

# Historical fragmentation of islands and genetic drift in populations of Galápagos lava lizards (*Microlophus albemarlensis* complex)

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## Abstract

The formation of islands following a rise in sea level at the end of Pleistocene is expected to disrupt the equilibrium between genetic drift and gene flow in species with limited ability to disperse. Here, we test the hypothesis that genetic drift in isolation has caused the differentiation of Galápagos lava lizards (*Microlophus albemarlensis* complex) found on 12 islets that are likely to have been connected to a larger island, Isla Santa Cruz, during the late Pleistocene. Using 11 microsatellite loci, screened on 524 individuals from 17 localities distributed among and within 15 islands, we found marked differences in allelic richness and heterozygosity. Genetic differentiation was strong (global  $F_{ST} = 0.44$ ), with pairwise differences found among populations on islets being larger than differences among three localities sampled within Isla Santa Cruz. As expected under a scenario of drift in isolation, there was a positive correlation of genetic diversity with island size, no relationship between genetic and geographical distance and a strong negative correlation between heterozygosity and measures of genetic differentiation. We conclude that seawater is a significant barrier to gene flow in lava lizards on this timescale. Our results suggest that the shallow diversification of the *M. albemarlensis* complex is not due to recent gene flow and that genetic drift may have played a substantial role in observed patterns of phenotypic variation among islands.

**Keywords:** Galápagos Islands, genetic differentiation, genetic diversity, microsatellite, Pleistocene, sea level

Received 2 August 2007; revision accepted 25 November 2007

## Introduction

Island systems provide critical insight to the processes underlying evolutionary diversification of species (Grant 1998). Archipelagos originating from volcanic activity have been and continue to be at the forefront of such efforts due to their examples of adaptive diversification, well characterized geological history and relatively simple ecologies (Grant 1986; Baldwin & Robichaux 1995; Losos *et al.* 1998; Roderick & Gillespie 1998; Juan *et al.* 2000). In this context, a central focus of molecular genetic analyses has been the relationship between phylogeny and island formation (reviewed in Emerson 2002). As expected, older

groups and greater genetic structure tend to be found in the older regions of volcanic archipelagos. While such phylogenetic investigation is important for revealing the broad pattern of colonization and diversity within and among closely related species, studies that focus on the population level are needed to test hypotheses concerning mechanisms underlying diversification in its initial stages (e.g. Beheregaray *et al.* 2004; Petren *et al.* 2005; Ciofi *et al.* 2006).

A shorter-term process involving island origin that has received less attention is fragmentation due to the rise in sea levels that occurred as a result of glacial melting at the end of the Pleistocene. The study of such systems is of interest because they allow analysis of the relative roles of genetic drift and gene flow after populations are established on nascent islands. The patterns of neutral genetic variation revealed at this level can then provide insight to the

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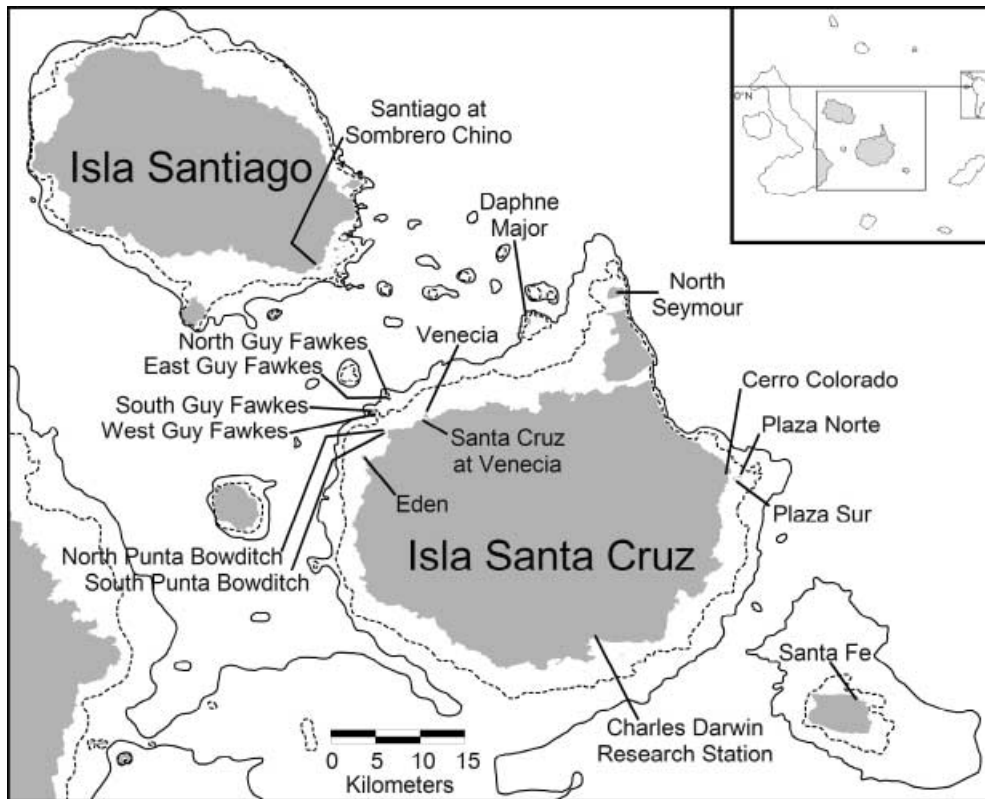


Fig. 1 Map of sample localities of Galápagos lava lizards (*Microlophus albemarlensis* complex) within the Galápagos Archipelago. Sea depth contours surrounding current islands (shaded) are shown at depths of 130 m (solid line) and 60 m (dashed line) (H. Snell, unpublished data).

processes leading to phenotypic diversification and possible speciation (e.g. Jordan *et al.* 2005; Petren *et al.* 2005). Due to their high densities (Rodda *et al.* 2001) and intermediate dispersal ability over seawater (relative to birds and amphibians), lizards are an attractive group in which to focus such study. In many cases, patterns of genetic diversity among lizard populations on islands suggest that dispersal is limited and that genetic drift has driven divergence of neutral markers on small islands (Ciofi *et al.* 1999; Malone *et al.* 2003; Nichols & Freeman 2004; MacAvoy *et al.* 2007). The generality of this pattern however, is subject to further investigation. Both circumstantial and direct evidence suggests that lizards are capable of colonizing islands either on their own (Schoener & Schoener 1984; Schoener *et al.* 2001) or on rafts of vegetation (Censky *et al.* 1998), and this potential for resulting gene flow has been suggested to have a strong role in the evolution of some groups (Calsbeek & Smith 2003). Furthermore, the systems where genetic drift has been identified are complicated by significant anthropogenic effects that have occurred subsequent to island fragmentation.

