Genetic Differentiation between Marine Iguanas from Different Breeding Sites on the Island of Santa Fé (Galápagos Archipelago)

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Abstract

We studied patterns of genetic diversity within and among 5 populations (318 individuals) of Galápagos marine iguanas (*Amblyrhynchus cristatus*) from the island Santa Fé. Populations were separated by distances of 0.2 to 9.9 km. We sequenced 1182 base pairs of the mitochondrial control region and screened 13 microsatellite loci for variability. We also added data from 5 populations (397 individuals) sampled on 4 neighboring islands (Santa Cruz, Floreana, Espanola, and San Cristobal). The 5 Santa Fé populations, revealed as genetically distinct from populations on other islands, present relatively low levels of genetic diversity, which are similar for both microsatellite (average observed heterozygosity from 0.7686 to 0.7773) and mitochondrial DNA (mtDNA) markers (haplotypic and nucleotide diversity from 0.587 to 0.728 and from 0.00079 to 0.00293, respectively), and comparable with those observed in similar-sized sampling sites on other islands. There was frequency-based evidence of genetic structure between northern and southern sites on Santa Fé (F_{st} of 0.0027–0.0115 for microsatellite and 0.0447–0.2391 for mtDNA), but the 4 southern sites showed little differentiation. Most of the intra-island genetic variation was allocated within rather than between sites. There was no evidence of sex-biased dispersal or population substructuring due to lek-mating behavior, suggesting that these 2 observed behaviors are not strong enough to leave an evolutionary signal on genetic patterns in this species.

Key words: fine-scale genetic diversity, dispersal, gene flow, microsatellite, mtDNA, marine iguanas, Galápagos

Marine iguanas (*Amblyrhynchus cristatus*) are endemic to the Galápagos archipelago and are widespread, inhabiting all 13 major islands as well as many smaller islands and islets. They have been subject of many behavioral, physiological, and ecological investigations (e.g., Trillmich 1983; Trillmich KGK and Trillmich F 1986; Mackenzie et al. 1995; Dellinger 1996; Wikelski and Bäurle 1996; Partecke et al. 2002; Hayes et al. 2004; Wikelski et al. 2005; Vitousek, Mitchell, et al. 2007; Vitousek, Adelman et al. 2007; Vitousek et al. 2008) that have typically been centered at the fine spatial scale, focusing on populations from a small number of localities

and islands. Several phylogeographic studies of marine iguanas have also been carried out using immunological (Higgins and Rand 1974, 1975; Higgins et al. 1974; Higgins 1977) and genetic data (Wyles and Sarich 1983; Rassmann et al. 1997; Steinfartz et al. 2007, 2009), but these studies have concentrated on large-scale patterns, examining major evolutionary relationships among a limited number of populations from each island. However, the understanding of many biological and behavioral aspects of marine iguanas (e.g., dispersal, aggregation, and mating choice) could benefit from a fine-scaled genetic study focusing on

population dynamics and differentiation within a single island or locality. Marine iguanas feed exclusively on aquatic algae along the coastline, making them susceptible to passive dispersal by ocean currents (Carpenter 1966; Drent et al. 1999; Hobson 1965; Shepherd and Hawkes 2005; Trillmich 1983; Vitousek, Rubenstein et al. 2007; Wikelski and Hau 1995). However, such dispersal is not likely to be equal among different sex and age classes because larger iguanas (about 5% of the population; Wikelski and Trillmich 1994), which tend to be males, feed offshore in the subtidal zone, whereas the typically smaller females and juveniles more often feed on or near the shoreline (Buttemer and Dawson 1993; Shepherd and Hawkes 2005; Trillmich KGK and Trillmich F 1986; Wikelski and Trillmich 1994; Vitousek et al. 2008; Wikelski et al. 1997). Thus, large males are more likely exposed to marine currents and may cause genetic exchange between populations (see Rassmann et al. 1997).

Marine iguanas are also patchily distributed across the Galápagos coastal landscape, which may lead to the formation or maintenance of discrete population units over time. The species exhibits a unique aggregation behavior, with groups ranging from several to thousands of individuals (Figure 1C). The density of such aggregations depends on the patchiness of food resources in the intertidal (Rubenstein and Wikelski 2003), overall population density (which can vary drastically; Laurie 1989), and time of year. In the nonbreeding season, aggregation size appears to be determined by algal productivity, with the largest groups of animals inhabiting regions with abundant resources and accessible foraging grounds (Wikelski and Trillmich 1997; Wikelski and Romero 2003; Wikelski 2005). During the breeding season, clustering increases as the largest males defend small display territories that attract many visiting females (Wikelski 1996; Wikelski et al. 1996; Wikelski and Nelson 2004). Low-quality males cluster their territories near high-quality males according to the "hot-shot mechanism" (Partecke et al. 2002), resulting in particularly dense aggregations during the reproductive season. The fine-scale location of territories is largely determined by geographical features (e.g., crevices or small lava boulders), and the location of territory clusters is fairly consistent between years (Dellinger 1991; Partecke et al. 2002). Individual males show high fidelity to specific breeding sites between years (Dellinger 1991), but territory location has little effect on mate choice (Partecke et al. 2002). Instead, females choose mates based on their rate of head-bob display, an energetically costly behavior that varies substantially both between individuals and between years (Wikelski et al. 2001; Vitousek, Mitchell et al. 2007; Vitousek et al. 2008). Thus, although males gather generally in the same lekking arena from year to year, females chose the best male in any given year and do not rely on previous year's choice (Wikelski et al. 1996; Partecke et al. 2002). Recent fine-scale field observations depicted a marked movement of males from their original locations to new sites over short distances (e.g., between sites within an island), immediately following the breeding season, with about 25% of study males dispersing up to several kilometers (e.g., between the 2 sites

S1 and S3 on the island of Santa Fé; Figure 1). In contrast, females seem to be very site-faithful, usually to within 0.2 km of the coastline (Rauch 1985; Laurie and Brown 1990; Wikelski et al. 2001; Vitousek, Mitchell et al. 2007). However, the long-term breeding site fidelity of both males and females suggests that this end-of-season dispersal may only be temporary, with males traveling to areas of high algal productivity in order to regain the mass lost during the energetically costly reproductive period (Partecke et al. 2002; Vitousek et al. 2008). As suggested by these aspects of marine iguana biology, it is predicted that the boundary of populations should be heavily influenced by breeding and foraging behavior and that dispersal between populations should be male biased. Genetic data (e.g., highly polymorphic genetic markers such as microsatellite loci) on patterns of population differentiation could therefore reveal much about the interplay between biological and demographic processes over time.

