkevold et al. 2020; Dudaniec et al. 2018; Frichot et al. 2013; Guerrero et al. 2018), lower proportions are not entirely unprecedented. For example, Caye et al. (2019) tested their LFMM package on a set of 345,067 CpG sites collected from patients with the autoimmune inflammatory disease rheumatoid arthritis (RA) and found only nine of the sites to be significantly correlated with tobacco consumption. These nine included genes with clear ties to RA, demonstrating that analyses returning low proportions of significant loci can still yield meaningful and accurate results. Of the three loci we identified as significant, all were located in currently unannotated regions of the *A. carolinensis* genome, preventing characterization of their potential functions. A nearly significant locus noted from the model of canopy cover was part of the coding sequence for the catalytic subunit of an enzyme that plays a critical role in the translation process (Stelzer et al. 2016; Wang et al. 2001); though there is no

clear link between this enzyme and thermal conditions—aside from the possibility that a mutation may impact the temperature at which it denatures (Goward et al. 1994)—its regulation of translation could impact virtually any other coding sequence. No locus was significantly associated with both canopy cover and impervious surface area, and none of the significant loci displayed fixed differences between parental species (preventing comparison with AIMs identified as genomic cline outliers). Allele frequency patterns displayed by the three significant loci suggest that these markers correspond to mutations that arose in the hybrid population after the initial hybridization event, and thus that alleles unique to the hybrid anoles themselves may play a greater role in environmental adaptation than those associated with either parental species. However, each of the hybrid-specific minor alleles were observed at relatively low frequencies, so parental populations would need to be sampled in greater numbers to confirm that these alleles are indeed unique to the hybrid population. While these results do not disprove our hypothesis that increased temperatures in urban habitats have facilitated the spread of *A. porcatus* alleles, they also provide little support for such a conclusion. Next steps in addressing this hypothesis will require the collection of live anoles from both hybrid groups, for which thermal tolerance—in the form of critical thermal maximum (CT_{max}) as described by Leal and Gunderson (2012)—can be measured and compared both between discrete groups and across the continuous hybrid index spectrum. CT_{max} values should also be incorporated into a genome-wide association study to identify specific loci that may be involved in the genetic regulation of thermal

tolerance. A significant association between CT_{max} and *A. porcatus* allele frequency at any such locus would provide strong support for the thermal facilitation hypothesis.

The observed association of hybrid genotypes with environmental factors indicative of urbanization was further illuminated by our investigation of the simpler relationship between hybrid group assignment and habitat type. Group 2 individuals (those displaying relatively higher proportions of *A. porcatus* ancestry) were found in habitats with, on average, 20% less canopy cover and 10% more impervious surface area than habitats associated with group 1 individuals. These differences may result in relatively warmer microhabitats for group 2 hybrids. After accounting for spatial autocorrelation, this relationship held true for canopy cover, but not for impervious surface area. The association of group 2 hybrids with low canopy cover values supports the hypothesis that invasive genotypes are more abundant in anthropogenically disturbed habitats, though the lack of a significant association with impervious surface area indicates some uncertainty in the mechanism driving this pattern. Ultimately, we provide preliminary evidence for a relationship between urbanization and hybridization in the *A. carolinensis* x *A. porcatus* system, though both the ecological cause and underlying genomic basis of this pattern remain to be elucidated.

Conservation implications and conclusions

Native *A. carolinensis* is both abundant and widely distributed across the southeastern United States (Campbell-Staton et al. 2012; Losos 2009), so the

isolated hybridization scenario reported here is not a conservation concern in and of itself. However, our observations should be applied to inform conservation action in systems where hybridization does constitute a genuine concern. We demonstrate that genetic material from an invasive species introgresses rapidly and remains established in native populations even after (1) pure parental individuals of the invasive species are no longer present and (2) immigration of additional invasive individuals has ceased. Such introgression may result from positive selection favoring foreign alleles but can also occur randomly as an unpredictable outcome of genetic drift (McFarlane et al. 2021). These findings suggest that, in situations where a rare native species with a restricted range is threatened by hybridization with a non-native species, management should focus on identifying pure native individuals and preventing these individuals from breeding with hybrids—rather than simply trying to eliminate the invader.

Conversely, we also report an association of hybrid genotypes with environmental variables indicative of urbanization, demonstrating that hybridization can lead to adaptive introgression when occurring in an anthropogenically disturbed habitat. Though the genetic swamping that occurs in a hybrid swarm is generally considered undesirable (Fitzpatrick and Shaffer 2007; Huxel 1998; Vuillaume et al. 2015), adaptive introgression is increasingly being viewed as a positive hybridization outcome (Hamilton and Miller 2015; Pardo-Diaz et al. 2012). Adaptive alleles inherited from a resilient invader have the potential to facilitate the persistence of native species in the face of ever-accelerating global change (Oziolor et al. 2019) and thus may constitute a

valuable conservation tool. Rather than immediately dismissing all forms of hybridization as dangerous, we should carefully consider both the benefits and risks of admixture from an ecological perspective—particularly in terms of long-term persistence when facing multiple dimensions of global environmental change.

Our study ultimately provides a detailed description of the genetic structure of the *A. carolinensis* x *A. porcatus* hybrid system, revealing that rapid, directional introgression of foreign alleles into a native background can occur even when immigration of the invasive parent is negligible. While this finding is significant, much further study will be necessary to fully understand the dynamics of *A. carolinensis* x *A. porcatus* hybridization. Next steps should prioritize identifying hybrid range boundaries, which will require broad, systematic sampling of green anole genetic material ranging from the southeast coast of Florida into the Everglades and north along the heavily developed east coast of Florida. Such a sampling design will also accommodate the application of additional forms of environmental association analysis and will permit the collection of microhabitat and thermal data that can be used to more rigorously support (or oppose) our preliminary evidence for a relationship between non-native alleles and urbanization. While various studies have reported similar associations between anthropogenic disturbance and the spread of invasive genotypes via hybridization, the underlying causes of such associations are rarely discussed beyond general statements that invasive species are typically resilient, and thus better able to withstand habitat disturbance (Beninde et al.

2018; Riley et al. 2003; Walters et al. 2008; but see Fitzpatrick and Shaffer 2004). We suggest that the identification of specific mechanisms underlying these patterns should be a primary goal of future research both in this system and in the field of invasion genetics as a whole.

Table 1. Outlier status of 8,551 fixed, ancestry-informative loci, as assigned by Bayesian estimation of genomic clines. Alpha outliers display a greater-than-expected contribution of genetic material from one parental species (positive = excess *A. carolinensis* ancestry, negative = excess *A. porcatus* ancestry), while beta outliers display unusual rates of introgression (positive = reduced introgression, negative = rapid introgression). The first value within each cell represents the total number of loci assigned to the corresponding outlier status, while the second represents the subset of those loci currently mappable to a known chromosomal location.

alpha		**beta**		**both**		**neither**	**total**
+	-	+	-	$+\alpha$ $-\beta$	$-\alpha$ $-\beta$	3980 / 2445	**8551 / 6234**
298 / 3	2595 / 2501	0 / 0	811 / 431	11 / 0	856 / 854		

