

On the origin of bar codes

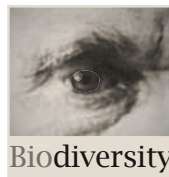
Genetic sequences in a cell's mitochondria can be used to accurately determine species. Could this be because they are responsible for creating what they identify? **Nick Lane** investigates.

Mitochondria, the cell's energy producers, keep a low profile in terms of their genome. Descended from free-living bacteria that took up residence within other cells some 2 billion years ago, they've maintained a modest genetic repertoire — a mere 37 genes in vertebrates, compared with more than 20,000 in a nucleus.

Yet within this little genome, researchers have pinpointed a 648-nucleotide stretch as the ultimate identifier of species, dubbed the DNA bar code. The sequence can distinguish between closely related species such as humans and chimps and even classify new species from identical-looking ones, such as the blue-flasher butterfly (*Astraptes fulgerator*), which has since been divided into ten separate species, verified by the habitats, lifestyles and diets of their caterpillars.

The DNA bar code has been both praised and attacked for its simplicity. Many assume that it misses taxonomic subtleties that can be revealed only through traditional systematics or more extensive sequencing. However, proponents take the criticisms in their stride. "The fact is these short sequences yield surprisingly accurate information about the composition of the entire genome," says Donal Hickey, an evolutionary biologist at Concordia University in Montreal, Canada.

A part of a mitochondrial gene was chosen simply because it worked better than other sequences, and researchers assumed that the sequences became unique after two species split from their common ancestor. But what if DNA bar codes work for a deeper reason? Molecular biologist Dan Mishmar and his colleagues at the Ben-Gurion University of the Negev in Beer-Sheva, Israel, have been collecting evidence to support a new hypothesis: that mitochondrial sequences — such as those that give rise to each species's unique bar code — might actually be powerful drivers in the process of speciation¹. Rather than simply going along



Biodiversity

Genes in the nucleus must adapt quickly to partners in the mitochondria.

for the ride, they say, they could be responsible for undermining the reproductive compatibility within a species when they conflict with sequences in the nucleus.

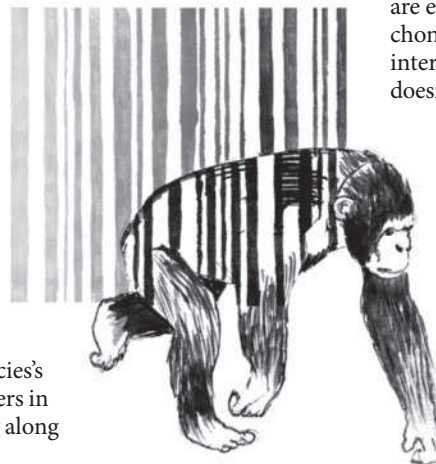
Although it is largely — some would say almost entirely — speculative, some evidence supports the idea, including data from a project called the Barcode of Life Initiative, says founder Paul Hebert from the University of Guelph in Canada. The initiative is a collection of research projects, organizations and individuals devoted to developing DNA bar-coding as a global standard for identifying species. So far, it has described the bar codes of almost 65,000 species. If true, Mishmar's hypothesis could connect the origins of species with biodiversity in a satisfying way. "Reproductive isolation may arise through a complex genomic ballet — a *pas de deux* between the mitochondrial and nuclear genomes," proposes Hebert.

Mitochondria matter

Cell survival depends on respiration, which takes place in the mitochondria through a series of large protein complexes, each built from as many as 43 subunits. These subunits are encoded by genes that reside in both the mitochondrial and the nuclear genome, and they must interact intimately with each other or respiration doesn't work.

Take the enzyme cytochrome oxidase, for example, which handles the final step of cell respiration. In mammals, the complex is composed of 13 subunits, three of which — including subunit 1, the bar-code gene — are encoded by mitochondrial DNA, and ten by nuclear genes. If the subunits of cytochrome oxidase don't work together properly, electrons are not passed to oxygen and respiration fails, triggering the death of the cell.

Making this cooperation even trickier is that fact that the two genomes evolve in dif-



ferent ways. Nuclear genes are mixed by sexual reproduction, every generation different alleles are introduced, whereas the mitochondria divide in a simple asexual fashion. Moreover, the mitochondrial gene sequences generally change much faster from generation to generation than the nuclear ones — typically 10–30 times faster.

Given the penalty for failure, it is hardly surprising that the two genomes have adapted to work together. Every generation the mitochondrial genes are tested against the new nuclear background of the offspring. If they don't work, there can be a developmental failure or a serious reduction in fitness after birth, referred to as hybrid breakdown. The outcome is that selection acts to ensure that the two genomes function properly together; and there is plenty of evidence showing that, despite their different modes of evolution, changes in one genome bring about a strong selection for compensatory changes in the other².

