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Genetic, Demographic and Ecological Factors Contributing to the Evolution of *Anolis* Lizard Populations

Johanna E. Wegener
University of Rhode Island, johanna_wegener@uri.edu

GENETIC, DEMOGRAPHIC AND ECOLOGICAL FACTORS CONTRIBUTING TO THE EVOLUTION OF *ANOLIS* LIZARD POPULATIONS

BY

JOHANNA E. WEGENER

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JOHANNA E. WEGENER

APPROVED:

Dissertation Committee:

Major Professor Jason Kolbe

Chris Lane

Dina Proestou

Nasser H. Zawia
DEAN OF THE GRADUATE SCHOOL

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ABSTRACT

Geographic and environmental factors can be catalysts of evolutionary processes and central drivers of genetic and phenotypic differentiation. Characteristic patterns of genetic and phenotypic variation elucidate whether predominately adaptive or non-adaptive processes are involved in shaping variation among populations. Such genetic and phenotypic signatures can provide insight in how specific extrinsic factors relate to adaptive and non-adaptive processes. This dissertation aims to investigate whether and how disturbance events, intraspecific competition and secondary contact after a species introduction contribute to genetic and phenotypic differentiation among populations of *Anolis* lizard.

In chapter 1, I tested whether patterns of genetic diversity and differentiation of island populations in the Bahamas were consistent with disturbance-driven demographic fluctuations and strong genetic drift. I used ten microsatellite markers to measure genetic diversity and population differentiation. Population genetic structure and differentiation were consistent with expectations of strong genetic drift after founder events. Genetic bottlenecks were prevalent across the island populations and coalescent-based demographic models supported a colonization scenario for most islands, rather than scenarios for population bottlenecks or relative stability of populations. Low rates of gene flow among island populations have likely preserved the genetic signatures of colonization and extinction events. This study provides evidence that disturbance events such as hurricanes are catalysts of long-term, non-adaptive evolution when gene flow is limited.

In chapter 2, I examined whether intraspecific competition is a potential driver of directional morphological change in male and female lizards (*Anolis sagrei*) among island populations in the Bahamas. I used measures of population density and injuries, as a proxy for aggressive encounters, to test whether body size and head size vary in the direction predicted by natural selection. I found that lizards in high-density populations had a higher proportion of injuries as compared to low-density populations, suggesting that the former experience higher levels of intraspecific aggression. Additionally, lizards in these higher density populations had larger heads (longer and wider, corrected for body size). In both sexes, relative head size and frequency of injury increased with population density, suggesting that females may play an active role in intraspecific competition.

In chapter 3, I examined whether the introduction of the Cuban green anole (*Anolis porcatus*) to South Florida has resulted in local hybridization with its closely related sister species, the native green anole (*Anolis carolinensis*), or whether reproductive barriers prevent gene exchange across species boundaries. I used one mtDNA marker and 18 microsatellite loci to test for cyto-nuclear discordance, which is indicative of hybridization, and whether population-genetic patterns are consistent with recent or historic gene flow. I found mtDNA haplotypes of two species in South Miami, local *A. carolinensis* and *A. porcatus* from West Cuba. Contrary to expectations of recent hybridization, I did not find intermediate nuclear genotypes. Instead, the population in South Miami forms a separate, genetically homogeneous cluster, which is differentiated from both parental species. A historic gene flow analysis confirms that ~33% of the nuclear ancestry of the South Miami population is

derived from West Cuban *A. porcatus*. Thus, reproductive isolation between *A. porcatus* and *A. carolinensis* is weak or absent, despite considerable divergence time in allopatry, which reinforces a proposal to revise the taxonomy of *A. carolinensis* and *A. porcatus* from West Cuba according to guidelines of the biological species concept.

In summary, my dissertation evaluated how demographic and ecological factors relate to patterns of genetic and phenotypic variation in populations of *Anolis* lizards. My research shows that hurricanes can act as evolutionary catalysts by altering non-adaptive evolutionary processes when gene flow is limited. The same island populations vary morphologically in the direction predicted by natural selection as a consequence of intraspecific competition. Lastly, historic hybridization resulting from secondary contact between formerly allopatric introduced and native species can locally erode species boundaries and alter the genetic composition of native populations. By characterizing patterns of genetic and phenotypic variation, my research provides insight in to how extrinsic factors and long-term evolutionary processes are interconnected and potentially shape future trajectories of these populations.

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PREFACE

This thesis is submitted in manuscript format. All three chapters are in preparation for submission to peer-reviewed journals. The first chapter "Genetic diversity and differentiation among small-island lizard populations in relation to hurricane-driven disturbance" is in preparation for publication in Molecular Ecology. The second chapter "Head size of male and female lizards increases with intraspecific competition among small island populations in the Bahamas" is in preparation for publication in Breviora. The third chapter "Hybridization and rapid differentiation after secondary contact between the native green anole (*Anolis carolinensis*) and the introduced Cuban green anole (*A. porcatus*)" is in preparation for publication in Evolutionary Applications. All references are formatted according to the reference guidelines of the proposed journal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENETIC DIVERSITY AND DIFFERENTIATION AMONG SMALL-ISLAND LIZARD POPULATIONS IN RELATION TO HURRICANE-DRIVEN DISTURBANCE

This chapter is in preparation for publication in Molecular Ecology

Johanna E. Wegener[1], Kevin P. Mulder[2], Robert M. Pringle[3], Jonathan B. Losos[4], Jason J. Kolbe[1]

[1]*Department of Biological Sciences, University of Rhode Island, Kingston RI, 02881*

[2]*CIBIO/InBIO, Research Center in Biodiversity and Genetic Resources, Rua Padre Armando Quintas 7, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*

[3]*Department of Ecology and Evolutionary Biology, Princeton University, Princeton NJ, 08542*

[4]*Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, 02138, USA*

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Abstract

The Bahamas are located in a high-frequency hurricane trackway, which has caused repeated extinctions of animal populations on small islands. Whereas hurricane-driven disturbance is known to drive rapid ecological change in populations and communities, the longer-term population-genetic and evolutionary consequences are far less understood. As the intensity and frequency of catastrophic disturbance events is increasing with global climate change, there is an urgent need to understand the biological impacts of such events. We used naturally and experimentally colonized island-populations of the widespread brown anole (*Anolis sagrei*) in the Bahamas to evaluate the hypothesis that the legacy of past hurricanes has shaped patterns of genetic diversity and differentiation. Our results suggest that population structure and genetic differentiation on most islands is the result of strong genetic drift associated with founder events. Coalescent-based simulations show that populations on relatively small and low-elevation islands have undergone recent demographic changes consistent with colonization, whereas this signal of colonization weakens somewhat for populations on larger and higher-elevation islands. Low levels of gene flow have likely preserved signatures of hurricane-driven extinction and recolonization in this system. Our study provides evidence that disturbance events such as hurricanes are catalysts of long-term, non-adaptive evolution when gene flow is limited.

Introduction

The frequency and intensity of catastrophic disturbance events such as fires, floods and hurricanes is increasing as a result of global change (Aponte *et al.* 2016; Arnell & Gosling 2016; Bender *et al.* 2010; Goldenberg *et al.* 2001; Westerling *et al.* 2006). Disturbance events can catalyze ecological change by rapidly restructuring ecosystems and species communities (Turner 2010). Such disturbance-driven ecological changes are relevant for evolution when they alter environmental conditions, meta-community structure, and population sizes (Banks *et al.* 2013; Hanski 2012; Pelletier *et al.* 2009; Schoener 2011). Disturbance can shift the direction and strength of natural selection and consequently drive adaptive evolution (Grant & Grant 2002; Grant *et al.* 2017). Alternatively, changes in genetic diversity following population size reductions and altered gene-flow patterns (Apodaca *et al.* 2013; Spear *et al.* 2012) can increase the relative importance of genetic drift as a driver of non-adaptive evolution (Banks *et al.* 2013; Davies *et al.* 2016). However, empirical studies that highlight the relative importance of disturbance events as non-adaptive driver are rare, because genetic data are often limited to a small number of populations or lack undisturbed reference populations (Apodaca *et al.* 2013; Fleming & Murray 2009), making it difficult to distinguish the effects of population size reductions from preexisting population structure and post-disturbance gene flow (Banks *et al.* 2013). We took advantage of a replicated small-island system in the Bahamas with a well-understood disturbance regime and used genetic data to study the non-adaptive evolutionary consequences of reoccurring colonization and extinction events. We include a subset of islands with known colonization history as reference populations

and compared patterns of genetic diversity and differentiation of experimental islands to islands with natural colonization histories.

The Bahamas are located in a major hurricane trackway and previous work has shown that hurricanes are an important driver of extinction-recolonization dynamics in populations of the brown anole, *Anolis sagrei* (Losos *et al.* 2003; Schoener *et al.* 2001a, 2004; Spiller *et al.* 1998; Spiller & Schoener 2007). Hurricane-induced extinction and subsequent recolonization follows a complex pattern in which island size, elevation, and timing of reproduction determine population survival and natural recolonization (Schoener *et al.* 2001a, 2004). Previous studies have focused primarily on the various ecological effects of hurricanes, but the underlying population-genetic effects and thus long-term evolutionary consequences have not previously been studied in this system. A total of 22 tropical storms and hurricanes of category 1-5 have occurred within a 50-km radius of our focal island system near Staniel Cay, Bahamas (24.169492, -76.438746), records started in 1842 (US National Hurricane Center, www.coast.noaa.gov). Four storms passed directly over the islands, including a category 2 hurricane in 1891, two unnamed tropical storms in 1928 and 1936 and the tropical storm Gilda in 1973.

In this study, we systematically investigated whether and how repeated population-size reductions, extinctions, and recolonizations have affected the genetic diversity and differentiation of 17 island populations of brown anoles near Staniel Cay. We hypothesized that hurricane-induced extinction and natural recolonization is frequent in this system and that strong genetic drift is a central driver of population diversity and differentiation. We include four reference islands, that were

experimentally colonized in 1977 with 5-10 lizards from Staniel Cay, a larger source island for an unrelated study (Schoener & Schoener 1983); the remainder are all thought to have been occupied continuously since before 1977. Because the timing of colonization and the number of colonizers is known, these islands serve as valuable comparisons for our analysis decreasing the ambiguity associated with inferring demographic processes from patterns. We tested whether patterns of genetic diversity and differentiation across these 17 islands were consistent with disturbance-driven population demography. First, we used overall genetic diversity, population structure, and differentiation to test for signatures of strong genetic drift. Second, we used demographic models to reconstruct the potential processes leading to the observed patterns of genetic diversity. Finally, we compared population-genetic parameters and demographic models of the reference populations to naturally established populations with unknown demographic histories to gain insight in whether similar demographic processes gave rise to similar patterns of genetic diversity and differentiation.

In the case of strong drift, we predict low genetic diversity, random genetic differentiation and high inbreeding coefficients. In contrast, if an alternative process, such as vicariance due to rising sea levels has occurred, we predict genetic differentiation to be related to geographic distance among islands (i.e., isolation by distance) and genetic diversity to be a function of island size. If hurricanes are major drivers of recolonization and extinction dynamics, we predict widespread signatures of genetic bottlenecks for populations on islands susceptible to hurricanes, but not on larger, less-susceptible islands; we used estimated elevation above sea level and island size as a proxy for hurricane susceptibility (Schoener *et al.* 2001a, 2004). Finally, if

natural colonization is prevalent in our system, we predict that patterns of genetic diversity and differentiation of the experimentally colonized reference islands will mimic patterns of similar, but naturally colonized islands.

Material and methods

Study system

Anolis sagrei occurs naturally on islands throughout the Bahamas and more widely throughout the Caribbean and beyond (Losos 2009). It is a trunk-ground habitat specialist, occupying a structural habitat including the ground as well as tree trunks and branches close to the ground. Female lizards become reproductively mature within 6-9 months and lay several single-egg clutches during the breeding season. Females are capable of storing sperm from multiple males for up to two month (Calsbeek *et al.* 2007), which might facilitate natural recolonization when mates are absent. Previous work on populations in the Bahamas has shown that viable populations can be founded with as few as two individuals (Kolbe *et al.* 2012).

Study site and sampling

In 2011, we sampled populations of *A. sagrei* on 16 small islands ranging in area from ~500 to 3320 m^2 and Staniel Cay, which is considerably larger at ~1.4 km^2 (Fig 1). The entire study area spans ~14 km (SE to NW) and is ~4.5 km wide (SW to NE) and the two closest neighboring islands (311 and 305) are ~150 m apart from each other (Fig 1A). We sampled a total of 469 adult individuals (12 - 35 per population; mean = 27.6 ± 6.0), including both males and females. Lizards were captured using a noose or

by hand. Tail tips were collected and preserved in ethanol for DNA extraction. Population sizes were estimated using log-linear capture-recapture methods based on a three-day census on each island (Heckel & Roughgarden 1979).

DNA extraction and microsatellite genotyping

Genomic DNA was extracted from tail tips using a standard isopropanol precipitation protocol. We amplified 10 microsatellite loci using PCR and fluorescently labeled primers. We combined primers from the literature (Bardeleben *et al.* 2004; Wordley *et al.* 2011) and newly designed primers (Table S1). A 10 μL reaction contained 0.8 μL of 10x standard PCR buffer (New England Biolabs®Inc.), 0.8 μL of 10x BSA, 0.6 μL of 10 mM dNTPs, 1.50 μL of 25 mM MgCl, 0.24 μL of 10 μM primer, 0.08 μL of 5 units/μl *Taq* polymerase (New England Biolabs®Inc.) and 2 μL of 20 ng/μL genomic DNA. Cycles started with initial denaturation at 94˚C for 2 min, followed by 35 cycles of 94˚C for 45 sec, T_m for 45 sec, 72˚C for 1 min and a final elongation step at 72˚C for 10 min. Samples were genotyped at the DNA Analysis Facility on Science Hill at Yale University. Markers for all samples were analyzed with the software GeneMapper® v4.1 and individually checked to ensure accurate peak calling. We genotyped a subset of loci and individuals (~ 5%) twice to ensure peaks were called consistently, with particular focus on individuals with private alleles.

Genetic diversity and population differentiation

To measure genetic diversity, we calculated allelic richness and expected and observed heterozygosity using GenAlEx v6.5 (Peakall & Smouse 2012). The inbreeding coefficient (F_{IS}) was calculated using GENEPOP web version (Raymond & Rousset 1995; Rousset 2008). Deviations from Hardy-Weinberg equilibrium (HWE) for each locus and each population were estimated in GenAlEx v6.5 (Peakall & Smouse 2012). Mean values of observed heterozygosity and allelic richness were compared among the experimentally colonized reference populations and the naturally colonized ones using Welch's t-test, adjusting alpha levels of *P*-values with the Bonferroni method using the R v3.3.2 package STATS (Team 2016).

Differentiation among island populations was estimated with pairwise F_{ST} values using Arlequin v3.5 (Excoffier & Lischer 2010). To test for isolation by distance, we used Mantel test correlations between linearized pairwise F_{ST} values ($F_{ST} / (1 - F_{ST})$) and geographic distance using the function *mantel* of the R package VEGAN v2.4 - 3 (Oksanen *et al.* 2013). The experimentally colonized reference populations were excluded when testing for isolation by distance. Geographic distance was calculated from island midpoints using *geodist* in the R package GMT v1.0 (Magnusson 2011). Mantel tests have been criticized for spatial analysis and have low power for detecting patterns when the correlation does not extend over the entire study area (Legendre *et al.* 2008). We therefore also used a distance-based redundancy analysis (dbRDA) with the *capscale* function in the R package VEGAN v2.4 - 3 (Legendre *et al.* 2008; Oksanen *et al.* 2013). We transformed geographic data to a rectangular vector using *pcnm* and tested for significance of the multivariate regression using *anova.cca* with

9999 permutations. We used Pearson's correlations to test for relationships between island characteristics and genetic diversity. Island variables were elevation (estimated to the nearest 0.5 m) and vegetated island area (measured in Google Earth Pro). Genetic variables were allelic richness, expected heterozygosity, and observed heterozygosity.

Population structure

We used two approaches to determine population structure among islands. First, we performed a Bayesian cluster analysis with STRUCTURE v2.3.4 (Rosenberg 2004), using an admixture model and correlated allele frequencies. We assumed gene flow among island populations and sequentially increased the number of clusters (K = 3 - 18). Iterations for each run were set to 10, with 10^6 generations and a burn-in of 500,000 steps. We used STRUCTURE HARVESTER (Earl & Vonholdt 2012) to determine the most likely number of clusters based on deltaK. To visualize the result, we used CLUMPP (Jakobsson & Rosenberg 2007) and DISTRUCT (Rosenberg 2004). Second, we used discriminant analysis of principal components (DAPC), a multivariate-distance based approach, to characterize population structure without the assumption of HWE using the R package ADEGENET (Jombart 2008). We retained the first 80 principal-component axes for the discriminant analysis and estimated genetic clusters rather than using population priors.

Contemporary gene flow

To test for contemporary gene flow between islands, we used a Bayesian method implemented in the software BayesAss v3 (Wilson & Rannala 2003). This method estimates the proportion of immigrants for each population from the past two generations and the present generation, without assuming HWE (Wilson & Rannala 2003). The program assumes well differentiated populations ($F_{ST} \geq 0.1$) and a low migration rate (m = 0.01) among populations (Faubet *et al.* 2007; Meirmans 2014). We conducted 10 independent runs starting from random seeds, $20*10^6$ iterations, a burn-in of 10^6 generations sampling every 2000^{th} generation. We optimized the mixing parameters *deltaA*, *deltaM* and *deltaF* according to the user manual. To assure convergence and to select the best run, we examined the tracer file for high effective sample size (EES) and calculated the Bayesian deviance (Faubet *et al.* 2007; Meirmans 2014). Because BayesAss can show biased results when the number of populations is greater than 10 (Faubet *et al.* 2007; Meirmans 2014), we repeated the analysis with three sub-groups based on geographic proximity: the five northernmost islands, the five central islands, and the six southernmost islands.

Genetic bottlenecks and demographic history

We used two complementary approaches to test for genetic bottlenecks and alternative demographic histories. First, we used Approximate Bayesian Computation to test for the presence of past genetic bottlenecks using the program DIYABC v2.0 (Cornuet *et al.* 2014). We constructed three historic demographic scenarios (Fig. 2): colonization (scenario 1), population bottleneck (scenario 2), and stable population

size (scenario 3). We used uniform prior distributions for all variable parameters (Table S2). The census population size of each population was used as an upper limit for the effective populations size (N_P). Time of the demographic event was allowed to vary between 10 and 100 generations and extended in 100-generation intervals up to 1000 generations (Table S2). Because *Anolis sagrei* becomes sexually mature in 6-9 months (Losos 2009), we assumed one generation per year to be a good estimate. Further, because lizard populations can grow rapidly after colonization (Schoener & Schoener 1983), the duration of the bottleneck (db) was allowed to vary between 1 and 5 generations. The colonization scenario (Scenario 1) models a demographic scenario, in which a small number of individuals (N_C) from an unknown source population (i.e., a ghost population) colonizes the island population. After 1-5 generations, the population reaches the current effective populations size (N_P). All study islands are relatively close ($\leq$ 2 km) to considerably larger islands in the east (Fig 1), which represent potential sources of founder individuals and were not sampled. We thus allowed the effective population size of the ghost population to vary between 1,000 and 10,000, which prior work suggests is a realistic range for large islands of these sizes. In the bottleneck scenario (Scenario 2), the island population prior to the bottleneck (N_B) is of similar size as the present population (N_P). During the bottleneck, N_B is reduced to half its size (N_H) for one to five generations. Scenario 3 models a population of size N_P that has remained constant or larger (N_U) for the past 100 generations. Some islands can potentially support larger lizard populations and we thus set the upper limit of the past effective population size to twice the size of the current population (upper limit for N_U=2*N_P). Parameters used to generate the

summary statistics were mean number of alleles, mean heterozygosity, mean allele size variance and the M index. We then calculated posterior probabilities for each scenario and estimated parameter values for the number of colonizers (N_C), timing of the event (T) and duration of the bottleneck (db). We used a Pearson's correlation to test for relationships between island characteristics (i.e. elevation and area) and timing of the demographic event for the best fitting model. Parameters were estimated from posterior distributions.

Second, we calculated the M-ratio using M_P_val software, based on the method described in Garza and Williamson (2001). The M-ratio uses the mean ratio of the number of alleles to allele size: M=k/r with k being the number of alleles in a population and r the range of allele sizes (e.g. repeat number for microsatellites used here). When populations undergo a bottleneck, the number of alleles is reduced to a greater extent than the size range. The program uses an equilibrium distribution of M assuming a two-phase mutation model and ranks that against the calculated M-ratio. An M-ratio < 0.68 is indicative of a genetic bottleneck (Garza & Williamson 2001). We followed the recommendations of the user manual to choose the parameters for the model (Garza & Williamson 2001).

