Electronic Supplementary Material

Kolbe et al. "Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species."

METHODS

Sampling

We sampled tail or liver tissue from 20 individuals from each of 10 non-native *Anolis sagrei* populations including Coral Gables, Gainesville, Lower Matecumbe Key, Orlando, St. Petersburg, and Tampa in Florida, Corpus Christi and Houston in Texas, New Orleans, Louisiana, USA, and George Town, Grand Cayman (Kolbe et al. 2007). We also sampled 52 individuals from eight putative native-range source populations previously identified using mtDNA sequences, including Belize (N=4), and Caibarien (N=5), Jicarita (N=5), La Habana & Lajas (N=8), Mariel (N=7), Portillo (N=11), Soroa (N=6), and S. Esmeralda (N=6) in Cuba (Kolbe et al. 2004). These represent all available samples from native-range source populations.

Mitochondrial DNA

For 199 of the 200 samples from non-native populations and 30 of the 52 samples from native-range source populations, we previously reported at least a 500 base-pair region of mtDNA including a portion of the gene encoding ND2 (Kolbe et al. 2004, 2007). These previously published mtDNA sequences were obtained from Genbank (#AY655164-AY655483 and #DQ846752-DQ846771). We sequenced the same mtDNA region for the remaining individuals from native-range sources. For mtDNA sequences, we estimated genetic diversity within populations using the mean within-population percent sequence divergence (uncorrected (p) distance) using MEGA 3.1 (Kumar et al. 2004). We also calculated the mean uncorrected (p) distance between populations and

used analysis of molecular variance (AMOVA) to calculate pairwise Φ_{ST} values (Excoffier et al. 1992). To visualize relationships among mtDNA haplotypes and their distribution among non-native populations, we constructed haplotype networks using TCS v. 1.21 (Clement at al. 2000).

Microsatellites

We used previously published primers developed specifically for *A. sagrei* to amplify nine microsatellites (loci 15, 61, 63, 68, 70, 77, 91, 94, and 95 from Bardeleben et al. 2004). We optimized buffer concentrations and annealing temperatures for each primer pair on our thermocycler, and amplified fragments with the following protocol: 180 s at 94°C followed by 30 cycles of 94°C for 45 s, 52-60°C for 45 s, 72°C for 30 s, and finally 300 s at 72°C. Fluorescently labeled PCR products were separated on an ABI 3730 capillary sequencer (Applied Biosystems) and allele sizes scored by comparison to the ROX size standard using GeneMapper software.

For each population-locus combination, we estimated the number of alleles (N_A), observed heterozygosity (H_O), and unbiased expected heterozygosity (H_E) using GENEPOP (GENEPOP ON THE WEB at http://wbiomed.curtin.edu.au/genepop/; Raymond & Rousset 1995). We tested for linkage disequilibrium by comparing locus pairs both within and across populations using GENEPOP (Raymond & Rousset 1995). Deviations from Hardy-Weinberg equilibrium (HWE) were tested using separate one-tailed tests for heterozygote excess and deficiency for each locus within a population using GENEPOP (Raymond & Rousset 1995). *P*-values for multiple comparisons were adjusted using sequential Bonferroni correction (Rice 1989). We also used the program MICRO-CHECKER (van Oosterhout et al. 2004) to determine if deviations from HWE might be due to null alleles.

To determine the distribution of population-genetic variation within and among populations, we tested for significant geographic differentiation among populations with analysis of molecular variance (AMOVA) using ARLEQUIN 3.0 (Excoffier et al. 1992; Excoffier et al. 2005). We conducted AMOVAs for the mtDNA and microsatellite datasets separately.