In 2006, Mishmar found evidence for this co-adaptation while working with Doug Wallace, a geneticist at the University of California, Irvine. They found that the primate nuclear genes that encoded mitochondrial proteins had a similar number of changes to the mitochondrial genes and that these genes evolved ten times faster than other genes in the nucleus³. In other words, the mitochondrial and nuclear genes adapt to each other within a population, and the process must happen quickly because the mutation rate is so high in mitochondrial DNA.

Mishmar started thinking about the implications of this. If one set of genes with a critical effect on survival evolves ten times faster than other genes, two populations of the same species could quickly diverge, possibly even driving a wedge between them reproductively.

Where's the evidence?

Perhaps the best evidence for reproductive incompatibility comes from marine biologist Ron Burton and his colleagues at the Scripps Institution of Oceanography in La Jolla, California. They have shown that incompatibilities between mitochondrial and nuclear genes can seriously undermine the fitness and fertility of the intertidal copepod *Tigriopus californicus*⁴ — a crustacean whose small size and abundance often earns it the name 'insect of the sea'.

Burton cross-bred individuals from nearby populations of *T. californicus* that don't usually interbreed so that the mitochondria from one population ended up paired with the nuclear genes of the other — a process known as introgression. The mismatch suppressed reproduction and cellular respiration of offspring by as much as 40%. Low respiration was also linked to slow juvenile development and poor survival — altogether, a serious reduction in fitness.

If, in effect, all these hybrid offspring are runts with low fertility, then they are less likely to survive and reproduce than the offspring of pairings within the same population. Over time, what started as partial reproductive incompatibility ends up as a total failure for the two populations to produce viable offspring when crossbreeding — which is to say, speciation.



In copepods, a mitochondrial gene mismatch makes hybrids less fit.

Burton, however, is careful not to over-claim. And although others praise his work, they too caution against over-interpretation. "Just because a genetic interaction causes hybrid problems doesn't mean it was responsible for speciation in the first place," says Jerry Coyne, an evolutionary biologist at the University of Chicago in Illinois. At least 200 genetic interactions are known to cause inviability when *Drosophila simulans* is crossed with *Drosophila melanogaster*. But when the common ancestor originally split into these two species, only one of them was probably the driver, because any one alone causes inviability. Other differences would only have emerged after the species had diverged.

Pinning down exactly which of these 200 interactions is responsible for speciation is an arduous task, and Coyne has seen no hint that mitochondrial interactions have a role. Far from it — he believes that the existing evidence refutes the postulated role of mitochondria in speciation.

"Closely related species living together often have identical or very similar mitochondrial DNA, even when their nuclear DNA is more diverged," Coyne says. Mitochondrial genes seem to flow between closely related species more easily than nuclear genes do, for unknown reasons — although this does not happen frequently enough to totally derail bar codes.

David Rand, a molecular evolutionist at Brown University in Providence, Rhode Island, has come across similar problems. With Brown's Colin Meiklejohn and Kristi Montooth, now at Indiana University in Bloomington, Rand has transplanted mitochondrial DNA from different species of fruitfly into the nuclear background of *D. melanogaster*, and found little evidence of the hybrid breakdown that would support the idea of mitochondria controlling speciation.

"I'm sure there are cases in which mitochondrial–nuclear interactions have had roles in speciation" says Rand, "but how common is it? Few people have dissected this carefully enough yet."

As one of the few who has approached the topic, Burton is not surprised that examples are scarce as yet. The degree of hybrid breakdown, he says, depends on how fast the mitochondrial DNA mutates — and that is under tight genetic control, and varies from species to species. Fruitflies and other heavily studied model systems don't have particularly high rates of mutation, he says. "That makes them the last place to look for evidence."

The best places to look, according to Burton, are taxa that have high mitochondrial DNA mutation rates, which range widely from rodents to Galapagos tortoises, from snails to copepods — even yeast. Populations from these taxa are much more likely to break down if crossed with other populations of the same species, as a first step towards reproductive isolation and speciation — as happens in Burton's copepods, and in yeast⁵.

But why do some organisms have a much faster mutation rate than others? The mitochondrial DNA of birds, for example, changes at a quarter of the speed it does in mammals⁶. So what is the benefit of a fast mutation rate; and what, if anything, does it say about speciation?

Wallace says the answer is simple: rapid mitochondrial

mutation is adaptive. “Reproductive success requires adapting to different food sources — carbohydrates, proteins or fats — and climates from icy cold to intense heat or humidity. A lot of this adaptation goes on in the mitochondria,” he says.

A fast mutation rate can quickly produce variants that are suited to the changing environmental conditions. The only drawback is that a high rate of change should also lead to lots of negative mutations, and ultimately a meltdown for some populations. It’s hard, on the face of it, to see how that could be adaptive.

Last year, however, Wallace’s group came up with an answer to this conundrum⁷. In mice, severe mitochondrial mutations are eliminated in the germ line — eggs with mitochondrial mutations fail to develop, meaning that the eggs that do develop are more likely to be healthy. The fast mutation rate generates variation coupled to a developmental filter that gets rid of the most detrimental mutations before they have the chance to undermine the health of an animal’s offspring.