The lava lizards (*Microlophus* spp.) of the Galápagos Archipelago represent a novel system for analysis of the genetic consequences of the historical fragmentation of

islands. Although there is evidence of human impact on lava lizards (Stone *et al.* 1994), no population is known to have experienced decline or have become extinct since humans arrived in 1535. Nine putative species have radiated from two colonization events (Wright 1983; Lopez *et al.* 1992; Kizirian *et al.* 2004; Benavides *et al.* 2007) but individual islands have a single representative. Like several other groups (Snell *et al.* 1984; Rassmann *et al.* 1997; Caccone *et al.* 2002; Parent & Crespi 2006), the phylogeny of lava lizards broadly mirrors the progression of island formation resulting from the southeasterly shift of the Nazca plate over the Galápagos hotspot. Populations found on the islands of the western part of the archipelago however, are not well resolved and are conservatively treated as a species complex (*Microlophus albemarlensis* complex, Kizirian *et al.* 2004; but see Benavides *et al.* 2007). In addition to the possibility of overwater dispersal, gene flow during periods of lower sea level may constrain genetic divergence among populations within this closely related group. Islands were larger, forming land bridges with current satellite islands (hereafter referred to as islets), and distances among islands were shorter in the Pleistocene (Fig. 1 and maps in: Simpson 1974; Geist 1996; Grant & Grant 1998), when sea level was below its current level

**Table 1** Microsatellite loci amplified in multiplex PCR reactions and the combinations used in subsequent multiloading of products for genotyping. Reactions were conducted in a total volume of 11  $\mu$ L using the conditions described below along with: 1  $\times$  PCR buffer [10 mM Tris-HCl (pH 9.0 at 25 °C), 50 mM KCl and 0.1% Triton®X-100], 100  $\mu$ M each dntp, 1  $\mu$ g/ $\mu$ L bovine serum albumin, 0.5 units *Taq* DNA polymerase (Promega) and ~2.5 ng of template. Loci and thermocycling parameters are described in Jordan *et al.* (2002)

Multiload	Multiplex	MgCl <sub>2</sub> concentration (mM)	Locus	Label (5' forward primer)	Primer concentration ( $\mu$ M)	Observed number of alleles
Mix 1	A	2.5	Mic8	6FAM	0.12	8
	A		Mic5	HEX	0.24	16
	B	4.0	Mic6	TET	0.24	9
Mix 2	B	2.5	Mic2	6FAM	0.36	78
	C		Mic3	TET	0.09	5
	C	Mic10	HEX	0.30	10	
Mix 3	—	2.5	Mic4	6FAM	0.24	56
	D	1.5	Mic1	6FAM	0.24	8
	D		Mic12	HEX	0.24	6
	E	2.5	Mic7	TET	0.24	15
	E		Mic9	FAM	0.24	14

(Fairbanks 1989; Lambeck & Chappell 2001). In apparent contrast to the existing genetic data, there is a large degree of phenotypic diversity within and among populations of the *M. albemarlensis* complex (Carpenter 1966; Schluter 1984; Snell *et al.* 1988; Stone *et al.* 1994, 2002; Miles *et al.* 2001).

Here, we test the hypothesis that genetic drift in the absence of gene flow has caused the differentiation of populations within the *M. albemarlensis* complex that occur on islets created following a rise in sea level. We use analyses of allelic variation from 11 microsatellite loci to describe genetic variation and test for genetic structure within and among 17 putative populations. We then rely on several correlational analyses to assess the relative roles of genetic drift and gene flow in shaping genetic variation among sample localities. If drift is not counteracted by gene flow we will expect a positive correlation between island size, a proxy for population size, and genetic diversity (e.g. Petren *et al.* 2005). Also, drift in isolation should lead to strong pairwise differentiation among populations ( $F_{ST}$ ) that is negatively correlated with differences in heterozygosity (Hedrick 1999). Conversely, we expected little differentiation and a pattern of isolation by distance if dispersal is frequent and results in gene flow.

## Materials and methods

### Geography and sampling

Lava lizards were sampled near the centre of the archipelago on and around Isla Santa Cruz (Fig. 1). Latitude and longitude of each sample locality was taken in the field with global positioning system receivers and used in the measurement of geographical distance. Island nomenclature, area, and isolation distances are

available in Snell *et al.* (1996). Reconstruction of the Pleistocene distribution of islands within Galápagos is based on sea level change derived from the dating of coral cores in the Caribbean (Fairbanks 1989; Bard *et al.* 1990) and the bathymetry of the archipelago (Geist 1996; Grant & Grant 1998; Snell, unpublished data). In addition to the 130-m contour corresponding to the last glacial maximum (17 000 YBP), we show a 60-m contour which suggests the configuration of islands 12 000 YBP. All islands in the sample, other than Islas Santa Fe and Santiago, are likely to have been part of Isla Santa Cruz as the Pleistocene ended.

As part of a large mark–recapture study, toe-clips were collected and stored in silica gel. Except in one case where the sample was limited to 14 lizards (Islote South Guy Fawkes), 32 individuals per locality collected between 1991 and 1995 were used in the analysis. Although the sample was taken across years, most individuals from a particular locality were collected on a single day and the year of collection was haphazardly distributed across islands of varying size and ecology. For these reasons and the fact that the period of sampling approximated one generation for these lizards (Snell, unpublished data), we assume that the temporal nature of the sample did not affect the spatial patterns investigated here.

### Screening of microsatellite variation

A single toe (ca. 5 mg) from each individual was dissected with a scalpel prior to digestion in proteinase K for 48 h at 55 °C. We extracted genomic DNA using columns lined with a silica membrane (Promega Wizard® SV 96 system). Eleven microsatellite loci (Table 1) were amplified using fluorescently labelled primers in a polymerase chain reaction (PCR) prior to their visualization on an

automated DNA sequencer (cf. Jordan *et al.* 2002). Multiplex reactions, similar to the original protocols developed for single loci, were designed to streamline the screening process (Table 1). Additionally, to facilitate the determination of genotypes, we forced the adenylation of PCR products by adding a linker sequence (GTTTCTT-) to the 5' end of each reverse primer (PIG tailing, Brownstein *et al.* 1996). PCR reactions were run on an Eppendorf Mastercycler and resulting fragments were characterized using an ABI 31000 Genetic Analyser (Applied Biosystems) and GENEMAPPER 3.7 (Applied Biosystems). All loci within each population were screened for allelic dropout and genotyping error due to stuttering in MICRO-CHECKER (van Oosterhout *et al.* 2004) using Bonferroni (Dunn-Sidak) adjusted confidence intervals derived from Monte Carlo simulation.

