provide extensive genome sequencing within dogs and cats to map their genetic diversity. The rewards of these efforts for evolutionary biology are considerable. Well-resolved phylogenetic trees of most carnivore families were early accomplishments. Efforts to map genome diversity have found signals of selection in regions that can be related to specific phenotypes. In the dog, the genetic basis of skeletal, pelage, and behavioral differences among dog breeds has been revealed. The challenges may be greater in natural populations where the signals of selection are more diffuse and compromised by population history and demography, but initial findings seem promising. For example, researchers were able to show that the gene for black coat color in dogs was transferred to North American wolves and swept to high frequency in many populations. This study exemplified how findings and techniques developed for model species can be applied to their 'genome-enabled' close relatives.

Many carnivorans are endangered by climate change and habitat loss, and some lineages tend to have high extinction rates when confronted with environmental change. The poster children for these problems are the polar bear and giant panda but the challenges are not well summarized by these two species alone. Aside from habitat loss and climate change, carnivorans are uniquely challenged by the loss of population connectivity because many species, especially large ones, disperse over great distances. Some, such as large east African carnivorans and gray wolves of the high Arctic, migrate >1000 kilometers each year with their prey, and roads, development, and climate change threaten to sever critical ties between areas. Further, the high trophic position of predators makes them vulnerable to compounded bottom-up effects that may be initiated by climate change. Other challenges unique to carnivorans include interactions with humans and their livestock, and the trade for fur and body parts in traditional medicine. These issues demand an integrative approach focused on education and human attitudes, and a redirection of the focus to restoration rather than population control. The gray wolf of the American West provides important lessons in this regard, as the reintroduction of the western wolf is the most successful effort ever for a wild carnivoran. This success hinged on the involvement and education of stakeholders, as well as the fact that the listing of the western gray wolf as an endangered species under the U.S. Endangered Species Act required specific recovery actions. However, this alone is not enough, as a parallel reintroduction effort with the Mexican wolf was a dismal failure. Here, political inaction, compromised science, and weak enforcement collided such that the population remained stagnant through the 10 year history of reintroduction. If we wish to preserve carnivores — and we should given their ecological significance — changed attitudes and focused actions are prerequisites.

Further reading

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A spliceosomal intron of mitochondrial DNA origin

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The origin of spliceosomal introns is one of the most enduring mysteries in molecular biology. In nuclear genomes such as our own, the protein-coding regions of genes (exons) can be separated from one another by hundreds of thousands of base pairs (bp) of intronic (non-coding) DNA, and while they are often considered 'junk', introns are increasingly ascribed important regulatory functions [1]. Here we present evidence that an intron in a GTPase superfamily gene in the unicellular alga *Bigelowiella natans* is derived from — and was created by — the insertion of a fragment of mitochondrial DNA. Organelle-to-nucleus DNA transfer is an increasingly well-understood phenomenon, one that has the potential to greatly influence genome structure [2,3]. Our data suggest that such transfers could represent a hitherto underappreciated source of new spliceosomal introns.

First discovered in 1977 [4], introns have become a textbook feature of nuclear protein genes. Because their sequences evolve so rapidly, the origin and evolution of introns remain obscure despite decades of study and a wealth of nuclear genome sequence data [5]. At least six distinct mechanisms have been suggested to play a role in intron creation [5,6], including intron transposition through reverse transcription and conversion of newly inserted transposons. Significantly, these mechanisms each pertain to the generation of introns from pre-existing ones or the intronization of exons by recruitment of cryptic splice sites, rather than integration of exogenous DNA. Farlow *et al*. [7] recently explored the possibility of novel intron gain via DNA insertion but did not address the question of where such DNA might come from.