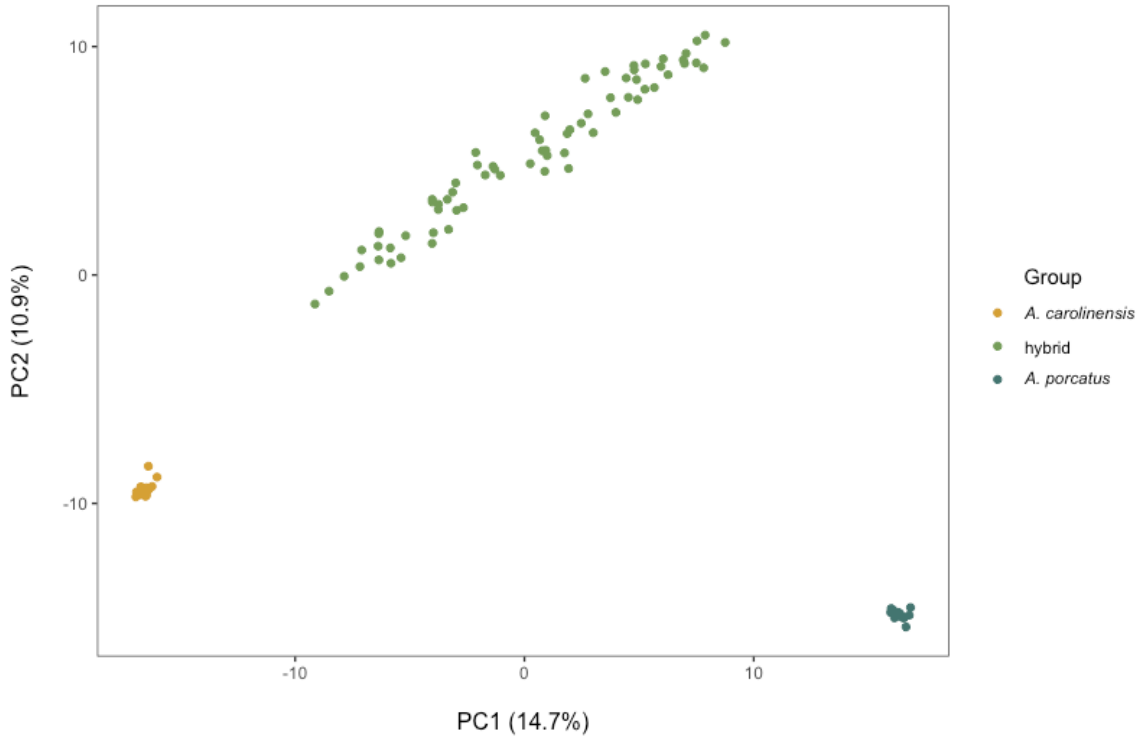

Figure 1. Principal component plot displaying genetic differentiation between *Anolis carolinensis* (n = 16), *A. porcatus* (n = 15), and their hybrids (n = 70). Hybrid individuals collected from South Miami are intermediate to the parental species along PC1, and extreme relative to both parental species along PC2.

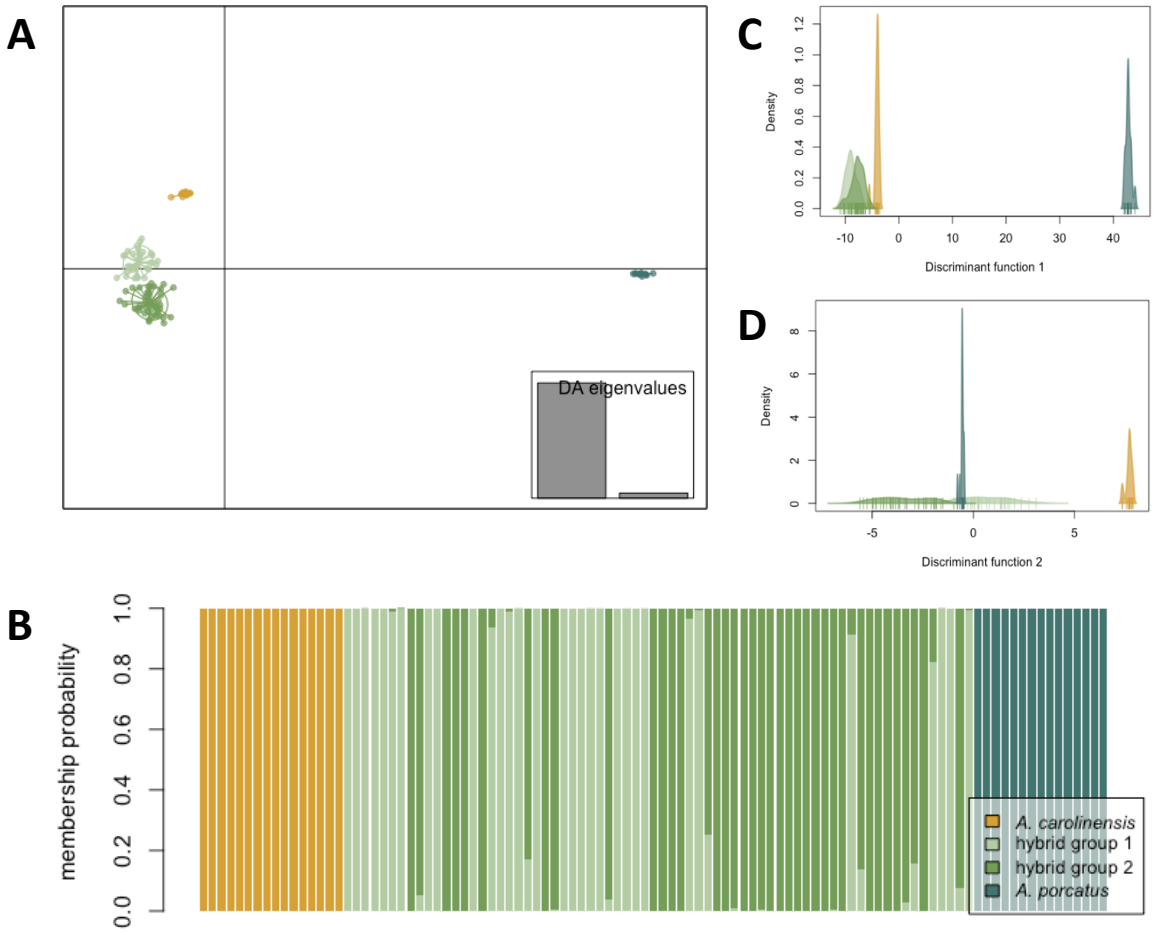

Figure 2. Results of discriminant analysis of principal components (DAPC) performed on hybrid anoles and parental species. DAPC identified K = 4 groups, subdividing the hybrids among two separate genetic clusters (A, B). The first discriminant function groups both hybrid clusters with *A. carolinensis* (C), while the second discriminant function differentiates among *A. carolinensis*, hybrid group 1, and hybrid group 2 (D).

