THE BARCODE OF LIFE PROJECT

2010 International Year of Biodiversity

At Ontario's Biodiversity Institute, scientists are using cutting-edge technology to catalogue every plant and animal on earth in what may be one of the world's most ambitious biodiversity conservation initiatives.

By Paul Christopher Webster

Paul Hebert's breakthrough in biology started with a flick of a light switch on the island of Papua New Guinea more than 30 years ago. Armed with plenty of notebooks, moth catchers, and a recently acquired PhD in genetics, Hebert had come to the lush island to catalogue its teeming moth and butterfly populations. As his eyes adjusted to the harsh glare in the tropical night, he saw a dense cloud of moths swarming toward the light. "I realized that there were thousands of separate species of moths, and that I'd never be able to catalogue them all using traditional methods," he recalls from behind his desk at the Biodiversity Institute of Ontario, a research centre he directs at the University of Guelph. "It was just overwhelming. I went home and eventually gave away all my moth samples."

From that flash of insight – defeating as it may have seemed – Hebert has spun a career that has placed him at centre stage in one of the world's most ambitious biodiversity conservation initiatives. Backed by $80 million in funding from the Ontario and federal governments, he now directs the Barcode of Life project, the largest effort in history to catalogue animal and plant species. Using sophisticated DNA sequencing technologies that are vastly more powerful than the traditional tools of the taxonomic trade, the project promises to improve the enforcement of regulations that apply to endangered species. It may also transform biodiversity research, says Ahmed Djoghlaf, executive secretary of the United Nations' Secretariat of the Convention on Biological Diversity in Montreal, by allowing scientists to trace familial links between species, some of them newly discovered or little known, that share biological characteristics but live great distances apart. Although Hebert's project is just getting started, Djoghlaf says it is already "contributing to a deeper understanding of the evolution of species, and hence, of biodiversity."

To identify a species, Hebert notes, you first need to know it exists, which is where the Barcode of Life Data Systems comes in – along with almost 300 barcoding projects worldwide that Hebert's work has helped spawn. "What we're building, really, is a sort of global positioning system for plant and animal research," he explains. "And we're doing it at exactly the time when humanity is threatening to provoke the sixth mass extinction."

The 95 moths pinned to a foam-rubber backing inside a wooden box are part of a consignment of 40 such boxes that have just arrived from Australia, explains Natalia Ivanova, who is lead DNA scientist at the Canadian Centre for DNA Barcoding, a large laboratory within the Biodiversity Institute. Each moth is given an identity tag and photographed using a digital camera attached to a microscope. Then, in a suite of rooms stuffed with high-tech robotic gear and computers, samples of the moth's DNA are extracted and sequenced in a series of chemical processes known as polymerase chain reaction, or PCR. This analysis yields a genetic "barcode" composed of 650 bands in four colours. Seen together on the computer screen at the end of Ivanova's barcoding production line, the DNA sequences look like an unusually intricate and colourful tapestry.

If you hazard to ask Ivanova to explain the PCR process, she will probably deliver a bombardment of brain-straining scientific phrases. But Ivanova, who completed her doctorate in molecular systematics in Russia before joining Hebert's team, will graciously assure you that all you really need to know is that this lab is capable of deducing the genetic codes of more than 200,000 samples taken from vertebrates, invertebrates and plants each year. So far, it has about 840,000 barcodes on file, mostly of insects. They can be all accessed via the Internet, as can a photo of the

Through DNA barcoding, conservation efforts can move beyond relatively easily monitored species such as whales and polar bears, toward lesser known species – such as the Cercropia moth (left) and the underwing moth (right) – that may be vitally important to an ecosystem.

specimen and information on where it was collected, where it currently resides and other details.

The Australian moths are just a few of the roughly 25,000 samples the lab receives and analyzes every month, submitted from around the world for the Barcode of Life project. With an estimated 10 million species to be catalogued, Hebert's goal is nothing less than recording a DNA barcode for every living species.

Hebert, 64, has always been interested in classifying nature, a painstaking process known as taxonomy, in which biologists study specimens and produce carefully written descriptions that are then classified according to a complex hierarchy. From the days when, as a child, Hebert collected bugs on summer holidays in Ontario forests, the obsession propelled him first to summer jobs cataloguing insects at the Royal Ontario Museum, then to a doctorate in genetics at Cambridge University and eventually into the vastly diverse forests of New Guinea.

