ScienceWatch Home
Inside This Month...
Interviews

Featured Interviews
Author Commentaries
Institutional Interviews
Journal Interviews
Podcasts

Analyses

Featured Analyses
What's Hot In...
Special Topics

Data & Rankings

Sci-Bytes
Fast Breaking Papers
New Hot Papers
Emerging Research Fronts
Fast Moving Fronts
Corporate Research Fronts
Research Front Maps
Current Classics
Top Topics
Rising Stars
New Entrants
Country Profiles

About Science Watch

Methodology
Archives
Contact Us
RSS Feeds

INSTITUTIONAL INTERVIEWS - 2009

June 2009

PDF

Zoological Society of San Diego

Second of three parts | (Part 1)

A featured institution selection from *Essential Science Indicators*SM

Essential Science Indicators *from Thomson Reuters recently named the Zoological Society of San Diego a Rising Star in the field of Plant & Animal Science, meaning that the Zoo had the highest percent increase in total citations in this field from August to October 2008. The Zoo's current record in this field includes 228 papers cited a total of 1,545 times between January 1, 1999 and February 28, 2009.*

View a giraffe video from the Zoological Society of San Diego.

In the second part of this feature, ScienceWatch.com's *Jennifer Minnick explores the Zoo's dedication to the psychological well-being and breeding success of the animals in its collection, by both natural and artificial means, as well as some unique animal communications studies.*

Mating Mechanics in the Lab...

One way the Zoo's Institute for Conservation Research helps in the reproductive success of its animals, particularly its endangered species, is by studying their physiology and endocrinology in the Reproductive Physiology Division, headed up by Dr. Barbara Durrant.

In terms of the endocrinology aspect of the division, Durrant is currently looking at hormone receptors, in particular, the global effect of pollutants and environmental toxins on reproduction at the endocrine level. The work is important because, Durrant says, "We see this all over the world, happening in a number of species. Many reports have documented the decline of reproductive parameters in humans as well, and some think that this is directly attributed to environmental toxins and pollutants. We're studying the effects of these substances in female reproduction."

So far, her labs have cloned hormone receptors for three rhino species and the polar bear, something that has never been done before. These cloned receptors will then be challenged *in vitro* with phytoestrogens, pollutants, toxins, and pesticides—all things known or suspected to affect reproduction. The outstanding benefit of this research is the fact that in doing in *in-vitro*, there is no need to expose the animals themselves to these substances, and it is high-throughput in that they can screen toxins at all different concentrations and see results almost immediately.

Durrant has a lot of experience with *in-vitro* studies. Some of her most-cited papers deal with reproductive studies in domestic dogs, for which her team obtained ovaries and testes from local spay and neuter clinics. Their aim was to use the much more common domestic dogs as models for endangered carnivores, especially canids like wolves, foxes, jackals, etc. Of the research, Durrant comments, "We were able to describe the dynamic of follicle growth in the ovary and actually classify follicles that hadn't been classified before, to look at how follicles or eggs die in the ovary; we looked at age effects, breed effects, and we looked at all kinds of culture conditions. So we were teaching ourselves about the ovaries, the follicles, and the eggs of carnivores by working with the dogs, but we also learned new things about the domestic dog itself in the course of our studies."

One unexpected benefit of this research is that the domestic dog is also an excellent model for ursids (bears). Canids and ursids are not too far apart on the taxonomic tree, and there are marked similarities between some of their reproductive strategies. At the time, Durrant and her group were looking for a model for the giant panda. They settled on the American black bear, which is the most common bear in North America. They had a test group of six free-ranging, well-managed American black bears in South Dakota, and from their research with this group, it was discovered that the domestic dog could also serve as a model for bears. "The ovaries are very similar, the way that the eggs die inside the ovaries are very similar, even the way the eggs look are identical. It turned out to be an excellent model, and of course, using the domestic dog, over the course of the year, hundreds of sets of ovaries and testes from local spay clinics increase our numbers, so that really gets some statistical power and show us which way to go when we had to go to a model species."

