

MARY ASHLEY

DM: And so our next talk. And I also pointed out before the break that the listing in the program schedule is not correct. So if you'll just turn the page from the program schedule, you'll see this is the appropriate listing. So our next talk is Dr. Mary Ashley, from the University of Illinois at Chicago, and the title of that talk is Finding Families in the Field: Molecular Methods for Studying Breeding and Behavior.

FINDING FAMILIES IN THE FIELD: MOLECULAR
METHODS FOR STUDYING BREEDING AND BEHAVIOR

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MA: I'm feeling so low-tech, because I have slides.... But look they come up right away. (Laughter) And I'm going to try to rush through some of these. They made me relinquish my slides before I'd completed triage, so I may have a few too many slides. So let me get started.

The point today that I'm going to try to make is a rather simple one, but I think one that hasn't come up exactly yet in the conference, or the symposium, and that is I think that one of the most important contributions that can be made by molecular genetics or biotechnology and conservation is simply to supply a new tool to ecologists and population biologists that are doing work on the species that we're trying to protect.

By combining molecular-genetic data with that obtained from more standard methods of ecological research such as measuring dispersal, survival, recruitment, life history and so on we can get some new information, and sometimes important information useful for management. And that's what I think an important contribution is.

This is one of the study subjects that I'll get to in a moment.... On this slide I've just listed some things that came to the top of my head, that are the kinds of things that managers might want to know. To, say, develop a nature reserve or preserve for a particular species, or to come up with a population viability analysis for a threatened species something like coming up with a sustainable harvest for a particular species you'd like to know to know something about the ecology of the species.

And I think some of these things that you need to know really, I can't see any role for molecular genetics. And I've put those in white. I mean, maybe somebody can come up with some, but I couldn't. Things like habitat requirements; interactions with interspecific interactions; competition; predation; and so on. Usually population size it's better to just count the animals. Maybe not always.

But in yellow I've listed some things where I think genetics can, at least in some cases, make a contribution. Things like effective population size; reproductive rates; reproductive skew among breeding individuals; dispersal pattern; and gene flow.

Sometimes fieldwork alone can get at these issues better than genetics and, in that case, I don't think there's any reason to do genetics. But sometimes genetics will add a new dimension new information.

Some of the studies we do in the lab involve paternity assignment, and we heard a little bit about that in an earlier talk. One thing we're doing more and more of in our lab is looking for sib relationships among individuals in the field. And the reason we're doing this for some species, it's a lot easier to sample juveniles than it is to sample adults and sharks are an example. It's a lot easier to sample a baby shark than an adult shark.

Some of you may be old enough to remember when DNA sequences looked like this. (Laughter) The technique we're using is microsatellite analysis. We've heard about that already this morning. Here's some examples of some actual microsatellites. Some of the advantages you can amplify these by PCR. The applications that we're using are a little bit different than what we heard about this morning. We're mostly doing this for pedigree reconstruction. But you can use preliminary chain reaction, and the size of the allele will reflect the number of repeats in that core unit. And then we saw a gel like this from Steve O'Brien. This is genotypes of a whole bunch of individual sharks, I believe, that are scored at three microsatellite loci. Each lane has one individual, and there's a size standard that's run internally.

The first study that I'm going to talk about is sort of an extreme example, where the behavioral and ecological is massive. There's a mountain of information. These are the Gombe chimps. In fact, this is Fanny and her son Fudge. And Jane Goodall began studying the chimpanzees in Gombe 40 years ago. And since that time that they were habituated, these chimpanzees have had no privacy at all. There's data on copulations; on births, deaths; behaviors, diets, so on. Just a tremendous amount of valuable information on the behavior of these animals.

So you might ask: Well, what, possibly, more could we learn from using molecular genetics? And it turns out, as many of you know, there's one aspect of chimp biology that's always been a problem, and that is paternity assignment. Our closest relatives are highly promiscuous. Females may copulate 100 times during an estrus cycle. She may copulate with every adult male in the community. So, really, without genetic testing, it's been difficult, or impossible, to determine who's actually fathering the babies.

So using molecular techniques, we were interested in asking some questions about chimpanzees: What are the reproductive skew among males? What implications are there for effective population size? If there's only one alpha male that's siring most of the offspring, then that will have important implications for effective population size. Alternatively, do strategies that are maybe used by low- or middle-ranking males lead, sometimes, to paternity so that there are more fathers in the community? Alternatively, maybe females move outside of the community to mate, and avoid inbreeding that way. Or are there alternative strategies for females to avoid mating with, say, dominant male relatives that might be in the community?

So we weren't the first ones to attempt paternity assignment. And, actually, Phil Morin is here at the conference. And he, and Dave Woodruff and colleagues, actually did some initial paternity assignments, and assigned at least a couple of the paternities and we've built on that. And by we I mean Julie Constable, who is the graduate student who did genotyping in my lab. And she's actually a graduate student of Ann Pusey at the University of Minnesota. And, of course, Jane Goodall and all the individuals who have been working at Gombe need to be acknowledged, certainly.

