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Recently introduced invasive geckos quickly reach population genetic equilibrium dynamics

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Abstract Invasive species are spreading at high rates, yet fundamental processes allowing them to progress through the stages of invasion are unclear. The establishment stage is a critical point because this is when exotic species can survive, reproduce, and begin to spread. Unfortunately, inference of population dynamics during this stage may be impossible if historical and observational data are incomplete. Nonetheless, critical inferences on population dynamics during the establishment stage can be acquired indirectly by characterizing demographic history via the population genetics of recently introduced populations. Geckos have been introduced at a global scale and are one of the most successfully establishing families of alien reptile known. Here we conduct a series of population genetic analyses among five close subpopulations of the introduced Mediterranean gecko *Hemidactylus turcicus*. We tested for non-equilibrium genetic signatures, a pattern expected during early stages of invasion if there were few founders or repeated introductions led to population turnover. Genetic analyses showed no evidence of non-equilibrium dynamics such as genetic bottlenecks. Moreover, we found strong support for population genetic equilibrium dynamics. The observed results may have been generated via an introduction that involved high propagule pressure. However, given the life history of *H. turcicus* including generation time and dispersal potential, we favor the hypothesis that the invasive metapopulation has rapidly reached the establishment stage as indicated by relatively constant effective sizes and migration rates among introduced subpopulations. The ability to rapidly pass through the establishment stage may in part explain the invasion success of these geckos.

Keywords Invasive species · Gecko · *Hemidactylus turcicus* · Bottleneck · Migration–drift equilibrium · Fine-scale genetic structure

Introduction

Due to globalization and climate change, invasive species are spreading at a high rate (Kraus 2009). While many population genetic studies focus on determining the source and pathways of invasion, fewer examine the fundamental processes that allow an invasive species to successfully progress from the transport stage to the establishment and subsequent

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Table 1 Sampling information for *Hemidactylus turcicus* collected within Brazos County, TX

Sampling area	General location	Building name, GPS coordinates	Date building originated	Estimated surface building area[a] (ha)	Total building area (ha)	Census size for geckos in sampling area[b] (N_c)
1	Texas A&M University main campus	Floriculture greenhouse, 30°36′55.30″N, 96°20′16.53″W	1954	0.0456	0.259	271
		Horticulture greenhouse, 30°36′56.31″ N, 96°20′15.03″W	1949	0.0315		
		Forest genetics greenhouse, 30°36′57.34″N, 96°20′13.37″W	1954	0.0523		
		TAES Annex Building, 30°36′59.35″ N, 96°20′09.45″W	1933	0.1294		
2	Texas A&M University main campus	Cain Hall, 30°36′42.07″N, 96°20′36.63″W	1975	0.4779	0.4779	500
3	Texas A&M University west campus	Borlaug Center for Southern Crop Improvement, 30°36′29.32″N, 96°20′58.07″W	1993	0.2471	0.9675	1,014
		Southern Crop Improvement greenhouses, 30°36′27.51″N, 96°21′00.22″W	2001	0.1201		
		Horticulture/Forest Science, 30°36′27.51″N, 96°21′00.22″W	1984	0.6003		
4	Texas A&M University main campus	Sanders Corps of Cadets Center, 30°36′43.58″N, 96°20′15.02″W	1992	0.1731	0.1731	181
5	Suburban neighborhood	House 1, 30°37′14.95″N, 96°17′13.36″W	1980	0.0254	0.2129	223
		House 2, 30°37′14.21″N, 96°17′12.68″W		0.0721		
		House 3, 30°37′13.27″N, 96°17′13.38″W		0.0274		
		House 4, 30°37′12.96″N, 96°17′13.99″W		0.0306		
		House 5, 30°37′12.37″N, 96°17′14.69″W		0.0296		
		House 6, 30°37′13.69″N, 96°17′16.29″W		0.0278		

Building information indicates when geckos could possibly first colonize a sampling area (date when building originated), though it should be noted that the first museum record for *H. turcicus* in Brazos County was 1970

[a] Surface building area was calculated by estimating the perimeter area (m) with the polygon tool in Google Earth Pro and then multiplying that number by 4 m for each each above ground floor (e.g., 8 m for a two-story building)

[b] Census size was calculated by multiplying 1,048 geckos/ha [an average based on the results of Selcer (1986) and Locey and Stone (2006)] by the estimated building area

spreading stages within the introduced range (Blackburn et al. 2011; Handley et al. 2011). Successful establishment means that the alien species survives, reproduces, and maintains a self-sustaining population (i.e., C3 category in Table 1 and Fig. 1 of Blackburn et al. 2011). Establishment is often dependent upon the propagule pressure of the introduction, including the number of individuals invading and the number of introduction attempts (Kolar and Lodge 2001; Hayes and Barry 2008; Fauvergue et al. 2012). Theoretical modeling has shown that in small populations, demographic stochasticity increases population fluctuations