This means that a high mutation rate in mitochondrial DNA can be adaptive. It is beneficial and can be selected for. But equally, it affects speciation. A fast mutation rate means that the genes controlling respiration change quickly over generations. And that, in turn, increases the chance of mismatch between mitochondrial and nuclear gene sequences if and when individuals outbreed with other populations.

This scenario paints a radically new picture of mitochondrial genes as being tightly regulated by selection. Until recently, most had thought of them as little more than ‘neutral markers’.

Evolutionary biologist Nicolas Galtier and his colleagues at the University of Montpellier 2 in France have been chipping away at this neutral view, and have found that in most species, the variation in mitochondrial DNA is surprisingly restricted⁸. If mitochondrial DNA really is a neutral marker, mutations should build up quickly over time, giving plenty of variation. But if mitochondrial DNA is subject to periodic bouts of selection then much of this variation would get purged.

That’s what Galtier sees in the data. Each such ‘selective sweep’ wipes out the common ground between species, and leaves little variation within a species — a nifty trick if you’re looking for a bar code.

From bar codes to species

By definition, a DNA bar code is a unique identifier. Between any two humans, it varies at no more than two positions². By comparison, humans differ from chimpanzees at approximately 60 sites, and from gorillas at about 70.

Much the same is true of the other 65,000 or so species that have had their bar codes sequenced. Just as Galtier has found in mitochondrial DNA in general, DNA bar codes vary remarkably little within a species, but have little or no overlap between species. Given the characteristic mitochondrial combination of rapid mutation and limited variation, only two processes can generate such a pattern: natural selection, or genetic drift after a population ‘bottleneck’.

The human genome seems to be a result of the latter. As mitochondria are inherited only through egg cells, human mitochondrial DNA can be traced back to a shared

common female ancestor, thought to have lived in Africa 170,000 years ago, and named Mitochondrial Eve. But Mitochondrial Eve did not live alone, so why has all humanity inherited her mitochondrial DNA? The standard answer invokes a bottleneck that reduced the number of humans down to a few thousand individuals, whose descendants took over the world. From a limited repertoire of mitochondrial DNA, one type happened to become fixed through genetic drift — no selection necessary.

Like Galtier’s findings, data from the Barcode of Life Initiative raise doubts about this interpretation. All species show the same lack of bar-code diversity. Although it is easy to imagine that humans passed through a bottleneck 170,000 years ago, it’s hard to believe that exactly the same thing happened in all species. “Did herrings really pass through an equally recent population bottleneck? Anchovies too?” asks Hebert. In his view, the only explanation is heavy selection across whole populations.

That makes a lot of sense in the context of Wallace’s ideas on mitochondrial adaptation to climate or food. Mitochondrial genes mutate rapidly, generating variation that is subject to selection whenever environmental conditions change. That’s suggestive of a selective sweep. And as Mishmar has been noticing, mitochondrial sequences place a premium on compatible nuclear genes, forcing fast changes on them. If individuals from nearby populations are then mated, the outcome is hybrid breakdown — the serious loss of fitness chronicled in Burton’s crossed copepods. The rate at which any of this happens depends on the mitochondrial mutation rate — fast rates leads to fast divergence and a greater likelihood that mitochondria will have a role in speciation. In this view, the DNA bar code does not merely track species — it could very well create them.

Is this really a powerful driver of speciation? It’s still too early to say. Mishmar is about to embark on a series of studies on cellular respiratory function in mismatched populations, and he challenges others to follow. If bar codes are fundamental, then the best place to look will be in closely related species with distinct bar codes. Mismatches in nuclear and mitochondrial genes between such populations should lead to hybrid breakdown that can be rescued simply by backcrossing. There is already a database of 65,000 species out there — and everything to play for. ■

Nick Lane is the first Provost’s Venture Research Fellow at University College London and author of *Life Ascending: The Ten Great Inventions of Evolution*.

1. Gershoni, M., Templeton, A. R. & Mishmar, D. *Bioessays* **31**, 642–650 (2009).
2. Blier, P. U., Dufresne, F. & Burton, R. S. *Trends Genet.* **17**, 400–406 (2001).
3. Mishmar, D. *et al. Gene* **378**, 11–18 (2006).
4. Burton, R. S., Ellison, C. K. & Harrison, J. S. *Am. Nat.* **168**, S14–S24 (2006).
5. Lee, H. Y. *et al. Cell* **135**, 1065–1073 (2008).
6. Nabholz, B., Glémin, S. & Galtier, N. *BMC Evol. Biol.* **9**, 54 (2009).
7. Fan, W. *et al. Science* **319**, 958–962 (2008).
8. Bazin, E., Glémin, S. & Galtier, N. *Science* **312**, 570–572 (2006).

See Editorial, page 251, and the whole biodiversity special at www.nature.com/darwin.



Mitochondrial DNA's high mutation rate may help some organisms adapt to changing environments.