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GENETIC DIVERSITY AND DIVERGENCE OF ENDANGERED GALÁPAGOS AND HAWAIIAN PETREL POPULATIONS[1]

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Abstract. The genetic diversity and divergence of populations of Galápagos and Hawaiian Petrel (*Pterodroma phaeopygia* and *sandwichensis*, respectively) were investigated using allozyme electrophoresis. Within the Galápagos Islands, *P. phaeopygia* samples were monomorphic at 12 of 13 loci. The Hawaiian population *P. sandwichensis* was monomorphic at all 13 loci. One fixed allelic difference was found between *P. phaeopygia* and *P. sandwichensis*. Eleven loci were fixed for the same allele in both populations. Our results indicate that there has been no recent gene flow between Galápagos and Hawaiian Petrels, but gene flow occurs among Galápagos populations of *P. phaeopygia*. The existence of a unique genetic variant discriminating Galápagos and Hawaiian Petrels, in addition to previously documented morphological and behavioral differences, supports the recent elevation of these two taxa to species status.

Key words: Pterodroma, *Dark-rumped Petrel, Galápagos Petrel, Hawaiian Petrel, allozyme, allele frequency.*

Pterodroma petrels breeding in the Galápagos and main Hawaiian archipelagos present taxonomic and conservation difficulties. Until recently, the populations in these two archipelagos were considered subspecies of the Dark-rumped Petrel, *P. phaeopygia phaeopygia* in Galápagos and *P. p. sandwichensis* in Hawaii (Warham 1990). However, Sibley and Monroe (1993) used geographical separation and morphological and behavioral differences to elevate these taxa to species status. This change in taxonomy, if generally accepted, would have implications for the conservation of these populations. We provide molecular data to complement the existing non-molecular information regarding taxonomic status of these groups. Given the current taxonomic uncertainty, we refer to the *Pterodroma* complex subsuming these Galápagos and Hawaiian populations as "Dark-rumped Petrels," collectively.

Within the Galápagos, breeding colonies of Dark-rumped Petrels are known to exist on the islands of Floreana, Santiago, Santa Cruz, San Cristobal (Cruz and Cruz 1987a), and Isabela (F. Cruz, unpubl. data). Although they occur on a number of Hawaiian Islands, albeit at low densities, only the Maui population has been studied (Harris 1970). These archipelagos are separated by ~5,000 km of open water. All breeding populations have been seriously reduced as a result of predation by introduced mammals (Simons 1984, Cruz and Cruz 1987a) and destruction of nesting habitat (Cruz and Cruz 1987a, 1987b). Although predator control programs have had some success in both Hawaii and the Galápagos (Simons and Whittow 1989, Tomkins and Milne 1991), the low reproductive rate of Dark-rumped Petrels has slowed recovery of these populations. Even under ideal circumstances it would take the Hawaiian population over a century to double (Simons and Whittow 1989). Because of their small population sizes and endangered status, determining the genetic relationships among breeding colonies of the Dark-rumped Petrel is a critical part of developing an effective conservation strategy.

In contrast to the traditional view that Dark-rumped Petrels from the two archipelagos are subspecies (Warham 1990), phenotypic divergence and the possibility of genetic isolation suggest a higher level classification. Tomkins and Milne (1991) found the Galápagos Dark-rumped Petrels to be 16% larger than Hawaiian birds, with significant differences in culmen length, bill depth, tarsus length, wing length, and tail length. The population in Hawaii has shorter, wider bills, and larger total wing area and wing loading (Simons 1985). Plumage markings also distinguish the Hawaiian and Galápagos populations. The variable black markings found on the foreheads of the Galápagos birds are

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lacking on the Hawaiian birds (Tomkins and Milne 1991). Tomkins and Milne (1991) also found a larger difference between the calls (sonograms) from Hawaii and the Galápagos than among those from different islands in the Galápagos archipelago. Smaller differences in morphology and calls also differentiate island populations within the Galápagos (Cruz and Cruz 1987b, Tomkins and Milne 1991). These differences are consistent with at least partial genetic isolation among the different islands, and especially between archipelagos. However, environmental effects remain an alternative explanation for some of these patterns.

Pitman (1982) proposed that the Hawaiian and Galápagos populations may be socially and genetically continuous between archipelagos because birds from different islands have been observed to join mixed species foraging flocks. The proximity of the breeding colonies also indicates a high likelihood of gene flow among the Galápagos populations. However, nine years of banding and recovery within the Galápagos indicate complete natal island fidelity (Cruz and Cruz 1987b, 1990, Tomkins and Milne 1991). Even movement between colonies on the same island is rare.