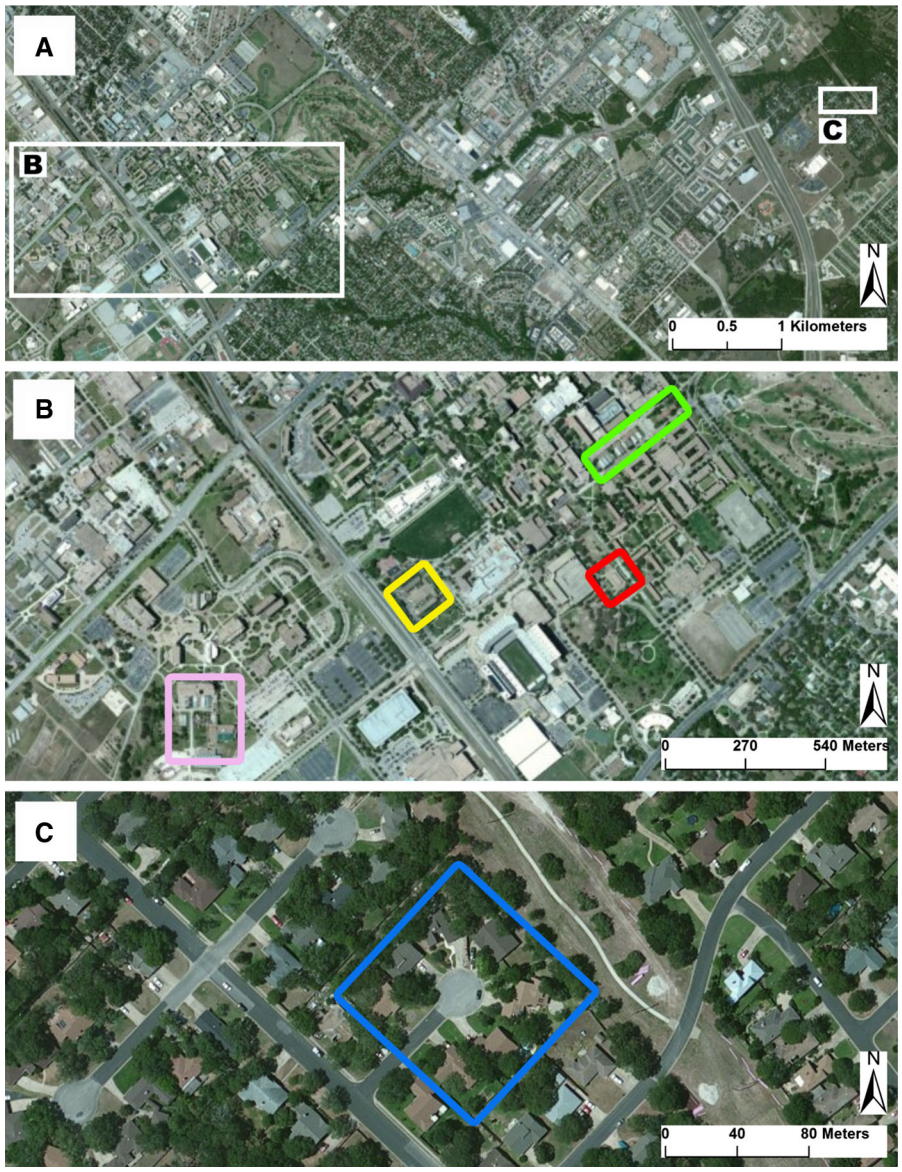

Fig. 1 Maps of sampling area in College Station, TX, USA. **a** Depicts distance between campus locations (*white box b*) and the suburban neighborhood (*white box c*). **b** Enlarged view of the four campus sampling areas color coded to match Fig. 2**d**. Sampling area 1 = *green*, sampling area 2 = *yellow*, sampling area 3 = *pink*, sampling area 4 = *red*. **c** Enlarged view of the neighborhood. Sampling area 5 = *blue*

and extinction risks (Fauvergue et al. 2012). Moreover, founder events result in genetic bottlenecks, which may in turn decrease the likelihood of establishment success via inbreeding depression or loss of allelic diversity through drift (Roman and Darling 2007). Populations that can overcome this founding event rapidly will have a better chance for establishment because extensive genetic diversity will not be lost (Hanski and Gaggiotti 2004). In addition, many invasive species have likely experienced repeated introductions, which may alleviate the negative consequences associated with small founding populations (Roman and Darling 2007; Allendorf and Luikart 2007; Uller and Leimu 2011).

Unfortunately, for most invasions, inference of the population dynamics during the establishment stage is impossible as historical and observational data are incomplete (Bomford et al. 2009; Fauvergue et al.

2012). While knowledge on propagule pressure is ideal for understanding successful establishment, for most species, the number of introduced individuals or number of introductions is unknown (Uller and Leimu 2011). Fortunately, critical inferences on population dynamics during the establishment stage can be acquired indirectly by characterizing demographic history via the population genetics of recently introduced populations. In the early stages of invasion, one would expect non-equilibrium genetic signatures such as bottlenecks if there were few founders or if small, non-self-sustaining local populations experienced repeated introductions leading to high population turnover, i.e., the introduced population is a sink that is only replenished by a new set of founders (Cornuet and Luikart 1996; Hanski and Gaggiotti 2004). However, inference of demographic establishment vs non-establishment is not possible with genetic data alone as enough time may not have passed in an established population to erode the bottleneck signature. Therefore, a signature of a bottleneck only leads to the inference that the introduced population had a small founding population relative to its source population and thus, had to persist or is persisting through a period of reduced genetic diversity. On the other hand, the finding of constant effective sizes and migration rates (i.e., genetic equilibrium) among introduced subpopulations would necessarily indicate that the introduced subpopulations have been demographically stable, i.e., reached the establishment stage. It is possible that a single non-established population fails to show a bottleneck signature due to high propagule pressure. However, high propagule pressure that results in migration–drift equilibrium among introduced, genetically subdivided subpopulations seems unlikely because propagule pressure would have to be similar (in terms of amount and genetic diversity) across all subpopulations.

One group of vertebrates, the geckos, has been introduced at a global scale and is one of the most successfully establishing families of alien reptile or amphibian known (Bomford et al. 2009; Kraus 2009). The large introduced range in the southeastern USA for the Mediterranean gecko, *Hemidactylus turcicus* (Linnaeus, 1758), illustrates how successful these invasive geckos can be. The native range of *H. turcicus* includes the Mediterranean regions of Africa, Asia, and Europe. A molecular phylogenetic study indicates that this species originated in the Middle East and then moved west around the Mediterranean (Carranza and Arnold 2006). No additional population genetic studies have been done on *H. turcicus* in its native range. Due to human-mediated dispersal, the gecko has now invaded the southern USA, Mexico, Panama, Cuba, Puerto Rico, Argentina, and Chile (Rödder and Lötters 2009). Introductions are recent as suggested by observational records from USA port cities [Key West, Florida in 1910 (Fowler 1915; Stejneger 1922), New Orleans, Louisiana circa 1945 (Etheridge 1952; Kraus 2009) and Brownsville, Texas circa 1945 (Conant 1955; Kraus 2009)] and also by the low genetic divergence (maximum of <2 % for concatenated partial gene sequences of cytochrome oxidase and 12S rRNA) between isolates from the native and introduced range (Carranza and Arnold 2006). There is some genetic evidence to suggest that introduced *H. turcicus* populations have experienced founder events. Schwaner et al. (2008) showed that for two allozyme loci, the frequencies of one of two alleles generally decreased in the gecko's invasive range going from coastal southeastern cities to the western USA. However, with the exception of a population that was deliberately introduced repeatedly on the campus of the University of Central Oklahoma (Locey and Stone 2006), levels of propagule pressure are unknown for most introduced populations of *H. turcicus*. Thus, little is known about the establishment stage of this highly successful invasive species. In particular, it is not known if during the period from the introduction stage through the establishment stage, gecko populations experienced prolonged periods of reduced genetic diversity that could have resulted from having few founders. In the absence of complete observational data, we used population genetic methods to infer the demographic history of *H. turcicus* from a relatively recently introduced location (College Station, TX, USA). Our primary goal was to determine whether several subpopulations have experienced genetic bottlenecks or whether they have already achieved genetic equilibrium (through constant effective population size and migration).

