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RESEARCH ARTICLE

Floreana Island re-colonization potential of the Galápagos short-eared owl (*Asio flammeus galapagoensis*)

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Abstract Non-native invasive species threaten Galápagos' endemic biodiversity, and increasing efforts are underway to protect its species from further harm. One such project is focused on the eradication of invasive rodents using rodenticide bait on Floreana, the archipelago's sixth largest island. Short-eared owls (*Asio flammeus galapagoensis*) that consume poisoned rodents will, therefore, be at risk of secondary poisoning. If negatively impacted, it is not known to what degree the Floreana Island short-earned owl population is isolated, and whether potential re-colonization exists from its closest neighboring large population on Santa Cruz. Based on eight microsatellite loci and mtDNA control region sequence data from museum and contemporary samples, the short-eared owl populations on Floreana and Santa Cruz are not isolated from each other. However, gene flow is asymmetric from Floreana to Santa Cruz and not in the opposite direction. Morphometric data, including tarsus and bill size, and behavioral observations corroborate the genetic results and suggest that the Floreana population may possess unique traits compared to neighboring populations. For example, Floreana short-eared owls are more crepuscular than neighboring islands, which are predominately nocturnal, and were also non-responsive to inter-island call back recordings. Therefore, these results have important management implications concerning short-eared owl persistence on Floreana following rodenticide application. We recommend that managers implement additional precautions to protect the short-eared owl population until the risk of secondary poisoning has passed such as maintaining individuals in captivity. This study provides no evidence to suggest that short-eared owls are likely to disperse from Floreana's closest large population on Santa Cruz if the local population is negatively impacted by rodenticide exposure, and the observed morphological and behavioral traits argue against translocating owls between islands.

Keywords Galápagos Short-eared Owl · Asio flammeus · Floreana Island · Santa Cruz · Conservation · Galápagos

Introduction

A high proportion of species extinctions recorded since 1400 have occurred on islands (68% of mammal and 82% of bird extinctions) and many have been attributed to invasive species (Bellard et al. 2016). The impact of non-native invasive species on ecosystems in general is a major threat

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to biodiversity (Vitousek et al. 1997; Ricciardi 2007; Ehrenfeld 2010), and therefore, their eradication is advocated as a useful tool for conserving global biodiversity (Jones et al. 2016). A minimum of 1224 successful non-native species eradications on 808 islands worldwide have been documented, the majority (87%) of which have been vertebrate eradications (Glen et al. 2013). More recent attempts to eradicate invasive species have focused on larger islands that also possess higher levels of biodiversity. Globally, invasive rats have been eradicated from over 447 islands (Russell and Holmes 2015). Current rodent eradication methods for large islands rely on the second-generation anticoagulant brodifacoum, a broad-spectrum toxicant that is not species specific (Campbell et al. 2015). Consequently, the likelihood that eradication methods for non-native species may negatively impact endemic taxa exists, and care should be taken to identify a priori at-risk taxa so that any potential negative impacts can be addressed by managers prior to the eradication event.

The Galápagos Islands are an oceanic archipelago located approximately 1000 km west of mainland Ecuador with a rich history of biodiversity research. Non-native invasive species have been introduced to the islands and are considered a major threat to Galápagos' endemic terrestrial biodiversity (Phillips et al. 2012). Feral ungulate populations have caused massive destruction of native vegetation, and feral cats, rodents, and dogs have depredated many naïve island endemics (Phillips et al. 2012), together leading to extirpations of multiple Galápagos endemic species (Steadman 1986).

There is a relatively long history of invasive species eradication campaigns in the Galápagos Islands (Phillips et al. 2012). Large-scale campaigns have successfully removed feral goats, pigs, and donkeys from multiple islands (Cruz et al. 2005; Carrion et al. 2007, 2011). While a few recent efforts have successfully removed rats from several small islands throughout the archipelago (Campbell et al. 2013; Rueda et al. 2016), feral cats and invasive rodents remain on many of the larger islands. Plans currently exist to initiate the eradication of invasive rodents (*Rattus rattus* and *Mus musculus*) and feral cats in 2018 from Floreana Island, the sixth largest island in the Galápagos (17,125 ha; Fig. 1). The eradication effort will involve aerial application of rodenticide bait containing the second-generation anticoagulant

