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Population genetics

Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species

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Molecular genetic analyses show that introduced populations undergoing biological invasions often bring together individuals from genetically disparate native-range source populations, which can elevate genotypic variation if these individuals interbreed. Differential admixture among multiple native-range sources explains mitochondrial haplotypic diversity within and differentiation among invasive populations of the lizard *Anolis sagrei*. Our examination of microsatellite variation supports the hypothesis that lizards from disparate native-range sources, identified using mtDNA haplotypes, form genetically admixed introduced populations. Furthermore, within-population genotypic diversity increases with the number of sources and among-population genotypic differentiation reflects disparity in their native-range sources. If adaptive genetic variation is similarly restructured, then the ability of invasive species to adapt to new conditions may be enhanced.

Keywords: introduced species; microsatellite; mitochondrial DNA; multiple native-range sources

1. INTRODUCTION

Studies on a wide variety of taxa reveal extensive geographical genetic differentiation among conspecific populations (Avise 2000). This spatial genetic heterogeneity makes possible the identification of native-range sources for introduced populations during biological invasions. The existence of genetically differentiated populations, some of which come into contact within their native range without merging, raises the question of whether intrinsic reproductive barriers prevent interbreeding between them. If these populations are reproductively isolated and therefore represent cryptic species, then we have underestimated biodiversity. Alternatively, if these populations lack intrinsic reproductive isolation, then an explanation other than genetic incompatibility is needed for their extensive genetic differentiation and apparent lack of gene flow in the native range. Additionally, whether these geographical populations can admix has significant implications for conservation purposes: genetic admixture of previously isolated populations can increase genetic variation and create novel genetic combinations within non-native populations, which may enhance adaptation during biological invasions (Lavergne & Molofsky 2007).

Genetic analyses of biological invasions reveal two related patterns: introductions from multiple native-range source populations and increased levels of genetic variation within non-native populations compared with native ones (Wares *et al.* 2005). An implicit assumption is that individuals from different native-range sources interbreed, causing admixture that combines genetic variation from multiple genetically differentiated source populations to increase genetic variation within introduced populations. Most previous studies rely on a single marker, such as mtDNA or cpDNA, to identify native-range source populations. These markers, however, cannot distinguish genetic admixture from coexistence of cryptic species within non-native populations; thus, the assumption that admixture produces increased genetic variation is rarely tested.

Phylogenetic analyses of mtDNA sequences reveal at least nine geographically and genetically distinct native-range source populations from the Bahamas, Belize and Cuba contributing to invasive populations of *Anolis sagrei* in the southeastern USA and Caribbean and Pacific islands. As many as five native-range sources contribute to a single non-native population, and mtDNA sequence divergence among haplotypes from these sources ranges from 1.4 to 11.1% (Kolbe *et al.* 2004, 2007). These mtDNA haplotypes coexist within non-native *A. sagrei* populations, indicating that lizards from different native-range sources co-occur and have the opportunity to interbreed. Given its invasion history, *A. sagrei* provides an opportunity to examine whether individuals from geographically and genetically distinct native-range sources interbreed and how admixture affects genetic diversity within and differentiation among non-native populations during a biological invasion. We use mtDNA sequence haplotypes to identify native-range sources and nuclear microsatellite genotypes to identify mixing of genetic variation in introduced populations.

2. MATERIAL AND METHODS

(a) *Sampling*

We sampled 20 individuals from each of 10 non-native *A. sagrei* populations in Florida, Texas, Louisiana, USA and Grand Cayman, and 52 individuals from eight putative native-range source populations (Kolbe *et al.* 2004, 2007). We sequenced the mitochondrial gene *ND2* and genotyped nine microsatellite loci for each individual (Bardeleben *et al.* 2004).