Despite a broad introduced range in the southern USA (Rödder and Lötters 2009), several mark–recapture studies indicate very low dispersal at a local scale. A secondary goal for our study was to evaluate local-scale structure to determine whether genetic data corroborate the low dispersal found in population-based studies. The Mediterranean gecko is primarily

found in urban and suburban areas on human structures (e.g., Rose and Barbour 1968; Meshaka et al. 2006; Stabler et al. 2012). Mark–recapture studies have estimated high densities on buildings (721–896 per ha, Stabler et al. 2012; 544–2,210 per ha, Selcer 1986), and very limited dispersal between buildings (<6 m, Selcer 1986; 20 m/year, Locey and Stone 2006). Although dispersal can be directly measured with mark–recapture methods, this approach can be time intensive, interpretation of data may be difficult due to low recapture rates, and long-distance dispersal may be underestimated due to the decreasing probability of detection within a finite sampling area (Rose and Barbour 1968; Selcer 1986; Koenig et al. 1996). As a complimentary alternative, genetic data can be used to indirectly infer dispersal by estimating among-population gene flow, i.e., the successful dispersal events that lead to reproduction among migrants and residents. The finding of significant genetic structure on a local scale would verify the results of the mark–recapture studies whereas the finding of panmixia among subpopulations suggests that mark–recapture methods underestimate dispersal.

Materials and methods

Sampling and microsatellite genotyping

Geckos (*H. turcicus*) were hand-collected from five sampling areas within College Station (Brazos County), TX (Table 1; Fig. 1). These sampling areas are likely relatively recent introductions as the earliest record of *H. turcicus* in Brazos County, TX is 1970 (Biodiversity Research and Teaching Collections, Texas A&M University) and most of the sampling locations did not exist until several years after 1970 (Table 1). Four areas were sampled on the Texas A&M University campus, each of which corresponded to at least one building, or several adjacent buildings (Fig. 1). A fifth area comprised a set of six adjacent houses in a suburban neighborhood (Fig. 1). Geckos from several buildings were collected within some sampling areas to approximate equal sampling areas within the limitations that not all buildings were conducive to thorough sampling. The maximum straight line distance among the four campus areas was 1.7 km (areas 1 and 3), and the maximum straight line distance between the most distant sampling areas was 6.2 km (areas 3 and 5). The minimum distance between two sampling locations was 0.584 km (areas 2 and 4). Approximately 50 individuals were collected from each of the five sampling areas from May 2011-October 2011 (Table 2). Geckos were dissected for parasite studies to be reported on in the future. Because geckos were sacrificed to assess parasitism, mark–recapture was not possible. Tail muscle was preserved in 70 % ethanol until DNA extractions were performed following the protocol outlined in Owusu et al. (2012). Genotyping was conducted with the microsatellite loci characterized in Owusu et al. (2012). For all loci, a 10 µl PCR reaction with 1.6 µl of extraction supernatant (genomic DNA) was used. PCR conditions, reagent concentrations, and genotyping followed protocols described in Detwiler and Criscione (2011), but with a 95° initial denaturation temperature as reported in Owusu et al. (2012). We omitted three loci reported in Owusu et al. (2012): di001 and tet015 because they were monomorphic and di020 because null alleles were suspected after some individuals failed to amplify despite repeated polymerase chain reactions (PCRs) from both originally extracted and re-extracted DNA. Thus, our study included 18 microsatellite loci.

Data analyses

For each sampling area, deviations from Hardy–Weinberg equilibrium (HWE) (per locus and multilocus) were tested by permuting alleles among individuals 20,000 times in SPAGEDi v1.3 (Hardy and Vekemans 2002). Genotypic disequilibrium (GD) between pairs of loci within sampling areas was evaluated with 5,000 dememorizations, 5,000 batches, and 5,000 iterations in GENEPOP v4.2 (Rousset 2008). The effect of multiple tests on significance values was corrected with sequential Bonferroni. We estimated pairwise population differentiation among the sampling areas with F_{ST} (Weir and Cockerham 1984) in FSTAT v2.9.3 (Goudet 1995). Standardized F_{ST} was also calculated by dividing raw F_{ST} by $F_{ST\text{-max}}$ (Meirmans 2006). The latter parameter reflects the maximum possible divergence among populations and was obtained using RECODEDATA (Meirmans 2006).

To further evaluate genetic structuring among sampled locations, we used Bayesian clustering as implemented in STRUCTURE v2.1, which allows the

Table 2 Results of population genetic tests for *Hemidactylus turcicus* sampling areas

Sampling area	Full dataset	No migrants	F_{IS}	Contemporary estimates				Historical estimates	
	N	*N*		LD method Full dataset	LD method No migrants	SA method Full dataset	SA method No migrants	Mode *F*	θ
1	54	50	0.008	108.3 (59–357)[a]	141.6 (68–1,403)[a]	57 (38–91)[b]	50 (33–79)[b]	0.054 (0.032–0.077)	0.222 (0.128–0.294)
2	51	46	0.042	148.8 (59–infinite)	288.7 (62–infinite)	54 (36–86)	65 (43–101)	0.088 (0.057–0.116)	0.171 (0.094–0.241)
3	50	47	0.045	115.3 (57–670)	202.7 (76–infinite)	58 (40–89)	54 (35–85)	0.050 (0.030–0.070)	0.175 (0.094–0.248)
4	50	44	0.045	65 (40–133)	99.3 (45–2,179)	42 (27–69)	45 (29–75)	0.069 (0.043–0.094)	0.189 (0.108–0.261)
5	49	41	0.0008	11.4 (6–19)	46.3 (20–293)	16 (9–32)	26 (15–50)	0.220 (0.165–0.272)	0.074 (0.014–0.128)

Hardy–Weinberg tests, contemporary effective population size (N_e) estimates derived from linkage equilibrium (LD) and sibship assignment (SA) methods, and historical population size estimates (mode *F* and mean θ) from migration–drift equilibrium testing in 2MOD and MIGRATE analysis, respectively

N is the number of geckos (*Hemidactylus turcicus*) collected from each location either with the full dataset or excluding migrants as identified by GENECLASS2

F_{IS} measures the deviation from Hardy–Weinberg equilibrium across 16 loci. Multilocus estimates were not significantly different than 0 (2-tailed $P > 0.05$)

Mode *F* was estimated from *F* values generated by 2MOD and is the probability that two alleles in a population share a common ancestor. In parentheses are the 90 % HPD intervals

θ is equal to $4N_e\mu$ and was estimated with the mean of the posterior distribution in MIGRATE. Parentheses indicate the 25th to 75th percentile

[a] Parentheses enclose jack-knifed estimated 95 % confidence intervals at an allele frequency cutoff of 0.02

[b] Parentheses denote 95 % confidence intervals

estimation (assuming Hardy–Weinberg and linkage equilibrium) of the number of genetic clusters (k) without a priori delineation of populations (Pritchard et al. 2000). Ten replications were run for k values 1–8 using the admixture model and correlated allele frequencies with burn-in of 100,000 followed by 100,000 iterations of Markov Chain Monte Carlo. Although we sampled five locations, we evaluated higher k values in case there was structuring within sampling areas.

