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SUSTAINABLE ECOSYSTEMS INSTITUTE

SCIENCE REVIEW

Volume I

Thursday, July 6, 2006

8:50 a.m.

Colorado State University Campus

Lory Student Center

Fort Collins, Colorado

MORNING SESSION, THURSDAY, JULY 6, 2006

DR. COURTNEY: I want to thank you for coming to this meeting. My name, as I said, is Steven Courtney, and I'm the moderator for this panel meeting. But before I kick into the business of the meeting proper, I wanted to spend a few minutes kind of setting the tone for what we're going to do, maybe talk a little bit about the code of conduct, the things that we need to address, and how we're going to address it.

But also to start by -- a few minutes by saying, well, who the heck am I and how come we're being brought into this issue and what are we trying to achieve here. So if you'll forgive me, you hear that I have a bit of a cold here; so if you have trouble in the back hearing me -- is this okay? Can you cope with that?

AUDIENCE MEMBER: You can speak up a bit.

DR. COURTNEY: Okay. I'll try my best. When we -- I'm not going to go behind the microphones, but the panelists will be sitting up front, and so they will have the use of the microphones.

Let me start by telling you a little bit about the organization SEI, who we are. We're a public-benefit, nonprofit organization. We were founded, what, 14 years ago now by Dr. Deborah Brosnan

who's still the president. And we're kind of a strange duck, kind of like me. I don't -- you can tell from my accent I don't really belong here, right. We're not an advocacy group. We're not a lot of things. What we are is we're a group of scientists who attempt to bring scientific expertise into the public arena and make it useful.

And as I mentioned, we're public benefit; definitely nonprofit, trust me; and we do a lot of things that we try to do for the public good. Many of us have positions at other institutions, but we have, of course, a small core staff. Essentially what we do in meetings like this or other things where we try to bring science to bear. So we're kind of a means by which interested scientists often in academia or in the private sector, wherever, can bring their talents and bring them to public good. So we do a lot of scientific support work like this or advising folks who look for help, such as the environmental community and others.

We also carry out research. We have a research program. And then, as I said, we do a fair amount of pro bono work; and over the last year or so, we've been actually putting a large proportion of our effort into helping in Southeast Asia on tsunami relief issues. I'm giving you the big picture because I want

you to understand that we're fundamentally trying to be good citizens. We're not advocacy. We don't do lawsuits. We don't do -- essentially try to change anyone's mind if they don't want to be changed. And we do put an awful lot of our resources into this sort of thing.

As I said, we have a small core staff, but we have some 400-plus folks who have joined the organization in some form or another often to act as reviewers, like the panelists will be doing today or who, you know, carry out programs through us. This large group of 400-plus formed a thing called the Conservation of Science Network who have all volunteered their time to help with peer review and -- particularly peer review of the endangered species and other issues, where, again, expert scientific help can really move things along.

So that's kind of who we are, who I am. This is -- this sort of meeting thing I'm doing today -- although I do have a research background, in fact, still do research -- this is the sort of thing I do most of -- most of the time. And we carry out panel processes -- peer-review panels where we ask people to come in to a meeting to focus on the science of an issue and to attempt to resolve somewhat technical but still

thorny issues which have maybe gotten a little bit out of hand. We try to bring them into a meeting like this, set up a panel process where the panel is able to interact with the scientists involved, and we try to essentially determine what the information really says.

And I just wanted to show you that, you know, we've done a number of these things. On the Columbia River, for instance, we worked with -- on a mediation between three different federal agencies. It wasn't even like there were any other folks involved, just three different members of the federal family where there was a 15-year controversy on how to deal with some salmon issues. And essentially through this whole series of these structured workshops, we were able to help them -- the participants come to a point where they could agree on what the science actually said.

We've done similar things in the Everglades. We're currently working in Missouri. Some of you may know that we actually helped the Department of Interior with the science ethics policy that's recently been adopted by the department, and several of us here in the room are also involved in the review of information for the northern spotted owl.

And you-all think -- I know that everyone thinks that Colorado is the center of the universe.