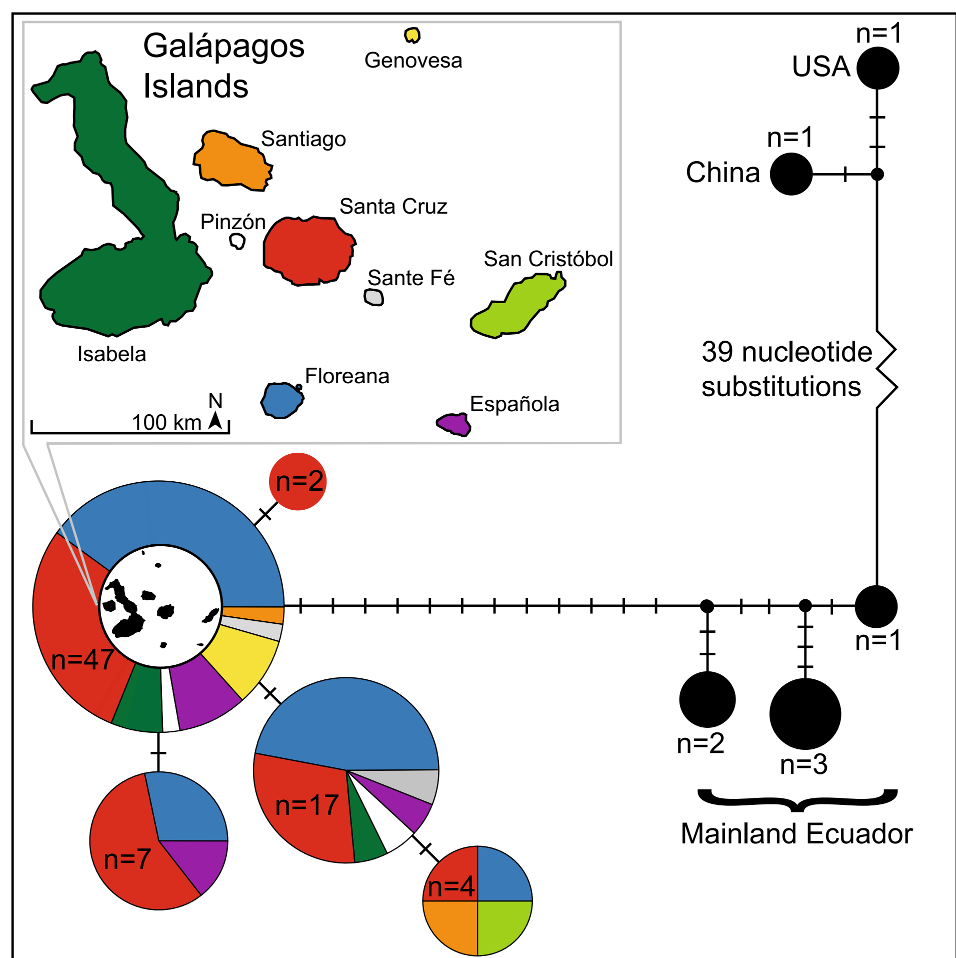

Fig. 1 Median-joining haplotype network based on short-eared owl mtDNA control region domain I haplotypes. *Circles* represent unique haplotypes and the size of the *circle* corresponds to the number of individuals sharing a haplotype (sample size also indicated). For Galápagos Island samples, *colors* correspond to geographic location sampled and correspond to the map. Nucleotide substitutions indicated with *hash marks* or stated

brodifacoum (Island Conservation 2013). However, the Galápagos short-eared owl (*Asio flammeus galapagoensis*) is the only native raptor currently on Floreana Island, and has been identified as a species at high risk from secondary exposure to rodenticide (Fisher and Campbell 2015). Further management actions are therefore required if secondary poisoning is to be avoided.

The Galápagos short-eared owl is currently distributed on multiple islands in the Galápagos, and it is unknown if any island populations are isolated from neighboring islands. The aim of this study was to determine the genetic distinctiveness of the short-eared owl population on Floreana Island based on mitochondrial (mtDNA) control region sequence data, and further quantify the level of connectivity (or gene flow) between neighboring island populations using microsatellite DNA loci. The results of this study have important management implications by providing information concerning the natural re-colonization potential of short-eared owl to Floreana from neighboring islands if negatively impacted by rodenticide exposure.

Methods

Sampling and DNA extraction

Museum samples

Toe pad tissues were obtained from a total of 37 short-eared owl specimens from three museum collections (California Academy of Sciences, San Francisco, CA; American Museum of Natural History, New York, NY; and the Museum of Comparative Zoology, Cambridge, MA; Online Appendix 1). The samples originated from 31 Galápagos short-eared owls from nine islands collected originally between 1891 and 1933 throughout the Galápagos archipelago, and six short-eared owls from mainland Ecuador (*A. f. bogotensis*) between 1913 and 1922 (see online Online Appendix 1).

To reduce the potential for contamination with contemporary samples, genomic DNA from toe pad samples was extracted in batches of ten or fewer samples in a facility exclusively reserved for ancient DNA work at the University of North Texas. Filtered pipette tips were used throughout the extraction procedure, and blank extraction controls were included with each subset, all of which were negative following polymerase chain reaction (PCR).

Contemporary samples

Short-eared owls were sampled on Santa Cruz (n = 26), Plaza Sur (satellite of Santa Cruz, n = 1; grouped with Santa Cruz samples hereafter), Floreana (n = 23), and Isabela (n = 3) Islands (Fig. 1; Table 1) between May 2014 and March 2015. All trapped birds were banded with a unique alphanumeric color band and morphometric data were collected, which included bill length, bill height, bill width, and tarsus length. Blood was collected by puncturing the metatarsal vein with a 24-gauge syringe, and stored in 2.0 ml sterile tubes with Queen's lysis buffer (Seutin et al. 1991) until DNA extraction. In addition, we opportunistically sampled fresh muscle tissue from a salvaged short-eared owl in north Texas, USA (subspecies *A. f. flammeus*). DNA was extracted from blood and fresh tissue samples using QIAGEN DNeasy Blood and Tissue kit according to manufacturer's protocol.

Sequencing and genotyping

Mitochondrial DNA

Mitochondrial ND6 (NADH dehydrogenase subunit 6), tRNA-Glu and control region domain I and a portion of domain II were amplified using PCR with primers N1 and D16 (Barrowclough et al. 1999). Each 10 µL reaction contained 2 µL of extracted DNA and final concentrations of 1× GoTaq Flexi PCR Buffer, 1.5 mM $MgCl_2$, 1.0 µM each primer, 0.4 mM total dNTPs, and 0.1 µL of GoTaq Flexi DNA Polymerase (Promega). Following a 2 min 94 °C hot start, each PCR consisted of 35 cycles of 94 °C (30 s), 50 °C (30 s), and 72 °C (60 s), followed by a 10 min final extension at 72 °C. In addition, we amplified mtDNA ND2 (NADH dehydrogenase subunit 2) for the short-eared owl sampled in Texas and a subset of 11 contemporary Galápagos samples (Santa Cruz, n = 6; Floreana, n = 5) using primers L5219 and H6313 (Sorenson et al. 1999). Each 25-µL reaction contained 2 µL of extracted DNA

Table 1 Genetic diversity indices for sampled short-eared owl subspecies and populations based on 433 bp of mitochondrial control region

	n	S	h	Hd	π
A. f. flammeus	2	3	2	1.000 (0.500)	0.007 (0.003)
A. f. bogotensis	6	8	3	0.733 (0.155)	0.010 (0.002)
A. f. galapagoensis	77	4	5	0.575 (0.052)	0.002 (0.000)
Floreana	29	3	4	0.552 (0.079)	0.002 (0.000)
Santa Cruz	27	4	5	0.652 (0.083)	0.002 (0.000)
Española	6	2	3	0.600 (0.215)	0.002 (0.001)
Isabela	4	1	2	0.500 (0.265)	0.001 (0.001)
Genovesa	4	n/a	1	n/a	n/a

n number of individuals sampled, *S* number of segregating sites, *h* number of haplotypes, *Hd* haplotype diversity, *π* nucleotide diversity, n/a insufficient polymorphism to calculate

Standard deviation indicated in parentheses

and final concentrations of 1× GoTaq Flexi PCR Buffer, 2.5 mM $MgCl_2$, 0.5 µM each primer, 0.4 mM total dNTPs, and 0.25 µL of GoTaq Flexi DNA Polymerase. Following a 2 min 94 °C hot start, each PCR consisted of 35 cycles of 94 °C (15 s), 55 °C (20 s), and 72 °C (60 s), followed by a 10 min final extension at 72 °C.