### Statistical analyses

Tests for deviation from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium among all loci across localities were carried out using GENEPOP on the Web (Raymond & Rousset 1995). Exact tests using a Markov Chain method to estimate  $P$ -values were applied (Guo & Thompson 1992) with 200 batches of 1000 iterations. To determine the statistical significance of the individual tests ( $\alpha = 0.05$ ), we used  $q$ -values, derived from the table of  $P$ -values, estimated in the software QVALUE (Storey & Tibshirani 2003). As in the sequential Bonferroni procedure (Rice 1989), the use of  $q$ -values is aimed at minimizing the probability that the null hypothesis is falsely rejected, but it does so by directly considering the number of tests that appear to be statistically significant but are in fact not ('false discovery rate', Storey & Tibshirani 2003; Verhoeven *et al.* 2005). We calculated allelic diversity ( $A$ ), observed ( $H_O$ ) and unbiased expected heterozygosity ( $H_E$ , Nei 1978) for each sample site using MICROSATELLITE ANALYSER (MSA version 4.05, Dieringer & Schlötterer 2003). Differences among samples in the means of each of the measures of genetic diversity were tested with ANOVA using SPSS version 15.0.1 (SPSS 2006).

We principally relied on  $F_{ST}$ , as estimated by  $\theta$  (Weir & Cockerham 1984), to assess the level of genetic differentiation among localities. Although measures based on allele size ( $R_{ST}$  & Slatkin 1995) rather than allele frequency may be appropriate under scenarios of drift (Balloux & Goudet 2002), three of the 11 loci we examined (Mic2, Mic4 and Mic8) had alleles of unexpected size under a strict stepwise mutation model. Such differences further increase the already substantial variance in  $R_{ST}$  that tends to be found in analyses using the number of loci, individuals per locality, and timescale of divergence we consider in this study (see reviews in Balloux & Moulin-Lugon 2002; Estoup *et al.* 2002).

Differences in heterozygosity among populations, however, can be a considerable source of bias in the evaluation of  $F_{ST}$ . The analyses of genetic diversity described above suggested that such bias might be manifested in our estimates in two ways. First, there was some evidence that null alleles may occur in the dataset. Null alleles can inflate estimates of genetic differentiation by reducing heterozygosity within populations (Chapuis & Estoup 2007). We therefore investigated population structure with and without consideration of the presence of null alleles using FREENA (Chapuis & Estoup 2007). FREENA estimates a null allele frequency for each locus within each population using the Expectation Maximum algorithm (Dempster *et al.* 1977). Adjusted data are then available for analyses of genetic differentiation. Although it is likely that some missing data was due to technical error, to be conservative in this analysis we assumed all missing data was due to null homozygotes. Global and pairwise values of  $F_{ST}$  were estimated from both the raw data using MSA and data corrected for null alleles by exclusion in FREENA ( $F_{ST(ENA)}$ ). Confidence intervals (95%) for global differentiation were calculated from 1000 bootstrap resamples across loci in both procedures. Second, comparisons between localities with high heterozygosities, often characteristic of microsatellite loci, are constrained to have a maximum  $F_{ST} \ll 1$  (Hedrick 1999). To assess this potential effect in the dataset, we calculated a standardized measure of  $F_{ST}$  (Hedrick 2005). Such standardized measures (here denoted  $F'_{ST}$ ) are made by determining the maximum  $F_{ST}$  for a given level of heterozygosity ( $F_{ST(MAX)}$ ) and then dividing this value into the observed  $F_{ST}$ . We determined  $F_{ST(MAX)}$  in MSA using a dataset adjusted to make alleles unique across localities using RECODEDATA (Meirmans 2006). This approach preserves observed heterozygosity within localities while maximizing it across them.

Population structure was further investigated with principle components analysis (PCA) and Bayesian cluster analysis using the full dataset. Multilocus genotypes were ordinated to visualize patterns of similarity among sample localities using PCA-GEN (Goudet 1999). Bayesian clustering was performed using BAYESIAN ANALYSIS OF POPULATION STRUCTURE (BAPS version 4.14, Corander *et al.* 2003). A mixture analysis was conducted at the individual level to determine the posterior probability of different numbers of clusters ( $K$ ) assuming a maximum of 17 sampling localities. The analysis was run 10 times to account for possible variation in the results due to stochasticity inherent to the procedure's algorithm. Once  $K$  was identified by assigning genotypes, an admixture analysis was performed to assess the proportion of each lizard's genome originating from a particular cluster (population) (Corander & Marttinen 2006). In the admixture analysis, admixture coefficients for individuals were derived from 100 iterations and compared to 200 reference individuals iterated 20 times.

We assessed the relative roles of drift and gene flow in shaping population structure using two correlational methods. First, we assumed a null hypothesis by which drift and gene flow are in equilibrium in a stepping-stone framework. Under such circumstances, there should be a pattern of isolation by distance whereby the mean and variation in genetic differentiation increases with geographical distance (Hutchison & Templeton 1999). In contrast, there should be no relationship with uniformly large variation at all values of geographical distance if drift alone is the primary force shaping genetic variation among populations. Second, we correlated genetic differentiation with the mean of the observed heterozygosity between population pair. Differences in genetic diversity due to fluctuation in population size are expected to magnify measures of genetic differentiation (Chakraborty & Nei 1977; Hedrick 1999; Goodman *et al.* 2001). We therefore expected a negative correlation between these variables under a scenario of genetic drift in isolation. Both types of relationships were tested using Mantel tests with 1000 permutations of matrices in GENALEX 6 (Peakall & Smouse 2006). Because of the likely separate history of Islas Santiago and Santa Fe from Isla Santa Cruz (Fig. 1.; Simpson 1974; Geist 1996; Benavides *et al.* 2007), we excluded the former localities from these analyses.

## Results

### Tests of assumptions

Of 187 combinations of loci and populations, 146 were polymorphic. One locus (Mic7) did not amplify in Islote West Guy Fawkes. Among the polymorphic loci, there was no suggestion of allelic dropout or error due to stutter bands. In 621 tests of linkage equilibrium, 19 had  $P$ -values  $< 0.05$  but none had  $q$ -values at this level. Tests of heterozygote deficiency revealed 37 of 146 tests with  $P < 0.05$ . Among those, four had  $q$ -values  $< 0.05$  (Mic4 CC, Mic7 SF, Mic10 DM and Mic12 DM). Inspection of the table of  $P$ -values suggested that no single locus or population was predominant in these tests. We therefore considered the possibility that null alleles were the source of deviation from HWE rather than Wahlund effect or assortative mating.

