Phenotypic divergence despite high levels of gene flow in Galápagos lava lizards (*Microlophus albemarlensis*)

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Abstract

The extent of evolutionary divergence of phenotypes between habitats is predominantly the result of the balance of differential natural selection and gene flow. Lava lizards (*Microlophus albemarlensis*) on the small island of Plaza Sur in the Galápagos archipelago inhabit contrasting habitats: dense vegetation on the western end of the island thins rapidly in a transitional area, before becoming absent on the eastern half. Associated with these habitats are phenotypic differences in traits linked to predator avoidance (increased wariness, sprint speed, and endurance in lizards from the sparsely vegetated habitat). This population provides an opportunity to test the hypothesis that reduced gene flow is necessary for phenotypic differentiation. There was no evidence of any differences among habitats in allele frequencies at six out of seven microsatellite loci examined, nor was there any indication of congruence between patterns of genetic variability and the change in vegetation regime. We infer that gene flow between the habitats on Plaza Sur must be sufficiently high to overcome genetic drift within habitats but that it does not preclude phenotypic differentiation.

Keywords: Galápagos Islands, microsatellite, morphology, performance, predator escape

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Introduction

The extent of evolutionary divergence of phenotypes between habitats is mainly the result of the balance of differential natural selection and gene flow (Ehrlich & Raven 1969; Endler 1977; Lenormand 2002). Theoretical work suggests that under a wide range of conditions, as few as one reproducing migrant per generation may prevent differentiation between populations at neutral loci as a result of genetic drift (Slatkin 1985). However, the impact of gene flow on the evolution of putatively adaptive traits is less clear and has generated considerable controversy. While natural selection may favour phenotypes particular to certain habitats, theory suggests that gene flow should act to homogenize such differences (reviewed in: Slatkin 1985, 1987), and can lead to situations where individuals have features that are detrimental to their fitness (Stearns & Sage 1980; Reichert 1993; Storfer & Sih 1998; Hendry et al. 2002). Gene flow, however, does not stop natural selection from occurring.

Although geographical (and thus genetic) isolation is traditionally considered fundamental to phenotypic divergence (Mayr 1963), a growing body of empirical work suggests that strong natural selection can still lead to divergence in spite of high levels of gene flow (e.g. Smith et al. 1997; Schneider et al. 1999; Brown et al. 2001; Calsbeek & Smith 2003).

The population of Galápagos lava lizards (*Microlophus albemarlensis*) that occurs on Isla Plaza Sur, Galápagos Islands, provides an opportunity to test the hypothesis that genetic isolation is critical to phenotypic divergence. Plaza Sur is characterized by two habitats. The western end of the island has 30-fold more vegetative cover than the barren eastern end (Snell et al. 1988). Phenotypes associated with predator escape in lava lizards are correlated with this habitat difference. Male lava lizards sprint faster, and adults of both sexes are warier (will flee sooner and further when approached) in the sparsely vegetated habitat (Snell et al. 1988). Recent work has extended these results by showing that both sexes also have higher endurance in the sparse habitat (Miles et al. 2001). Neither study demonstrated differences between habitats in expected morphological correlates of performance (cf. Garland & Losos 1994).
Nevertheless, vegetative cover appears to provide greater access to refugia for lizards subject to attack by avian predators and may allow individuals to allocate fewer resources to antipredatory behavior (Lima & Bednekoff 1999) and performance (e.g. Krupa & Sih 1999). There are no physical barriers that could prevent the movement of lizards between the two habitats.

To test the hypothesis that microgeographical differentiation in phenotypes is correlated with genetic isolation, we assessed the degree of genetic differentiation among lava lizards on Plaza Sur using microsatellite markers. Following the general assumption that microsatellites evolve in a neutral fashion, we are able to evaluate the level of genetic differentiation within the island independent of natural selection. If gene flow is low, we expect that there will be genetic structure associated with the vegetation regime. Otherwise, high levels of gene flow may indicate that phenotypic plasticity and / or natural selection contribute to the maintenance of observed phenotypic differences in ecological performance. In addition to the genetic analysis, we present analyses of morphological traits to further evaluate patterns described in past work and explore phenotypic variation within the island.