RESULTS

Cytonuclear disequilibrium

As an exploratory analysis of potential factors affecting the extent of admixture within non-native populations, we tested for a relationship between the significance values for the correlation between microsatellite and mtDNA genetic distances (an estimate of cytonuclear disequilibrium within introduced populations) and mtDNA divergence among individuals from different sources within introduced populations, number of native-range sources, and time since introduction. Note all correlations were non-significant (table S5), hence the exploratory nature of this analysis. Significance values show a negative relationship with mean pairwise within-population mtDNA sequence divergence ($r^2 = 0.753$, $p = 0.001$) and number of sources ($r^2 = 0.567$, $p = 0.012$), but no relationship with time since introduction ($r^2 = 0.000$, $p = 0.957$). This suggests that introduced populations with more sources and larger mean mtDNA sequence divergences among individuals from different sources (these two values that are not independent) approach significant values for the correlation between microsatellite and mtDNA distances, which would indicate cytonuclear disequilibrium. Potential explanations may include reproductive incompatibilities between individuals from some

sources, assortative mating between individuals from some sources, or that more time is needed to come to equilibrium if individuals from more native-range sources are present.

Genetic diversity

The aligned mtDNA dataset for the non-native range includes 488 sites for 37 unique sequences derived from the 199 individuals sampled from 10 non-native *A. sagrei* populations (Kolbe et al. 2004, 2007; figure S1). Mean within population mtDNA uncorrected (p) distance ranges from 0.6% in Corpus Christi to 6.7% in Tampa and mean mtDNA sequence divergence among populations ranges from 0.6 % (Corpus Christi-Houston) to 8.6 % (St. Petersburg-New Orleans & St. Petersburg-George Town; table S1). Construction of haplotype networks gives seven separate networks (figure S2), corresponding to the native-range source populations previously identified (see Kolbe et al. 2007 for phylogenetic relationships among these haplotype networks). For the native-range source populations, the aligned mtDNA dataset includes 1196 sites for 35 unique sequences derived from the 52 individuals. Mean within population mtDNA uncorrected (p) distance ranges from 0.2% in Belize to 2.0% in Soroa and mean mtDNA sequence divergence among populations ranges from 1.4% (Mariel-La Habana) to 11.1% (Belize-S. Esmeralda; table S2). Newly collected mtDNA sequences from the native range are available from Genbank (Genbank #s).

In the non-native range, the nine microsatellite loci have 6-28 alleles per locus (table S3). The total number of alleles across loci within a population ranges from 45 (George Town) to 74 (Gainesville) with a single private allele in each of the Corpus Christi, Houston, and George Town populations, and 5 private alleles in Lower Matecumbe Key (table S3). All loci are polymorphic within each population except

locus 63, which is monomorphic in three populations: Corpus Christi, Houston, and New Orleans.

Little evidence exists for linkage disequilibrium with only one locus pair across populations (loci 91 and 94) in significant disequilibrium. The following 11 of 360 locus pairs within populations exhibit significant disequilibrium: St. Petersburg, 61-63, 63-91, 63-95; Coral Gables, 70-91, 77-91, 91-94; Orlando, 61-91; Gainesville 61-91; Corpus Christi, 70-91, 61-95; Houston, 68-77. After Bonferroni correction ($P < 1.39 \times 10^{-4}$), only one comparison (Coral Gables, loci 91-94) is significant.

No loci show a significant excess of heterozygotes, whereas five loci (15, 61, 63, 68, and 94), while controlling for multiple comparisons (Bonferroni $P < 5.56 \times 10^{-4}$), show a significant deficit of heterozygotes within one or more populations. The following 23 of 90 locus-population combinations are heterozygote deficient: Tampa, 63, 68; St. Petersburg, 15, 68; Lower Matecumbe Key, 15, 68, 94; Coral Gables, 15, 63, 68; Orlando, 15, 63; Gainesville, 61, 68, 94; New Orleans, 15, 68; Corpus Christi, 15; Houston, 15, 68, 94; George Town, 15, 68. MICRO-CHECKER detects a general excess of homozygotes at loci 61, 63, 68, and 94, suggesting that null alleles may be present at these loci. Therefore, we repeated some analyses excluding these four loci to determine whether null alleles could bias our results. Using only 5 loci, a significant correlation between H_O and the number of sources ($r^2 = 0.721$, $p = 0.001$, one-tailed) still exists. The deficiency of observed heterozygotes probably represents a Wahlund effect resulting from the recent mixing of multiple native-range sources, whose interbreeding has not yet homogenized them completely.