### Genetic variation

A total of 236 alleles were observed in the 11 loci across the 17 sampling locations. Loci differed in allelic variation, ranging from five to 78 alleles in Mic3 and Mic2, respectively (Table 1). Sampling locations varied in genetic diversity (Table 2). Between 116 and 121 alleles were observed in the three sites sampled on Isla Santa Cruz. By contrast, the Guy Fawkes islets had 16–24 alleles. This result is due in part

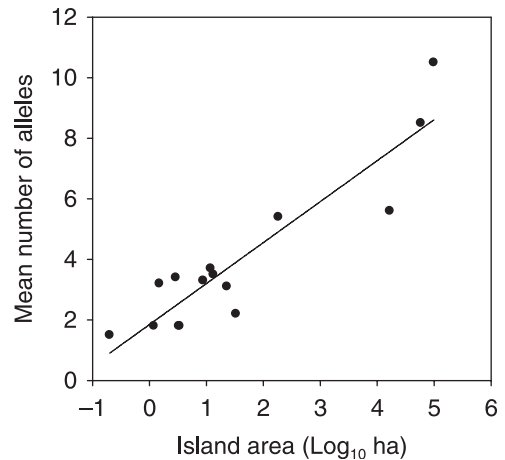


Fig. 2 Relationship between mean number of alleles per locus and island area.

to the large number of monomorphic loci observed in these populations (Table 2). Means of  $H_O$  were found to be different among samples (ANOVA;  $F = 9.152$ ,  $P < 0.0001$ ) with multiple comparisons confirming the pattern of the Guy Fawkes islets, and also Daphne Major, having less variation than the sample sites on Santa Cruz. Similar results were observed for  $A$  and  $H_E$  (not shown). All measures of genetic diversity were correlated with one another ( $r = 0.81$ – $0.97$ ,  $P \leq 0.0001$ ) and with island area (Fig. 2;  $r = 0.69$ – $0.92$ ,  $P \leq 0.004$ ). The former result suggests a prominent role for drift in shaping genetic variation among islands. Private alleles were uncommon on the islets, with the greatest number found on the two largest islands in the sample (Santa Cruz and Santiago, Table 2).

### Population differentiation

Global  $F_{ST}$  was 0.44 (95% CI:  $\pm 0.05$ ) and ranged from 0.31 to 0.61 among loci. Pairwise  $F_{ST}$  varied from 0.01 to 0.90, and the only estimate that was not statistically significant among 136 tests was between two sites on Santa Cruz, CDRS and CC (Table 3). The largest values of  $F_{ST}$  were found between the Guy Fawkes islets and all other sites. The alternative measures of  $F_{ST}$  showed the same overall pattern of strong differentiation but with differences in magnitude. Because Mic7 did not amplify in GFO, it was necessary to remove this locus from the dataset to calculate  $F_{ST(ENA)}$ . While  $F_{ST}$  using the 10 remaining loci did not change (0.44),  $F_{ST(ENA)}$  was lower (0.32; 95% CI  $\pm 0.04$ ), with pairwise comparisons ranging from 0.01 to 0.67. Given the variation in genetic diversity among localities, both the global (0.78) and pairwise values of  $F'_{ST}$  (Table 3) were elevated relative to  $F_{ST}$  as expected.

The results of the PCA and Bayesian clustering were broadly similar to the  $F_{ST}$  analysis. The first three axes of the PCA explained 46% of the overall variation with Axes 1 and 2 explaining 20% and 13%, respectively.

**Table 2** Sample location, sample size ( $n$ ), measures of genetic diversity [mean  $\pm$  standard error: number of alleles per locus ( $A$ ), expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ )], number of monomorphic loci and number of private alleles of Galápagos lava lizards

Island/Site	Site ID	Latitude	Longitude	$n$	$A$	$H_E$	$H_O$	Number of monomorphic loci	Number of private alleles
Isla Santa Cruz									
Charles Darwin Research Station	CDRS	0°44'33.1"S	90°18'13.2"W	32	10.5 $\pm$ 2.0	0.73 $\pm$ 0.05	0.68 $\pm$ 0.05	0	8
Cerro Colorado	CC	0°34'40.2"S	90°10'24.0"W	32	11.0 $\pm$ 2.7	0.75 $\pm$ 0.05	0.69 $\pm$ 0.06	0	8
at Islote Venecia	SCV	0°31'21.1"S	90°28'54.2"W	31	10.9 $\pm$ 2.1	0.71 $\pm$ 0.06	0.66 $\pm$ 0.06	0	12
Isla Plaza Sur	PS	0°35'0.1"S	90°9'55.2"W	32	3.7 $\pm$ 0.6	0.50 $\pm$ 0.06	0.40 $\pm$ 0.07	1	1
Isla Plaza Norte	PN	0°34'48.9"S	90°9'31.7"W	32	3.3 $\pm$ 0.5	0.45 $\pm$ 0.06	0.40 $\pm$ 0.07	1	2
Isla North Seymour	NS	0°23'20.4"S	90°16'40.9"W	32	5.4 $\pm$ 1.6	0.55 $\pm$ 0.06	0.55 $\pm$ 0.08	0	2
Islote Venecia	VE	0°31'4.8"S	90°28'33.3"W	32	3.5 $\pm$ 0.6	0.52 $\pm$ 0.06	0.41 $\pm$ 0.09	2	0
Islote North Punta Bowditch	NPB	0°32'0.8"S	90°31'3.1"W	32	3.4 $\pm$ 0.4	0.48 $\pm$ 0.06	0.46 $\pm$ 0.06	0	1
Islote South Punta Bowditch	SPB	0°32'9.6"S	90°31'2.5"W	32	3.2 $\pm$ 0.4	0.48 $\pm$ 0.05	0.40 $\pm$ 0.08	2	0
Isla Eden	ED	0°33'42.9"S	90°32'13.3"W	32	3.1 $\pm$ 0.4	0.50 $\pm$ 0.05	0.46 $\pm$ 0.07	1	1
Islote North Guy Fawkes	GFN	0°29'46.7"S	90°30'55.7"W	32	1.5 $\pm$ 0.3	0.20 $\pm$ 0.07	0.02 $\pm$ 0.02	8	2
Islote East Guy Fawkes	GFE	0°29'57.1"S	90°30'49.1"W	32	1.8 $\pm$ 0.4	0.23 $\pm$ 0.06	0.06 $\pm$ 0.04	7	1
Islote West Guy Fawkes*	GFO	0°30'52.1"S	90°31'43.7"W	32	1.8 $\pm$ 0.3	0.19 $\pm$ 0.04	0.09 $\pm$ 0.04	6	0
Islote South Guy Fawkes	GFS	0°30'59.4"S	90°31'34.1"W	14	1.8 $\pm$ 0.2	0.35 $\pm$ 0.05	0.22 $\pm$ 0.01	4	0
Isla Daphne Major	DM	0°25'20.4"S	90°22'21.7"W	31	2.2 $\pm$ 0.5	0.35 $\pm$ 0.07	0.13 $\pm$ 0.06	5	5
Isla Santa Fé	SF	0°48'12.0"S	90°2'28.2"W	32	5.6 $\pm$ 1.7	0.52 $\pm$ 0.08	0.44 $\pm$ 0.09	1	4
Isla Santiago (near Isla Sombrero Chino)	SSC	0°21'58.6"S	90°35'9.2"W	32	8.5 $\pm$ 2.7	0.62 $\pm$ 0.09	0.52 $\pm$ 0.10	2	24