Materials and methods

Natural history and field data

Plaza Sur is located 440 m off of the northeast coast of the larger island, Santa Cruz (Fig. 1; Snell et al. 1996). The island is small (11.9 ha, approx. 1 km long), positioned along a roughly east–west axis. The area of dense vegetation on Plaza Sur is characterized by trees, shrubs, and tall Opuntia cactus. This vegetation thins rapidly in a transitional area, before becoming absent on the eastern half of the island.

Lava lizards are distributed in high densities across the island regardless of habitat. Their principal predators, egrets and herons, use vision to detect lava lizards during their daytime period of activity (Snell et al. 1988). Like many species of lizard (Greene 1988), lava lizards will flee to nearby refugia when attacked (Werner 1978). Males are larger, faster, and have higher stamina than females in all habitats on Plaza Sur (Snell et al. 1988; Miles et al. 2001).

There are no direct studies of dispersal between habitats on the island. However, work on home range size of adults on Plaza Sur (Stone 1995; Stone et al. 2002), and dispersal of hatchlings and juveniles in a closely related species endemic to the archipelago (Microlophus delanonis, Werner 1978; Jordan 1999), suggests that most lava lizards exhibit site fidelity. While there are observations of movements of up to 200 m, hatchlings disperse less than 100 m from their natal site prior to settling in an area within the first few weeks of life. Most adults occupy home ranges that have a maximum linear distance of less than 100 m. These values are probably larger for a relatively small group of males that have low dominance status or in situations where novel food resources cause shifts in the use of space in both sexes (Stone et al. 2002). Whether individuals that are involved in these larger movements establish new home ranges and reproduce during or subsequent to relocating is unknown.

From 26–30 June 2001, we captured 300 lava lizards by noose across the length of the island. Latitude and longitude of the capture site of each lizard was estimated with handheld global positioning receivers (Garmin GPS 12). We used a dial caliper (0.1 mm) to measure snout-vent length (SVL) and hindlimb length (HL, taken from the anterior edge of the preacetabular process to the distal point of the longest digit) on captured lizards. Hindlimb length was included in our analysis because of its expected functional relationship to locomotor performance (Snyder 1954; Garland & Losos 1994). Body mass was measured using a Pesola spring scale (0.2 g). The reproductive status of females was assessed by palpating the abdomen to detect the presence of eggs (cf. Snell et al. 1988). Following measurement, two toes were clipped and preserved in 95% ethanol for subsequent

Fig. 1 Map of Plaza Sur showing the location of vegetative cover (shaded grey) and the position of sampled individuals (circles, genotyped; dots, captured but not genotyped).
Samples and genetic screening

Because of logistical constraints, a subsample of 211 individuals (Fig. 1) was screened for genetic variation at a series of microsatellite loci. Individuals were chosen for the subsample with respect to their geographical position and not by morphology. Most individuals within the subsample were selected to maximize the geographical distance between capture sites in the vegetated ($n = 82$) and sparse habitats ($n = 80$) to provide a conservative test of gene flow. Remaining individuals (transition, $n = 49$) were chosen to occupy a longitudinal position intermediate to the samples from the two predominant habitats. Thus, individuals from the transition habitat were defined by their geographical position and not by the amount of vegetative cover.

One toe from each individual was dissected with a scalpel prior to digestion in proteinase K for 48 h at 55 °C. We extracted DNA using columns lined with a silica-gel membrane (QIAGEN DNAeasy Tissue Kit). To equalize DNA concentrations between sexes, DNA was eluted in 200 and 150 µL of TE (10 mM Tris-HCl, pH 7.6; 1 mM EDTA) for males and females, respectively. Seven microsatellite loci (Mic1, Mic2, Mic3, Mic4, Mic5, Mic6, and Mic8) 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). A further subsample of 32 individuals was genotyped a second time to test the veracity of genotype assignments. No differences were found between the two sets of scores.

