

Comparative phylogeography of Floreana’s lizards supports Galápagos Pleistocene paleogeographical model and informs conservation management decisions

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Abstract

Terrestrial squamate reptiles from the Galápagos archipelago have limited gene flow among islands, providing an opportunity to test paleogeographic models. Previous work suggests that Pleistocene glaciations had a strong influence on the evolution of Galápagos’ land-locked vertebrates, such as lizards and snakes, by allowing dispersal and contact among populations from different islands or islets through land connections. One prediction of this model is that extant populations, despite being isolated at present, are genetically similar due to recent (Pleistocene) gene flow. Here we test this prediction with a simple comparative phylogeographic analysis of two sympatric lizards from Floreana island and surrounding islets. Based on two mitochondrial genes, we show that Floreana lava lizards (*Microlophus grayii*) and leaf-toed geckos (*Phyllodactylus baurii*) from Floreana Island are very similar genetically to conspecifics from Champion, an islet in the Floreana group that was connected to Floreana during Pleistocene glacial maxima. Moreover, they are significantly less similar to conspecifics from Gardner, an islet in the Floreana group that was not in contact with Floreana during Pleistocene glacial maxima. Thus, our results support the idea of Pleistocene glaciation-driven contact among populations from different islands in the Floreana cluster with no identifiable subsequent dispersal. These results also show that Floreana and Champion populations are part of the same evolutionary significant unit for both species, which might be at risk due to an upcoming invasive mammal eradication program in Floreana. Therefore, Champion represents a reasonable source for potential reintroductions of both lava lizards and leaf-toed geckos into Floreana.

Introduction

Archipelagos provide unique opportunities for phylogeographic analyses. They are geographically isolated and dispersal-mediated gene flow among individual islands is limited for some taxa (Emerson & Hewitt, 2005), such as terrestrial squamate reptiles. Located in the Tropical Eastern Pacific 960 km off the coast of Ecuador, the Galápagos archipelago has long been recognized as a model system for evolutionary and biogeographic studies (Darwin, 1859; Emerson, 2002; Grant & Grant, 2014; Losos & Ricklefs, 2009; Parent, Caccone, & Petren, 2008).

At present, the Galápagos archipelago includes 123 islands, only 13 of which are larger than 10 km² (Snell, Stone, & Snell, 1996). Among the larger islands, Floreana is one of the few inhabited islands, and lies on the southern limit of the archipelago (Fig. 1). Only three years before Darwin’s historical arrival to the Galápagos in 1835, the first human colony of the Galápagos was established in Floreana under the term of Ecuador’s first president, Juan José Flores, after whom the island was named in Spanish. The new inhabitants introduced domestic animals, mice and rats, and the domestic animals established feral populations. Floreana’s terrestrial biodiversity was thereby impacted; several vertebrate species including

Floreana's giant tortoise (*Chelonoidis niger* Quoy & Gaimard, 1824), racer snake (*Pseudalsophis biserialis* Günther, 1860) and mockingbird (*Mimus trifasciatus* Gould, 1837) went extinct in the following 40-50 years (Broom, 1929; Steadman, 1986). Of these, Floreana's racer snake and mockingbird still survive on adjacent islets (Grant, Curry, & Grant, 2000; Thomas, 1997), such as Champion and Gardner, which lack introduced mammals. In contrast, the Floreana lava lizards *Microlophus grayii* Bell, 1843 and the Floreana leaf-toed geckos *Phyllodactylus baurii* Garman, 1892 have survived human impacts both on Floreana and adjacent islets, although both species are frequently predated by feral and domestic cats in Floreana (Paula Castaño pers. obs. 2018; Rodriguez & Fessl, 2016; Steadman 1986). A population viability analysis of Floreana lava lizards showed that lack of recruitment was the primary factor restricting population size on Floreana and suggested that eradication of feral cats and invasive rodents might have a positive effect (Rodriguez & Fessl, 2016).