As part of an ongoing investigation of the nuclear genome of the

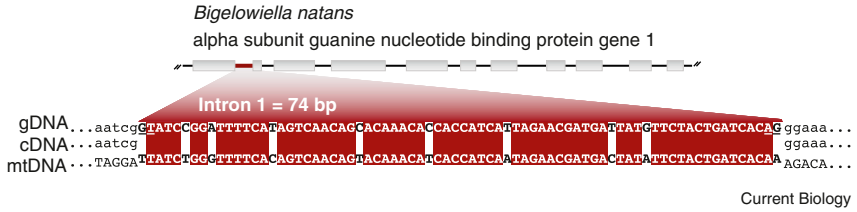

Figure 1. mtDNA-derived spliceosomal intron in *Bigelowiella natans.*
(Top) Schematic representation of the *B. natans* alpha subunit guanine nucleotide binding protein gene 1. (Bottom) Schematic showing a sequence alignment corresponding to the first intron of this gene (gDNA), its corresponding cDNA (determined by RT-PCR) and the region of the *B. natans* mitochondrial genome (mtDNA) from which the intron is derived. Differences between the nuclear genomic sequence and the mitochondrial sequence are denoted by black bold typeface while identical bases are colored red. Nuclear exonic bases are lowercase while the intron is uppercase. Canonical donor (GT) and acceptor (AG) positions are underlined.

chlorarachniophyte *B. natans* (http://www.jgi.doe.gov/sequencing/why/50026.html), we discovered a fragment of mitochondrial DNA located in an alpha subunit paralog of a guanine nucleotide-binding protein gene. The complete gene is 1,792 bp and contains ten predicted introns between 47 and 111 bp in size, each of which possesses canonical 5′-GT…AG-3′ boundaries, and seven of which have expressed sequence tag (EST) support. Remarkably, intron 1 is 86% identical across its entire length (64 of 74 bp) to a *B. natans* cytochrome c oxidase (*cox1*) mitochondrial gene fragment. Existing EST data did not span the region of the putative intron. We therefore extracted *B. natans* mRNA and, using site-specific primers, successfully amplified the region of interest using reverse transcription polymerase chain reaction (RT-PCR; see Supplemental Information, published with this article online). Subsequent cloning and sequencing of RT-PCR products showed that the putative intron is indeed spliced. None of the 33 other genes for guanine nucleotide-binding protein alpha subunits in the *B. natans* genome have an intron at the same site. Collectively, these data indicate that (i) the mitochondrion-to-nucleus DNA transfer occurred recently and (ii) the mitochondrial DNA fragment was very likely not inserted into an existing intron but rather created the intron *de novo*. Interestingly, the *cox1* gene fragment in the *B. natans* mitochondrial genome does not possess GT or AG sequences at the sites corresponding to the intron–exon boundaries (Figure 1). In *Drosophila* it has been shown that introns of recent origin are more likely to possess ‘weak’ splicing signals and premature stop codons [7]. This is consistent with the hypothesis that nonsense-mediated decay (NMD) could play a role in the establishment of novel, initially poorly spliced, introns by buffering them from natural selection. In the case of *B. natans,* it is not clear when and how the nucleus-localized mitochondrial gene fragment acquired the 5′-GT…AG-3′ splicing elements it now possesses. However, close examination of the gene in question reveals that all ten introns, including the mtDNA-derived intron 1, possess premature termination codons and/or frame shifts, and would thus presumably be subject to NMD if improperly spliced. In fact, ten sequenced cDNA clones covering the first four exons of the gene showed incomplete splicing involving various combinations of the three introns (Figure S1).

How important a factor is organelle-to-nucleus DNA transfer in the origin and evolution of spliceosomal introns? This question is difficult to assess, but it should be noted that two cases of organellar DNA-derived introns have recently been reported in animals. Ricchetti *et al*. [8] identified a 74-bp intron in the human nuclear genome that was 100% identical to a region of the mitochondrial genome encoding ATP synthase FO subunit 6, while a 64-bp intron with 96% identity to a portion of the mitochondrial 16S ribosomal RNA gene was discovered in the crustacean *Daphnia* [9]. Together with our results, these data are consistent with the possibility that the random transfer of DNA from organelles to the nucleus could be an important source of intronic DNA, particularly in organisms such as animals and plants where such transfer is rampant [2]. In addition to significantly impacting the size, structure and regulatory function(s) of existing introns [10], such transfers could also create new ones, as shown here for *B. natans.*

Supplemental Information

Supplemental Information includes one figure and experimental procedures and can be found with this article online at doi:10.1016/j.cub.2010.09.038.

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