At the larger scale, a past genetic study of marine iguanas (Rassmann et al. 1997) showed that populations throughout the archipelago were fairly undifferentiated according to nuclear genetic markers (3 microsatellite and 3 minisatellite loci), mainly in the form of low F_{st} values even among populations from distant islands. Conversely, mitochondrial DNA (mtDNA) phylogeographic patterns in the same study showed significant genetic structure, especially among the older eastern and younger western islands of the archipelago. This discrepancy between nuclear and mitochondrial markers led to the interpretation that dispersal between populations is male biased, a conclusion supported by the tendency for males to feed offshore in greater numbers than females. However, a more recent study (Steinfartz et al. 2009) using a larger set of microsatellite markers and more sensitive methods of genetic analysis revealed that marine iguanas are highly differentiated between islands and that most islands are host to single evolutionary genetic clusters. As a consequence, male-biased dispersal based on high levels of nuclear gene flow as in the study of Rassmann et al. (1997) was not supported at the inter-island level. Both genetic studies on marine iguanas examined only one or a few localities from most islands (exceptions were Fernandina and Isabela) and therefore say little about within-island population structure. Such information, however, could not only serve as a basis for understanding marine iguana biology, but also for defining units of conservation.

In this study, a major goal was not only to complement prior genetic studies that have been undertaken on a larger (archipelago-wide) scale iguana populations (Rassmann et al. 1997; Steinfartz et al. 2009) but also to test for sex-biased dispersal or population substructuring due to lek-mating behavior in a finer scale perspective. Here, we investigate fine-scale genetic patterns of population structure in marine iguanas from 5 sites (318 individuals) of a single island, Santa Fé, a relatively small island (~2400 ha) located near the center of the Galápagos archipelago. The 5 sampling sites were separated by distances ranging from 0.2 to 9.9 km of coastline and have been well studied from an ecological
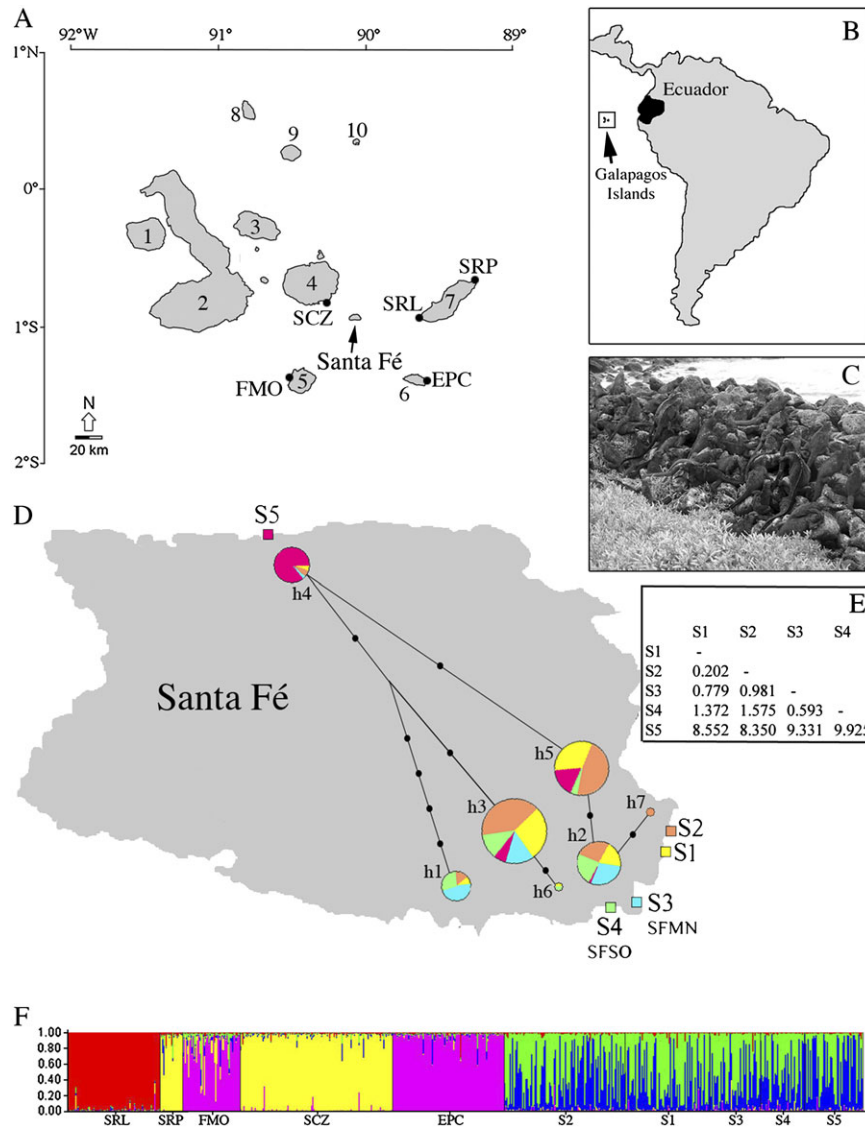
Figure 1. Map of the Galápagos archipelago (Ecuador) (**A**), in relation to South America (**B**), with a zoom on Santa Fé Island (**D**) where marine iguanas were collected (5 sites, S1–S5). Marine iguanas live nonaggressively most part of the year (**C**). The pairwise distances (±0.01 km) along the coastline between sampling sites were calculated from georectified marine navigational charts (Instituto Oceanografico de la Armada, Ecuador) via ArcView 3.3 (**E**). Individual-based mixture analysis of Galápagos marine iguanas to 5 genetic clusters as determined by the program Structure version 2.0 (Pritchard et al. 2000) (**F**), following the estimation of true number of genetic clusters (K) by maximizing the estimated log-likelihood (ln Pr(X/K)) of the data for different values of K (see Supplementary Figure 1A). Each individual is represented by a column, and membership coefficients are color-coded according to the cluster of origin ($K = 1$–5). The median-joining network of CR mtDNA was drawn on the map of Santa Fé (branch lengths are not proportional to the number of changes) (D). Sampled haplotypes (h1–h7) are represented by circles and drawn in a size proportional to their frequency (number of individuals harboring that haplotype). Each connection between haplotypes represents one mutational step between haplotypes and small black circles (nodes) are inferred (unsampled or extinct) haplotypes. The 5 sampling locations (S1–S5) are color coded. If more than one population harbored a given haplotype, its frequency in each is indicated by the pie diagrams. Numbers 1–10 (A) represent the different islands: (1) Fernandina, (2) Isabela, (3) Santiago, (4) Santa Cruz, (5) Floreana, (6) Espanola, (7) San Cristobal, (8) Pinta, (9) Marchena, and (10) Genovesa. Three letter symbols on the map identify the location of the sampling sites on Espanola (EPC), Floreana (FMO), San Cristobal (SRP and SRL), and Santa Cruz (SCZ).