Statistical analyses

The central goal of the analysis was to assess the amount of phenotypic and genetic differentiation associated with the shift in vegetative cover. Standard parametric statistics are described below for the analysis of morphological variation. To analyse the genetic data, we used a range of statistical methods characteristic of the emerging field of ‘landscape genetics’ (Manel et al. 2003). We divided the genetic analysis into three groups. First, we performed global tests for population substructure on the entire sample. Secondly, we made use of the subsample categories described above (habitats = vegetated, transition, or sparse) to test for the presence of population structure and the degree of differentiation. In the third approach, we sought to identify possible geographical patterns in genetic variation across the island.

Analyses of morphological variation

Each morphological variable was log transformed prior to analysis to fit assumptions of analysis of variance (ANOVA). We used habitat and sex as factors in two-way ANOVA to test for differences in SVL and HL. Several females that we captured were gravid or had recently nested. Because females can vary up to 30% in body mass while producing eggs (Jordan & Snell 2002), we tested only males for differences among habitats in body mass using one-way ANOVA.

Snout–vent length correlates strongly with morphometric traits in lizards. To determine whether variation in body mass or hindlimb length varied out of proportion with body size, we used SVL as a covariate (ANCOVA) in the models described previously. The assumption of equal slope in the relationship between the covariate and dependent variable among factors was satisfied in each ANCOVA (Sokal & Rohlf 1995). Multiple comparisons were conducted using Bonferroni adjustment and analyses were performed in ssrs (version 8).

Global tests of population structure

We used GENEPOP (http://wbiomed.curtin.edu.au/genepop/index.html; Raymond & Rousset 1995) to test for deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between loci as an initial indication of population substructure within the island.

Levels of genetic differentiation among habitats

We also used GENEPOP to make exact tests of overall and pairwise differences in allele frequencies among habitats, and to test for deviation from HWE (at individual loci and all loci combined) and LD between loci within habitats. To visualize genetic differentiation among habitats, we calculated multilocus scores for individuals using correspondence analysis as implemented in genetix (Belkhir et al. 2002).

Because there were no apparent barriers to gene flow within the island, we also used a Bayesian clustering program (structure version 2.1, Pritchard et al. 2000) to search for cryptic population structure. STRUCTURE finds subpopulations ($K$) by looking for deviations from HWE and LD when assuming different values for $K$. Following runs of the program at these different values, the probability that the data fit each hypothesized number of subpopulations is calculated using Bayes’ rule. Then, if subpopulations are identified, it is possible to determine if individuals originating from one habitat or another are assigned to the prescribed subpopulations with high likelihood. This allows us to determine if possible genetic differentiation is linked with habitats within the island.

Individuals within Plaza Sur were likely to have mixed ancestry with respect to the habitat shift. Therefore, we used a model that assumed admixture and the possibility of correlations in allele frequencies among subpopulations (Falush et al. 2003). Structure also relies on the assumptions...
that loci are unlinked, at linkage equilibrium, and HWE within putative subpopulations. The data conformed to these assumptions. Five runs of the model were made at each presumed value of K (1–4). Estimates of the probability of K in each run were taken following 1 000 000 iterations that were preceded by a burn-in of 150 000 iterations.

**Levels of inbreeding**

To test for differences in level in inbreeding in different habitats (perhaps related to variation in population sizes or mating patterns) we calculated internal relatedness (IR, Amos et al. 2001) for each genotyped individual. Differences in average IR among subsamples were tested using one-way ANOVA.