The mitochondrial control region was sequenced in both directions for all contemporary samples using a nested primer approach with L16758 modified from Sorenson et al. (1999) for short-eared owl (see Online Appendix 2) and D16. In addition, we sequenced ND6 and tRNA-Glu for 11 contemporary Santa Cruz individuals using the N1 primer and ND2 in both directions with the same primers used for PCR reaction. Mitochondrial samples were sequenced using ABI BigDye Terminator chemistry and run on an ABI 3130xl automated sequencer.

The contemporary mtDNA control region sequences were used to design internal primer sets (Online Appendix 2) for amplifying aDNA samples of smaller fragment size. Primers were designed using Primer3web version 4.0.0 (Koressaar and Remm 2007; Untergrasser et al. 2012) to produce short (i.e., 200–250 bp) overlapping (i.e., by 20–30 bp) sequences that collectively covered the mitochondrial control region domain I and a portion of domain II.

During PCR preparation with aDNA samples, we followed precautions to prevent contamination with contemporary DNA (Shapiro and Hofrieter 2014). Specifically, PCR reactions were prepared in a facility designated for aDNA and in a laminar flow hood that was exposed to UV light for 10+ minutes and cleaned with a bleach solution and/or soapy water before and after each use. Additionally, PCRs were performed in small batches ($n \leq 6$ samples), each in individual PCR tubes. Blank controls were included in all reaction sets.

PCR reactions contained 4 µL of extracted aDNA in a final volume of 25 µL. Reactions had final concentrations of 1x High Fidelity PCR Buffer, 2.0 mM $MgSO_4$, 0.5 µM each primer, 0.8 mM total dNTPs, and 0.1 µL of Platinum Taq DNA Polymerase High Fidelity (Life Technologies). Following a 2 min 94 °C hot start, each PCR consisted of 40 cycles of 94 °C (15 s), primer specific annealing temperature (30 s; see Online Appendix 2), and 68 °C (60 s), followed by a 5-min final extension at 68 °C. Some of the more highly degraded samples required PCR with all 10 overlapping primer sets, while, for less degraded samples, forward and reverse internal primers were paired to create longer fragments.

Corresponding mtDNA regions were also obtained from the complete mtDNA genome for a short-eared owl sampled from a specimen collected at the Jiuhua Mountain Airport in Anhui Province, China (*A. f. flammeus;* Zhang et al. 2015; GenBank Accession: NC_027606.1).

Microsatellite DNA

We screened contemporary samples for polymorphism at sixteen microsatellite loci using primer sets previously shown to amplify *A. f. flammeus* (i.e., SneA012, SneA004, SneA127, Oe3-7.1, Oe054.1, Oe128.1, BUOW16.1, FEPO43.1, and HRU2, Dial et al. 2012; Oe053, Tgu06, GgaRBG18, TG13-016-Tal, BOOW19-Tal, TG04-061, and FEPO42-Tal; Klein et al. 2009).

Each 10-µL reaction contained 2 µL of extracted DNA and final concentrations of 1× GoTaq Flexi PCR Buffer, 2.5 mM $MgCl_2$ (2.0 mM for Tgu06 and 3.0 mM for TG13-016-Tal and BOOW19-Tal), 0.5 µM each primer, 0.4 mM total dNTPs, and 0.1 µL of GoTaq Flexi DNA Polymerase (Promega). Following a 3-min hot start at 94 °C, each PCR consisted of 34 cycles of 94 °C (30 s), specified annealing temperature (30 s), and 72 °C (30 s), followed by a 10-min final extension at 72 °C. Annealing temperatures were 52 °C (GgaRBG18), 53 °C (SneA127, Oe3-7.1, Oe054.1, and Oe128.1), 58 °C (Tgu06), and 62 °C (Oe053). The annealing temperature for all remaining loci was 56 °C. We genotyped each amplified product using a 3130xl Applied Biosystems Genetic Analyzer and analyzed results using the program GeneMarker v.1.6 (SoftGenetics).

Statistical Analysis

Mitochondrial DNA

Genetic diversity indices, including number of segregating sites (*S*), number of haplotypes (*h*), haplotype diversity (*Hd*), and nucleotide diversity (π), were calculated for subspecies as well as for islands with four or more samples using DNAsp v. 5.10.01. To visualize the phylogenetic relationships among sampled taxon, we constructed a median-joining haplotype network for the control region sequence data using the program Network v.4.610 (http://www.fluxus-engineering.com; Bandelt et al. 1999). To assess genetic differentiation between subspecies and also between island populations within the Galápagos, pairwise φst using the Tamura and Nei (1993) distance method were calculated in Arlequin v. 3.11 (Excoffier et al. 2005) with 10,000 permutations. Significant population differentiation was assessed using an exact test of sample differentiation with a Markov chain of 100,000 steps in Arlequin v. 3.11. Average pairwise genetic distances between subspecies and between island populations were calculated between groups using the Kimura 2-parameter model in MEGA version 5.10 (Tamura et al. 2011). We calculated standard error using a bootstrap resampling approach with 500 replicates.

For an approximate estimate of divergence time between *A. f. flammeus* and *A. f. galapagoensis*, we employed a strict molecular clock approach based on the number of nucleotide

differences observed in the two ND2 and ND6 mtDNA protein-coding regions and 95% HPD fossil calibrated substitution rate estimates for the corresponding loci reported in Lerner et al. (2011; ND2, 2.4–3.3% per site per million years; ND6, 2.0-2.8% per site per million years).