(b) *Genetic diversity and population differentiation*

For each population–locus combination, we estimated the number of alleles (N_A), observed heterozygosity (H_o) and unbiased expected heterozygosity (H_e) in GENEPOP (Raymond & Rousset 1995). For each population, the total number of alleles, mean number of alleles per locus and number of private alleles were calculated in GENALEX v. 6 (Peakall & Smouse 2006), the inbreeding coefficient (F_{IS}) was estimated with GENEPOP and allelic richness (A) was estimated with FSTAT v. 2.932 (Goudet 2001). See the electronic

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Table 1. Genetic diversity at nine microsatellite loci for introduced *A. sagrei* populations. (N_A, total number of alleles; N_{MA}, mean number of alleles per locus; N_{PA}, number of private alleles; A, allelic richness; F_{IS}, inbreeding coefficient; H_e, unbiased expected heterozygosity; H_o, observed heterozygosity.)

population	N_A	N_{MA}	N_{PA}	A	F_{IS}	H_e	H_o
Tampa, FL	59	6.556	2	3.143	0.232	0.669	0.515
St Petersburg, FL	59	6.556	3	3.165	0.171	0.632	0.524
Lower Matecumbe Key, FL	67	7.444	5	3.180	0.251	0.623	0.469
Coral Gables, FL	62	6.889	2	3.006	0.218	0.614	0.478
Orlando, FL	66	7.333	2	3.457	0.221	0.694	0.540
Gainesville, FL	74	8.222	3	3.512	0.219	0.717	0.555
New Orleans, LA	51	5.667	2	3.044	0.288	0.711	0.431
Corpus Christi, TX	53	5.889	1	2.970	0.190	0.703	0.512
Houston, TX	54	6.000	1	3.066	0.279	0.694	0.432
George Town, GC	45	5.000	1	2.815	0.104	0.584	0.489

Table 2. Estimates of pairwise genetic differences among introduced *A. sagrei* populations using nine microsatellite loci. (Nei's D is above the diagonal and F_{ST} is below the diagonal.)

	Tampa	St Petersburg	Lower Matecumbe	Coral Gables	Orlando	Gainesville	New Orleans	Corpus Christi	Houston	George Town
Tampa	—	0.201	0.368	0.273	0.110	0.127	0.360	0.382	0.386	0.478
St Petersburg	0.067	—	0.476	0.442	0.220	0.207	0.480	0.598	0.623	0.693
Lower Matecumbe Key	0.126	0.169	—	0.191	0.173	0.250	0.131	0.128	0.167	0.189
Coral Gables	0.096	0.161	0.073	—	0.160	0.153	0.219	0.187	0.136	0.197
Orlando	0.020	0.071	0.054	0.049	—	0.082	0.170	0.221	0.212	0.277
Gainesville	0.024	0.062	0.079	0.044	0.003	—	0.232	0.264	0.276	0.322
New Orleans	0.119	0.165	0.040	0.080	0.048	0.068	—	0.155	0.167	0.168
Corpus Christi	0.129	0.200	0.040	0.069	0.070	0.081	0.049	—	0.141	0.168
Houston	0.132	0.204	0.060	0.045	0.068	0.087	0.055	0.045	—	0.145
George Town	0.167	0.230	0.076	0.080	0.099	0.110	0.059	0.064	0.051	—

supplementary material for tests of linkage disequilibrium and Hardy–Weinberg equilibrium. To evaluate population differentiation among the non-native *A. sagrei* populations, we calculated pairwise F_{ST} values (Weir & Cockerham 1984) using the microsatellite data in GENEPOP and pairwise Φ_{ST} values using mtDNA sequence data in ARLEQUIN v. 3.1 (Excoffier *et al.* 2005).

(c) *Hypothesis testing*

First, we conducted an indirect test of interbreeding among individuals from different native-range source populations within introduced populations by comparing the concordance between multilocus microsatellite (Nei's D) and mtDNA (uncorrected (p) distance) genetic distances at both the individual and population levels. Such interbreeding will quickly erode cytonuclear disequilibrium generated by initial founders coming from different sources. Thus, no correlation is expected between microsatellite and mtDNA distances at the individual level within an introduced population. A correlation between microsatellite and mtDNA distances should, however, persist at the population level because both microsatellite alleles and mtDNA haplotypes from founding individuals still exist within a population, even if no longer correlated within individuals.

Alternatively, if individuals from distinct sources are reproductively isolated or outbreeding depression exists, and successful breeding occurs only or predominantly among individuals from the same source, then correlations between microsatellite and mtDNA distances should persist at both the individual and population levels. To test this alternative, we used genetic distances among individuals sampled from eight putative native-range sources to mimic the cytonuclear disequilibrium that would be generated by initial founders coming together within an introduced population prior to any potential interbreeding.

