

OLIVER RYDER

ES: (Announcements).... What we're working on this afternoon is continuing to think about the future of conservation genetics. We have two speakers this afternoon.

I was panning the room, talking to people who've known Ollie since a long time ago. In fact, I knew Ollie a long time ago. He didn't even know that. When I was an undergraduate I worked at the San Diego Zoo, and watched Ollie putting together the fantastic program that he has there. But he also, just as an aside, is really active in community environmental efforts in San Diego. And I think we should all follow his lead on that, as an individual being involved in our own backyard in conservation efforts.

But one of the things that I learned today, that I thought was very interesting—and would, I think, give you a perspective on his ability to talk to us from a global overview of conservation—is the fact that a few years ago Ollie Ryder took up skydiving. And it's a really important thing for him. He felt that he suddenly had a different view of the earth—a much broader, more comprehensive view of the earth. And we hope that will give him some insights for us in thinking about where we're going with the future of conservation genetics. Ollie?...

(Applause)

CONSERVATION AND GENOME RESOURCE BANKING

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OR: Thank you very much. And I want to join the others in thanking the organizers for putting together what's a very remarkable meeting.

I'm taking an idea from Steve O'Brien. I've never shown slides before, side by side—this is the first time I've done that. It's not the first time I've taken a good idea from Steve O'Brien, and I could acknowledge that, too, I suppose.

I work at The Center for Reproduction of Endangered Species—CRES—which is the research and conservation arm of the Zoological Society of San Diego. And a year ago we organized a conference—there's the logo: Genetic Resources for the New Century—anticipating many of the same issues that are brought up at this meeting. And we were fortunate to have George Amato present at that meeting.

And I do think that this group, collectively, I hope, appreciates that we've found an appropriate enterprise for this time—an appropriate focus. And something that can help really come up with some of the reflection—undertake that work as is necessary for the strategic thinking. Many of the talks here have addressed that very elegantly—including the one that we just heard from John Robinson.

What I want to talk about today is—well, the first thing I want to say is that the way to save species is to save them in their habitats. All of the work that we undertake must reflect that fundamental notion—that the way to conserve biological diversity is to conserve it *in situ*.

Now, we also know that we are vastly ignorant of what we're trying to conserve. We don't know how many species there are—this point has been raised. I just draw attention to this International Biodiversity Observation Year, or IBOY—we're just in the midst of beginning right now.

IBOY is an initiative of Diversitas, and Diversitas' sponsors include major scientific organizations around the world: Interreligion Council for Science; International Union of Biological Sciences; Scientific Committee for Problems of the Environment; UNESCO. It's a global effort to focus attention on biological diversity in a way that happened several decades ago for physical data about the planet—the International Geophysical Year. Now we're in time to examine the biological diversity, and its evolution on a global basis. And some of what I talk about, I think, should be related in that context.

I'm always interested in what the breakthrough of the year is in science, and this year—last year, that is ... the last time it was named—it was sequence to genomes, and the fact that there's going to be diversity of that. And that just underscores the point that the future of biology is going to be seen through the glasses of genomics, and proteomics, and bio-informatics. I think analogous to the way that, centuries ago, people started to look through microscopes. It's going to change our view of the biological world.

Now, I'm to talk about genetic resources. And I know you can't read the legend over there, but it says that—Noah's saying down there: So, actually, we're only taking tissue samples. (Laughter) That's not what this is about. (Laughter) But, as we're talking about these archetypes—about these models—everything that we do is, in a certain sense, first filtered through our own needs, and our own aspirations, and our own interests. And the solution, of course—all of these conservation solutions have to be considered in the human context. And for those locals—you know, on the right—that's Coney Island a few years ago.

So it's the era of genomics, and so much attention has been drawn to the human genome. And we're at the dawn of this—of annotating our own genome; of understanding it. Of course, we won't really understand what is human about the human genome until we know how we're different from other species. And that's one of, I think, the most interesting intellectual problems of our time.