The Frozen Zoo®

Using model species is nothing new to Durrant. Her team has also done research in the Chinese pheasant, with the domestic chicken as a model. These same principles apply to her work with the Frozen Zoo®, a repository of genetic resources, including DNA, cell cultures, blood and tissue specimens, sperm, oocytes, ova, and embryos, from over 8,400 individual animals from more than 800 species or subspecies. It is the job of Durrant and her team to develop preservation protocols for eggs, sperm, and embryos. It's a challenging task, because every single species, no matter how closely related they are to each other, has different requirements, and using model species from their own experiences and from the literature is the best way to go about developing these protocols.

For instance, when a rhino at the Zoo died of old age, the team wanted to be able to do *in-vitro* maturation and fertilization, but needed to know how to preserve the rhino's genetic material, because such a thing had never been tried. Because rhinos are closely related to horses, the team used the published literature on horses as a springboard for the rhino, and developed the protocol from there.

"The process of developing cryopreservation techniques for genetic material can be very hit-or-miss, and there appears to be no rhyme or reason—just a lot of trial and error." Durrant relates. "When we were looking to develop the protocol for the pheasant, we first looked to the literature for chickens and turkeys, because they are closely related. But it turned out that the requirements for the pheasant were a closer match to cranes, which aren't nearly as close on the taxonomic tree. More recently, we obtained ovaries from the camel. We isolated the eggs, but no one has done this work in the camel, and so we used what we had been developing as a bovine protocol for gazelles, antelopes, etc., based on what's done in domestic cattle, and it worked extremely well. So we were surprised that we could use this protocol for the camel, and that we had succeeded on the first try. That's why they call it **re**search you know!" Durrant jokes.

...And in the Collection

Perhaps the division's most publicized success story was the 1999 birth of giant panda Hua Mei—her mother, Bai Yun, was artificially inseminated by father Shi Shi's sperm. Hua Mei was the first panda born in captivity in the US to survive to adulthood.

More recently, the Wild Animal Park celebrated the birth of a male African elephant. Durrant's team is very involved with monitoring the Park's herd of elephants, specifically their hormone levels. Better still, they have seen enough pregnancies and births in the herd to be able to make predictions about things like when the elephants are likely to give birth, making it easier on keepers anxiously awaiting said births. Currently, Durrant's team is working on a non-invasive method for extracting sperm from one of the bull African elephants in the collection.

"Semen collection phantoms, which are crude dummies of females, have been in use with domestic bulls, horses, and pigs, but no one's ever tried it with an elephant," Durrant explains. "We had an artificial vagina designed, and we're currently training one of our bull elephants to use it so that we can do non-invasive semen collection. He's learning to climb on top of the phantom and get in position, and he's starting to understand the process, what we want from him. We haven't put it all together yet, but all the pieces are coming together one by one."

"It's quite a project," she continues, "This is a male who's actively breeding, and he has lots of real live females that he can breed with pretty much whenever he wants, so it's taking some time to get him to transfer his attention to an inanimate object, but he's extremely smart and he will figure it out. Our elephant keepers are all excellent trainers as well and so they're taking it step by step and he's doing very well."

Getting Pandas to Do What Comes Naturally

Quite possibly the most famous animal at the San Diego Zoo is the giant panda, due to well-publicized efforts by the Zoo and other organizations throughout the world to help the species survive. Dr. Ron Swaisgood, who is the Institute's Director of Applied Animal Ecology, has worked on the panda recovery projects for many years.

Swaisgood's work with the pandas involves aiding them in breeding success as well as supervising enrichment programs for the animals in captivity both at the San Diego Zoo and at the Wolong Panda Reserve in China. "When you look at pandas in the wild, if they're left alone, they do just fine at perpetuating themselves. In captivity, we knew there wasn't something fundamentally wrong with the pandas, so there had to be something wrong with the way the pandas were being managed, and we set out to answer that question," says Swaisgood of the program's rationale.

One of the first theories the team came up with was that the pandas in captivity were experiencing some form of a communications breakdown, that the Zoo wasn't providing the proper environment to communicate. Giant pandas are solitary animals that rarely interact face-to-face except for aggression and mating. "Three-hundred-sixty days a year, pandas avoid one another or perhaps even respond aggressively to one another," Swaisgood relates, "The challenge then, was to try, for a very brief window of time, to turn off that aggressive/avoidance response and turn on the sexual stimulation. We thought the key might be in communication opportunities."