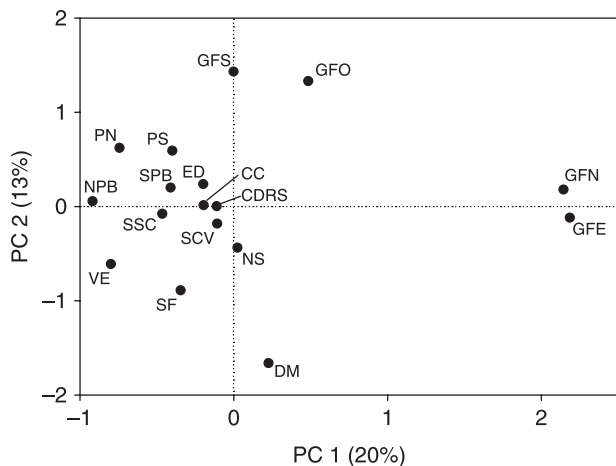
\*Mic 7 did not amplify.

**Table 3** Pairwise  $F_{ST}$  values among sample locations. Values in the upper triangle are derived from Weir & Cockerham's (1984) estimator ( $\theta$ ). The lower triangle is the standardized value of the same estimator calculated as the ratio of  $F_{ST}$  to  $F_{ST(MAX)}$  for each locality (Hedrick 2005; Meirmans 2006). † indicates lack of statistical significance ( $q \geq 0.05$ ) in the upper triangle only

CDRS	CC	SCV	PS	PN	NS	VE	NPB	SPB	ED	GFN	GFE	GFO	GFS	DM	SF	SSC	
CDRS		0.0122†	0.0518	0.2098	0.2707	0.1565	0.2291	0.2351	0.3007	0.2064	0.4284	0.3939	0.4092	0.2874	0.3648	0.2227	0.1480
CC	0.0482		0.0454	0.1989	0.2583	0.1476	0.2423	0.2384	0.2979	0.2361	0.4454	0.4143	0.4246	0.2792	0.3646	0.2376	0.1596
SCV	0.1941	0.1754		0.2490	0.3004	0.1534	0.2835	0.2882	0.2898	0.1890	0.4649	0.4218	0.4475	0.3166	0.3344	0.2422	0.1570
PS	0.5308	0.5103	0.6203		0.2988	0.3024	0.4361	0.4145	0.4849	0.4636	0.6479	0.6207	0.6072	0.4808	0.5864	0.4247	0.4105
PN	0.6346	0.6172	0.6924	0.5305		0.4114	0.4889	0.4577	0.5323	0.4927	0.7216	0.6967	0.6654	0.5510	0.6205	0.4643	0.4315
NS	0.4407	0.4241	0.4232	0.6163	0.7897		0.3928	0.3880	0.4239	0.3889	0.5570	0.5329	0.5803	0.4534	0.4448	0.3141	0.3263
VE	0.5567	0.5959	0.6788	0.7851	0.8221	0.7688		0.4491	0.4992	0.4502	0.7349	0.6876	0.6762	0.5723	0.5688	0.4446	0.3304
NPB	0.6078	0.6260	0.7328	0.7916	0.8242	0.8080	0.8324		0.4369	0.4337	0.6880	0.6580	0.6477	0.5431	0.5904	0.4063	0.3837
SPB	0.6944	0.7006	0.6596	0.8558	0.8914	0.8076	0.8448	0.7845		0.4054	0.7086	0.6783	0.7125	0.5972	0.6603	0.4794	0.3786
ED	0.5278	0.6157	0.4759	0.8839	0.8860	0.8008	0.8223	0.8397	0.7195		0.6447	0.6184	0.5983	0.4817	0.5709	0.4321	0.3062
GFN	0.7119	0.7495	0.7657	0.8780	0.9288	0.8041	0.9397	0.9426	0.9149	0.8838		0.7257	0.8958	0.8208	0.8175	0.6477	0.5995
GFE	0.6878	0.7300	0.7289	0.8692	0.9270	0.7949	0.9254	0.9381	0.9001	0.8776	0.7663		0.8727	0.7710	0.7563	0.6178	0.5736
GFO	0.7015	0.7324	0.7489	0.8554	0.8845	0.8619	0.9188	0.9100	0.9336	0.8319	0.9649	0.9591		0.6060	0.7996	0.6651	0.5155
GFS	0.6259	0.6191	0.6803	0.7774	0.8414	0.7991	0.8817	0.8991	0.9046	0.7954	0.9130	0.8911	0.7084		0.7106	0.5347	0.4415
DM	0.7083	0.7176	0.6394	0.9037	0.9131	0.7307	0.8327	0.9225	0.9631	0.8882	0.9444	0.9013	0.9353	0.9315		0.5319	0.5134
SF	0.6152	0.6681	0.6563	0.8437	0.8607	0.6826	0.8501	0.8253	0.8851	0.8681	0.8998	0.8927	0.9410	0.9171	0.8440		0.3447
SSC	0.4333	0.4781	0.4524	0.8568	0.8489	0.7471	0.6595	0.8202	0.7397	0.6524	0.8787	0.8711	0.8052	0.7945	0.8670	0.7740	

**Table 4** Average proportion of a lava lizard's genome originating from the 17 clusters selected in Bayesian mixture analysis. Values in bold indicate proportions exceeding 50%