Microsatellites

We tested microsatellite genotypes for linkage disequilibrium and departure from Hardy Weinberg Equilibrium (HWE) with each population and locus using the program GDA v. 1.1 (Lewis and Zaykin 2001). Sequential Bonferroni corrections were used to correct for multiple simultaneous comparisons (Rice 1989). Percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*), number of private alleles, and observed (H_o) and expected (H_e) heterozygosity were calculated for the Floreana and Santa Cruz populations using GDA. Allelic richness (*AR*) provides an estimate of allelic diversity while controlling for differences in population sample size, and was calculated with FSTAT v. 2.9.3.2 (Goudet 1995; Leberg 2002). Due to small sample size, these diversity measures were not calculated for Isabela Island or Texas samples.

Overall genetic differentiation using global F_{ST} was assessed in Arlequin v. 3.11 (Excoffier et al. 2005) using the method of Weir and Cockerham (1984). Significance was assessed with 10,000 permutations. We calculated two global F_{ST} values with the Galápagos short-eared owl samples with and without the short-eared owl sample from Texas. Only polymorphic loci were used in the estimate (n = 12 and 8 loci with and without the Texas sample, respectively). Pairwise F_{ST} as implemented in Arlequin was calculated to assess genetic differentiation between island populations. Significance of F_{ST} estimates were assessed using 1000 permutations with Fisher's exact test.

Additionally, we used the Bayesian assignment method of Pritchard et al. (2000) as implemented in Structure v. 2.3.2 to estimate population genetic differentiation. This method determines the most likely number of genetically distinct clusters (*K*) based on maximizing HWE and linkage equilibrium among sampled individuals. The method assigns each individual a probability of belonging to a detected cluster. Each simulation was performed 20 times for $K = 1$ to 6 using a burn-in of 100,000 followed by 500,000 iterations while allowing for admixture and an individual alpha for each locus. Analyses were also conducted using the LOCPRIOR model that allows for prior sampling location information to maximize population assignment (Hubisz et al. 2009). The number of genetic clusters was determined using the ΔK method (Evanno et al. 2005) implemented in Structure Harvester web v0.6.94 (Earl and vonHoldt 2012) as well as by comparison of the log likelihood values as proposed by Pritchard et al. (2000). We used the programs Clumpp (Jakobsson and Rosenberg 2007) to compile replicate runs from Structure and Distruct v 1.1 (Rosenberg 2004) to visualize results.

The program BayesAss v. 3.0.3 (Wilson and Rannala 2003) was used with the microsatellite data to calculate contemporary migration rates between sampled populations and proportion of individuals derived from the source population. In this Markov Chain Monte Carlo based program, proposed parameter values for migration rate, allele frequencies, and inbreeding coefficient can be adjusted at each iteration using mixing parameter adjustments. For each of the parameters in the model, mixing rates were adjusted according to user's manual to obtain acceptance rates between 20 and 60%. Specifically, delta values for migration rates, allele frequencies, and inbreeding were 0.15, 0.30, and 0.45. The MCMC method was run for 2×10^7 iterations with a sampling frequency of every 2000 iterations after a burn-in of 1×10^6 iterations. To ensure consistent estimates, we performed ten runs with different starting seeds. To select the run with the best fit, we calculated the Bayesian deviance from the trace file produced by BayesAss, and report the run with the lowest value (Faubet et al. 2007; Meirmans 2014).

Morphometric data

Only data for adults from Santa Cruz (n = 23) and Floreana (n = 17) were included in analysis of morphometric data using SPSS. Levene's test for equality of variance were performed to determine if the variation in bill height, bill width, bill length, and tarsus was different for Floreana and Santa Cruz individuals. Non-parametric Mann–Whitney U tests were performed to determine if differences existed for these morphometric measures between the two island populations.

Results

Mitochondrial DNA

A total of 433 base pairs from the mtDNA control region were obtained for 85 individuals representing *A. f. flammeus* (n = 1), *A. f. galapagoensis* (n = 78), and *A. f. bogotensis* (n = 6). When comparing all samples including the short-eared owl from China (Zhang et al. 2015), a total of 52 variable sites were observed resulting in a total of ten haplotypes (Fig. 1; Online Appendix 1 and 3; GenBank accession numbers: MF769776-MF769784). Of these ten, five control region haplotypes were identified among the sampled Galápagos short-eared owls, four of which were observed in both museum and contemporary samples (Fig. 1; Table 1). The fifth haplotype was shared by two contemporary Santa Cruz samples. Of the ten total identified haplotypes, three haplotypes were identified from the six short-eared owl (*A. f.*

bogotensis) samples collected from mainland Ecuador. The *A. f. flammeus* sample from Dallas, TX and Anhui Province, China (Zhang et al. 2015) were unique, comprising the final two haplotypes. Though haplotypes were shared within subspecies, no control region haplotypes were shared between subspecies (Fig. 1).

Pairwise φ_{ST} values, pairwise genetic distance, and the median-joining haplotype network showed strong differentiation between the three subspecies (Table 2; Fig. 1); however, the φ_{ST} value for *A. f. flammeus* and *A. f. bogotensis* was not statistically significant (Table 2). Within *A. f. galapagoensis*, the majority of the control region haplotypes were shared among several islands, and most islands had three or more haplotypes (Fig. 1). Accordingly, pairwise φ_{ST} values and pairwise genetic distance between islands were low and not significantly different from zero (Table 3), suggesting little mtDNA differentiation among Galápagos short-eared owl populations on different islands.

Only a partial sequence was obtained for ND2 with the Galápagos and Texas samples (880 and 705 base pairs total, respectively; GenBank accession numbers MF769785-MF769786). ND2 sequences were identical among Galápagos short-eared owl samples, and identical between the two birds sampled in Texas and China. A total of 25 nucleotide differences were observed between the two ND2 haplotypes within the overlapping 705 bp sequence. No nucleotide differences were observed between the eleven Galápagos samples for ND6 and adjacent tRNA-Glu region (598 bp; Online Appendix 3; GenBank accession number MF769787), whereas 13 nucleotide substitutions were identified when compared to the short-eared owl sampled in China. Based on a strict molecular clock using mtDNA ND2 and ND6 protein-coding regions (1273 bp total) and their corresponding fossil calibrated substitution rates from Lerner et al. (2011), the two subspecies diverged between 890,000 and 1.7 million years ago using the equation $\mu = L\lambda t$, where μ is the number of nucleotide substitutions between taxa, L is the sequence length in bp, λ is the mean rate of nucleotide substitutions in substitutions per site per year, and t is the time period considered in years.

