
Multiple Sources, Admixture, and Genetic Variation in Introduced *Anolis* Lizard Populations

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Abstract: *Invasive species are classically thought to suffer from reduced within-population genetic variation compared to their native-range sources due to founder effects and population bottlenecks during introduction. Reduction in genetic variation in introduced species may limit population growth, increase the risk of extinction, and constrain adaptation, hindering the successful establishment and spread of an alien species. Results of recent empirical studies, however, show higher than expected genetic variation, rapid evolution, and multiple native-range sources in introduced populations, which challenge the classical scenario of invasive-species genetics. With mitochondrial DNA (mtDNA) sequence data, we examined the molecular genetics of 10 replicate introductions of 8 species of Anolis lizards. Eighty percent of introductions to Florida and the Dominican Republic were from multiple native-range source populations. MtDNA haplotypes restricted to different geographically distinct populations in the native range of a species commonly occurred as intrapopulation polymorphisms in introduced populations. Two-thirds of introduced populations had two or more sources, and admixture elevated genetic variation in half of the introduced populations above levels typical of native-range populations. The mean pairwise sequence divergence among haplotypes sampled within introduced populations was nearly twice that within native-range populations (2.6% vs. 1.4%). The dynamics of introductions from multiple sources and admixture explained the observed genetic contrasts between native and introduced Anolis populations better than the classical scenario for most introduced populations. Elevated genetic variation through admixture occurred regardless of the mode or circumstances of an introduction. Little insight into the number of sources or amount of genetic variation in introduced populations was gained by knowing the number of physical introductions, the size of a species' non-native range, or whether it was a deliberate or accidental introduction. We hypothesize that elevated genetic variation through admixture of multiple sources is more common in biological invasions than previously thought. We propose that introductions follow a sequential, two-step process involving a reduction in genetic variation due to founder effects and population bottlenecks followed by an increase in genetic variation if admixture of individuals from multiple native-range sources occurs.*

Keywords: anoles, biological invasion, invasion history, invasive species, mtDNA, native-range source populations

Fuentes Múltiples, Mezcla y Variación Genética en Poblaciones de Lagartijas *Anolis* Introducidas

Resumen: *La reducción en la variación genética en especies introducidas puede limitar el crecimiento poblacional, incrementar el riesgo de extinción y limitar la adaptación, lo que dificulta el establecimiento exitoso y dispersión de una especie exótica. Sin embargo, los resultados de estudios empíricos recientes muestran mayor*

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variación genética que la esperada, evolución rápida y múltiples fuentes nativas en poblaciones introducidas, lo que cuestiona el escenario clásico de la genética de especies invasoras. Con datos de secuencia de ADN mitocondrial (ADNmt) examinamos la genética molecular de 10 introducciones replicadas de 8 especies de lagartijas Anolis. Ochenta por ciento de las introducciones en Florida y la República Dominicana partieron de poblaciones fuente con múltiples rangos de distribución. Los haplotipos de ADNmt restringidos a poblaciones geográficamente distintas en el rango de una especie nativa a menudo ocurrieron como polimorfismos intrapoblacionales en poblaciones introducidas. Dos tercios de las poblaciones introducidas tenían dos o más fuentes, y la mezcla incrementó la variación genética en la mitad de las poblaciones introducidas por arriba de niveles típicos de poblaciones nativas. La divergencia en la secuencia pareada promedio entre haplotipos muestreados en poblaciones introducidas fue casi el doble que en las poblaciones nativas (2.6% vs. 1.4%). La dinámica de las introducciones desde fuentes múltiples explicó los contrastes genéticos observados entre las poblaciones nativas e introducidas de Anolis mejor que el escenario clásico para la mayoría de las poblaciones introducidas. La variación genética elevada por la mezcla ocurrió independientemente del modo o circunstancias de la introducción. Se obtuvo poco entendimiento del número de fuentes o de la cantidad de variación genética en las poblaciones introducidas al conocer el número de introducciones físicas, el tamaño del rango de distribución no nativa de la especie o si fue una introducción deliberada o accidental. Proponemos la hipótesis que la elevada variación genética por mezcla de fuentes múltiples es más común en las invasiones biológicas que lo que se pensaba anteriormente. Proponemos que las introducciones siguen un proceso secuencial de dos etapas que implica la reducción de la variación genética debido a efectos de fundador y cuello de botella poblacionales seguida por un incremento en la variación genética si ocurre una mezcla de individuos provenientes de fuentes múltiples.

Palabras Clave: ADNmt, *Anolis*, distribución nativa, especies invasoras, invasión biológica, historia de invasión, poblaciones fuente

Introduction

In the classical scenario of biological invasion, populations of alien species have reduced within-population genetic variation compared to native-range source populations (Sakai et al. 2001; Allendorf & Lundquist 2003; Novak & Mack 2005). Variation is lost through genetic drift associated with a small number of initial colonists and continued small size of the introduced population in the initial generations of its establishment (Nei et al. 1975; Frankham et al. 2002; Novak & Mack 2005). Reduced genetic variation is expected to inhibit the successful establishment and spread of alien species for two reasons (Allendorf & Lundquist 2003). First, reduced genetic diversity and increased relatedness among individuals can cause inbreeding depression, thus limiting population growth and increasing the probability of population extinction (Newman & Pilson 1997; Frankham & Ralls 1998; Nieminen et al. 2001). Second, reduced genetic variation can constrain the evolution of a population by natural selection in the potentially novel non-native environment (Fisher 1930; Falconer & Mackay 1996).

In contrast to these classical expectations, recent evidence shows that some introduced populations contain higher genetic variation than populations in their native range (reviewed in Novak & Mack 2005 and Wares et al. 2005) and that adaptation may play an important role in their invasion success (Sakai et al. 2001; Lee 2002). Indeed, rapid life history and morphological evolution in introduced populations (e.g., Hendry et al. 2000; Huey et al. 2000), novel hybridization between previously iso-

lated species or populations (e.g., Ellstrand & Schierenbeck 2000; Gaskin & Schaal 2002), and increased genetic variation within introduced populations (e.g., Kolbe et al. 2004; Voisin et al. 2005) are not consistent with predictions of the classical scenario and require an alternative model of the dynamics of population genetic variation during an introduction.

The classical scenario does not explain one observation in particular: elevated levels of genetic variation in introduced versus native populations. The simplest explanation is that admixture of genetic variation from multiple sources within introduced populations overcomes the loss of genetic variation incurred through founder effects and population bottlenecks within each introduction (e.g., Kolbe et al. 2004; Voisin et al. 2005). Repeated introductions from the same local source cannot explain the elevated levels of haplotype diversity in many introduced populations because such introductions merely curb the potential loss of haplotypes but cannot introduce new ones.