All this work was done noninvasively. And we used mostly fecal samples and, in some cases, we had hair samples. And we obtained microsatellite genotypes from these fecal and hair samples. We used paternity assignment using a very nice program, that was written by Marshall and

colleagues, called Service which uses a likelihood approach for paternity assignment. And then, once we assigned paternities, we went back and looked at what was going on at the time of conception.

I don't want to go into the details of this, but this noninvasive sampling you know, it sounded so great when we first learned about it. And, let me tell you, it's a lot of trouble, and you have to be very careful. And we took a number of precautions and I won't go into them. We used human microsatellites tetra repeats, mostly for this genotyping. One of the precautions we had to take was make sure that Julie Constable wasn't coming out as father as any of the Gombe chimps. (Laughter) Because the problem of contamination is just extreme.

But we took a lot of precautions, and we assigned paternity to 14 of the Gombe chimps and so patterns now begin to emerge. Of the 14, we assigned paternity to seven of them. Exactly half were fathered by either the alpha male or the second-most high-ranking male.

So, clearly, being male can lead to or a high-ranking male can lead to reproductive success. The most common strategy for these successful males was guarding of a female, or not letting any other males get near her when she was in estrus. Maybe more surprisingly was the fact that seven of the paternities were assigned to either middle- or low-ranking males. So these alternative strategies do also sometimes lead to paternity maybe to a higher extent than we would have thought.

Sometimes these opportunistic strategies of this was just, you know, a male that happened to win out in sperm competition when there were lots of males mating with a female. And, also, this tendency to form consortships, where a male and a female would go off, away from the community. This also successfully led to some paternities, and was a strategy that low-ranking males had some success with. Of course, there's a whole story behind each one of these, that I don't have time to go into.

Another issue was: Do females go outside the community to mate? This is important for inbreeding or knowing the threat of inbreeding for chimpanzees. We didn't find any cases of that at all. Fourteen offspring were assigned to community males. And I'll skip that next point, but it looks like extragroup paternity is not a common strategy.

So what about strategies when your brother or your son is a dominant male? It turns out that, in most cases, females were successful in using alternative strategies, so that they did not mate with their high-ranking males. And, in some cases, these consortships they'd go away from the group with a low-ranking male. The strategies were successful except in one case, and this is the saga of Frodo, Fifi and Fred.

This is Frodo. He's currently the dominant male in this community, and he's the largest male on record there. He's very aggressive, and he was seen copulating with his mother, Fifi. This is much more melodramatic than I should say. But this is Fred, the baby and it turned out that Frodo was the father and brother of Fred. Fred happened to die during a scabies epidemic. Here he is very sick, right before his death. And whether or not that had anything to do with inbreeding. You know, we have a sample size of one here, so we can't draw any conclusions. But it's at least an interesting possibility.

Okay. Let me move right along here. That's just too morose to dwell on.... Okay, here's a completely different situation. This is not a species that we want to protect. This is a brown-headed cowbird it's a big pest. It's a problem. And we've heard a lot about invasive species and problems with them. And if you go back think about that first list of things that managers might want to know about a species in order to properly manage it, or promote it I think that you could

also argue that those are all of the things that we'd like to know about, say, an invasive species, or a problem species, in order to come up with ways of controlling it. We need to know more about the ecology of invasive species, as well as threatened species.

Now, cowbirds are not invasive. They are native to the Great Plains of North America. But because of anthropogenic changes in the landscape, they've they have been very conducive to cowbirds, and they've spread their range dramatically in the last 200 years. They've moved all the way to the West Coast; more recently into the Southeast and Florida.

As most of you know, they are brood parasites they lay their eggs in the nests of other birds. Here we have two cowbird eggs in I think this is song sparrow nest. And so many songbirds throughout North America are busily raising cowbirds, and not raising their own babies. The cowbird babies grow very quickly. This is a cowbird nestling with a same-age song sparrow nestling. A lot of times the fitness, or the reproductive output, is greatly reduced. And there are at least a couple species, including the Kirtland's warbler, that probably would be gone now, if it weren't for massive cowbird control measures. So these are a problem.

Bill Straussberger is a student in my lab. He's a very excellent field ornithologist, and he wanted to do a combination of a long-term field study with a genetic analysis to look at patterns of cowbird parasitism. Interested in the community-wide patterns of parasitism at our site, which hosts are most vulnerable to parasitism? And those are two questions that come from the field data alone.

But, again, in yellow I have some questions that can be added to this by adding a genetic component to the study: What is the fecundity of individual female cowbirds? Are female cowbirds host-specialist? We know they parasitize over 200 species of hosts, but what are individual females doing? And, finally: Do females return to breed at the same sites year after year?

This is our study site this is The Morton Arboretum which is actually a lovely place, even though it doesn't look like this. But you'll see why I wanted to give this aerial shot. This is about 30 miles west of Chicago, and it's a clearly urban-suburban mosaic habitat. And it's these kinds of habitats where cowbirds do really well, and these sorts of areas are probably a tremendous source for cowbirds, in a sink for other bird species that are trying to breed there.