Specifically? Scent, the study and use of which are the subjects of several highly cited papers by Swaisgood and his colleagues. Essentially, in the wild, pandas have overlapping home ranges, and although they might not have physical contact or vocal communication, they do leave scent messages for one another in the common areas of their home ranges.

Giant Panda

So what the team did was to tease apart what the chemical signals in the scents meant to the pandas. They set up a series of experiments to gauge what kind of information pandas can extract from these signals and how it affects their motivation. The results showed a clear effect on their sexual motivation after they were exposed to opposite sex odors. "They showed a heightened sexual arousal as evidenced in their vocal behavior—they began to bleat and chirp—which is a sign that they are certainly interested and perhaps willing to mate," says Swaisgood.

The next step was to experiment with mimicking the ways pandas use scent in the wild: researchers let the pandas mark pieces of wood, which were then delivered as "scent postcards" to pandas of the opposite sex. The pairings were selected by qualities that indicated they would be good breeders.

"We tried to encourage familiarity through scent a few weeks before the female was going to come into heat," Swaisgood explains. "We found that when we did this, we had increased their libido and decreased their aggressive tendencies. The other method that we used, which was actually more powerful, was a simple process we call pen swapping, where you pull a female out and let the male come in her pen, which has her scent, and we do the reverse in the male's pen. We found that had the desired effect on their motivation."

Thought scent was important, it wasn't the only component to engaging the pandas' interest in mating. Various enrichment tools were also employed to engage the panda's interest, because, as Swaisgood says, "a happy panda is a breeding panda." These enrichment devices included things like food poles, which make the pandas work for their food, and in doing so, more closely imitate their experiences in nature. Though the project started small, the team was eventually able to build bigger and better enclosures for the pandas at Wolong.

Swaisgood and his colleagues are also working with pandas in the wild. The best estimate of the number of pandas in the wild is around 1,600. For the last three years, Swaisgood has been working with his colleagues and collaborators at the Chinese Academy of Sciences, tracking a population of wild pandas in the Foping Nature Reserve, using GPS satellite collars and other methods to understand their ecology and behavior with regard to conservation issues. Of the fifty-odd pandas in the reserve, researchers have four animals presently fitted with collars and they are in the process of collaring six more.

Species Survival through Maternal Investment

Animal behavior is Fred Bercovitch's *raison d'être* as the Director of Behavioral Biology, a position he has held since coming to the Zoo in 2001. The main focus of his division is to determine why different animals breed at different rates.

"That's the quick and dirty issue," Bercovitch says by way of introduction. "We look at the factors that are involved in promoting reproductive success. Those factors can include things like behavior of the animal, physiology of the animal, the demography or social structure of their communities, what they eat, etc. We try to take a whole-organism approach to ferreting out the reasons why they don't breed at the same rate, or, in a captive situation, why some animals are not breeding, and others are. So it all comes down to working with animals in the collection and in the field to focus in on what the main reasons are for the different rates of breeding."

Prior to coming to the Zoo, Bercovitch worked with rhesus monkeys, trying to determine the key factors in their reproductive success from an evolutionary biology standpoint. He found it a natural transition to go from exploring reproductive success in a common animal like the rhesus monkey to studying more exotic species, about which much less is known. Coming from primatology to studying other mammals also gave him and other researchers the chance to approach things from a new perspective.

For instance, he had done many studies on first-time mothers in rhesus monkeys, and this continued to be an interest of his at the Zoo. In rhesus monkeys, he had looked at how, in first-time mothers, the rank of the mother affected the birth weight of the baby. At the Zoo, he has examined why some first-time mothers seem to have trouble raising their babies. Given the fact that in certain species in the wild, first-time mothers also have this same difficulty, Bercovitch hypothesizes that the difficulty is more a function of being a mother for the first time and not of being in captivity. "It's a lot more difficult to be a mother than most people think," he jokes.

"The differences in reproductive success can come from differences in breeding, as some animals may mate more than others. It also comes from differences in rearing success, which is why we do a lot of work on maternal investment," Bercovitch explains. "Do these moms partition resources differently to sons vs. daughters, does it depend upon the rank of the mother, the age of the mother, and also looking across species, which is really fascinating, of how these different patterns of maternal investment act as a mechanism to push their genes onto the next generation."