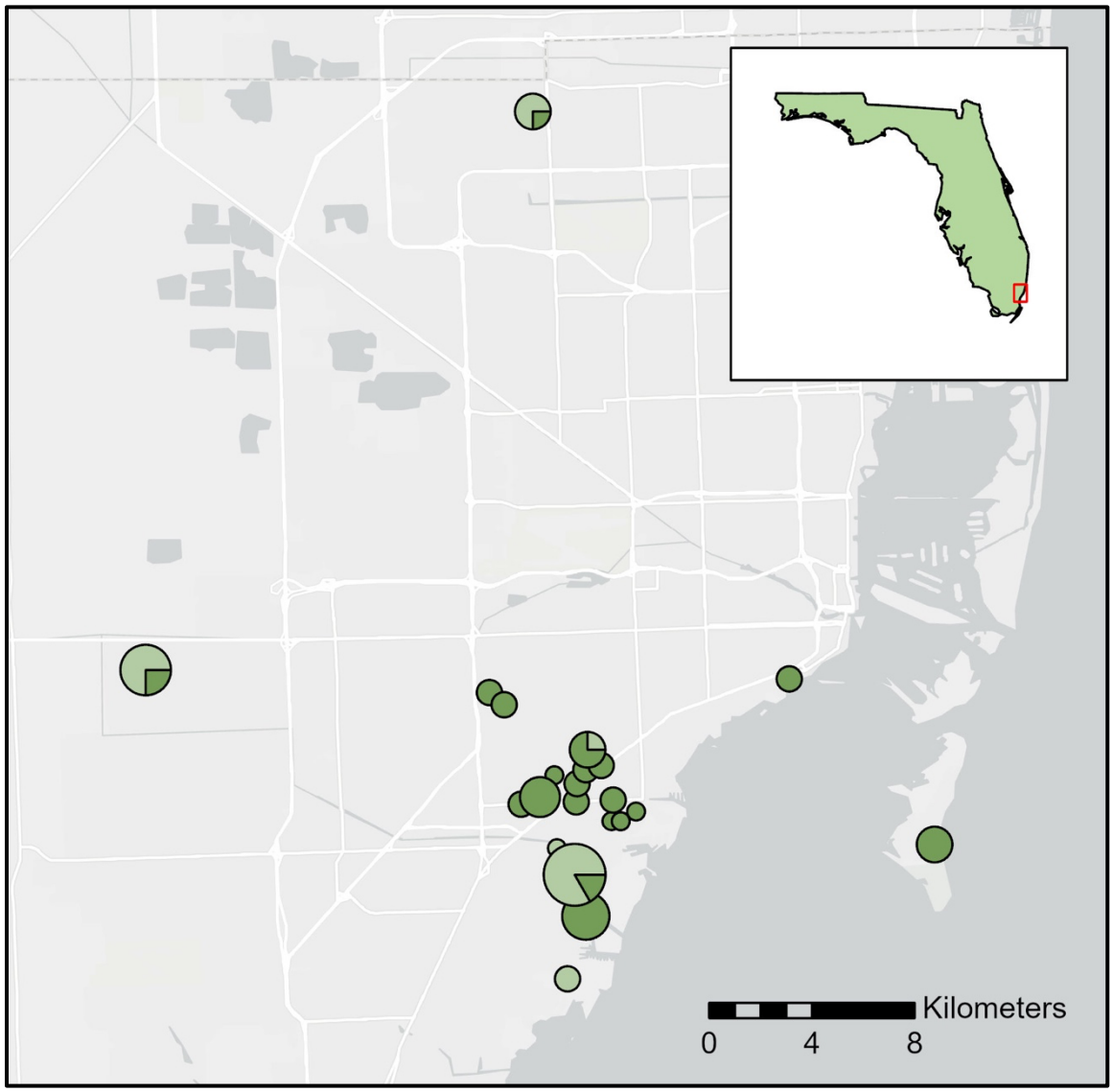

Figure 3. Geographic distribution of hybrid groups in South Miami, FL. Pie chart area corresponds to the number of individuals sampled at each site, ranging from n = 1 to n = 12. Group 1 individuals are depicted in light green, while group 2 individuals are depicted in dark green.

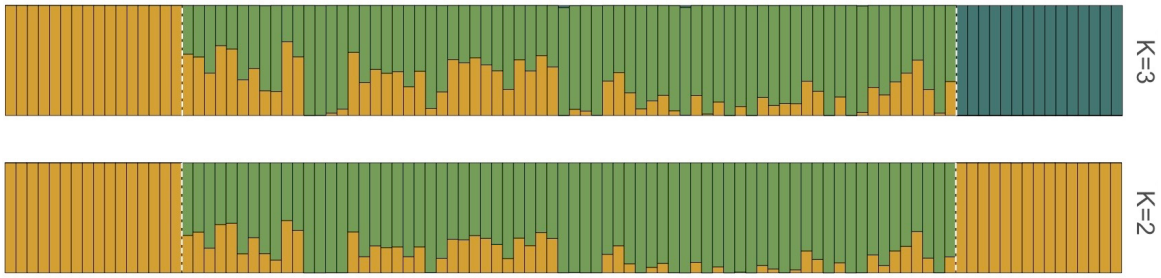

Figure 4. Individual ancestry proportions for hybrid anoles and parental species as inferred by STRUCTURE. Twenty replicate runs for K values 1-8 identified K = 3 as the optimal number of groups. For K = 3, gold bars signify *A. carolinensis* ancestry, while teal bars indicate *A. porcatus* ancestry. For K = 2, gold bars signify parental (combined *A. carolinensis* and *A. porcatus*) ancestry. For both K values, green bars indicate an additional group associated only with hybrid individuals.

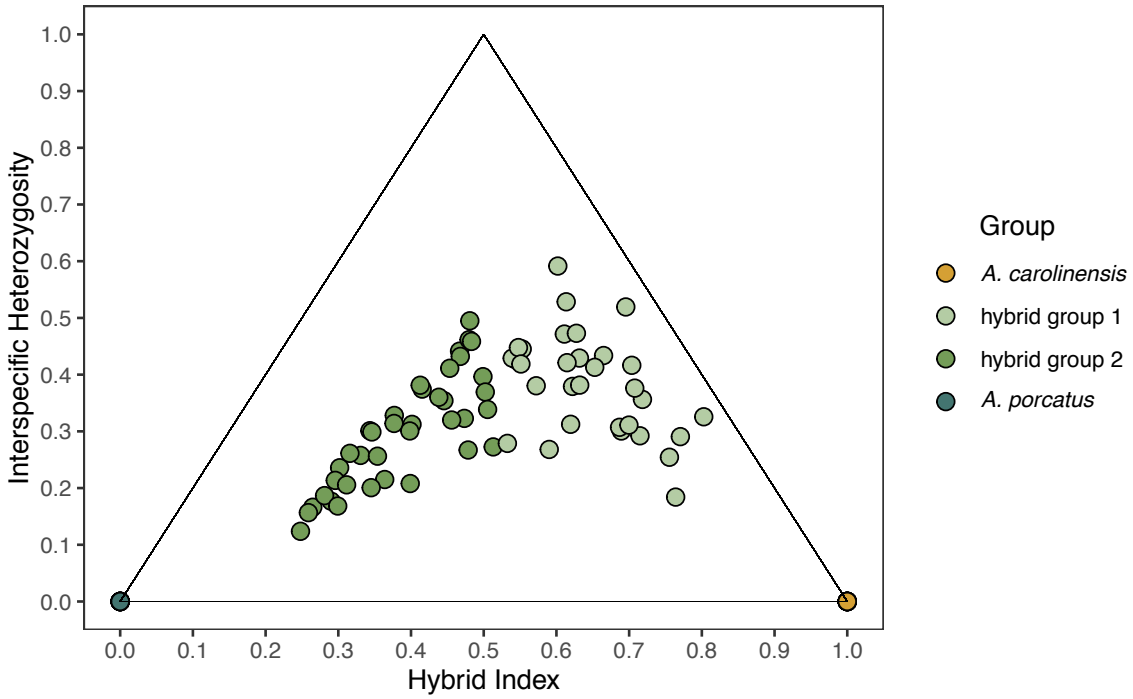

Figure 5. Triangle plot of hybrid class as determined by the relationship between hybrid index and interspecific heterozygosity at the individual level. These values were calculated using only loci displaying fixed differences between parental *Anolis* species (n = 10,269).