Floreana has an area of 173 km² and is surrounded by several satellite islets, including Caldwell, Enderby, Champion, Gardner and Watson. With an estimated age of 1.5–2.3 million years (Geist, Snell, Snell, Goddard, & Kurz, 2014; White, McBirney, & Duncan, 1993), Floreana is of intermediate age of islands within the archipelago. Noting that some Galápagos islands are broadly connected by shallow seabed, some authors have proposed that Pleistocene changes in sea level had a strong influence on the evolution of land-locked vertebrates by allowing contact among populations from different islands during glaciation periods (Ali & Aitchison, 2014; Geist et al., 2014; Poulakakis, Russello, Geist, & Caccone, 2012). For example, Fernandina, Isabela, Pinzón, Rábida, Santiago, Baltra and Santa Fe are connected by shallow water (<200 m); therefore, these islands were possibly connected by land bridges during Pleistocene glaciations, when sea level fell to between -90 and -130 m below the present level, for 5–10 kyr intervals (Ali & Aitchison, 2014). In contrast, other major islands are isolated from one another by a deeper (>500 m) ocean floor and were thus not connected to other large islands in the recent past. Nonetheless, these islands were presumably connected to satellite islets separated by depths <200 m. According to this paleogeographical model, Floreana was connected to the satellite islets of Caldwell, Champion and Enderby, whereas Gardner and Watson were separated and formed a single islet during Pleistocene glaciations (Fig. 1; Geist et al., 2014). Consequently, present land organisms with limited dispersal abilities from Floreana should be more similar genetically to those from Caldwell, Champion and Enderby than they are to organisms from Gardner and Watson due to gene flow during Pleistocene low sea-level periods.

If the paleogeographic model holds true, it has great implications for the conservation of Floreana's terrestrial vertebrates. Plans currently exist to implement an invasive rodent and feral cat eradication program using a rodenticide bait containing the second-generation anticoagulant brodifacoum (Island Conservation, 2013). Brodifacoum is highly toxic to mammals and birds; therefore, it could represent a serious threat for non-target wildlife species through primary or secondary exposure pathways (Broome, et al., 2014; Rueda et al., 2016). Fisher, Campbell, and Castaño (2017) identified that Floreana lava lizards and Floreana leaf-toed geckos were at low risk (island population may be reduced by up to 10%) if exposed to brodifacoum, in agreement with a previous study showing low toxicity of this rodenticide in the fence lizard *Sceloporus occidentalis* (Weir et al., 2015), as well as findings from previous successful invasive rodent eradications implemented in the Galápagos Islands (Rueda et al., 2019). However,

However, as both species are endemic to Floreana and are considered evolutionary significant units (Young, 2016), mitigation actions (e.g. captive holding *in situ* or translocation from satellite islets) should be considered if the invasive rodent and feral cat eradication program is to be implemented. Thus, knowing the genetic affinities among lizard populations of Floreana and surrounding islets is of great importance for their conservation. Moreover, if a future goal is to recover Floreana's extirpated endemics using the closest genetic counterparts, then reintroduction strategies should consider translocating individuals from islets containing populations recently (Pleistocene) connected with Floreana.

The aim of this study was to test the hypothesis that haplotypes of Floreana lava lizards and Floreana leaf-toed geckos from Floreana are more similar to those from Champion than they are to those from Gardner, thereby supporting the Pleistocene paleogeographic model. If the invasive rodent and feral cat eradication

program on Floreana Island is implemented, our results will help identify the most appropriate risk mitigation action for both lizard species.

Materials and Methods

Sampling and laboratory protocols

We sampled tail tips of 29 specimens of the Floreana lava lizard and 33 of the Floreana leaf-toed gecko from Floreana (n = 10 and 10, respectively), Champion (n = 9 and 10), and Gardner (n = 10 and 13) islands in August 2018. In addition to the newly generated DNA sequences, we obtained from GenBank sequences of taxa belonging to the same radiations as *M. grayii* and *P. baurii*, with *M. occipitalis* and *P. reissii*, respectively, as outgroups (Benavides, Baum, Snell, Snell, & Sites, 2009; Torres-Carvajal et al., 2014). Lava lizards were captured by noosing or by hand, while geckos were captured by hand. Samples were stored in Longmire buffer. Floreana island (173 km²) lies in the south of the Galápagos archipelago; the islet of Champion (0.094 km²) lies 700 m NE of Floreana, whereas Gardner (0.81 km²) lies 8 km E of Floreana (Fig. 1). Maximum sea depth between Floreana and these islets is 50 m and 200 m, respectively.

We generated novel DNA sequences of NADH dehydrogenase subunit 2

(ND2) for lava lizards and NADH dehydrogenase subunit 4 (ND4) for leaf-toed geckos (Genbank accession numbers to be provided) following published protocols (Kizirian, Trager, Donnelly, & Wright, 2004; Torres-Carvajal, Barnes, Pozo-Andrade, Tapia, & Nicholls, 2014).

