Wildlife forensics

Genomics has become a powerful tool to inform conservation measures

Howard Wolinsky

You might call him Queequeg. Like Herman Melville's character in the 1851 novel *Moby Dick*, Howard Rosenbaum plies the seas in search of whales following old whaling charts. Standing on the deck of a 12 m boat, he brandishes a crossbow with hollow-tipped darts to harpoon the flanks of the whales as they surface to breathe (Fig 1). "We liken it to a mosquito bite. Sometimes there's a reaction. Sometimes the whales are competing to mate with a female, so they don't even react to the dart," explained Rosenbaum, a conservation biologist and geneticist, and Director of the New York City-based Wildlife Conservation Society's Ocean Giants programme. Rosenbaum and his colleagues use the darts to collect half-gram biopsy samples of whale epidermis and fat—about the size of a human fingernail—to extract DNA as part of international efforts to save the whales.

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Like Rosenbaum, many conservation biologists and wildlife managers increasingly rely on DNA analysis tools to identify species, determine sex or analyse pedigrees. George Amato, Director of the Sackler Institute for Comparative Genomics at the American Museum of Natural History in New York, NY, USA, said that during his 25-year career, genetic tools have become increasingly important for conservation biology and related fields. Genetic information

taken from individual animals to the extent of covering whole populations now plays a valuable part in making decisions about levels of protection for certain species or populations and managing conflicts between humans and conservation goals.

Moreover, Amato expects the use and importance of genetics to grow even more, given that conservation of biodiversity has become a global issue. "My office overlooks Central Park. And there are conservation issues in Central Park: how do you maintain the diversity of plants and animals? I live in suburban Connecticut, where we want the highest levels of diversity within a suburban environment," he said. "Then, you take this all the way to Central Africa. There are conservation issues across the entire spectrum of landscapes. With global climate change, techniques in genetics and molecular biology are being used to look at issues and questions across that entire landscape."

Rosenbaum commented, "The genomic revolution has certainly changed the way we think about conservation and the questions we can ask and the things we can do. It can be a forensic analysis." The data translates "into a conservation value where governments, conservationists, and people who actively protect these species can use this information to better protect these animals in the wild."

Rosenbaum and colleagues from the Wildlife Conservation Society, the American Museum of Natural History and other organizations used genomics for the largest study so far—based on more than 1,500 DNA samples—about the population dynamics of humpback whales in the Southern hemisphere [1]. The researchers analysed population structure

and migration rates; they found the highest gene flow between whales that breed on either side of the African continent and a lower gene flow between whales on opposite sides of the Atlantic, from the Brazilian coast to southern Africa. The group also identified an isolated population of fewer than 200 humpbacks in the northern Indian Ocean off the Arabian Peninsula, which are only distantly related to the humpbacks breeding off the coast of Madagascar and the eastern coast of southern Africa. "This group is a conservation priority," Rosenbaum noted.

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He said the US National Oceanographic and Atmospheric Administration is using this information to determine whether whale populations are recovering or endangered and what steps should be taken to protect them. Through wildlife management and protection, humpbacks have rebounded to 60,000 or more individuals from fewer than 5,000 in the 1960s. Rosenbaum's data will, among other things, help to verify whether the whales should be managed as one large group or divided into subgroups.

He has also been looking at DNA collected from dolphins caught in fishing nets off the coast of Argentina. Argentine officials will be using the data to make recommendations about managing these populations. "We've been able to demonstrate that it's not one continuous population in Argentina. There might be multiple populations that merit conservation protection," Rosenbaum explained.

Fig 1 | Howard Rosenbaum with a crossbow to obtain skin samples from whales. © Wildlife Conservation Society.

The sea turtle is another popular creature that is high on conservationists' lists. To get DNA samples from sea turtles, population geneticist and wildlife biologist Nancy FitzSimmons from the University of Canberra in Australia reverts to a simpler method than Rosenbaum's harpoon. "Ever hear of a turtle rodeo?" she asked. FitzSimmons goes out on a speed boat in the Great Barrier Reef with her colleagues, dives into the water and wrangles a turtle on board so it can be measured, tagged, have its reproductive system examined with a laparoscope and a skin tag removed with a small scissor or scalpel for DNA analysis (Fig 2).

Like Rosenbaum, she uses DNA as a forensic tool to characterize individuals and populations [2]. "That's been a really important part, to be able to tell people who are doing the management, 'This population is different from that one, and you need to manage them appropriately,'" FitzSimmons explained. The researchers have characterized the turtle's feeding grounds around

Australia to determine which populations are doing well and which are not. If they see that certain groups are being harmed through predation or being trapped in 'ghost nets' abandoned by fishermen, conservation measures can be implemented.