Studies using molecular markers have documented multiple native-range source populations in biological invasions of disparate taxa, including grasses (Novak & Mack 1993), algae (Voisin et al. 2005), snails (Facon et al. 2003), fishes (Collins et al. 2002), and lizards (Kolbe et al. 2004). The frequency with which introduced populations contain genetic contributions from single versus multiple native-range source populations nonetheless remains largely unmeasured (Novak & Mack 2005), and whether admixture of genetic variation from multiple source populations facilitates invasion success is little known (but

see Ellstrand & Schierenbeck 2000; Lavergne & Molofsky 2007). We assessed the prevalence of multiple sources in successful introductions of Caribbean *Anolis* lizards outside their native ranges.

To estimate the frequency of multiple sources in introductions, one needs systematic studies of introductions of multiple, closely related species under similar circumstances (Vellend et al. 2005). Nearly 20 species of *Anolis* lizards are established outside their native ranges (Lever 2003), and eight of these species show replicate introductions to and from the same geographic regions. These species of *Anolis* lizards are well established in southern Florida (Lever 2003; Meshaka et al. 2004), and all are native to the northern Caribbean (Schwartz & Henderson 1991). In addition, independent introductions for two of these *Anolis* species occurred to the Dominican Republic, for a total of 10 independent introductions.

One of these introductions, the *Anolis sagrei* invasion into Florida, already has been studied. Kolbe et al. (2004) found that *A. sagrei* populations in Florida derive from at least eight distinct native-range sources, and most introduced populations have elevated haplotype diversity compared with native-range populations. By extending this analysis to all introduced *Anolis* species in Florida and the Dominican Republic, we addressed two questions: How common are introductions from multiple native-range source populations? and What effects do multiple source populations have on genetic variation within introduced populations? We used phylogenetic and population-genetic analyses of mitochondrial DNA (mtDNA) haplotypes to identify the native-range source populations of introduced *Anolis* lizards and to determine the effects of single versus multiple native-range sources for within-population genetic variation in introduced populations.

Methods

Introduced Species and Sampling

Eight species of *Anolis* lizards endemic to the northern Caribbean—the Bahamas, Cuba, Hispaniola, Jamaica, and Puerto Rico—are established in Florida (*A. chlorocyanus*, *A. cristatellus*, *A. cybotes*, *A. distichus*, *A. equestris*, *A. garmani*, *A. porcatius*, and *A. sagrei*; Schwartz & Henderson 1991; Lever 2003; Meshaka et al. 2004). Some species are known from a single (*A. garmani*) or few (*A. chlorocyanus*, *A. cristatellus*, *A. cybotes*, and *A. porcatius*) localities in the greater Miami area, whereas others are more widespread in Florida (*A. distichus*, *A. equestris*, and *A. sagrei*). Two of these species (*A. cristatellus* and *A. porcatius*) are established also in the Dominican Republic.

We collected from their native and introduced ranges individuals of the eight *Anolis* species introduced to Florida. For *A. sagrei*, we used data in Kolbe et al. (2004,

2007). For the other seven *Anolis* species, we collected 17–167 individuals per species from 12–48 native populations and 4–31 individuals per species from 1–6 introduced populations. Within each species, sampling ranged from 1 to 14 individuals per population with a mean of 3.8 individuals per population. Previously published sequences used in this study are available from Genbank (Supplementary Materials).

DNA Isolation and Sequencing

We extracted genomic DNA from liver or tail tissue with Viogene extraction kits (Viogene, Taipei, Taiwan). We used the following polymerase chain reaction (PCR) protocol to amplify gene products: 300 seconds at 95 °C followed by 30 cycles of 95 °C for 35 seconds, 53–60 °C for 35 seconds, and 72 °C for 150 seconds. The PCR reaction volumes varied from 25 to 50 µL and included 1–5 µL genomic DNA and a mixture of 49.5% H₂O, 10% M190G thermophilic DNA polymerase 10× buffer, 10% 25 mM MgCl₂, 10% dNTPs, and 10% 2 pmol of each primer, and 0.5% Promega *Taq* DNA polymerase. We purified PCR products with Viogene Gel-M purification kits (Viogene). We sequenced an approximately 1200 base-pair region of mtDNA, including the genes encoding ND2, tRNA^{Trp}, and tRNA^{Ala} for each species with primers H5730, L4882c, and L4437 (Macey et al. 1997). Sequencing reactions were run with Big-Dye Terminator Ready-Reaction Kits (Perkin-Elmer, Waltham, Massachusetts) on either a BaseStation automated sequencer (MJ Research, Waltham, Massachusetts) or an ABI 3130 capillary sequencer (Applied Biosystems, Forest City, California). Sequences were aligned manually with secondary-structural models (Kumazawa & Nishida 1993). All new sequences were deposited in GenBank (Supplementary Materials).

Phylogenetic and Population-Genetic Analyses

We used phylogenetic analysis to determine whether haplotypes sampled in introduced populations were derived from multiple native-range source populations. Phylogenetic analyses included all unique sequences for each introduced *Anolis* species, including haplotypes from both native and introduced populations, closely related species, and two more distant outgroups for each analysis based on previous phylogenetic studies of *Anolis* (Glor et al. 2003; Nicholson et al. 2005). We used MRMODELTEST 1.1B (<http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html>), a modified version of MODELTEST (Posada & Crandall 1998) to conduct hierarchical hypothesis testing to choose the appropriate model of evolution for subsequent Bayesian phylogenetic analyses. We implemented Bayesian phylogenetic analyses in MRBAYES 3.1 (Huelsenbeck & Ronquist 2001). These analyses ran four chains for 2,000,000 generations and sampled trees every 10,000 generations. We determined

the burn-in trees by plotting the ln-likelihood score of each sampled tree against its generation. Trees generated before an asymptotic ln-likelihood score was reached were discarded (the first 20% of trees to be conservative). Using the remaining trees, we calculated a 50% majority-rule consensus tree in PAUP* 4.0b10 (Swofford 2002). We repeated the analysis for each species twice to avoid searching within local optima. These trees were used to identify well-supported, geographically restricted, native-range haplotype clades that also contained haplotypes from introduced populations nested within them (Kolbe et al. 2004). Posterior probabilities indicate support for nodes leading to clades with haplotypes from introduced populations.

If the sequence divergence of haplotypes within introduced populations exceeds that normally found within native populations, then these haplotypes may be from different native-range sources. To test this prediction we calculated the sequence divergence (%) among mtDNA haplotypes sampled in introduced populations but derived from different native-range sources. These results, combined with phylogenetic analyses, were used to identify introductions from multiple native-range source populations for all species of introduced *Anolis* lizards.

To identify changes in the distribution of population-genetic variation in these introductions, we tested for significant geographic structure among both native and introduced populations with analysis of molecular variance (AMOVA; Excoffier et al. 1992). Comparison of separate native and introduced-range AMOVAs for a given species can identify changes in the distribution of haplotype diversity that occurred during introduction. We conducted a third AMOVA for species in which multiple source populations were detected to determine the effect of admixture on haplotype diversity in these introduced populations. This AMOVA used all the mtDNA haplotypes detected in the introduced range of a species, but instead of partitioning haplotype diversity within and between introduced populations as in the previous AMOVA, we partitioned the total amount of haplotype diversity found in the introduced range into components derived from within native-range source populations versus variation created by admixture of these distinct sources. We conducted a second set of AMOVAs for *A. sagrei* that included only the nine populations from the Miami area. We also tested for a difference between introduced and native populations in mean within-population sequence divergence with a Wilcoxon rank-sum test.