Table 1 List of sampling sites on Santa Fé and surrounding islands, as well as their location, the year of collection, and the number (N) of collected samples

Islands	Sampling sites	N	Year of collection	Coordinates
Santa Fé	S1 – Bahia Paraiso	75	2004	90°01′44″W, 0°49′52″S
	S2 – Bahia Paraiso	107	2004	90°01′41″W, 0°49′46″S
	S3 – Miedo North	45	2004	90°01′51″W, 0°50′11″S
	S4 – Miedo South	39	1991–1993[a]	90°02′02″W, 0°50′18″S
	S5 – North	52	1991–1993[a]	90°04′00″W, 0°48′00″S
Santa Cruz	SCZ – Estacion/Camano	135	1991–1993[a], 2004	90°18′15″W, 0°44′35″S
Floreana	FMO – Punta Montura	60	1991–1993[a], 2004	90°29′53″W, 1°17′49″S
Espanola	EPC – Punta Cevallos	99	1991–1993[a], 2004	89°37′18″W, 1°23′38″S
San Cristobal	SRP – Punta Pitt	20	1991–1993[a]	89°37′04″W, 0°55′21″S
	SRL – Loberia	83	1991–1993[a], 2004	89°36′00″W, 0°56′00″S

[a] Rassmann et al. (1997).

standpoint. We collected data from 13 highly polymorphic microsatellite loci as well as the complete mitochondrial control region (CR) in order to examine the relationship of individuals within and between sites. In order to provide a wider geographic context for interpreting genetic patterns within the island, we also added data from 5 populations (397 individuals) sampled on 4 neighboring islands (Santa Cruz, Floreana, Espanola, and San Cristobal).

Materials and Methods

Collection of Samples

Marine iguanas were captured (in 1991–1993 and 2004–2005) either by hand or with the aid of a noose at the end of a bamboo pole. Approximately 1–2 ml of blood were taken from the caudal vein of each individual and placed in storage buffer (100 mM Tris, 100 mM ethylene diaminetetraacetic acid, 2% sodium dodecyl sulfate) until processing. Iguanas were released after sampling, unharmed, at the point of capture. Blood samples were collected in 5 sites (S1–S5) on the island of Santa Fé in Galápagos Archipelago (Figure 1; Table 1). Bahia Paraiso (S1 and S2) and Miedo (S3 and S4) are 0.8–1.6 km apart (Figure 1E). The S1 and S2 sites are separated by approximately 200 m of coastline, whereas the northern S5 site is approximately 8.3–9.9 km from the southern sites (Figure 1E). These sites have been chosen because most have been extensively studied from an ecological and behavioral standpoint, and mating aggregations are known to occur at 4 of the 5 sites (S1–S4) (Laurie 1990; Laurie and Brown 1990; Wikelski et al. 2001; Partecke et al. 2002; Vitousek, Mitchell, et al. 2007; Vitousek et al. 2008). The pairwise distances (±0.01 km) along the coastline between sampling sites were calculated from georectified marine navigational charts (Instituto Oceanográfico de la Armada, Ecuador) via ArcView 3.3 (ESRI, Redlands, CA, USA).

To place the genetic data from the Santa Fé population in a broader geographic context, we analyzed these samples together with population samples from 4 islands surrounding Santa Fé (Santa Cruz, Floreana, Espanola, and San Cristobal). We used one population sample for each island

with the exception of San Cristobal (2 sampling sites; Figure 1A; Table 1). Sample sizes for each site ranged from 20 to 135 individuals (Table 1). Samples were collected during 1993–1994 or 2004–2005. Rassmann et al. (1997) and Steinfartz et al. (2007, 2009) presented the genetic results of the archipelago-wide population survey.

DNA Extraction, Microsatellite Loci Genotyping and Mitochondrial CR Sequencing

Total genomic DNA from 318 marine iguanas from Santa Fé was extracted from the blood samples using the QIAGEN DNeasy Tissue kit and according to published protocols (Steinfartz et al. 2007). Thirteen species-specific microsatellite loci were amplified by polymerase chain reaction (PCR) and scored for alleles following protocols previously described (Steinfartz and Caccone 2006; Steinfartz et al. 2007). Samples were analyzed on an ABI 3730 DNA analyzer (Applied Biosystems). Locus-specific heterozygosity of samples was estimated with the program Arlequin version 2.0 (Excoffier et al. 2005). Complete mitochondrial CR sequences (1182 base pairs; bp) were generated for 315 of the 318 marine iguanas screened for variation at microsatellite loci. PCR and DNA sequencing were carried out according to Steinfartz et al. (2007). Electropherograms were checked by eye using the editing program Sequencher (GeneCodes), and then aligned using default parameters in ClustalX (Thompson et al. 1997). The mitochondrial haplotypes of Santa Fé have been deposited in Genbank under the accession numbers (HM245604-HM245610).

Data Analysis

We used genetic data to investigate population history at 2 different levels: 1) global tests for population structure between the 5 islands (715 marine iguanas and 10 sampling sites), Santa Fé and the 4 surrounding islands (i.e., Santa Cruz, Floreana, Espanola, and San Cristobal), and 2) tests between and within the 5 sites on Santa Fé (318 iguanas and 5 sampling sites).

MtDNA genetic variability within populations was estimated in terms of haplotype diversity (H_d) and nucleotide diversity (π) that were calculated for each sampling site using

the program DnaSP version 3.51 (Rozas and Rozas 1999). Identical haplotypes among the 318 Santa Fé sequences were found using Collapse version 1.2 (Posada 2004). To examine the evolutionary relationships among haplotypes and illustrate the phylogeographic structure on the map (Figure 1), we inferred a median-joining graph (Bandelt et al. 1999) using the program Network version 2.0 (available at http://www.fluxus-engineering.com/sharenet.htm). The network shows the frequency of each haplotype and the number of changes.

Statistics of nuclear genetic diversity were reported as the number of alleles per locus (N_a), allele frequencies, and observed and expected heterozygosities (H_o and H_e, respectively). These values were computed according to Nei (1987), calculated per locus as well as averaged over the 13 microsatellite loci using the software Arlequin 2.0 (Schneider et al. 2000). A one-tailed probability of departure from the Hardy–Weinberg equilibrium was also calculated to test for deficiency of heterozygotes. Deviation from Hardy–Weinberg was tested using a Markov chain approximation with 100 000 steps and 1000 dememorization steps. The software FSTAT version 2.9.3.2. (Goudet 1995, 2001) was used to estimate the allelic richness (AR), a standardized measure of the number of alleles per locus that takes into account unequal sample sizes. AR was calculated based on the minimum sample size (i.e., the smallest number of individuals typed for a locus; 39 individuals assigned to the S4 sampling unit).