If an invading population went through a founding event or if the invading population was a sink and thus subject to turnover during repeated introductions, we would expect a signature of a bottleneck. Thus, we tested for the signature of bottlenecks at each of our sampled locations. Recent reductions in effective population size (N_e) lead to deviations from mutation–drift equilibrium. BOTTLENECK v1.2.02 detects recent ($2N_e$–$4N_e$ generations past) deviations from the mutation–drift equilibrium by simulating equilibrium gene diversity from the observed number of alleles (Piry et al. 1999). The expectation is that a bottleneck causes the number of alleles to decrease faster than gene diversity (i.e., expected heterozygosity), thus the program tests if observed gene diversity is greater than gene diversity at mutation–drift equilibrium (Piry et al. 1999). In BOTTLENECK we employed the infinite-alleles mutation model because locus tet019 had alleles that were off the tandem repeat and locus di021 had large gaps in allele sizes that prevented ascertainment of a tandem repeat mutation process. Thus, we did not use stepwise mutation models. Following 10,000 simulations, statistical significance was calculated with Wilcoxon's sign-rank test because fewer than 20 loci were used (Piry et al. 1999).

We suspected that dispersal (especially the possibility of human mediated) was possible because our sampling was at a very local scale. Thus, treating each location as a separate population likely violates the assumption of a closed population for the method implemented in BOTTLENECK. As an alternative to test for non-equilibrium dynamics, we used the program 2MOD (Ciofi et al. 1999). For a set of sampled populations, the software 2MOD tests between a non-equilibrium model of population fragmentation followed by drift with no gene flow among subpopulations versus an immigration–drift equilibrium model (Ciofi et al. 1999). The former non-equilibrium model could result from an invasion processes if each of the sampled locations were founded independently by a subset of individuals from the larger pool of potential colonizers. Support for the drift model would suggest that gecko populations are isolated and under non-equilibrium population dynamics. In contrast, support for the immigration–drift model would indicate that the observed levels of genetic differentiation among sampled locations have largely reached a level of equilibrium, which is a pattern one would expect if subpopulations are firmly established with relatively constant effective sizes and migration rates. Using 2MOD, we performed 100,000 MCMC iterations and then discarded 10 % of the data (as burn-into discard sample values unlikely to occur in samples from the true distribution) before calculating the probabilities for each model. The proportion of iterations supporting each model was summarized between two independent runs, and the Bayes factor was derived from the ratio of iterations supporting each model.

As another means to assess demographic stability, contemporary and historical patterns of drift occurring within populations and gene flow between populations (estimated as the effective number of migrants per generation, N_em) were assessed by comparing contemporary and long-term estimates of N_e and N_em, respectively. Contemporary N_e was determined with two single sample estimators. We used the bias-corrected linkage disequilibrium (LD) method (Waples 2006), which assumes that drift alone generates nonrandom associations between alleles at unlinked loci, as implemented in LD*Ne* v1.31 (Waples and Do 2008). The LD method estimates N_e in the previous generation, though residual LD via a recent bottleneck could influence the estimate for a few generations (Waples and Do 2010). LD*Ne* v1.31 was used to calculate LD with the random-mating model. As recommended by Waples and Do (2010), we reported values at an allele frequency cutoff of 0.2 as our sample sizes were >25 and used the jackknife method to determine 95 % confidence intervals. The second contemporary estimate was generated via the sibship assignment (SA) method of Wang (2009), which also estimates the N_e of the previous generation, as implemented in COLONY v2.0.3 (Jones and Wang 2010). We used the full-likelihood method, medium run length, updated allele frequency, and complexity prior options. Both contemporary estimators can be influenced by recent migration events (Wang 2009; Waples and England 2011). Thus, we ran each of the five sampled locations that included all sampled

individuals and as a reduced dataset that lacked putative first-generation migrants as identified by GENECLASS2 (Piry et al. 2004). In the reduced dataset, migrants were excluded to determine if they had an impact on the contemporary estimates of N_e. We estimated the number of first-generation migrants by using the L_home test statistic (likelihood of the individual genotype within the population where the individual was sampled) because this test statistic is best to use when not all source populations are sampled (Piry et al. 2004). The latter point was true for our study as clearly not all buildings (i.e., possible gecko subpopulations as discussed in the results) were sampled within or around the Texas A&M campus or in the suburban neighborhood. We employed the Bayesian criterion of Rannala and Mountain (1997) to calculate the likelihood, and for the probability computation, we used the resampling method of Paetkau et al. (2004). A total of 100,000 simulations was run and a critical value of 0.05 was used.

Long-term N_e was evaluated via estimates of θ ($4N_e\mu$) from the program MIGRATE; thus we assumed mutation rates (μ) were the same among locations. These estimates should reflect a longer time span of $4N_e$ generations in the past (Beerli 2009). We ran MIGRATE three times in order to find convergence and optimal parameter estimates for θ. The first two runs were shorter with 50,000 recorded steps and one or two replicates. The prior distribution for the parameters was uniform with θ bounded between 0.001 and 10, and M (immigration rate divided by the mutation rate μ) bounded between 0.001 and 100. The full migration matrix model was specified and θ was calculated from an independent initial estimate of F_{ST} and four-chain heating at temperatures of 1, 1.5, 3, and 1,000,000. Final estimates were combined over ten replicates and were computed in parallel using WestGrid supercomputing resources (https://www.westgrid.ca/about_westgrid). Historical inference about genetic drift can also be obtained from 2MOD via estimation of the parameter F, the probability that two genes share a common ancestry within a population (Ciofi et al. 1999). Increasing values of F indicate a greater influence of drift. The posterior distribution of F was obtained by simulating points with local density estimation in R with the function locfit (Loader 1999). We report the mode and 90 % highest probability density (HPD) limits for each sampling area.

