

their sperm. As mentioned above, if sperm were not adapted to survive (sperm longevity) inside the female's reproductive tract, the female would be limited in her ability to maintain sperm. Males may face a trade-off between sperm competitive ability (fast-swimming sperm) and ability to be stored (long-lived and slow-swimming sperm). Sperm pre-adaptations for storage include a decreased sensitivity to sperm activation specifically, acrosomal reaction desensitization, and the presence of antioxidative enzymes. Also commonly observed and apparently important for storage is a decrease in sperm motility. In addition to changes to the spermatozoa, males may produce chemicals that increase sperm viability during their stay in the female's reproductive tract. The notion that mate choice only occurs prior to copulation has been rejected for well over 40 years. However, the complexity of post-copulatory sexual selection and the potential for sexual conflict may be even greater than currently appreciated if the time-frames over which events occur are lengthened by weeks, months or even years through the storage of sperm. Sperm storage remains an exciting area for future research, including the refinement of associated terminology as well as studies relating to co-evolution.

#### Where can I learn more?

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## Correspondence

### Genetic rediscovery of an 'extinct' Galápagos giant tortoise species

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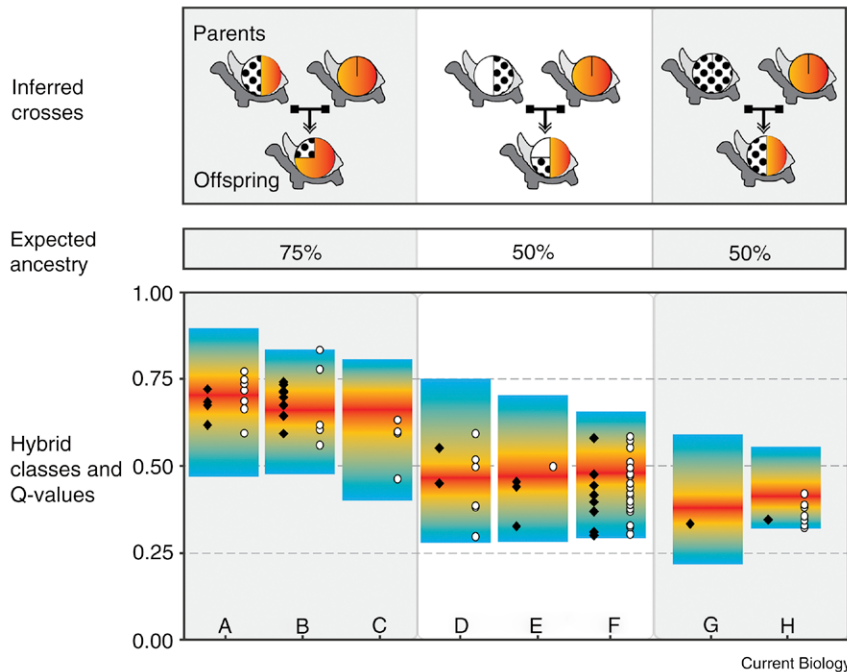
Genes from recently extinct species can live on in the genomes of extant individuals of mixed ancestry. Recently, genetic signatures of the giant Galápagos tortoise once endemic to Floreana Island (*Chelonoidis elephantopus*) were detected within eleven hybrid individuals of otherwise pure *Chelonoidis becki* on Volcano Wolf, Isabela Island [1]. Movement of tortoises between islands by pirate and whaling ships was not uncommon during the 1800s [2], representing a likely mechanism by which individuals from Floreana were translocated to northern Isabela, despite being presumed extinct soon after Charles Darwin's historic voyage to the Galápagos Islands in 1835. These eleven hybrid individuals with *C. elephantopus* ancestry were thought to be the last genetic vestiges of a unique evolutionary lineage in the wild. Here, we report that reproductively mature purebred tortoises of the recently 'extinct' *C. elephantopus* from Floreana Island are very likely still alive today, as identified and tracked through the genetic footprints left in the genomes of very recent hybrid offspring on Volcano Wolf. If found, these purebred *C. elephantopus* individuals could constitute core founders of a captive breeding program directed towards resurrecting this species.