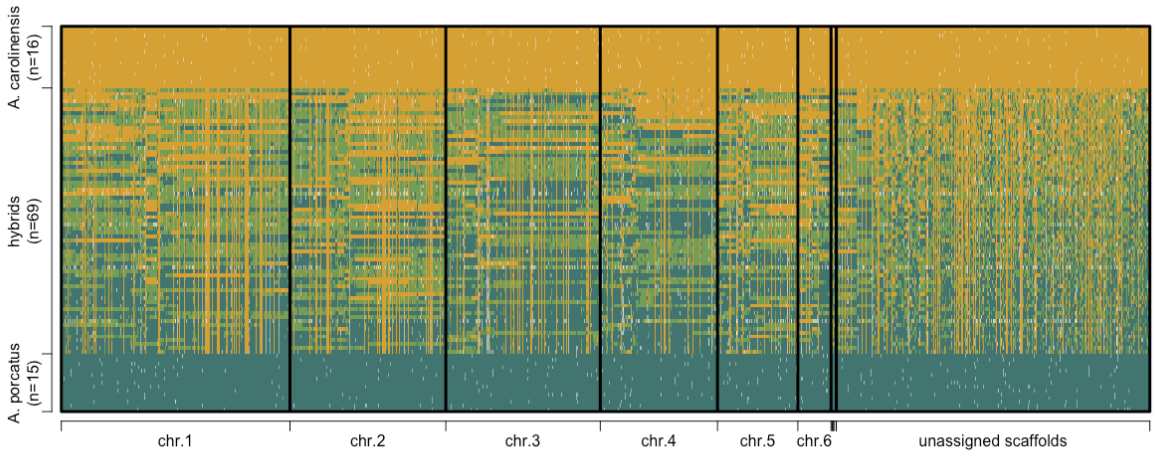

Figure 6. Ancestry plot of observed hybrid genotypes at loci displaying fixed differences between parental species. Columns correspond to loci (ordered by chromosome), while rows correspond to individual lizards. Gold indicates a homozygous *Anolis carolinensis* genotype, teal indicates a homozygous *A. porcatus* genotype, green indicates a heterozygous genotype, and white indicates missing data. Loci mapped to the “unassigned scaffolds” bin are currently unplaced within the *A. carolinensis* reference genome, and thus are not meaningfully ordered.

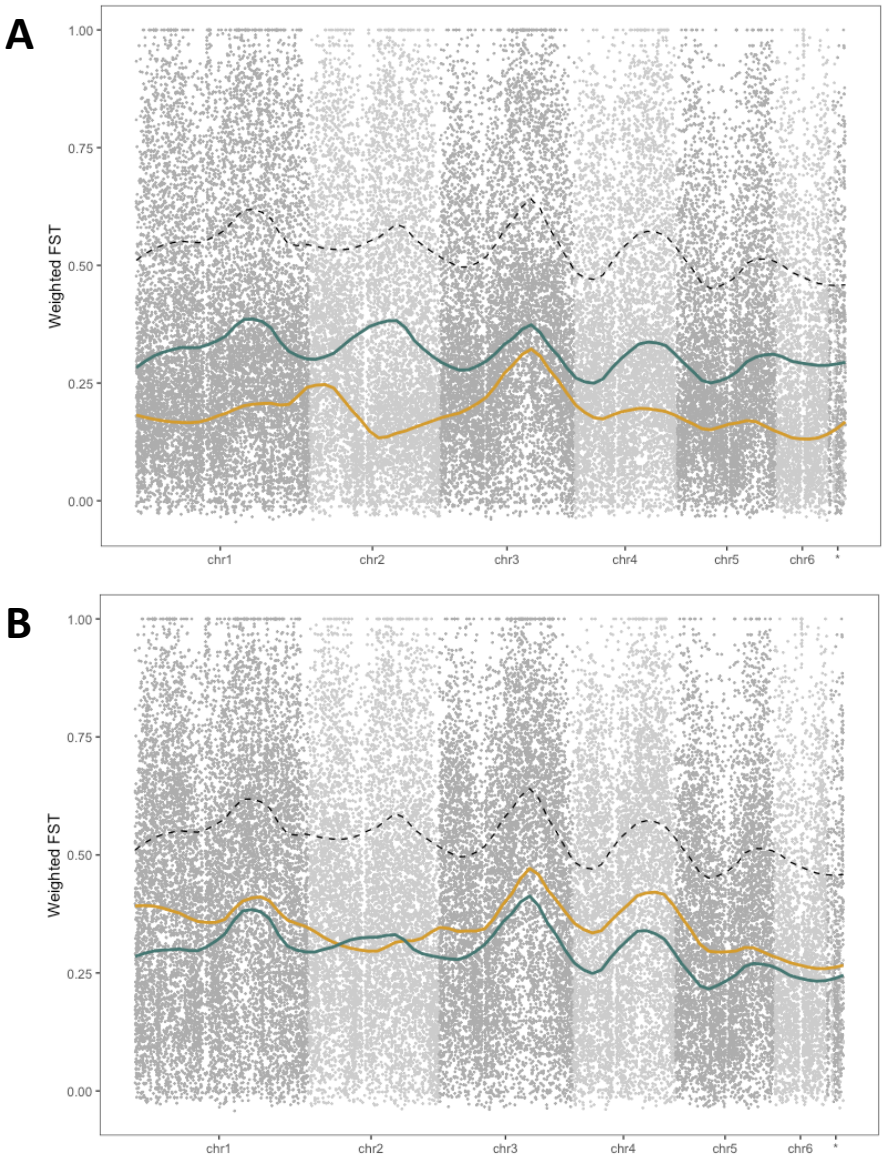

Figure 7. Weighted fixation index (F_{ST}) values calculated for windows (width = 50,000 base pairs) across the genome. Pairwise F_{ST} values for hybrid-*Anolis carolinensis* are plotted in gold, hybrid-*A. porcatus* in teal, and *A. carolinensis*-*A. porcatus* in black. Calculations were performed separately for hybrid groups 1 (A) and 2 (B). The asterisk (*) identifies loci mapped to any of the seven *A. carolinensis* microchromosomes (linkage groups a, b, c, d, f, g, and h).