Table 2 Mitochondrial control region pairwise φ_{ST} (below diagonal) and average Kimura 2-parameter genetic distances (above *diagonal*) between short-eared owl subspecies (*A. f. flammeus*, n = 2; A. *f. bogotensis*, n = 6; *A. f. galapagoensis*, n = 77)

	A. f. flammeus	*A. f. bogotensis*	*A. f. galapagoensis*
A. f. flammeus		0.099 (0.015)	0.106 (0.016)
A. f. bogotensis	0.904 (0.143 ± 0.005)		0.039 (0.008)
A. f. galapagoensis	0.984 (0.001 ± 0.000)*	0.939 (0.000 ± 0.000)*	

P-values with their standard deviation for each pairwise φ_{ST} value are shown in parentheses. Significant φ_{ST} values (alpha < 0.008) are indicated by an *asterisk*. Kimura 2-parameter standard error values are given in parentheses

Table 3 Mitochondrial control region pairwise φst (below diagonal) and average Kimura 2-parameter genetic distances (above diagonal) between Galápagos Island short-eared owl populations

	Floreana (n = 29)	Santa Cruz (n = 27)	Española (n = 6)	Isabela (n = 4)	Genovesa (n = 4)	Santa Fe (n = 2)	Pinzon (n = 2)	Santiago (n = 2)
Floreana		0.002 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.002 (0.002)
Santa Cruz	−0.009 (0.553 ± 0.008)		0.002 (0.001)	0.001 (0.001)	0.001 (0.001)	0.002 (0.001)	0.002 (0.001)	0.003 (0.002)
Española	−0.055 (0.695 ± 0.007)	−0.098 (1.000 ± 0.000)		0.001 (0.001)	0.001 (0.001)	0.002 (0.001)	0.002 (0.001)	0.003 (0.002)
Isabela	−0.146 (1.000 ± 0.000)	−0.120 (1.000 ± 0.000)	−0.178 (1.000 ± 0.000)		0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.002 (0.002)
Genovesa	0.032 (0.691 ± 0.004)	−0.042 (0.846 ± 0.003)	−0.081 (1.000 ± 0.000)	0.000 (1.000 ± 0.000)		0.001 (0.001)	0.001 (0.001)	0.002 (0.002)
Santa Fe	−0.256 (1.000 ± 0.000)	−0.167 (0.715 ± 0.006)	−0.164 (1.000 ± 0.000)	−0.379 (1.000 ± 0.000)	0.385 (0.331 ± 0.001)		0.001 (0.001)	0.002 (0.002)
Pinzon	−0.256 (1.000 ± 0.000)	−.167 (0.703 ± 0.007)	−0.164 (1.000 ± 0.000)	−0.379 (1.00 ± 0.000)	0.385 (0.333 ± 0.002)	−1.000 (1.000 ± 0.000)		0.002 (0.002)
Santiago	0.096 (0.302 ± 0.005)	0.087 (0.353 ± 0.010)	0.095 (0.638 ± 0.002)	−0.018 (0.595 ± 0.003)	0.385 (0.333 ± 0.001)	−0.500 (1.000 ± 0.000)	−0.500 (1.000 ± 0.000)	

P-values ± standard deviation for each pairwise φ_{ST} value are shown in parentheses

No pairwise φ_{ST} values were significant (alpha < 0.002). Kimura 2-parameter standard error values are given in parentheses

Table 4 Genetic diversity indices based on eight polymorphic microsatellite loci in the Floreana and Santa Cruz Galápagos short-eared owl populations

Population	*n*	*P*	*A*	*AR*	H_e	H_o	Private Alleles
Floreana	22.9	0.5	3.4	3.4	0.33	0.34	2
Santa Cruz	27.0	1.0	4.6	4.3	0.48	0.47	12

n number of samples, *P* percentage of polymorphic loci, *A* number of alleles, *AR* allelic richness, H_e expected heterozygosity, H_o observed heterozygosity

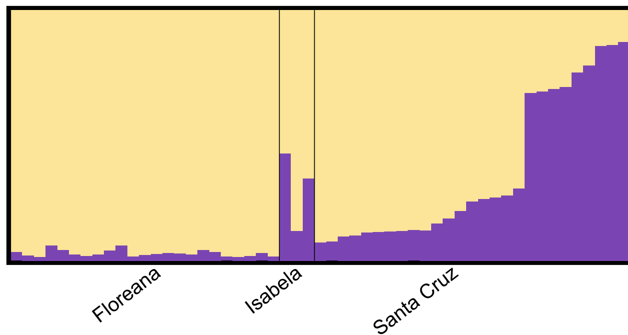

Fig. 2 Bayesian clustering of Galápagos short-eared owls using STRUCTURE based on sample location and $K=2$. *Each bar* represents a single individual sample and presented in groups based on island sample location. The proportion of a *particular color* represents the likelihood of an individual belonging to that cluster

Microsatellites

Loci HRU2 and FEPO42-Tal failed to amplify with the short-eared owl samples. BOOW19 and GgaRBG18 were monomorphic among all samples, while, SneA004, SneA127, BUOW16.1, and TG04-061 were monomorphic among the Galápagos samples. Of the eight loci that were polymorphic among Galápagos samples, TG13-016 possessed a single nucleotide repeat (see also Klein et al. 2009) and SneA012, Tgu06 and Oe054 were dinucleotide repeats (but see Dial et al. 2012). All remaining microsatellite loci were tetranucleotide repeats.

The Floreana short-eared owl population had reduced genetic diversity compared with Santa Cruz based on eight polymorphic loci (Table 4). Two private alleles were observed in the Floreana population, and twelve private alleles in Santa Cruz (Table 4). In contrast to the mtDNA control region analyses, the global F_{ST} among Galápagos Island populations based on microsatellite data using eight polymorphic loci was low/moderate at 0.029, but statistically significant ($p=0.011 \pm 0.001$). Pairwise F_{ST} was significant between Floreana and Santa Cruz ($F_{ST}=0.023$, $p=0.009 \pm 0.001$) and between Floreana and Isabela ($F_{ST}=0.119$, $p=0.009 \pm 0.001$), but not between Santa Cruz and Isabela ($F_{ST}=0.008$, $p=0.410 \pm 0.004$).