Sample site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Isla Santa Cruz																	
Charles Darwin Research Station	0.030	0.021	0.054	0.023	0.028	0.203	0.104	0.028	0.025	0.030	0.026	0.262	0.039	0.038	0.036	0.033	0.020
Cerro Colorado	0.035	0.016	0.030	0.035	0.017	0.018	0.041	0.032	0.028	0.036	0.055	<b>0.537</b>	0.018	0.030	0.022	0.014	0.036
near Islote Venecia	0.081	0.004	0.022	0.009	0.011	0.021	0.005	0.025	0.022	0.020	0.028	<b>0.548</b>	0.078	0.055	0.017	0.028	0.025
Isla Plaza Sur	0.003	0.001	<b>0.928</b>	0.043	0.000	0.000	0.001	0.002	0.000	0.000	0.014	0.000	0.001	0.000	0.000	0.008	0.000
Isla Plaza Norte	0.002	0.000	0.004	<b>0.981</b>	0.000	0.001	0.000	0.001	0.002	0.006	0.002	0.000	0.001	0.000	0.000	0.002	0.000
Isla North Seymour	0.002	0.005	0.010	0.005	0.001	0.002	0.001	0.001	0.001	0.002	<b>0.952</b>	0.000	0.000	0.000	0.003	0.012	0.003
Islote Venecia	0.014	0.001	0.000	0.014	0.001	0.005	0.003	0.000	0.003	<b>0.951</b>	0.000	0.000	0.003	0.004	0.000	0.000	0.000
Islote North Punta Bowditch	0.000	0.008	0.002	0.000	0.002	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.006	<b>0.963</b>	0.014
Islote South Punta Bowditch	0.001	0.002	0.007	0.000	0.006	0.000	0.002	0.000	0.000	0.000	0.003	0.000	0.002	0.000	0.000	0.000	<b>0.977</b>
Isla Eden	0.001	0.000	0.001	0.000	0.000	0.000	0.002	0.002	0.000	0.000	0.000	0.001	<b>0.977</b>	0.002	0.001	0.000	0.013
Islote North Guy Fawkes	0.000	<b>0.991</b>	0.000	0.001	0.000	0.000	0.000	0.000	0.006	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.000
Islote East Guy Fawkes	0.000	0.060	0.002	0.000	<b>0.898</b>	0.031	0.000	0.003	0.000	0.002	0.001	0.000	0.000	0.000	0.001	0.000	0.001
Islote West Guy Fawkes	0.002	0.009	0.000	0.002	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	<b>0.983</b>	0.000	0.000
Islote South Guy Fawkes	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	<b>0.939</b>	0.000	0.000	0.000	0.001	0.001	0.049	0.000	0.008
Isla Daphne Major	<b>0.992</b>	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.001	0.002	0.000	0.000
Isla Santa Fé	0.004	0.003	0.000	0.002	0.002	0.004	0.002	<b>0.938</b>	0.005	0.021	0.004	0.001	0.001	0.004	0.000	0.005	0.005
Isla Santiago	0.004	0.002	0.004	0.000	0.001	0.012	0.006	0.002	0.003	0.001	0.010	0.001	0.004	<b>0.935</b>	0.010	0.003	0.004

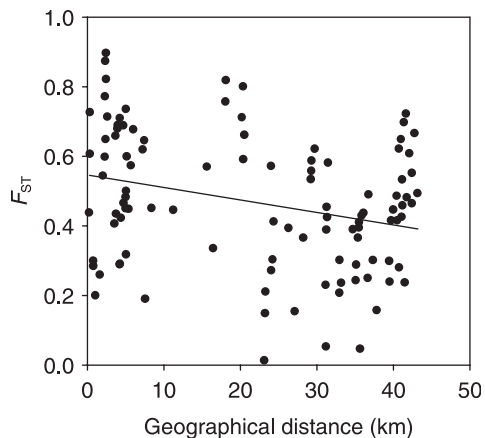


**Fig. 3** Plot of the first two axes resulting from a principle component analysis on multilocus genotypes of Galápagos lava lizards.

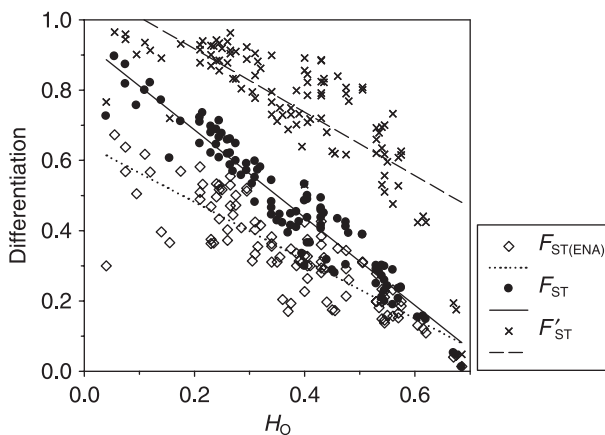
Axis 1 revealed the separation of North and East Guy Fawkes from the other sample localities (Fig. 3). The other two Guy Fawkes islets and Daphne Major were shown to be distinct along the second axis. Under Bayesian clustering, the mixture analysis identified the maximum number of possible clusters as being most likely [ $K = 17$ ,  $P(K|X) = 1$ ]. Table 4 shows the average proportion of individual genomes originating from each of the 17 clusters. Except for the sites on Santa Cruz, the proportional membership of genotypes to particular clusters was high ( $> 89\%$ ). The genotypes of individuals from CDRS were not strongly associated with any cluster. The highest proportional membership of genotypes from CDRS (26%) was found in a cluster shared with half of the genome of lizards from each of the two other sample localities on Santa Cruz.

#### Correlation analysis

We correlated all measures of genetic differentiation with geographical distance using data from the 15 sampling locations hypothesized to be linked to Santa Cruz. The null hypothesis of equilibrium between gene flow and drift as indicated by a positive relationship between the two matrices was rejected in each case (Table 5, Fig. 4). Geographical distance explained little variation in genetic differentiation, and only the relationship between  $F_{ST}$  and geographical distance was marginally significant but in the direction opposite of expectation (Fig. 4). Given the possibility that the Guy Fawkes islets and Daphne Major have a longer history of separation from Isla Santa Cruz (Fig. 1) and the low genetic diversity observed (Table 2), we ran the correlations in the absence of these five localities. The relationships were weaker in these analyses (Table 4). Overall, the data fit the case-III pattern described



**Fig. 4** The relationship between pairwise genetic differentiation ( $F_{ST} = \theta$ ) and geographical distance. Statistics for the correlations are given in Table 5.



**Fig. 5** The relationship between the pairwise average of observed heterozygosity ( $H_O$ ) and different measures of genetic differentiation. Statistics for the correlations are given in Table 5.

by Hutchison & Templeton (1999), indicating a strong role for genetic drift in the absence of gene flow as the cause of differentiation.

To further investigate the importance of drift in isolation we correlated genetic differentiation with the pairwise average of observed heterozygosity using both datasets as above (Table 5, Fig. 5). In general, there were strong negative correlations between these variables observed in both datasets. Adjusting  $F_{ST}$  for genetic variation within populations ( $F'_{ST}$ ) weakened but did not eliminate the relationship with  $H_O$ .