Contemporary and historical rates of migration (N_em) were estimated with BAYESASS (Wilson and Rannala 2003) and MIGRATE (Beerli 2013), respectively. For the former, we conducted five short runs (3,000,000 iterations, 1,000,000 burn-in, and 2,000 thinning interval) to identify delta values that resulted in 20–60 % acceptance rate as recommended in the BAYESASS manual. The delta values for the final run had 32–38 % acceptance rates with allele frequency, migration rate, and inbreeding delta values set to 0.30, 0.13, and 0.4, respectively. We increased the number of iterations for the final run to 21,000,000 with 2,000,000 as burn-in and 2,000 as the thinning interval. Convergence was verified visually with TRACER (Rambaut and Drummond 2007). Using the BAYESASS migration rate matrix values (m), we calculated N_em from the product of m_{ij} (the proportion of individuals in population i that are migrants derived from population j per generation) and the harmonic mean of the LD and SA contemporary N_e estimates of population i. Waples and Do (2010) suggest that if two single-sample N_e estimators are independent and are estimating the same parameter from a population, then a more precise or "best" estimate of N_e can be obtained by taking the harmonic mean of the two single-sample estimators. We did this for both the full and no migrant data sets that were used to estimate contemporary N_e (Table 2). Tables S1 and S2 contain the calculated N_em estimates and the m_{ij} estimates, respectively. As an example with population 1, the harmonic mean from the two contemporary N_e estimates of the full data set was 74.7. From BAYESASS, $m_{12} = 0.0376$ (Table S2), hence N_em from population 2–1 was 2.81 (Table S1). Historical N_em was calculated as the product of θ_i and M_{ij} (each estimated as the mean of the posterior distribution from MIGRATE with conditions explained above) divided by 4. We then tested for a correlation between contemporary and historical N_em estimates using the regression-based randomization (10,000 randomizations) procedure implemented in FSTAT. It should be noted that we do not necessarily expect the contemporary and historical estimates to be equal, just correlated if there is stability in migration patterns between populations across generations. The reason for the latter is that estimates of N_em from BAYESASS might be larger than historical estimates because the estimation of m in BAYESASS includes first-generation migrants, which may not necessarily contribute genes to the next generation. Historical estimates only include genes incorporated into populations via reproduction involving migrants.

Results

As indicated by the non-significant multilocus F_{IS} values, each sampling area was in HWE (Table 2), so combining neighboring buildings did not result in Wahlund effects. On a per-locus basis, no more than two loci were out of HWE in any of the populations, and only locus di021 was out of HWE in two sampling areas (1 and 4). After Bonferroni correction within populations, only di021 deviated from HWE in sampling area 4.

If drift was solely responsible for GD, then the pairs of loci that test significant will be random among the populations. Among the 18 loci, 2 locus pairs (di010 × tet005 and di003 × tet005) tested significant ($P < 0.05$) for GD in five and three of the sampling locations, respectively. Therefore, we assumed there was possible strong linkage among these loci and subsequently removed di010 and di003 from downstream analyses. The inclusion of these latter two loci does not qualitatively change any of the downstream results or conclusions (data not shown). For the remaining 16 loci, it was expected that six pairwise combinations (0.05 × 120 pairwise combinations) would test significant for GD by chance within each sampled location. For sampling areas 1–5, there was an excess of GD observed in which 11, 9, 7, 10, and 18 combinations tested significant below $P < 0.05$, respectively. As none of the remaining significant locus pairs were significant in more than 2 populations, we considered the excess GD to be driven by drift. Indeed, we detected small N_e with the SA method (results below), which does not rely on linkage disequilibria to estimate N_e. Thus, the remaining 16 loci were used for all later analyses.

Pairwise genetic differentiation was highest between Texas A&M sampling areas (1–4) and the suburban neighborhood (area 5) (raw multilocus F_{ST}: 0.221–263) (Table 3). Among the campus locations (sampling areas 1–4), the degree of subdivision was lower (raw multilocus F_{ST}: 0.036–0.097). In accordance with the F_{ST}-based analyses, the Bayesian clustering method also provided evidence of genetic structuring among sampling areas. There was an increase in the mean posterior probability, ln P(D), until the number of clusters (k) reached $k = 5$ and $k = 6$ (−6,569.4, −6,552.1, respectively) after which mean ln P(D) decreased (Fig. S1). Although $k = 6$ had the higher ln P(D) the variance among runs was substantially higher than at $k = 5$ (Fig. S1). Moreover, at $k = 5$ the five clusters largely reflected the five sampling locations (Fig. 2). For example, approximately 60 % of the total individuals within our study were assigned with high fractional membership (Q-value ≥ 0.7) to the location from which it was sampled. As noted above, we did not sample all possible subpopulations. Thus, the increase in the ln P(D) at $k = 6$ could possibly reflect that we sampled migrant individuals belonging to other genetic clusters. However, our goal was not to estimate k, per se, but rather ascertain genetic structuring without delimiting populations. Indeed, the results from the STRUCTURE analyses mirrored that of the pairwise F_{ST} analyses. For example, Fig. 2a shows that at $k = 2$ the four campus locations remained clustered together, while the suburban neighborhood (area 5), which had high pairwise F_{ST} to the other locations, formed a separate cluster. The last populations to separate out from $k = 4$ to $k = 5$ (areas 1 and 3) had the lowest pairwise F_{ST} (Fig. 2c, d; Table 3). Clearly there is substructure on this small scale that is largely defined by sampling location, thus subsequent analyses treat each sampling area as a subpopulation.

Inference of demographic history via genetic data shows no evidence for a founder event or population turnover (e.g., extinction-recolonization as a result of repeated introductions). We did not detect signatures of genetic bottlenecks in our sampled areas. If the gecko subpopulations experienced founding events, the genetic signatures are now eroded. In part, the lack of a bottleneck signature may be due to recurrent migration among subpopulations (a violation of the closed population assumption in BOTTLENECK). Indeed, the test in 2MOD comparing the non-equilibrium dynamic of isolated populations following fragmentation versus an immigration–drift equilibrium among subpopulations overwhelmingly supported the immigration–drift equilibrium P(immigration–drift equilibrium) = 1.

Similarly, comparison between contemporary and historical estimators of drift suggests that the among-population pattern of drift has remained stable over time. In particular, the contemporary N_e estimates of areas 1–4 were similar (low hundreds for the LD method and around 50 for the SA method) where the point estimate of a population typically fell within the confidence interval of other populations. In contrast, area 5 for both the LD and SA method had much lower

Table 3 Multilocus estimates of F_{ST} between pairs of *Hemidactylus turcicus* subpopulations

	1	2	3	4	5
1	–	0.053	0.036	0.057	0.221
2	0.094	–	0.081	0.097	0.239
3	0.067	0.147	–	0.076	0.259
4	0.102	0.170	0.136	–	0.263
5	0.375	0.400	0.444	0.436	–

All comparisons were significant even after Bonferroni correction ($P < 0.01$). Raw F_{ST} is above and standardized F_{ST} is below the diagonal. Further descriptions of the sampling areas can be found in Table 1

estimates (Table 2). When looking at the historical inference of drift, a similar among-population pattern was observed. Theta estimates for populations 1–4 were similar and larger than the estimate for area 5 (Table 2). Likewise, the mode F values indicated that the campus populations were less affected by drift than the suburban neighborhood (Table 2) as sampling areas 1–4 had smaller mode F values than the area 5.