Some of us, you know, have other views in thinking coming from the northwest. The northern spotted owl is seen as being the -- you know, the big, emotionally charged issue. We worked extensively with the owl, and a couple years ago now, we helped with the Fish and Wildlife Services' standards review. And I think, again, you'll find that the work we did there, many -- some of which parallels the discussions we'll be having here today. The work we did there was again able -- we were able to get folks to agree on what the science actually said.

I mentioned the spotted owl because it's -- one of the issues in the spotted owl review was, well, are the three subspecies that have been named on the spotted owl good subspecies. That becomes an issue with the spotted owl because only two of those are listed, and so if we were to synonymize everything, perhaps the spotted owl would no longer stay on the endangered species list. So there was, as you can see, a fairly charged issue.

We resolved it by looking at the information. Some of the panelists -- one of them is here -- we looked at all the information, we brought people forward, and I think everyone pretty much agreed at the end of the process what the science actually

said. So I see that as being kind of a model for what we may do here.

I should also mention that we have a long-term relationship with the Fish and Wildlife Service to provide peer review for them. As I mentioned, we do that largely on a voluntary basis, without payment, and that was something we set up in the previous administration. So in many service actions, you know, they set a science component; and so they send some of those materials to us, and we ship it out to our reviewers who take a look at that, and then we ship those reviews back to the service.

There's been some question and issues raised about, well, why have you gotten involved in the Preble's jumping mouse, and certainly at various points I've asked myself that question. I want to give you a chance in verse on how we came to get involved. I think it's important for the transparency that I think is important in this process.

I was -- in January, I was back talking to the director of the Fish and Wildlife Service, Dale Hall, and telling him about what we've been doing on the peer reviews and saying, you know, here's how we can make it better, here's how things don't work, here's how things do work, what would make this process work

better. And I talked to him also about what we do with panels; and he said, you know, that's what we need to do with Preble's jumping mouse, and I went. So it was at that meeting that he said that he wanted to apply this sort of panel process to mouse issues. Well, it would be nice if, you know, he could write a check and write a contract and I could get on with it, but that's not the way the federal government works, as you know.

So once that decision had been made by the service, they wanted a panel process. They had to put out a request for proposals. I had to bid on that along with anybody else who wanted to bid. The service was squeaky clean about making sure that that process was carried out impartially; in fact, they responded back to external folks to review our capabilities, and ultimately on June 2, I think we were awarded the contract. But I want to emphasize, again, this is how the process worked, it's how we came to get involved. Yes, the review was set up to look like our panel, but we had to win the contract. And that's -- that's how it came to be.

The contract says we are to assess and evaluate the different studies that have been carried out on taxonomic status of Preble's meadow jumping mouse, and we're to provide peer reviews of those

studies. I want to emphasize that's our stated task, not a whole bunch of other things you might like us to do. Our task is to focus on trying to understand and evaluate and weight the reasons why a number of these studies maybe have come to different conclusions.

We're going to provide peer reviews, and those will be individual panelist's opinions. It's not our task to come up with one group consensus; however, I have often found that when you have a panel working together, they bounce ideas off each other, that they tend to, you know, work things out; and you will see maybe some consensus amongst them. But that's not part of our process, but we -- we insist that happens.

So we will recall the individual panelist's opinions, and there will be a final report. And I believe that we have to deliver that draft depressingly soon and that the final be delivered to the service certainly by the end of this month. Now, how that plays into the service's decisions about this mouse is frankly not my job, okay. That's the service's prerogative, and it's important that you recognize that clear distinction between our activities and the activities of the Fish and Wildlife Service. If you have questions for them, you know, several of them are sitting in the audience, it's maybe appropriate for you

to talk to them, but remember that it's not part of our process.

Some of the other things we're going to deliver, you'll see that there's a court reporter sitting in the corner here. She's going to be taking down verbatim every last joke I make, every last comment that's made during this process. That transcript will become part of our report; and it's part of the record which will be delivered to the service. And our intent by doing that is simply to be completely transparent about what went on and why we might reach the evaluations that we do.