Results

Genetic diversity and population differentiation

The small island populations had low measures of genetic diversity as compared to Staniel Cay (Table 1). Average allelic richness was lower in all 16 small-island populations (mean $A_R = 4.2 \pm 1.14$; range 1.9 - 5.9) than in the largest island

population, Staniel Cay (A_R = 7.5). Observed heterozygosity was lower than expected heterozygosity in all populations, suggesting deviations from HWE that might be the result of inbreeding or small population size. Eleven island populations had relatively high inbreeding coefficients (F_{IS} > 0.25), five islands had moderate inbreeding coefficients (0.25 > F_{IS} > 0.13), and only island 931 had a low inbreeding coefficient of 0.08. All but two populations (i.e., 305 and WBC) had five-or-more loci deviating significantly from HWE, suggesting widespread non-equilibrium.

Genetic diversity did not differ between the naturally colonized and experimentally colonized reference populations. Average observed heterozygosity was 0.52 ± 0.13 for naturally colonized islands and 0.56 ± 0.1 for the reference populations (Welsh's t-test: t = -0.68; DF = 6.9; P = 0.52). Allelic richness was slightly higher in experimentally colonized populations (mean A_R: 5.1 ± 0.74) than natural ones (mean A_R: 3.4 ± 1.1), but this difference was only marginally significant after accounting for multiple comparisons (Welch's t-test: t = -2.54; DF = 7.9; P = 0.04; adjusted P = 0.07).).

Pairwise F_{ST} values showed moderate-to-high differentiation, with values ranging from 0.03 to 0.39 (mean = 0.13 ± 0.08). Island 204 had the overall highest pairwise F_{ST} (mean = 0.27 ± 0.05). After accounting for multiple comparisons, pairwise F_{ST} values were significantly different in 130 of 136 comparisons (Table S3).

We did not find evidence for isolation by distance: genetic differentiation between island populations was not correlated with geographic distance between islands (Mantel test: r = 0.03; P = 0.37; dbRDA: DF = 10; F = 1.46; P = 0.27). In addition, we detected no relationship between island characteristics and measures of

genetic diversity: island area was not related to genetic diversity (Pearson's correlation: A_R r = -0.04; *P* = 0.90; He r = 0.24; *P* = 0.46; Ho r = -0.01; *P* = 0.97), and we found no support for a relationship between island elevation and genetic diversity (Pearson's correlation: A_R r = -0.10; *P* = 0.75; He r = 0.19; *P* = 0.78; Ho r = 0.04; *P* = 0.90).

Population structure and gene flow

Population structure largely reflected geographic separation of island populations. The STRUCTURE analysis identified 13 genetic clusters (Fig. S1) grouping individuals according to sampling islands with little evidence of gene flow among populations (Fig. 1A). We found only two instances of different islands being assigned to the same genetic cluster: islands 305 and 311 formed one genetic cluster and island 332 and 314, respectively. Individuals from Staniel Cay, islands 1 and 6, and WBC shared alleles with other islands from this group, likely reflecting the experimental colonization history 34 years prior to our study. The DAPC analysis likewise grouped individuals in 13 genetic clusters (Fig. 1B and C), reassigning individuals to their original sampling population. Exceptions were individuals from island 1, island 6, Andrew, Staniel Cay, WBC and island 312, which were assigned to two admixed clusters (Table S4).

Contemporary gene flow among islands was low overall, with an average migration rate among the 17 islands of m = 0.01 ± 0.0001 (combining the three best runs of BayesAss; Table S5). Migration rates were slightly but significantly higher when restricting the analyses to smaller groups of islands based on geography (North:

m=0.03 ± 0.03; Central: m = 0.04 ± 0.04; South: m=0.03 ± 0.03; Table S6). Overall, migration rates were lower than 0.1 and these populations can accordingly be considered independently evolving units (Wilson & Rannala 2003).

Demographic history and population bottlenecks

Evidence for genetic bottlenecks was prevalent across island populations. For the ABC analysis, we distinguished between two types of genetic bottlenecks: population-size reduction and founder event. Twelve island populations had high posterior probabilities for the founder event scenario, including the four experimentally colonized populations (Fig. 3). Four other island populations (931, 311, 930 and 936) showed support for the founder-event scenario and a scenario with stable population size (absence of genetic bottleneck), albeit with lower posterior probabilities. The average number of founder individuals was 6.21 ± 0.87 (Table 2), based on posterior parameter estimation. For Staniel Cay, the bottleneck scenario was supported with relatively high posterior probabilities, followed by the scenario with stable population size (Fig. 3).

Results of the ABC analysis were largely consistent with genetic bottlenecks identified with the M-ratio. Fifteen populations had an M-ratio < 0.68, suggesting genetic bottlenecks (Table 1). The only discrepancy was island 332, which showed evidence for a genetic bottleneck in the ABC analysis, but not in the M-ratio test. Island 931 showed evidence for a genetic bottleneck in the ABC analysis, but not in the M-ratio test.

Colonization-time estimates from the ABC analysis suggest that the majority of colonization events occurred within the last 200 generations. Twelve islands had median colonization times between 18.4 - 108 generations and an upper limit 95% CI of < 200 generations (Table 2). Three islands had older colonization times (island 922 T = 220; island 930 T = 126; island 936 T = 300). Island 931 had the oldest colonization time with a 95% CI upper limit close to 1000 generations (island 931 T = 401 95% CI 34.7 - 963). Staniel Cay did not support the colonization scenario and was not included in the time estimates. We found a significant correlation between island area and timing of the colonization event (Pearson's correlation: r = 0.65; $P = 0.03$; Fig. S2), indicating that smaller islands had been colonized more recently. Island 931 was excluded from the regression, due to the exceptionally large confidence interval. We found no correlation between island elevation and timing of colonization events (Pearson's correlation: r = 0.09, $P = 0.78$).

Discussion

Multiple lines of evidence support the hypothesis that hurricanes shape genetic diversity and population structure by driving extinction-recolonization dynamics of *Anolis* lizard populations on small islands in the Bahamas. We found that smaller, lower-lying islands (which should be most vulnerable to hurricane disturbance; Schoener *et al.* 2001a, 2004) have undergone population size reductions as a consequence of founder events during recolonization. Timing of the founder events suggests that populations on larger islands have persisted for longer timespans than populations on smaller islands. Low genetic diversity, population differentiation not

related to geographic distance, and high inbreeding coefficients are characteristic signatures of founder effects and provide support for the prediction that strong genetic drift is a major driver of non-adaptive evolution in this system.

Principal factors determining presence or absence and timing of genetic bottlenecks are likely related to island characteristics. Population persistence and recolonization depends, among other factors, on island size and elevation relative to storm surges (Schoener *et al.* 2001a, 2004). During natural restoration in times between storms, larger islands are likelier targets for immigration and are less susceptible to extinction when hurricanes reoccur (Losos & Ricklefs 2009; Schoener *et al.* 2001a). Islands smaller than 1860 m^2 show evidence for complete extinction and recolonization, whereas islands larger than 2000 m^2 (311, 930, 936, Staniel Cay) support multiple demographic scenarios including relative stability as well as colonization (Fig. 2, Table 2). The only exceptions to this pattern are islands 931 (1070 m^2) and 926 (3320 m^2). Island 931 has the highest elevation (9 m), the lowest inbreeding coefficient, and the oldest colonization time, and its population has likely persisted during past hurricanes when more low-lying islands were submerged by storm surges. The relatively low elevation of island 926 (1.5 m, among the three lowest values among all islands), might explain the presence of a genetic bottleneck despite its large size. Alternative causes of population size reduction, such as predation, disease and resource depletion, are unlikely explanations for the widespread occurrence of genetic bottlenecks in this system. Once colonized, lizard populations can grow rapidly and persist for at least several decades, and seem to be relatively unaffected by avian predators, resource depletion and disease (Schoener & Schoener

1983). Rodent predators occur occasionally on offshore islands in the Bahamas, but only one study was able to link rat predation to population size reduction (Gasc *et al.* 2010). Reduced population size prior to hurricanes, however, does not explain population survival or lack of it alone (Schoener *et al.* 2001b). Predation may have a synergistic effect on survival during hurricanes by making populations more susceptible to extinction and by reducing the likelihood of subsequent population recovery (Schoener *et al.* 2001b).However, curly tail lizards (*Leiocephalus carinatus*) were not present on any of the 16 small islands at the time of sampling.

The average number of colonizers from the ABC analysis (6.21 ± 0.87) is strikingly similar to the number of lizards that were used to found the experimentally colonized reference islands (5-10 lizards) in 1977 (Schoener & Schoener 1983). Lizards are capable of overwater dispersal and females can store sperm from multiple males (Calsbeek *et al.* 2007), which might facilitate establishment after colonization even in the absence of mates (Schoener & Schoener 1983). *Anolis sagrei* can survive up to 24 h floating in sea water, but evidence for the actual occurrence and frequency of overwater dispersal is scarce (Logan *et al.* 2016; Schoener & Schoener 1984). Migration rates in our study were lower (m = 0.01 ± 0.0001) than previously reported rates of *A. sagrei* on a similar archipelago in the Bahamas. Logan et al. (2016) attribute gene flow to thermal similarity among islands and proposes that physiological preadaptation is a more important factor than geographic distance. Thus, factors unrelated to geographic distance might promote or constrain rates of gene flow in this system. In our case, rates of gene flow are so low that populations can be considered independently evolving units (Faubet *et al.* 2007).

Patterns of genetic diversity and differentiation in this study are in agreement with the general predictions that disturbance events reduce genetic diversity within populations and increase differentiation among populations as a result of strong genetic drift (Banks *et al.* 2013; Davies *et al.* 2016). Low genetic diversity, increase in population structure and differentiation, absence of isolation by distance and inbreeding have been observed frequently immediately after disturbance events (Apodaca *et al.* 2013; Banks *et al.* 2015; Evanno *et al.* 2009; Fleming & Murray 2009). However, a number of studies found that migration from undisturbed areas led to rapid recovery of genetic diversity and thus erased characteristic signatures of disturbance events (Brown *et al.* 2013; Schrey *et al.* 2011; Spear *et al.* 2012). In some instances, disturbance events such as hurricanes have translocated individuals and thus even facilitate migration (Apodaca *et al.* 2013; Censky *et al.* 1998). When gene flow is limited, as in our case, disturbance events become catalysts of long term non-adaptive evolution (Beheregaray *et al.* 2003).

Conclusion

Our study highlights the evolutionary significance of disturbance events as driver of non-adaptive evolution (Grant *et al.* 2017). We show that disturbance events can catalyze evolution by driving extinction and recolonization dynamics. In the absence of gene flow, random genetic differentiation persists as a consequence of small population size during recolonization. The finding that timing of the colonization events is related to island size is consistent with the prediction that larger islands are less susceptible to disturbance-induced extinction and better targets for recolonization

(Losos & Ricklefs 2009; Schoener *et al.* 2001a). The only exceptions to this pattern provide further support that hurricanes are major drivers of extinction: the highest island at 9 m above sea level shows no genetic evidence of recolonization and likely has not experienced complete extinction since low sea levels connected the island populations ~1000 years ago. The second largest island 926 with an elevation of only 1.5 m shows evidence for recent colonization despite its large size. As hurricanes become more frequent, the time intervals between extinction events likely become shorter, leaving less time for recolonization, population recovery and adaptive evolution. Thus, random, non-adaptive processes will increasingly play an important role in the evolution of island populations and coastal ecosystems that are susceptible to hurricane disturbance.

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Table 1. Microsatellite summary statistics for all 17 island populations. In 15 out of 17 populations the majority of loci (≥50%) deviate from Hardy-Weinberg equilibrium. All but two populations show evidence of past reductions in population size indicated by an M-ratio < 0.68 (Garza & Williamson 2001). N = number of individuals, H_O = observed heterozygosity ± standard error, H_E = expected heterozygosity ± standard error, A_R = allelic richness, HWE = number of loci deviating from Hardy-Weinberg Equilibrium, F_{IS} = inbreeding coefficient. Asterisks denote populations that were experimentally introduced in 1977.

Population	N	Ho		He		A_R	HWE	F_{IS}	M-ratio
1	30	0.55	±0.04	0.81	±0.01	5.2	10-Sep	0.33	0.57
5*	35	0.45	±0.04	0.72	±0.04	4.6	10-Sep	0.39	0.66
6*	32	0.58	±0.07	0.76	±0.03	5.6	10-Jun	0.24	0.57
204	19	0.4	±0.09	0.51	±0.06	4.1	10-Oct	0.24	0.49
305	26	0.68	±0.06	0.8	±0.03	4.2	10-Feb	0.15	0.53
311	22	0.55	±0.08	0.72	±0.05	4.7	10-Jun	0.25	0.38
312	29	0.58	±0.06	0.72	±0.03	5.4	10-May	0.21	0.59
314	32	0.36	±0.07	0.6	±0.04	3.3	10-Aug	0.43	0.5
332	12	0.31	±0.05	0.61	±0.04	1.9	10-Jun	0.53	0.82
922	22	0.39	±0.06	0.51	±0.06	2.8	10-Jun	0.28	0.52
926	30	0.58	±0.05	0.76	±0.02	4.9	10-Aug	0.25	0.55
930	26	0.57	±0.07	0.74	±0.03	3.9	10-Jun	0.24	0.56
931	35	0.72	±0.07	0.77	±0.03	2.5	10-May	0.08	0.72
936	30	0.55	±0.09	0.76	±0.02	3.8	10-Jun	0.26	0.58
Andrew*	28	0.54	±0.05	0.76	±0.02	4.4	10-Jul	0.31	0.45
WBC*	32	0.68	±0.07	0.78	±0.03	5.9	10-Mar	0.14	0.63
Staniel Cay	29	0.5	±0.05	0.79	±0.02	7.5	10-Sep	0.39	0.62

Table 2. Posterior parameters for DIY-ABC analysis and island characteristics. Estimated parameters are shown for the colonization scenario. N_P = effective population size, T = median number of generations since colonization, db = duration genetic bottleneck, N_C = number of colonizers, E = island elevation in meters, A = and vegetated area in m^2. Experimentally colonized islands are indicated with *.

Population	N_P	T	95% CI	db	N_C	E	A
1	234	38	[11.70 - 85.30]	1.07	5.62	7	1429
5*	94.2	18.4	[10.03 - 43.5]	1.86	6.75	3.5	1333
6*	289	36.5	[11.2 - 92.1]	1.73	6.74	2	1851
204	53.5	18.5	[10 - 37.1]	2.02	6.73	2	487
305	159	55	[13.8 - 96.5]	1.61	6.87	1	603
311	401	85.2	[15.8 - 180]	1.06	5.75	3.5	2241
312	113	21.8	[10.6 - 45.0	1.37	6.44	5	640
314	122	108	[28.4 - 191]	2.92	6.41	5.5	1400
332	50.2	104	[22.3 - 192]	3.4	4.22	3	1450
922	174	220	[45.9 - 383]	4.07	4.9	4	1648
926	332	77.5	[14 - 172]	1.95	7.1	1.5	3320
930	294	126	[20.6 - 280]	1.32	6.44	7	2582
931	226	401	[34.7 - 963]	2.97	5.7	9	1070
936	333	300	[64.3 - 575]	1.6	7.12	2.5	2772
Andrew*	173	50.4	[12.4 - 96.1]	2.4	7.29	1.5	1758
WBC*	132	18.2	[10 - 38.6]	1.04	5.72	3	1575

Table S1. Primers and amplification conditions for microsatellite loci.

Primer	Forward	Reverse	Reference	Tm
Sag_C11	5'-AGGGAAGAAGGTGGCATCAT-3'	5'-GCCAGAGTCTTTGCAGTTCC-3'		60°C
Sag_B09	5'-GCTTATTTTAACCAGCGGAAG-3'	5'-TGCTTTCAGAAGGACAAGTTCA-3'		60°C
S15	5'-GCTTGACAGCCCAAAGC-3'	5'-GCCACGGATAGACACCATC-3'	Bardeleben et al., 2004	60°C
Acar19	5'-GAAAAGTAGTGGGGCATTGG-3'	5'-AGTTTCCCAAGAAAACCCGT-3'	Wordley et al., 2011	Touchdown
Acar11	5-'AGTTTCCCAAGAAAACCCGT-3'	5'-GGGTTGCTCGTTCTGGACTA-3'	Wordley et al., 2011	Touchdown
Acar8	5'-CCCAATAGAGGAAAGGGACC-3'	5'-AGAATCACGCCTTCTGCTTT-3'	Wordley et al., 2011	Touchdown
S77	5'-GAGTAAAGGTCTGGGTCAGG-3'	5'-GCAGTACAAATACCACAGAGC-3'	Bardeleben et al., 2004	Touchdown
S94	5'-GAAAATCCTGTGAATCCTGTG-3'	5'-GATACTAATCAAAGCCACTGT-3'	Bardeleben et al., 2004	57°C
S63	5'-CCTTTAGCCTTGCCAGAGTC-3'	5'-CCTAAGCACTAAACAGATGCC-3'	Bardeleben et al., 2004	57°C
S68	5'-CTTCAGAGCAAGCGCAGGCAC-3'	5'-TCCCTCCTTCTTTTCCCTCCGAG-3'	Bardeleben et al., 2004	57°C

Table S2. Prior settings and posterior probabilities with 95% confidence intervals for the three competing demographic scenarios in the ABC analysis. A uniform distribution was used for all parameters. N_P = effective population size in the present, N_H = half of the census population size, N_U = effective population size for scenario 3, T = time in generations.

PRIOR SETTINGS					POSTERIOR PROBABILITY					
Island	**N_P**	**N_H**	**N_U**	**T**	**Scenario 1**		**Scenario 2**		**Scenario 3**	
1	200, 265	123, 133	265, 530	10, 100	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
5*	60, 120	50, 60	120, 240	10, 100	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
6*	250, 324	152, 162	324, 648	10, 100	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
204	40, 64	22, 32	64, 128	10, 100	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
305	120, 190	70, 80	190, 380	10, 100	1	[0.99, 0.99]	0	[0.00, 0.00]	0	[0.00, 0.00]
311	350, 445	200, 220	445, 890	10, 200	0.74	[0.36, 1.00]	0.06	[0.00, 0.27]	0.2	[0.00, 0.55]
312	100, 124	47, 57	124, 248	10, 100	1	[0.00, 0.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
314	110, 144	112, 122	144, 288	10, 200	0.98	[0.86, 1.00]	0	[0.00, 0.00]	0.02	[0.00, 0.14]
332	40, 63	22, 32	63, 126	10, 200	0.96	[0.79, 1.00]	0	[0.00, 0.00]	0.04	[0.00, 0.21]
922	150, 203	92, 102	203, 406	10, 400	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
926	300, 363	172, 182	363, 726	10, 200	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
930	280, 307	144, 154	307, 614	10, 300	0.7	[0.29, 1.00]	0.06	[0.00, 0.27]	0.24	[0.00, 0.61]
931	200, 250	115, 125	250, 500	10, 1000	0.56	[0.12, 0.99]	0.18	[0.00, 0.51]	0.26	[0.00, 0.64]
936	300, 362	171, 181	362, 724	10, 600	0.62	[0.19, 1.00]	0.1	[0.00, 0.36]	0.28	[0.00, 0.67]
Andrew*	150, 195	88, 98	195, 390	10, 100	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
WBC*	100, 156	68, 78	156, 312	10, 100	1	[1.00, 1.00]	0	[0.00, 0.00]	0	[0.00, 0.00]
Staniel	1000, 10000	200, 500	1000, 20000	10, 1000	0.1	[0.00, 0.36]	0.58	[0.15, 1.00]	0.32	[0.00, 0.73]

Table S3. Pairwise F_{ST} values and *P*-values after Bonferroni correction, where $P \leq 0.05$ are shown as + sign and $P > 0.05$ as - sign.