Average and individual pairwise relatedness between iguanas within the 5 sites were estimated from microsatellite allele frequencies using the programs Relatedness 5.0.8 (Goodnight and Queller 1995) and Kinship 1.2 (Goodnight and Queller 1999). Individuals were weighted equally, frequency bias was corrected by site, and standard errors for average relatedness values were estimated by jackknifing over loci (Queller and Goodnight 1989). Estimated relatedness values may vary between −1 and +1 with positive values signifying 2 individuals share more alleles that were identical by descent than expected by chance, whereas negative R values were indicative of 2 individuals sharing fewer such alleles than expected by chance. Distributions corresponding to the different relationship types are centered on their theoretical values (Figure 3): full siblings, half siblings, and unrelated individuals are expected, on average, to have a mean relatedness of 0.5, 0.25, and 0.0, respectively (Blouin et al. 1996). We compared the observed distribution of pairwise relatedness values between individuals within each site with the expected distributions obtained from simulations (Kinship 1.3.1; Goodnight and Queller 1996). We estimated the expected relatedness of 1000 full siblings ($R_m = 0.5$, $R_p = 0.5$; these variables define the probabilities that individuals in the pair share an allele by direct descent from their mother or father, respectively), 1000 half siblings ($R_m = 0.5$, $R_p = 0$ or $R_m = 0.5$, $R_p = 0$), and 1000 unrelated ($R_m = 0.0$, $R_p = 0.0$). For the half-sibling simulation, we used the first of the 2 possibilities ($R_m = 0.5$ and $R_p = 0$), as the data set does not include information about maternal or paternal origin).

Pairwise F statistics were calculated to estimate mitochondrial and microsatellite genetic differentiation among the 5 breeding sites using Arlequin 2.0 (Schneider et al. 2000). For the microsatellite data, we used 1000 permutations for the significance level. Estimates of pairwise F_{st} values for each microsatellite locus were calculated using Genepop version 1.2 (Raymond and Rousset 1995).

Patterns of genetic structure across Santa Fé were investigated using a hierarchical analysis of molecular variance (AMOVA) implemented in Arlequin 2.0 (Schneider et al. 2000). Total genetic variance was partitioned into covariance components (see Excoffier 2003) that were used to compute fixation indexes as measures of the degree of genetic differentiation. We estimated significance of fixation indexes using permutation tests (1000 permutations of individual genotypes among sampling sites or sampling sites among groups, as appropriate; Schneider et al. 2000). Sampling sites among groups were defined by pooling.

Analyses of the relationship between geographical (coastline distances) and genetic distance (F_{st}) between the 5 Santa Fé sites were performed using Mantel tests (Mantel version 2.0; Liedloff 1999). One thousand iterations were used to determine significance.

A Bayesian method implemented in the program Structure version 2.0 (Pritchard et al. 2000) was used to determine whether (i) our a priori sampling sites by island and (ii) the identification of distinct potential breeding sites on Santa Fé (i.e., S1–S5) were consistent with genetic information. The number of groups (K) most compatible with the observed data can be obtained by maximizing the estimated log likelihood (ln Pr($X|K$) or ln $P(D)$) of the data for different values of K. To examine patterns of genetic differentiation of the Santa Fé samples in the context of samples from surrounding islands (5 islands, 10 sampling sites), we performed a series of independent runs with K varying between 1 and 12, assuming correlated allele frequencies and an admixture model, with a burn-in of 50 000 iterations and a data collection period of 500 000 iterations. Five runs for each value of K were performed to check for convergence and determine the optimal value of K. The same method was used to analyze patterns of genetic differentiation among sampling sites on Santa Fé (i.e., S1–S5) by applying a series of independent runs with K values between 1 and 10, a burn-in of 50 000 iterations, and a data collection period of 250 000 iterations.

Five tests for sex-biased dispersal were calculated for males and females separately, for sets of individuals sampled in 1991–1993 and 2004, using FSTAT 2.9.3 (Goudet 2001): F_{is}, F_{st} (Weir and Cockerham 1984), relatedness, (R), mean Assignment Index (mean AIc), and variance of Assignment Indices (vAIc). The AIc statistics calculate the probability for each genotype to be represented in the sampled population. Allele frequencies at each locus, after a correction for multilocus probabilities, provide a corrected AIc value (centered around 0) for each individual (l being the number of loci) (Goudet et al. 2002). FSTAT version 2.9.3 (Goudet 2001) was used to calculate individual AIc values and to test for significant differences in vAIc between the sexes.

Table 2 Genetic diversity measures of Galápagos marine iguanas from Santa Fé

Island	Sampling site	Microsatellite loci					Mitochondrial DNA				
		Sample size	A	AR	H_o	H_e	Sample size	n	H_d	π	p
Santa Fé	S1	75	9.00	7.95	0.7695	0.7760	73	6	0.663 (0.032)	0.00167	9
	S2	107	9.31	8.32	0.7553	0.7773	104	7	0.661 (0.026)	0.00173	9
	S3	45	8.85	8.41	0.7621	0.7698	45	4	0.690 (0.024)	0.00293	8
	S4	39	8.38	6.27	0.7412	0.7686	37	5	0.728 (0.038)	0.00275	9
	S5	52	8.28	8.28	0.8019	0.7690	52	4	0.587 (0.053)	0.00079	4

A, mean number of alleles; AR, mean allelic richness; H_d, haplotype diversity (standard error values are in parentheses); H_e, mean expected heterozygosity; H_o, mean observed heterozygosity; n, number of haplotypes; π, nucleotide diversity; p, number of polymorphic sites.

We also implemented the isolation with migration model (IMa; Hey and Nielsen 2004) to estimate the joint posterior probabilities of 6 demographic parameters: effective population sizes of 2 descendant populations (Θ_1 and Θ_2), a single ancestral effective population size (Θ_A), an estimated time since divergence (t), and 2 migration rates (m1 and m2). The populations sampled on Santa Fé correspond to a single island clade (Steinfartz et al. 2009), and we defined 2 populations: a southern clade including 4 nearby demes (S1–S4), which show evidence of mating aggregations, and a single more isolated northern population (S5). We focused on the estimation of migration rates to examine sex-biased dispersal and potential patterns of symmetrical versus asymmetrical migration. Analyses were run on 3 data sets: mtDNA CR sequences, microsatellite loci, and the 2 data sets combined. Our demographic estimates were based on a Markov chain Monte Carlo run for 2 000 000 steps, following a 100 000 step burn-in with sampling every 100 steps. We assumed a 2-step heating scheme with 10 coupled chains and heating parameters set to $g_1 = 0.08$ and $g_2 = 0.01$. We used the appropriate inheritance scalars and slightly different priors for each of the 6 estimated parameters. We inspected effective sample sizes and autocorrelation values to assess adequate chain mixing and convergence of the Markov chain. Posterior probabilities (HiSmth) and 95% confidence intervals were recorded from the posterior distribution of each parameter.

Results

Genetic Diversity

Mitochondrial DNA

Complete mitochondrial CR sequences were compared among 315 marine iguanas from the 5 sampling sites on Santa Fé (S1–S5) (Table 2; Figure 1D). Out of a total of 1182 bp, only 10 sites were polymorphic (9 parsimony informative sites). These sites defined seven distinct haplotypes found in the 5 Santa Fé sampling sites. Genetic variability in terms of haplotypic and nucleotide diversity is similar among the 5 Santa Fé sites, but slightly higher in S4 for H_d and S3 for π (Table 2). S5 presents a lower H_d and π compared with the 4 other sites (Table 2).