We also will deliver to the service a record of all our phone conversations or all our emails, all the things that we've done, why we've made the evaluations we have. So again, I want to emphasize this is a very transparent and perhaps unusual process for many of you; but it's our intent by making clear our decision process, what information we used and to give everybody the opportunity to partake in that process. It's our intent to make sure that this is an entirely scientific and transparent process. That's our job. This is not our job.

And I want to emphasize, again, to -- the limitations. It's not our job -- and I will prevent the

panelists here from making any statement if I have any way of doing that -- it's not our job to make any recommendation on the listing status of the Preble's jumping mouse; and in fact, you know, we don't want to make any comments on those things. And I want you to understand that when, you know, you engage with us or have comments for us or questions for us -- because where you may have strong opinions on some of those policy or management issues, it's simply not part of our job, it's not part of our view, and we will not be commenting on that.

So if you want us to listen to things about, well, you know, it's really important that I be allowed to develop my land or not, or the mouse be protected, remember that we're not going to comment on those. We're not going to make any recommendation. And so simply that's just going to pass us by and won't affect what we are evaluating, which are, frankly, the arcane of, you know, sampling design, of experimental technique. Those are the things that we're going to be focusing on.

I want to also remind everybody that this little thing here, FACA, Federal Advisories Committees Act, which is an important law governing how the service or any government entity can receive information, which

is -- we're not here to advise them. They have contracted with SEI to provide a peer review; that is what we're doing. We're not here to advise the service or any government entity on what to do. And in fact, if they -- if they were to take that advice, it would be a violation of FACA. And so it's important, again, that you realize that my job as moderator and mediator is to make sure that that line is kept very clean. We're focused entirely on the scientific issues, it's a peer-review meeting.

Also I want to emphasize again that we're not even looking at all the science. It is not our job. It's not in the contract for us to be looking at the threats to this mouse or the extinction risks or anything else other than a fairly limited brief to do with the genetics taxonomy of the mouse. I'd like you all to read this. This is really not our job. Previous stuff is not our job, this is truly not our job. We are really not interested in the politics.

I have selected a panel who have committed to listening, evaluating, and giving you a clear technical evaluation of the scientific materials. N. Machiavelli said it very well -- I hope you find this amusing -- there's always a history to hypothesis than to actually believe the truth because the hypothesis has

often been tailored to fit what we think that truth should look like. And his conclusion was never discuss the truth, stick to the hypothesis.

You should remember where -- you know, you laugh too much about what -- the sentiment that's been applied here. Remember that in Machiavelli's world, if you disagreed with the prevailing hypothesis, you could be burned at the stake; and people were being burned to the stake a hundred years after he wrote this statement.

I think it's important that we not adhere to those sorts of things. I believe that what we're going to be doing is not looking at clean, simple statements. We're going to be looking at what I think is appropriate, which is the clumsy truth. Truth -- scientific truth in all its messiness, the difficulties. You know, well, things don't always work out right; people don't always agree; you know, things aren't always clear, right? I mean, and isn't that what science often entails. You know, I see the decision-makers in the audience going -- you know, they're used to dealing with scientists; and they never get a clean answer out of scientists, right.

We are going to be focusing on the clumsy truth. We're trying to get to the heart of

understanding difficult scientific issues. And I think it's important to recognize that and to talk a little bit about, well, how does science operate, because it operates by critique. It operates by criticism and by people changing their minds and people redoing other people's work. That's normal, and it's normal that people don't agree. Okay. And I think it's important that we acknowledge that up-front. And you know, there's nothing abnormal about the discussion that's going on here.

So what is our job. I want to emphasize then the sort of things we're going to be doing. We're going to be talking about the risk -- reasons for disagreement about some of the studies that have been put forward. And I think originally we're tasked with looking at Dr. King's work and Dr. Ramey's work who are in the audience here; but also, you know, as we look into this and we feel like it's more and more important to look at the array of data that pertains to this issue, so we have a phone call coming in from Dr. Vignieri, who's also participating in this discussion. I hope we'll be able to engage with some of the other protagonists, if you want to call them that, in this work. So there will be a large scope, basically, to look at these issues concerning taxonomist

status and particularly genetics.