Island	1	5	6	204	305	311	312	314	332	922	926	930	931	936	Andrew	WBC	Staniel
1	0	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5*	0.03	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6*	0.04	0.08	0	+	-	+	+	+	+	+	+	+	+	+	+	-	+
204	0.25	0.27	0.21	0	+	+	+	+	+	+	+	+	+	+	+	+	+
305	0.04	0.08	0.01	0.2	0	+	+	+	+	+	-	+	+	+	+	+	+
311	0.06	0.13	0.08	0.28	0.06	0	+	+	+	+	+	+	+	+	+	+	+
312	0.12	0.17	0.12	0.29	0.1	0.07	0	+	+	+	+	+	+	+	+	+	+
314	0.22	0.26	0.17	0.37	0.17	0.23	0.16	0	-	+	+	+	+	+	+	+	+
332	0.19	0.23	0.13	0.34	0.14	0.21	0.13	0.01	0	+	+	+	+	+	+	+	+
922	0.19	0.24	0.15	0.39	0.17	0.23	0.24	0.25	0.23	0	+	+	+	+	+	+	+
926	0.09	0.14	0.08	0.22	0.03	0.09	0.12	0.2	0.18	0.21	0	+	+	+	+	+	-
930	0.09	0.13	0.08	0.26	0.09	0.15	0.17	0.2	0.17	0.2	0.11	0	+	+	+	+	+
931	0.07	0.13	0.04	0.25	0.05	0.08	0.12	0.2	0.17	0.21	0.12	0.1	0	+	+	+	+
936	0.07	0.1	0.05	0.21	0.06	0.12	0.14	0.19	0.16	0.21	0.12	0.05	0.07	0	+	+	+
Andrew*	0.07	0.1	0.06	0.26	0.06	0.11	0.13	0.2	0.16	0.17	0.1	0.07	0.06	0.05	0	+	+
WBC*	0.03	0.09	0.01	0.25	0.02	0.06	0.12	0.18	0.15	0.17	0.09	0.09	0.04	0.07	0.06	0	+
Staniel	0.06	0.11	0.05	0.24	0.05	0.06	0.06	0.16	0.14	0.17	0.07	0.05	0.07	0.08	0.05	0.06	0

Table S4. Group membership of individuals obtained from the DAPC analysis and reassignment to sampling populations.

	Cluster												
Island	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0	0	1	0	0	0	0	0	0	3	26	0	0
5*	0	0	0	0	31	2	0	0	0	0	2	0	0
6*	0	0	1	0	3	0	1	0	0	14	12	1	0
204	0	0	0	19	0	0	0	0	0	0	0	0	0
305	0	0	25	0	0	0	0	0	1	0	0	0	0
311	0	0	0	0	0	0	1	0	20	0	1	0	0
312	0	0	0	0	0	0	0	0	0	3	26	0	0
314	0	0	0	0	0	0	0	0	0	2	0	30	0
332	0	0	0	0	0	0	0	11	1	0	0	0	0
922	22	0	0	0	0	0	0	0	0	0	0	0	0
926	0	29	1	0	0	0	0	0	0	0	0	0	0
930	0	0	0	0	0	0	26	0	0	0	0	0	0
931	0	0	0	0	0	0	0	0	0	0	0	0	35
936	0	0	0	0	0	29	0	0	0	0	1	0	0
Andrew*	0	0	8	0	0	0	0	0	0	20	0	0	0
WBC*	0	1	0	0	1	0	0	0	0	16	14	0	0
Staniel	0	0	1	0	0	0	1	0	0	12	15	0	0

Table S5. Migration rates among islands are consistently low. The migration rate is the proportion of individuals with immigrant ancestry on islands in rows, immigrating from islands in columns. Migration rates >0.1 are shown in italics.

Island	1	5*	6*	204	305	311	312	314	332	922	926	930	931	936	Andrew*	WBC*	Staniel
1	*0.73*	0.04	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.01
5*	0.06	*0.79*	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01
6*	0.01	0.01	*0.73*	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.03	*0.11*	0.01
204	0.01	0.01	0.01	*0.83*	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01
305	0.01	0.01	0.01	0.03	*0.67*	0.01	0.01	0.01	0.01	0.01	0.06	0.01	0.01	0.01	0.01	*0.12*	0.01
311	0.01	0.01	0.01	0.01	0.01	*0.78*	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.07	0.01
312	0.01	0.01	0.01	0.01	0.01	0.08	*0.77*	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
314	0.01	0.01	0.01	0.01	0.01	0.01	0.01	*0.82*	0.01	0.07	0.01	0.01	0.01	0.01	0.01	0.01	0.01
332	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1	*0.68*	0.04	0.01	0.01	0.01	0.01	0.03	0.01	0.01
922	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	*0.84*	0.01	0.01	0.01	0.01	0.03	0.01	0.01
926	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	*0.81*	0.04	0.01	0.01	0.01	0.01	0.01
930	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	*0.76*	0.01	*0.11*	0.01	0.01	0.01
931	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	*0.84*	0.03	0.02	0.01	0.01
936	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	*0.8*	0.09	0.01	0.01
Andrew*	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.03	0.01	*0.83*	0.01	0.01
WBC*	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	*0.82*	0.01
Staniel	0.03	0.01	0.02	0.01	0.01	0.05	0.04	0.01	0.01	0.01	0.01	0.09	0.01	0.01	0.01	0.01	*0.67*

Table S6. Migration rates for separate analysis of the northern islands, central islands and southern islands.

North

Island	204	305	311	312	314
204	*0.92*	0.04	0.02	0.01	0.01
305	0.04	*0.89*	0.01	0.01	0.04
311	0.01	*0.12*	*0.83*	0.03	0.01
312	0.01	0.01	*0.11*	*0.81*	0.06
314	0.01	0.01	0.01	0.01	*0.96*

Center

Island	332	922	931	936	930
332	*0.84*	0.10	0.02	0.02	0.02
922	0.01	*0.95*	0.01	0.01	0.01
931	0.01	0.02	*0.92*	0.04	0.01
936	0.01	0.01	0.07	*0.89*	0.02
930	0.01	0.01	0.01	*0.15*	*0.81*

South

Island	1	5	6	Andrew	926	Staniel	WBC
1	*0.75*	0.04	*0.13*	0.01	0.01	0.01	0.05
5	0.07	*0.82*	0.06	0.01	0.01	0.01	0.03
6	0.01	0.01	*0.76*	0.05	0.01	0.04	*0.13*
Andrew	0.01	0.02	0.01	*0.89*	0.02	0.02	0.03
926	0.01	0.01	0.01	0.01	*0.85*	*0.11*	0.01
Staniel	0.04	0.01	0.02	0.01	0.02	*0.88*	0.02
WBC	0.01	0.01	0.07	0.02	0.01	0.02	*0.86*

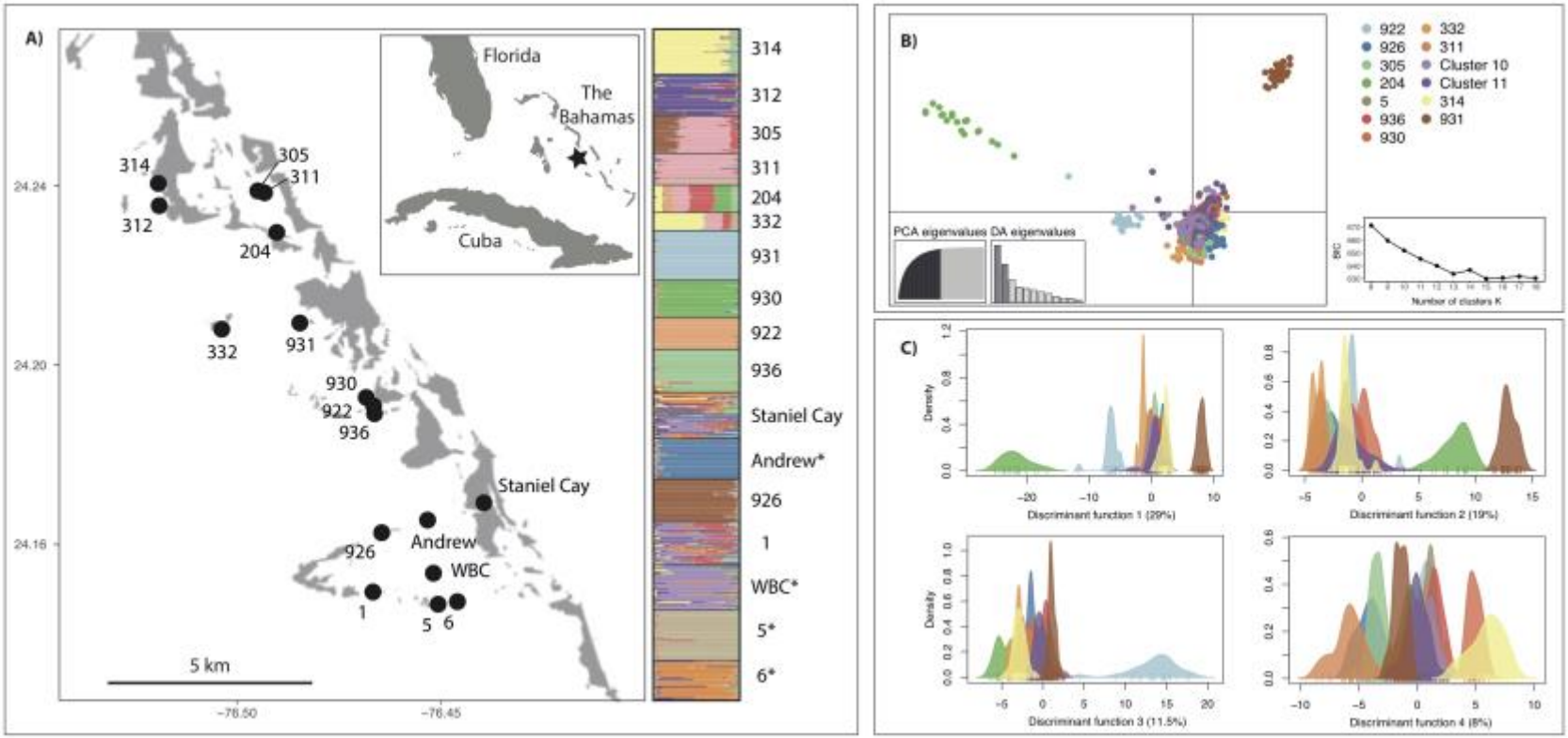
A)
Florida
The Bahamas
Cuba
314
312
305
311
204
332
931
930
922
936
Staniel Cay
Andrew
926
WBC
1
5
6
5 km
24.24
24.20
24.16
−76.50
−76.45
314
312
305
311
204
332
931
930
922
936
Staniel Cay
Andrew*
926
1
WBC*
5*
6*
B)
922
926
305
204
5
936
930
332
311
Cluster 10
Cluster 11
314
931
PCA eigenvalues
DA eigenvalues
BIC
Number of clusters K
C)
Density
Discriminant function 1 (29%)
Discriminant function 2 (19%)
Discriminant function 3 (11.5%)
Discriminant function 4 (8%)

Figure 1. A) Study site near Staniel Cay, Bahamas. Black circles indicate sampled island populations of *Anolis sagrei*. Bar plot on the right shows result from Bayesian cluster analysis. Individual genotype data from 10 microsatellite markers are arranged in horizontal lines and populations are arranged matching the geographic distribution from north to south (top to bottom). The STRUCTURE analysis revealed 13 genetic clusters. Colors correspond to the assignment probability of individual genotypes as a proportion of genetic clusters. Experimentally colonized populations are indicated with asterisks. B) DAPC analysis groups the 17 island populations in 13 genetic clusters (96.9% of total variance). Scatter plot of the first two axes and BIC values for varying number of clusters. Individuals from multiple islands were assigned to cluster 10 and 11 (Table S4). C) Density plots of the first four discriminant functions of the DAPC analysis. Percentage indicates the variance explained by each axis.

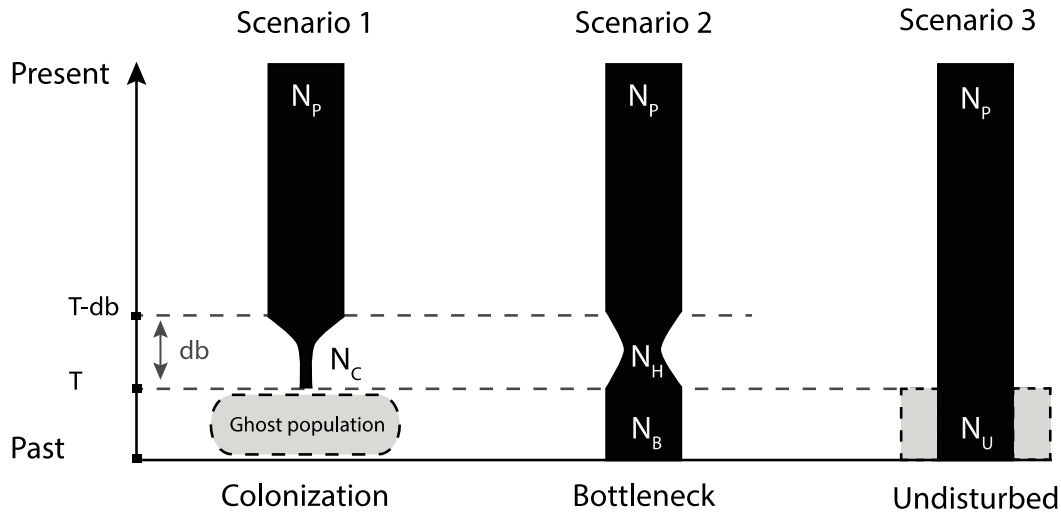

Figure 2. Schematic graphical representation of the three demographic scenarios used for ABC analysis. Scenario 1 simulated a colonization event from a larger unsampled population (ghost population) at time T. The effective number of colonizers N_C was set to 1-10 individuals. The effective population size of the ghost population was set to 1000-10000. The duration of the bottleneck (db) following the colonization event was set between 1-5 generations. Scenario 2 simulated a bottleneck event in which the effective population size (N_B) is reduced to half (N_H) at time T. N_B is equal to the effective population size of N_P, the present population. The census population size estimates were used as upper bound for N_P. Scenario 3 simulated a stable population, in which the past effective population size (N_U) can be as large as double the current effective populations size (N_P).

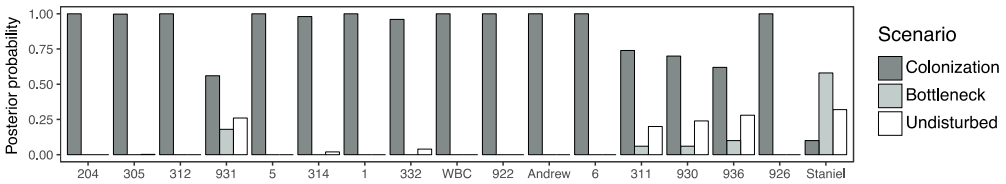

Figure 3. Posterior probabilities for the three demographic scenarios of 17 island populations using Approximate Bayesian Computation, indicating a preponderance of support for colonization scenarios, with sporadic support for bottlenecks and relative stability of populations. Islands are ordered by island size (vegetated area), starting with the smallest island on the left.

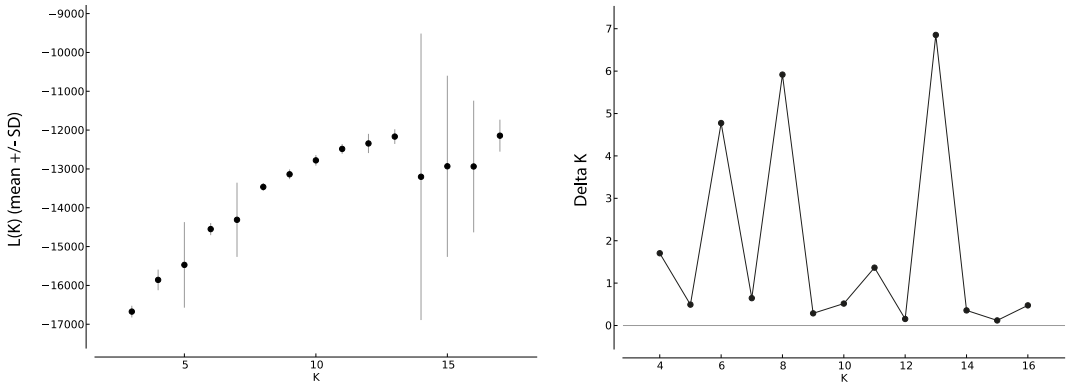

Figure S1. Model comparison of the STRUCTURE analysis for a sequentially increasing number of clusters (K). left: large log likelihood L(K) values indicate a good fit of the model. Shown are mean values and standard deviation for L(K) of 10 independent runs per K clusters. Right: large delta K values indicate that 13 clusters are the best fitting model, using the Evanno method.

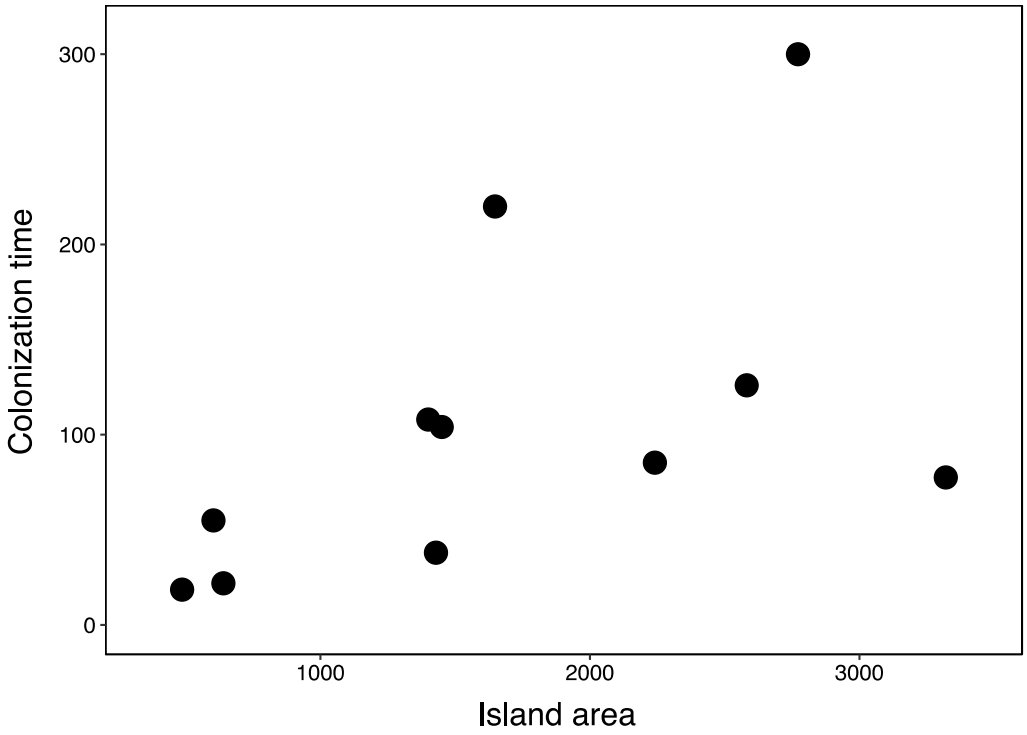

Figure S2. Colonization time increases with island area, in that larger islands have persisted for longer and smaller islands were colonized more recently. Spearman correlation r = 0.65, $P = 0.03$. The colonization time for island 931 was substantially older than the remaining populations with a 95% CI of 34.7 – 963 generations and was excluded from the analysis.

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CHAPTER 2

HEAD SIZE OF MALE AND FEMALE LIZARDS INCREASES WITH INTRASPECIFIC COMPETITION AMONG ISLAND POPULATIONS IN THE BAHAMAS

This chapter is in preparation for publication in Breviora

Johanna E. Wegener[1], Kevin P. Mulder[2], Robert M. Pringle[3], Jonathan B. Losos[4], Jason J. Kolbe[1]

[1]*Department of Biological Sciences, University of Rhode Island, Kingston RI, 02881*

[2]*CIBIO/InBIO, Research Center in Biodiversity and Genetic Resources, Rua Padre Armando Quintas 7, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*

[3]*Department of Ecology and Evolutionary Biology, Princeton University, Princeton NJ, 08542*

[4]*Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, 02138, USA*

Key-words: head size, male - male competition, intraspecific aggression, sexual dimorphism, Bahamas, Staniel Cay

Abstract

In polygynous lizards, male-male competition is an important driver of morphological traits associated with dominance during intraspecific encounters. The extent to which females engage in aggressive behavior and thus contribute to phenotypic evolution is not well studied. Most previous studies rely on population density as a proxy for intraspecific competition and lack measures that may better reflect aggressive behavior. We used injury frequency of male and female brown anoles (*Anolis sagrei*) from 16 island populations to test whether aggressive encounters increase with population density. We further asked whether intraspecific competition is a potential driver of phenotypic traits related to dominance. We found that populations with higher densities had higher proportions of injuries, consistent with increased intraspecific competition. Relative head size (both length and width) of male and female lizards increased with population density, suggesting that larger heads might be advantageous when competition is higher. Morphological changes and injuries were detected in both males and females and show that females play a more active role in intraspecific competition than previously appreciated. More studies are needed to determine whether female aggressive encounters are restricted to intrasexual competition and how morphological traits of females are related to dominance and reproductive success.