#### Discussion

Fragmentation of islands following a rise in sea level is expected to impact genetic differentiation in resident populations. In our analysis of Galápagos lava lizards subject

**Table 5** Correlations between genetic differentiation, and either geographical distance (km) or observed heterozygosity ( $H_O$ ). Analyses are presented for all localities on or hypothesized to have been connected with Isla Santa Cruz during the Pleistocene ( $n = 15$ , pairwise  $n = 105$ ) and those same localities excluding distant islets [Guy Fawkes islets and Daphne Major ( $n = 10$ , pairwise  $n = 45$ )]. Statistics and their inference were calculated using the Mantel procedure implemented in GENALEX version 6 (Smouse *et al.* 1986; Peakall & Smouse 2006)

Genetic differentiation	Measure of differentiation	Linear fit	R	R <sup>2</sup>	P
vs. Geographical distance	$F_{ST}$	$y = -0.0036x + 0.55$	-0.29	0.08	0.045
	(Excluding distant islets)	$y = -0.0001x + 0.31$	0.02	0.0002	0.46
	$F_{ST(ENA)}$	$y = -0.0018x + 0.38$	-0.19	0.04	0.13
	(Excluding distant islets)	$y = 0.0005x + 0.22$	0.08	0.006	0.26
	$F'_{ST}$	$y = -0.0020x + 0.80$	-0.18	0.03	0.13
	(Excluding distant islets)	$y = 0.0004x + 0.64$	-0.03	0.001	0.34
vs. $H_O$	$F_{ST}$	$y = -1.25x + 0.94$	-0.96	0.92	0.001
	(Excluding distant islets)	$y = -1.57x + 1.11$	-0.94	0.89	0.002
	$F_{ST(ENA)}$	$y = -0.83x + 0.65$	-0.86	0.75	0.001
	(Excluding distant islets)	$y = -1.11x + 0.80$	-0.88	0.78	0.001
	$F'_{ST}$	$y = -0.90x + 1.10$	-0.79	0.62	0.010
	(Excluding distant islets)	$y = -2.10x + 1.73$	-0.86	0.73	0.003

to this late Pleistocene process, we observed high levels of genetic differentiation regardless of the measure used, as well as marked variation among populations in genetic diversity. Our results strongly point to drift in isolation as the principle cause of these patterns as shown by:

- 1 A strong relationship between island size and genetic diversity.
- 2 Greater differentiation among than within islands.
- 3 Lack of a positive correlation and a large degree of scatter in the relationship between geographical and genetic distance.
- 4 A strong negative relationship between genetic differentiation and diversity.

It appears that the small size of the islets has accelerated drift by limiting population size shortly after isolation and/or making populations more susceptible to demographic stochasticity. We therefore reject the hypothesis that gene flow has had a significant role in shaping genetic differentiation on this timescale either by overwater dispersal or by the maintenance of ancestral polymorphism in microsatellites.

#### *Null alleles, heterozygosity and population differentiation*

Measures of genetic differentiation, such as  $F_{ST}$ , are highly sensitive to the amount of genetic variation found within populations, especially when migration rates are presumed to be low (Hedrick 1999; Balloux *et al.* 2000). Because high mutation rates in microsatellites create the potential for high levels of heterozygosity, there is increasing concern about the biological interpretation of  $F_{ST}$  when heterozygosity varies among populations (Balloux & Moulin-Lugon 2002).

The large differences in heterozygosity and differentiation we observed among populations of lava lizards caused us to investigate the relationship between these two variables in more detail.

One possible source of bias in measures of genetic differentiation is null alleles. Null alleles cause a reduction in observed heterozygosity and this can inflate  $F_{ST}$  and genetic distances between populations (Chapuis & Estoup 2007). We suspected the presence of null alleles in the dataset due to lower-than-expected heterozygosity in four combinations of populations and loci, and one locus that did not amplify in a single population. Our analysis of their effect, however, suggests that they are not the underlying cause of patterns of differentiation among populations. Although accounting for them did lead to the expected reduction in  $F_{ST}$ , global levels of differentiation remained high and the pattern of differentiation among populations was unchanged. This lack of effect on the overall pattern was further reflected in subsequent analyses involving correlations with both geographical distance and observed heterozygosity.

In a related way, comparisons between localities with high heterozygosity were expected to underestimate measures of  $F_{ST}$  (Hedrick 1999). For example, samples from Santa Cruz which had the highest heterozygosities should have had maximum values for  $F_{ST}$  much lower than the theoretical maximum for fixation of different alleles between populations (i.e.  $F_{ST} = 1$ ). Indeed,  $F_{ST(MAX)}$  averaged 0.26 within the island and  $F'_{ST}$  was notably higher than  $F_{ST}$  in these pairwise comparisons ( $F_{ST} \rightarrow F'_{ST}$ : Charles Darwin Research Station – Cerro Colorado = 0.01 → 0.05, Charles Darwin Research Station – near Venecia = 0.04 → 0.19, Cerro Colorado – near Venecia = 0.05 → 0.18). This suggests that the Santa Cruz population near Venecia may be more

differentiated than was otherwise indicated by the standard measure. When applied to all pairwise comparisons,  $F'_{ST}$  was elevated overall and had the largest effect on populations with the high heterozygosities (Fig. 5). The negative relationship between observed heterozygosity and genetic differentiation remained when using  $F'_{ST}$  but was not as strong. The maintenance of this relationship when  $F_{ST}$  was adjusted for genetic variation suggests that differences in allele frequency were correlated with levels of heterozygosity among populations. Thus, while the effect of heterozygosity on  $F_{ST}$  did not lead to a substantial change in the inferences we make herein, it reaffirms the caution called for when interpreting analyses of populations that appear to be minimally differentiated (Hedrick 1999).

#### *Genetic diversity and history of populations*

Stone *et al.* (2002) classified islands in the archipelago based on geographical and vegetative characteristics. Here, we follow that classification in developing hypotheses for the post-Pleistocene history of lava lizard populations.

*Barren islets (includes the Guy Fawkes islets).* The Guy Fawkes islets are the most isolated (except currently Daphne Major, see Snell *et al.* 1996) and ecologically depauperate in the sample. Above-ground vegetation on these islands is lacking and food availability appears to be low (Stone *et al.* 2002). Our results show that resident populations of lava lizards have extremely low allelic diversity, a large number of monomorphic loci, few private alleles and are highly differentiated (Tables 2 and 3, Fig. 3). Given the bathymetric pattern and their small size, these islets are likely to harbour populations that have lost genetic diversity through founder events and/or demographic stochasticity during a relatively long period of isolation. This is further supported by the observation that the two northern Guy Fawkes islets (GFN and GFE) not only had populations with the greatest differentiation in the sample (Fig. 3) but also appear to be isolated from Santa Cruz by a deeper channel (> 65 m) than the two southern islets (GFO and GFS) (Fig. 1).