Sample size, both the numbers of individuals and populations, could affect our ability to detect multiple native-range source populations. Therefore, we tested for a relationship between the number of populations sampled and the number of sources detected for each species and between the mean number of individuals sampled per population and the number of sources detected.

Results

Aligned mtDNA data sets ranged from 1102 to 1251 base pairs and were composed of 504 previously published sequences and 167 new sequences (Supplementary Materials). The program MRMODELTEST selected the GTR+I+ Γ model of evolution for Bayesian phylogenetic analyses for every mtDNA data set except *A. equestris*, for which the HKY+I+ Γ was selected.

For all species of introduced *Anolis*, phylogenetic analyses of mtDNA sequences revealed strong patterns of geographic genetic structure in the native range of each species (Figs. 1a-g), providing unambiguous assignment of haplotypes sampled from introduced populations to source populations in the native range. For all species except *A. chlorocyanus* (Figs 1a & 2), haplotypes from introduced Florida population(s) were nested within more than one well-supported native-range clade. For five species—*A. cristatellus*, *A. cybotes*, *A. equestris*, *A. garmani*, and *A. porcatius*—haplotypes from introduced populations were derived from two geographically and genetically distinct native-range source populations (Figs. 1b, c, e, f, g, & 2). In addition to including haplotypes from two native-range sources, over 85% of *A. porcatius* individuals sampled from introduced Florida populations possessed mtDNA haplotypes nested within the southern Florida clade of the native anole, *A. carolinensis* (Fig. 1g). Two species had more than two native-range sources: *A. distichus* populations in Florida were likely from four geographically and genetically distinct native-range sources (Figs. 1d & 2), whereas Miami-area introduced populations of *A. sagrei* had five native-range source populations (Kolbe et al. 2007). For introductions in the Dominican Republic, the introduced *A. cristatellus* population contained haplotypes from two different native-range clades (Figs. 1b & 2), whereas the introduced *A. porcatius* population contained haplotypes from only one native-range clade (Figs. 1g & 2). These *A. cristatellus* and *A. porcatius* haplotypes in introduced populations in the Dominican Republic were phylogenetically distinct from haplotypes detected in introduced Florida populations of these two species (Figs. 1b & 1g), and they originated from geographically distinct areas of their native ranges (Fig. 2).

For nearly every species these phylogenetic results were corroborated by comparison of the sequence divergence between haplotypes sampled within introduced populations but derived from different native-range sources. These sequence divergences ranged from 1.8% to 9.9% (Table 1). For all species except *A. garmani* this sequence divergence exceeded the mean within-population sequence divergence for native populations, suggesting that haplotypes within introduced populations were derived from distinct native-range populations. For *A. garmani* the sequence divergence within

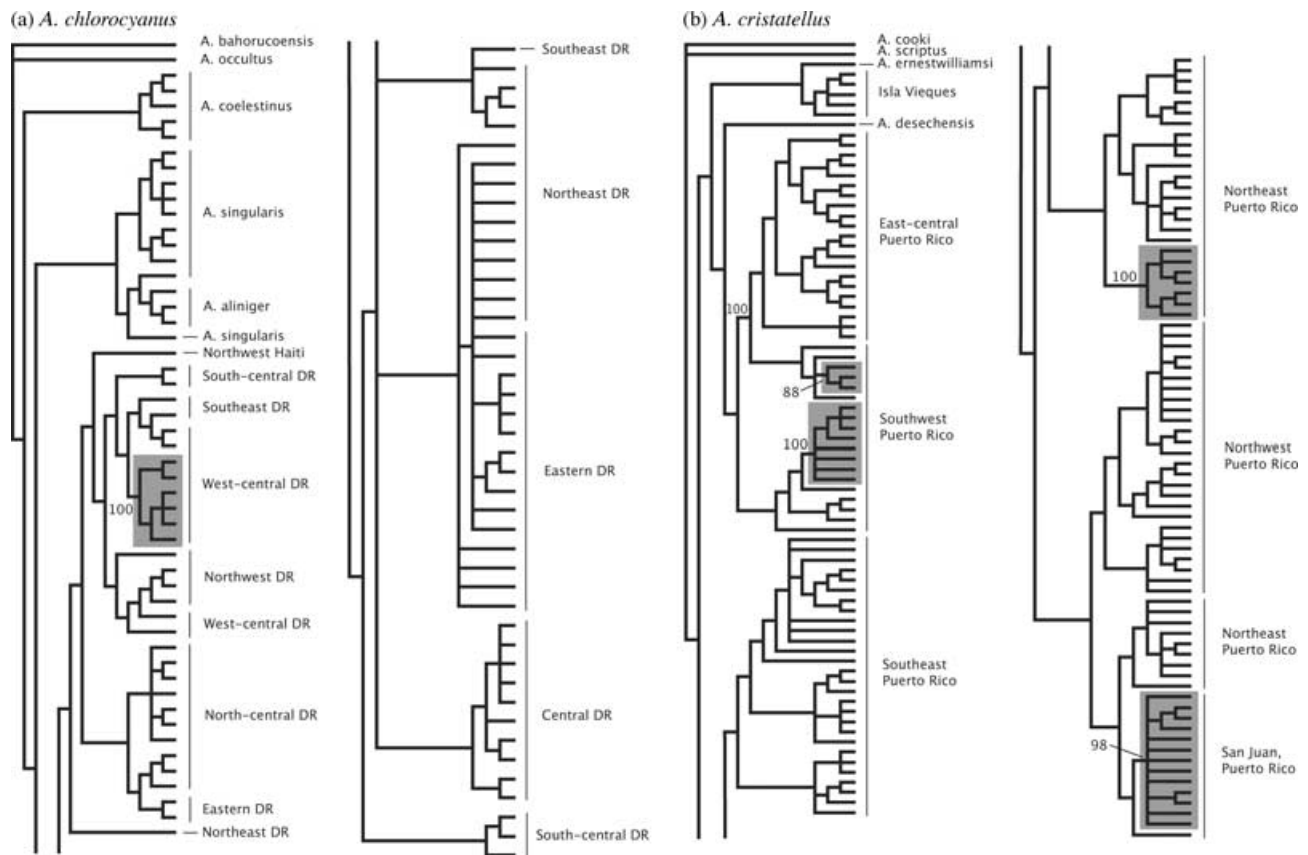


Figure 1. (a–g) Majority-rule consensus trees from Bayesian analyses of all unique mtDNA haplotypes sampled from introduced and native-range populations of (a) *A. chlorocyanus*, (b) *A. cristatellus*, (c) *A. cybotes*, (d) *A. distichus*, (e) *A. equestris*, (f) *A. garmani*, and (g) *A. porcatius*. Background shading indicates well-supported clades that are geographically distinct in the native range and collectively contain all haplotypes sampled from the introduced populations in Florida or the Dominican Republic (DR). Bayesian posterior probabilities are shown on branches leading to these clades. Geographic regions in the native range, closely related species, and more distant outgroups included in the phylogenetic analysis are shown to the right.

the introduced population was similar to the average within-population sequence divergence for native populations, although the well-supported phylogeographic structure in its native range suggests multiple distinct source populations.