Table 5 Migration rates among Floreana and Santa Cruz Galápagos short-eared owl population using the program BAYSASS

	Migration from:	
Migration into.	Floreana	Santa Cruz
Floreana	**0.986 (0.958–1.000)**	0.014 (0.000–0.042)
Santa Cruz	0.310 (0.236–0.344)	**0.690 (0.656–0.723)**

Values shown are means of the posterior distributions of migration rates, m, into each population and their 95% credible intervals in parentheses from the analysis with the lowest calculated Bayesian deviance (10 runs total). Values along the diagonal in bold are the proportion of individuals derived from the source population each generation

STRUCTURE analyses identified two genetic clusters (K = 2) based on the log likelihood values and Evanno et al. 2005 ΔK method (Fig. 2). All Floreana individuals were assigned with high likelihood to a single genetic cluster. However, multiple individuals from Santa Cruz were also assigned to the same cluster as the Floreana birds, with the remaining individuals identified as either admixed or to a second genetic cluster.

Migration estimates from each of the ten BayesAss runs were highly consistent, with estimates deviating by ≤0.01 between runs. Further supporting the results obtained from the STRUCTURE analyses for Santa Cruz, the results from BayesAss indicated that the Floreana and Santa Cruz short-eared owl populations were not isolated, but possessed an asymmetric migration rate from Floreana into Santa Cruz (mean = 0.310, 95% credibility interval 0.236–0.344; Table 5). The migration rate from Santa Cruz into Floreana was negligible and not significantly different from zero (mean = 0.014, 95% credibility interval 0.000–0.042).

Morphometric data

Significant differences existed for several morphometric measurements between adult short-eared owls from Floreana (n = 17) and Santa Cruz (n = 23; Fig. 3; Table 6). Bill length (Mann–Whitney test, $U=100$, 2 sided $p=0.009$), width ($U=90.5$, 2 sided $p=0.004$), and height ($U=31.5$, 2 sided $p<0.001$) were significantly different between the two island populations. These patterns can be partly explained

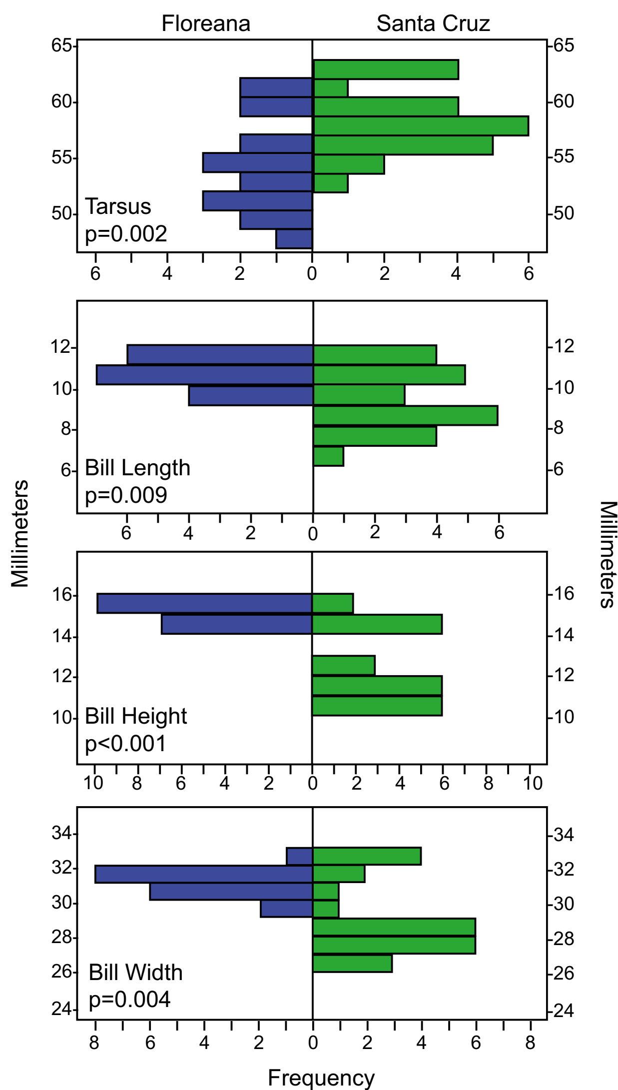

Fig. 3 Frequency size distributions for tarsus, bill length, bill height, and bill width for sampled adult Galápagos short-eared owls on Floreana (n = 17) and Santa Cruz (n = 23). Mann–Whitney test *P*-values (alpha < 0.05) for differences in size for each measurement between island populations

Table 6 Bill measurements (mean ± SD) for Galápagos short-eared owls sampled on Floreana (n = 17) and Santa Cruz (n = 23)

	Floreana	Santa Cruz
Length (mm)	30.8 ± 0.74	28.7 ± 2.11
Width (mm)	10.7 ± 0.83	9.1 ± 1.66
Height (mm)	15.2 ± 0.48	12.2 ± 1.93

due to significantly less variation observed among individuals on Floreana compared to Santa Cruz for bill length (Levene's test for equality of error variance; $F = 20.4$, $p < 0.001$), width ($F = 15.2$, $p < 0.001$) and height ($F = 40.0$, $p < 0.001$). Although tarsus length was significantly different between islands with larger tarsi among Santa Cruz individuals (Mann–Whitney test, $U = 307$, 2 sided $p = 0.002$), the observed variation on the two islands was not significantly different ($F = 2.7$, $p = 0.107$); Fig. 3.

Discussion

While the Galápagos short-eared owl has been observed on most of the major islands in the Galápagos archipelago with the exception of Wolf (Harris 1973; De Groot 1983), this is the first study conducted to determine if any island populations are distinct or connected by gene flow. Genetic and morphometric evidence presented here provide no evidence to suggest that contemporary immigration of short-eared owls to Floreana exists from its closest neighboring large population in Santa Cruz. Specifically, Floreana individuals represent a single unique genetic cluster, while Santa Cruz possessed a mixed population with individuals assigned to either Floreana or a second genetic cluster, or identified as admixed individuals (i.e., assigned to both clusters; Fig. 2). Because of logistical constraints and lack of prior information about short-eared owl long-distance movements between islands within the Galápagos, our sampling was limited to Floreana, Santa Cruz, and Isabela. Therefore, it remains possible that dispersal exists between Floreana and other unsampled islands.