Based on this evidence, Tomkins and Milne (1991) and Cruz and Cruz (1990) raised the possibility that island populations of Dark-rumped Petrels are genetically distinct, and Sibley and Monroe (1993) reclassified the group into two separate species. However, none of the evidence points conclusively to genetic divergence. Banding studies of breeding adults, which suggested the lack of interchange among islands, were most likely conducted on pairs which had already established nesting sites. Such birds are not as likely as prebreeding subadults to disperse to a new island (Cruz and Cruz 1990). Furthermore, the observed phenotypic differences could be due to environmental differences. We analyzed variation in protein allozyme frequencies to test the hypotheses of genetic divergence of Hawaii and Galápagos populations, and of populations within the Galápagos archipelago.

METHODS

Blood samples were collected from nestlings which were found in their burrows on Floreana, Santa Cruz, and Santiago (Galápagos, Ecuador), and on Haleakala Crater (Maui, Hawaii) (Table 1). We extracted birds from their burrows and drew a blood sample of ~1 cc with a hypodermic syringe from the brachial vein of the wing. We also sampled quill tissue from Santa Cruz only (Marsden and May 1984, Browne et al. 1993) by removing one growing feather from each bird. Blood and quill samples were each placed into a separate microfuge tube on dry ice for shipment to the laboratory at Wake Forest University. Tissues were kept at −70° C until analyzed. When the blood samples were thawed, multiple sample tabs were made from each to minimize the need to thaw and refreeze the blood again. Continuous (Tris-Borate-EDTA) or discontinuous (Poulik's) buffers were used, depending upon the resolution of each locus. The techniques employed followed standard electrophoretic procedures after Selander et al. (1971). After the allozymes were separated by electrophoresis, the gels were sliced and stained for the following allozymes: Alcohol dehydrogenase (ADH) EC 1.1.1.1; Creatine kinase (CK) EC 2.7.3.2; Cytosol aminopeptidase (CAP) EC 3.4.11.1; Esterase (EST) EC 3.1.1.–(Colorimetric); Glucose-6-phosphate dehydrogenase (G6PDH) EC 1.1.1.49; Glucose-6-phosphate isomerase (GPI) EC 5.3.1.9; Hemoglobin (HGB) (no EC no.); Isocitrate dehydrogenase (IDH) EC 1.1.1.42; Lactate dehydrogenase (LDH) EC 1.1.1.27; Malate dehydrogenase (MDH) EC 1.1.1.37; Peptidase (PEP) EC 3.4.___ using leucylalanyl/phenylalanyl-leucine as substrates; Phosphoglucomutase (PGM) EC 5.4.2.2; Sorbitol dehydrogenase (SDH) EC 1.1.1.14; Superoxide dismutase (SOD) EC 1.15.1.1; and Xanthine dehydrogenase (XDH) EC 1.1.1.204. Attempts to adequately resolve 15 additional presumptive loci were unsuccessful.

TABLE 1. Percent of loci polymorphic (P), percent average individual heterozygosity (H), and sample sizes (n) for Hawaiian and Galápagos populations of *Pterodroma*.

Island	P(%)	H%	n
Hawaii			
Maui	0	0	32
Galápagos			
Santa Cruz	7.7	0.85	18
Floreana	0	0	32
Santiago	0	0	3
Total for:			
Galápagos	7.7	0.29	53
Hawaii and Galápagos	15.4	0.18	85

Chi-square tests were used to assess conformance of genotypic frequencies to Hardy-Weinberg expectations. Wright's weighted F-statistic F_{ST} (Wright 1978, Nei 1986) was used as one measure of genetic differentiation among populations. The significance of F_{ST} was determined by the procedures described in Workman and Niswander (1970; see also Chesser 1983). A second measure of genetic differentiation among populations used Nei's unbiased genetic identity (Nei 1977).

RESULTS

Of the 15 loci examined, one (EST) was scorable only in blood samples, two (ADH and CAP) only in quill samples, and 12 in both blood and quill samples. Because quill tissue was obtained only from individuals on Santa Cruz, data from ADH and CAP were not included in the calculations. The PEP locus was identified using two different amino acid substrates (phe-leu and leu-ala), which resolved in identical positions and are presumed to represent the same locus. Of the 13 loci examined in blood samples, 11 were monomorphic for all 85 birds sampled. The 11 monomorphic loci were: CK, EST, HGB, IDH, LDH, MDH, G6PDH, PGM, SDH, SOD, and XDH. One locus, PEP, exhibited a fixed allozymic difference between the collective Galápagos populations (slow allele) and the Hawaiian population (fast allele). Results are summarized in Table 1.

The Hawaiian population was monomorphic at all loci. The collective Galápagos populations were mono-

morphic at 12 loci, with only the GPI locus polymorphic, due to two heterozygotes from the Santa Cruz population. For the Santa Cruz population, the slow and fast allele frequencies were 0.944 and 0.055, respectively. GPI, the only locus with heterozygotes, conformed to Hardy-Weinberg expectations ($P > 0.9$). *F*-statistics estimate the amount of genetic differentiation among populations; F_{ST} for the PEP locus was 1.00, and was significantly different from zero ($\chi^2_3 = 157.6$, $P < 0.001$). Nei's unbiased genetic identity (*I*) for the Hawaii collective-Galápagos comparison was 0.92, with *D* (genetic distance) equal to 0.08.