After the island's phenomenal fecundity thwarted his taxonomic ambitions, Hebert returned to Canada, took teaching jobs that led him to the University of Guelph and focused his research on the evolution of a handful of insect species in the Arctic – a place where the diversity had no chance of overwhelming him. But he stayed abreast of DNA research, and through the 1990s he watched with fascination as ever more efficient and inexpensive DNA sequencing techniques emerged. That same decade, he got involved in DNA cataloguing by building a prototype lab capable of sequencing gene regions for a dozen or so specimens a week – in essence, molecularly decoding how life forms are programmed. But it was not until the invention of fully automated sequencing systems, able to process thousands of samples daily, that Hebert had his "Eureka!" moment: using this rapid, low-cost DNA sequencing technology, he could develop an inventory of the vast number of species facing extinction. He has been doing that ever since.

Hebert's project is rooted in millennia of research. Scientists have been trying to catalogue nature at least since the days of Aristotle, who first proposed a "great chain of being" based on rudimentary taxonomies about 2,400 years ago. But it was the 18th-century Swedish naturalist Carolus Linnaeus, whose taxonomic hierarchy divides nature into progressively smaller categories, who showed that careful classification opens the way for major discoveries. As Charles Darwin demonstrated with the theory of evolution that drew from the catalogues Linnaeus and his followers assembled, taxonomy can yield utterly transformative breakthroughs.

How the Barcode of Life may eventually help science is impossible to predict, but assembling a database that in time yields a planetary species count should greatly advance "biological literacy," says Hebert. Most immediately, it will make harvesting of endangered species for commercial use more difficult. After all, he notes, if a fisheries investigator can run a quick DNA test on a boat's haul and cross-reference it with the barcode database, illegal practices can be far more easily policed.

Charles Francis, manager of species abundance and distribution at Environment Canada, says the potential of Hebert's work is significant. "DNA barcodes could be used to confirm the identities of birds or other animals involved in an enforcement case," he explains. For example, officials could test any meat product, even a sausage (provided it has not been cooked), to find out what animals went into its making. Researchers have used barcodes to confirm

PHOTO (LEF) ROBERTMCCAW.COM; (RIGHT) ANDREW MCLAUGHLAN

Birds and barcodes

The All Birds Barcoding Initiative (ABBI), held its first meeting at Harvard University's Museum of Comparative Zoology in September 2005, has a mighty ambitious goal: to collect DNA barcodes from each of the approximately 10,000 known species of birds. Even after several hundred years of work on identifying bird species, genetic surveys – including those using DNA barcoding – show that hundreds of avian species have yet to be fully described and understood. "Our barcode surveys reveal cases of deep [DNA] sequence divergence in some bird species, suggesting that the members of a species actually have not been interbreeding for a long time," explains Paul Hebert, director of the Biodiversity Institute of Ontario. Detailed studies of birds such as the eastern meadowlark have revealed significant differences in the species' physical characteristics and song patterns in various locations. In fact, there are two species: eastern and western medowlarks.

The ABBI aims to speed up the discovery of new species and provide a practical tool for specimen identification. To date, more than 17,000 specimens from almost 3,000 species have been barcoded. By depositing these records in Hebert's Barcode of Life database, investigators are establishing an electronic library of avian DNA, reference specimens and other collection data. Conservation planners, ornithologists, ecologists, public health officials and interested amateurs will then be able to consult this database to easily confirm – from as little as a single feather – a given bird's identity, regardless of its age, sex or plumage.

The ABBI is a testing ground for DNA barcoding, the larger initiative to catalogue all animal and plant life. As part of the ABBI, Environment Canada is supporting the development of a reference database for all North American bird species. Using samples in the Royal Ontario Museum and Environment Canada's National Wildlife Research Centre tissue bank, along with U.S. sample sources, the project has produced DNA barcodes for more than 95 percent of recognized species on the continent.

Visit the ABBI website www.barcodingbirds.org for more information.