AMOVA showed significant geographic genetic structure in the native ranges of all eight *Anolis* species, and more haplotype diversity was partitioned among rather than within populations for all species except *A. garmani* (Table 2). Significant geographic genetic structure also existed among introduced-range populations of *A. cristatellus*, *A. distichus*, and *A. sagrei*. In contrast to the higher among-population haplotype diversity in the native range, however, higher within-population haplotype diversity existed for all species in the introduced range except *A. cristatellus* (Table 2). For that species, although phylogenetic analysis indicated introductions from two geographically distinct native-range sources, we detected no admixture between the two sources in the introduced range (Fig. 2). We could not perform an AMOVA for intro-

duced populations of *A. chlorocyanus* and *A. garmani* because only one population was sampled for each species. Analyses to partition the haplotype diversity in the introduced range into that derived from native-range source populations versus the admixture of multiple sources revealed that admixture explained the vast majority of the total haplotype diversity for all introduced *Anolis* species analyzed (Table 2). Some species were not included in this analysis because of insufficient sample size (*A. chlorocyanus* and *A. porcatius*) or because multiple introductions were not admixed (*A. cristatellus*).

Introduced populations had significantly higher mean within-population haplotypic sequence divergence than native-range populations (Wilcoxon rank-sum test: $z = 3.508$; $p = 0.0005$; Fig. 3). The mean within-population sequence divergence for introduced populations was nearly twice that of native-range populations (2.6% vs. 1.4%). A significant positive relationship existed between the number of introduced populations sampled for each species and the number of native-range source populations

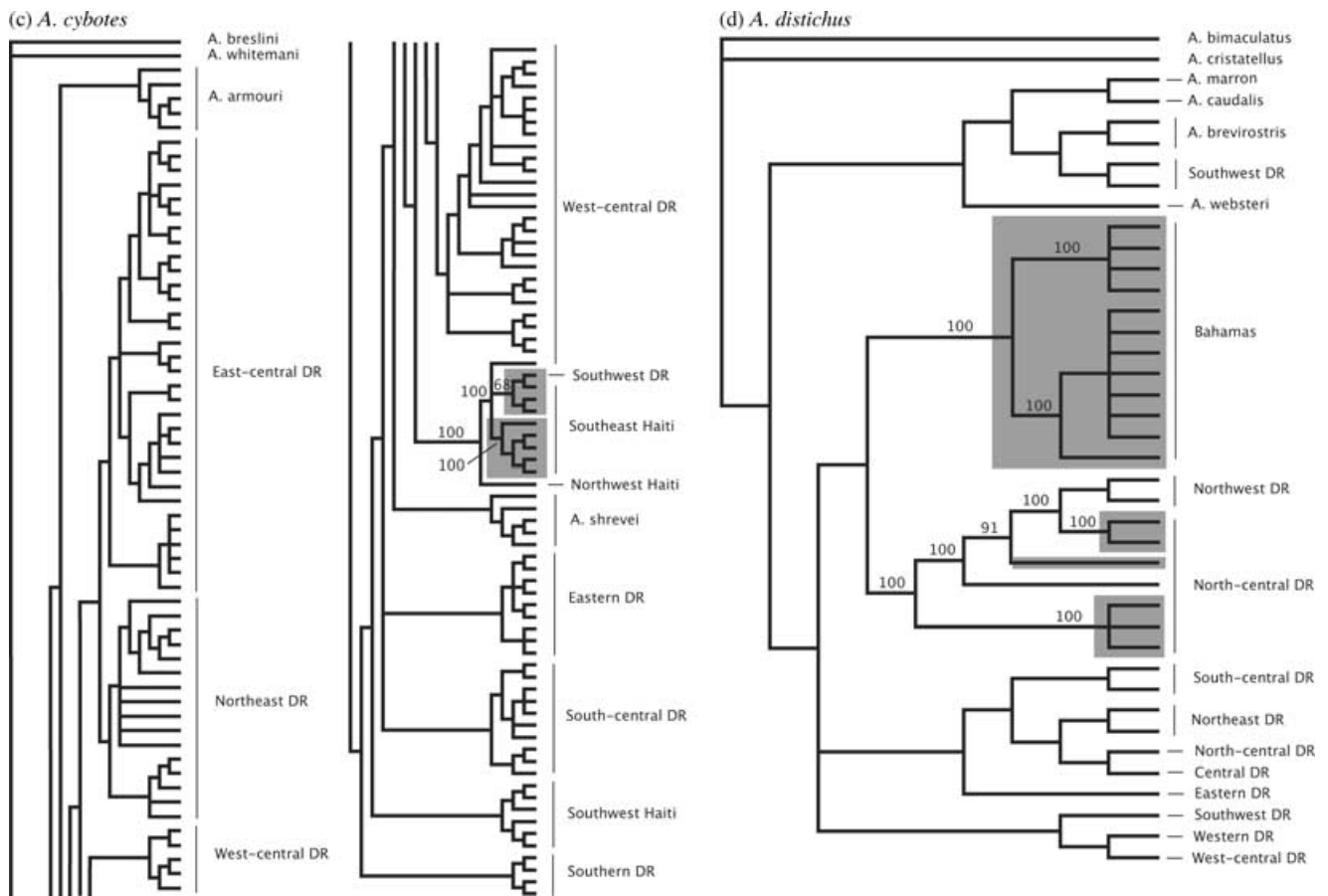


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detected ($R^2 = 0.842$; $p = 0.0002$; $n = 10$; Fig. 4). The two introduced *Anolis* with single sources detected (*A. chlorocyanus* in Florida and *A. porcatius* in the Dominican Republic) had the smallest sample sizes, suggesting that more extensive sampling might detect additional sources for these species. The mean number of individuals sampled per population and the number of sources detected ($R^2 = 0.003$; $p = 0.8741$; $n = 10$) were not related.

Discussion

Phylogenetic and population-genetic analyses revealed that 80% of *Anolis* lizard introductions were from multiple native-range source populations (Fig. 2). Seven of eight introduced species of *Anolis* lizards in Florida were derived from multiple native-range sources, and one of two introductions to the Dominican Republic had multiple sources (Figs. 1a-g; Kolbe et al. 2004). Although previous researchers detected multiple sources in biological invasions of single species (e.g., Collins et al. 2002; Facon et al. 2003; Voisin et al. 2005), ours is the first multi-species analysis to determine the frequency with which introductions occurred from multiple sources, and our

results suggest that introductions from multiple sources are a general phenomenon.

