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# Lonesome George is not alone among Galápagos tortoises

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The loss of biological diversity continues at an astounding rate, recently punctuated by the reported extinction of the Baiji or Yangtze River Dolphin (*Lipotes vexillifer*) [1]. The conservation landscape is dotted with similarly charismatic species that have become flagships for protection because of their increasing rarity. Although a miniscule fraction of biological diversity is at risk, flagship species effectively demonstrate the critical status of an ever-increasing number of taxa worldwide, potentially justifying the disproportionate amount of conservation resources directed towards their protection. Lonesome George, “the rarest living creature” according to the Guinness World Records, is perhaps the most renowned. The apparent sole survivor of the *Geochelone abingdoni* species of giant Galápagos tortoises from Pinta Island, Lonesome George is a potent conservation icon with much publicity surrounding the search for a mate [2,3]. Here, we report finding an individual of Pinta ancestry in a population on Volcano Wolf on neighboring Isabela Island.

Volcano Wolf harbors two populations, Puerto Bravo (PBR) and Piedras Blancas (PBL), of *G. becki*, one of the eleven extant species of Galápagos tortoises (Figure 1A) [4,5] (see Supplemental data available on-line with this issue for more details of the taxonomic history). Unlike other species that exhibit distinctive

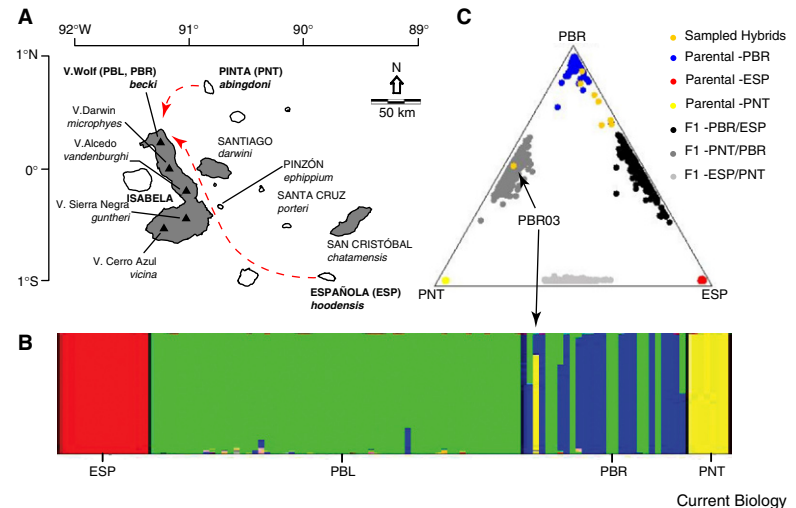


Figure 1. Giant tortoises of the Galápagos.

(A) Distribution of giant tortoises in the Galápagos archipelago. Shaded islands indicate presence of extant tortoise populations and italicized names indicate current taxonomic designations [4,5]. Island names are capitalized with triangles representing volcanoes on Isabela Island. Bold names designate the primary populations of focus in the current study. Red arrows highlight direction of transport/colonization consistent with the observed pattern of hybridization revealed for individual PBR03. (B) A STRUCTURE bar plot indicating the genetic composition of the principal populations in the current study, highlighting the mixed ancestry recovered in the PBR population. The analysis was run according to parameters specified in the supplemental data for all extant populations in Galápagos, but, for the purposes of display, only the clustering of the principal populations are shown above. Colors represent the relative contribution of each of four genetic partitions recovered from the data for each individual (column) in each sampled population. Population acronyms are as in (A). (C) A STRUCTURE triangle plot revealing patterns of clustering of simulated parental and F1 genotypes for all possible pairwise comparisons involving the Volcano Wolf Puerto Bravo (PBR), Pinta (PNT) and Española (ESP) populations. Colors for the parental populations are as in (B), with simulated F1s according to the legend. Clustering of the eight observed PBR individuals with the *G. hoodensis* (ESP)-like mtDNA haplotype (orange) are overlaid on top of the simulated parental and F1 distributions. The likely PNT/PBR F1 hybrid (PBR03) is indicated by arrows in (B) and (C).