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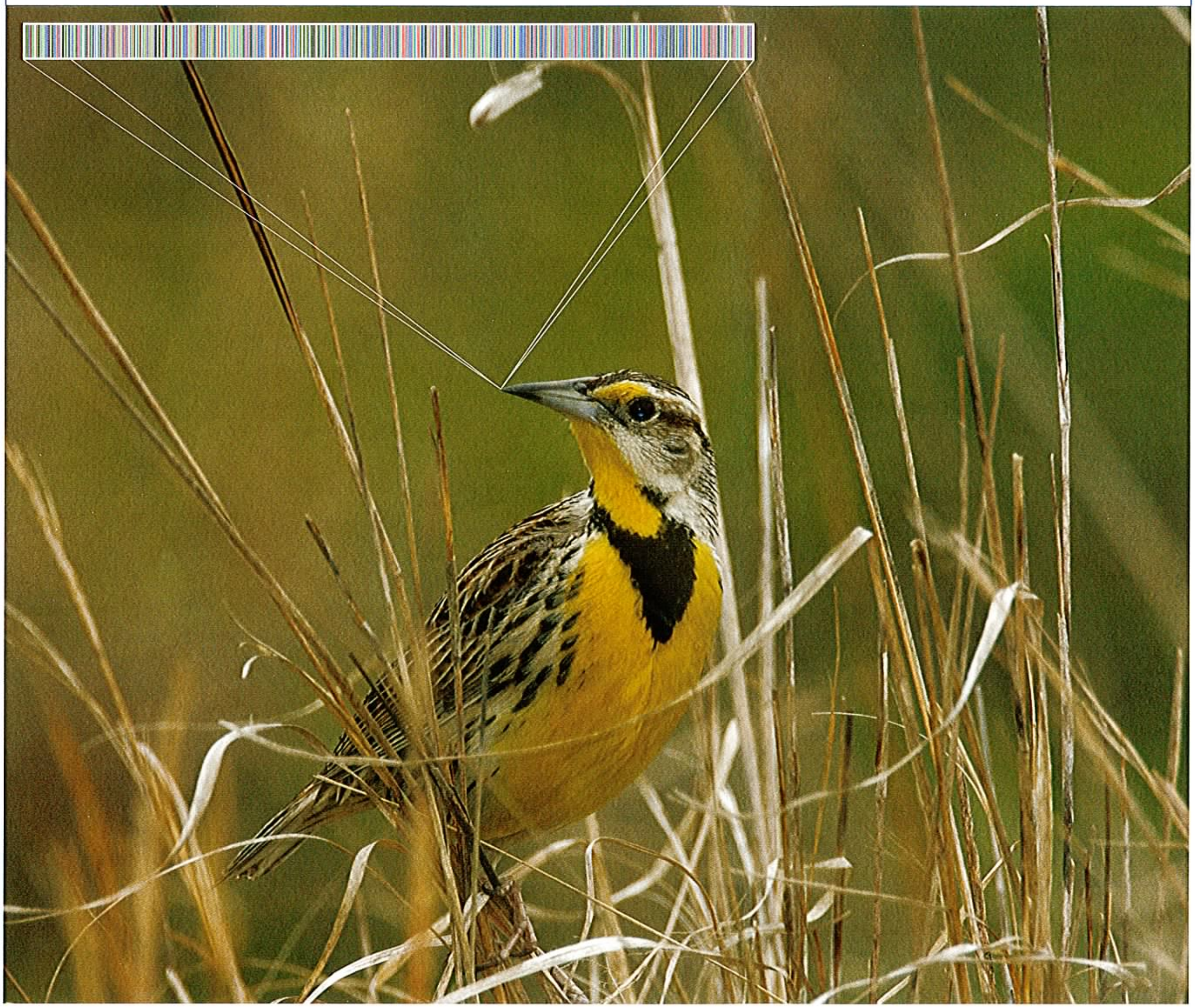

PHOTO KEN NEWCOMBE

Researchers have discovered significant differences in the eastern meadowlark's physical characteristics and song patterns in different locations.

Natalia Ivanova, lead DNA scientist at the Canadian Centre for DNA Barcoding, helps oversee the lab where scientists can deduce the genetic codes from the more than 200,000 samples taken from vertebrates, invertebrates and plants every year.

PHOTO BERNARD BOHN

the identities of birds so heavily covered in oil that their plumage patterns were obscured, he says. "They have also been used to identify blood smears and feathers stuck to aircraft that have hit birds, which can help with understanding the risks to both birds and planes, and avoiding them," he adds.

Francis argues that DNA barcodes will help enforce international trade regulations under the Convention on International Trade in Endangered Species. "It is difficult for customs officers to have expertise in identifying all types of endangered species from throughout the world," he points out. As long as the product they are inspecting contains a DNA sample, such as hair or skin or feather, DNA barcodes could quickly confirm its source.