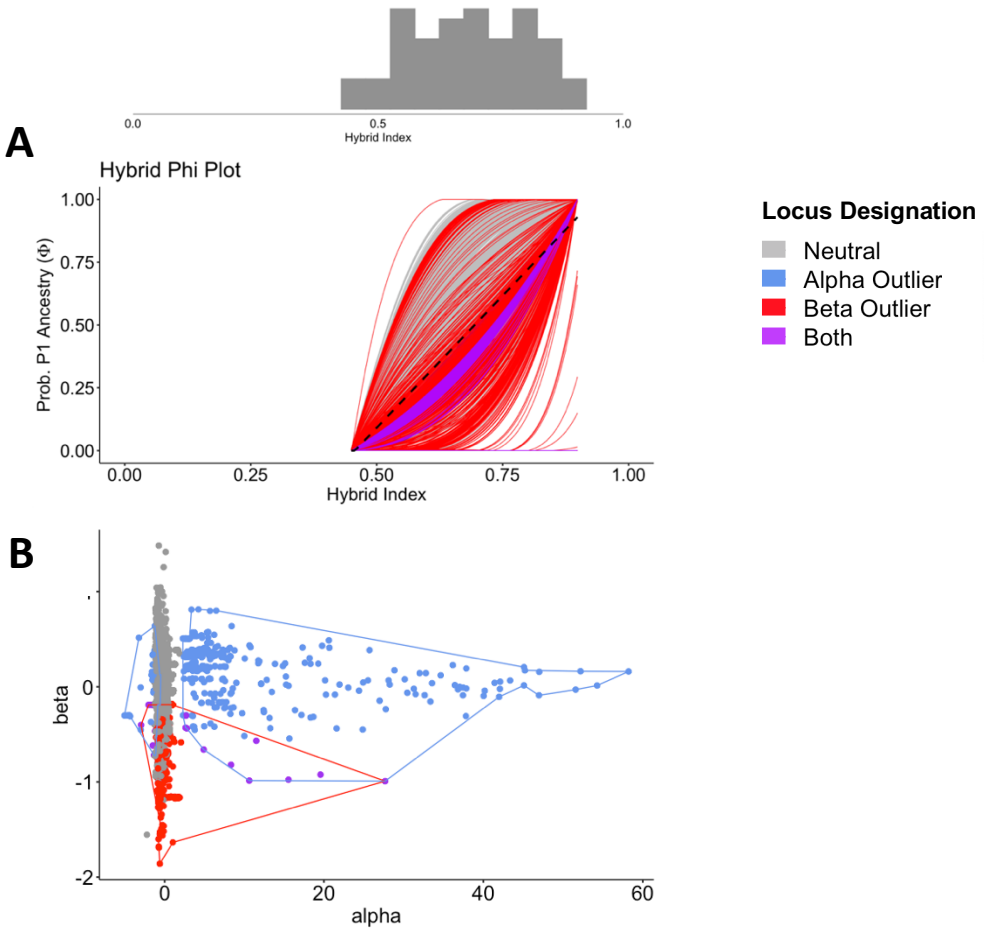

Figure 8. Phi plot of hybrid index values (A) and scatterplot of alpha-beta scores (B) for 8,551 loci displaying fixed differences between *Anolis carolinensis* and *A. porcatus*. Significance was assessed conservatively, assigning outlier status only to loci for which the credible interval did not contain zero and the alpha and/or beta values fell beyond the default quantile interval. In panel A, solid lines represent the genomic clines observed for individual loci, while the dashed line represents a neutral cline. In panel B, points indicate alpha and beta scores for individual loci, and polygons enclose regions within which loci are classified as outliers. In both panels, blue indicates an alpha outlier, red indicates a beta outlier, and purple indicates loci that are outliers for both alpha and beta.

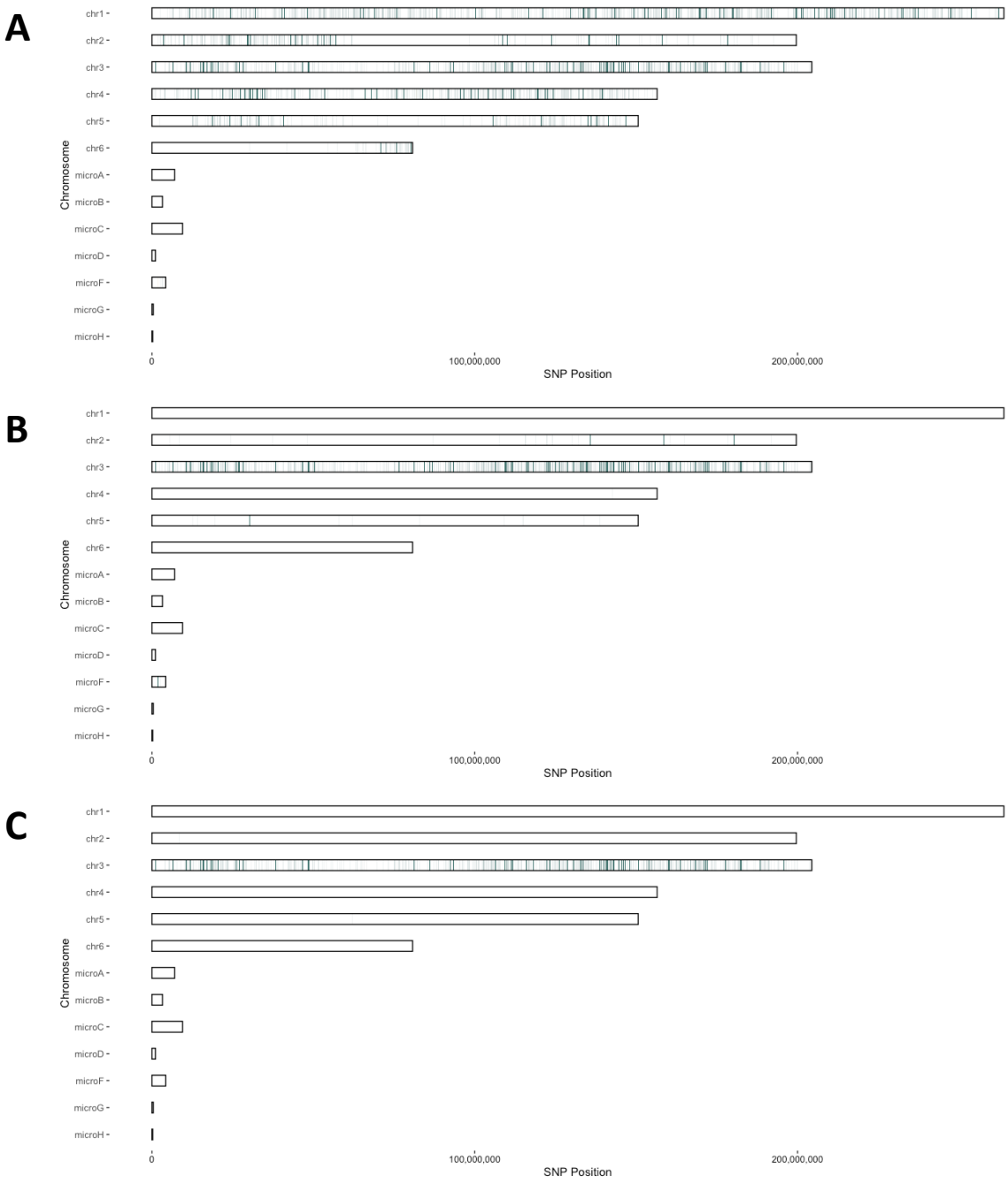

Figure 9. Ideograms of alpha (A), beta (B), and alpha-beta (C) outliers as identified through genomic cline analysis of *Anolis carolinensis* x *A. porcatus* hybrids. Positive alpha outliers are plotted in gold (n = 3), while negative alpha outliers are plotted in teal (n = 3,355). All beta outliers are negative.