Introduction

In many vertebrate species, males compete for mating opportunities and engage regularly in aggressive male-male interactions (Knell 2009; Kokko & Rankin 2006). In polygynous species, dominant males mate with the majority of females in their territory, whereas subordinate ones reproduce substantially less, if at all (Kokko & Rankin 2006). Thus, the ability to obtain and defend territories disproportionally increases reproductive success for males. One key prediction is that sexual selection shapes phenotypic traits associated with dominance and performance during aggressive male-male interactions.

In lizards, morphological traits associated with dominance and performance in male-male competition are well characterized. Larger males hold larger territories in the wild (Lappin & Husak 2005) and tend to "win" over smaller ones in staged encounters (Jenssen *et al.* 2005; Lailvaux *et al.* 2004). When equally sized males are matched, head size and bite force are key predictors of dominance (Gvozdík & Damme 2003; Lailvaux *et al.* 2004; Perry *et al.* 2004). In the majority of species, body size is correlated with head size and bite force and thus is the primary trait linked to reproductive success of males (Herrel *et al.* 2001; Herrel *et al.* 2007; Herrel *et al.* 2010; Verwaijen *et al.* 2002; Wittorski *et al.* 2016; but see Lappin & Husak 2005). While the idea that sexual selection shapes body size and head size has been supported empirically in several lizard species (Donihue *et al.* 2015; Jenssen *et al.* 2000; Lailvaux *et al.* 2004; Perry *et al.* 2004), most studies rely on population density as a proxy for intraspecific competition and lack more direct measures for aggressive behavior. Additionally, the extent to which females engage in aggressive behavior and

contribute to phenotypic evolution is not well studied because past studies mostly focus on males.

Females are often described as less aggressive (Claessen *et al.* 2000) and less territorial (Nunez *et al.* 1997; Schoener & Schoener 1982), devoting more time to resource acquisition than competition with conspecifics. In several species, females have smaller body and head size and disproportionally weaker bite force than males, even when corrected for body size (Lappin *et al.* 2006; Wittorski *et al.* 2016). The observed sexual dimorphism led to the interpretation that intraspecific competition plays a minor role in shaping morphological characters of female lizards (Herrel *et al.* 2010; Lappin *et al.* 2006; Wittorski *et al.* 2016). However, the few available studies that include females suggest that aggressive behavior might be a relevant driver of morphological characters (Calsbeek & Smith 2007; Comendant *et al.* 2003).

Injuries are a frequent consequence of intraspecific interactions and can be used as a proxy for aggressive behavior (Donihue *et al.* 2015; Gvozdik 2000). Biting and jaw locking during agonistic encounters leave bite scars and amputated body appendages, such as claws, digits, limbs and tails (Brock *et al.* 2015; Donihue *et al.* 2015; Vervust *et al.* 2009). While limb related injuries are associated with intraspecific aggression, tail amputations are more ambiguous (Brock *et al.* 2015; Gvozdik 2000; Schoener & Schoener 1980). The frequency of tail amputations can vary with species richness and type of predators as well as intraspecific aggression (Brock *et al.* 2015; Cooper *et al.* 2004; Pafilis *et al.* 2009). Tails are shed more easily (i.e., requiring lower bite force) in populations with predators as compared to predator-

free populations, making interpretations of tail loss more complex (Brock *et al.* 2015; Cooper *et al.* 2004; Pafilis *et al.* 2009).

In this study, we use brown anoles (*Anolis sagrei*) from 16 island populations to test whether intraspecific competition is a potential driver of phenotypic traits in males and females. We use the frequency of injuries as a proxy for intraspecific aggressive encounters. If intraspecific competition is higher in populations with higher densities, we expect the proportion of injuries to increase with population density. Larger body and head size enhance dominance and performance during male–male competition (Gvozdík & Damme 2003; Jenssen *et al.* 2005; Lailvaux *et al.* 2004; Perry *et al.* 2004), but little is known about female-female interactions. Therefore, if dominance related traits are advantageous during aggressive encounters, we expect larger lizards with larger heads in higher density populations.

Material and Methods

Sampling and morphological measurements

Male and female brown anoles were collected in 2011 on 16 small islands in the Bahamas near Staniel Cay where they were the only lizard species present (N = 419; N_{female} = 177; N_{male} = 242, Table 1). We took x-ray and scanner images of live lizards. We scored injuries as the number of missing claws, digits and limbs from the x-ray and scanner images. We measured body size (snout-vent length, or SVL), head length, and head width from x-ray images using the plug-in ObjectJ for the software package Image J (Abràmoff *et al.* 2004). We used Mosimann's geometric mean (Mosimann 1970) to correct for the effect of body size following Butler & Losos (2002). We

calculated the geometric mean of all measured traits as an overall measure of size (SIZE) and used the log transformed ratio of each trait to SIZE (log(trait/SIZE)).

Population density was calculated using the estimated number of individuals per vegetated island area. We used vegetated area, rather than island size, to better represent the actual usable habitat of the lizards. We estimated population size with a log-linear capture-recapture method (Heckel & Roughgarden 1979) based on a three-day census on each island.

Injury and intraspecific competition

As a proxy for intraspecific aggressive encounters, which we predict will increase with population density, we calculated the proportion of injured lizards on each island. We used a linear model to test whether the percentage of injured lizards is related to population density. We then compared injury percentages between males and females using Welch's t-test to test for sex differences in aggressive encounters. Percentages were arcsine transformed prior to the analysis. All statistical analyses were conducted in R v3.3.2 (Team 2016).

Morphological differentiation and intraspecific competition

To test whether variation in body size and head shape is related to population density, we used separate linear mixed models for each trait. We included sex and population density as fixed effects and island as a random effect (Pinheiro *et al.* 2015). Since the morphology of males and females might be affected differently by population density, we included sex*density as interaction term. For instance, if

selection on phenotypic traits is stronger for males, we would expect a steeper slope for the relationship between a given trait and population density. If morphological traits of the sexes are similarly affected, we expect no differences in slope between males and females, resulting in a non-significant interaction term. Non-significant interaction terms were excluded from the analysis.

Results

We tested whether the percentage of lizards injured, a proxy for aggressive encounters, increases with population density. Injury percentage was higher in populations with higher densities ($P = 0.05$; t = 2.15; DF = 14; $R^2 = 0.25$; Fig 1), suggesting an increase in intraspecific aggression. Injury percentage for females was more variable among populations and on average higher than for males (Females = 0.29% ± 0.20%; Males = 0.24% ± 0.12%), however, the difference was not significant ($P = 0.62$; t = 0.49; DF = 26.12; Fig 2).

We tested whether body size and head shape varies with population density in the direction predicted by natural selection. Overall, males were larger than females ($P < 0.001$; t = 41.03; DF = 402; Table 2, Fig 3). Body size did not vary with population density ($P = 0.31$; t = -1.04; DF = 14; Table 2) and the interaction term (sex*density) was not significant. The random effect of island accounted for 13.8 % of the total variance in body size. Females had relatively longer heads than males, but relative head width did not differ between the sexes (Fig 3). Relative head length and head width increased with population density (head length: $P = 0.003$; t = 3.68; DF = 14; head width: $P = 0.05$; t = 2.19; DF = 14; Table 2; Fig 4), but the interaction terms were

not significant. The random effect of island explained 12.2% of the total variance for relative head length and 20.9% of variance for relative head width.

Discussion

How morphological traits of males and females relate to intraspecific competition is critical for advancing our understanding of non-random evolutionary processes in polygynous mating systems (Kokko & Rankin 2006). Using morphological data from 16 island populations, we found evidence that intraspecific competition plays a role in shaping head proportions of male and female *A. sagrei*. Lizards in high-density populations had a higher proportion of injuries as compared to low-density populations, suggesting the former experience higher levels of intraspecific aggression (Donihue *et al.* 2015). Lizards in these high-density populations had larger heads (longer and wider, corrected for body size), a trait associated with dominance during intraspecific interactions (Gvozdík & Damme 2003; Lailvaux *et al.* 2004; Perry *et al.* 2004). Relative head size increased for both sexes and injuries were detected in males and females, suggesting that females play an active role in intraspecific interactions (Calsbeek 2009; Calsbeek & Smith 2007; Donihue *et al.* 2015). Thus, dominance related traits are likely advantageous for both males and females when intraspecific competition is high.

The relationship between head proportions and dominance during agonistic encounters in males is well documented (Gvozdík & Damme 2003; Lailvaux *et al.* 2004; Perry *et al.* 2004). In several lizard species, larger head proportions increase bite force, which is associated with larger territory size and reproductive success (Donihue

et al. 2015; Herrel *et al.* 2010). Our data are in agreement with this prediction and show that both sexes have larger heads (longer and wider heads relative to their body size) in populations with higher densities. In males, natural selection might drive head shape variation due to the beneficial effect of bite force, similar to patterns observed in other lizard species (Donihue *et al.* 2015; Herrel *et al.* 2010). Whether head size in females increases social dominance and reproductive success remains to be determined in future studies.

In agreement with previous studies, our data show that the number of aggressive encounters leading to injuries increases with population density (Donihue *et al.* 2015; Vervust *et al.* 2009). However, since populations with higher densities have larger heads, and head size typically increases bite force (Herrel *et al.* 2001; Herrel *et al.* 2007; Herrel *et al.* 2010; Verwaijen *et al.* 2002; Wittorski *et al.* 2016), we acknowledge that injuries might be more frequent in high-density populations because of greater bite force rather than increased aggressive encounters. Future studies could measure bite force in these populations to evaluate this possibility.

The overall proportion of injuries in our study did not differ between males and females, suggesting that both sexes engage in aggressive behavior. Our findings are inconsistent with the assumption that males engage substantially more in aggressive behaviors than females, in this polygynous mating systems (Kokko & Rankin 2006). Moreover, empirical studies fail to establish any clear pattern related to sex and injury, with some studies showing no difference between the sexes (Donihue *et al.* 2015; Vervust *et al.* 2009), others showing a higher proportion of injuries in males (Gvozdik 2000) or a higher proportion in females (Passos *et al.* 2013). Whether, and if so how,

the sexes differ in aggressive behavior needs to be determined in future studies by including behavioral observations of natural populations or behavioral experiments including females.

Conclusion

Our findings highlight that the current understanding of polygynous mating systems in *Anolis* lizards is incomplete with respect to females (Kamath & Losos 2017) and their role in intraspecific competition. Sexual dimorphism and head shape variation have been attributed to sexual selection, enhancing male performance during agonistic encounters (Donihue *et al.* 2015; Jenssen *et al.* 2005; Lailvaux *et al.* 2004; Perry *et al.* 2004). This conclusion is mainly based on the assumption that females engage substantially less in aggressive and territorial behavior. Our data show that relative head size, a trait related to dominance, increased in both males and females with increasing population density. Injuries were detected in both sexes, suggesting that females may engage in aggressive intraspecific encounters as well. Previous associations of head size and bite force indicate that both males and females might benefit from enhanced performance when levels of competition are high (Calsbeek 2009; Calsbeek & Smith 2007; Donihue *et al.* 2015; Herrel *et al.* 2010). Our data suggest that females play a more active role in intraspecific competition than previously assumed. More studies are needed to examine whether female aggressive encounters are restricted to intrasexual interactions or involve both sexes, and whether female dominance increases reproductive success, similar to the pattern observed in males.

Acknowledgments

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Table 1. Sample sizes of male and female lizards, island area, population density and injury percent for the 16 island populations of brown anoles. N = number of individuals, F = female, M = male, area = vegetated area in m^2, density = population density.

Island	N_F	N_M	Area	Density	Injury %
1	16	12	1429	0.19	0.36
5	15	15	1333	0.09	0.32
6	15	16	1851	0.18	0.32
204	7	10	487	0.13	0.41
305	9	15	603	0.31	0.50
311	7	14	2241	0.20	0.43
312	15	16	640	0.19	0.26
314	17	13	1400	0.10	0.20
332	6	5	1450	0.04	0.17
922	4	18	1648	0.12	0.18
926	17	13	3320	0.11	0.03
930	12	16	2582	0.12	0.07
931	12	18	1070	0.23	0.17
936	7	23	2772	0.13	0.20
Andrew	8	18	1758	0.11	0.26
WBC	10	20	1575	0.10	0.37

Table 2. Generalized linear mixed model with density and sex as fixed factors and island as random factor. Non-significant interactions were removed for the final models. SE = standard error, DF = degrees of freedom, *t* = *t*-value, *P* = *P*-value.

	Value	SE	DF	*t*	*P*
SVL					
Density	-0.88	0.84	14	-1.05	0.31
Sex	13.54	0.33	402	41.04	<0.001
HEAD LENGTH					
Density	0.02	0.01	14	3.68	0.003
Sex	-0.05	0.00	402	-23.80	<0.001
HEAD WIDTH					
Density	0.01	<0.01	14	2.19	0.05
Sex	<0.01	<0.01	401	-0.01	0.99

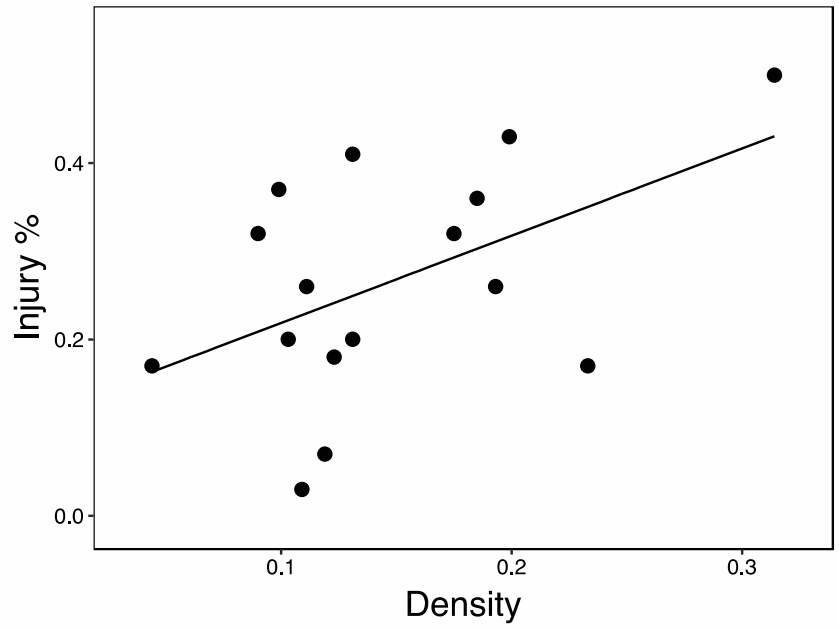

Figure 1. Injury percent increases with population density among the 16 island populations of brown anoles. Injury percent includes missing claws, digits and limbs, and males and females are combined for each island.

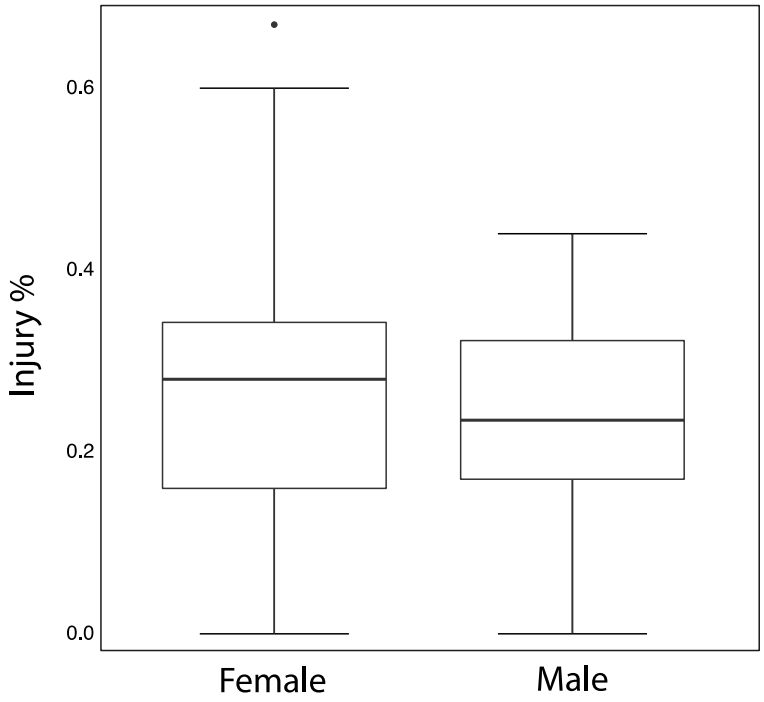

Figure 2. Boxplots showing the injury percentage for males and females from the 16 island populations.

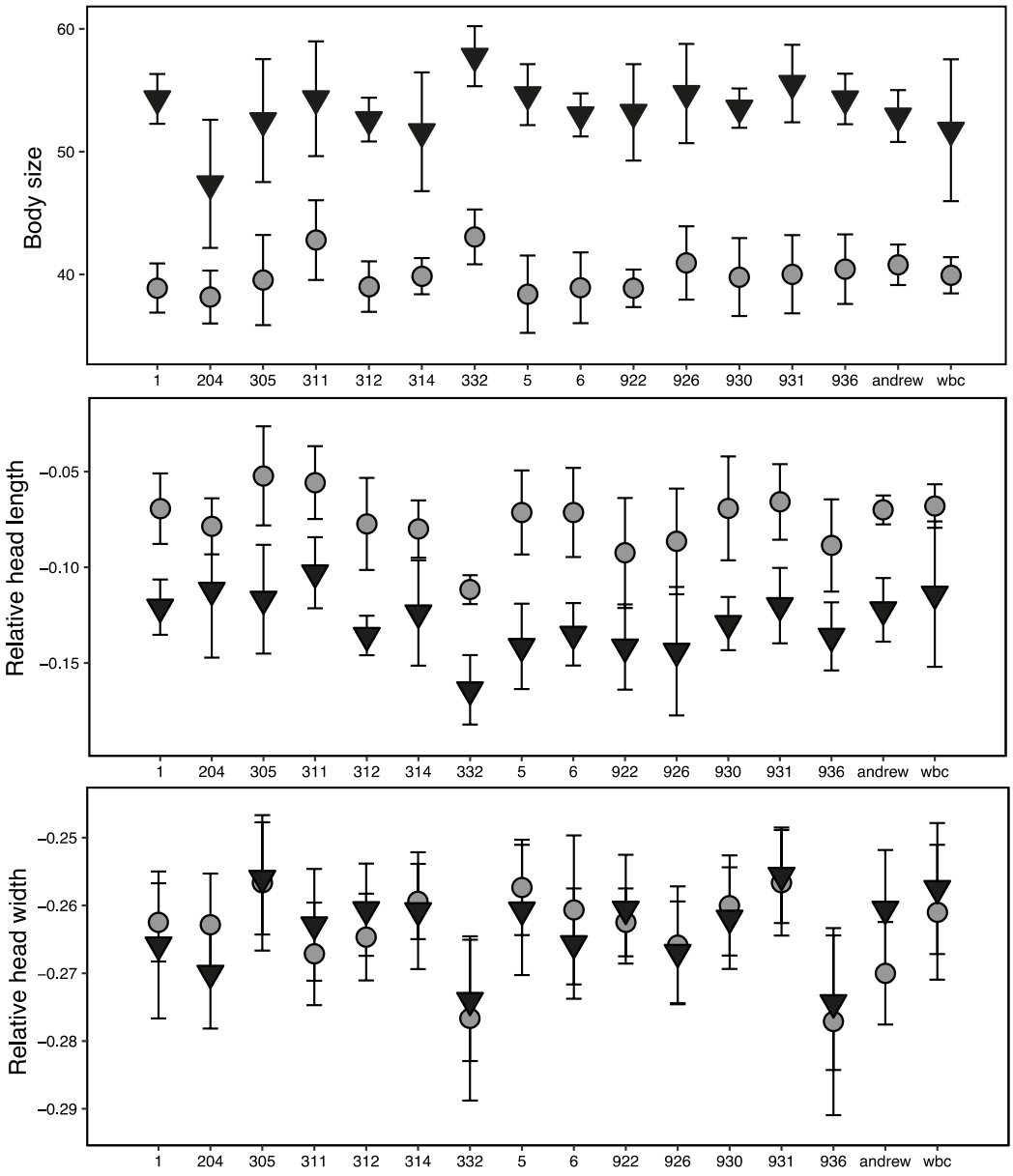

Figure 3. Variation in body size (SVL) and relative head shape for males (black triangles) and females (gray circles) among the 16 island populations. Males are larger than females (top). Relative head length is longer in females (middle). Relative head width did not differ between males and females (bottom).