domed versus saddle-backed carapace morphology, the PBR population displays relatively high morphological diversity [6]. This diversity is consistent with molecular studies that revealed a complex history for the PBL and PBR populations on Volcano Wolf, including individuals with extremely divergent mitochondrial (mt)DNA haplotypes that are more similar to haplotypes found in other species than to those from the populations in which they were sampled [7]. The PBR population exhibits the highest proportion of individuals with divergent haplotypes: eight of 27 individuals have mtDNA haplotypes closely related to the sole haplotype from *G. hoodensis* on Española Island (Figure 1A) [7]. This non-native mtDNA haplotype differs by 27–30 substitutions from the

endemic *G. becki* haplotypes in PBR. Likely shaped by both natural and human-mediated dispersal [7], the degree of nuclear introgression represented within these PBR individuals with divergent mtDNA haplotypes and their relationships to the former inhabitants of Pinta have never been assessed. To date, the ability to detect Pinta ancestry has been precluded by the lack of genotypic information regarding historical population allele frequencies from this locality.

We explored the complex evolutionary history of the *G. becki* PBR population on Isabela Island by analyzing variation at ten nuclear microsatellite loci relative to a genotypic database including 354 individuals from all extant populations of Galápagos tortoises [8]. The nearly extinct *G. abingdoni* on Pinta was included

in this reference population database for the first time by way of genotypic data collection from six museum specimens. Bayesian clustering revealed a widespread pattern of mixed ancestry in the PBR population (Figure 1B and Supplemental data). Of particular note, one of eight PBR individuals with a *G. hoodensis* (Española)-like haplotype (PBR03) exhibited a strong signature of *G. abingdoni* ancestry and an assignment to the Pinta population ( $q$ -value = 0.743; Figure 1B). The other seven assigned to the PBR population from which they were collected ( $q$ -values  $\geq 0.994$ ).

The triangle plot in Figure 1C depicts a fine-scale examination of the history of mixed ancestry in the PBR population, obtained through  $q$ -value distributions of 500 simulated genotypes each of parental, F1 hybrids, F2 hybrids, and B2 and B3 backcrosses for all pairwise comparisons between *G. becki* (PBR, Isabela), *G. hoodensis* (Española), and *G. abingdoni* (Pinta). PBR03 falls in the center of the PBR–Pinta F1  $q$ -value distribution (Figure 1C). Combined with the results from previous mtDNA analyses [7], these data suggest a hybrid origin of PBR03 resulting from a mating between a *G. becki* female from PBR with a *G. hoodensis* (Española)-like haplotype and a male from Pinta. Unfortunately PBR03 is a male. Our results also indicate that the seven additional PBR individuals with the divergent mtDNA haplotype are most likely at least second-generation backcrosses of PBR–Española F1s to the resident PBR population on Isabela Island.

The detection of Pinta ancestry on Volcano Wolf on Isabela Island provides evidence that Lonesome George is not the only living descendent of *G. abingdoni*. The identification of eight individuals of mixed ancestry among only 27 individuals sampled (estimated Volcano Wolf population size 1,000–2,000) [9] suggests the need to mount an immediate and comprehensive survey of the PBR population to search for additional individuals of Pinta

ancestry. Given the failure of Lonesome George to reproduce despite considerable efforts over the past 30 years, it is clear that recovery of the genetically unique *G. abingdoni* will require identification of Pinta-native genotypes elsewhere; our data indicate that this goal may now be attainable. In the event that additional individuals of Pinta ancestry are discovered on Volcano Wolf or among the large number of individuals of unknown origin in captivity [10], an interactive *in situ* and *ex situ* conservation and repatriation program may be enacted for species recovery. This conservation strategy has had demonstrated success for a similarly imperiled sister species (*G. hoodensis*) [11], providing a model program for guiding future management of *G. abingdoni*. More generally, our results highlight the importance of historical DNA analysis, simulation approaches and reference population databases for reconstructing evolutionary patterns, revealing cryptic diversity, and informing conservation management.

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#### Supplemental data

Supplemental data are available at <http://www.current-biology.com/cgi/content/full/17/9/R317/DC1>

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