Koala

When asked if, because he works with such a wide variety of species, he has noticed similarities across species, Bercovitch replies that it's not a yes or no type of answer, but that it's fascinating to see what the similarities—and the differences—are. For example, he has noticed that the biggest similarity among mammals is that the mothers are very invested in insuring the survival of their young. This is especially true for mammals that bear a single offspring at a time, but also holds true for mammals that bear litters.

The biggest difference comes in the time spent with the offspring to ensure that they can survive. "For me," Bercovitch relates, "it was really surprising to watch koalas, because they kind of have a reputation for being these old-fashioned evolutionary hangovers that haven't kept pace with the times, and yet they are with their young for about the same amount of time that Patas monkeys are. Patas monkeys are about the same size as koalas. As primates, they're supposed to be really clever, and the idea was that the offspring were dependent on the mom for a long amount of time because they have so much to learn—all the social nuances and the feeding habits and the rest of group and how to get along, etc. So why do these two animals, with seemingly different rearing needs, have roughly the same rearing time?"

"It gets even stranger when you look at giraffes," continues Bercovitch, "because here you have a much bigger animal, but with a much shorter rearing period. Giraffe calves have a really quick growth trajectory—

at three to four months they can actually run around, probably to avoid predators and keep up with their moms and their herds in the wild."

Another curious animal is the Nile lechwe, an endangered antelope native to Southern Sudan. It's unknown how the females do it, but as they get older, they appear to put more effort into bearing sons, often at their own expense, since sons tend to be larger and harder for older females to bear, so they may die making the effort.

In essence, it all comes down to the fact that the mothers ultimately want to increase the chance of their kids' survival, and all the similarities and differences in breeding and rearing times serve a common purpose. "There's no simple, easy way to figure out what's going on," Bercovitch remarks. But that doesn't mean he won't keep trying. Bercovitch praises two Zoo resources as being key to his research: the meticulous records that the Zoo archives, and the keepers of the animals themselves. With the records kept on so many animals, not to mention being able to study the animals themselves, Bercovitch likens himself to a kid in a candy store, "an amazing academic challenge as well as great fun," he adds.

Bioacoustics: Listening to the Animals

Taking a whole-organism approach to behavior and conservation leads to untapped areas of research. One of these that Bercovitch finds very exciting is bioacoustics—how sound influences the various aspects of reproduction. He and colleague Dr. Matthew Anderson are starting to unravel the mysteries of how animals "talk" to each other and what they're saying. Anderson, who trained as a primatologist specializing in the evolution of reproduction, is particularly interested in how animal communication affects reproduction.

One of the acoustics projects involves koalas. "Koalas have this sound called a bellow," Bercovitch explains. "The males make this bellowing noise which sounds like a cross between a pig snorting and lawnmower or motorcycle revving. The males use the bellow to both attract females and repel other males from mating. Are these sounds related to their hormone levels? How far can it travel in the wild? What time to they give them—is it better to give a call when no one else is calling to say 'Hey, here I am,' to try to target females to mate with, or when other males are calling to say 'Hey, I'm a better male than he is?' How much competition is mediated by calling? These are the questions we're trying to answer."

"Over on the rearing side of things, we're also looking at mom-infant acoustic communications," Bercovitch continues, "for instance, the high shrieking noises lots of young animals make. They tend to be easy to localize so one of the common things among little guys is that while it's easy for moms to locate them, it's easy for predators too. But the other advantage to the noise is that you get your mom and the community involved in your protection."

Bercovitch is intrigued by parallels across species—that loud booming calls seem to be cries for mates and high shrieking noises are infants in need. "What we try to do here is see if we can't get a handle on the meaning of the various calls, to use them as tools in colony management here at the zoo," he explains, "For example, what we'd like to try is if you do have a first-time mom who's not very involved, and you have a little baby that has to be brought up in the nursery, maybe if you understood more about the vocal communications, when the baby's in the nursery you could play its mom's calls to calm them down."

Cheetah

Other studies are underway in cheetahs, okapis, and elephants, but the premise behind each is slightly different. In the cheetahs, for instance, the acoustic studies are directed at breeding. "We've had good historical success for breeding in the Wild Animal Park, but we're a little hit-or-miss as to how that breeding success has come about," Anderson relates. "Cheetahs are relatively difficult to breed at the best of times. A few years ago I was involved in a project that looked at cheetah vocabulary, for want of a better term, in order to examine what calls cheetahs make and thereby discern their meaning."