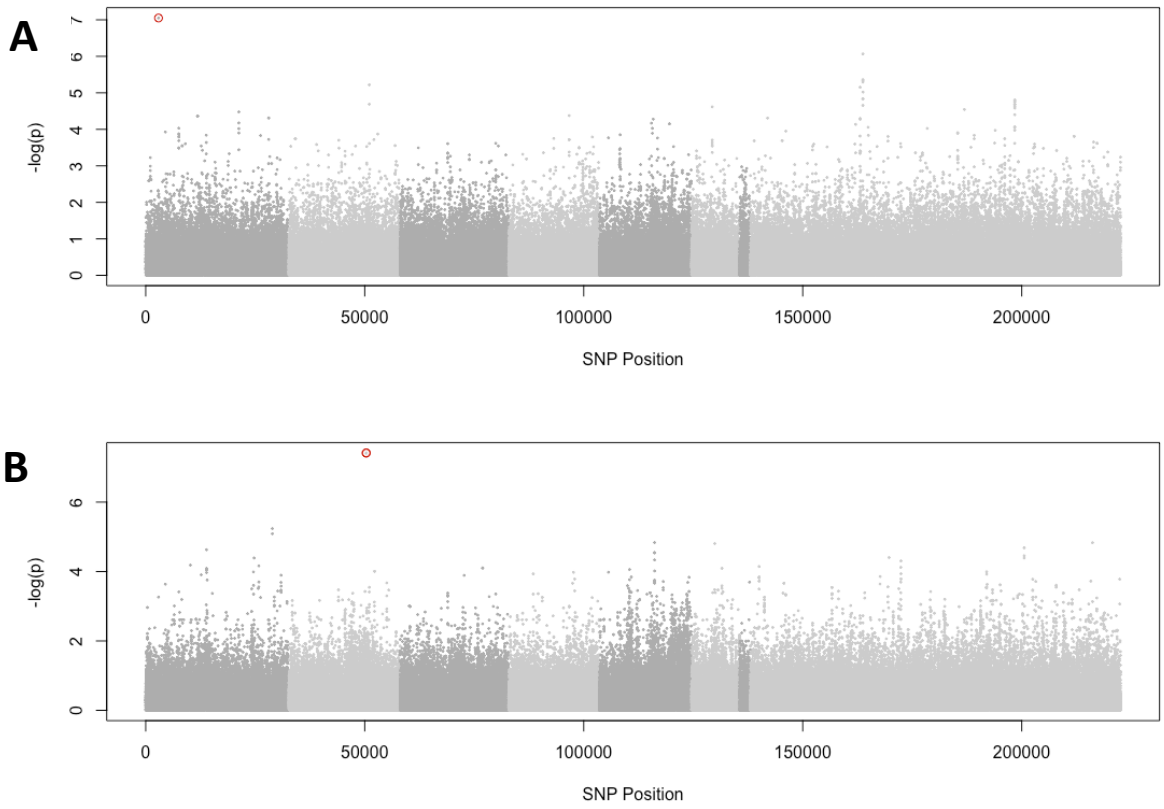

Figure 10. Manhattan plots displaying LFMM results for comparison of *Anolis carolinensis* x *A. porcatus* hybrid genotypes (n = 222,567 loci) to canopy cover (A) and impervious surface area (B). Significant loci are circled in red (panel A: n = 1; panel B: n = 2 overlapping loci). Shaded blocks correspond to chromosomal location (1, 2, 3, 4, 5, 6, or micro), with the final bin containing loci mapped to unassigned scaffolds.

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SUPPLEMENTAL MATERIALS

Table S1. Group assignment, sex, year, latitude, longitude, and environmental data for all hybrid anoles (n = 70) included in our study.

Sample ID	Group	Sex	Year	Latitude	Longitude	Canopy	Impervious SA
H_AC12	1	female	2017	25.664444	-80.280833	66	3
H_AC13	1	male	2017	25.664444	-80.280833	66	3
H_AC14	1	female	2017	25.664444	-80.280833	66	3
H_AC16	1	female	2017	25.750445	-80.434731	64	0
H_AC17	1	female	2017	25.750445	-80.434731	64	0
H_AC18	1	male	2017	25.750445	-80.434731	64	0
H_AC19	1	male	2017	25.750445	-80.434731	64	0
H_AC29	1	male	2017	25.750445	-80.434731	64	0
H_AC30	1	female	2017	25.750445	-80.434731	64	0
H_AC4	1	female	2017	25.664444	-80.280833	66	3
H_AC5	1	male	2017	25.664444	-80.280833	66	3
H_AC7	1	male	2017	25.664444	-80.280833	66	3
H_AC8	1	female	2017	25.664444	-80.280833	66	3
H_JJK1836	1	male	2005	25.678712	-80.285042	16	36
H_JJK1838	1	male	2005	25.678712	-80.285042	16	36
H_JJK1874	1	male	2005	25.678712	-80.285042	16	36
H_JJK1875	1	male	2005	25.678712	-80.285042	16	36
H_JJK1876	1	male	2005	25.678712	-80.285042	16	36
H_JJK1877	1	male	2005	25.678712	-80.285042	16	36
H_JJK1945	1	NA	2005	25.724025	-80.279876	20	0
H_JJK1948	1	female	2005	25.679004	-80.284428	0	20
H_JJK1949	1	male	2005	25.679004	-80.284428	0	20
H_JJK1951	1	male	2005	25.679004	-80.284428	0	20
H_JJK3423	1	female	2010	NA	NA	NA	NA
H_MIA195	1	male	2010	25.642500	-80.287222	70	0
H_MIA196	1	male	2010	25.642500	-80.287222	70	0
H_MIA713	1	male	2014	25.679004	-80.284428	0	20
H_MIA802	1	male	2017	25.945920	-80.299370	0	35
H_MIA803	1	female	2017	25.945990	-80.299380	0	35
H_MIA806	1	female	2017	25.946220	-80.299370	0	44
H_MIA82	1	female	2010	25.688169	-80.290931	21	17
H_AC20	2	male	2017	25.750445	-80.434731	64	0
H_AC21	2	female	2017	25.750445	-80.434731	64	0
H_AC32	2	male	2017	25.689444	-80.158889	0	44
H_AC34	2	female	2017	25.689444	-80.158889	0	44
H_AC36	2	male	2017	25.689444	-80.158889	0	44
H_AC40	2	female	2017	25.689444	-80.158889	0	44
H_JJK1837	2	male	2005	25.678712	-80.285042	16	36
H_JJK1860	2	male	2005	25.722331	-80.280485	0	21
H_JJK1861	2	female	2005	25.722331	-80.280485	0	21

H_JJK1947	2	male	2005	25.679004	-80.284428	0	20
H_JJK3581	2	female	2010	25.706206	-80.296669	80	1
H_JJK3582	2	female	2010	25.706206	-80.296669	80	1
H_MIA172	2	male	2010	25.747347	-80.209697	36	24
H_MIA173	2	female	2010	25.747347	-80.209697	36	24
H_MIA49	2	male	2010	25.705000	-80.271056	21	3
H_MIA50	2	female	2010	25.704983	-80.271492	17	1
H_MIA636	2	male	2014	25.704328	-80.284173	0	27
H_MIA637	2	male	2014	25.704328	-80.284173	0	27
H_MIA644	2	male	2014	25.705848	-80.297061	0	10
H_MIA647	2	female	2014	25.705848	-80.297061	0	10
H_MIA651	2	female	2014	25.705848	-80.297061	0	10
H_MIA669	2	male	2014	25.703548	-80.303452	0	16
H_MIA670	2	female	2014	25.703548	-80.303452	0	16
H_MIA677	2	female	2014	25.713663	-80.291831	13	28
H_MIA678	2	female	2014	25.710727	-80.283879	0	55
H_MIA681	2	male	2014	25.710727	-80.283879	0	55
H_MIA694	2	female	2014	25.715572	-80.280804	0	41
H_MIA701	2	male	2014	25.715631	-80.280835	0	41
H_MIA705	2	male	2014	25.717062	-80.275447	0	43
H_MIA706	2	female	2014	25.717062	-80.275447	0	43
H_MIA725	2	male	2014	25.721350	-80.280275	0	70
H_MIA744	2	male	2014	25.738302	-80.309376	63	0
H_MIA745	2	female	2014	25.738302	-80.309376	63	0
H_MIA748	2	male	2014	25.742569	-80.314544	21	36
H_MIA750	2	female	2014	25.742569	-80.314544	21	36
H_MIA76	2	male	2010	25.697631	-80.271878	56	4
H_MIA77	2	female	2010	25.697575	-80.268622	13	23
H_MIA80	2	female	2010	25.700972	-80.263261	30	10
H_MIA808	2	male	2017	25.944610	-80.299320	0	40

Table S2. Sample source, sex, year, and location data for all parental (n = 16 *Anolis carolinensis*, n = 15 *A. porcatus*) and outgroup (n = 2 *A. smaragdinus*) anoles included in our study.