But going back to the strictly scientific issues, that is, about sequenced genomes, we can say that for those species whose genomic biology we can grasp—we're more likely to be able to make informed choices about their conservation. So one might say that we, under ideal circumstances, would like to have genomic information about the endangered species we're trying to conserve. This is an extrapolation from the kind of data that has been gradually developed in the last three or four decades—just information, first, about genetic variation; then a finer structure; then it's geographic distribution; then it's evolutionary interpretation. I think this can be seen as an extendable concept—that we're going to want to have this kind of information about many other species.

Thepo'ouli, a Hawaiian honeycreeper, is a species that is going to be hard to consider under this paradigm. So far as we know there are only three birds remaining. And I've been involved peripherally in this project, because they don't know how to sex them. And there are no museum specimens of assigned sex. And, so, efforts to try to find out whether the species is, in fact, already extinct, because they're all of the same sex.

It looks like—if I remember correctly, there is one male and two females—and they don't live near to each other. So one strategy may be to see if you could encourage the male to go over to another—so that there would be a possibility of a mating. But I've also been involved in discussions beyond determining the sex of the birds. It's interesting. The managers—it's hard for them to say it. But, you know, as a biologist you say: Well, you know, someday these birds are going to die. And what's going to happen? And a very real possibility, you know, is that, if we're lucky, the only thing we may have from the po'ouli is a carcass. The only thing we may be able to get from it is DNA.

Well, the California condor is another species that went down to very low numbers. And, of course, it's on the rebound now. And we waited very long, but not as long as for the po'ouli. It was right down to the nubs. And we know that—well, I want to point out that condors are also externally monomorphic, and you have to undertake a genetic test to sex them. And it's very nice now, because we have these very nice specific primers that we can sex the birds from eggshell, without ever touching the bird. Which is a lot easier than karyotyping, which required a blood draw and intense lab work.

But the issue for the condor is one that's poignant and pertinent. Because its numbers came down so low, it had to be brought into captivity to save it. It's being reintroduced back into the wild. But we now know that there's a genetic disease segregating in California condors. It appears to segregate as an autosomal recessive trait its chondrodystrophy. These chicks do not hatch; they die in the egg, with skeletal abnormalities.

Now, there are a number of genes that are known to cause chondrodystrophy, and they have a finer diagnosis and naming—there is a kind of a taxonomy of the disease in humans. And, of course, in humans these genes are now mapped. And this is an example where, as condors are being reintroduced back into the wild—the first condor egg to be laid in the wild since this hiatus - when they were all brought into captivity - was laid last week. And what we would like to see that population in the wild do is grow. What we would like to not have happen is have that population growth be compromised by the problem of parental investment in chicks that don't hatch, and by an unacceptably high frequency of this disease.

And we can only identify carriers by their being the parents of affected offspring. So right now we only have an affected-offspring test to identify the carriers. And wouldn't it be nice if we had a carrier test? If we simply had a linked marker, we could do this. If we had a genome project for condors. If this were cystic fibrosis, or some other inherited disease in humans, one could rather easily imagine receiving funding from NIH and track this down. And we could sort of imagine saying: Okay—what's the fastest way to do this project? So that we could get this done with condors. And it would be a matter of genomic biology, and it would clearly have an impact on the monitoring and ability to manage this endangered species.

And when I think that—Bob Lacy can correct me on this. But I think it would be possible, with knowledge of the carriers—and with some of the demographic flexibility we have in the population—to maintain its full genetic diversity, while reducing—or almost its full genetic diversity—while reducing the frequency of this gene in the population. At least in the early years, as we try to expand California condor populations. So I think that I would really love to do a genome project on the condor—we'll see.

Of course, the intense proliferation of information about genomics is most readily applied to species that are closest related to the object of all this attention, ourselves—and the great apes are our close relatives, the gorillas, chimpanzees and orangutans. And, as our closest relatives, these species can both benefit from the knowledge of the human genome—in the sense of our understanding—using tools to understand aspects of their biology, indeed, broad aspects of their biology. As an example performing paternity analysis in chimps, using primers that were available from humans. (Mary Ashley talked about this today.)

And it also goes the other way around—that these animals have something to directly benefit us. And I'd like to explore this context with you. I want to talk about single-nucleotide polymorphisms (SNPs), and I thought I had a little slide in there that said something about SNPs.