DISCUSSION

An F_{ST} value of 1.00 for the PEP locus is statistically highly significant, indicating genetic separation between Hawaiian and collective Galápagos populations. A broader estimate, based on numerous loci, is Nei's genetic distance (*D*), which is estimated from our data to be 0.08 for the Hawaiian vs. collective Galápagos populations, indicating a lower level of genetic differentiation than indicated by the F_{ST} values. *D* values among the Aves are known to be conservative (Sheldon and Bledsoe 1993, Avise 1994) with speciation often marked by *D* values of 0.05 or less (Zink 1982, Corbin 1983). The relatively large *D* value and fixed allozymic difference between the Hawaiian and Galápagos populations support the species status recently assigned to the Hawaiian population (*P. sandwichensis*) by Sibley and Monroe (1993).

Our results resemble those found for seven populations of the Spotted Owl (*Strix occidentalis*). Barrowclough and Gutíerrez (1990) found no genetic variation at 23 loci in six populations from Oregon and California. At one locus there was a major allele frequency difference between the Pacific coast populations (*S. o. caurina* and *S. o. occidentalis*) and the allopatric taxon (*S. o. lucida*). Their F_{ST} value of 0.55, based on a single polymorphic locus, while not as high as the 1.00 value found in this study, is large by avian standards. They attribute the paucity of variation to a small overall effective population size or past bottlenecks, and believe that the two allopatric populations have long been isolated and probably represent two species. This interpretation is equally applicable to our data on *Pterodroma* petrels.

As with the Spotted Owl, the data presented here strongly suggest that there is currently no gene flow between the Hawaiian and Galápagos populations of *Pterodroma* petrels. The Galápagos and Hawaiian populations apparently have independent evolutionary trajectories and consequently should be considered separate species within the "evolutionary species concept" (*sensu* Wiley 1978). Within the "biological species concept" (e.g., Mayr 1970), the genetic data show that the populations are not in contact. Given the relatively high frequency of hybridization between avian species (Grant and Grant 1992), it is not unlikely that the Galápagos and Hawaiian populations are capable of hybridizing if their breeding grounds were sympatric. However, the genetic evidence documents that the populations are reproductively allopatric. Within the framework of the "phylogenetic species concept" (Cracraft 1983), the Galápagos and Hawaiian populations can be diagnosed by the presence of a slow or fast PEP allele, respectively. Thus, the genetic data, in addition to the previously described morphological and behavioral differences, suggest that the Hawaiian and Galápagos populations should be regarded as separate species by these widely recognized species concepts.

The effort and cost of protecting the remaining Galápagos and Hawaiian Petrel nests are large relative to the resources available. One motivation for our study was to answer Tomkins' (1985) call for a scheme to prioritize different populations for protection. Our results suggest the following:

(1) The Hawaiian and collective Galápagos populations contain unique gene pools, and that loss of either population will result in a loss of genetic variability.

(2) The Hawaiian and collective Galápagos populations are discrete demographic units, with interchange between archipelagos currently absent. If one population is lost, and threats at that location are subsequently removed, natural recolonization from the other population is unlikely.

(3) This study evaluated a relatively small number of loci, and most of the loci showed no variation. As a result, our ability to rank the Galápagos populations in terms of genetic diversity is limited. Our data offer a suggestion that the Santa Cruz population contains more genetic variability than does the Floreana population, but the data are not sufficient to warrant a prioritization on that basis. Additional information (e.g., mtDNA and/or microsatellite analyses or additional allozyme loci) is needed before firm conclusions can be made regarding the genetic diversity of the collective Galápagos populations.

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INTRA- AND INTERSPECIFIC SEQUENCE VARIATION IN A PORTION OF THE MITOCHONDRIAL ND6 GENE IN CUCKOOS[1]

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Abstract. A 474 base pair segment of the NADH dehydrogenase subunit 6 (ND6) mitochondrial gene was sequenced for four cuckoo species (family Cuculidae). To assess the potential of this little-studied gene for intra- and interspecific genetic analyses, we examined sequence variation between (1) individuals from sympatric populations of the Common Cuckoo (*Cuculus canorus canorus*) collected in Great Britain, (2) two subspecies of Common Cuckoo (*C. c. canorus* and *C. c. telephonus*) collected at the geographical extremes of this species' range, and (3) four representative Cuculids: the Common Cuckoo, the Rusty-breasted Cuckoo (*Cacomantis sepulcralis*), the Fan-tailed Cuckoo (*Cacomantis flabelliformis*), and Klaas's

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