We calculated Nei's D (Nei 1978) in GENALEX v. 6 and uncorrected (p) distance in MEGA v. 3.1 (Kumar *et al.* 2004) and compared distance matrices with a Mantel test in PASSAGE (Rosenberg 2001). For the individual level in the non-native range, we used a partitioned Mantel test because only comparisons between individuals within each non-native population were relevant (Melville *et al.* 2006).

Second, we predicted a positive relationship between within-population genetic diversity (allelic richness and H_o) and the number of native-range source populations contributing to a non-native population. We previously identified two to five sources derived from Belize and Cuba for the 10 non-native populations in this study (Kolbe *et al.* 2007).

Third, we tested for a relationship between population differentiation (microsatellite F_{ST} and mtDNA Φ_{ST}) and geographical distance with Mantel tests. We also tested for a relationship between microsatellite F_{ST} and mtDNA Φ_{ST}. We predicted a positive relationship between population differentiation and native-range source disparity, testing microsatellite F_{ST} against both the number of sources and frequency of haplotypes from different sources. See Kolbe *et al.* (2007) for details on calculating the source number and source frequency matrices from mtDNA haplotype frequencies.

3. RESULTS

Genetic diversity and differentiation for introduced populations are described in tables 1 and 2. A strong correlation exists between microsatellite and mtDNA distances at the population level in both the native and introduced ranges (table 3). By contrast, no correlation exists at the individual level for the introduced populations (partitioned Mantel test: within populations, all $p > 0.150$, mean $p = 0.711$), as predicted if individuals from different native-range sources are interbreeding within introduced populations. Consistent with the null prediction for cytonuclear disequilibrium and no interbreeding, microsatellite and mtDNA genetic distances are correlated for individuals from genetically divergent native-range sources (table 3). See the electronic supplementary material for an exploratory analysis of

Table 3. Mantel results for matrix correlations. (Correlation coefficients (r) significantly different from zero (p-value <0.05 are in italics.)

matrix 1	matrix 2	r	p-value
native range			
μsat-Nei's D (individuals)	mtDNA p-distance (individuals)	*0.285*	*<0.001*
μsat-Nei's D (populations)	mtDNA p-distance (populations)	*0.561*	*0.036*
introduced range			
μsat-Nei's D (individuals)	mtDNA p-distance (individuals)	—	0.711[a]
μsat-Nei's D (populations)	mtDNA p-distance (populations)	*0.615*	*<0.001*
μsat-F_{ST}	geographical distance	0.134	0.218
mtDNA-Φ_{ST}	geographical distance	0.169	0.170
μsat-F_{ST}	mtDNA-Φ_{ST}	0.074	0.620
μsat-F_{ST}	source number	*0.680*	*<0.001*
μsat-F_{ST}	source frequency	*0.614*	*<0.001*

[a] Mean p-value for introduced populations from a partitioned Mantel test (see table S5 in the electronic supplementary material for details).
μsat, microsatellite.

the effect of mtDNA sequence divergence within introduced populations, number of sources and time since introduction on cytonuclear disequilibrium.

Allelic richness and source number show a nearly significant positive relationship ($r^2=0.244$, $p=0.074$, one tailed), and a positive relationship exists between heterozygosity and the number of sources ($r^2=0.504$, $p=0.011$, one tailed; figure 1). No relationship exists between population differentiation and geographical distance among introduced populations (table 3), providing no evidence for isolation-by-distance among non-native populations. A highly significant relationship exists between population differentiation and native-range source disparity, using both source-number and source-frequency matrices (figure 1; table 3). The less differentiated they are genetically, the more similar two populations are in their native-range sources. A general deficiency of heterozygotes (table 1; table S3 in the electronic supplementary material) suggests a Wahlund effect; the historical mixing of non-native sources is not yet obliterated by interbreeding among them.