So we've heard something about SNPs. And this information is being mined. This is the raw information about variation in the human genome. And we would like to ask—this is work that was done by Joe Hacia, in Francis Collins' lab: Can we use DNA chips to assess the character state of the same nucleotides in our common ancestor, and, thereby, identify what the ancestral character state is for human SNPs?

And the idea for this is to use these DNA chips- an Affymetrix® chip was produced in cooperation between The National Human Genome Research Institute and Eric Lander at MIT—Whitehead—that could call 397 human SNPs. It was used to interrogate chimpanzee and gorilla DNA's.

Anyway, you can see there that most of the sites were able to be scored on the other species. And one of the things that turned out is that the same sites were not polymorphic in the apes. And it was possible to call ancestral character states for many of these SNPs. And the chips could also be used to identify new alleles. Some SNPs were apparently held in common between these species, but these are really likely to be identical by state, not by descent. Because they occur at CpG nucleotides, and we don't need to go into that in great detail.

But assigning those ancestral character states then says something about—can be used to infer the age of the alleles, and to be used in information about linkage disequilibrium. And about whether these alleles—whether these sites linked to these SNPs are actively under selection in the human genome. And this information is then valuable for understanding—for developing diagnostics for susceptibility to human SNPs that are linked to disease.

In other words, there's a great value added to determining the ancestral character state of the human genome, and that value added, you know, basically comes from these close relatives of ours. Which, as I hasten to add—and I'm sure you know—are very highly endangered.

Additionally, it's possible—some experiments that Joe Hacia and I now are collaborating on—to look at relative expression of genes in humans and gorillas, will identify genes that are expressed differently in our model system of the two species. This involves this microarray analysis. Ultimately, we would want to confirm that we're seeing expression-level differences. And then, because most of the changes between the human genome and the genome of chimps and gorillas is anticipated not to be primary coding-sequence differences, but rather more likely to be evolution of regulatory sequences.

At least, that's the dominant paradigm right now. It will be possible to identify some of those elements, and to begin to, I really think, approach

some understanding about which genes—the DNA sequences that differ between chimps, and gorillas and humans—may be adaptive. And, of course, that's going to be very important and useful information in human medicine, and it will be information we can only derive by access to the genomes of the great apes.

Now, there's no advocacy for these apes in terms of their genomic information. I think we're at a pivotal time, when we should ask: What about the stewardship of this information? How should the benefits that come from this information be distributed? There is clearly humanitarian value in this information that we can anticipate; in fact it is accruing now. Can some of these benefits go back to conserve the species in the wild? This may be a new chapter in the paradigm of sustainable utilization and conservation. And if it could contribute to efforts to conserve these populations *in situ*, I think it merits pursuing.

I would want to talk briefly about gorillas now. These are the mountain gorillas, that George Schaller was among the first to study, and they're found in the Virunga regions, and also in the Impenetrable Forest in Uganda. The other two kinds of gorillas are the western lowland gorilla, that you see in zoos, and the Grauer's gorilla—or eastern lowland gorilla—that lives in the eastern part of the species' range to the west of the Virunga gorillas. And these animals are largely distributed in the Democratic Republic of the Congo, where their plight is growing more grim, with the civil conflict that goes on there.

Here is a tree of mitochondrial diversity—pruned through a topiary-pruning algorithm - that shows the relatively low mitochondrial genetic diversity of humans compared to that of our closest relatives, the chimpanzees and the bonobos, and the enormous genetic diversity of the gorillas. And this context—understanding the genetic differences that are sex-specific—those that are transmitted in the male lineages or in female lineages provides new insights. And the understanding of diversity in nuclear DNA sequences will surely enrich our understanding of the influence of culture of these animals—of their sociobiology - on the structure of gene pools. We suspect this will also enrich our knowledge about human origins and human evolution.

On the right here are some of the famous mountain gorillas that starred in the movie "Gorillas in the Mist." And one of the things—like the chimpanzees that Mary Ashley talked about, paternity isn't known in the gorillas. This was a pilot study done to look at the feasibility of doing paternity analysis on gorillas. And it was able to qualify some of these males and exclude others. This animal, long-deceased—there was one young in the cohort here that could be qualified to none of the males in the group. And so it remains possible that, you know, the favored and beloved silverback male, Beethoven, was an effective sire.