The above conclusion is largely based on results generated from nuclear microsatellite loci because the mtDNA control region sequence data lacked sufficient resolution to investigate population genetic structure. While fixed mtDNA haplotype differences were observed among the three sampled short-eared owl subspecies, four of the five mtDNA haplotypes identified for the Galápagos subspecies were shared among multiple islands, including Floreana (Fig. 1). This pattern may be due to dispersal events between islands, but incomplete lineage sorting and ancestral polymorphism are also likely to account for the observed pattern where more slowly evolving gene regions (i.e., mtDNA) can persist for longer periods of time among isolated populations compared to genomic regions with higher mutation rates (i.e., microsatellites). Due to the comparatively slower mutation rate compared to microsatellites, mitochondrial DNA has been the marker choice for phylogeography studies for investigating historic demographic processes that have shaped genetic structure (e.g., Nicholls and Austin 2005; Ursenbacher et al. 2006; Höglund et al. 2009), whereas microsatellites are preferred for population genetic studies investigating demographic patterns such as gene flow and population size (e.g., Delaney et al. 2010). Discrepancies between mtDNA and microsatellite results are not uncommon in studies with

recently divergent taxa or populations (e.g., Johnson et al. 2003, 2007; Farrington et al. 2014).

Interestingly, contemporary migration estimates based on microsatellite data using the program BayesAss indicate asymmetric gene flow between Floreana and Santa Cruz. Although the migration point estimates should be treated with caution (see Meirmans 2014), the Floreana short-eared owl population may serve as a source population for dispersing individuals, but has received few if any individuals in return, at least from Santa Cruz. In fact, a male short-eared owl that was banded as an adult in August 2014 on Floreana, was observed the following May on Isabela, more than 50 km away immediately west of Santa Cruz (D. Mosquera pers. obs.; see Fig. 1). Floreana short-eared owl displayed reduced microsatellite genetic diversity compared with Santa Cruz. Increased habitat isolation or fragmentation is associated with increased genetic structure and reduced genetic diversity across bird populations (e.g., Segelbacher et al. 2003; Lindsay et al. 2008). These results further support that the contemporary Floreana population has received few, if any, immigrants from its nearest neighboring large population on Santa Cruz.

Other studies indicate that some movement of short-eared owls between islands does occur. For example, short-eared owl pellets collected on Daphne Major contained prey item remains from animals (e.g., cuckoos, mice, rats, wading birds, and a finch) that do not live on that island, suggesting that they were obtained elsewhere, possibly the islands of Baltra or Santa Cruz, both approximately 8 km from Daphne Major (Grant et al. 1975). Further, Harris (1973) stated that short-eared owls were commonly seen on small islands with nesting seabirds, yet the owls were not observed breeding or raising young on those islands suggesting transient individuals. However, records of long-distance movements are rare. A single observation exists from the early 1970s that documented a short-eared owl banded on Sante Fe that was recaptured on Floreana, ~68 km to the southwest (Grant et al. 1975 cited De Vries pers. comm.).

While the above cases indicate that short-eared owls can move between islands, it is not known how often such events occur. The microsatellite data reported here suggest that at least the contemporary migration rate from Santa Cruz to Floreana is negligible. More research is needed to explore the frequency of short-eared owl dispersal between other islands not sampled for this study, and identify the demographic or environmental factors that may stimulate dispersal events between islands. Other studies investigating adaptive radiations among bird species within the Galápagos archipelago suggest a predominant colonization direction from south to north in agreement with wind-based dispersal models (e.g., Galápagos mockingbirds, *Nesomimus* spp.; Arbogast et al. 2006). A predominant south to north colonization pattern is in agreement with our genetic data indicating asymmetric dispersal from Floreana to Santa Cruz in the north.

The morphometric differences observed between Floreana and Santa Cruz short-eared owl adults also supports the conclusion that asymmetrical dispersal exists between the two islands. Specifically, the Floreana population had significantly smaller tarsi than the Santa Cruz population and possessed significantly reduced variation in bill sizes for all measurements (i.e., length, width, and height). Despite possessing an overlapping range of bill sizes, those on Floreana were skewed toward a larger size compared to the measurements obtained from the Santa Cruz short-eared owl population (Fig. 3). Therefore, these results provide no evidence to suggest that owls disperse from Santa Cruz to Floreana given the lack of smaller bill size variation on Floreana. Similarly, Floreana displayed reduced microsatellite genetic diversity compared with Santa Cruz. As other studies have found that increased isolation or fragmentation is associated with increased genetic structure and reduced genetic diversity across bird populations (e.g., Segelbacher et al. 2003; Lindsay et al. 2008), these results further suggest that the contemporary Floreana population has received few, if any, immigrants from its nearest neighboring large population in Santa Cruz.

These results were further supported based on field observations when sampling owls for this study. Early attempts to attract and capture short-eared owls on Floreana were less frequent than on Santa Cruz while using recorded calls obtained from the Santa Cruz population. In contrast, however, recordings made subsequently from the Floreana population were much more successful in attracting owls to the traps on the same island, suggesting that differences may exist in call characteristics between the two islands. Grant and Grant (2002) have shown that song divergence has developed between finch species on different islands in the Galápagos, which has likely reduced interisland breeding frequency. For example, in several Darwin's finch species (*Geospiza* spp.), individuals are significantly more aggressive when responding to playback recordings from conspecifics on the same island compared to those from different islands (Ratciliffe and Grant 1985; Grant and Grant 2002) or even from different geographic locations on the same island (Podos 2007). Although vocal development in short-eared owls may be innate rather than learned (Clark 1975; Wiggins et al. 2006) in contrast to Darwin's finches (see Goodale and Podos 2010), vocalizations used during owl courtship (Wiggins et al. 2006) may be important for maintaining genetic differentiation between islands if inter-island movement exists. A more systematic investigation using a reciprocal design based on behavioral responses to call recordings and a more detailed sonogram comparison is warranted to explore how vocalizations may differ among short-eared owl populations.