The contemporary N_e estimates can be affected by recent migrants. The SA method is expected to produce upwardly biased estimates in N_e due to the presence of more unrelated individuals (Wang 2009). The LD method may overestimate local N_e if there is a migration rate greater than 0.1 or underestimate N_e if a there are a few migrants from a highly diverged population (Waples and England 2011). With the exception of the LD estimate in area 5, the removal of putative migrants did not strongly impact the N_e estimates because the confidence intervals conducted with and without migrants overlapped. It is possible that divergent migrants were causing additional linkage disequilibria in population 5 and thus, driving the N_e estimate down. Nevertheless, the N_e estimate without migrants is still substantially lower than it is at sites 1–4.

To compare our single-sample N_e estimates to demographic estimates, we calculated N_e/N_c ratios. As noted above, we used the harmonic mean of the LD and SA N_e estimates. N_c is the census population size and was estimated for each of our sampled areas based on the surface areas of the buildings (Table 1) and a demographic density estimate of 1,048 geckos/ha [an average of the results from Selcer (1986) and Locey and Stone (2006)]. The single-sample estimators we used here would reflect uneven sex ratios and variation in reproductive success of the previous breeding generation. We observed a N_e/N_c range of 0.06–0.28 among our sampled areas. In an extensive review by Frankham (1995), the mean N_e/N_c ratio was 0.35 (95 % CI 0.28–0.42) among species for which variation in reproductive success and uneven sex ratios were taken into account to obtain demographic estimates of N_e. Thus, the values for *H. turcicus* fall on the lower edge for what is known from single generation N_e/N_c estimates of other species.

Just as N_e was temporally stable, patterns of gene flow as estimated by N_em between subpopulations were similar between the contemporary and historical estimates. The vast majority of contemporary estimates of N_em between subpopulations from BAYESASS were low (0.108–2.808) no matter if they included the full dataset or excluded migrants (Table S1). The one exception was $N_em = 15$ from subpopulation 3 to 1. However, simulations by Faubet et al. (2007) show that when F_{ST} is less than 0.05, there can be upward bias in estimates of F and m in BAYESASS (Faubet et al. 2007). Indeed, F_{ST} between populations 1 and 3 was only 0.036 (Table 3). Hence, we removed this data point from further analysis as it was a clear outlier and likely a reflection of the inaccuracy of BAYESASS to estimate N_em with low F_{ST}. Historical N_em estimates from MIGRATE were also low and, as expected (noted earlier), generally lower than the contemporary estimates (0.052–1.334; Table S1). As shown in Fig. 3, there was a significant positive correlation between contemporary and historical estimates, thus indicating patterns of gene flow between gecko populations are relatively similar over time (full dataset: $P = 0.02$, $r = 0.55$; no migrants, $P < 0.04$, $r = 0.48$).

Discussion

We find little to no evidence for non-equilibrium population genetic signatures within or among several recently introduced subpopulations of the

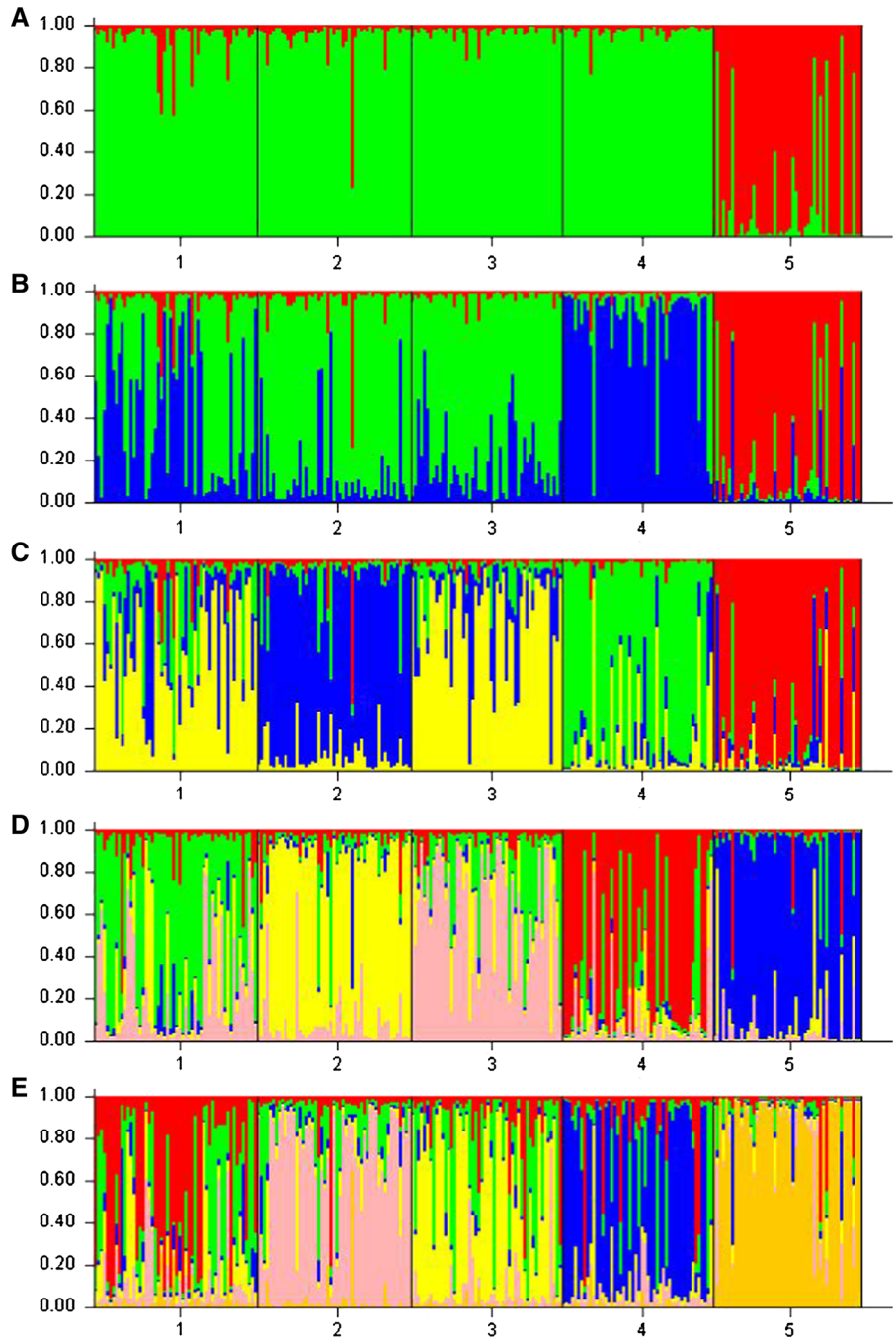

Fig. 2 Population structure at increasing values of k illustrates the degree of genetic differentiation among the five sampling areas. **a** $k = 2$. Individuals from sampling area 5 form a cluster, while the four remaining areas cluster together. **b** $k = 3$. Sampling areas 4 and 5 are now differentiated from the three remaining areas. **c** $k = 4$. Sampling areas 2, 4, and 5 each cluster separately from the two remaining areas. **d** $k = 5$. Each sampling location clusters separately. **e** $k = 6$. Each sampling location largely remains a distinct cluster

invasive Mediterranean gecko. Thus, these results do not support recent founder events or population turnover from repeated introductions. We are not suggesting these events did not occur, only that if they did, these populations have recovered. Overall, we propose that the recent population history of *H. turcicus* in College Station, TX is best explained as a metapopulation consisting of firmly established subpopulations with relatively constant N_es and low migration rates. This conclusion is based on the large support for the equilibrium immigration–drift model in 2MOD and that among-population patterns in N_e and N_em were similar between contemporary and historical estimators.