Population differentiation

Using the microsatellite dataset, global F_{ST} across all populations is 0.089. The highest pairwise F_{ST} values involve comparisons between Tampa or St. Petersburg and the other populations, especially Lower Matecumbe Key, New Orleans, Corpus Christi, Houston, and George Town (table 2). The largest value (pairwise $F_{ST} = 0.230$) involves St. Petersburg and George Town, whereas the smallest value (pairwise $F_{ST} = 0.003$) involves Gainesville and Orlando. Pairwise Φ_{ST} values are generally higher than microsatellite-based F_{ST} values as expected (Allendorf and Luikart 2007; table S1). Some negative Φ_{ST} values exist, most often involving comparisons with Houston, whereas the highest value (pairwise $\Phi_{ST} = 0.642$) involves Corpus Christi and St. Petersburg (table S1). AMOVA results indicate more genetic variation is partitioned within rather than among non-native populations for both datasets with over 90% of variation within populations for microsatellites compared to only 59% within populations for mtDNA (table S4). Results of the partitioned Mantel test are given in table S5. Sampling within native-range source populations (4-11 individuals per population) is somewhat low for traditional population genetic estimates of diversity and differentiation using microsatellites. For this reason, we limit our use of the native-range population microsatellite data to calculating multi-locus genotypic distances for comparisons among individuals and populations in conjunction with the mtDNA genetic distances.

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Table S1. Genetic diversity and estimates of pairwise population divergence among non-native populations using mtDNA sequence data. Mean percent sequence divergence (p-dist) between populations is above the diagonal and pairwise Φ_{ST} below the diagonal. Bold values on the diagonal are the mean within population p-dist. † indicates pairwise Φ_{ST} values not significantly different from zero.

	Tampa	St. Petersburg	Lower Matecumbe	Coral Gables	Orlando	Gainesville	New Orleans	Corpus Christi	Houston	George Town
Tampa	**0.067**	0.071	0.070	0.071	0.060	0.062	0.073	0.071	0.071	0.073
St. Petersburg	0.142	**0.055**	0.083	0.085	0.068	0.072	0.086	0.085	0.085	0.086
Lower Matecumbe	0.374	0.541	**0.021**	0.023	0.073	0.054	0.021	0.018	0.019	0.028
Coral Gables	0.413	0.578	0.186	**0.017**	0.075	0.054	0.013	0.011	0.012	0.019
Orlando	-0.013†	0.201	0.485	0.524	**0.054**	0.057	0.077	0.076	0.076	0.076
Gainesville	0.034†	0.253	0.314	0.352	0.054†	**0.053**	0.055	0.052	0.053	0.057
New Orleans	0.471	0.620	0.252	-0.018†	0.582	0.419	**0.010**	0.008	0.009	0.016
Corpus Christi	0.489	0.642	0.264	0.013†	0.605	0.440	0.033†	**0.006**	0.006	0.016
Houston	0.477	0.630	0.255	-0.015†	0.592	0.428	-0.005†	-0.041†	**0.007**	0.016
George Town	0.429	0.580	0.311	0.091	0.528	0.381	0.138	0.300	0.235	**0.017**

Table S2. Genetic diversity and estimates of pairwise population divergence among putative native-range source populations using mtDNA sequence data. Mean percent sequence divergence (p-dist) between populations is below the diagonal and bold values on the diagonal are the mean within population p-dist.

	La Habana	Portillo	S. Esmeralda	Caibarien	Jicarita	Soroa	Mariel	Belize
La Habana	**0.011**							
Portillo	0.099	**0.011**						
S. Esmeralda	0.084	0.106	**0.009**					
Caibarien	0.094	0.098	0.087	**0.006**				
Jicarita	0.045	0.101	0.080	0.095	**0.019**			
Soroa	0.019	0.096	0.081	0.091	0.044	**0.020**		
Mariel	0.014	0.101	0.084	0.095	0.049	0.016	**0.008**	
Belize	0.104	0.031	0.111	0.105	0.103	0.101	0.106	**0.002**

Table S3. Genetic diversity at nine microsatellite loci for 10 non-native *A. sagrei* populations. N, number of individuals; N_A, number of alleles; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity

Population		**15**	**61**	**63**	**68**	**70**	**77**	**91**	**94**	**95**
Tampa	N	19	20	20	19	20	20	20	20	20
	N_A	5	4	5	7	11	8	7	8	4
	H_O	0.526	0.150	0.300	0.263	0.750	0.900	0.800	0.550	0.400
	H_E	0.583	0.387	0.628	0.758	0.786	0.826	0.849	0.792	0.414
St. Petersburg	N	20	20	20	19	20	20	20	20	20
	N_A	10	2	5	9	8	7	9	5	4
	H_O	0.350	0.100	0.450	0.368	0.900	0.950	0.950	0.350	0.300
	H_E	0.678	0.097	0.691	0.838	0.846	0.755	0.876	0.594	0.310
Lower Matecumbe	N	20	19	20	19	20	20	20	20	20
	N_A	7	2	4	7	11	8	6	18	4
	H_O	0.150	0.053	0.100	0.421	0.900	0.700	0.800	0.700	0.400
	H_E	0.809	0.053	0.235	0.646	0.840	0.781	0.733	0.949	0.560
Coral Gables	N	20	18	20	15	20	20	20	20	20
	N_A	8	3	4	8	9	8	7	11	4
	H_O	0.250	0.056	0.050	0.400	0.850	0.750	0.700	0.650	0.600
	H_E	0.592	0.160	0.314	0.809	0.754	0.805	0.703	0.746	0.645
Orlando	N	19	20	20	18	20	20	20	20	20
	N_A	7	4	5	8	10	8	7	13	4
	H_O	0.263	0.200	0.200	0.444	0.950	0.850	0.850	0.650	0.450
	H_E	0.768	0.234	0.491	0.826	0.880	0.852	0.814	0.873	0.507
Gainesville	N	17	18	20	17	20	20	20	20	19
	N_A	7	5	5	8	9	11	8	16	5
	H_O	0.412	0.167	0.350	0.353	0.950	0.850	0.900	0.700	0.316
	H_E	0.715	0.459	0.608	0.729	0.890	0.839	0.812	0.936	0.469
New Orleans	N	17	18	20	13	20	20	20	20	18
	N_A	5	7	1	4	7	8	5	9	5
	H_O	0.176	0.389	---	0.077	0.800	0.750	0.750	0.550	0.389
	H_E	0.775	0.473	---	0.686	0.745	0.784	0.761	0.786	0.678

Corpus Christi	N	18	19	18	13	20	19	20	18	14
	N_A	5	6	1	7	7	8	6	10	3
	H_O	0.111	0.158	---	0.692	0.900	0.842	0.650	0.611	0.643
	H_E	0.744	0.415	---	0.763	0.832	0.838	0.698	0.757	0.579
Houston	N	16	17	19	15	20	20	20	19	16
	N_A	7	3	1	7	6	8	7	10	5
	H_O	0.188	0.000	---	0.333	0.700	0.900	0.800	0.526	0.438
	H_E	0.694	0.221	---	0.816	0.771	0.827	0.757	0.882	0.587
George Town	N	12	13	17	10	19	20	18	18	15
	N_A	5	2	2	8	7	8	4	6	3
	H_O	0.083	0.154	0.059	0.400	0.947	0.900	0.778	0.611	0.467
	H_E	0.764	0.148	0.059	0.784	0.794	0.830	0.708	0.789	0.384
Total	**N_A**	**14**	**11**	**8**	**13**	**12**	**14**	**12**	**28**	**6**

Table S4. AMOVA results partitioning genetic variation among and within non-native populations using both microsatellite and mtDNA sequence data.

Source of variation	d.f.	% of variation	*P* value
Microsatellites:			
Among	9	9.33	< 0.0001
Within	390	90.67	
Total	399		
mtDNA:			
Among	9	40.92	< 0.0001
Within	189	59.08	
Total	198		

Table S5. Partitioned Mantel test results for comparisons between individuals using microsatellite and mtDNA genetic distances.