In nearly every case of an *Anolis* introduction from multiple sources, genetic admixture was indicated by the presence of haplotypes from multiple native-range sources within at least some introduced populations. This was true for *A. cristatellus* (Dominican Republic), *A. cybotes*, *A. distichus*, *A. equestris*, *A. garmani*, and *A. sagrei* (Fig. 2; Kolbe et al. 2004). In fact, 62% (18 of 29) of introduced populations in the Miami area and the Dominican Republic were admixed (see Fig. 2 & Kolbe et al. 2004, 2007 for *A. sagrei*). Alternatively, despite introductions from multiple sources, admixture did not occur for *A. cristatellus* in Florida, where one population is established on the mainland and the other on Key Biscayne, an offshore island (Fig. 2). A similar situation exists for *A. sagrei* in Jamaica, where introductions from genetically distinct native-range sources occurred in the eastern and western parts of the island; nevertheless, these introductions eventually merged in central Jamaica (Kolbe et al. 2004). Given the lack of admixture, strong founder effects or bottlenecks experienced by *A. cristatellus* in Florida reduced mean within-population mtDNA sequence divergences to 0% and 0.4% for the two introduced populations

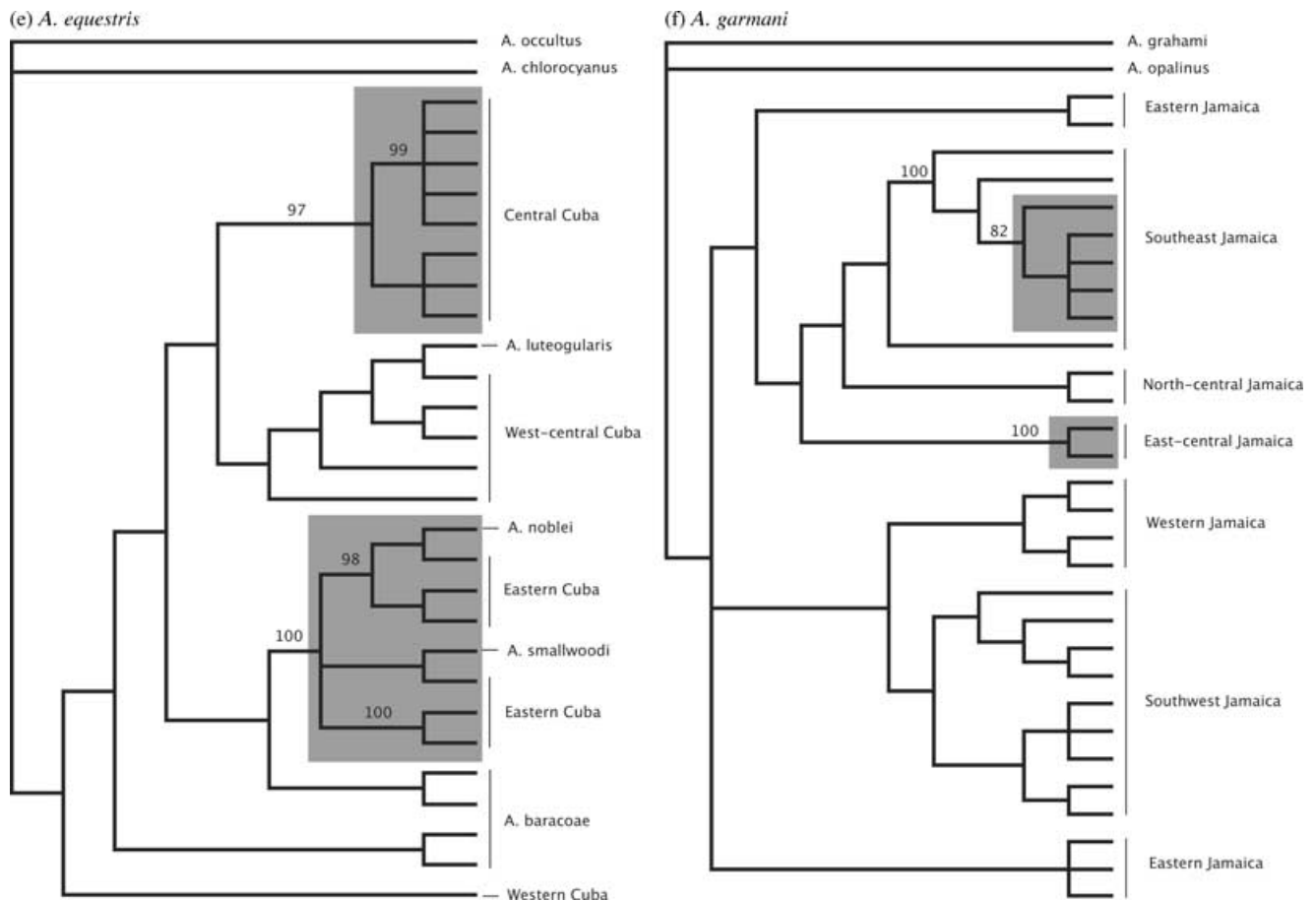


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from a mean of 2.5% for their native-range sources (1.7% for all native populations in Puerto Rico). Thus, the classical scenario in which genetic variation decreases through founder effects and population bottlenecks during introduction held for *A. cristatellus*, although the potential for future spread and subsequent admixture exists.

Multiple sources were detected also for *A. porcatius* in Florida; most putative *A. porcatius* sampled, however, had haplotypes nested within the native *A. carolinensis* clade (Fig. 1g). This result suggests hybridization between introduced *A. porcatius* and native *A. carolinensis*, which is likely given that *A. carolinensis* is derived from within the western clade of *A. porcatius* (Glor et al. 2005), that western Cuba is the source of Florida introductions (Fig. 2), and that hybridization occurs between *A. porcatius* and the closely related *A. allisoni* along contact zones in Cuba (Glor et al. 2004). Misidentification of some native *A. carolinensis* as “introduced *A. porcatius*” is also possible because these species are distinguished only by slight differences in size and shape of the snout (Schwartz & Henderson 1991; Rodríguez-Schettino 1999); nevertheless, this does not discount the occurrence of introduced *A. porcatius* in Florida from multiple native-range sources (Figs. 1g & 2). Despite these situations for

A. cristatellus and *A. porcatius*, admixture of genetic variation from geographically and genetically distinct source populations was the most common outcome in *Anolis* introductions.

Admixture of genetic variation from multiple native-range source populations usually caused elevated genetic variation within introduced populations compared with native-range populations of *Anolis* lizards (Table 1; Fig. 2). Nearly two-thirds of the introduced *Anolis* populations in the Miami area were admixed, and within-population genetic variation was elevated compared with mean values in the native range for 52% (15 of 29) of introduced populations and was even higher considering introduced *A. sagrei* populations elsewhere (Table 1; Fig. 2; see Kolbe et al. 2004, 2007 for *A. sagrei*). Admixture transformed among-population genetic variation from the native range to within-population genetic variation in the introduced range (Table 2).