The ability to read and quickly sequence DNA has transformed species scholarship and protection, Hebert says. "We can do forensics on life." In a recently published study on eastern red bats, for instance, Hebert and a team of biologists from the University of Guelph and the University of Western Ontario used his techniques to identify fragments of prey animals in red bat guano, gaining insight into the bats' food sources and the workings of the entire ecosystem.

Hebert believes, however, that it is in the field of biodiversity conservation that the Barcode of Life will yield the greatest advances. "Simply put, you can't conserve something if you can't recognize it," he says. The database will enable scientists to trace the evolutionary patterns and cross-relationships of innumerable as yet unstudied species. A Quebec colleague of Hebert's, for example, has used barcoding to examine fish parasites in a new way, and discovered that, for the most part, the parasites are highly specialized.

Hebert says that, through barcoding, conservation strategies will move beyond large, relatively easily monitored species such as whales, eagles and polar bears toward lesser-known or currently unknown species of insects and biota that may be vitally important within their own ecologies and may offer valuable medicinal or other properties. Currently, vast numbers of species are in danger of disappearing without ever having been identified. In tackling this knowledge gap, the Barcode of Life project is already yielding rich results; new life forms have been discovered on every continent except Antarctica. "New species have been found just about everywhere we have looked and in every group: birds, fish, insects, crustaceans," says Hebert. "We used barcoding to reveal that the commonest butterfly that one sees along roadsides in Central America is actually 10 different species whose adults happen to look much alike, but their larvae have different colour patterns and they feed on different plants."

Indeed, projects like the Barcode of Life are helping scientists realize just how little we really know about bio-diversity. "As a consequence, conservation plans are largely a shot in the dark," Hebert argues, because different species we cannot yet tell apart may require different strategies. To protect a species, Hebert warns, "you need to know where it sleeps at night. You need to know what it eats. You need to know it intimately. Every bit of knowledge is crucial."

Barcode projects around the world

The Barcode of Life Initiative, under the guidance of the University of Guelph's Biodiversity Institute of Ontario, brings together a range of projects that all target a very short gene sequence from a standardized position in the genome as a means of establishing a global standard for assigning specimens to the correct species. Numerous studies on insects, birds, fish, algae and many other taxonomic groups are under way; many more are being planned. Some are global research campaigns involving scores of contributors. Others involve small teams tackling small taxonomic groups. Taken from the Barcode of Life Initiative website, projects include the following:

FISH-BOL: The Fish Barcode of Life campaign is collecting barcodes for the 30,000-plus species of marine, freshwater and estuarine fish of the world.

TBI: The Tephritid Barcode Initiative is a two-year demonstration project that will create a system for identifying fruit flies around the world. It will barcode at least five representatives of all tephritid fruit flies, ranging from agricultural pests to beneficial species used for biological control of other pests.

MBI: The Mosquito Barcode Initiative is another demonstration project aimed at producing a global operational system for identifying mosquitoes within two years. The MBI plans to barcode at least five specimens from 80 percent of the 3,200 known mosquito species. Disease-bearing species and their closest relatives will be the highest priority.

All-Leps: This barcoding project tackles moths and butterflies. Two of the six All-Leps campaigns are global in scope and focus on two families – the Sphingidae and Saturnidae. The other four are biotic surveys of all moths and butterflies found in North America, Australia, and Costa Rica's Area de Conservación Guanacaste.

PolarBol: The Canadian Arctic initiative is part of an international collaboration aimed at barcoding the northern biota of Canada and other circumpolar countries. Intensive collecting and barcoding efforts to date have focused on the region around Churchill, Man.

CMarZ: The Census of Marine Zooplankton is one of 14 field projects launched by the Census of Marine Life, a global effort to inventory the marine biota. CMarZ focuses on marine zooplankton, a diverse community of approximately 6,800 species. MarZ was the first research project to produce DNA sequences in a shipboard laboratory.

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He first realized that in his encounter with the myriad moths that started his quest. The first thing he did after the barcoding lab was built was buy lights for his backyard out in the countryside near Guelph. "Then I started collecting. Then, we barcoded them. And we can now tell them apart," says Hebert. "I finally found a way to understand those moths."

***Paul Christopher Webster** is a Toronto-based writer and radio and TV documentary director. To see his latest work, please go to www.paulcwebster.com.*