	P-value
Among populations	0.000
Within populations:	
Tampa	0.177
St. Petersburg	0.832
Lower Matecumbe Key	0.999
Coral Gables	0.999
Orlando	0.670
Gainesville	0.434
New Orleans	0.999
Corpus Christi	0.999
Houston	0.999
George Town	0.999

Fig. S1 Map of the 10 non-native *A. sagrei* populations (black circles) showing the year of introduction in parentheses, and putative native-range source populations in Cuba and Belize (open circles). Citations for dates of introductions are Oliver (1950) for Tampa; Garman (1887) for the Florida Keys (Lower Matecumbe Key); Bell (1953) for Miami (Coral Gables); Godley et al. (1981) for Orlando; Wygoda & Bain (1980) for Gainesville; Thomas et al. (1990) for New Orleans; King et al. (1987) for Texas (Corpus Christi); Dixon (1987) for Houston; Minton & Minton (1984) for George Town.

Fig. S2 Haplotype networks of mtDNA sequence data reconstructed using TCS version 1.21. Rectangles are unique haplotypes, open circles are inferred haplotypes, and lines connecting shapes indicate one mutational step (1 step = 0.2% sequence divergence). Dashed black lines indicate seven or more steps that are not shown. Dashed grey boxes surround haplotype networks identified in previous phylogenetic analyses to be derived from the same native-range source population (Kolbe et al. 2004, 2007), which is indicated in each box. Total sample size (N) is given for each haplotype. The introduced population where the haplotype was sampled is also shown with the sample size for each population in parentheses. See Fig. 1 in Kolbe et al. (2007) for the phylogenetic relationships among these haplotype networks.

Fig. S1

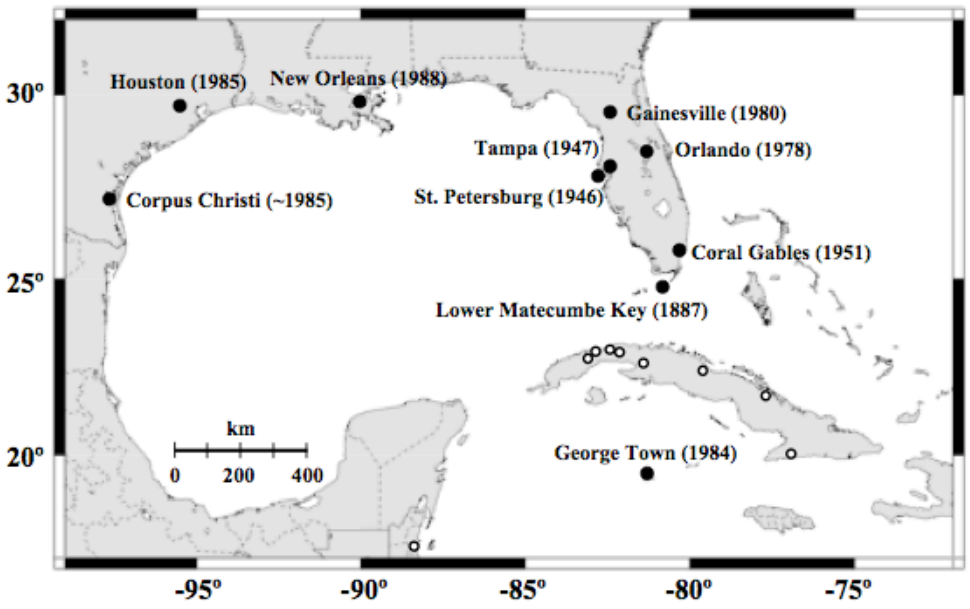
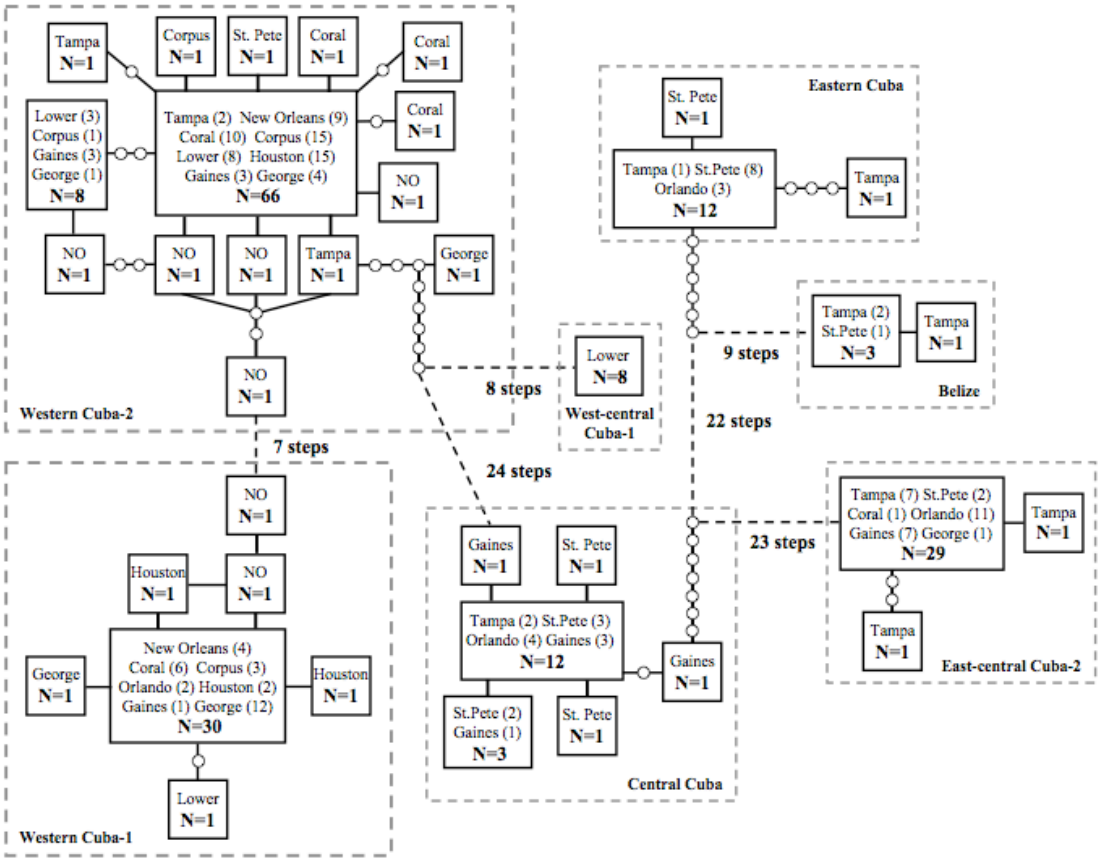