The classical scenario of invasive-species genetics (Sakai et al. 2001; Allendorf & Lundquist 2003) cannot account for the higher within-population genetic variation of introduced populations relative to their native-range sources (Fig. 3). The AMOVAs in which haplotypes were partitioned into those derived from different

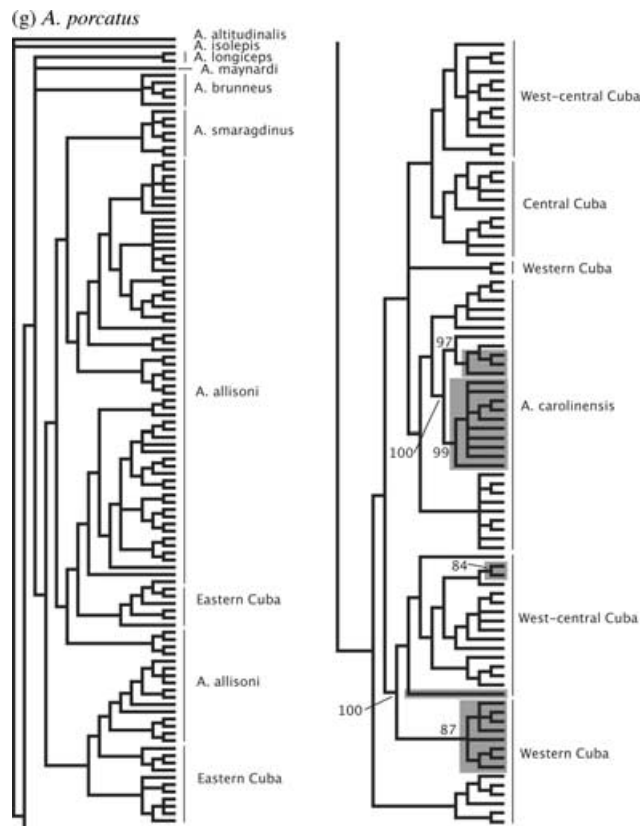


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native-range sources illustrates this point well (Table 2); for each of the five *Anolis* species in this analysis, over 94% of genetic variation was derived from admixture of haplotypes from different sources as opposed to variation from within single sources. If individual introductions had not admixed for a given species, such as the *A. cristatellus* case in Florida, then a severe reduction in within-population genetic variation due to genetic drift would have resulted. Thus, the reduction in haplotypic variation caused by a small number of founders from each native-range source population was overcome by admixture of multiple sources (Table 2), causing haplotypic variation in most introduced populations to exceed that of native-range populations. These results for human-mediated introductions contrast with natural range expansions, which do not combine geographically disparate sources. Indeed, many natural range expansions show reduction of genetic variation (Hewitt 2000).

In addition to these changes in within-population genetic diversity, genetic disparity also increased among introduced populations. Large changes in the frequency of haplotypes from different native-range sources occurred over extremely short distances in Miami, on the order of 10 km or less. For example, three of six introduced *A. distichus* populations were from a single source in the Bahamas, whereas adjacent populations had high frequencies of haplotypes from Maziel and San Francisco de Ma-

coris, Dominican Republic (Fig. 2). Similar changes in allele frequency over short distances are reported in a study of allozyme variation in introduced *A. distichus* populations in Miami (Miyamoto et al. 1986) and in other introduced species (Voisin et al. 2005). Thus, even when multiple sources occur in an introduction, substantial variation in the extent of admixture may exist among introduced populations. This result is in sharp contrast to most native-range populations of *Anolis* lizards in which populations tend to be genetically similar to their geographic neighbors (Glor et al. 2003, 2004; Kolbe et al. 2004).

Evidence of multiple sources, admixture, and elevated genetic variation rejected the classical scenario in which introduction decreases within-population genetic variation for half of the introduced *Anolis* populations that we surveyed. In the absence of data on failed invasions, a common problem in invasion biology, we could not estimate the proportion of all introduced populations that resulted from single versus multiple sources or statistically evaluate whether the amount of genetic variation was associated with introduction success.

We used molecular markers strictly to reconstruct the historical dynamics of introduced populations, not to quantify selectively important variation. Nevertheless, if haplotypic variation found in introduced populations is related to beneficial genetic variation in ecologically relevant traits, then populations would have greater ability