Sample ID	Source	Species	Sex	Year	Site ID
C_CRYO4393	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4394	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4395	Harvard MCZ	*A. carolinensis*	female	2012	Hobe Sound, FL
C_CRYO4397	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4398	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4399	Harvard MCZ	*A. carolinensis*	female	2012	Hobe Sound, FL
C_CRYO4400	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4401	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4402	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4403	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4404	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4405	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4406	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
C_CRYO4407	Harvard MCZ	*A. carolinensis*	male	2012	Hobe Sound, FL
H_JJK1932	Kolbe Lab - URI	*A. carolinensis*	male	2005	Parkland, FL
H_JJK1933	Kolbe Lab - URI	*A. carolinensis*	female	2005	Parkland, FL
P_JJK2793	Kolbe Lab - URI	*A. porcatus*	male	2008	La Habana - near Institute
P_JJK2794	Kolbe Lab - URI	*A. porcatus*	male	2008	La Habana - near Institute
P_JJK2795	Kolbe Lab - URI	*A. porcatus*	female	2008	La Habana - near Institute
P_JJK2796	Kolbe Lab - URI	*A. porcatus*	female	2008	La Habana - near Institute
P_JJK2797	Kolbe Lab - URI	*A. porcatus*	female	2008	La Habana - near Institute
P_JJK2799	Kolbe Lab - URI	*A. porcatus*	female	2008	La Habana - near Institute
P_JJK2800	Kolbe Lab - URI	*A. porcatus*	female	2008	La Habana - near Institute
P_JJK2825	Kolbe Lab - URI	*A. porcatus*	male	2008	La Habana - near Institute
P_JJK2827	Kolbe Lab - URI	*A. porcatus*	male	2008	La Habana - Bosque Park
P_JJK2832	Kolbe Lab - URI	*A. porcatus*	male	2008	La Habana - Bosque Park
P_JJK2830	Kolbe Lab - URI	*A. porcatus*	male	2008	La Habana - Bosque Park
P_JJK3066	Kolbe Lab - URI	*A. porcatus*	female	2008	Guanabo, Cuba
P_JJK3068	Kolbe Lab - URI	*A. porcatus*	female	2008	Guanabo, Cuba
P_JJK3070	Kolbe Lab - URI	*A. porcatus*	male	2008	Guanabo, Cuba
P_JJK3071	Kolbe Lab - URI	*A. porcatus*	female	2008	Guanabo, Cuba
S_JBL4329	Kolbe Lab - URI	*A. smaragdinus*	male	2013	Marsh Harbor, Abaco
S_JBL4330	Kolbe Lab - URI	*A. smaragdinus*	female	2013	Marsh Harbor, Abaco

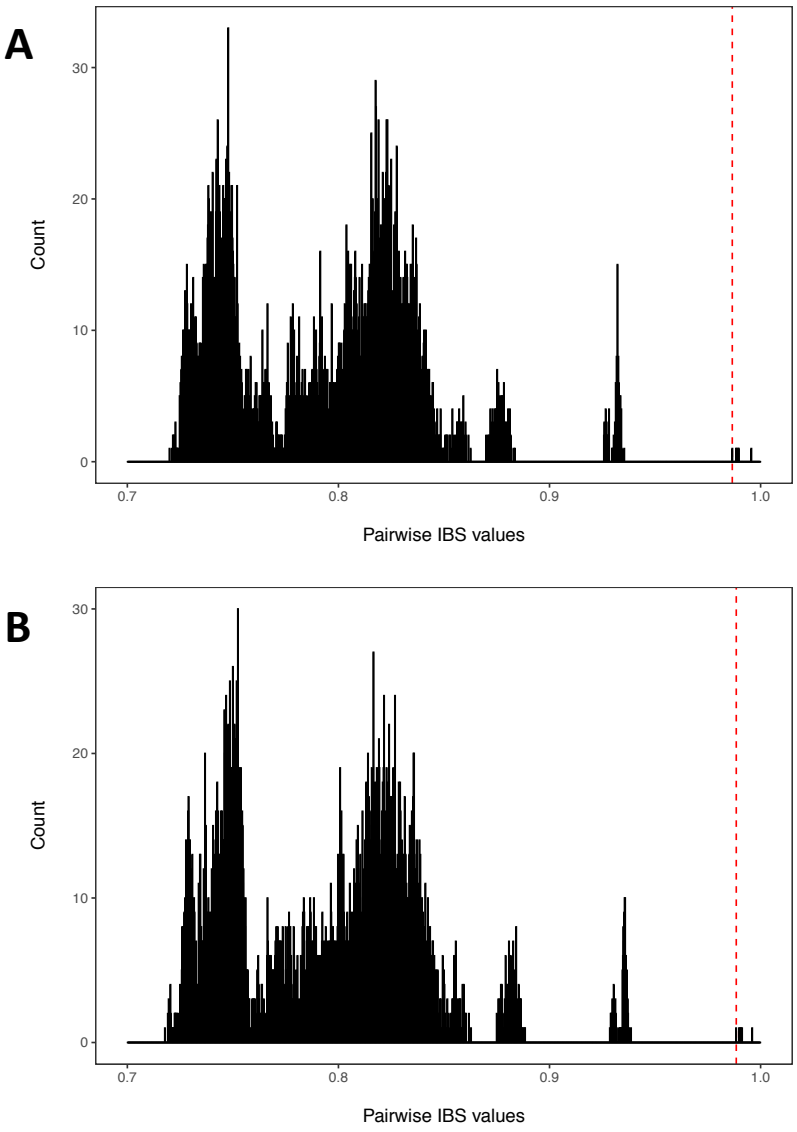

Figure S1. Pairwise IBS values for all *Anolis* samples. These values were calculated for SNPs filtered at call rates of both 70% (A) and 95% (B) to check for potential library effects. The red dashed line indicates the lowest IBS value observed among replicate sample pairs. Beyond values associated with paired replicates, only the value comparing the two outgroup *A. smaragdinus* samples crossed this boundary.