Also I think it is part of our job as good citizens and as good scientists to be clear about ambiguities -- does that sound like an Irishism -- to be clear about the uncertainties in the data. It's tempting, you know, to take the Manchurian root, give you a clean answer; but it's accurate to tell you about the clumsy truth. It's accurate to tell you about, well, you know, sometimes you just can't tell. Sometimes things are on the fence.

And if those uncertainties are present in the materials or if we simply cannot tell from looking at things what's the best interpretation, I believe it's incumbent on us to tell you that rather than to say, you know, this is the right answer.

So you will hear us repeatedly through the course of the meeting probably saying, well, you know, this is fairly ambiguous or the weight of the evidence may be leaning this way, but it's not really clear. Or maybe, you know, maybe we can resolve things. But I believe it's important for us to be as articulate as we can about uncertainty, and typically I find that decision-makers rest more comfortably knowing what the uncertainties are rather than having to guess what they are.

The agenda is a work in progress. It's been changing a little bit. The latest agenda I think you all have is pretty good, but there's still a few changes to it. Dr. Bergstrom is not going to be able to call in, as I understand it; so when we come on to talk about morphology, we're going to have to think about how best to do that.

The way that we'd like to structure things though is to keep this as a conversation between the panel and the scientists who are present. We've still -- we're still kind of figuring out how best to carry out that conversation, and we've had some dialogue with the various parties who've asked us to make various changes; and we're still thinking about how best to do that. So I want to beg your indulgence by the fact that we may not keep strict adherence to this time line, in fact, we might change it around a little bit.

One of the things, for instance, that we'd like to do -- and we've asked a couple of the scientists to bring some raw data. The panel want to look at the actual original information. They want to look at the chromatograms that have been produced. And I think it's very likely they're going to want to go away and do that, so they'll go away and look at those things. Maybe we'll take a half an hour break once they

do that.

So I want to beg your indulgence. There may be times when I just say, hey, go away for a half hour or even two hours and come back at this particular time. And I know that that may seem a little unstructured; but hey, science is somewhat, you know, flexible and fluent and essentially this is what we may do in order to get to the materials we need. So understand, though, that it is part of our intent to allow everybody to be heard, scientists at least to be heard. And we, you know, fully intend to be able to cover all the materials that are at hand.

I want to talk a little bit then about the clumsy truth, and I want to tell you a story about a misguided scientist who I will call Mike. He's a good friend of mine. And this is a real person. My own research was to do with -- a large part, at least, to do with the evolution of behavior, and I'm interested in the evolution of diet and why things eat the things they do. I think it's the most important topic in the world, but everybody has their own.

Mike is a diluted individual who has -- while he agrees on the importance of that topic, essentially he and I disagree on absolutely everything about the study. He works on butterflies, I work on

butterflies. And we have a long history, it's about 20 years now, of criticizing each other's papers. And sometimes, you know, I didn't get my rebuttal into print before he even got his criticism into print. People who read the literature think we must be sworn enemies. I can only tell you that Mike is also a Brit, and when I made the big leap to come to America a long time ago now, despite the fact that he and I agree on almost nothing, he called me up and said, well, do you need a loan in order to make that transition.

I'm telling you that story because to me that illustrates the high moral tone that I think scientists can have and that I'd like you all to try to adopt, which is there's a distinction between the personal and the academic. And in Mike's case, you know, he's a really good guy, he just happens to be wrong a lot of the times. He's still wrong.

So I think the press, in particular -- and I know you're sitting here, some of you, so don't take this the wrong way -- I think there's a tendency to totalize and personalize things. It makes good press, but often science works by critique. Like I say, that's part of who we are. It's by being a scientist, you make your ideas and your data and your models available for criticism. Trust me, most everything I've written is

wrong; and you know, I've come to realize that over the years; and I've changed my ideas. Most scientists do that. You know, we don't believe the things we did 20 years ago. So getting things wrong is actually part of science. Getting things, you know, snarled up and then figured out, that's how we all do things and often that takes place because other people will come in and criticize you, you know. And I'm still trying to get Mike to see the light, and I've spent 20 years trying to criticize him and getting him straight, but it takes a while.