A total of 37 polymorphic sites (34 parsimony informative sites) and 28 distinct haplotypes were found for the data set including Santa Fé and the 4 surrounding islands. Haplotypic diversity within Santa Fé is similar to the other islands, except for Santa Cruz (SCZ) and San Cristobal (SRP), which present the lowest haplotypic diversity (0.0 and 0.017), whereas it ranged from 0.616 in San Cristobal (SRL) to 0.786 in Floreana (FMO). Nucleotide diversity varied from 0.00000 in San Cristobal (SRL) to 0.00422 in Floreana (FMO).

Microsatellite Loci

Heterozygote deficiencies appeared in 6 loci but were considered as rare as they were restricted to single populations (Supplementary Table 1). Within the sites on Santa Fé, all 13 microsatellites loci were highly polymorphic, with up to 19 alleles at the most polyallelic locus in one population. Mean number of alleles (A), mean AR, and mean heterozygosity were very similar across the 5 Santa Fé sites, ranging from 8.38 to 9.31 for A, 6.27–8.41 for AR, and 0.7412–0.8019 and 0.7686–0.7773 for H_o and H_e, respectively (Table 2). All sampling sites presented a mean observed heterozygosity lower than expected, except for S5 (0.8019/0.7690; Table 2). As no locus showed significant heterozygote deficiencies for more than one population, we included all 13 loci in all analyses. Mean number of alleles (A), mean AR and mean heterozygosity (across all 13 loci) were quite different between the 5 neighboring islands, ranging from 3.85 to 10.85 (A), from 3.74 to 8.01 (AR), and from 0.5073 to 0.8020 and from 0.6080 to 0.8242 (H_o and H_e, respectively).

Genetic Variation Between Individuals: Relatedness Estimates

Table 3 shows the average relatedness R values (and Jacknife standard errors) calculated among all individuals in each of the 5 Santa Fé sites. The average R values range from −0.0015 (±0.0114) to 0.0265 (±0.0136). The average R value for the whole population (across the 5 sites) is 0.0079 (±0.0039). Average relatedness values do not vary significantly among sites on Santa Fé (Figure 2). Figure 3 compares the observed distributions of relatedness for the 5 sites to the expected distributions for 1000 simulated pairs of full siblings ($R_m = R_p = 0.5$), half siblings ($R_m = 0.5$, $R_p = 0$),

Table 3 Comparison of average relatedness values calculated within the 5 sampling sites (S1–S5), and kinship simulation results for full siblings, half siblings, parent–offspring and unrelated individuals

| | S1 | S2 | S3 | S4 | S5 | Simulations | | | |
						Full	Half	Parent–offspring	Unrelated
Sampling size	75	107	45	39	52	1000	1000	1000	1000
Minimum R	−0.3742	−0.4734	−0.3602	−0.3238	−0.3918	−0.0198	−0.1383	0.2294	−0.3451
Maximum R	0.4559	1.0	0.4171	1.0	1.0	0.8868	0.6325	0.7397	0.4369
Median R	0.0008	−0.0085	0.0121	0.0010	0.0208	0.5044	0.2454	0.4872	0.0017
Average R	0.0042	−0.0015	0.0135	0.0111	0.0265	0.49719	0.24384	0.48928	0.00165
Standard deviation	0.1353	0.1440	0.1414	0.1425	0.1474	0.1465	0.1329	0.0844	0.1331
Jackknife standard error	0.0152[a]	0.0114[a]	0.0139[a]	0.0128[a]	0.0136[a]	—	—	—	—
95% confidence interval	0.0034[b]	0.0022[b]	0.0041[b]	0.0040[b]	0.0037[b]	0.0091	0.0082	0.0052	0.0082

[a] Calculated in Relatedness 5.0.8 (Goodnight and Queller 1995).

[b] Calculated based on the Jackknife standard error. Kinship simulations were calculated using Kinship 1.2 (Goodnight and Queller 1999).

and unrelated individuals ($R_m = 0.0$, $R_p = 0.0$). For the simulated pairs, average R (±SD) were 0.4972 (±0.1465), 0.2438 (±0.1329), and 0.0017 (±0.1331), for full siblings, half siblings, and unrelated, respectively. Pairwise R distributions for each of the 5 sites overlap tightly with the expected distribution obtained using 1000 simulated unrelated pairs.

Phylogeographical Patterns

The median-joining network (Figure 1D) reveals that haplotype frequencies vary between the northern and southern

Average relatedness within sites

Figure 2. Comparisons of average relatedness within the 5 sampling sites. Average values are reported ± jackknife standard error calculated in Relatedness 5.0.8 (Goodnight and Queller 1995). Whole population relatedness (R ± SE) = 0.0079 ± 0.0039. Numbers 1–5 refer to the 5 sampling sites.

sites. Two haplotypes (H3 and H5) are more common than others (37% and 26%, respectively), whereas 3 haplotypes (H1, H2, and H4) are less common (8%, 17%, and 11%, respectively), and 2 haplotypes (H6 and H7) are relatively low in frequencies (0.6% and 0.3%). Most haplotypes were found in multiple locations on Santa Fé, except haplotype H7, found only in 1 individual (site S2). Haplotypes H3 and H5 are the most represented in S1 and S2, whereas H1, H2, and H3 are similarly abundant in the southern sites (S3 and S4). H4 is the most common haplotype (58%) in the northern site (S5). Three of the 7 haplotypes found on Santa Fé also occur on surrounding islands: H2 is also found in the population from Floreana, H3 in Espanola and Floreana, and H5 in Santa Cruz and Floreana. We also extended the comparison to populations in the whole archipelago. The 2 latter haplotypes (H3 and H5) are also shared with more distant islands: H3 is also found in the population from Pinzon and H5 in Santiago and Isabela (in lower proportion) (Steinfartz et al. 2009), whereas H1, H4, H6, and H7were only found on Santa Fé.