Furthermore, differences in short-eared owl hunting behavior among Galápagos Islands may also support reduced breeding opportunities between individuals originating from different islands. First, short-eared owl hunting behavior differs on islands depending on the presence of two other native raptors, the Galápagos hawk (*Buteo galapagoensis*) and barn owl (*Tyto alba*; De Vries 1973; De Groot 1983). On islands where all three species occur (Isabela, Santiago, Santa Cruz), the Galápagos hawk is the dominant diurnal predator, the barn owl is mostly nocturnal, and the short-eared owl is present in relatively small numbers and also nocturnal (De Vries 1973; De Groot 1983; Bollmer et al. 2006). Similarly, on islands where the barn owl is absent (Pinta, Marchena, Pinzon, Santa Fe, and Española), the short-eared owl is strictly nocturnal and aggressively attacked by the Galápagos hawk during daylight hours (De Vries 1973; Bollmer et al. 2006; David Anderson pers. comm.). On islands where the Galápagos hawk and barn owl are both absent (Floreana, Plaza Sur), the short-eared owl is crepuscular (De Groot 1983; Paula Castaño, pers. obs.). It is not known if the observed behavioral differences among short-eared owl populations have resulted in morphological differences, but recent studies with over 500 non-passerine species, including the short-eared owl and other owl species, have documented a correlation between daily activity patterns (i.e., diurnal, crepuscular, or nocturnal) and eye shape and other eye morphology indices (Lisney et al. 2012; Hall and Ross 2007). Such findings indicate that a genetic mechanism may underlie the timing of daily behavior patterns in birds due to differences in eye morphology. Whether this pattern holds within species, such as among island populations of Galápagos short-eared owl, is unknown and deserves further investigation.

The Galápagos Islands are replete with examples of species evolution. Multiple bird species on different islands in the Galápagos show varying degrees of genetic, morphologic, and behavioral differentiation. For example, four mockingbird species (*Mimus* spp.) are each endemic to a different island in the Galápagos (Arbogast 2006; Hoeck et al. 2010), and one of the species, the Galápagos mockingbird (*M. parvulus*), has six recognized subspecies, all of which are endemic to a particular island or islands with levels of population genetic differentiation distributed in an isolation by distance pattern (Hoeck et al. 2010). Levels of divergence among five Galápagos ground finch species (*Geospiza* spp.) differ based on geographic location within the archipelago. Ground finch species showed strong genetic distinction among centrally located islands, but differed on peripheral islands where co-occurring species possessed near-complete admixture (Farrington et al. 2014). The sharp-beaked ground finch (*Geospiza difficilis*) was shown to represent a distinct ground finch species (Farrington et al. 2014) possessing genetic differences correlated with both geographic distance and morphological differentiation (Grant et al. 2000). The Galápagos hawk has also been shown to possess significant genetic differentiation (Bollmer et al. 2005), morphological differences, and even mating system variation (i.e., monogamous and polyandrous populations) among islands (Bollmer et al. 2003). Similarly, morphological differentiation exists among island populations of Galápagos dove (*Zenaida galapagoensis*) despite documented dispersal between islands (Santiago-Alarcon et al. 2006).

Conservation implications

The results of this study suggest that the Galápagos short-eared owl population on Floreana does not receive immigrants from its closest neighboring large population on Santa Cruz, yet dispersal does exist in the opposite direction from Floreana to Santa Cruz. The Santa Cruz population also possessed a larger number of private alleles and a significantly larger variation in bill sizes compared to the Floreana population further supporting a pattern of asymmetric dispersal. Therefore, until additional evidence suggests otherwise, we recommend that conservation management treat the contemporary Floreana short-eared owl population as a distinct population receiving very few immigrants from other island populations.

Consequently, these results suggest that natural immigration from Santa Cruz is unlikely to occur if the local population on Floreana is reduced in size from secondary exposure to rodenticide used for invasive rodent eradication. Until more work is completed to further investigate the unique behavioral and vocalization observations made during this study, we also recommend that managers not translocate short-eared owls from Floreana to neighboring islands or vice-versa. Future efforts should be made to sample the short-eared owl population on Española to investigate connectivity with Floreana. However, the Galápagos hawk population on Española appeared isolated compared to other island populations to the north such as Santa Fe and Santiago based on mtDNA and minisatellite data (Bollmer et al. 2006), and is absent on Floreana. Therefore, we recommend that an adequate number of owls from Floreana be kept in captivity until the risk of secondary poisoning has passed (i.e., ≥2 years; Rueda et al. 2016), similar to efforts made with the Galápagos hawk when rodenticide was used to remove invasive rodents on Rábida, Bartolomé and Bainbridge islands (Campbell et al. 2013).

The results of this study also have important taxonomic implications for the short-eared owl species complex further highlighting the importance of protecting locally adapted populations within the Galápagos. Globally, ten short-eared owl subspecies have been identified based on differences in morphology and plumage characteristics, but this is the first study to investigate genetic differentiation and divergence

between subspecies. Strong genetic differentiation was documented for the three sampled subspecies (*A. f. flammeus, A. f. bogotensis*, and *A. f. galapagoensis*). In agreement with previous research, the taxonomy of this group deserves revision with several subspecies elevated to species status (e.g., Hoffman et al. 1999; Garrido 2007), particularly the Galápagos subspecies due to distinct differences in diet, morphology, plumage, and behavior (Beebe 1926; DeGroot 1983, Johnsgard et al. 1988; Garrido 2007; König and Weick 2008).

Based on an approximate divergence estimate using a strict molecular clock approach with two mtDNA protein coding genes (ND2 and ND6), the Galápagos short-eared owl subspecies, *A. f. galapagoensis*, diverged between 890,000 and 1.7 million years ago from its common ancestor with the much broader geographically distributed nominate subspecies *A. f. flammeus* (North America, Europe, north Africa and Asia). The estimated divergence between the two short-eared owl subspecies is much greater than the divergence between the Galápagos hawk and its sister taxon, the Swainson's hawk (*B. swainsoni*, 51,000-254,000 ybp; Bollmer 2006). With that said, however, additional divergence estimates are required between the Galápagos short-eared owl and geographically proximate subspecies such as those distributed on mainland South America (*A. f. bogotensis, A. f. pallidicaudus*, and *A. f. suinda*).

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