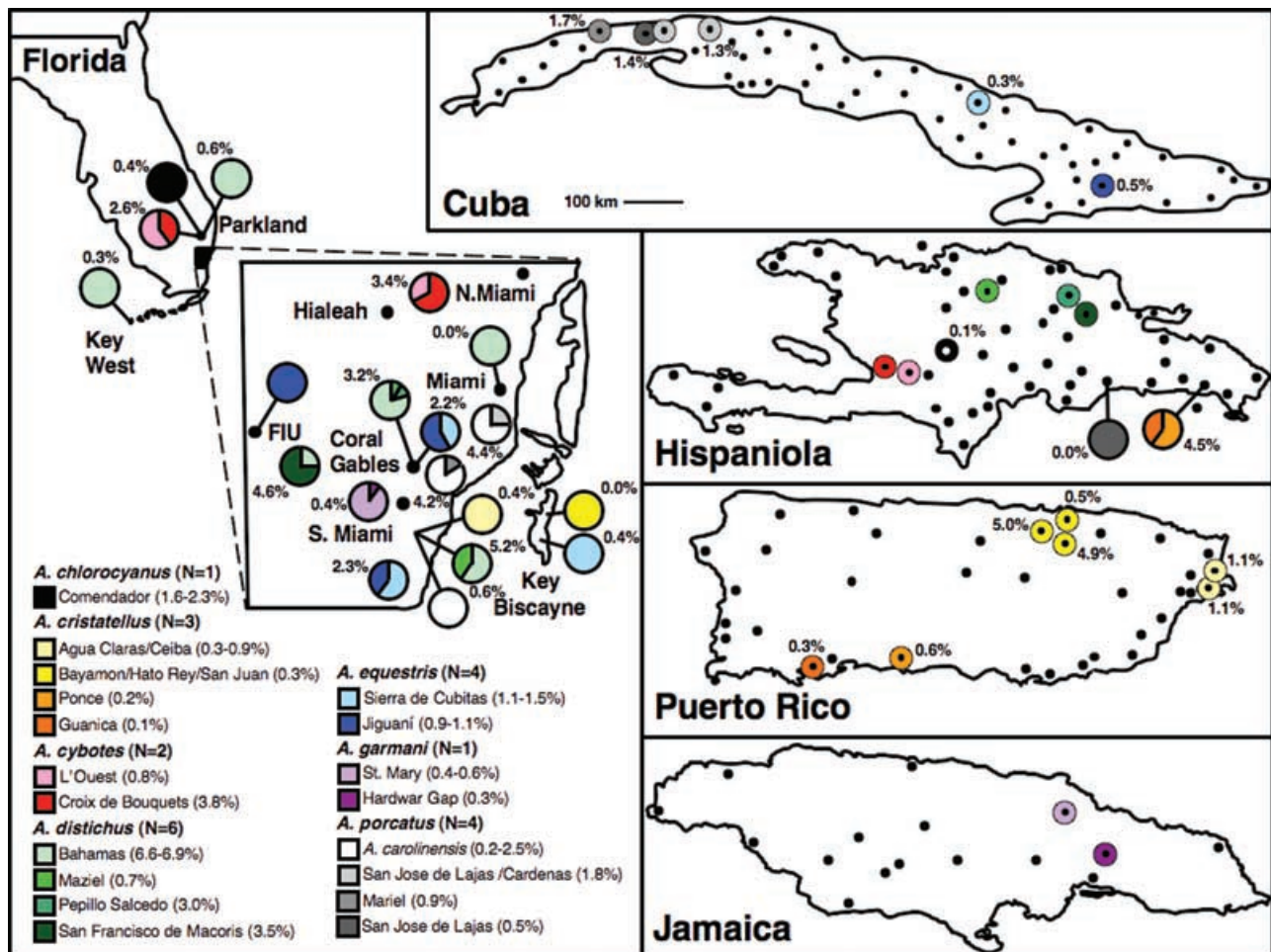


Figure 2. Distribution of mitochondrial haplotypic variation in introduced populations of seven *Anolis* species in Florida (enlarged section, greater Miami area) and the Dominican Republic (eastern portion of Hispaniola) and the source of this genetic variation from native populations in Cuba, Hispaniola, Puerto Rico, and Jamaica. Black dots indicate native populations sampled. Colors encircling some black dots denote native-range populations containing haplotypes to which introduced-population haplotypes are most closely related. Pie charts representing each introduced population indicate the frequency of haplotypes from different native-range source populations (only one individual was sampled from the FIU population of *A. equestris*; thus, this pie chart indicates only its native-range source population, not frequency). Percentages next to each pie chart give the mean pairwise mtDNA sequence divergence within that population. Sample sizes in the key indicate the number of introduced populations sampled. Percentages in parentheses after the native-range source populations in the key indicate the pairwise sequence divergence (or range of divergences) between introduced haplotype(s) and the most closely related native-range haplotype(s).

to adapt to environmental conditions that differ from their native range, increasing the likelihood of persistence (e.g., Lavergne & Molofsky 2007). Under the conditions of recent colonization, “molecular genetic variation is likely to be a reliable indicator for invasive species of the potential for adaptive change because of the genetic effects of recent colonization [i.e., population bottlenecks and admixture]” (Allendorf & Luikart 2007: 494), although a similar relationship is not expected for populations lacking a recent history of population bottlenecks or admixture (Frankham 1999; Reed & Frankham 2001; McKay &

Latta 2002). The degree to which molecular genetic variation correlates with additive genetic variance in fitness for introduced populations is a direction for future research.

Mode of Introduction, Multiple Source Populations, and Genetic Variation

Contrary to prior expectations, the mode or circumstances of introduction had no obvious consequences for the general finding of increased haplotype variation through admixture in introduced populations. Multiple sources

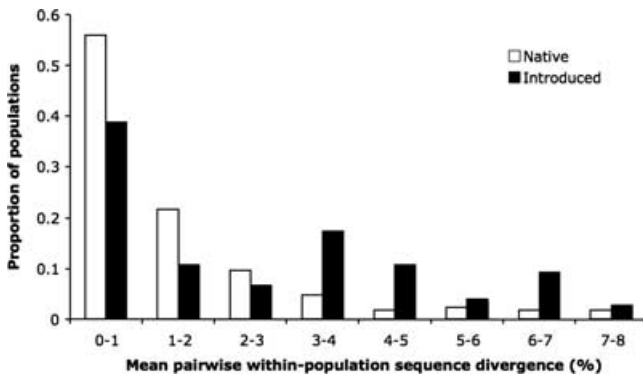


Figure 3. Sequence divergences among haplotypes sampled within introduced and native-range populations of *Anolis*. Mean pairwise within-population sequence divergences were pooled for introduced ($n = 75$) and native ($n = 166$) populations of all eight *Anolis* species.

and high levels of genetic variation are expected for some types of introductions. For example, intentional introductions of plants for food, forage, or ornamental use, and seed contaminants, likely have high genetic variation due to repeated introductions and multiple sources (Mack & Erneberg 2002; Mack 2003; Novak & Mack 2005). In contrast, it is more likely that geographically restricted introductions of a small number of individuals are derived from single-source populations and have reduced genetic variation. *Anolis* introductions to Florida and the Dominican Republic occurred both intentionally (*A. cybotes*, *A. distichus*, *A. equestris*, and *A. garmani*), often as released pets, and accidentally via shipments of produce, plants, or freight (*A. cristatellus* in the Dominican Republic, *A. distichus*, and *A. sagrei*) (Lever 2003). Despite these varied modes of introduction, most introduced *Anolis* populations have multiple sources and elevated levels of within-population genetic variation (this study; Kolbe et al. 2004, 2007).

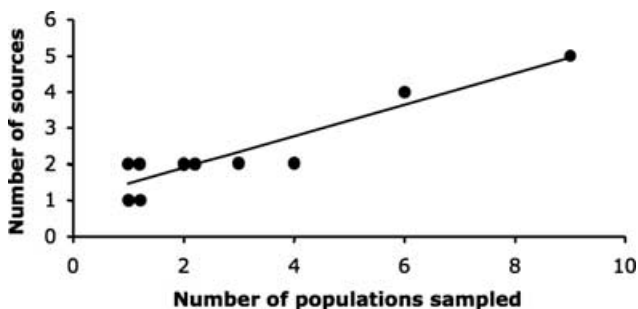


Figure 4. The relationship between the number of source populations detected and the number of introduced populations sampled for introduced *Anolis* species ($n = 10$).

One reason that single and multiple introductions may not differ in their genetic effects is that even single introductions may contain individuals from multiple native-range populations. Documented introductions of *A. cybotes* and *A. garmani* in Florida suggest single-introduction events of a small number of individuals for each species (Ober 1973; M. Cooper, personal communication), yet multiple sources were detected within introduced populations of both species. Although subsequent, undocumented introductions are possible, a single introduction of *A. cybotes* in northeastern Dade County in 1967 derived from the Petionville area of Haiti (Ober 1973) is entirely consistent with the genetic data (Figs. 1c & 2). Phylogenetic analyses reveal that haplotypes from this introduced population are most closely related to native-range haplotypes from L'Ouest and Croix de Bouquets, Haiti, which are the closest Haitian populations to Petionville that we sampled. Thus, it is possible that individuals were collected from several localities in this region of Haiti during a single trip and then subsequently released in Florida. The same explanation may hold for *A. garmani* because both source populations are near Kingston, Jamaica, and only a single introduction is documented (M. Cooper, personal communication; Figs. 1f & 2).

The distinction between the number of introductions and the number of sources included in an introduction is important when discussing colonization and transport aspects of species introductions versus the evolutionary consequences of introductions from multiple sources. The evolutionary potential of an introduced population is more strongly associated with the amount of genetic and phenotypic variation found within introduced populations than with the number of introduction events (Kolbe et al. 2007; Lavergne & Molofsky 2007). Multiple introductions from the same source may contribute less genetic variation to an introduced population than a single introduction that contains multiple sources, and even geographically restricted introductions can harbor high levels of genetic variation.