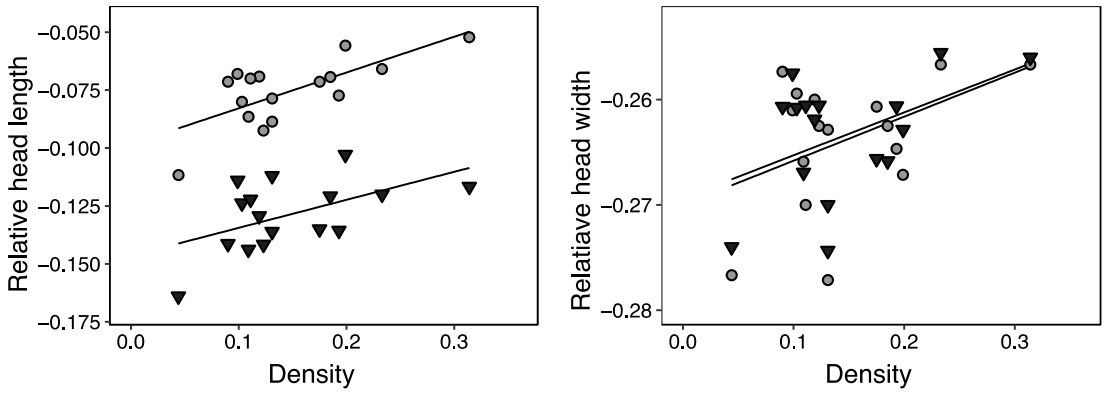

Figure 4. Head proportions increase with population density in males (black triangles) and females (gray circles). Head proportions are relative head length (left) and relative head width (right).

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CHAPTER 3

HYBRIDIZATION AND RAPID DIFFERENTIATION AFTER SECONDARY CONTACT BETWEEN THE NATIVE GREEN ANOLE (*ANOLIS CAROLINENSIS*) AND THE INTRODUCED CUBAN GREEN ANOLE (*A. PORCATUS*)

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Johanna E. Wegener[1], Adam Moreno[2], Jessica Atutubo[1], Jessica N. Pita-Aquino[3] and Jason J. Kolbe[1]

[1]*Department of Biological Sciences, University of Rhode Island, Kingston, RI, 02881*

[2]*Department of Biology, Brown University, Providence, RI, 02912*

[3]*Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, PR, 00931*

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Abstract

In allopatric species, reproductive isolation evolves through the accumulation of genetic incompatibilities. The degree of divergence required for complete reproductive isolation is highly variable across taxa, which makes the consequences of secondary contact between allopatric species unpredictable. Since the Pleistocene, two species of *Anolis* lizards, *A. carolinensis* and *A. porcatus*, have been allopatric, yet this period of independent evolution has not led to substantial species-specific morphological differentiation and therefore they might not be reproductively isolated. In this study, we determined the genetic consequences of localized, secondary contact between the native green anole, *A. carolinensis*, and the introduced Cuban green anole, *A. porcatus*, in South Miami. Using one mitochondrial marker and 18 microsatellite loci, we found cyto-nuclear discordance for lizards sampled in South Miami, which is indicative of hybridization. Thirty-five percent of mitochondrial haplotypes were derived from *A. porcatus* from near Havana in West Cuba and 65% from *A. carolinensis* from South Florida. On the nuclear level, the South Miami population is homogeneous and genetically distinct from reference populations of the parental species. Maximum likelihood simulations confirm historic gene flow and suggest that ~33% of the nuclear ancestry in South Miami is derived from *A. porcatus*. Our results suggest that reproductive barriers between *A. porcatus* and *A. carolinensis* are weak or absent and that secondary contact has led to hybridization in South Miami. Thus, genetic evidence for successful hybridization, as well as morphological similarities between the two species reinforces a proposal to revise the taxonomy of *A. carolinensis* and *A. porcatus* from West Cuba.

Introduction

In allopatric species, reproductive isolation evolves through the accumulation of genetic incompatibilities in geographically separated lineages (Dobzhansky and Dobzhansky 1937; Orr 1995). As divergence time increases, negative epistatic interactions that reduce hybrid viability become more likely. However, the degree of divergence required for complete reproductive isolation is highly variable across taxa (Bolnick and Near 2005; Wiens, Engstrom, and Chippindale 2006; Stelkens, Young, and Seehausen 2010; Martin et al. 2017), making the consequences of secondary contact between allopatric species unpredictable. When reproductive barriers are weak, secondary contact between previously isolated lineages (e.g. native and introduced species) can lead to hybridization (Prentis et al. 2008; Schierenbeck and Ellstrand 2009), rapidly homogenize parental genotypes, erode species boundaries (James and Abbott 2005; Ward et al. 2012; Hasselman et al. 2014; Glotzbecker, Walters, and Blum 2016) and threaten the genetic integrity of native species (Jiggins and Mallet 2000; Brennan et al. 2015). Because species introductions are often pulse-like and localized, recombination and repeated backcrossing can result in complete admixture of parental genotypes and erase genetic signatures of hybridization within only few generations (Lombaert et al. 2011; Ward et al. 2012; Hasselman et al. 2014; Roy et al. 2015; Glotzbecker, Walters, and Blum 2016).

Empirical studies that document genetically cryptic hybridization patterns are rare (James and Abbott 2005; Kronforst et al. 2006; Mims et al. 2010; Keller et al. 2014; Lavretsky et al. 2015) and strong inferences often require sampling of reference populations of parental species as well as cytoplasmic and nuclear markers. After

several generations of backcrossing and recombination, reference populations of parental genotypes are necessary to distinguish hybrid populations from subpopulations of parental species and trace the origin of individual nuclear markers (Della Croce, Poole, and Luikart 2016). For introduced species, this can be challenging since the geographic location of the source population is often unknown or includes multiple source locations, and pure native populations might be genetically swamped by introduced genotypes (Caracristi and Schlötterer 2003; Kolbe et al. 2004; Kronforst et al. 2006; Kolbe et al. 2007; Della Croce, Poole, and Luikart 2016). In addition to multi-locus nuclear markers, non-recombining cytoplasmic markers are useful for identifying hybrid ancestry when the nuclear genomes of hybrid populations are completely homogenized (Della Croce, Poole, and Luikart 2016). A mismatch between nuclear genotypes and cytoplasmic haplotypes is often the first step to identifying hybrid populations (Toews and Brelsford 2012). Subsequent population genetic analyses are then needed to distinguish between cytoplasmic introgression, past (including ancient) hybridization and incomplete lineage sorting (Della Croce, Poole, and Luikart 2016). In this study, we aim to reconstruct the invasion history of the Cuban green anole, *Anolis porcatus* and determine the genetic consequences of localized, secondary contact with the native Green anole, *A. carolinensis*, in south Florida (USA). We use multilocus nuclear genotypes and a mitochondrial haplotypes to distinguish between contemporary and past gene flow, allowing us to test whether secondary contact has eroded species boundaries or whether the two sister species are reproductively isolated and genetically distinct coexisting taxonomic units.

Anolis porcatus and *A. carolinensis* are allopatric species and have been

geographically separated since the Pleistocene (Campbell-Staton et al. 2012; Tollis and Boissinot 2014; Manthey et al. 2016). *Anolis carolinensis* is nested within a clade of *A. porcatus* from West Cuba, making the latter species paraphyletic (Glor et al. 2004; Glor, Losos, and Larson 2005). After the initial colonization of the Florida Peninsula, *A. carolinensis* has undergone substantial range expansion and differentiation resulting in five major clades. The current distribution ranges from south Florida to North Carolina and west to Texas (Campbell-Staton et al. 2012; Tollis and Boissinot 2014; Manthey et al. 2016).

The introduction of *A. porcatus* was first documented in Florida in the 1990s, based on morphological characters (Meshaka et al. 1997) and later confirmed genetically (Kolbe et al. 2007). Two individuals collected in Miami were genetically similar to *A. porcatus* in West Cuba, indicating the putative source population of the introduction (Kolbe et al. 2007). Since the 1940s, seven other non-native anole species from various locations in Cuba and in the Caribbean have established in Miami, leading to admixture among genetically distinct source populations in several cases (Kolbe et al. 2007). Despite widespread intraspecific admixture, hybridization between recognized species is considered rare among anoles (Losos 2009). A few cases are documented between closely related species, including *A. porcatus* x *A. allisoni* in Central Cuba (Glor et al. 2004) and *A. pulchellus* x *A. krugi* in Puerto Rico (Jezkova, Leal, and Rodríguez-Robles 2013). Hybridization between *A. carolinensis* and *A. porcatus* has been suggested repeatedly, mainly because the two species have no species-specific morphological characters despite considerable divergence time (Kolbe et al. 2007; Camposano 2011; Tollis 2013). Sufficient evidence for

reproductive isolation or lack thereof has not been shown.

In this study, we examine whether *A. porcatus* and *A. carolinensis* are reproductively isolated species, and characterize the genetic consequences of secondary contact in South Miami. We used one mtDNA marker and 18 nuclear microsatellite loci to test whether hybridization has occurred between the two species. We distinguished between contemporary and historic gene flow and estimated the timing of the admixture event. Discordance between nuclear and cytoplasmic markers is characteristic of hybridization and commonly used to identify hybrid individuals (Toews and Brelsford 2012). If the two species interbreed in South Miami, we expect a high frequency of individuals with mismatches between nuclear genotypes and mtDNA haplotypes.

Materials and Methods

Sample collection

We sampled 32 *Anolis carolinensis* individuals from the J.W. Corbett Wildlife Management in South Florida ~200 km north of Miami, 92 green anole individuals from the putative hybrid population in South Miami and 54 *A. porcatus* individuals from Western Cuba (Table S1). Genomic DNA was extracted from tail tips and liver tissue using a modified ethanol precipitation protocol.

Molecular methods

We amplified a region of 343-571bp of the mtDNA NADH dehydrogenase subunit 2 using primers from Campbell-Staton *et al*. (2012) and two newly designed primers (TableS2). A 50 μL reaction contained 5.0 μL of 10x standard PCR buffer

(New England Biolabs®Inc.), 3.0 μL of 10 mM dNTPs, 5.0 μL of 25 mM MgCl, 1.0 μL of 10 μM primer, 0.1 μL of 5 units/μl *Taq* polymerase (New England Biolabs®Inc.) and 4 μL of 50 ng/μL genomic DNA. Cycles started with initial denaturation at 94˚C for 2 min, followed by 35 cycles of 94˚C for 45 sec, T_m for 45 sec, 72˚C for 1 min and a final elongation step at 72˚C for 10 min. PCR products were purified and sequenced at the Rhode Island Genomics and Sequencing Center.

We amplified 18 microsatellite markers using PCR with fluorescently labeled primers. We used seven newly designed primers (Table S1) and 11 previously published primers (Wordley, Slate, and Stapley 2011). A 10 μL reaction contained 0.8 μL of 10x standard PCR buffer (New England Biolabs®Inc.), 0.8 μL of 10x BSA, 0.6 μL of 10 mM dNTPs, 1.50 μL of 25 mM MgCl, 0.24 μL of 10 μM primer, 0.08 μL of 5 units/μl *Taq* polymerase (New England Biolabs®Inc.) and 2 μL of 20 ng/μL genomic DNA. Cycles started with initial denaturation at 94˚C for 2 min, followed by 35 cycles of 94˚C for 45 sec, T_m for 45 sec, 72˚C for 1 min and a final elongation step at 72˚C for 10 min. Samples were genotyped at the DNA Analysis Facility on Science Hill at Yale University. Markers for all samples were analyzed with the software GeneMapper® v4.1 and visually checked to ensure accurate peak calling.

Phylogenetic analysis and haplotype divergence

To determine the species identity of mtDNA haplotypes for individuals sampled in South Miami and the geographic origin of the introduction, we constructed a maximum likelihood phylogeny including samples from the geographic range of both species (Fig. 1; Table S3). We included 111 individuals of *A. porcatus* from East and

West Cuba spanning the entire native range, 83 individuals of *A. carolinensis* sampled throughout Florida and 86 individuals from South Miami (Fig. 1A). *Anolis loysiana* was used as outgroup taxon. Sequences were aligned and visually inspected for accuracy using the MUSCLE plugin in Geneious v7.1.9 (Kearse et al. 2012). We collapsed individual sequences into distinct haplotypes using DNAcollapser implemented in FaBox v1.41 (Villesen 2007). To retain individuals with short mtDNA sequences, we generated two separate alignments. One alignment consisted of 571bp for 200 individuals, resulting in 156 haplotypes. The second alignment was 343bp long and included all 280 samples resulting in 182 haplotypes. We used RAxML v8.0 (Stamatakis 2006) implemented in the CIPRES Science Gateway v3.3 (Miller, Pfeiffer, and Schwartz 2011) to generate maximum likelihood phylogenies. Bootstrap values were obtained from 1000 iterations using rapid bootstrapping.

We used pairwise sequence divergence to determine the degree of nucleotide divergence between native and introduced *A. porcatus* haplotypes. We identified the genetically most similar individuals between introduced-range and Cuban haplotypes based on the fewest number of pairwise nucleotide differences. Pairwise sequence divergence was calculated as the number of nucleotide differences divided by the sequence length.

Population genetic statistics

In addition to one mtDNA locus, we genotyped 18 microsatellite loci for lizards sampled from the putative hybrid population in South Miami (MIA), five sampling locations of *A. porcatus* from West Cuba (WCU) and *A. carolinensis* from one

sampling location 200 km north of Miami (SFL; Fig 1). We calculated deviations from Hardy-Weinberg equilibrium (HWE) and pairwise F_{ST}-values in Genepop v1.2 (Rousset 2008). Allelic richness and heterozygosity was calculated using the R package *Poppr* v2.2.0 (Kamvar, Tabima, and Grünwald 2014; Kamvar, Brooks, and Grünwald 2015).

Population structure and differentiation

First, we performed a Bayesian cluster analysis with STRUCTURE v2.3.4 (Rosenberg 2004), using the admixture model and correlated allele frequencies. We allowed for gene flow among populations and modeled six different clustering scenarios, sequentially increasing the number of clusters K (1-6). We conducted 10 independent runs for each scenario with a burn-in of 500 000 and 1000 000 MCMC iterations. We used delta K to determine the most likely number of clusters following the Evanno method (Evanno, Regnaut, and Goudet 2005) implemented in STRUCTURE HARVESTER v0.6.94 (Earl and Vonholdt 2012). We combined the genotype proportions of each cluster (q-matrix) from 10 independent runs with CLUMPP (Jakobsson and Rosenberg 2007) and visualized the results with the R package ggplot2 v2.1.0 (Wickham 2011). We repeated the Bayesian cluster analysis with population pairs (SFL–MIA and WCU–MIA) and WCU separately to identify potential population substructure. Model parameters were used as described above. Second, we used discriminant analysis of principal components (DAPC) to determine the degree of differentiation between clusters using the R package *adegenet* (Jombart 2008). To characterize and find genetic clusters, DAPC uses a multivariate approach

and PCA transformed allele frequencies. In contrast to the Bayesian clustering approach, DAPC does not rely on specific population model assumptions, such as HWE. The number of clusters (K) was sequentially increased starting with one cluster. The model fit for K clusters was determined with the Bayesian Information Criterion (BIC).

Maximum likelihood and ABC modeling of historic admixture

To detect historic gene flow, we used a tree-based maximum likelihood approach with the program TreeMix (Pickrell and Pritchard 2012). This approach uses allele frequencies to model relatedness among populations as a non-bifurcating tree. Migration edges are added as additional branches to a bifurcating tree allowing for population ancestry from more than one parental populations. Migration edges are added stepwise to the tree model until the covariance of the model best matches the covariance of the data. Residual matrices were used to determine the model fit. Positive residuals indicate greater genetic variation in the population than explained by the simple tree model suggesting admixture (Pickrell and Pritchard 2012). The model assumes migration within a single generation. The fraction of alleles derived from migration is represented as weight of the migration edge.

To infer the timing of the admixture event, we used approximate Bayesian computation to model the demographic history of the three populations using DIY-ABC v2.0 (Cornuet et al. 2014). A set of summary statistics was used to assess the fit between simulated datasets and empirical data. We used mean number of alleles, mean genetic diversity, pairwise F_{ST}-values and the maximum likelihood coefficient of

admixture λ (Choisy, Franck, and Cornuet 2004). The demographic scenario simulates divergence between SFL and WCU and a more recent admixture event that gave rise to the MIA population. The prior for the divergence between SFL and WCU was set between [6000 000–13 000 000] generations, based on previous divergence time estimates (Campbell-Staton et al. 2012; Tollis and Boissinot 2014). We set the prior for the effective population size as [100–10 000] using a uniform prior distribution. To estimate timing of the admixture event, we used a prior of [1–4000] generations assuming one generation per year. We simulated 1000 000 datasets and used the 1000 dataset with the smallest Euclidean distance to the empirical data for parameter estimation.

Results

Phylogenetic analysis and mtDNA haplotype divergence

We constructed a maximum likelihood phylogeny from mtDNA haplotypes to determine the haplotype identity of individuals sampled in South Miami. Individuals sampled in South Miami (N=86) were not monophyletic. Thirty samples (35%) representing six haplotypes were nested within a well-supported clade of *A. porcatus* from West Cuba. Fifty-six samples (65%) clustered with *A. carolinensis* from South Florida, representing 20 haplotypes (Fig. 1C). Haplotypes from *A. carolinensis* and *A. porcatus* were co-distributed across the study area in South Miami (Fig. 1B).

The 571bp alignment resulted in a total of 155 haplotypes, 41 from South Florida, 43 from Central and north Florida, 52 from West Cuba and 19 from East Cuba. *Anolis porcatus* from West Cuba is sister to the monophyletic *A. carolinensis* (Fig S1). Since,

the 343bp alignment resulted in an overall similar tree topology (Fig. S2), we focus on the 571bp in the main text. Individual haplotypes and sampling locations for the 343bp alignment can be accessed in the supplementary material.

Introduced *A. porcatus* haplotypes were nested in a well-supported clade of *A. porcatus* from seven sampling locations near Havana in West Cuba (Figure 1A). Branches within the clade were not well supported (bootstrap < 95), which limits more specific identification of potential source locations of the introduction. Average sequence divergence between introduced *A. porcatus* haplotypes and the genetically most similar ones from Cuba ranged from 0.0–1.75% divergence (mean = 1.14 ± 0.68%; Table 1). One individual from South Miami (MIA640) shared the same haplotype with one individual from Havana (JJK2796).

Genetic diversity and differentiation using microsatellite loci

Genetic structure and diversity was assessed for populations from WCU, SFL and MIA using nuclear microsatellite markers. Three microsatellite loci (Ac2, F06, g01) deviated significantly ($P < 0.05$) from HWE. Excluding those loci from the analysis did not affect the results and we thus included them in subsequent analyses. Allelic richness was similar across populations (mean $A_R = 10.62 \pm 0.55$; Table 2). Observed heterozygosity was lower than expected heterozygosity in all populations (mean Ho = 0.70 ± 0.03; mean He = 0.81 ± 0.03). F_{ST}-values showed similar degrees of differentiation between populations (mean pairwise $F_{ST} = 0.08 \pm 0.01$, Table 2). Individual allele frequencies of all markers are shown in Fig. S2.

Population structure and differentiation

The genetic cluster analysis recovered three distinct genetic clusters (Fig. 2B and C; model comparison Fig S4). Individual genotypes were correctly reassigned to their sampling locations and had genotype proportions >90% consistent with their own cluster. The MIA population shared a larger genotype proportion with WCU than with SFL, but accounted for less than 5% of ancestry. The average genotype proportion was 0.03 ± 0.05, and 0.01 ± 0.01 assigned to WCU and SFL, respectively. Three MIA individuals share genotype proportions greater than 20% with WCU (MIA647 q = 0.21 MIA719; q = 0.86 and MIA725 q = 0.70).

When the number of clusters was set to K=2 in the STRUCTURE analysis, the MIA population formed a distinct cluster. SFL and WCU grouped together in the second cluster (Fig. 2B). Analysis of separate population pairs recovered all three populations and did not suggest population substructure (Fig. S6). Similarly, genotypes from five sampling locations in WCU show no evidence for population structure when analyzed separately (Fig. S7).

Consistent with results from the STRUCTURE analysis, the DAPC analysis revealed three distinct genetic clusters. All individuals except one (JJK2783) grouped according to the three sampling locations WCU, SFL and MIA. Similar distances between cluster centroids indicate equal degrees of genetic differentiation among the populations (Fig. 2C; PCA is shown in Fig. S5). On the first axis, the MIA cluster is intermediate between SFL and WCU. On the second axis, MIA is distinct with respect to SFL and WCU, which have similar values. Individual JJK2783 from WCU was assigned to the MIA cluster (posterior probability = 0.83).