As it turns out, male cheetahs make a very distinctive call, known as a "stutter-bark," which has been found to be an acoustic cue to stimulate hormone levels in the females and bring about ovulation. In the cheetahs they are observing, researchers have noticed that it takes a few days for the male cheetahs' stutter-barking to reach full peak, and then a few days after that, the females will show an upswing in their levels of reproductive hormones. The goal of the study is to determine the evolution, the mechanisms, and the exact function of the call, with the idea of using the call in the collection. Anderson hopes the stutter-bark recordings will be particularly useful for naïve or never-before-bred animals—getting males to perform the stutter-bark themselves, and in turn stimulating females.

Anderson is also working with African elephants and the myriad communications they use. Female elephants have been known to put out very low-pitched calls that have long frequencies, which are thought to serve as an announcement to males that they have come into season. Interestingly, this behavior is the diametric opposite in elephants than in cheetahs. In elephants, females have a tendency to stick together in the wild, and males are solitary, whereas with cheetahs, the opposite is the norm.

"From my point of view, in trying to understand the evolution of these signals, different problems play into their development," Anderson hypothesizes. "For cheetahs, the females don't cycle regularly, and the males have evolved this signal with which they can stimulate the regulation of hormones and thereby initiate mating. Whereas with the elephants, it's just a matter of distance being problematic, and the low-frequency calls have evolved for attraction of the males."

Elephants—and other species—also have a great deal of communication that can't be heard by the human ear. Infrasound, a sort of secret language various animals have evolved, is of great interest to Anderson. The Zoo has had great success with breeding its African elephants, and, just as Barbara Durrant and her team have been able to discern much about the reproductive physiology of these elephants, so too does Anderson have extensive opportunities to study mother-calf interactions and vocalizations. Currently, he's looking into the idea of mother elephants using low-frequency calls to make announcements of biological significance to the herd, such as an imminent birth.

Another animal Anderson is quite keen to study is the okapi, a threatened African mammal closely related to the giraffe. "They're rather like the next step up in that they've evolved completely infrasound-based communication," Anderson enthuses. "They communicate all the time using calls we simply can't hear. Until very, very recently, they were viewed as being a non-vocal species, but in fact, it turned out they've got a whole secret language we just weren't aware of. In the last five or six years, we thought this secret language might exist, but it was only a few months ago that we did the first recording to show that it's really happening."

The process of recording turned out to be quite simple in the end. "You record the animals for many hours and hope you're capturing the calls as you can't actually hear them during observation," explains Anderson, "but later you can play the recordings into a computer and listen at varying speeds. By playing things three to four times faster, you can push the calls up into a frequency range we actually can hear. It sounds strange at first , but it allows us to say that there is definitely communication going on outside of our hearing range." How okapis make these sounds and why are questions Anderson is hoping to answer with these studies. Similar studies are also getting started in the giraffe.

Bioacoustics may also prove to be a useful tool in the wild, particularly as an extension of population censusing studies. Anderson is optimistic about its use. "By developing a separate modality such as sound we can get much more information than if we used just traditional methods. This will be a boon in the field and I believe we will have great success with it by setting up automated recording units, we'll be able to get an idea of how many species there are in the area—males and females, etc. We'll get a much better idea of the levels of biodiversity within certain habitats," he says.

Next Month: In the Field and Educating the Public

The Institute for Conservation Research is devoted to the health, well-being, and reproductive success of not only the animals in their collection, but also endangered animals and threatened habitats worldwide. Next month, we'll talk with some of the teams who oversee these field projects. We'll also take a look at the education efforts of all the teams.

Zoological Society of San Diego's current most-cited paper in *Essential Science Indicators*, with 515 cites:

Murphy WJ, *et al.*, "Molecular phylogenetics and the origins of placental mammals," *Nature* 409 (6820): 614-8, 1 February 2001. Source: *Essential Science Indicators* from Thomson Reuters.

Additional Information:

The Zoological Society of San Diego was a Rising Star in Plant & Animal Science in March 2009.

View Part 1 of this interview from May 2009.

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