**Geographic patterns of genetic differentiation**

We analysed the relationship between pairwise genetic distance and the linear geographical distance of individuals to determine if isolation by distance (cf. Wright 1943) might explain the phenotypic differences between habitats. We calculated individual genetic distance by estimating relatedness (r) among all pairwise combinations of individuals using Wang’s method (2002) as implemented within the program SPAGEDI (Hardy & Vekemans 2002). We chose this estimator because of its robustness to assumptions associated with the use of samples from individuals of unknown ancestry taken from a wild population (Wang 2002). Pairwise geographical distances were calculated in ARCVIEW (version 8). We expected a negative slope between the two measures as low estimates of relatedness should be found between two geographically distant individuals if gene flow is restricted across the island. Statistical significance of negative correlation was assessed via a one-tailed Mantel’s test using 1000 permutations of localities among individuals (implemented in SPAGEDI).

**Results**

**Patterns of morphological differentiation**

Compared to females, males had greater SVL and HL regardless of habitat (Tables 1 and 2). Males retained longer hindlimbs than females after adjusting for differences in SVL (Table 2). Both males and females had greater SVL in the sparse habitat (Tables 1 and 2). Variation in mean HL among habitats differed between the sexes with and without adjusting for SVL. Relative HL of males did not differ among habitats (one-way ANCOVA: P = 0.7), while females had relatively longer hindlimbs in the vegetated side of the island (one-way ANCOVA: F_{2,120} = 16.7, P < 0.0001). Males were heavier in the sparse habitat both before

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<th>Table 1</th>
<th>Means (±s) of morphological traits among habitats and between sexes</th>
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<td>Vegetated</td>
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<td>Snout–vent length (cm)</td>
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<tr>
<td>Male</td>
<td>8.86 ± 0.42</td>
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<td>(n = 63)</td>
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<td>Female</td>
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<td>Hindlimb length (cm)</td>
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<td>Male</td>
<td>6.57 ± 0.23</td>
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<td>(n = 56)</td>
<td>(n = 37)</td>
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<tr>
<td>Female</td>
<td>5.40 ± 0.17</td>
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<td>Body mass (g)</td>
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<td>Male</td>
<td>23.6 ± 4.49</td>
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<td>(n = 38)</td>
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<tr>
<th>Table 2</th>
<th>F-statistics and P values of tests of variation in snout–vent length and hindlimb length between sexes and among habitats</th>
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<tr>
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<td>Habitat</td>
<td>F_{2,272} = 16.6, P &lt; 0.0001</td>
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<td>Sex</td>
<td>F_{2,272} = 1963.2, P &lt; 0.0001</td>
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<td>Habitat × sex</td>
<td>F_{2,272} = 0.29, P = 0.74</td>
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<td>Covariate (SVL)</td>
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<td>Habitat</td>
<td>F_{1,271} = 11.8, P &lt; 0.0001</td>
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<tr>
<td>Sex</td>
<td>F_{1,271} = 168.6, P &lt; 0.0001</td>
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<tr>
<td>Habitat × sex</td>
<td>F_{2,272} = 7.5, P &lt; 0.001</td>
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</tbody>
</table>

(one-way ANOVA: F_{2,156} = 16.2, P < 0.0001) and after (one-way ANCOVA: F_{2,155} = 7.0, P = 0.001) adjusting for SVL.

**Levels of genetic differentiation**

A total of 41 alleles were found for use in the analysis of genetic variation (Table 3). Males and females differed neither in allele (P = 0.98) nor genotype (P = 1.0) frequencies and were therefore pooled for all analyses. There was no evidence of deviation from HWE (over all loci, P = 0.34) or of LD between any pair of loci when the sample was considered in its entirety. However, when the habitat subsamples were tested separately, there was a deficiency

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of heterozygotes (Wahlund effect) in the transition habitat (over all loci, \( P = 0.013 \)), but not in the two others.

A multilocus test indicated that there was highly significant heterogeneity in allele frequencies across habitats (\( P = 0.0007 \)). However, it transpired that this overall result was driven mainly by differences at the Mic3 locus (\( P = 0.0002 \)). The multilocus heterogeneity was not significant when the data from the Mic3 locus was excluded (\( P = 0.08 \)). It should be noted, however, that data from Mic3 did not produce the apparent Wahlund effect in the transition habitat as this result was still significant when the test was repeated without Mic3 data (\( P = 0.02 \)). There were no significant differences in allele frequencies at any of the other loci in pairwise tests of differentiation between habitats.