Maximum likelihood and ABC modelling of historic admixture

Tree-based maximum likelihood analysis of microsatellite markers supported one migration event between WCU and MIA (Fig. 2A). The weighted migration edge suggested that ~33% of the nuclear genetic ancestry in the MIA population is derived from WCU. Including the migration edge significantly improved the fit of the model as compared to a strictly bifurcating tree model ($P < 0.001$, Table S4). The migration model explained 99% of the total variance in the data, whereas the strictly bifurcating the tree model accounted for 80% (Fig. S8).

Time estimates from the ABC analysis suggest that the admixture event between MIA and WCU occurred within the last 245 – 2670 generations (median $T_A = 887$, mode $T_A = 554$, 95% CI 245–2670; Table 3, Fig. 3). The median rate of admixture was $R_A = 0.24$ (95% CI 0.14–0.35), which is consistent with the maximum likelihood coefficient of admixture $\lambda = 0.31$ obtained from the summary statistics (Table S5). Estimates for the remaining parameters used in the model are shown in Table S5. Summary statistics generated from the posterior probability distribution show similar values compared to the observed data and are largely non-significant, suggesting that modeled parameters provide a good fit for the data (Table S5). Performance measures for parameter estimates were consistently low and indicate accurate estimates (Table S6).

Discussion

In an effort to characterize the genetic consequences of secondary contact between the native *A. carolinensis* and the closely related introduced *A. porcatus*, our data show evidence for past hybridization followed by differentiation of the hybrid population. We found discordance between nuclear microsatellite markers and mtDNA haplotypes in the South Miami population, which is indicative for hybridization (Hailer et al. 2012; Miller et al. 2012; Roy et al. 2015). Thirty-five percent of mitochondrial haplotypes in the South Miami population are derived from *A. porcatus* from West Cuba and 65% from the native *A. carolinensis* in south Florida. Genetic cluster analyses of nuclear markers show that the South Miami population is homogeneous and genetically distinct from populations of both parental species, which is characteristic of hybrid ancestry rather than ongoing hybridization (James and Abbott 2005; Kronforst et al. 2006; Mims et al. 2010; Keller et al. 2014; Lavretsky et al. 2015). Tree-based maximum likelihood analysis confirms that ~33% of the nuclear genetic ancestry is derived from West Cuba. Strikingly, the proportion of nuclear ancestry derived from West Cuba (~33%) was remarkably similar to the proportion of *A. porcatus* mtDNA haplotypes in South Miami (~35%). Thus, reproductive barriers between *A. porcatus* and *A. carolinensis* are weak or absent despite considerable divergence in allopatry. Thus, secondary contact after species introduction has led to a genetically distinct hybrid population.

Time estimates from ABC analyses suggest that hybridization occurred between 245 – 2670 generations ago with a skewed distribution towards the present (Fig. 3), suggesting relatively recent introduction and rapid differentiation of the hybrid

population. Surprisingly, the differentiation of the hybrid population in South Miami from both parental species is similar in magnitude to the differentiation between the parental species, *A. porcatus* and *A. carolinensis*. Potential factors facilitating differentiation include reduced gene flow with populations of the parental species (James and Abbott 2005; Hasselman et al. 2014; Roy et al. 2015; Schumer et al. 2016), assortative mating of hybrid individuals (Mavárez et al. 2006), increased hybrid fitness (e.g. heterosis; Schwarz *et al*. 2005) and genome incompatibility (Schumer et al. 2015). Whether mainly adaptive or neutral evolutionary processes are involved in driving differentiation of the hybrid population and to what extent ongoing introgression exists in locations where hybrid and pure individuals overlap remains to be determined in future studies. However, pre-existing population structure might have contributed to differentiation of the South Miami population in addition to historic hybridization.

Our study provides genetic evidence that the formerly independent lineages *A. carolinensis* from South Florida and *A. porcatus* from West Cuba are not reproductively isolated and interbreed successfully after secondary contact, leading to a fusion of the previously distinct lineages. The species status of *A. porcatus* and *A. carolinensis* has changed repeatedly over the last decades based on morphological traits (Voigt 1831; Gray 1840; Powell 1992). *Anolis porcatus* was considered a subspecies of *A. carolinensis* (Gray 1845) until described as a distinct taxonomic unit (Powell 1992). However, a thorough evaluation of morphological differences between the species concluded that morphological characters are inadequate for species delimitation (Camposano 2011). Genetic evaluation of the *A. carolinensis* species

complex revealed paraphyly of *A. porcatus*, dividing this species into an eastern and western clades in Cuba, with the western clade being sister to *A. carolinensis* (Glor et al. 2004; Glor, Losos, and Larson 2005). Our study provides a thorough genetic evaluation of species boundaries between *A. carolinensis* and *A. porcatus*. According to the biological species concept, populations of distinct species are incapable of effectively interbreeding with one another (Mayr 1982), which is inconsistent with the findings of our study. Thus, genetic evidence for successful hybridization, as well as morphological similarities between the two species (Camposano 2011) reinforces a proposal to revise the taxonomy of *A. carolinensis* and *A. porcatus* from West Cuba, according to guidelines of the biological species concept (Mayr 1982).

Several *Anolis* species have been introduced to Florida and some from multiple native-range source populations in Cuba (Kolbe et al. 2007). In agreement with previously collected *A. porcatus* haplotypes from Miami (Kolbe et al. 2007), the phylogenetic analysis identified sampling sites located near Havana in West Cuba as potential source of the introduction. Haplotypes from these locations did not show evidence for further spatial structure (Fig. 1A), which is consistent with a single West Cuban populations based on microsatellite data (Fig S6). Thus, the source locations likely resemble a single panmictic population. However, a more comprehensive sampling approach of West Cuban populations is needed to clarify whether population structure exists and whether the introduction of *A. porcatus* involves a single or multiple independent introductions.

Conclusion

The aim of this study was to characterize the genetic consequences of secondary contact between *A. porcatus* and *A. carolinensis* and to test whether weak or absent reproductive barriers have led to hybridization and erosion of species boundaries in South Miami. Mismatch between cytoplasmic and nuclear DNA as well as genetic evidence for gene flow support that *A. porcatus* and *A. carolinensis* are not reproductively isolated and that secondary contact has led to hybridization and fusion of formerly independent lineages. A major finding was that a temporally restricted hybridization event resulted in strong differentiation between the hybrid population and populations of the two parental species, *A. porcatus* and *A. carolinensis,* with no evidence of ongoing gene flow.

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Table 1. Introduced mtDNA haplotypes of *A. porcatus* sampled in South Miami and the genetically most similar haplotypes of *A. porcatus* from West Cuba. Haplotypes are shown for mtDNA haplotype length of 571bp.

mtDNA Haplotype			
South Miami	**West Cuba**	**Sampling location West Cuba**	**% Divergence**
H102	H101	8 (Glor et al. 2004)	1.23
H103	H101	8 (Glor et al. 2004)	0.7
H105	H100	10, 11 (Glor et al. 2004)	1.4
H106	H122 & H126	Havana & 9 (Glor et al. 2004)	1.75
H107	H122	Havana	1.75
H123	H123	Havana	0

Table 2. Microsatellite summary statistics. SFL = individuals from J.W. Corbett Wildlife Management in South Florida, MIA = South Miami, WCU = West Cuba. N = number of individuals, A_R = allelic richness, Ho = observed heterozygosity, He = expected heterozygosity and pairwise F_{ST}.

Population	N	AR	Ho	He	M-ratio	F_{ST} - SFL	F_{ST} - MIA
SFL	32	10.22	0.68	0.79	0.81		
MIA	92	10.38	0.68	0.81	0.85	0.09	
WCU	54	11.24	0.73	0.85	0.83	0.08	0.07

Table 3. Posterior parameter estimates from the ABC demographic scenario. N = effective population size, T_A = time of the admixture event in units of generations, R_A = admixture rate, T_{MRCA} = time of the split between SFL and WCU.

Parameter	Median (95% CI)	
N_{SFL}	4980	[2130 - 9030]
N_{MIA}	4410	[2230 - 7270]
N_{WCU}	8570	[5670 - 9920]
T_A	887	[245 - 2670]
R_A	0.24	[0.14 - 0.35]
T_{MRCA}	$1.03 * 10^6$	[$1.28 * 10^6$ - $1.29 * 10^7$]

Table S1. Sample ID, mtDNA haplotype, sampling location and mtDNA clade membership of samples included in the phylogenetic analysis. Haplotypes were generated from two alignments S = 343bp, H = 571bp.

Sample ID	mtDNA Haplotype	Sampling location	Latitude	Longitude	mtDNA clade
JJK2782	S164	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2784	S105	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2786	S147	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2787	S104	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2788	S123	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2789	S105	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2790	S163	Varadero, Cuba	23.19039	-81.16894	*A. porcatus*
JJK2793	S142/H118	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2794	S144/H119	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2795	S148	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2796	S148/H122	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2797	S131/H122	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2800	S131/H113	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2825	S149/H123	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2826	S139	Havana, Cuba	23.08706	-82.36572	*A. porcatus*
JJK2827	S126	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2828	S143	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2829	S128/H110	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2832	S129	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2833	S145/H120	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2834	S146/H121	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2835	S130/H111	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2859	S99	Havana, Cuba	23.11684	-82.38881	*A. porcatus*
JJK2984	S121	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK2985	S132	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK2986	S123	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK2989	S100	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK2991	S151	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK2992	S122	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK3003	S116	Mariel, Cuba	22.98575	-82.75347	*A. porcatus*
JJK3026	S125	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*
JJK3027	S137	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*
JJK3028	S135	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*

JJK3029	S124	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*
JJK3030	S134	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*
JJK3031	S113	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*
JJK3032	S152	San Jose de las Lajas, Cuba	22.96503	-82.16134	*A. porcatus*
JJK3066	S136	Guanabo, Cuba	23.15459	-82.10105	*A. porcatus*
JJK3067	S152	Guanabo, Cuba	23.15459	-82.10105	*A. porcatus*
JJK3068	S130	Guanabo, Cuba	23.15459	-82.10105	*A. porcatus*
JJK3069	S150	Guanabo, Cuba	23.15459	-82.10105	*A. porcatus*
JJK3070	S140	Guanabo, Cuba	23.15459	-82.10105	*A. porcatus*
JJK3071	S127	Guanabo, Cuba	23.15459	-82.10105	*A. porcatus*
MIA637	S118	South Miami, FL	25.703825	-80.284162	*A. porcatus*
MIA640	S194/H123	South Miami, FL	25.732598	-80.245682	*A. porcatus*
MIA641	S118	South Miami, FL	25.732598	-80.245682	*A. porcatus*
MIA642	S118	South Miami, FL	25.705713	-80.293224	*A. porcatus*
MIA643	S117	South Miami, FL	25.705713	-80.293224	*A. porcatus*
MIA644	H103	South Miami, FL	25.705892	-80.295908	*A. porcatus*
MIA645	S111	South Miami, FL	25.705892	-80.295908	*A. porcatus*
MIA648	S118/H107	South Miami, FL	25.705892	-80.295908	*A. porcatus*
MIA652	S118/H107	South Miami, FL	25.705892	-80.295908	*A. porcatus*
MIA656	S111/H103	South Miami, FL	25.707552	-80.299120	*A. porcatus*
MIA657	S118/H107	South Miami, FL	25.707552	-80.299120	*A. porcatus*
MIA660	S118/H107	South Miami, FL	25.705713	-80.293224	*A. porcatus*
MIA664	S118/H107	South Miami, FL	25.705713	-80.293224	*A. porcatus*
MIA670	S111	South Miami, FL	25.703554	-80.303461	*A. porcatus*
MIA674	S111	South Miami, FL	25.701835	-80.303370	*A. porcatus*
MIA681	S111	South Miami, FL	25.710900	-80.284038	*A. porcatus*
MIA683	S120	South Miami, FL	25.715213	-80.283312	*A. porcatus*
MIA685	S111/H103	South Miami, FL	25.717840	-80.273581	*A. porcatus*
MIA694	S118/H107	South Miami, FL	25.715330	-80.280858	*A. porcatus*
MIA699	S118/H107	South Miami, FL	25.715779	-80.281151	*A. porcatus*
MIA701	S118/H107	South Miami, FL	25.715779	-80.281151	*A. porcatus*
MIA703	S118	South Miami, FL	25.717406	-80.276231	*A. porcatus*
MIA705	S118/H107	South Miami, FL	25.717406	-80.276231	*A. porcatus*
MIA713	S118/H107	South Miami, FL	25.706499	-80.285600	*A. porcatus*
MIA743	S114/H105	South Miami, FL	25.728767	-80.300729	*A. porcatus*
MIA749	S115	South Miami, FL	25.740831	-80.311349	*A. porcatus*
MIA750	S114/H105	South Miami, FL	25.740831	-80.311349	*A. porcatus*
MIA725	S115	South Miami, FL	25.721547	-80.279833	*A. porcatus*
MIA636	S63/H56	South Miami, FL	25.703825	-80.284162	*A. carolinensis*
MIA646	S29/H31	South Miami, FL	25.705892	-80.295908	*A. carolinensis*

MIA647	S37/H38	South Miami, FL	25.705892	-80.295908	*A. carolinensis*
MIA649	S50/H49	South Miami, FL	25.705892	-80.295908	*A. carolinensis*
MIA653	S28/H30	South Miami, FL	25.707552	-80.299120	*A. carolinensis*
MIA654	S52	South Miami, FL	25.707552	-80.299120	*A. carolinensis*
MIA655	S78/H67	South Miami, FL	25.707552	-80.299120	*A. carolinensis*
MIA659	S36/H36	South Miami, FL	25.706803	-80.295817	*A. carolinensis*
MIA661	S39/H41	South Miami, FL	25.705713	-80.293224	*A. carolinensis*
MIA662	S61	South Miami, FL	25.705713	-80.293224	*A. carolinensis*
MIA665	S36/H36	South Miami, FL	25.705077	-80.289196	*A. carolinensis*
MIA667	S50	South Miami, FL	25.699681	-80.301318	*A. carolinensis*
MIA668	S62	South Miami, FL	25.703554	-80.303461	*A. carolinensis*
MIA671	S62	South Miami, FL	25.703554	-80.303461	*A. carolinensis*
MIA672	S45/H46	South Miami, FL	25.703554	-80.303461	*A. carolinensis*
MIA673	S68	South Miami, FL	25.703554	-80.303461	*A. carolinensis*
MIA675	S37/H39	South Miami, FL	25.701835	-80.303370	*A. carolinensis*
MIA676	S51	South Miami, FL	25.703554	-80.303461	*A. carolinensis*
MIA677	S48	South Miami, FL	25.713857	-80.292350	*A. carolinensis*
MIA680	S53	South Miami, FL	25.710900	-80.284038	*A. carolinensis*
MIA682	S58	South Miami, FL	25.710900	-80.284038	*A. carolinensis*
MIA684	S49	South Miami, FL	25.764939	-80.291341	*A. carolinensis*
MIA686	S71	South Miami, FL	25.717840	-80.273581	*A. carolinensis*
MIA688	S34/H35	South Miami, FL	25.717840	-80.273581	*A. carolinensis*
MIA689	S79	South Miami, FL	25.717840	-80.273581	*A. carolinensis*
MIA690	S30	South Miami, FL	25.715330	-80.280858	*A. carolinensis*
MIA692	S62	South Miami, FL	25.715330	-80.280858	*A. carolinensis*
MIA695	S60	South Miami, FL	25.715330	-80.280858	*A. carolinensis*
MIA696	S53	South Miami, FL	25.715330	-80.280858	*A. carolinensis*
MIA697	S66	South Miami, FL	25.715779	-80.281151	*A. carolinensis*
MIA700	S45	South Miami, FL	25.715779	-80.281151	*A. carolinensis*
MIA702	S59	South Miami, FL	25.718418	-80.279209	*A. carolinensis*
MIA709	S50/H53	South Miami, FL	25.703825	-80.284162	*A. carolinensis*
MIA710	S50/H53	South Miami, FL	25.703825	-80.284162	*A. carolinensis*
MIA712	S46/H47	South Miami, FL	25.706499	-80.285600	*A. carolinensis*
MIA714	S47/H48	South Miami, FL	25.706499	-80.285600	*A. carolinensis*
MIA715	S65/H58	South Miami, FL	25.706499	-80.285600	*A. carolinensis*
MIA716	S50/H53	South Miami, FL	25.706275	-80.285502	*A. carolinensis*
MIA717	S78/H69	South Miami, FL	25.706275	-80.285502	*A. carolinensis*
MIA718	S38/H40	South Miami, FL	25.706275	-80.285502	*A. carolinensis*
MIA719	S68/H59	South Miami, FL	25.706275	-80.285502	*A. carolinensis*
MIA720	S78/H69	South Miami, FL	25.706275	-80.285502	*A. carolinensis*

MIA721	S32/H33	South Miami, FL	25.706275	-80.285502	*A. carolinensis*
MIA722	S62/H55	South Miami, FL	25.706499	-80.285600	*A. carolinensis*
MIA723	S69	South Miami, FL	25.720751	-80.279788	*A. carolinensis*
MIA724	S57	South Miami, FL	25.721547	-80.279833	*A. carolinensis*
MIA729	S35	South Miami, FL	25.718768	-80.281233	*A. carolinensis*
MIA731	S67	South Miami, FL	25.717773	-80.294104	*A. carolinensis*
MIA732	S78	South Miami, FL	25.717773	-80.294104	*A. carolinensis*
MIA733	S50	South Miami, FL	25.722233	-80.297597	*A. carolinensis*
MIA734	S75	South Miami, FL	25.722233	-80.297597	*A. carolinensis*
MIA735	S56	South Miami, FL	25.724167	-80.298412	*A. carolinensis*
MIA740	S31	South Miami, FL	25.724167	-80.298412	*A. carolinensis*
MIA744	S42	South Miami, FL	25.736254	-80.308689	*A. carolinensis*
MIA747	S54	South Miami, FL	25.736254	-80.308689	*A. carolinensis*
MIA748	S48	South Miami, FL	25.740831	-80.311349	*A. carolinensis*

Table S2: Primer sequences and annealing temperatures (Tm) for the 18 microsatellite loci and partial mtDNA region of the NADH dehydrogenase subunit 2.

Primer	Forward	Reverse	Reference	Tm
f06	GCCTTCCCTAAGCTATCCAAA	TGGCATTGAACCATCAGAA		60
g01	CAGATGGTTGACTCGATGTGTT	TTCAATAAAGTTGTGGCTGGTG		61
Ac2	TGTAAAACGACGGCCAGTGGCCACATAGTTGTGCCTCT	TTCACAATGTTTGTGGGTGT		60
Ac5	TGTAAAACGACGGCCAGTTGCTGGATTTCGTATCACAA	GTGGCCCATGAGTCACATCT		60
Ac6	TGTAAAACGACGGCCAGTTATTGTGATGTTGGGCAAGG	TGCTTCATGGTGATCTTGGA		60
Acar1	CCAAAAACCAAAAAGGCTGA	TGGACACACATACACCCACA	Wordley et al. 2011	57
Acar4	ACAGGGTACTGTGGACAGGG	AGGAGCGTGGAGCTACAAAA	Wordley et al. 2011	58
Acar10	GGATGTGTGTGTTTGTGTTGG	GGCTGTTGAGGGATTCTTGA	Wordley et al. 2011	57
Acar11	AGTTTCCCAAGAAAACCCGT	GGGTTGCTCGTTCTGGACTA	Wordley et al. 2011	59
Acar14	TATGTTGGGAGAAAGACGGG	CCTGAGCTACGTGACATGGA	Wordley et al. 2011	59
Acar16	CCAGAAAGCTTATTTCGGGTT	ATGTTGGATGAGCAAGGAGG	Wordley et al. 2011	58
Acar19	GAAAAGTAGTGGGGCATTGG	AGTTTCCCAAGAAAACCCGT	Wordley et al. 2011	57
Acar22	AACCACCTTTGTTCTGGTGC	AAGATGGCATTTCAGTGTTGC	Wordley et al. 2011	58
Acar23	TAATGGGGAGCAATTCAAGG	GAGCCCTATCTTTGGAAGGC	Wordley et al. 2011	58
Acar28	AACCCCATACATCGCCAATA	GAACTTGCATGAGGCTGTCA	Wordley et al. 2011	58
Acar30	CATCTCTTCAGGCTTTTGCC	CTGTCTCTTCCTCCACCTGC	Wordley et al. 2011	57
Acar32	ATCTGTGCTACACTGGCCCT	TCCCCACAGTCAAAAGAAGC	Wordley et al. 2011	58
Acar43	GAGAGGCCACCAGCATTTAC	GCATAAAGTGGGAATTGCTTC	Wordley et al. 2011	59
ND2	CCCACGATCTACAGAAGCAG	AGTAGGGAGGATGCGGCTAT		57

Table S3. Published sequences of mtDNA NADH dehydrogenase subunit 2 and haplotypes from two alignments. S = 343bp, H = 571bp.