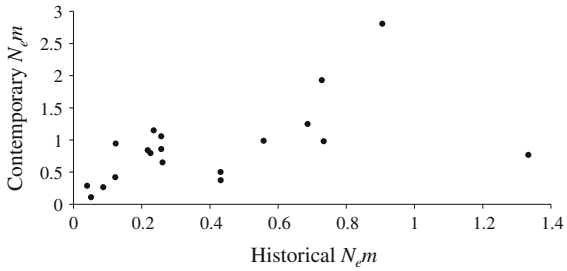

Fig. 3 Positive correlation between contemporary and historical estimates of the effective number of migrants (N_em) suggests relatively stable patterns of gene flow among the five gecko subpopulations. The graph shows contemporary N_em estimates based on the full data set ($P = 0.02$, $r = 0.55$). The contemporary estimates based on the data set with no migrants was similar and also significant ($P < 0.04$, $r = 0.48$). The main text describes how contemporary and historical N_em were calculated

There are two possible explanations for the observed genetic equilibrium patterns. First, the non-detection of non-equilibrium could suggest that a large number of individuals were introduced or repeatedly introduced without population turnover (i.e., there was large propagule pressure). Unfortunately, in our study area, there are no historical reports on propagule pressure that allow us to refute or support the latter hypothesis. However, given that mark–recapture studies in other populations found very limited gecko-mediated dispersal, we suspect that geckos do not often disperse to new areas in large numbers. Indeed, the low contemporary and historical estimates of N_em support the latter argument (Fig. 3; Table S1). Even in the case of a known purposeful introduction at the University of Central Oklahoma, 12 or fewer geckos were used as a founding population (Locey and Stone 2006). Thus, we suspect that geckos do not often disperse to new areas in large numbers and that large founding populations seem unreasonable. We cannot definitively rule out repeated introductions without turnover. In fact, the University of Central Oklahoma population involved repeated introductions from 1962 to 1997 (Locey and Stone 2006). However, even in the latter situation, a breeding population was not achieved until the late 1980's (Locey and Stone 2006), thus indicating there was population turnover in the early stages of invasion. Thus, it would be interesting to see if the University of Central Oklahoma population shows a genetic signature of a bottleneck. We also believe that large propagule pressure is unlikely because repeated introductions, which would continually add genetic variation, would not likely be constant among all sampled subpopulations. Therefore, the among-population patterns of drift or gene flow would not remain stable over time. Because we observed stability in the latter patterns, we lean towards the following hypothesis.

A second explanation for the population genetic equilibrium is that geckos have the ability to rapidly pass through or recover from unstable demographic events (e.g., bottlenecks). Populations that recover quickly after a bottleneck lose little genetic variation even if the population was reduced to a few individuals (Hanski and Gaggiotti 2004). The key to recovery is growth rate, and even at modest levels such as of an intrinsic rate of growth of 0.5, a population of two individuals can retain 50 % of the original population's genetic variability (Nei et al. 1975; Hanski and Gaggiotti 2004). In addition, generation overlap can preserve genetic variability as the same adults contribute to the population over several years. Although population growth rate has never been estimated for *H. turcicus*, a life-history study for a population in south Texas, USA found that the geckos had relatively high adult and egg survivorship, and thus undoubtedly overlapping generations within a population (Selcer 1986). Life span was estimated at 3 years or longer and 1–3 clutches of two eggs each can be produced per year (Selcer 1986). In addition, populations in Texas and Louisiana have been described as having low predation and competition pressure, which may further allow rapid recovery if colonization involved a founder event (Rose and Barbour 1968; Selcer 1986). Overall, these life-history traits could have helped the gecko reach the establishment stage of invasion in a relatively short time. Assuming generation length is 1 year, we estimate a maximum of 41 generations (1970–2011) in our study area. However, given the gecko can live three or more years with the first clutch not being produced until the end of the first year (Selcer 1986), generation time will be closer to 2 years if not more. Therefore, population genetic equilibrium, which we infer as meaning the subpopulations have reached the establishment stage, could have happened in as few as 20 generations or less.

With regards to corroborating dispersal potential estimated from mark–recapture studies, we find strong support that indeed local dispersal of *H. turcicus* is limited. We found significant genetic structure on a local scale that best corresponds to individual sampling areas, which were comprised largely of

individual buildings or sets of adjacent buildings (Figs. 1, 2). Though sample sizes were small, a similar pattern was observed by Trout and Schwaner (1994) who found allele frequencies differences (two biallelic allozyme loci) between two buildings in Mobile, AL, USA. We did not perform an isolation-by-distance analysis across all areas as the results would have been weighted by sampling area 5. Qualitatively, geographic distance appears to play a role in isolation in that both the F_{ST} and STRUCTURE analyses show that all four campus locations were more similar compared to the more spatially distant neighborhood area (~4.8 km away). Significant genetic differentiation and population genetic structure probably arise because without human mediation, geckos typically disperse only a few meters within a year (Selcer 1986; Locey and Stone 2006). However, there was no isolation-by-distance among the four campus locations ($P = 0.26$, Mantel test between $F_{ST}/(1 - F_{ST})$ and geographic distance). In fact, the two most distant campus locations (sampling areas 1 and 3) had the lowest pairwise F_{ST} and were the last to separate out in the STRUCTURE analysis (compare Fig. 2c, d). Interestingly, both of these locations contain greenhouses where materials have been transported (e.g., during plant sales) between these locations. When humans transported items between the two areas, this may have dispersed hitchhiking geckos or their eggs. This type of dispersal, often called "jump dispersal", has been described as a means of gecko introduction (Locey and Stone 2006).

Other recently introduced gecko species in the mainland USA have shown a different pattern of fine-scale genetic structure. In Florida, *H. mabouia* was first documented in the early 1990s and has since expanded its introduced range from south to north (Short and Petren 2011a). Little to no genetic structure was found between subpopulations located near the origin of invasion, while greater levels of genetic structure were observed among more recently colonized subpopulations (Short and Petren 2011b). The authors argued that the structure in the more recent colonizations resulted from founder events while dispersal over time likely eroded the structure at the invasion origin. We too do not find evidence of founder events in our populations. However, we observe much more genetic structure at a similar scale (e.g., buildings on a campus) among subpopulations that are equal to or older (as early as 1970 and more recent as late as 1992, Table 1) than the originating introductions of *H. mabouia*. These differences could be due to (1) different invasion histories, (2) differences in local dispersal potential, or (3) sample sizes (~10 geckos per building, Short and Petren 2011b; ~50 per building, current study).