Houston (1985)
New Orleans (1988)
Gainesville (1980)
Tampa (1947)
Orlando (1978)
Corpus Christi (~1985)
St. Petersburg (1946)
Coral Gables (1951)
Lower Matecumbe Key (1887)
George Town (1984)
km
0
200
400
30°
25°
20°
-95°
-90°
-85°
-80°
-75°

Fig. S2


Tampa N=1
Corpus N=1
St. Pete N=1
Coral N=1
Coral N=1
Lower (3) Corpus (1) Gaines (3) George (1) N=8
Tampa (2) New Orleans (9) Coral (10) Corpus (15) Lower (8) Houston (15) Gaines (3) George (4) N=66
Coral N=1
NO N=1
NO N=1
NO N=1
NO N=1
Tampa N=1
George N=1
NO N=1
Western Cuba-2
7 steps
NO N=1
Houston N=1
NO N=1
George N=1
New Orleans (4) Coral (6) Corpus (3) Orlando (2) Houston (2) Gaines (1) George (12) N=30
Houston N=1
Lower N=1
Western Cuba-1
8 steps
Lower N=8
West-central Cuba-1
24 steps
Gaines N=1
St. Pete N=1
Tampa (2) St.Pete (3) Orlando (4) Gaines (3) N=12
Gaines N=1
St.Pete (2) Gaines (1) N=3
St. Pete N=1
Central Cuba
Eastern Cuba
St. Pete N=1
Tampa (1) St.Pete (8) Orlando (3) N=12
Tampa N=1
9 steps
Tampa (2) St.Pete (1) N=3
Tampa N=1
Belize
22 steps
23 steps
Tampa (7) St.Pete (2) Coral (1) Orlando (11) Gaines (7) George (1) N=29
Tampa N=1
Tampa N=1
East-central Cuba-2