The lack of apparent differentiation was also reflected in the correspondence analysis (Fig. 2). There was no separation of individuals by habitat when plotting their multilocus genotypes from the first two axes estimated by the analysis. This pattern held after removing various combinations of outliers that are evident in the plot.

**Bayesian clustering**

A component of the STRUCTURE algorithm is the assumption that allele frequencies are independently drawn from a distribution determined by a parameter called \( \lambda \). It can be set a priori at an arbitrary value or estimated from the data. We found that the program better converged on a probability for \( K \) when \( \lambda \) was estimated for \( K = 1 \) (\( \lambda = 0.78 \)) and then applied to runs assuming different numbers of subpopulations.

The most likely number of populations on the island was 1 (Table 4). For other values of \( K \), there was large variation in estimates of the probability. This lack of convergence for larger values of \( K \) is typical of populations that lack structure because the algorithm has difficulty finding clusters.

### Table 3 Allele frequencies at microsatellite loci in the Galápagos lava lizard population of Isla Plaza Sur

<table>
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<tr>
<th>Locus</th>
<th>Allele</th>
<th>Vegetated</th>
<th>Habitat transition</th>
<th>Sparse</th>
<th>Locus</th>
<th>Allele</th>
<th>Vegetated</th>
<th>Habitat transition</th>
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<td>0.244</td>
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</tbody>
</table>


---

![Fig. 2 Plot of multilocus scores of individuals derived from the first two axes of a correspondence analysis.](image)
Table 4 The average and range of the likelihood of allelic data from within Plaza Sur fitting different assumed numbers of sub-populations (K) is given from five runs of structure (Pritchard et al. 2000). The posterior probability of K \(P(X|K)\) is computed from the likelihoods using Bayes’ rule.

| K  | Average ln \(P(X|K)\) | Range ln \(P(X|K)\) | \(P(X|K)\) |
|----|-------------------------|----------------------|------------|
| 1  | −3717                   | −3716 to −3718       | 1          |
| 2  | −3743                   | −3730 to −3756       | −0         |
| 3  | −3736                   | −3713 to −3770       | −0         |
| 4  | −3760                   | −3716 to −3762       | −0         |

Table 5 Relationship between relatedness \((r)\) and geographical distance

<table>
<thead>
<tr>
<th>Locus</th>
<th>Slope ((b_{obs}))</th>
<th>(P) ((b_{obs} &lt; b_{exp}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mic1</td>
<td>−5.3 × 10⁻²</td>
<td>0.11</td>
</tr>
<tr>
<td>Mic2</td>
<td>−7.1 × 10⁻⁶</td>
<td>0.30</td>
</tr>
<tr>
<td>Mic3</td>
<td>−8.8 × 10⁻⁵</td>
<td>0.03</td>
</tr>
<tr>
<td>Mic4</td>
<td>−1.6 × 10⁻⁵</td>
<td>0.73</td>
</tr>
<tr>
<td>Mic5</td>
<td>−1.4 × 10⁻⁶</td>
<td>0.95</td>
</tr>
<tr>
<td>Mic6</td>
<td>5.6 × 10⁻⁵</td>
<td>0.94</td>
</tr>
<tr>
<td>Mic8</td>
<td>9.7 × 10⁻⁵</td>
<td>0.96</td>
</tr>
<tr>
<td>Overall</td>
<td>−0.3 × 10⁻⁷</td>
<td>0.73</td>
</tr>
</tbody>
</table>

\(b_{obs}\): the observed slope of the relationship.
\(b_{exp}\): the mean slope following 1000 permutations of locations among all individuals.

et al. 2000). This was confirmed when analysing the assignment of individuals to clusters identified by the analysis. Contrary to the expectation when distinct structure is found, individuals were equally likely to be assigned to one cluster or another. Because the clustering of data into sub-populations was improbable we abandoned the association of clusters with habitat.