Accession number	mtDNA Haplotype		Reference	Species
AY654025	S2	H2	Glor et al. 2004	*A. porcatus*
AY654026	S96	H91	Glor et al. 2004	*A. porcatus*
AY654027	S95	H90	Glor et al. 2004	*A. porcatus*
AY654028	S3	H3	Glor et al. 2004	*A. porcatus*
AY654029	S119	H108	Glor et al. 2004	*A. porcatus*
AY654030	S93	H88	Glor et al. 2004	*A. porcatus*
AY654031	S97	H92	Glor et al. 2004	*A. porcatus*
AY654032	S94	H89	Glor et al. 2004	*A. porcatus*
AY654033	S98	H93	Glor et al. 2004	*A. porcatus*
AY654034	S109	H101	Glor et al. 2004	*A. porcatus*
AY654035	S133	H115	Glor et al. 2004	*A. porcatus*
AY654036	S138	H116	Glor et al. 2004	*A. porcatus*
AY654037	S132	H114	Glor et al. 2004	*A. porcatus*
AY654038	S123	H109	Glor et al. 2004	*A. porcatus*
AY654039	S112	H104	Glor et al. 2004	*A. porcatus*
AY654040	S141	H117	Glor et al. 2004	*A. porcatus*
AY654041	S141	H117	Glor et al. 2004	*A. porcatus*
AY654042	S153	H126	Glor et al. 2004	*A. porcatus*
AY654043	S108	H100	Glor et al. 2004	*A. porcatus*
AY654044	S108	H100	Glor et al. 2004	*A. porcatus*
AY654045	S102	H96	Glor et al. 2004	*A. porcatus*
AY654046	S106	H98	Glor et al. 2004	*A. porcatus*
AY654047	S101	H94	Glor et al. 2004	*A. porcatus*
AY654048	S101	H95	Glor et al. 2004	*A. porcatus*
AY654050	S172	H144	Glor et al. 2004	*A. porcatus*
AY654051	S103	H97	Glor et al. 2004	*A. porcatus*
AY654052	S151	H125	Glor et al. 2004	*A. porcatus*
AY654053	S108	H100	Glor et al. 2004	*A. porcatus*
AY654055	S107	H99	Glor et al. 2004	*A. porcatus*
AY654056	S108	H100	Glor et al. 2004	*A. porcatus*
AY654057	S166	H137	Glor et al. 2004	*A. porcatus*
AY654058	S165	H136	Glor et al. 2004	*A. porcatus*
AY654059	S162	H135	Glor et al. 2004	*A. porcatus*
AY654060	S161	H134	Glor et al. 2004	*A. porcatus*
AY654061	S159	H132	Glor et al. 2004	*A. porcatus*

AY654062	S159	H132	Glor et al. 2004	*A. porcatus*
AY654063	S158	H131	Glor et al. 2004	*A. porcatus*
AY654064	S160	H133	Glor et al. 2004	*A. porcatus*
AY654065	S157	H130	Glor et al. 2004	*A. porcatus*
AY654066	S154	H127	Glor et al. 2004	*A. porcatus*
AY654067	S154	H127	Glor et al. 2004	*A. porcatus*
AY654068	S155	H128	Glor et al. 2004	*A. porcatus*
AY654070	S156	H129	Glor et al. 2004	*A. porcatus*
AY654071	S167	H138	Glor et al. 2004	*A. porcatus*
AY654072	S168	H139	Glor et al. 2004	*A. porcatus*
AY654073	S169	H141	Glor et al. 2004	*A. porcatus*
AY654074	S170	H142	Glor et al. 2004	*A. porcatus*
AY654075	S168	H140	Glor et al. 2004	*A. porcatus*
AY654076	S171	H143	Glor et al. 2004	*A. porcatus*
AY654077	S181	H155	Glor et al. 2004	*A. porcatus*
AY654078	S182	H156	Glor et al. 2004	*A. porcatus*
AY654079	S179	H152	Glor et al. 2004	*A. porcatus*
AY654081	S180	H154	Glor et al. 2004	*A. porcatus*
AY654082	S180	H154	Glor et al. 2004	*A. porcatus*
AY654083	S180	H154	Glor et al. 2004	*A. porcatus*
AY654084	S180	H153	Glor et al. 2004	*A. porcatus*
AY654085	S173	H145	Glor et al. 2004	*A. porcatus*
AY654086	S173	H145	Glor et al. 2004	*A. porcatus*
AY654087	S174	H146	Glor et al. 2004	*A. porcatus*
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AY654091	S177	H149	Glor et al. 2004	*A. porcatus*
AY654092	S177	H149	Glor et al. 2004	*A. porcatus*
AY654093	S177	H150	Glor et al. 2004	*A. porcatus*
AY654094	S176	H148	Glor et al. 2004	*A. porcatus*
AY902428	S70	H60	Glor et al. 2005	*A. carolinensis*
AY902429	S74	H63	Glor et al. 2005	*A. carolinensis*
AY902430	S12	H12	Glor et al. 2005	*A. carolinensis*
AY902431	S10	H10	Glor et al. 2005	*A. carolinensis*
AY902432	S19	H20	Glor et al. 2005	*A. carolinensis*
AY902433	S16	H21	Glor et al. 2005	*A. carolinensis*
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EU106324	S20	H22	Kolbe et al. 2007	*A. carolinensis*

EU106325	S16	H18	Kolbe et al. 2007	*A. carolinensis*
EU106326	S17	H17	Kolbe et al. 2007	*A. carolinensis*
EU106327	S18	H19	Kolbe et al. 2007	*A. carolinensis*
EU106328	S83	H72	Kolbe et al. 2007	*A. carolinensis*
EU106329	S89	H83	Kolbe et al. 2007	*A. carolinensis*
EU106330	S45	H51	Kolbe et al. 2007	*A. carolinensis*
EU106331	S76	H65	Kolbe et al. 2007	*A. carolinensis*
EU106332	S54	H52	Kolbe et al. 2007	*A. carolinensis*
EU106333	S30	H32	Kolbe et al. 2007	*A. carolinensis*
EU106334	S64	H57	Kolbe et al. 2007	*A. carolinensis*
EU106335	S78	H69	Kolbe et al. 2007	*A. carolinensis*
EU106336	S44	H45	Kolbe et al. 2007	*A. carolinensis*
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EU106338	S41	H43	Kolbe et al. 2007	*A. carolinensis*
EU106339	S55	H54	Kolbe et al. 2007	*A. carolinensis*
EU106340	S52	H50	Kolbe et al. 2007	*A. carolinensis*
EU106341	S43	H44	Kolbe et al. 2007	*A. carolinensis*
EU106342	S50	H53	Kolbe et al. 2007	*A. carolinensis*
EU106343	S110	H102	Kolbe et al. 2007	*A. porcatus*
EU106344	S118	H106	Kolbe et al. 2007	*A. porcatus*
JX524289	S33	H34	Campbell-Staton et al. 2012	*A. carolinensis*
JX524291	S24	H26	Campbell-Staton et al. 2012	*A. carolinensis*
JX524292	S25	H27	Campbell-Staton et al. 2012	*A. carolinensis*
JX524293	S22	H24	Campbell-Staton et al. 2012	*A. carolinensis*
JX524294	S26	H28	Campbell-Staton et al. 2012	*A. carolinensis*
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JX524296	S73	H62	Campbell-Staton et al. 2012	*A. carolinensis*
JX524297	S68	H59	Campbell-Staton et al. 2012	*A. carolinensis*
JX524298	S62	H55	Campbell-Staton et al. 2012	*A. carolinensis*
JX524299	S40	H42	Campbell-Staton et al. 2012	*A. carolinensis*
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JX524311	S4	H4	Campbell-Staton et al. 2012	*A. carolinensis*
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JX524313	S7	H7	Campbell-Staton et al. 2012	*A. carolinensis*
JX524314	S5	H5	Campbell-Staton et al. 2012	*A. carolinensis*

JX524315	S16	H16	Campbell-Staton et al. 2012	*A. carolinensis*
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JX524318	S14	H14	Campbell-Staton et al. 2012	*A. carolinensis*
JX524319	S13	H13	Campbell-Staton et al. 2012	*A. carolinensis*
JX524320	S15	H15	Campbell-Staton et al. 2012	*A. carolinensis*
JX524321	S88	H82	Campbell-Staton et al. 2012	*A. carolinensis*
JX524322	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
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JX524324	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
JX524325	S8	H8	Campbell-Staton et al. 2012	*A. carolinensis*
JX524326	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
JX524327	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
JX524328	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
JX524329	S84	H74	Campbell-Staton et al. 2012	*A. carolinensis*
JX524330	S87	H79	Campbell-Staton et al. 2012	*A. carolinensis*
JX524331	S86	H78	Campbell-Staton et al. 2012	*A. carolinensis*
JX524332	S82	H76	Campbell-Staton et al. 2012	*A. carolinensis*
JX524368	S82	H75	Campbell-Staton et al. 2012	*A. carolinensis*
JX524369	S85	H77	Campbell-Staton et al. 2012	*A. carolinensis*
JX524370	S82	H81	Campbell-Staton et al. 2012	*A. carolinensis*
JX524371	S82	H73	Campbell-Staton et al. 2012	*A. carolinensis*
JX524372	S82	H73	Campbell-Staton et al. 2012	*A. carolinensis*
JX524373	S91	H85	Campbell-Staton et al. 2012	*A. carolinensis*
JX524374	S91	H86	Campbell-Staton et al. 2012	*A. carolinensis*
JX524375	S21	H23	Campbell-Staton et al. 2012	*A. carolinensis*
JX524376	S90	H84	Campbell-Staton et al. 2012	*A. carolinensis*
JX524377	S92	H87	Campbell-Staton et al. 2012	*A. carolinensis*
JX524408	S72	H61	Campbell-Staton et al. 2012	*A. carolinensis*
JX524410	S74	H64	Campbell-Staton et al. 2012	*A. carolinensis*
JX524412	S77	H66	Campbell-Staton et al. 2012	*A. carolinensis*
JX524414	S27	H29	Campbell-Staton et al. 2012	*A. carolinensis*
JX524419	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
JX524420	S81	H70	Campbell-Staton et al. 2012	*A. carolinensis*
JX524421	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*
JX524422	S82	H71	Campbell-Staton et al. 2012	*A. carolinensis*

Table S4: Residual matrix of tree-based population models. Positive values indicate greater genetic variation in the true population than explained by the model. Lower positive residuals show improvement of the model after including migration.

No Migration			
	SFL	**MIA**	**WCU**
SFL	0.29		
MIA	**2.05**	-4.10	
WCU	-2.34	**2.05**	0.29
Migration			
	SFL	**MIA**	**WCU**
SFL	-0.32		
MIA	-0.07	-0.28	
WCU	**0.39**	**0.35**	-0.74

Table S5. Deviation of summary statistics between the observed data and simulated data from the posterior predictive distributions in the ABC analyses. A = mean number of alleles, H = mean gene diversity, FST = pairwise F_{ST} – value, λ = maximum likelihood coefficient of admixture.

Summary Statistics	Observed Value	Proportion (simulated<observed)	Significance
A_{SFL}	10.22	0.63	
A_{MIA}	13.17	0.06	
A_{WCU}	12.61	0.31	
H_{SFL}	0.80	0.42	
H_{MIA}	0.81	0.03	*
H_{WCU}	0.86	0.54	
$F_{ST\text{-}SFL \times MIA}$	0.09	0.11	
$F_{ST\text{-}SFL \times WCU}$	0.08	0.00	***
$F_{ST\text{-}MIA \times WCU}$	0.07	0.81	
$\bar{\lambda}$	0.31	0.87	

Table S6. Bias and precision of parameter estimates of the ABC analysis. Bias = the average relative bias, MMedAD = relative median absolute deviation, RMAE = relative median of the absolute error.

Parameter	Bias	MMedAD	RMAE
N_{SFL}	0.11	0.40	0.21
N_{MIA}	0.25	0.59	0.30
N_{WCU}	0.00	0.23	0.12
T_A	0.29	0.95	0.42
R_A	-0.07	0.30	0.17
T_{MRCA}	-0.02	0.31	0.20
µmic_1	-0.07	0.26	0.16
pmic_1	0.06	0.40	0.24
snimic_1	111.89	225.67	21.72

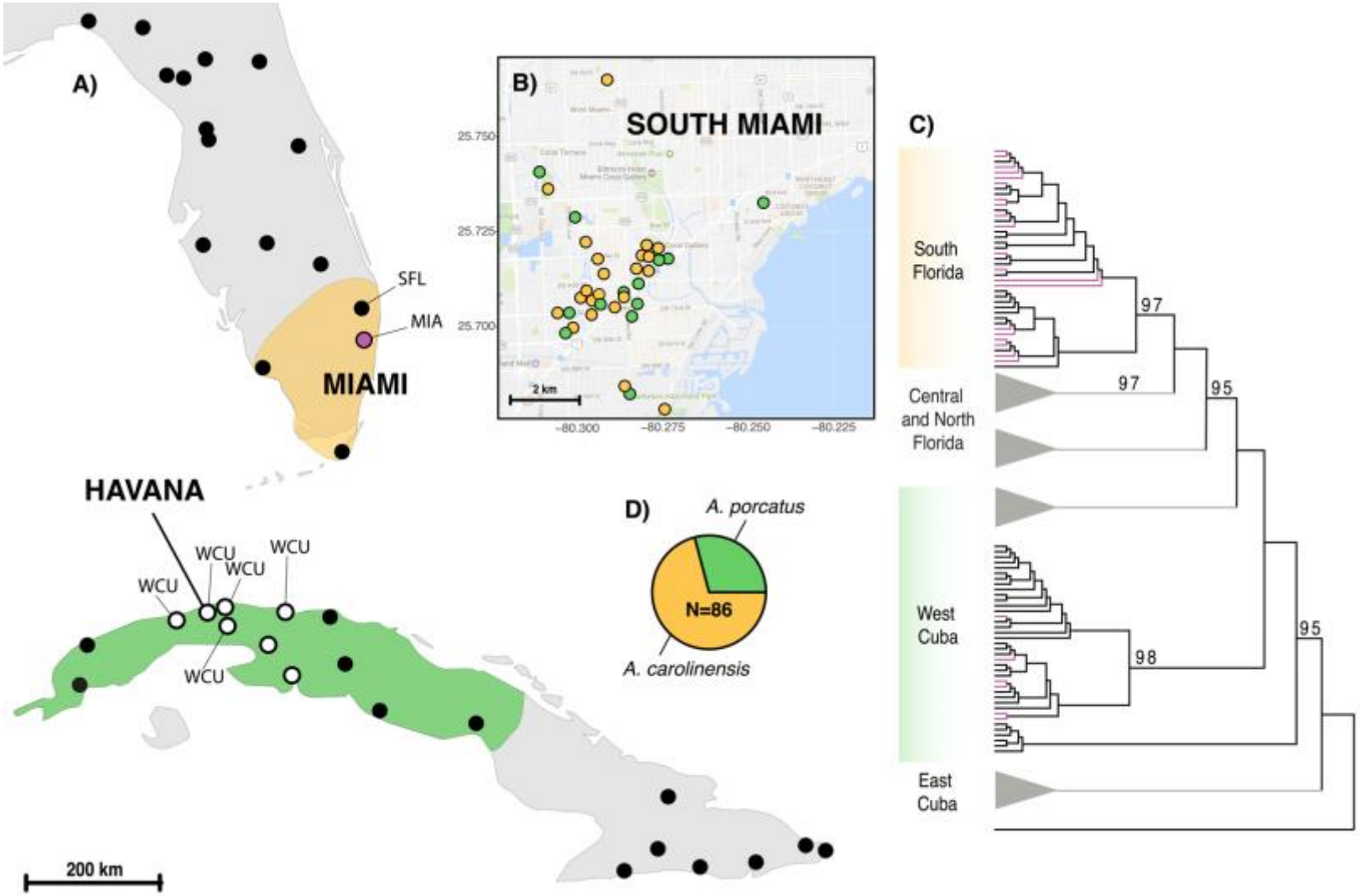
A)
SFL
MIA
MIAMI
B)
SOUTH MIAMI
25.750
25.725
25.700
2 km
-80.300
-80.275
-80.250
-80.225
C)
South Florida
Central and North Florida
West Cuba
East Cuba
97
97
95
98
95
HAVANA
WCU
WCU
WCU
WCU
WCU
D)
A. porcatus
N=86
A. carolinensis
200 km

Figure 1. Sampling locations of mtDNA haplotypes and microsatellite data. A) Black circles are sampling locations of mtDNA haplotypes. White circles indicate putative sources for introduced *A. porcatus*. Microsatellite data were sampled from five locations in West Cuba (WCU), from the putative hybrid population in South Miami (MIA) and ~200 km north of Miami (SFL). B) sampling sites in South Miami are colored by clade membership with yellow = *A. carolinensis*, green = *A. porcatus*. C) Maximum likelihood phylogeny based on 571bp mtDNA haplotypes. Haplotypes from South Miami (magenta colored branches) are nested with both *A. porcatus* and *A. carolinensis*. Bootstrap values are shown above branches for values >95. Clades without haplotypes from South Miami were visually collapsed. The full phylogeny can be accessed in Fig. S2. D) Frequency of mtDNA haplotypes in South Miami, the total number of individuals sampled was N = 86.

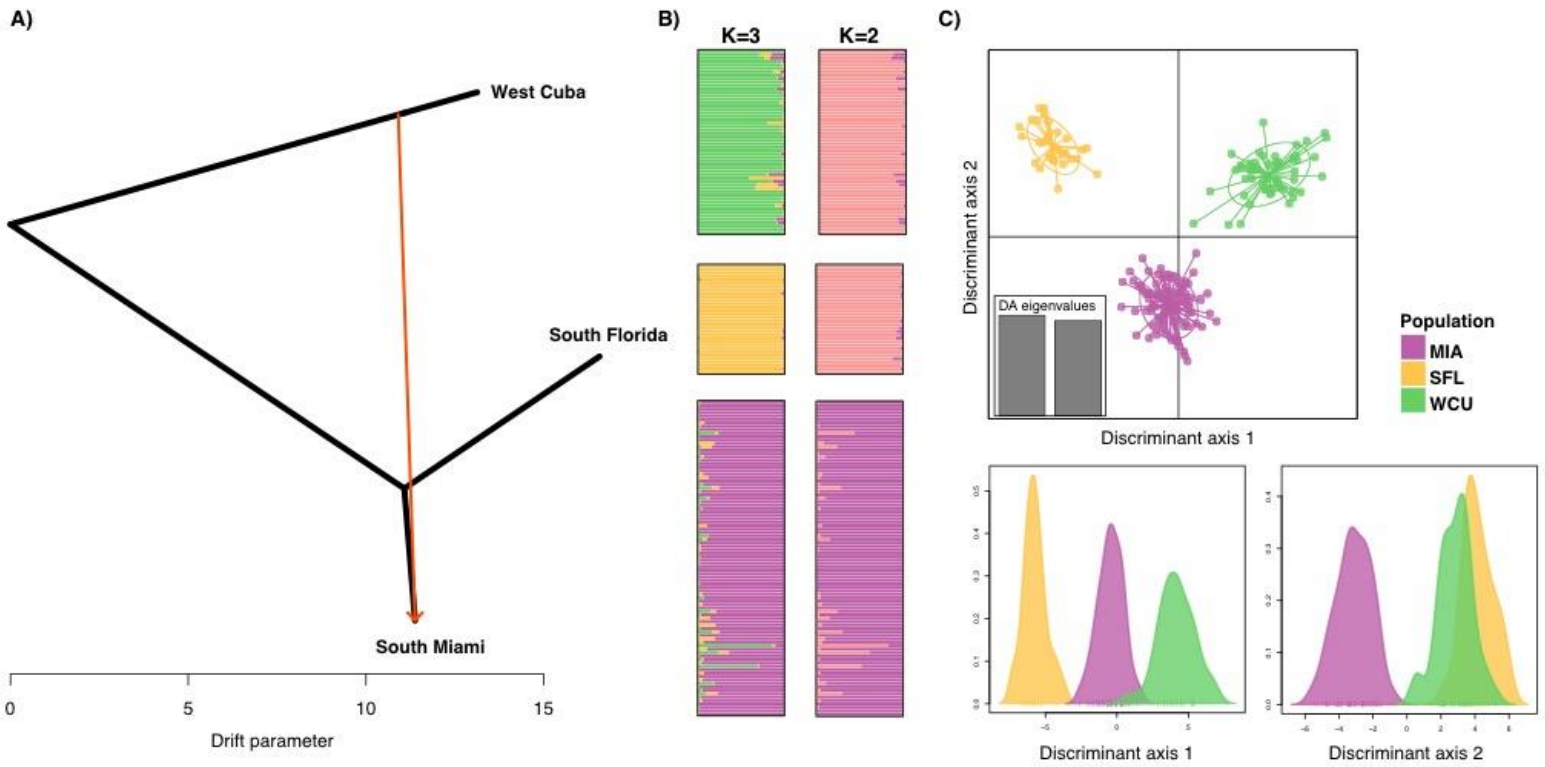
A)
West Cuba
South Florida
South Miami
0
5
10
15
Drift parameter
B)
K=3
K=2
C)
Discriminant axis 2
DA eigenvalues
Discriminant axis 1
Population
MIA
SFL
WCU
Discriminant axis 1
Discriminant axis 2

Figure 2. Historic gene flow and differentiation of the hybrid population in South Miami. A) Tree-based ancestry model with migration edge (red arrow) indicating gene flow between WCU and MIA accounting for 33% variation in the MIA population. B) Genetic clusters from the Bayesian cluster analysis for K = 2 and K = 3. The most likely number of cluster was K = 3. C) DAPC analysis with K = 3 clusters, axis 1 accounting for 51% variation and axis 2 for 49%. Bottom figures show the density of each cluster for axis 1 (left) and axis 2 (right).