Because introduced *Anolis* species generally have high haplotype variation from multiple sources despite differences in the number of introduction events, whether their non-native ranges are widespread or restricted and whether releases were accidental or intentional, these criteria have limited value for predicting the number of sources or amount of genetic variation of introduced populations. Nevertheless, species with larger non-native ranges are perhaps more likely to have multiple native-range source populations, suggesting a cause-and-effect relationship between the number of introductions and the size of the non-native range. The geographically widespread *A. sagrei* invasion, for example, represents many spatially and temporally distinct introductions from different native-range sources (Kolbe et al. 2004, 2007). In addition, more introductions to a single non-native area

Table 1. Number of source populations and mtDNA sequence divergences for *Anolis* introduced to Florida and the Dominican Republic.

Introduced species	Native range	Introduced range	Number of source populations	Mean within-population sequence divergence (%) —native	Mean within-population sequence divergence (%) —introduced	Inferred source populations	Mean sequence divergence (%) between introduced haplotypes from different native sources
<i>A. chlorocyanus</i>	Hispaniola	Florida	1	0.7	0.4	Comendador	—
<i>A. cristatellus</i>	Puerto Rico	Florida	2	1.7	0.2	Agua Claras-Bayamon	6.3
		Dominican Republic	2	1.7	4.5	Ponce-Guanica	7.6
<i>A. cybotes</i>	Hispaniola	Florida	2	1.1	3.0	L'Ouest-Croix de Bouquets	4.8
<i>A. distichus</i>	Hispaniola	Florida	4	0.5	2.3	Bahamas-Maziel	8.8
	Bahamas					Bahamas-San Francisco	8.9
						Bahamas-Pepillo Salcedo	8.5
						Maziel-San Francisco	4.9
						Maziel-Pepillo Salcedo	3.2
<i>A. equestris</i>	Cuba	Florida	2	0.3	1.6	San Francisco-Pepillo Salcedo	5.2
<i>A. garmani</i>	Jamaica	Florida	2	2.0	0.4	Sierra de Cubitas-Jiguani	4.0
<i>A. porcatus</i>	Cuba	Florida	2	2.0	3.1 ^a	St. Mary-Hardwar Gap	1.8
		Dominican Republic	1	2.0	0.0	San Jose de Lajas/Cardenas-Marial	2.8
<i>A. sagrei</i>	Cuba	Florida	5(8) ^b	1.7	3.5	San Jose de Lajas	—
	Bahamas					Bahamas-Western Cuba-1	9.9
	Caymans					Western Cuba-1-Western Cuba-2	1.9
	Mexico					Western Cuba-1-West-central Cuba-1	4.6
	Belize					Western Cuba-1-East-central Cuba-2	8.9
						Western Cuba-2-West-central Cuba-1	3.6
						Western Cuba-2-East-central Cuba-2	8.1

^a *Anolis carolinensis* mtDNA haplotypes were included in the within-population sequence divergence calculation for introduced populations of *A. porcatus*.

^b Number in parentheses for *A. sagrei* indicates the number of sources for all Florida populations.

Table 2. Results of analysis of molecular variance (AMOVA) for partitioning of haplotype diversity within and among native-range populations, introduced-range populations, and different native-range sources detected in introduced-range populations for eight introduced *Anolis* species.^a

Species	Native			Introduced			Source		
	source of variation	df	variation (%)	source of variation	df	variation (%)	source of variation	df	variation (%)
<i>A. chlorocyanus</i> ^b	among	25	81.5*						
	within	62	18.5						
	total	87							
<i>A. cristatellus</i> ^b	among	36	80.8*	among	1	95.9*			
	within	136	19.2	within	21	4.1			
	total	172		total	22				
<i>A. cybotes</i>	among	46	87.0*	among	1	0.0***	among	1	94.5*
	within	77	13.0	within	11	100.0	within	11	5.5
	total	123		total	12		total	12	
<i>A. distichus</i>	among	14	92.7*	among	5	26.5**	among	3	98.2*
	within	5	7.3	within	25	73.5	within	27	1.8
	total	19		total	30		total	30	
<i>A. equestris</i>	among	12	90.4*	among	3	1.8***	among	1	98.0*
	within	6	9.6	within	11	98.2	within	13	2.0
	total	18		total	14		total	14	
<i>A. garmani</i> ^b	among	17	41.2**				among	1	94.1***
	within	8	58.8				within	8	5.9
	total	25					total	9	
<i>A. porcatius</i> ^b	among	19	79.4*	among	2	0.0***			
	within	49	20.6	within	11	100.0			
	total	68		total	13				
<i>A. sagrei</i> ^c	among	56	79.9*	among	39	24.7*	among	8	97.0*
	within	198	20.1	within	159	75.3	within	190	3.0
	total	254		total	198		total	198	
<i>A. sagrei</i> (Miami only)				among	8	0.5***	among	3	82.6*
				within	30	99.5	within	35	17.4
				total	38		total	38	

^aSignificance: *p < 0.001, **p < 0.05, ***not significant.

^bAn introduced AMOVA was not possible for *A. chlorocyanus* and *A. garmani* because only one introduced population was sampled for each species and a source AMOVA was not possible for *A. chlorocyanus*, *A. cristatellus*, and *A. porcatius* due to a single source, lack of admixture, and insufficient sample size, respectively.

^cNative-range AMOVA for *A. sagrei* includes only Cuba.

may accelerate the spread of an invader (Lockwood et al. 2005). Further study is needed to resolve the relationship between the number of introductions and sources, and the geographic extent of introduced populations (Sakai et al. 2001).

Conclusions

Introduced *Anolis* lizard populations in Florida and the Dominican Republic vary in the number of native-range sources and haplotypic diversity (Fig. 2). Some single-source populations are monomorphic, whereas other multiple-source populations are more variable than native-range populations (Table 1). Allendorf and Luikart (2007) suggest that studies of the amount and patterns of genetic variation in introduced species reveal two contrasting patterns: reduced genetic variation associated with population bottlenecks or increased genetic variation due to admixture. Although our data on *Anolis* introductions are consistent with this dichotomy (Table 1;

Fig. 2), we suggest that introductions generally follow a sequential, two-step process with random genetic drift influencing initial introductions and admixture following in some instances. First, introduced populations may experience a reduction in genetic variation due to founder effects and/or population bottlenecks (e.g., *A. chlorocyanus*); this stage is equivalent to the classical scenario. Multiple, independent introductions may occur at this stage, remaining geographically isolated (e.g., *A. cristatellus* in Florida). Second, if individuals from multiple native-range sources come into contact, then admixture can increase within-population genetic variation despite the likely reduction in genetic variation already experienced by each founding propagule (e.g., *A. sagrei*). These steps may occur simultaneously if a single-introduction event contains individuals from multiple native-range sources and combines them prior to introduction (e.g., *A. cybotes*). By studying the introduction histories of multiple *Anolis* species, we illustrate how the evolutionary processes of genetic drift and admixture affect genetic variation in introduced populations.

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Supplementary Materials

The Genbank numbers for previously published (Appendix S1) and newly collected sequences (Appendix S2) are available as part of the on-line article from <http://www.blackwell-synergy.com/>. The author is responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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