Levels of inbreeding

There were no differences among habitats in internal relatedness (ANOVA: \(P = 0.29\)). Hence, inbreeding depression is unlikely to explain differences in lava lizard phenotypes between habitats.

Patterns of genetic differentiation

Because of missing localities for a few individuals, 21 736 of 22 155 pairwise geographical distances (mean = 359 m, range = 0–963 m) were available for correlation with genetic distance. Indicating that isolation by distance was unlikely between the two habitat types, there was no correlation between the two variables at six of the seven loci or for all loci combined (Table 5). Only Mic3 exhibited the expected negative correlation between the variables.

Discussion

Past studies on the lava lizards of Plaza Sur have shown greater levels of sprint speed, endurance, and wariness among individuals that occur in habitat with relatively low amounts of vegetative cover (Snell et al. 1988; Miles et al. 2001). Because each of these traits is thought to contribute to an individual’s ability to escape predation, this pattern is suggestive of adaptive differences that have arisen in response to selection pressures. We tested and rejected the hypothesis that reproductive isolation among lizards in different habitats is necessary for the maintenance of observed phenotypic variation as we found no evidence of allele frequency differences among habitats at six out of seven microsatellite loci examined.

Phenotypic variation

In addition to locomotor performance and behaviour, previous studies have also analysed potential morphological correlates of performance. Hindlimb length and snout–vent length were predicted to vary between habitats because of their expected positive relationship with sprint speed and endurance (Snyder 1954; Garland & Losos 1994). However, both of the past studies on Plaza Sur found no difference in either morphological trait between habitats (Snell et al. 1988; Miles et al. 2001). This suggests that causal mechanisms other than morphology underlie consistent differences in performance between habitats within the island.

Although we did detect differences in morphology among habitats, the patterns of difference were difficult to interpret. In contrast to the theoretical expectation that lizards in sparse habitats would have longer hindlimbs, there was no difference among males but surprisingly, females had relatively longer hindlimbs in the vegetated habitat. Meanwhile, as expected from theory but in contrast to the past work, individuals of both sex had greater SVL in the sparse habitat. The existence of this temporal variation in morphological trait values is intriguing and requires further study.

Genetic variation and the evolution of phenotypic differentiation

Neutral loci that undergo genetic drift produce genetic differences among populations that are reproductively isolated from one another. Apart from a deficit of heterozygotes in the transition habitat that might suggest an area where genetic mixing occurs between populations, our results show no evidence of differentiation at six of seven microsatellite loci in lava lizards sampled from habitats in which lizards are known to vary in traits linked to predator escape. We infer that gene flow between the habitats on Plaza Sur must be sufficiently high to overcome genetic drift but that it does not preclude phenotypic differentiation.
A further potential cause of the phenotypic differences among habitats that we can reject is differential inbreeding depression. Although there may be no differences among populations in allele frequencies, variation in population size and/or mating patterns in different habitats might influence levels of inbreeding and any associated depression in performance or fitness. Our results suggest that this is not the case.

While allowing us to test our initial hypothesis on gene flow and phenotypic differentiation, our results do not allow us to discern whether genetic or environmental factors, singly or in combination, determine the observed phenotypic differences. The lack of a genetic basis for the pattern is possible given that many of the phenotypic traits that we and others (Snell et al. 1988; Miles et al. 2001) have examined on Plaza Sur are known to be plastic in other species (e.g. Van Damme et al. 1992; Niewiarowski & Roosenburg 1993; Elphick & Shine 1998; Losos et al. 2000). Moreover, although not extensively studied among lizard taxa, maternal effects have been implicated as a source of variation in the locomotor performance of lizard offspring (Sorci & Clobert 1997). The scope for a purely environmental explanation of the observed pattern may be concentrated in early stages of the life history. For example, additional work on the island has shown that hatchlings differ in endurance capacity between the two habitats in the same manner as adults (D.B. Miles, personal communication). This suggests that common garden or reciprocal transplant experiments designed to test for environmental effects may profit from a focus on the role of incubation conditions during neonate development (Elphick & Shine 1998; Qualls & Shine 1998; Downes & Shine 1999). To date, few studies that have described the presence of gene flow among populations that exhibit phenotypic variation have tested for plasticity in a direct way (e.g. Hendry et al. 2002).