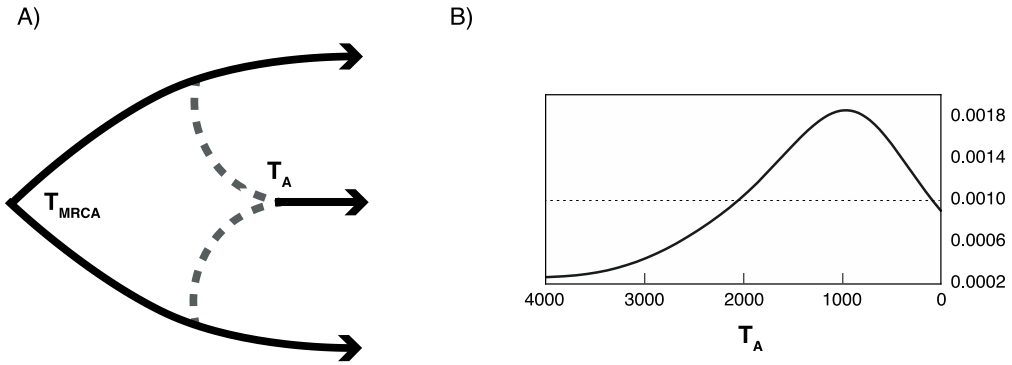

Figure 3. A) Demographic ABC model and time of admixture between *A. carolinensis* and *A. porcatus* in South Miami. B) Solid line shows the posterior distribution of T_A in units of generations and the uniform prior distribution as dotted line. Median time of the admixture event was 887 with 95% CI 245 - 2670.

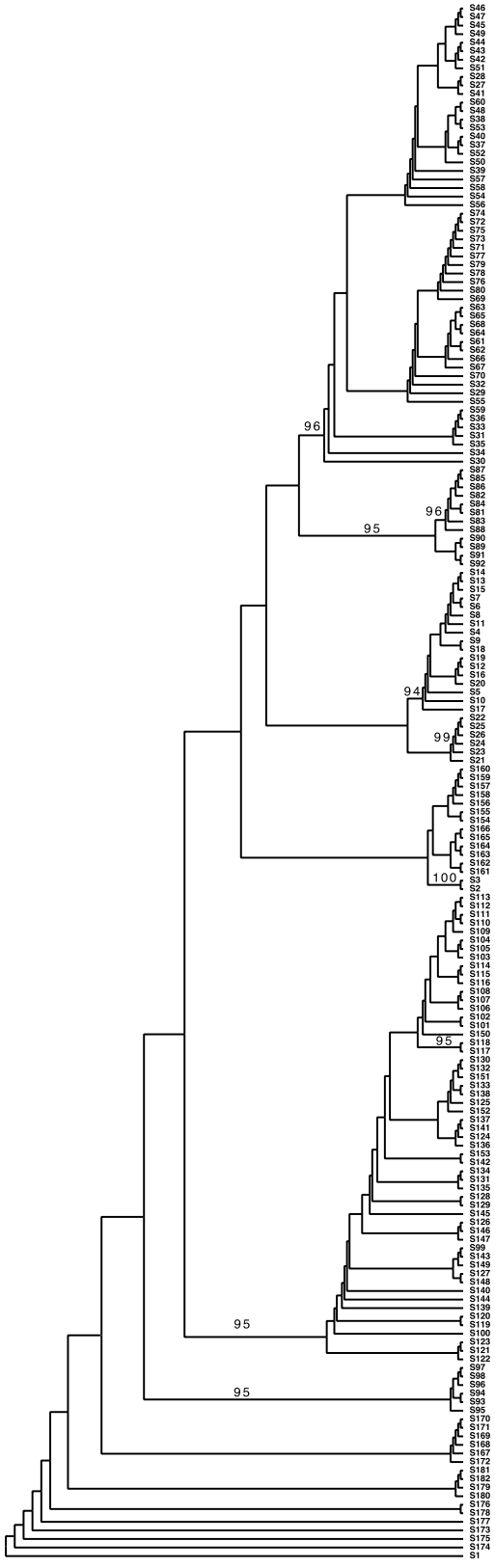

Figure S1. Maximum likelihood phylogeny of mtDNA haplotypes of the 343bp alignment. The phylogeny includes all 280 individuals, that were collapsed into 181 unique haplotypes. Bootstrap values are shown above branches for values >95.

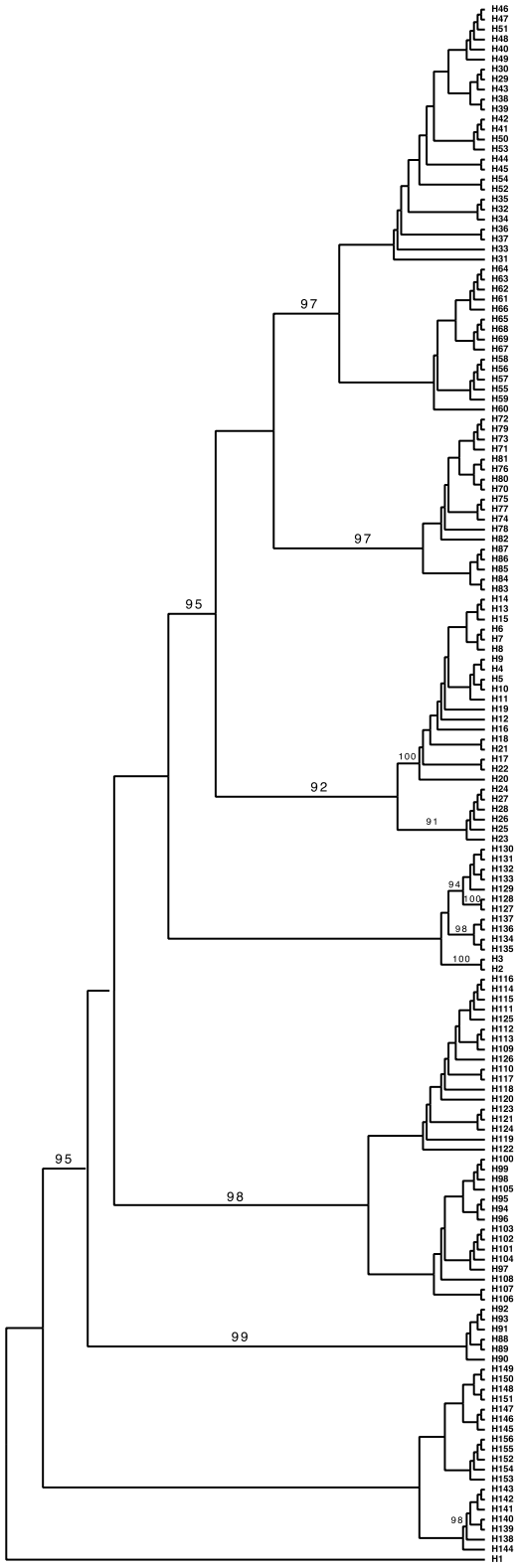

Figure S2. Maximum likelihood phylogeny of mtDNA haplotypes of the 571bp alignment. The phylogeny includes 200 individuals, that were collapsed into 156 unique haplotypes. Bootstrap values are shown above branches for values >95.

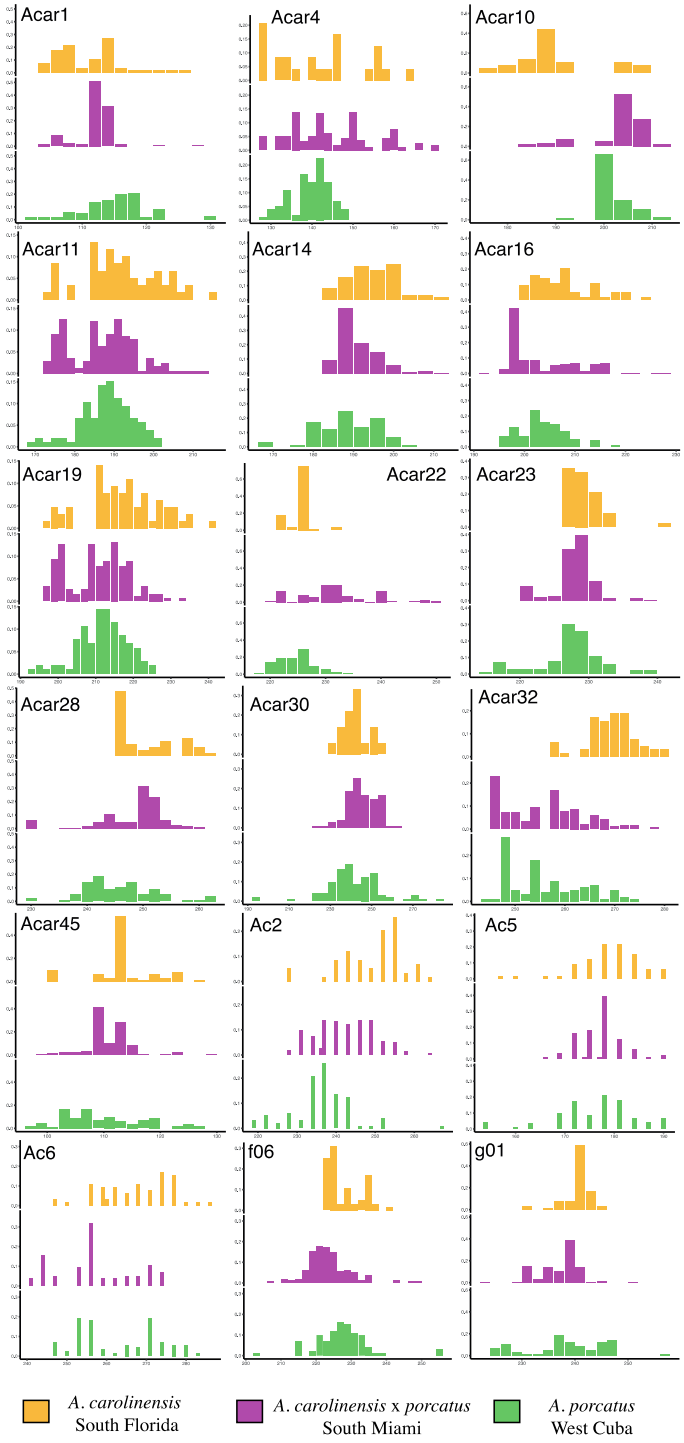

Figure S3. Allele frequency distributions for 18 microsatellite markers. *Anolis carolinensis* from South Florida is shown in yellow, *A. porcatus* is shown in green and the hybrid population is shown in magenta.

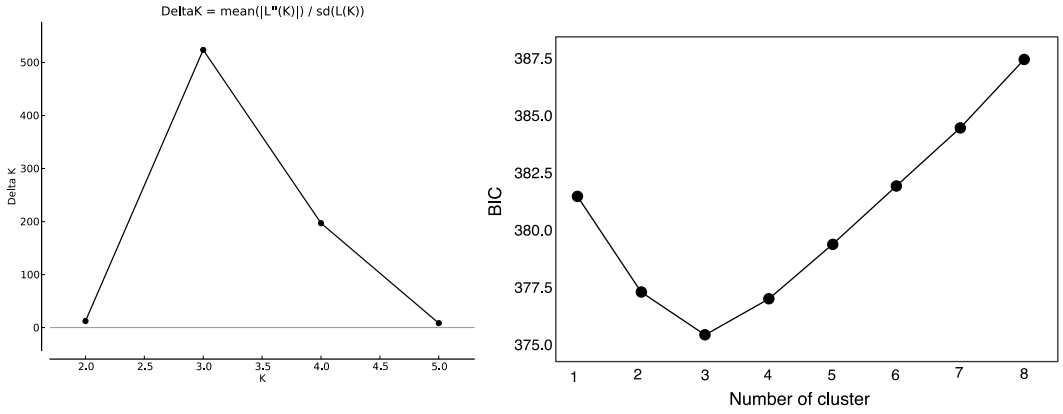

Figure S4. Model comparison for a sequentially increasing number of clusters (K). left: delta K for STRUCTURE models with varying number of clusters. Large delta K indicates that three clusters are the best fitting model. Right: BIC values for cluster models generated by DAPC. The best fitting model indicated by the smallest BIC has three clusters.

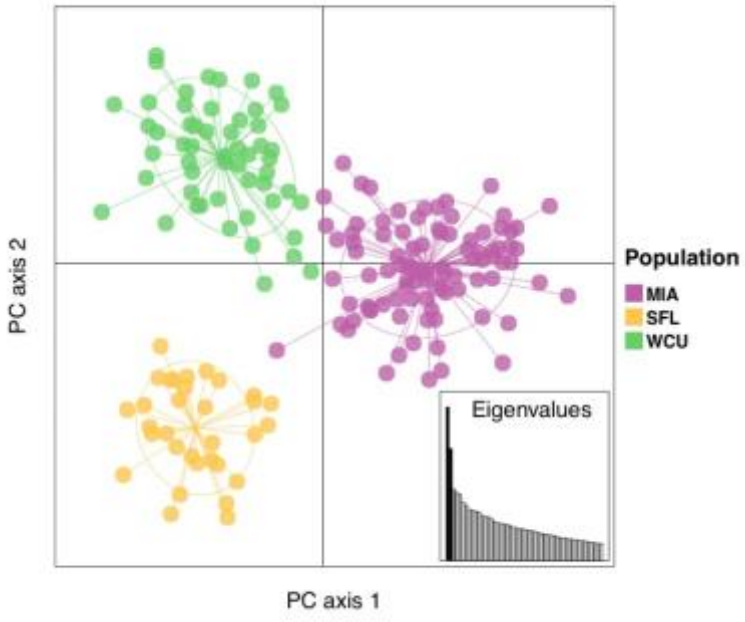

Figure S5. Principal component analysis of microsatellite genotypes from West Cuba (WCU), South Florida (SFL) and South Miami (MIA). The three sampling locations form separate genetic clusters.

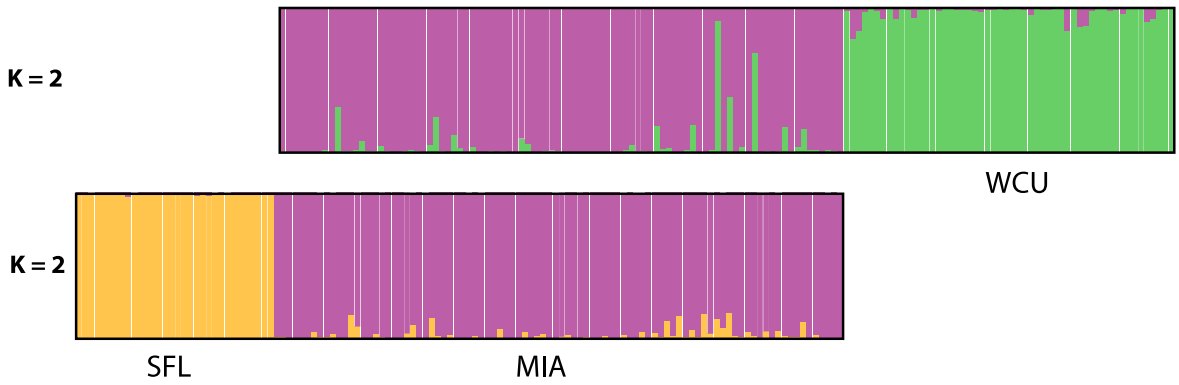

Figure S6. STRUCTURE analysis of population pairs. Populations cluster according to the sampling location and no further substructure was detected.

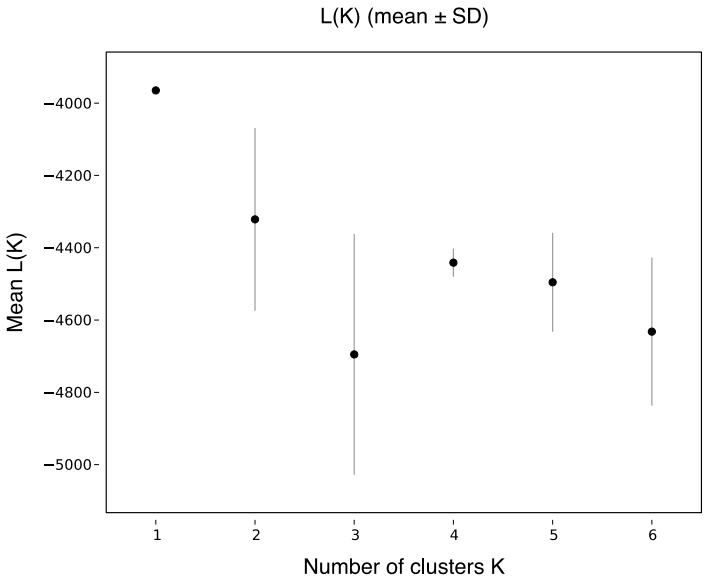

Figure S7. Log likelihood L(K) of STRUCTURE models with increasing number of clusters K for the WCU sampling locations. L(K) decreases with increasing number of clusters suggesting absence of population structure within WCU.

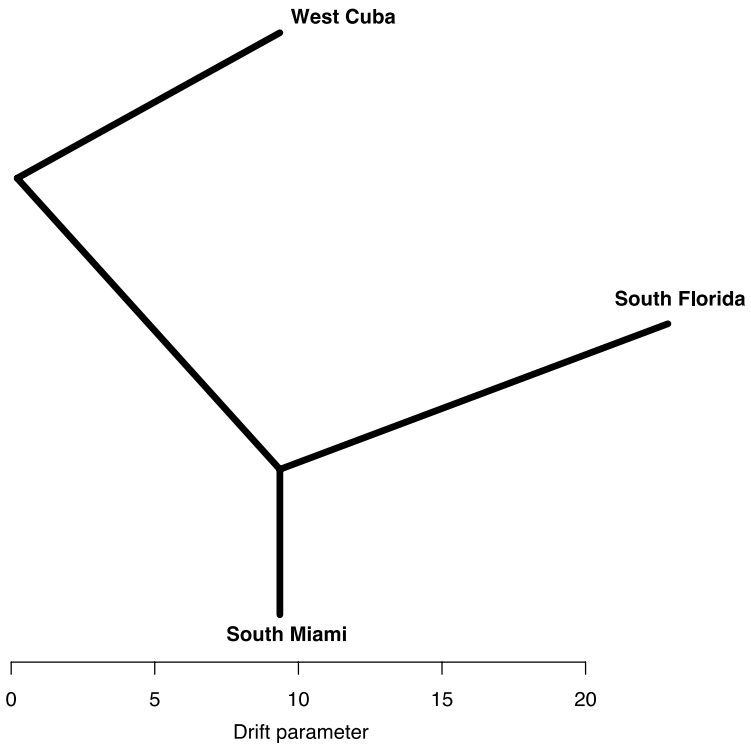

Figure S8. Simple tree model used to test for historic gene flow. The simple tree model accounted for 80% of the variance in the data.

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