FitzSimmons, who started her career studying the genetics of bighorn sheep, has recently been using DNA technology in other areas, including finding purebred crocodiles to reintroduce them into a wetland ecosystem at Cat Tien National Park in Vietnam. "DNA is invaluable. You can't reintroduce animals that aren't purebred," she said, explaining the rationale for looking at purebreds. "It's been quite important to do genetic studies to make sure you're getting the right animals to the right places."

Geneticist Hans Geir Eiken, senior researcher at the Norwegian Institute for Agricultural and Environmental Research in Svanvik, Norway, does not wrestle with the animals he is interested in. He uses a non-intrusive

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method to collect DNA from brown bears (Fig 3). "We collect the hair that is on the vegetation, on the ground. We can manage with only a single hair to get a DNA profile," he said. "We can even identify mother and cub in the den based on the hairs. We can collect hairs from at least two different individuals and separate them afterwards and identify them as separate entities. Of course we also study how they are related and try to separate the bears into pedigrees, but that's more research and it's only occasionally that we do that for [bear] management."

Eiken said the Norwegian government does not invest a lot of money on helicopters or other surveillance methods, and does not want to not bother the animals. "A lot of disturbing things were done to bears. They

Fig 2 | Geneticist Stewart Pittard measuring a sea turtle. © Michael P. Jensen, NOAA.

were trapped. They were radio-collared,” he said. “I think as a researcher we should replace those approaches with non-invasive genetic techniques. We don’t disturb them. We just collect samples from them.”

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Eiken said that the bears pose a threat to two million sheep that roam freely around Norway. “Bears can kill several tons of them everyday. This is not the case in the other countries where they don’t have free-ranging sheep. That’s why it’s a big economic issue for us in Norway.” Wildlife managers therefore have to balance the fact that brown bears are endangered against the economic interests of sheep owners; about 10% of the brown bears are killed each year because they have caused damage, or as part of a restricted ‘licensed’ hunting programme. Eiken said that within two days of a sheep kill, DNA analysis can determine which species killed the sheep, and, if it is a bear, which individual. “We protect the females with cubs. Without the DNA profiles, it would be easy to kill the females, which also take sheep of course.”

It is not only wildlife management that interests Eiken; he was part of a group

led by Axel Janke at the Biodiversity and Climate Research Centre in Frankfurt am Main, Germany, which completed sequencing of the brown bear genome last year. The genome will be compared with that of the polar bear in the hope of finding genes involved in environmental adaptation. “The reason why [the comparison is] so interesting between the polar bear and the brown bear is that if you look at their evolution, it’s [maybe] less than one million years when they separated. In genetics that’s not a very long time,” Eiken said. “But there are a lot of other issues that we think are even more interesting. Brown bears stay in their caves for 6 months in northern Norway. We think we can identify genes that allow the bear to be in the den for so long without dying from it.”

Like bears, wolves have also been clashing with humans for centuries. Hunters exterminated the natural wolf population in the Scandinavian Peninsula in the late nineteenth century as governments protected reindeer farming in northern Scandinavia. After the Swedish government finally banned wolf hunting in the 1960s, three wolves from Finland and Russia immigrated in the 1980s, and the population rose to 250, along with some other wolves that joined the highly inbred population. Sweden now has a database of all individual

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wolves, their pedigrees and breeding territories to manage the population and resolve conflicts with farmers. “Wolves are very good at causing conflicts with people. If a wolf takes a sheep or cattle, or it is in a recreation area, it represents a potential conflict. If a wolf is identified as a problem, then the local authorities may issue a license to shoot that wolf,” said Staffan Bensch, a molecular ecologist and ornithologist at Lund University in Sweden.

Again, it is the application of genomics tools that informs conservation management for the Scandinavian wolf population. Bensch, who is best known for his work on population genetics and genomics of migratory songbirds, was called to apply his knowledge of microsatellite analysis. The investigators collect saliva from the site where a predator has chewed or bitten the prey, and extract mitochondrial DNA to determine whether a wolf, a bear, a fox or a dog has killed the livestock. The genetic information potentially can serve as a death warrant if a wolf is linked with a kill, and to determine compensation for livestock owners.

Yet, not all wolves are equal. “If it’s shown to be a genetically valuable wolf, then somehow more damage can be tolerated, such as a wolf taking livestock for instance,” Bensch said. “In the management policy, there is genetic analysis of every wolf that has a question on whether it should be shot or saved. An inbred Scandinavian wolf has no valuable genes so it’s more likely to be shot.” Moreover, Bensch said that DNA analysis showed that in at least half the cases, dogs were the predator. “There are so many more dogs than there are wolves,” he said. “Some farmers are prejudiced that it is the wolf that killed their sheep.”