As I said, Bill is just really great at the fieldwork. He spent four seasons just basically trying to find every nest that was built at the Arboretum. And he found hundreds of nests and collected 349 cowbird eggs from those nests. We sexed all the eggs, or the embryos, with some nice new molecular markers that are very easy to use. And then, at least from 1995, we have the data from 175 eggs. All the eggs collected in 1995, and 27 adults. Again, this is a case where it's it's not easy to find those eggs, but it's easier than sampling the adults.

One sort of interesting new sort of technology we discovered is, that if you take a freshly laid egg you can get the maternal genotype from one part of the egg, and you can get the embryo genotype from the embryo. So I guess that would be noninvasive sampling of the mother but, you know, destructive sampling of the embryo. But this allowed us to reconstruct some maternal genotypes for individuals that we never even saw.

Then we tried to construct, or we did construct, sib groups, using sort of a hodgepodge of methods. This is something we're going to work on, the sib-group reconstruction, to do this a little more efficiently. But we used a combination of kinship which gives you a coefficient of relatedness and then the Service program, when we did have parents either genotyped, or their genotypes inferred.

Some of the results, quickly, for the field studies, alone, without the genetic studies. We found very high levels of parasitism. Eighteen of the 34 species were parasitized, but 80% of the eggs were found in just five hosts. The cowbird liked nests in the forest they liked nests on the edge. They avoided grassland-nesting species. Which is interesting, since they're thought to have evolved in the grassland. So maybe those species have evolved defenses. They avoided large hosts cavity nests and very aggressive hosts.

But, then, we can add the genetic data to this and get information about what individual females are doing. One interesting finding we found that cowbirds are laying female eggs in one kind of host and male eggs in another kind of host. And that had never been, you know, known before. When we placed these in full sib groups, one of the last things here is, we were really interested in the fecundity of cowbirds. There have been records of female cowbird producing over 40 eggs in captivity. But whether this is ever realized in natural situations is important to know. And the maximum fecundity we found was 13, with a mean of 4.2.

More results here. They were host generalists, is the main thing I want to show up that the females did not specialize on particular hosts. And then here's an example of that. And, you know, thank God for graduate students. Because this would have taken me a year to figure out how to do, but one of my students does GIS sorts of things.

And each of these is a cowbird egg, and the polygon represents all the eggs from a single female. And so we could map the laying territories for each cowbird. And this just shows five of them. They were largely overlapping territories. So if I showed them all, it'd be a big mess. But I think this illustrates what you can do with a combination of a field study with genetic studies the kind of data that you can get out.

And then, very quickly, I want to talk about the last system sharks. These are lemon sharks *Negaprion brevirostris*. Sharks are in big trouble. They are harvested for fins; they're caught as by-catch. Many species are listed, and many species are declining. And despite weeklong Shark Week on The Discovery Channel and incredible fascination by many, many little boys in sharks we really don't know very much about the basic biology of sharks.

What we do know suggests that management strategies that have been developed for teleost fish simply won't work. Sharks have very low reproductive rates maybe take 12 years or so to reach reproductive maturity. Lemon sharks might produce a litter of, you know, 10 or 12 offspring every two or three years we don't really know. But they simply are just not going to rebound from overharvesting, like a cod or a salmon might.

Particularly what we're interested in is the role of nursery lagoons for sharks. It seems like many species of coastal sharks require mangrove fringe lagoons, like this one. This is Marquesas Key, which is just west of Key West. The females go in there, they have their babies, and the babies stay in these protected lagoons sometimes for years. And this allows us the opportunity to go in and sample lots and lots of baby lemon sharks, and that's what we're doing. And so the idea is that we can reconstruct the breeding biology of the parents by exhaustively sampling cohorts of offspring.

And some of the questions we want to ask, just from field studies: How many young lemon sharks are in these nursery lagoons? How long do they stay there? What are the survival rates? What are the growth rates? And so on. And then, by adding genetic data, we can ask: Well, how many females produce this set of babies? What are the litter sizes? How often do females reproduce? We could look for maternally related sibs, say, in year one and year three or is it

year one and year four? to try and figure out whether the females are returning, and how often they re returning.

And this data is in a preliminary stage, but I thought it was a good example. We have three study sites. Our main one is in Bimini, Bahamas, and one of them is off of Brazil. This is Atol das Rocas, a very unusual site. Bimini has an interesting shape, where the island encircles a shallow lagoon. And, basically, we can go in here and fish out every baby lemon shark in this lagoon, year after year. And we re pretty confident that we re getting about 90% of the baby lemon sharks. And when we capture them we weigh them, sex them, measure them, and take a little fin clip for DNA analysis. And I won t spend any time on this....

Anyway, we ve been able to extensively find that flow is extensive throughout the Western Atlantic, and we ve found some new information about how many what the average litter size is, and how many females are giving birth in these lagoons each year.

So, just in conclusion, I ll very quickly You know, fieldwork is difficult, it s laborious. (Laughter) But we shouldn t just go out there and get our DNA samples, then come back to the lab. And the real strength, I think, of some of these molecular techniques is in combination with long-term field studies, and some new information can be obtained if we just combined these two methods together.

Thank you.

(Applause)