F Statistics

F_{st} values for all pairwise combinations between islands were statistically significant and varied from 0.0324 (Espanola/Floreana) to 0.1984 (Espanola/San Cristobal SRP) for the microsatellite loci (Table 4). The relative levels of genetic differentiation revealed by the mtDNA data is consistent with the nuclear markers results, but with a mitochondrial/microsatellite F_{st} ratio higher than 10, an expected result given the different rate and inheritance mode of the 2 markers (Table 4). Although some comparisons that are significant according to mtDNA are not significant according to microsatellite analyses, those microsatellite comparisons that are significant correspond to the largest mtDNA F_{st} values. For Santa Fé, the only F_{st} comparisons that are significant according to both microsatellite and mtDNA data are those between the northern and southern sites (0.0027–0.0115 for microsatellite and 0.0447–0.2391 for mtDNA; Table 4). However, according to the mtDNA data alone, all Santa Fé comparisons are
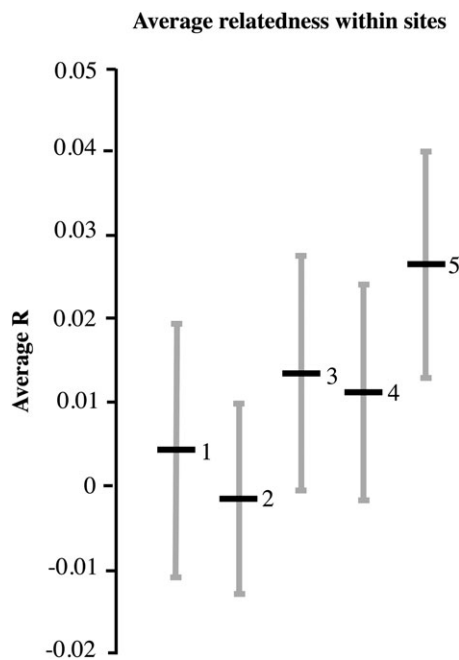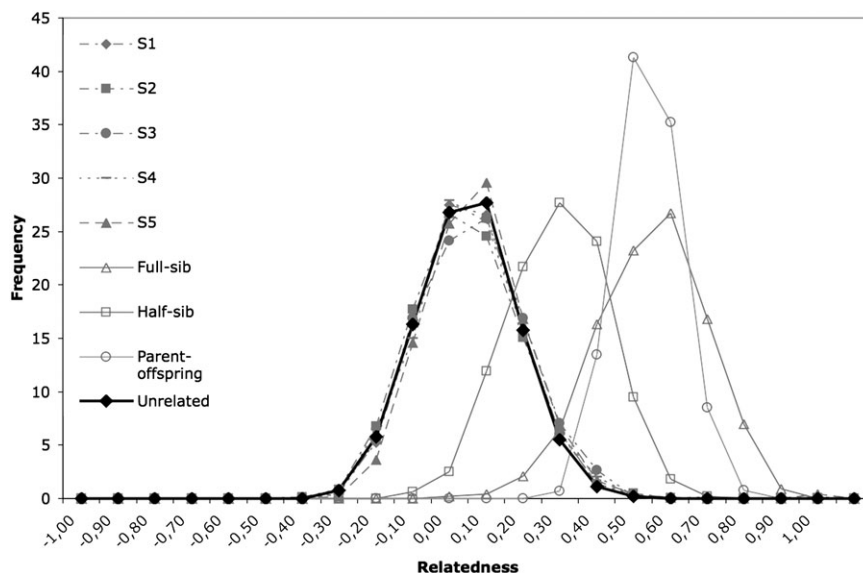
Figure 3. Observed distributions of relatedness pairwises within each of the 5 sampling sites (S1–S5) plotted against expected distributions for 1000 simulated pairs for each of the following relationship categories: unrelated, half siblings, full siblings and parent–offspring. *R* distributions are centered on their theoretical values: 0 for unrelated pairs, 0.25 for half siblings, and 0.5 for full siblings.

significant except for the one between the 2 Bahia Paraiso sites (S1 + S2) and the 2 Miedo sites (S3 + S4; Table 4).

AMOVA Analyses

The AMOVA analyses revealed low levels of nuclear and mitochondrial genotypic structuring across Santa Fé (Table 5). We tested for 2 grouping schemes. The first one included 3 groups: Bahia Paraiso (S1 and S2), Miedo (S3 and S4), and the North site (S5). The second scheme consisted of 2 groups: one group with all of the southern sites (S1–S4) and a second group with the northern site (S5). Most of the genotypic variation was distributed among individuals within sampling sites in both sampling schemes and for both genetic markers. Considering both microsatellites and mitochondrial data, the AMOVA analysis suggests that 99% and 86–89% of the variation, respectively, is best explained by variation

among individuals within sites (Table 5). No statistical support was obtained for any of the groups ($P > 0.05$).

Bayesian Clustering

Figure 1F shows the results of the Bayesian cluster analysis when all 10 sites are included. The analysis identifies 5 distinct clusters (Supplementary Figure 1A and Supplementary Table 2). One cluster includes the 2 southern islands of Espanola (EPC) and Floreana (FMO), another comprises 2 sites on separate islands (San Cristobal, SRP, and Santa Cruz, SCZ), a third one is made up of only the site from the southern coast of San Cristobal (SRL). The 5 sites from Santa Fé are included in a single cluster, although individuals in this cluster have mixed contributions from 2 distinct pools, suggesting some level of genetic differentiation between sites. However, even though the highest mean ln

Table 4 Estimates of population pairwise genetic distance (F_{st}) based on the analysis of 13 microsatellite loci (below diagonal) and the CR sequences (above diagonal)

	S1	S2	S3	S4	S5	SCZ	FMO	EPC	SRP	SRL
S1	*	*−0.0110*	0.1012	0.0474	0.1394	0.7688	0.3090	0.3245	0.8316	0.8562
S2	*0.0017*	*	0.0999	0.0447	0.1353	0.7266	0.3255	0.3260	0.8169	0.8570
S3	0.0050	*0.0020*	*	*−0.0150*	0.2391	0.7035	0.2235	0.3373	0.7539	0.8243
S4	0.0034	*0.0015*	*−0.0018*	*	0.2063	0.7470	0.2271	0.3185	0.7783	0.8291
S5	0.0115	0.0094	0.0027	0.0059	*	0.9102	0.3935	0.5202	0.9265	0.8829
SCZ	0.0800	0.0760	0.0809	0.0777	0.0842	*	0.5871	0.7069	0.9918	0.9319
FMO	0.0605	0.0620	0.0642	0.0639	0.0665	0.0626	*	0.3553	0.6413	0.7436
EPC	0.0771	0.0749	0.0720	0.0679	0.0726	0.0948	0.0324	*	0.7964	0.8465
SRP	0.1675	0.1612	0.1658	0.1721	0.1739	0.1211	0.1683	0.1984	*	0.8755
SRL	0.1070	0.0992	0.1116	0.1039	0.1092	0.0912	0.1207	0.1256	0.1705	*

F_{st} were calculated using Arlequin 2.0 (Schneider et al. 2000). All values were significant (significance level = 0.05) except bold italicized.