According to Dirk Steinke, lead scientist at Marine Barcode of Life and an evolutionary biologist at the Biodiversity Institute of Ontario at the University of Guelph in Canada, DNA barcoding could also contribute to conservation efforts. The technique—usually based on comparing the sequence of the mitochondrial *CO1* gene with a database—could help to address the growing trade in

Fig 3 | Bear management in Scandinavia. (A) A brown bear in a forest in Northern Finland © Alexander Kopatz, Norwegian Institute for Agricultural and Environmental Research. (B) Faecal sampling. Monitoring of bears in Norway is performed in a non-invasive way by sampling hair and faecal samples in the field followed by DNA profiling. © Hans Geir Eiken. (C) Brown-bear hair sample obtained by so-called systematic hair trapping. A scent lure is put in the middle of a small area surrounded by barbed wire. To investigate the smell, the bears have to cross the wire and some hair will be caught. © Hans Geir Eiken. (D) A female, 2.5-year-old bear that was shot at Svanvik in the Pasvik Valley in Norway in August 2008. She and her brother had started to eat from garbage cans after they left their mother and the authorities gave permission to shoot them. The male was shot one month later after appearing in a schoolyard. © Hans Geir Eiken.

shark fins for wedding feasts in China and among the Chinese diaspora, for example. Shark fins confiscated by Australian authorities from Indonesian ships are often a mess of tissue; barcoding helps them to identify the exact species. “As it turns out, some of them are really in the high-threat categories on the IUCN Red List of Threatened Species,

so it was pretty concerning,” Steinke said. “That is something where barcoding turns into a tool where wildlife management can be done—even if they only get fragments of an animal. I am not sure if this can prevent people from hunting those animals, but you can at least give them the feedback on whether they did something illegal or not.”

Steinke commented that DNA tools are handy not only for megafauna, but also for the humbler creatures in the sea, “especially when it comes to marine invertebrates. The larval stages are the only ones where they are mobile. If you’re looking at wildlife management from an invertebrate perspective in the sea, then these mobile life stages

are very important. Their barcoding might become very handy because for some of those groups it's the only reliable way of knowing what you're looking at." Yet, this does not necessarily translate into better conservation: "Enforcement reactions come much quicker when it's for the charismatic megafauna," Steinke conceded.

Moreover, reliable identification of animal species could even improve human health. For instance, Amato and colleagues from the US Centers for Disease Control and Prevention demonstrated for the first time the presence of zoonotic viruses in non-human primates seized in American airports [3]. They identified retroviruses (simian foamy virus) and/or herpesviruses (cytomegalovirus and lymphocryptovirus), which potentially pose a threat to human health. Amato suggested that surveillance of the wildlife trade by using barcodes would help facilitate prevention of disease. Moreover, DNA barcoding could also show whether the meat itself is from monkeys or other wild animals to distinguish illegally hunted and traded bushmeat—the term used for meat from wild animals in Africa—from legal meats.

Amato's group also applied barcoding to bluefin tuna, commonly used in sushi, which he described as the "bushmeat of the developed world", as the species is being driven

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to near extinction through overharvesting. Developing barcodes for tuna could help to distinguish bluefin from yellowfin or other tuna species and could assist measures to protect the bluefin. "It can be used sort of like 'Wildlife CSI' (after the popular American TV series)," he said.

In fact, barcoding for law enforcement is growing. Mitchell Eaton, assistant unit leader at the US Geological Survey New York Cooperative Fish and Wildlife Research Unit in Ithaca, NY, USA, noted that the technique is being used by US government agencies such as the FDA and the US Fish & Wildlife Service, as well as African and South American governments, to monitor the illegal export of pets and bushmeat. It is also used as part of the United Nations' Convention on Biological Diversity for cataloguing the Earth's biodiversity, identifying pathogens and monitoring endangered species. He expects that more law enforcement agencies around the world will routinely apply these tools: "This is actually easy technology to use."

In that way, barcoding as well as genetics and its related technologies help to

address a major problem in conservation and protection measures: to monitor the size, distribution and migration of populations of animals and to analyse their genetic diversity. It gives biologists and conservations a better picture of what needs extra protective measures, and gives enforcement agencies a new and reliable forensic tool to identify and track illegal hunting and trade of protected species. As helpful as these technologies are, however, they are not sufficient to protect severely threatened species such as the bluefin tuna and are therefore not a substitute for more political action and stricter enforcement.

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