Species limits and phylogeography

The species status of both lava lizards and geckos from Floreana and surrounding islets is strongly supported by morphological evidence (Van Denburgh, 1912; van Denburgh & Slevin, 1913; but see Discussion). To further test that populations from Floreana and surrounding islets represent the same species, we examined species limits of both *M. grayii* and *P. baurii* with phylogenetic analyses and genetic distances. We assume that monophyly and genetic distances relative to closely related species are strong criteria for delimiting species. Sequences were assembled and aligned in Geneious v9.1.8 (<http://www.geneious.com/>) under default settings for MAFFT (Algorithm: Auto, Scoring Matrix: 200PAM/k=2, Gap open penalty: 1.53, Offset value: 0.123; Katoh & Standley, 2013). Alignments were verified by translating sequences into amino acids to check for misplaced stop codons. After partitioning each dataset (i.e., *Microlophus* and *Phyllodactylus*) by codon position, we conducted maximum likelihood analyses using RAxML-HPC2 v.8.2.9 with a heuristic search that included 100 random addition replicates and 1000 thorough bootstrap pseudo-replications. We applied the GTR+GAMMA model to all partitions. Analyses were performed using the CIPRES platform (Miller, Pfeiffer, & Schwartz, 2010). The phylogenetic trees were visualized and edited using FigTree v1.4.2 (Rambaut, 2014). In addition, for each dataset we calculated uncorrected genetic distances in MEGA 7 (Kumar, Stecher, & Tamura, 2016) to address genetic differentiation within *M. grayii*, *P. baurii*, and their corresponding radiations.

The relationship among haplotypes from Floreana, Champion and Gardner was evaluated for both lizard species by inferring TCS networks (Templeton, Crandall, & Sing, 1992) in PopART (Leigh & Bryant, 2015). This method has been used widely with nucleotide sequence data to infer population level genealogies when divergences are low (Georgiadis et al., 1994; Gerber & Templeton, 1996; Vila et al., 1999). Because haplotype networks can be misleading in the presence of missing data (Joly, Stevens, & van Vuuren, 2007), two datasets were analyzed for each species: one including all sampled sequences, and the other removing those sequences containing significantly more undefined states than others.

Results

Phylogeny and genetic differentiation

Both *Microlophus* and *Phyllodactylus* phylogenetic trees are similar in that (1) they support the monophyly of the two study species, (2) the individuals from Gardner, Champion and Floreana form three separate clades, respectively, and (3) Champion and Floreana clades are closely related (Fig. 2). The only exception

to (2) and (3) is the only individual of *M. grayii* (AY625170) from Black Beach (Floreana), which is sister to the Champion clade. *M. grayii* is moderately supported (BS [bootstrap support] =62) as sister to a clade containing a paraphyletic *M. albemarlensis* (Isabela and Fernandina) and *M. pacificus* (Pinta). *P. baurii* is weakly (BS=40) supported as sister to a clade containing *P. galapagensis* (Santa Cruz), a paraphyletic *P. maresi* (Santiago), *P. simpsoni* (Isabela), and *P. duncanensis* (Pinzon).

Interspecific genetic distances (ND2) among species of the ‘*Microlophus* Western Radiation’ (Benavides et al., 2009) vary between 0.038–0.149 (average=0.094, SD=0.031), whereas intraspecific distances for *M. grayii* are 0.036 on average. Within *M. grayii*, the largest distances are between samples from Gardner and those from Floreana and Champion combined (0.068 on average). Similarly, interspecific genetic distances (ND4) among species of the ‘*P. galapagensis* Radiation’ (Torres-Carvajal et al., 2014) vary between 0.049–0.155 (average=0.108, SD=0.032), whereas intraspecific distances for *P. baurii* are 0.014 on average. Within *P. baurii*, the largest distances are between samples from Gardner and those from Floreana and Champion combined (0.026 on average).