Alerted by our previous discovery of hybrid individuals [1,3], we returned to Volcano Wolf, sampled its tortoise population intensively (1669 individuals, approximately 20% of

the estimated current population), and screened them for genetic variation using fast-evolving nuclear DNA markers (12 microsatellite loci). Each tortoise was then assigned to one or more parental gene pools by comparison to a genetic database of all extant and extinct Galápagos tortoise species. Genetic marker-based assignments can be challenging, however, in systems with a history of hybridization. In such cases, hybridization generates offspring with genomes that include parts of both parental gene pools, and subsequent crosses between hybrids and purebreds, or between two hybrids, lead to mosaic-like genomes. Indeed, there is an ever-increasing role of chance in shaping the genetic make-up of second-generation hybrids. To account for this complexity, we used computer simulations to determine the range of possibilities for the genetic make-up of hybrid tortoises resulting from an array of different hybridization scenarios that may be occurring on Volcano Wolf. Having quantified this inherent variability, we were able to identify hybrid tortoises as well as determine the most likely parental cross that generated them (Supplemental information).

We determined that the genotypes of 84 Volcano Wolf tortoises result from hybridization events that involved a purebred *C. elephantopus* as one of the immediate parents (Figure 1). Moreover, these events were very recent — 30 of the 84 tortoises are less than 15 years old (Supplemental information). Given the documented lifespan of Galápagos tortoises of more than 100 years, there is a good chance that purebred *C. elephantopus* tortoises are still alive. The minimum number of equally contributing *C. elephantopus* founders needed to produce the same genetic diversity observed in the 84 hybrids was 38, as estimated via founder genome equivalents (Supplemental information). Theoretically, 20 or more founder genome equivalents are the approximate genetic base necessary for a viable *ex situ* population [4]. Consequently, our findings offer hope for an attempt at species recovery via captive breeding.

Of the 84 hybrids identified from patterns of nuclear genetic



**Figure 1.** The parental gene pools of 84 Galápagos giant tortoise hybrids. Top panel: Genetic make-up of sampled hybrids for which one of the inferred parents is a purebred *Chelonoidis elephantopus* (pie charts show proportion of ancestry; orange: *C. elephantopus*, white or spotted: other gene pools). Middle. Expected proportion, on average, of the nuclear genome with alleles sourced from *C. elephantopus*. Bottom panel: Membership coefficients (Q-values) in *C. elephantopus* simulated for the eight hybridization classes (A–H; Supplemental information) where one of the parents is a purebred *C. elephantopus*. Colored gradients show simulated distributions (red: median, yellow: 50<sup>th</sup> quantile, upper and lower blue: maximum and minimum, respectively) with empirical data overlaid (solid diamonds and white circles are individuals with or without *C. elephantopus*-like mtDNA, respectively). This figure shows only the simulated Q-value range ( $Q_R$ ), which was used in conjunction with a second criterion (Q-value differences,  $Q_D$ ) to classify ‘unknown’ Volcano Wolf tortoises (Supplemental Information).

variation as being immediate descendents of a purebred *C. elephantopus*, 26 of these also had *C. elephantopus*-like mitochondrial DNA (mtDNA). Thus, despite being hybrids, these individuals are of high conservation value given that both biparentally- and maternally-inherited genetic markers from the imperiled tortoise species are represented in their genomes. Moreover, from microsatellite data we identified an additional 133 Volcano Wolf tortoises with non-negligible ancestry in the ‘extinct’ *C. elephantopus* lineage, i.e. there is a less than 1% chance that these are false positives. Our sampling also discovered eight tortoises that had *C. elephantopus*-like mtDNA, yet no membership in that lineage based on nuclear microsatellites. Simulations showed that four generations of backcrossing to the native *C. becki* are enough to obtain this outcome (90% chance).

Thus, hybridization on Volcano Wolf seems to have been a recurrent process over the past 200 years, ever since human-mediated translocation of hundreds of adult Galápagos giant tortoises became common [5].

To our knowledge, this is the first rediscovery of a species by way of tracking the genetic footprints left in the genomes of its hybrid offspring. These findings breathe new life into the conservation prospects for members of this flagship group. More broadly, despite hybridization often being considered largely deleterious to biodiversity conservation [6], in some cases as for Darwin’s finches in the Galápagos, it can also act as an important source of novel genetic variation [7,8]. Here, we have demonstrated another beneficial aspect of hybridization: its legacy may occasionally be the creation of opportunities to resuscitate imperiled species through targeted breeding efforts.

### Supplemental Information

Supplemental Information includes supplemental data and a table, and can be found with this article online at doi: [10.1016/j.cub.2011.12.004](https://doi.org/10.1016/j.cub.2011.12.004).

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