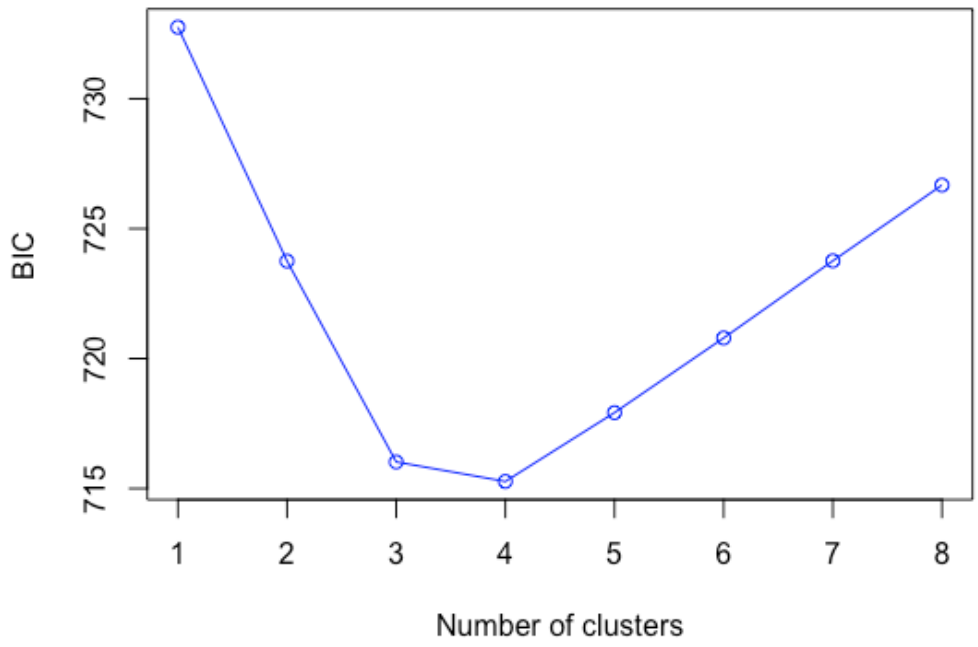

Figure S2. Bayesian information criterion (BIC) values indicating discriminant analysis of principal components model likelihoods for various numbers of genetic clusters within a group of samples including both hybrid and parental anoles. The lowest BIC value corresponds to the most likely number of clusters: K = 4.

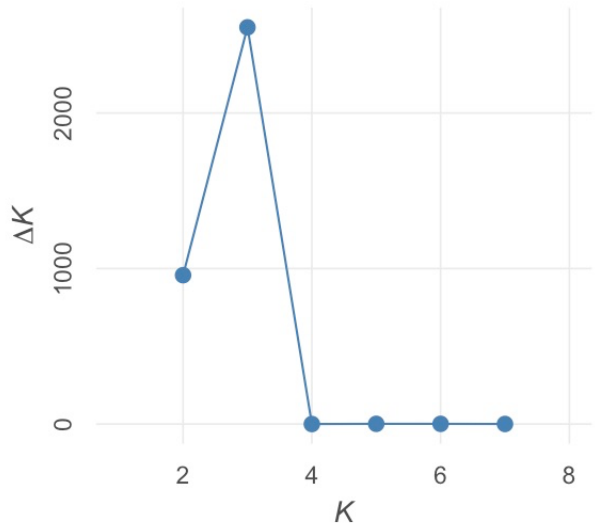

Figure S3. Plot of delta K values indicating cluster likelihoods for STRUCTURE models of hybrid *Anolis* ancestry (20 replicate runs per K value). The highest delta K value indicates the most likely number of genetic clusters: K = 3.

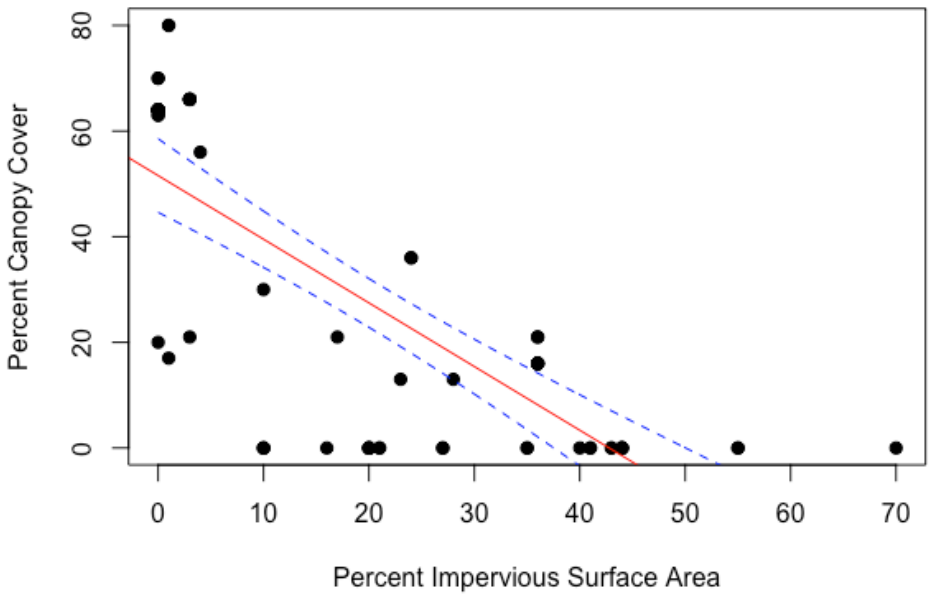

Figure S4. Linear regression with 95% confidence intervals displaying the negative correlation (r = -0.759) between canopy cover and impervious surface area. Points correspond to values associated with the habitats of n = 68 hybrid anoles.

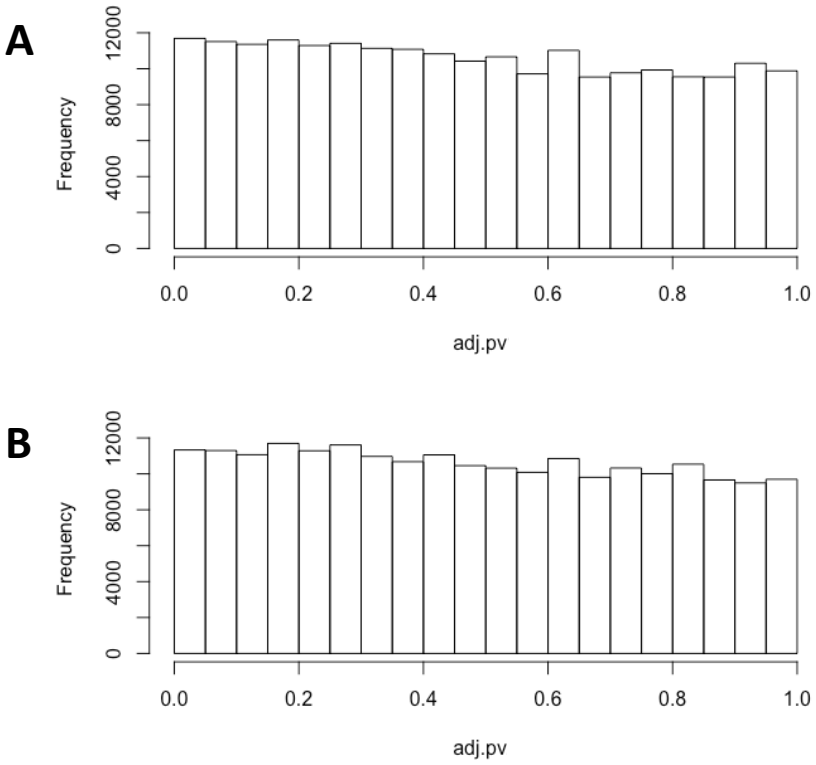

Figure S5. Histograms displaying the distribution of p-values for LFMMs comparing hybrid *Anolis* genotypes (n = 222,567 loci) to canopy cover (A; manually adjusted GIF = 0.96) and impervious surface area (B; manually adjusted GIF = 1.00).