As an increasing number of invasive species begin impacting native communities, it is becoming increasingly important to understand what factors allow invasions to progress from initial transport to the spreading stage. The establishment stage is particularly important as once a species reaches this stage, then this provides a foothold that makes subsequent range expansion possible. Given that observational data are often unavailable or difficult to collect during the early stages of invasion, population genetic data and evolutionary-based methods can be used to infer when species reach different stages of the invasion. For the invasive gecko *H. turcicus* in College Station, TX, we found no evidence of non-equilibrium dynamics. Rather, genetic inference of population demographic history suggested that these recently introduced gecko populations are firmly established with relatively constant N_es and migration rates; i.e., our sampling areas appear to function as a metapopulation at equilibrium, where each building largely functions as a subpopulation with small N_e (~50–100). Importantly, our study suggests that the Mediterranean gecko can reach the establishment stage rather quickly (maybe in as little as 20 generations), thus giving this invasive species a quick foothold to advance to the spreading stage.

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References

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, Oxford

Beerli P (2009) How to use MIGRATE or why are Markov chain Monte Carlo programs difficult to use? In: Bertorelle G, Bruford MW, Hauffe HC, Rizzoli A, Vernesi C (eds) Population genetics for animal conservation. Cambridge University Press, Cambridge, pp 42–79

Beerli P (2013) Migrate-N version 3.6: estimation of population sizes and gene flow using the coalescent. http://popgen.sc.fsu.edu/Migrate/Migrate-n.html

Blackburn TM, Pysek P, Bacher S, Carlton JT, Duncan RP, Jarosik V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339

Bomford M, Kraus F, Barry SC, Lawrence E (2009) Predicting establishment success for alien reptiles and amphibians: a role of climate matching. Biol Invasions 11:713–724

Carranza S, Arnold EN (2006) Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. Mol Phylogenet Evol 38:531–545

Ciofi C, Beaumont MA, Swingland IR, Bruford MW (1999) Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. Proc R Soc Lond B Biol Sci 266:2269–2274

Conant R (1955) Notes on three Texas reptiles, including an addition to the fauna of the state. Am Mus Novit 1726:1–6

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Detwiler JT, Criscione CD (2011) Testing Mendelian inheritance from field-collected parasites: revealing duplicated loci enables correct inference of reproductive mode and mating system. Int J Parasitol 41:1185–1195

Etheridge RE (1952) The Warty Gecko, *Hemidactylus turcicus turcicus* (Linnaeus), in New Orleans, Louisiana. Copeia 1952:47–48

Fauvergue X, Vercken E, Malausa T, Hufbauer RA (2012) The biology of small, introduced populations, with special reference to biological control. Evol Appl 5:424–443

Fowler HW (1915) Cold-blooded vertebrates from Florida, the West Indies, Costa Rica, and eastern Brazil. Proc Acad Nat Sci Phila 67:244–269

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genet Res Camb 66:95–107

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. J Hered 86:485–486

Handley LJL, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. Biocontrol 56:409–428

Hanski I, Gaggiotti OE (2004) Ecology, genetics and evolution of metapopulations. Elsevier Academic Press, San Diego

Hardy OJ, Vekemans X (2002) SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? Biol Invasions 10:483–506

Jones OR, Wang JL (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Koenig WD, VanVuren D, Hooge PN (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends Ecol Evol 11:514–517

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

Kraus F (2009) Alien reptiles and amphibians: a scientific compendium and analysis. Springer, Dordrecht

Loader C (1999) Local regression and likelihood. Springer, New York

Locey KJ, Stone PA (2006) Factors affecting range expansion in the introduced Mediterranean Gecko, *Hemidactylus turcicus*. J Herpetol 40:526–530

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60:2399–2402

Meshaka WE, Marshall SD, Boundy J, Williams AA (2006) Status and geographic expansion of the Mediterranean Gecko, *Hemidactylus turcicus*, in Louisiana: implications for the southeastern United States. Herpetol Conserv Biol 1:45–50

Nei M, Maruyama T, Chakraborty R (1975) Bottleneck effect and genetic variability in populations. Evolution 29:1–10

Owusu KA, Detwiler JT, Criscione CD (2012) Characterization of 21 microsatellite loci from the invasive Mediterranean gecko (*Hemidactylus turcicus*). Conserv Genet Res 4: 563–565

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetics assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13:55–65

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rambaut A, Drummond AJ (2007) Tracer v1.5. http://beast.bio.ed.ac.uk/Tracer

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94: 9197–9201

Rödder D, Lötters S (2009) Niche shift versus niche conservatism? Climatic characteristics of the native and invasive ranges of the Mediterranean house gecko (*Hemidactylus turcicus*). Glob Ecol Biogeogr 18:674–687

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Rose FL, Barbour CD (1968) Ecology and reproductive cycles of the introduced gecko, *Hemidactylus turcicus*, in the Southern United States. Am Midl Nat 79:159–168

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Schwaner T, DeVries A, Budge D, Schwaner D (2008) Genetic variation in founder populations of the Mediterranean gecko, *Hemidactylus turcicus*, across the southern United States. Amphib-Reptil 29:438–442

Selcer KW (1986) Life history of a successful colonizer: the Mediterranean Gecko, *Hemidactylus turcicus*, in southern Texas. Copeia 4:956–962

Short KH, Petren K (2011a) Multimodal dispersal during the range expansion of the tropical house gecko *Hemidactylus mabouia*. Ecol Evol 1:181–190

Short KH, Petren K (2011b) Fine-scale genetic structure arises during range expansion of an invasive gecko. PLoS One 6:e26258

Stabler LB, Johnson WL, Locey KJ, Stone PA (2012) A comparison of Mediterranean Gecko (*Hemidactylus turcicus*) populations in two temperate zone urban habitats. Urb Ecosyst 15:653–666

Stejneger L (1922) Two geckos new to the fauna of the United States. Copeia 1922:56

Trout L, Schwaner TD (1994) Allozyme evidence for insularity in exotic populations of the Mediterranean gecko (*Hemidactylus turcicus*). J Herpetol 28:391–393

Uller T, Leimu R (2011) Founder events predict changes in genetic diversity during human-mediated range expansions. Glob Change Biol 17:3478–3485

Wang JL (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Mol Ecol 18:2148–2164

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7:167–184

Waples RC, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8:753–756

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol Appl 3:244–262

Waples RS, England PR (2011) Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. Genetics 189:633–644

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191