But, from the scientific standpoint, of course, understanding the inheritance of genes through time—those vectors of transmission of genetic diversity—understanding those on the spatial landscape is going to be crucial to reserve design. And that's an area where genomic biology can also make contributions.

These studies that I've talked to you about would not have been possible without access to genetic resources to undertake the basic work. We couldn't have designed PCR primers for gorillas that could be used from hair, so that we could do the studies of the wild gorillas, without the prospect of cloning larger fragments of DNA and accurately determining this sequence.

The Center for Reproduction of Endangered Species (CRES), the conservation science branch of the Zoological Society of San Diego, has for 25 years been in the business of establishing cell lines from little skin biopsies—like the one you see on the right there, and growing up these cells in culture—this has been accomplished from a wide variety of taxa. And focusing on—we call it the Frozen Zoo[®]--last year we made kind of an end-of-the-year summary. In calendar year 2000 we had initiated over 700 new lines. We now have over 5,400. We're starting to add reptiles. Our focus—because we would like to save everything, but we don't have the resources—has been on the species that have been the intensive focus of conservation efforts of The American Zoo and Aquarium Association—the Species Survival Plan (SSP) species.

And we have as a goal to provide a bank that comprises the genetic diversity of these SSP programs as an off-site banked resource, in support of research efforts that are for conservation, and especially focused on these species. There, on the right, you can see some of the fun things we got to add this year.

This figure depicts the composition of the collection. You can find it on the web at www.sandiegozoo.org/conservation.zooproject.frozenspecies.html. This next figure depicts the representation of cells frozen from individuals of the SSP taxa. I'll just draw your attention to the fact that we have about 60 bonobos; we have about 30 or 40 chimpanzees now; we have about 150 gorillas. This is an irreplaceable resource. Most of these animals that are in this collection have been dead for years. And it represents a legacy of the genetic variation of these species. We acknowledge that our efforts are small in comparison to the challenge. Of course, it should be enlarged. It's going to be a very important resource for the future.

I think that we can trust that the technologies and the tools that people in the future will have are going to be better than what we have now. But I think it's difficult to see that they're going to have more access, or that the gene pool will be larger than it is now. Because, in fact, these are endangered species. Almost, by definition, it means their gene pools are shrinking.

Well, we all have a human penchant to try to make our own surroundings enjoyable, and we would all like to have the Garden of Eden in our own backyards. As conservationists, we appreciate this fundamental value, and it speaks to us deeply. We are cognizant of these myths that support both human and divine intervention, and say people have a role to play in conserving species.

I want to just briefly say that we need to apply conservation efforts everywhere. This image is a recent satellite photo of southern California—the region where I live. This is a fire, and this is a false infrared image—so that was the hot spot there that occurred recently in the hills east of the city of San Diego. This is the city of San Diego with the gray areas being urbanized and loss of native habitat. Surprisingly enough, there still are pristine native habitats—habitats that are relatively undisturbed—minimal disturbance from the time before Europeans arrived—that survived in this region. And they represented a global source of a hot spot of biological diversity—and we're challenged to save that, right in our own backyards.

In terms of, you know, computer speed going up, and human populations going up, so are endangered species going up—and they're

going up right in the Southern California bioregion, which those of us that live in that region ought to be addressing.

In the end, of course, I think that we have to recognize our links to these problems. Where I live is the site of so many endangered species. And how do my own patterns of living affect that? I think that the opportunities we have to utilize the resources that we have on hand—the genetic resources of our zoos, and the wonder of life we can engender, the curiosity that we can invoke, the sense of sharing how precious life is (I show this photo on purpose. For me, there's nothing cuter than a baby rhino.) (Laughter) - I think that we need to use all of these tools, to try to provide the best possible array of options to the future.

We are losing genetic diversity. How we can save it may not be totally obvious. And we may want to look at all imaginable arrays of possibilities in order to provide the best set of options for the future—whom, I believe, will thank us for what we can save.

Thank you.

(Applause)