Table 5 Hierarchical analysis of molecular variance based on microsatellite data and mitochondrial DNA of marine iguanas.

| | Microsatellites | | | | | | Mitochondrial DNA | | | | | |
Source of variation	df	Sum of squares	Variance components	% variation	Fixation indices (F value)	P value	df	Sum of squares	Variance components	% variation	F value	P value
Santa Fé only												
Design I												
Among groups	2	19.711	0.02512 V_a	0.51	F_{CT} = 0.00505	0.07527	2	27.722	0.14861 V_a	11.74	F_{CT} = 0.11739	0.06158
Among populations within groups	2	10.592	0.00270 V_b	0.05	F_{SC} = 0.00055	0.04985	2	0.781	-0.01166 V_b	-0.92	F_{SC} = -0.01043	0.00489
Among individuals within populations	631	3119.991	4.94452 V_c	99.44	F_{ST} = 0.00560	<0.001	307	346.597	1.12898 V_c	89.18	F_{ST} = 0.10819	<0.001
Design II												
Among groups	1	11.513	0.03097 V_a	0.62	F_{CT} = 0.00621	0.19257	1	14.742	0.12065 V_a	9.24	F_{CT} = 0.09238	0.20137
Among populations within groups	3	18.790	0.01050 V_b	0.21	F_{SC} = 0.00212	<0.001	3	13.761	0.05637 V_b	4.32	F_{SC} = 0.04755	<0.001
Among individuals within populations	631	3119.991	4.94452 V_c	99.17	F_{ST} = 0.00832	<0.001	307	346.597	1.12898 V_c	86.45	F_{ST} = 0.13554	<0.001

Santa Fé only, genetic differentiation was assessed between sampling sites and among geographic areas (groups). In design I, 3 groups were considered: one group including Miedo and the last one including the North site. In design II, only 2 groups were considered: north and south sites. P value (rand. value > obs. value) represents significant tests after 1000 permutations. V_a, V_b and V_c are the associate covariance components. FSC, FST and FCT are the F statistics.

$Pr(X/K)$ estimate is for $K = 5$ (Supplementary Figure 1) and only $K = 5$ and 6 estimates of $\ln Pr(X/K)$ reasonably converged across runs, these results need to be interpreted with caution (Supplementary Table 2). A Structure analysis (Pritchard et al. 2000) using only the 5 sampling sites on Santa Fé suggests $K = 1$ as the most likely grouping (Supplementary Figure 1B), confirming the previous Structure result.

Isolation by Distance

Genetic distances (F_{st} values) based on mtDNA and microsatellites data were plotted against the coastline distances between all Santa Fé sites. The graphs illustrate an overall potential relationship between geographical and genetic distances (regression: $R^2 = 0.7444$ and 0.4458, based on mitochondrial and microsatellites data analyses, respectively). The results of the Mantel tests revealed a significant correlation only between geographical and mtDNA genetic distances ($g = 1.9788$, $z = 13803.2568$, $r = 0.8628$, critical value $= 1.645$ with $P = 0.05$, for mtDNA; $g = 1.5205$, $z = 545.499$, $r = 0.6677$, critical value $= 1.645$, for microsatellites; significance level of $P < 0.05$). However, the absence of intermediate populations between northern and southern locations does not allow confirming that issue of isolation by distance and pattern of diversification.

Sex-Biased Dispersal

If sex-biased dispersal is occurring, the dispersing sex should present higher scores for F_{is}, Hs, vAlc, but lower scores for F_{st}, R, H_o, and mean Alc than the philopatric sex. Table 6 reports the results of the sex-biased analyses for the 4 sites on Santa Fé for which gender data were available (S1, S2, S4, and S5). We performed the sex-biased dispersal tests for 2 sets of individuals sampled in 1991–1993 (south, S4, vs. north, S5; Table 6A) and 2004 (south, S1, S2; Table 6B). For the comparison between the southern and northern sites (Table 6A), males showed lower F_{st}, relatedness and observed heterozygosity (H_o), and higher F_{is} and vAlc values, which may suggest a male-biased dispersal across the island. For the comparison between the 2 close southern sites (Table 6B), females showed higher F_{is}, higher Hs, higher vAlc, but lower F_{st}, lower R, and lower mean Alc values, which may suggest a female dispersal between breeding sites. However, these tests, as well as all the other tests reported in this table, are not statistically significant (Table 6).

Isolation with Migration

We estimated the high point and highest and lowest posterior densities for 2 migration rates in each of the 3 data sets (Supplementary Tables 3A–C). In each case, m1 refers to migration rates from population S1–S4 to S5 and m2 refers to the reversed case (S5 to S1–S4). We obtained convergence in the probability density curves for all (Supplementary Figures 2A–C) but one migration

Table 6 Test for sex-biased dispersal in marine iguanas

		n	F_{is}	F_{st}	R	H_o	H_s	Assignment indices Mean AIc	Variance vAIc
A: 1991–1993	Females	43	−0.0189	0.0067	0.0136	0.7849	0.7703	−0.09935	10.10823
(south, S4, vs. north, S5)	Males	48	−0.0129	0.0053	0.0106	0.7687	0.7589	0.08900	10.29360
	P value[a]		0.3784	0.4089	0.4061	0.7861	0.8604	0.6163	0.4680
B: 2004 (south, S1 and S2)	Females	0.0173	−0.0002	−0.0004	0.7637	0.7771	0.0173	−0.35275	16.57940
	Males	0.0165	0.0061	0.0120	0.7600	0.7727	0.0165	0.15678	12.96773
	P value[a]	0.4647	0.9115	0.9098	0.6262	0.6812	0.4647	0.7926	0.8759

F_{is}, F_{st}, relatedness (R), the observed heterozygosity (H_o), the within group gene diversity (H_s), and assignment indices (mean and variance) were calculated using FSTAT 293 (Goudet 2001). Probabilities were calculated using 10 000 permutations.

[a] One-sided test.

parameter, m1 for the microsatellite-only data set (Supplementary Figure 2B). Our results for the mtDNA and microsatellite dataset show asymmetrical migration but in reversed directions (north to south for mtDNA and south to north for microsatellites). The biological meaning of these results is unclear, given that in both cases their posterior distributions show some degree of overlap (Supplementary Figures 2 A,B; Supplementary Tables 3A,B). When analyzing the combined data set, we recover a stronger signature of asymmetrical migration (estimates with nonoverlapping posterior distributions), suggesting gene flow from south to north (Supplementary Figure 2C).

Discussion

Genetic Diversity of Santa Fé Marine Iguanas

Levels of genetic diversity were similar for both microsatellite and mtDNA markers across the 5 marine iguana populations sampled on Santa Fé. The apportionment of genetic variation within and between individuals is not suggestive of either inbreeding or substructuring within sites (Tables 4 and 5; Figure 3). Most of the intra-island genetic variation at both microsatellite and mtDNA markers was allocated within rather than between sites (Table 5). Generally, levels of variation within sites on Santa Fé (i.e., haplotypic and nucleotide diversity, mean number of alleles, mean AR, and mean heterozygosity) are comparable with those observed in similar-sized sampling sites on other islands, with the notable exception of 2 sites in the east and west of San Cristobal (see Steinfartz et al. 2009).