*Diverse islets (includes Plaza Sur, Plaza Norte, Seymour Norte, Venecia, Punta Bowditch Norte, Punta Bowditch Sur, Eden and Daphne Major).* This category includes islets that have above-ground vegetation similar to neighbouring large islands, a larger diversity of resident organisms than the barren islets, and the highest population densities of the three types of islands (Stone *et al.* 2002). The data suggest that although these islets are closer to Santa Cruz and show genetic diversity and differentiation less extreme than the Guy Fawkes islets, that restricted gene flow has also played a primary role in shaping genetic diversity in these

populations. For example, Islote Venecia is isolated from Santa Cruz by only 30 m but has one third the allelic diversity and  $F_{ST} = 0.28$  (compare to SCV, Table 2 and Table 3, respectively). Genetic diversity is intermediate in the category, but allelic diversity is lower relative to heterozygosity (Table 2) as expected under drift (Allendorf 1986). Moreover, removal of the Guy Fawkes islets and Daphne Major from the correlation analyses did not substantially alter the overall patterns, that implicate drift in isolation (Table 5). If more detailed bathymetric analyses support this assertion in the future, it would suggest that the process of allele loss on diverse islets may not have yet reached equilibrium.

One island that we include in this category due to its vegetation profile (see Boag & Grant 1984) is Isla Daphne Major. Despite the ecological similarity to the other diverse islets, the genetic diversity and differentiation of the resident lizard population is comparable to the barren islets (Table 2, Fig. 3). In addition to being the island most distant from Santa Cruz in our sample, the genetic data are consistent with the island's apparent early isolation and loss of size (Fig. 1).

*Large islands (includes Santa Cruz, Santiago and Santa Fe).* Most of the larger islands in the archipelago are thought to have persisted in isolation from one another, following their volcanic origin in spite of variation in sea level (Fig. 1, Simpson 1974; Geist 1996). The tendency toward relatively large numbers of private alleles in the large islands we sampled (Table 2) is consistent with this geological hypothesis. Although population densities are observed to be lower than either type of islet (Stone *et al.* 2002), genetic diversity on the large islands is relatively high (Table 2) and within the range of that observed in other microsatellite analyses of nonpiscine vertebrates (DeWoody & Avise 2000; Neff & Gross 2001). Given the small sample sizes and minimal spatial coverage of the large islands in the analysis, our estimates are probably lower than the true diversity. The lower sea levels that have occurred as a result of glacial advance every 100 000 years for the past 1 million YBP (see Fig. 9 in Grant 2001) cause large islands to periodically increase in size. Effective population sizes should increase due not only to this increase in size (Fig. 2) but also due to probable introgression, as formerly isolated islets become subsumed within the larger land mass.

#### *Evolutionary diversification*

Evolutionary diversification among populations associated with the *Microlophus albemarlensis* complex have been difficult to resolve (Van Denburgh & Slevin 1913; Wright 1983; Lopez *et al.* 1992; Kizirian *et al.* 2004; Benavides *et al.* 2007). While our analysis does not assist with resolving the *M. albemarlensis* complex, it does allow us to infer a temporal

constraint on the hypothesis that the lack of diversification is due to gene flow. The observation that rafts of vegetation wash out of freshwater systems following heavy rainfall in El Niño years has led to the supposition that dispersal of lava lizards (Kizirian *et al.* 2004) and other terrestrial taxa (Holmgren *et al.* 2001) is enhanced under such climatic conditions. Although El Niño events appear to have occurred every 2–15 years over the past 15 000 YBP (Rodbell *et al.* 1999), there is little evidence that there has been gene flow among current islands with resident lava lizards.

Alternative explanations for the shallow relationships among the *M. albemarlensis* complex probably include a combination of rare dispersal and incomplete lineage sorting among large islands, with introgression between large islands and islets occurring during glacial periods (e.g. Beheregaray *et al.* 2004; Morando *et al.* 2004; Ciofi *et al.* 2006). Unlike other groups that have radiated within the archipelago and show diversification within islands (Parent & Crespi 2006), lava lizard species diversity occurs only among islands. This, coupled with our observation that genetic differentiation is greater among-islands than within-localities on Santa Cruz, suggests that gene flow among populations likely occurs when islets are connected to the larger islands. Meanwhile, if dispersal across seawater is indeed as rare as we infer, colonization of the large islands may have taken place only once they have existed for a long period of time. The *M. albemarlensis* complex is found on the younger islands of the western portion of the archipelago, thus limiting the time for sequence divergence. Genetic tests of these hypotheses will require increased sample sizes (Funk & Omland 2003), comparison among several loci (Nichols 2001), and statistical approaches that consider both the phylogeography and population genetics of sequence variation (cf. Templeton 1998).

In spite of the lack of interspecific designation, intraspecific variation in phenotypic traits associated with fitness can be great in insular populations of lizards (Thorpe & Malhotra 1996; Wikelski & Trillmich 1997; Harmon & Gibson 2006). Our observation that genetic drift has been important in the neutral genetic differentiation of lava lizard populations has implications for study of such patterns. Analyses of morphology, physiology, and behaviour have revealed a notable amount of variation both within and among populations of the *M. albemarlensis* complex (e.g. Carpenter 1966; Snell *et al.* 1988; Stone *et al.* 2002), even in the presence of gene flow (Jordan *et al.* 2005). This type of pattern is suggestive of a prominent role for diversifying selection and/or phenotypic plasticity among habitats that vary in predation, food availability and parasitism (Stone *et al.* 2002). However, to the extent that the traits under consideration have a genetic basis, drift may be the direct cause of phenotypic differentiation (Mayr 1954) or can reduce the ability of populations to adapt to ecological change (reviewed in Templeton *et al.*

2001; Garant *et al.* 2007). Carpenter (1966) proposed drift as an explanation for inter-island variation in patterns of communicative display among lava lizards. Although empirical work has suggested that drift is unlikely to be the sole cause of phenotypic differentiation (Rice & Hostert 1993; Clegg *et al.* 2002), we suggest that it be considered in future comparisons among populations in this system.

## Conclusion

Archipelagos continue to be a rich resource for the study of the origin and maintenance of species. Although islands of volcanic origin can have a short life cycle in geological time (Christie *et al.* 1992), periodic change in sea level is a more rapid process that can impact the evolution of resident organisms. We have shown that a species with limited ability to disperse is subject to strong genetic drift as a result of island fragmentation. This is reflected in variation in genetic diversity and strong differentiation among populations on islands that have been recently isolated. Consideration of population history among multiple islands at this temporal scale can assist our understanding of the processes that cause variation in the rate of adaptive radiation within archipelagos.

## Acknowledgements

We are grateful to Heidi Snell and the many field assistants that helped to collect tissues over the years. Tom Giermakowski and the staff of the Museum of Southwestern Biology provided valuable assistance in organizing the sample. We thank J.C. Gutierrez, C. Hite, K. Ankenbruk and R. Naylor for help with the genotyping. Financial support for the research was provided by the Purdue Research Foundation (MAJ) and National Science Foundation grant IBN-920789 (HLS). The Servicio Parque Nacional de Galápagos provided permission to conduct the fieldwork. We thank TAME Airlines for their support of our research. Handling of lizards and collection of tissues was conducted under a protocol approved by the Animal Care and Use Committee of the University of New Mexico.

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