Phylogeography

The inferred networks show a close genetic similarity between Floreana and Champion populations of both lizard species. In contrast, samples from Gardner are markedly different (Fig. 3). For lava lizards, the full dataset resulted in a single haplotype each for Floreana (including the single specimen from Black Beach), Gardner and Champion; where Champion and Gardner are separated from Floreana by one and 23 mutations, respectively. After excluding identical sequences (five from Gardner— QCAZ 16769, 16770, 16773, 16776, 16781—and three from Champion— QCAZ 16760, 16762, 16764), two haplotypes each for Floreana (Black Beach and Post Office Bay) and Champion are recovered; the single Gardner haplotype is separated from the others by at least 41 mutations. For leaf-toed geckos, the full dataset resulted in four haplotypes for Floreana, one for Champion, and three for Gardner, which are separated from other haplotypes by a minimum of 11 mutations. Haplotypes from Floreana are separated from Champion’s haplotypes by at least three mutations. After excluding identical sequences (two from Champion— QCAZ 16704, 16707—, one from Gardner— QCAZ 16719), the leaf-toed gecko network is only different in that Gardner’s haplotypes are separated from other haplotypes by at least 12 mutations.

Discussion

Evolution and biogeography of Floreana’s lizards

Concordant phylogeographic patterns across codistributed species with similar dispersal abilities suggest that those species share historical biogeographic factors (Avice, 2000). In this study we show that two species of phylogenetically distant lizards share the same geographic pattern of genetic differentiation. Haplotypes from Floreana are significantly more similar to those from Champion, the closest islet with shallow water between, than they are to the more distant Gardner with deeper water between (Fig. 3). This genetic pattern was already noticed for *M. grayii* by Benavides et al. (2009). Pleistocene contact between Floreana and Champion (Fig. 1) most likely allowed gene flow between what are now separate populations of both lava lizards and leaf-toed geckos. Thus, our results support the model that Pleistocene changes in sea level had a strong influence on the evolution of land-locked vertebrates by allowing contact among populations from different islands during glaciation periods (Ali & Aitchison, 2014; Geist et al., 2014; Poulakakis et al., 2012). In contrast, lava lizard and leaf-toed gecko populations in Gardner have been isolated longer, possibly since they colonized this islet, which explains their strong genetic differentiation (Fig. 3). Although we have no data on the time of colonization of Gardner by any lizard species, exposed lava dating suggests that Gardner is at least one million years old (Geist et al., 2014); both lava lizard and leaf-toed gecko colonizers were present in the archipelago at that time (Benavides et al., 2009; Torres-Carvajal et al., 2014). The different haplotypes found in this study, as well as maximum sea depth (200 m), and long distance (8 km) between Gardner and Floreana suggest that Gardner’s populations have been isolated long enough to allow strong genetic differentiation through genetic drift. This is not a novel phenomenon for Galapagos organisms, as drift in isolation was identified as the main cause of genetic differentiation in populations of *M. albertmalensis*

on 12 islets that were connected to Santa Cruz island during the late Pleistocene (Jordan & Snell, 2008). Similarly, pronounced drift was reported in populations of Floreana’s mockingbirds (*Mimus trifasciatus*) from Champion and Gardner (Hoeck et al., 2010).

Genetic differentiation between islands or islets in the Galapagos for a suite of species with limited dispersal ability is observable due to the rarity of overwater dispersal, which reduces the chances of gene flow, and the time that has elapsed since colonization or separation of populations through sea level rise. Rafting dispersal in the Galapagos is less frequent because tree coverage is generally thin, with only sparse vegetation along coastal areas, and there are no rivers capable of transporting rafts to the sea (Ali & Aitchison, 2014). However, during El Nino or other severe flooding events, large logs and trees can be seen washed out to the ocean, and now floating debris like plastics are becoming more common. As in other archipelagoes, such as Cape Verde, the Canaries and the Mascarenes, reptile assemblages in the Galapagos have resulted mostly by intra-archipelago colonization and subsequent anagenesis (Ali & Meiri, 2019). Morphological (Van Denburgh, 1912) and molecular (this study) evidence suggest that populations of geckos in Gardner are the same species as in Floreana. However, our knowledge on the evolution of Galapagos leaf-toed geckos (Benavides et al., 2009; Kizirian et al., 2004; Torres-Carvajal, Rodriguez-Guerra, & Chaves, 2016) suggests that Gardner populations could become different species in the near ‘evolutionary’ future (assuming no extinction and limited or no gene flow). If this were true, we would be documenting a case of speciation at the earliest stages of separation of descendant lineages (de Queiroz, 2011). By contrast, the population of lava lizards from Gardner shows a greater genetic differentiation. Genetic distances between Gardner’s and Floreana + Champion’s populations (0.068 on average) overlap the range of interspecific distances within the Western Radiation clade (0.038–0.149). No morphological differences between specimens of lava lizards from Floreana and surrounding islets have previously been recognized (van Denburgh & Slevin, 1913); however, preliminary analyses suggest otherwise (O. Torres-Carvajal unpublished data). Thus, a closer examination of specimens of *M. grayii* from Floreana and surrounding islets is underway to clarify their taxonomic status.