Bayesian clustering methods using microsatellite data show that marine iguanas on Santa Fé are genetically distinct from populations on neighboring islands (see also Steinfartz et al. 2009) and the analysis assigned all Santa Fé individuals to a single cluster (Figure 1F). However, when frequency-based estimates of genetic differentiation (F_{st}) were considered (Table 4), microsatellite data revealed small but statistically significant F_{st} values between northern (S5) and southern sites (S1-S4), but not among the southern sites (except between S1 and S3, S1 and S4; Table 4). A similar picture of low genetic differentiation is obtained when looking at the results of the genealogical analyses based on

mtDNA CR sequences. The median-joining graph (Figure 1D) indicates that individuals from the north and the south do not cluster into 2 exclusive haplogroups. These data coupled with their low levels of divergence underscore their recent evolutionary origin and confirms the shallow genetic structuring revealed by the microsatellite data.

As for the microsatellite loci, mtDNA frequency data also show statistically significant genetic differences between northern and southern sites that were not revealed by genealogical analysis. Of the 7 haplotypes found on Santa Fé, the 3 most common (H2, H3, and H5) also occur on neighboring islands. Two of them (H3 and H5) are the most represented in the southern sites. H4 is the predominant haplotype in the northern site (58%), but is found in low frequency in the southern sites (1.5%) and is not found in any other populations in the archipelago. In contrast with microsatellite data, mtDNA reveals further structuring within southern sites, indicating that the Miedo (S3 + S4) and Bahia Paraiso (S1 + S2) sampling areas, which are located only 0.8–1.6 km apart, are genetically distinct from each other (Table 4).

The results of the isolation-migration analyses on the 3 data sets add another layer of information to understand the population structure pattern of the Santa Fé marine iguana populations. Migration rates based on the mitochondrial CR are very low, suggesting that isolated long-range female dispersal events in any direction have been a rare phenomenon over evolutionary time. The higher estimates of migration rates based on the microsatellite data set suggests higher levels of gene flow in the south to north direction for males rather than females but the results are not statistically robust. Migration rates estimated from the combined data set support and strengthen the results of the microsatellite data set: asymmetrical gene flow with higher migration rates from southern to northern sites. Given that the 2 marker types are best suited for studying different evolutionary time scales, with the mtDNA locus tracking older evolutionary events than microsatellites loci, we suggest that the apparent conflict between the 2 data sets is best explained by differences between historical and present demographic estimates rather than contrasting patterns of current gene flow.

Although we cannot argue for female-biased gene flow either in the past or present, our results provide evidence of

ongoing male-biased gene flow, which in addition, follows the prevailing southeast to northwest ocean currents typical of the Galápagos Islands (Caccone et al. 2002).

The significant, albeit low differences in gene frequencies between northern and southern sites, as well as between the 2 sets of southern sites in the case of mtDNA, suggests that gene flow can be limited even along a relatively small stretch of coastline on the same island. These findings parallel the results of a geographically broader study, showing that some marine iguana populations on the same island (i.e., San Cristobal) can be as genetically distinct from each other as from populations on other islands (Steinfartz et al. 2009). Notably, the geographic distance over which we observe genetic structuring on Santa Fé is generally smaller than the geographic distances separating distinct genetic units on other islands (e.g., San Cristobal).

Evolutionary Role of Mating and Foraging Behaviors

Previous studies of marine iguanas on Santa Fé and other islands in the Galápagos (Wikelski and Hau 1995; Wikelski et al. 1996) have shown that this species relies on rocky shores for breeding, and that breeding sites are relatively spaced from one another (e.g., 0.2 km between S1 and S2 on Santa Fé; Figure 1E). For Santa Fé, field observations from the same sites used in this study (S1–S4) suggest the existence of site fidelity among adults (Laurie 1990) and of lek-mating behavior with tendency of males to come back to their breeding sites (Dellinger 1991; Wikelski et al. 2001; Partecke et al. 2002; Vitousek, Mitchell, et al. 2007; Vitousek et al. 2008). However, field observations also reveal that site fidelity is far from complete, providing opportunities for long-range dispersal and genetic exchange among populations within and between islands. Females, juveniles, and small males are known to forage on algae in the intertidal zone at low tide, whereas large males and females (but in a much lower proportion) are able to swim farther from shore and feed in the subtidal zone independently of tides and weather conditions. This may potentially increase dispersal rates of these individuals through either active swimming or by being passively carried by currents (Trillmich 1983; Trillmich KGK and Trillmich F 1986; Buttemer and Dawson 1993; Wikelski and Trillmich 1994; Drent et al. 1999;).

Although lek-mating behavior should result in low dispersal rates for both sexes, sex-biased dispersal should lead to different levels and patterns of genetic structuring for males and females. A previous genetic study on marine iguana populations across the archipelago found evidence for males having higher dispersal rates than females (Rassmann et al. 1997). However, a recent, more comprehensive study on the same geographic scale (i.e., across the archipelago) indicated that this is not the case (Steinfartz et al. 2009): marine iguanas are highly differentiated between islands and most islands present single evolutionary genetic clusters.

Our genetic data allow us to evaluate the evolutionary impact of site fidelity and occasional long-range dispersal within a single island. Site fidelity should lead to similar genetic signatures in both sexes with genetic differentiation between sites, high relatedness values within sites, and relatively high levels of inbreeding. On the other hand, if sex-biased dispersal is frequently occurring, genetic signatures for the 2 sexes should be distinct (with positive F_{is} values and lower R relatedness and F_{st} for the dispersing sex) (Goudet et al. 2002). Our genetic data do not indicate elevated inbreeding levels or higher relatedness values than those expected in a group of unrelated individuals in any of the Santa Fé sites, and reveal low but significant genetic differentiation only between the most distant sites on a relatively large scale (northern vs. southern sites on Santa Fé Island). This suggests that, at least for the type of markers that we screened, lek-mating behavior is not strong enough to play a role in shaping the patterns of genetic diversity of the Santa Fé population and that gene flow is high enough to dilute kin relationships within and between potentially breeding sites. However, our data also indicate that the gene flow is not sex biased because the genetic data do not detect any statistically significant difference in dispersal rates between sexes (Table 6).

Conclusions

In conclusion, despite the low genetic differentiation observed between the 5 sampling sites of Santa Fé, our data allow insights into the fine-scale population structure of marine iguanas. Based on the actual genetic and behavioral data, Santa Fé appears as a separate genetic unit from the surrounding islands, suggesting restricted, although occasional, long-distance dispersal between islands. Within Santa Fé, the northern and southern regions, which are separated by almost 10 km of coastline, are significantly different based on frequency data for both nuclear microsatellite and mitochondrial data, but are not statistically different using genealogical mtDNA data or Bayesian clustering on microsatellites. Within the 4 southern sites, no genetic differentiation is statistically significant except for the mtDNA F_{st} between the Miedo versus Bahia Paraiso sites, 2 beaches separated by only 1.5 km.

The lack of genetic structure at the smallest scale and the presence of low levels of north/south genetic division suggest that long- and short-distance dispersal behavior is playing a long-term role in homogenizing genetic variation between sampling sites reducing the evolutionary impact of lekking and site fidelity behaviors in shaping patterns and levels of genetic diversity within sites.

Supplementary Material

Supplementary material can be found at http://www.jhered .oxfordjournals.org/.

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