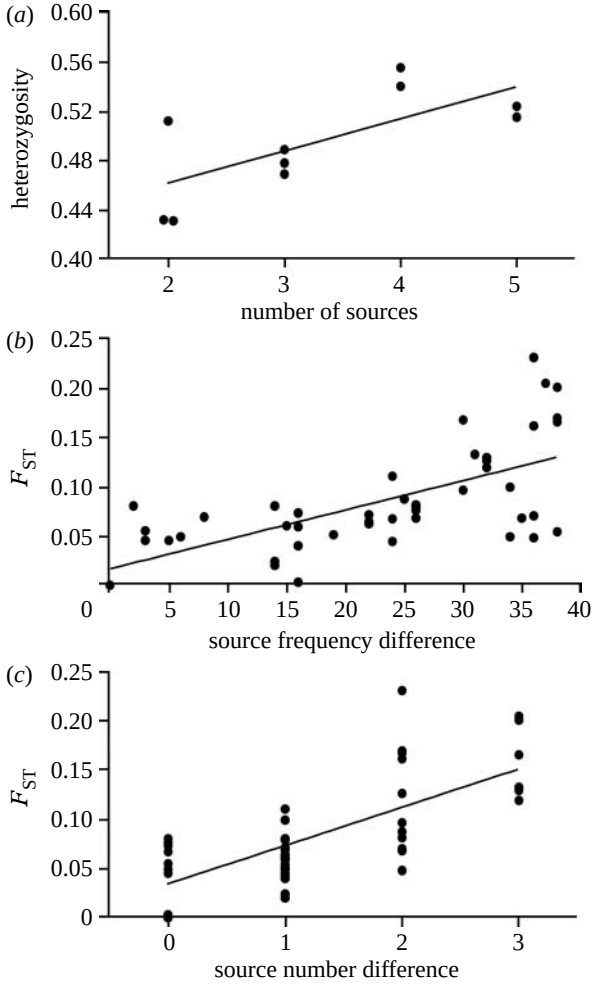

Figure 1. Introduced *A. sagrei* populations show (*a*) a positive relationship between genetic diversity (observed heterozygosity) and source number using linear regression, and significant Mantel test correlations between population differentiation (F_{ST}) and two measures ((*b*) source frequency difference and (*c*) source number difference) of native-range source disparity.

4. DISCUSSION

Our results reject the hypothesis that individuals from different native-range sources merely coexist without interbreeding within invasive *A. sagrei* populations. Genetic admixture produces a positive relationship between genetic diversity and source number over a range of values (figure 1), indicating that source number has an additive effect on within-population genetic diversity. An exception to this relationship is Corpus Christi, which has attained relatively high heterozygosity despite founding by only two sources (figure 1). If selectively important genetic variation also increases with source number, then introduced populations derived from more sources should undergo more rapid evolution by natural selection (Allendorf & Luikart 2007). Studies detecting multiple sources in biological invasions should test for an effect of admixture on genetic diversity, which is crucial for understanding the potential for adaptation in introduced populations (Gilchrist & Lee 2007; Lavergne & Molofsky 2007).

Our data do not exhibit the isolation-by-distance pattern of variation expected if post-introduction gene flow and genetic drift were the major factors structuring geographical genetic variation among populations (Herborg *et al.* 2007). Non-native populations more similar in source number and frequency are more

similar genetically regardless of geographical location; thus, genetic affinities among introduced populations are best explained by their sharing of colonists from distinct native-range sources (figure 1; table 3). In the case of Lower Matecumbe Key, private alleles from a unique source in west central Cuba (see Kolbe *et al.* 2007) contribute to population differences between it and all other non-native populations. Other factors, such as differential founder effects (Hawley *et al.* 2006), also may generate genetic differentiation among non-native *A. sagrei* populations.

By combining mtDNA and microsatellite data, we show that admixture of multiple genetically disparate native-range sources elevates genetic variation within non-native populations, which may enhance the response to natural selection and facilitate invasion success (Ellstrand & Schierenbeck 2000; Lavergne & Molofsky 2007). Additionally, this interbreeding of individuals from different native-range sources within non-native populations suggests that factors other than intrinsic reproductive isolation may be needed to explain the maintenance of historically and geographically distinct genetic lineages in the native range, a pattern found in many *Anolis* species (e.g. Glor *et al.* 2004; Kolbe *et al.* 2004).

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