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When is a Species not a Species?
Genetic techniques complicate taxonomy
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What, exactly, is a species? The answer to that question used to be fairly straightforward: a species was defined as a group of organisms that could naturally interbreed to produce fertile offspring. Bat species were usually described by appearance â€“ size, color, external characteristics, skeletal structure, etc. â€“ and by range.

But the past few decades have produced revolutionary techniques for exploring species at the genetic level. By analyzing chromosomes and sequencing the DNA of genes, evolutionary patterns were unraveled and the definition of species became much more genetic in nature. This led to the notion of "cryptic species" â€“ those that are nearly identical in appearance but genetically distinct. Quite a few cryptic species have been confirmed among bats and many other animals.

My research on the little brown myotis (Myotis lucifugus) suggests that this recent trend of taxonomy via genetics may have gone one step too far.

The little brown myotis, with six generally accepted subspecies, is one of the most widespread bats in North America and one of the most studied, yet we know surprisingly little about its taxonomy. With the exception of M. lucifugus occultus, the original definitions of its subspecies have not been reviewed. Because of the difficulty in telling these subspecies apart visually, biologists in the field tend to determine subspecies based on a 1981 map of their ranges.

In 1980, biologists M. Brock Fenton of the University of Western Ontario and Robert M.R. Barclay of the University of Calgary noted in their Mammalian Species account that the overlap zones of these subspecies' ranges needed additional study to better understand subspecies designations. Nearly 30 years later, we are finally starting to make progress in this area.

The genetics of the little brown myotis were not studied until 2006, when Tanya Dewey of the University of Michigan sequenced mitochondrial DNA and discovered surprisingly large differences among some of the subspecies. In fact, some of the differences were so great that she suggested certain subspecies might actually be separate species. These types of taxonomic conclusions based on mitochondrial DNA are becoming more common.

Mitochondrial DNA (mtDNA) is found inside structures called mitochondria, energy-producing units that exist outside the cell's nucleus. These generally small strands of mtDNA are distinct from nuclear DNA, which comprises the organism's chromosomes.

The International Barcode of Life Project, which originated in Canada, is dedicated to cataloguing all species on Earth by sequencing mitochondrial DNA and providing open access to the database of those genetic codes. While project founder Paul Hebert, a zoologist at the University of Guelph in Ontario, Canada, has cautioned biologists about using these sequences to draw taxonomic conclusions about species, such conclusions are

nonetheless being drawn. A quick scan through the scientific literature and media headlines reveals new species of fish, butterflies and, yes, even bats, being defined solely on mitochondrial DNA sequences. The suggested presence of cryptic species among little brown myotis based on differences in mtDNA is hardly unprecedented.

Under the traditional "Biological Species Concept," to suggest two groups are separate species implies limited interbreeding. So the suggestion that two of the little brown subspecies, M. lucifugus lucifugus and M. lucifugus carissima, could be cryptic species suggested limited interbreeding between these two groups. This is where research by me and Isabelle Delisle of the University of Alberta enters the picture.

We focused on these two subspecies, which are sympatric (their ranges overlap) in southern Alberta and northern MonÂ-tana. We investigated colonies of little brown myotis roosting along the Milk and Missouri Rivers and found several maternity colonies that included both subspecies.

It was immediately clear that little browns come in various colors in these areas: some had dark-brown fur, while others were almost blond. The original subspecies definitions for little brown bats describe M. l. carissima as pale brown and M. l. lucifugus as darker and larger.

In collaboration with Tanya Dewey and Jan Zinck of Portland State University, we investigated the genetic identities of these little brown myotis and found that color did not differentiate the two subspecies; both exhibit the full range of colors. Nor does size differentiate, as forearm length varied geographically â€“ generally increasing from south to north across our study area â€“ regardless of subspecies. Neither fur color nor size provided a reliable characteristic with which to differentiate these subspecies. In our data, the only thing that seemed to define the two groups of little brown myotis was mitochondrial DNA.

Previous research on other animals, such as grizzly bears, has demonstrated that taxonomic conclusions based solely on mitochondrial DNA can be very misleading. This is because mitochondrial DNA is inherited as a single unit, moving intact from mother to offspring. That's in stark contrast to nuclear DNA, which is shuffled like a deck of playing cards, with half coming from the mother and half from the father. So we turned our attention to nuclear DNA.

We wanted to know whether these two groups of little brown myotis interbreed. By analyzing nuclear DNA markers (repeated sequences called microsatellites), we showed that this was indeed occurring on the Missouri River. The two supposed cryptic species were not only roosting together, they were interbreeding.

How can two groups of interbreeding animals retain such large differences in mitochondrial DNA? Recall that mitochondrial DNA is inherited as a complete unit, rather than being mixed between generations. It is difficult to say with certainty when in the evolutionary past groups of little brown myotis were isolated from each other, but the mitochondrial DNA differences indicate a long-term separation that gave each group time to evolve its own unique set of mtDNA sequences. Now that these animals are able to move freely across the continent and to once again interbreed, their nuclear genes have been â€“ and still are â€“ shuffled with each new generation. But remnants of those long-ago separations are still seen in the mtDNA.

And now to put the whole story together. We determined that Myotis lucifugus carissima and M. lucifugus lucifugus are interbreeding in a zone where their ranges overlap. As such, can they be called separate species as Dewey speculated? Not according to the Biological Species Concept. But a newer Genetic Species Concept allows intact (nuclear) gene pools to retain species status despite some interbreeding in overlap zones. Therefore, as long as there is an area where we can find "pure lucifugus" individuals and an area where we can find "pure carissima" bats, then they could be designated as separate species.

There are, however, two complications that keep this from happening. First, there seem to be no morphological features that differentiate these two groups of bats, only differences in their mitochondrial genomes. And the currently recognized taxonomic system does not accommodate a designation based only on mtDNA. Perhaps a new category, such as a "Genetically Identifiable Unit," is in order, but that is beyond the scope of this discussion.

Second, and most importantly, Dewey recently found M. lucifugus lucifugus individuals spread across North America. Originally thought to be largely an eastern subspecies, it is now known that this subspecies is sympatric with most, if not all, of the other little brown subspecies in western North America. As we have shown, sympatry (overlapping ranges) seems to result in interbreeding, so widespread hybridization in the little brown myotis may be occurring. Without intact gene pools to define these supposedly different groups of little browns, it is unlikely that M. lucifugus will be divided into several species. In fact, it is not even clear whether subspecies designations should be retained. Certainly for the case of M. l. carissima and M. l. lucifugus, it seems appropriate that these distinctions be dropped.

The take-home lesson from the little brown myotis: mitochondrial DNA can be misleading. While substantial mtDNA differences between similar or identical-looking individuals might reveal cryptic species, it may instead provide only a glimpse into the evolutionary past of animals that are now one population of interbreeding individuals. While the Barcode of Life project will be able to categorize mitochondrial-genome diversity on Earth, species diversity still requires further investigation.

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