Puerto Rican Crested Toad © Dustin Smith, North Carolina Zoological Park

MOLECULAR MANAGEMENT

MANAGING SSP POPULATIONS WITH MOLECULAR GENETICS

BY ALINA TUGEND

Are they one species?
Are they two?
How closely related are they?
What's the parentage?
What sex are they?

Whether you're talking about the Puerto Rican crested toad, the fennec fox or the western pond turtle – all part of the Association of Zoos and Aquariums' (AZA) Species Survival Plan® (SSP) programs – these questions matter a lot.

And until relatively recently, it could be very difficult to get answers.

"Some of the historical attempts to collect data were not terribly effective," said Jamie Ivy, a population biologist with San Diego Zoo Global in San Diego, Calif. But recent advances in molecular techniques have changed that.

Puerto Rican Crested Toad

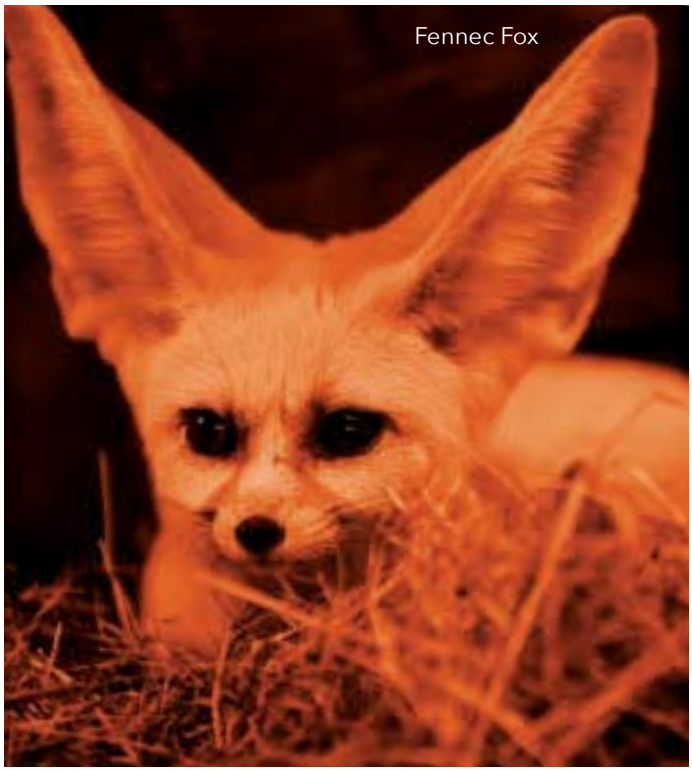
As the technology has become simpler to use and the cost has gone down, it has become more feasible and more desirable to use molecular genetics to help manage a wide variety of SSP populations, Ivy said.

"Genomics will allow us to rewrite pedigree information," said Budhan Pukazhenthi, a research physiologist at the Smithsonian Conservation Biology Institute and chair of AZA's Biomaterials Banking Scientific Advisory Group. "It will allow us to clean up animal relatedness information and shed light on who should be bred with whom."

Take fennec foxes, which often make their way to zoos from the commercial pet trade. "We had absolutely no idea how the animals were related to ours," Ivy said. "But using molecular data, we could incorporate information into their pedigrees about which foxes should be bred with others."

Ivy's colleague, Andrea Putnam, also a population biologist for the San Diego Zoo, said her experience with the Nubian ibex offers another example. Several were donated to her facility from an organization not accredited by the AZA and looked particularly small.

"There was a concern that they had been hybridized and were a cross between a Nubian ibex and a domestic goat," Putnam said. Not wanting to introduce a hybrid into the general Nubian ibex population, they did the DNA testing and discovered, it was in fact, just a small ibex.


Fennec Fox

And it's not only a matter of improving diversity, but of saving space. Diane Barber, curator of ectotherms at the Fort Worth Zoo in Fort Worth, Texas, manages the Puerto Rican Crested Toad SSP. The species lives in two very different habitats, in the north and the south of Puerto Rico. They were separated for at least one million years.

This particular toad is the only toad native to Puerto Rico and was the first amphibian SSP due to its rapidly declining population from habitat alteration and an invasive marine toad (*Bufo marinus*) that was introduced to Puerto Rico in the 1920s. The Fort Worth Zoo is part of the SSP program that breeds the toad and releases tadpoles into the wild in Puerto Rico.

"We were managing the northern and southern toads separately, but the northern population crashed – it only had 39 percent gene diversity," Barber said. Compare that with the southern toad, which was found to have 95 percent gene diversity.

"In 2011, we decided to do a genetic rescue and merge the two populations but manage them separately," as the northern x southern population and the southern population, Barber said.

DNA analysis demonstrated that the northern and southern groups were close enough that they could be merged and they wouldn't lose the traits necessary to adapt to their habitat upon reintroduction.

"Without genetic analysis of both managed and wild populations it would have been hard to merge the two," she said. "It helps us make informed decisions and makes everyone feel better as far as causing no harm." Now AZA-accredited facilities are struggling to find space to manage two separate crested toad groups and have proposed combining the two groups together into one to manage them as a single species.

While Barber used genetic testing as a tool to help merge two species, Jessie Bushell, director of conservation for the San Francisco Zoo and Gardens in San Francisco, Calif., said she wants to use genetic testing to manage one SSP species – the western pond turtle – that recently split into two.


Western Pond Turtle

"We are asking a simple question – is it northern or southern, and looking at three different areas of DNA," she said. Even so, the cost of testing the western pond turtles at the 23 zoos that hold them – 17 of which are accredited by the AZA – will run about $4,000 to $5,000, Bushell said.