Conservation Implications

One of the most conspicuous examples of ‘defaunation in the Anthropocene’ (Dirzo et al., 2014) is the extinction of Galapagos giant tortoises, such as *Chelonoidis abingdoni*, which became extinct with the death of Lonesome George in 2012. As in other islands, most species extinctions within the Galapagos have been caused by humans (Steadman, Stafford, Donahue, & Jull, 1991). Holocene vertebrate extinctions in Floreana, an island about the size of Washington DC with the most local extinctions in the Galapagos, include two species of reptiles, and 11 birds (Dvorak et al., 2017; Island Conservation, 2013; Steadman, 2006).

Eradication of invasive mammals from islands has proved to be an important tool to prevent extinctions, recover endangered populations, and restore ecosystem processes (Rueda et al., 2019; Russell & Holmes, 2015). As such the Galapagos National Park has eradicated dozens of invasive species populations around the archipelago (Campbell et al., 2013; Carrion et al., 2007; Carrion et al., 2011). Upcoming invasive mammal eradication projects include Floreana Island (Island Conservation, 2013) to prevent extinctions of endemic species such as the Medium tree-finch (*Camarhynchus pauper*), Floreana lava lizard, and Floreana leaf-toed gecko. Unfortunately, invasive mammal eradication efforts may result in non-target species mortality; therefore, it is important to identify appropriate mitigation actions (e.g., captive holding *in situ*, translocation) for species at higher risk, or species for which any risk is unacceptable. In this context, populations unexposed to the eradication process that are identified as the same evolutionary significant unit (ESU) as exposed populations are a great source for future reintroductions if necessary. Our results show that both lava lizard and leaf-toed gecko populations from Champion can be considered the same ESU as populations from Floreana (Fig. 3). Champion lacks introduced mammals and will therefore not be included in Floreana’s eradication program. Consequently, populations of lava lizards and leaf-toed geckos from Champion can be considered as safeguard populations for both species. Finally, if one of the goals is to use a population that was likely part of the same ESU, Champion or other islets with possible Pleistocene land connections to Floreana might be good sources for reintroducing other terrestrial organisms, such as

the racer snake *Pseudalsophis biserialis*, which became extinct in Floreana over a century ago. Nonetheless, we recognize that other factors like the viability of source populations (e.g., Bozzuto, Hoeck, Bagheri, & Keller, 2017) should also be considered as part of any reintroduction plan (Robert et al., 2015).

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Data Accessibility Statement

DNA sequences: Genbank accessions will be provided upon acceptance of manuscript.

Author Contributions OTC and PAC designed the research and wrote the manuscript; PAC and FM obtained tissue samples; OTC obtained DNA sequences and analyzed data.

Figure captions

Figure 1. Map of Floreana and surrounding islets showing study sites. Bathymetric contour lines delimit depths of 50 m (lighter blue), 100 m, 150 m, 200 m and >200m (darker blue). Inset map shows location of Floreana within the Galapagos archipelago.

Figure 2. Maximum likelihood gene trees of *Microlophus* (left) and *Phyllodactylus* (right) from the Galapagos Islands. Numbers next to branches correspond to bootstrap support values; for clarity, numbers in short branches are not shown. Species names followed by voucher (new sequences) or Genbank accession number and collection island are presented. Specimens from Floreana are in red, Champion in magenta, and Gardner in blue.

Figure 3. TCS networks of Floreana's lava lizards (A, B) and leaf-toed geckos (C, D) based on mitochondrial genes ND2 and ND4, respectively. Networks A and C include all sampled sequences, whereas B and D

exclude sequences containing significantly more undefined states than others as calculated by PopART (Leigh & Bryant, 2015). Numbers next to branches are number of mutations among haplotypes. Black circles are inferred haplotypes. Localities in Floreana are Black Beach (BB), Puerto Velasco Ibarra (PVI) and Post Office Bay (POB).