While environmental variation may play an important role in the development of antipredator traits, genetic explanations that provide the potential for an evolutionary response to natural selection are also possible. Experimental studies have suggested that locomotor performance, behaviour, and morphology are heritable (Tsuji et al. 1989; Garland 1994; Sorci et al. 1995) and appear to exhibit genetically based geographical variation (Sinervo & Losos 1991; Losos et al. 2000; Vanhooydonck et al. 2001; O’Steen et al. 2002). To the extent that the suite of antipredator traits documented in lava lizards has a genetic basis, it should be possible to detect genetic differences between lizards found within the two major habitats on the island. Gene flow and genetic drift affect all loci in the population while natural or sexual selection is typically directed toward a handful of loci (Lewontin & Krakauer 1973; Slatkin 1987). The observation that one of the seven loci (Mic3) screened within our sample shows allelic differences between habitats may be an example of a locus undergoing evolution via natural selection. Analysis of many more loci will be required to determine whether this result is more than a statistical anomaly and therefore indicative of genetic differentiation in response to natural selection. However, it is increasingly apparent that even relatively small-scale screens of the genome can reveal loci under selection (Beaumont & Nichols 1996; Wilding et al. 2001). Recent empirical work has begun to reveal the complexity of the evolutionary forces acting on the genome by comparing loci under selection (e.g. allozymes, major histocompatibility complex) with those that are not (Lemaire et al. 2000; Landry & Bernatchez 2001; Dufresne et al. 2002). Furthermore, there is increasing evidence that microsatellite loci are often closely linked to or epistatically interact with loci subject to selection. For example, a recent study (Dufresne et al. 2002) of the acorn barnacle (Semibalanus balanoides) compared spatial variation in six microsatellite loci with that found in two allozyme loci presumed to be under differential natural selection. While four of the microsatellites showed low levels of differentiation among sites, two of these presumably neutral loci showed low levels of polymorphism and patterns of divergence similar to the allozymes.

A final scenario that could explain the phenotypic pattern is that lava lizards are adaptively plastic in their response to perceived risk of predation or other correlated cues in their habitat (reviewed in: Travis 1994; Schlichting & Pigliucci 1998; Robinson & Parsons 2002). Lava lizards from across the island would have the ability to express genes that produce a phenotype which maximizes fitness in either habitat. Hence there would be a genetic basis for the phenotypic differences. However, it would not be possible to detect differences at the genomic level by comparing individuals from different habitats because the genetic architecture necessary for plasticity would be common to all lizards. A test for adaptive phenotypic plasticity in this system reiterates the need for an approach that utilizes common garden or reciprocal transplant experiments to understand the relative roles of genetic and environmental variation in producing phenotypic variation.

Conclusion

The focus of this study was to determine if microgeographical isolation was necessary for the divergence of putatively adaptive traits in Galápagos lava lizards between habitats on a single island. Phenotypic divergence associated with low gene flow may be the result of genetic drift alone or can be caused by founder events followed by selection. We conclude that gene flow is high within the island and therefore reject both of these hypotheses. Several studies (Smith et al. 1997; Vitt et al. 1997; Schneider et al. 1999; Hendry et al. 2002; Ogden & Thorpe 2002; Stenson et al. 2002) have found similar patterns of gene flow combined with phenotypic differences among habitats. Assuming that there is a genetic
basis to these traits, phenotypic differentiation on fine spatial scales such as these are excellent systems for the continued study of the early ecological processes that can lead to evolutionary divergence, parapatric speciation, and adaptive radiation (Schluter 2000).

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References


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


Niewiarowski PH, Roosenburg WM (1993) Reciprocal transplant program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes, 2, 618–620.


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