Genetic testing can also help in figuring out what happens to the turtles that are released into the wild since, during their juvenile phase, they essentially disappear from sight.

"It is manpower-intensive to track using transmitters, and GPS only lasts six to eight months," she said. "Looking at genetics, while it can't answer all the questions, will provide some highlights, such as how many males are contributing to the next generation."

But the testing isn't easy or cheap to do, Barber said. The cost can run around $35 to $50 a sample, which adds up if the population runs into the hundreds or thousands, and "it's money we just don't have," she added. And while it's great to find locals geneticists to do the work for free, that could mean it could fall to the bottom of the priority list. "It can take years to get results if they're doing it gratis for us," Barber said. "They have other things on their plate."

Ivy, of the San Diego Zoo, agreed that even though the cost and complexity of testing has decreased, it's still a problem. "We're looking to make molecular analysis more common and more effective within the zoo community," she said. "It's routinely used with bird sexing but not with parentage or relatedness."

There are two big hurdles now in genetic testing: getting the samples and funding the work. Obtaining blood or other tissue from animals, especially big ones, for DNA analysis may be difficult, and zoos often want to do it when they're already performing another procedure. That can mean waiting months or even years to get a sample, Ivy said.

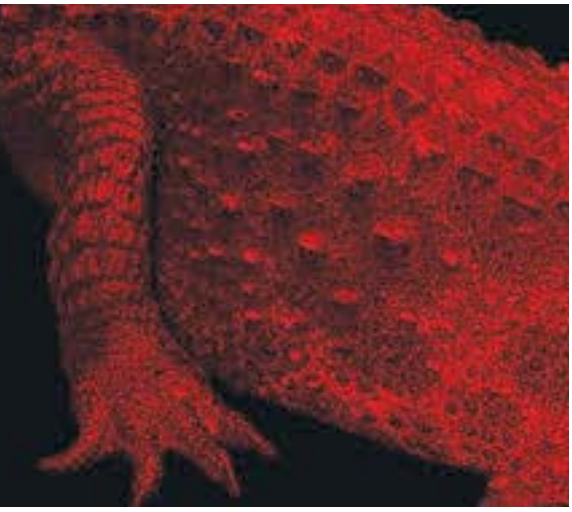
And, she added, "It's more difficult to get grants for this kind of work because, since it is applied work for animal management, it's often not considered research." So while individual zoos are now being asked to fund some of the work themselves, Ivy acknowledged, "A third hurdle is better demonstrating the benefits of this kind of work so that zoos know it's worth the cost."

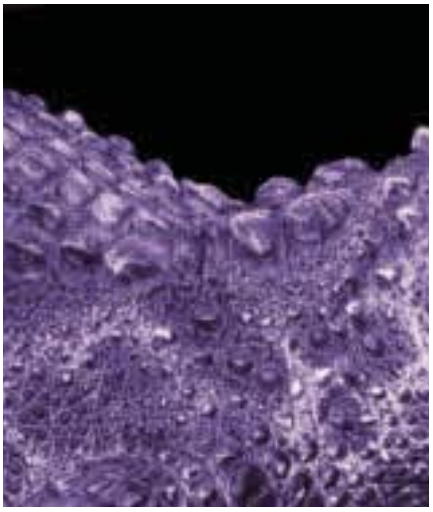
Klaus Koepfli, who works as a research associate with Pukazhenthi at the Smithsonian, said another issue is that the some taxa, such as amphibians, have genomes two to three times larger than humans.

"These are really challenging genomes and the cost of sequencing multiplies," he said.

Pukazhenthi said one way to address the difficulty in gathering samples is for all zoos and aquariums to collect blood or tissue samples every time they treat an animal. "Just put them in a freezer," he said. "Having biological material is the most important step in using genomics for species management."

Dr. Oliver Ryder, director of genetics at the San Diego Zoo Institute for Conservation Research, pointed to the California condor as a species where genetics have played an important role. People working with the species have been at the forefront of collecting and using genetic information.





At one point, the condor population had declined to 27 animals, and many were related. Through scientific management, the population is up to more than 400 now, and "we have the DNA from 99 percent of the entire species pedigree," Ryder said. "The California condor is the first species for which the DNA data were used to guide a breeding program. It's a model for using genome sequencing to manage endangered species."

Genetic information isn't just used to maintain genetic diversity. In the past, condor chicks that inherited a deleterious recessive gene would die without hatching. Now, it's possible to identify genetic markers for that gene to manage the population, Ryder said.

Kim Lovich, curator of herpetology & ichthyology for the San Diego Zoo, said genetic testing is important not only to better manage animals within a zoo but among zoos as well. For example, Lovich said, "We now understand that the dwarf crocodile is at least three species, when we thought it was one. There might be an opportunity for us to collaborate with the European Association of Zoos and Aquaria (EAZA) so we can each focus on one or two full species and avoid hybridization. It's exciting to be able to be considering this now that we understand the genetics."

She is also using genetic testing to manage the SSP populations of the Fiji banded iguana and the African slender-snouted crocodile.

"We're trying to find out exactly what species we have in our managed population," Lovich said.

Genetics is about managing animals in zoos and ensuring their survival in the wild.

"Amphibians and reptiles are unusual because so much new genetic work is being done on them, and so many different species are being described," she said. "But it's a race against time – species are going extinct as fast as we can describe them.

"Genetics can help us plan and prioritize, so we can focus on where animals are at greatest risk. Having the genetic baseline information, along with the field data, means we have the information to make sound conservation decisions."

Alina Tugend in a writer based in Larchmont, N.Y.

Dwarf Crocodile

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