So I want to attempt to persuade you all that some of the things you've seen or believe about how these things are played out are actually just part of the normal process of science and it's as well to recognize that and simply say, you know, okay, we get criticized, we move on.

With that in mind, I want to talk a little bit about how I want to run the meeting. I want to emphasize that this is again a science meeting. It's not a federal meeting, it's an SEI meeting. It's a science meeting. We're not going to be making any recommendations. We're not, essentially, interested in the policy or political ramifications of the decisions that the service ultimately will make. We're just

interested in trying to figure out what the science is.

We're also interested in the relevant science. So if you ask to raise issues about things that I or the panel sees irrelevant, it's just, you know, not a useful use of time. We structured this as a panel-driven process, although as I mentioned, I'm a scientist; in fact, half of my cases work was on genetics and taxonomy of these butterflies. It's not my processing. I'm not going to be the one designing questions. I'm not the one going to be writing the reports. I have no vote in what the panel actually finally says. It's a panel-driven process.

And mostly what I'd like to then do is have you see it's a panel-driven process where questions are mostly coming from the panel and being directed to the scientists that are here, okay. So you know, I'm not attempting to prevent dialogue or prevent you from partaking in what's going on. I am saying it's about science, guys; and moreover, I'd like you to allow the panel to drive this process as far as possible.

If, however, there are things that you feel are not being addressed that are appropriately addressed or you have questions or you have information, we will take that information. And the way I'd like to do it in this workshop, at least, is to have that come

to us in the form of written comments or questions; and they come to me, not to the panel. Now, I'll pass them on to the panel; and if they deem it something they want to talk about, then they'll address it, okay.

We may relax a little bit as we go along. Let's just see if that works. For now, at least, I want to work with this idea of information coming to us in the form of written comments or questions. You'll find that we're going to take lots of breaks. You will have lots of opportunity. I pledge that I will be available to each of you if you have questions you want to raise; that you will have access. And we will address the things that you need to address if they're germane to what we're discussing.

And lastly, in terms of the code of conduct, I want to mention that, you know, I've done some outreach for the various parties and, you know, I know you may all have suspicions and things about how this all works or about other parties here. Let me tell you, every single person that I've spoken to has committed to making this a scientific process and making it to -- respecting the integrity of that process and to maintain high professional standards throughout.

And I'll actually point out, for instance, that I didn't even have to ask some folks for

that. The environmental community -- Erin was there. Hi, Erin. When I reached out to the environmental community, Erin just volunteered that she committed to understanding the process and to respecting the scientific integrity of everyone who's here. So I've seen similar pledges from some of the other interested parties and from the individual scientists. So I want you all -- and I hope, let's really do our best. I received those commitments, I'm going to try and hold you to those commitments. And I expect, you know, that we will treat this as a purely technical scientific discussion.

Some housekeeping issues. As I mentioned, there will be complete transcripts of everything we say. That raises a problem for our poor court reporter, which is someone in the back says er-er-er. And there's two problems with that. Can you even spell that? It raises two problems. A, she can't hear you, so please speak up. And two, she doesn't know who you are. So when you make a comment or if we ask you -- you know, if I say, Dr. Crandall, who's sitting over here, Dr. Crandall, what do you think. You know, if I've addressed you that way, great. But if I say, Tim, what do you think? You might say, I'm Tim King and this is my opinion, okay. That works. Okay. So that's

a housekeeping issue.

The transcripts take a little while to get processed. I'm sure you'll -- some of you will at least want to get copies of this, so we will do our best -- they're part of our product for the service, so I'm not quite sure how we release all this stuff, but we'll try to get them done as quickly as we can and certainly over to the service. Likewise on digital recordings, we have somewhere --

MS. SZTUKOWSKI: Around.

DR. COURTNEY: Around us. We have recorders listening and recording everything. We're going to be shipping those recordings to some of the scientists who can't be present but we want to keep informed. And you know, if you need these things, if you want to share them with somebody during the course of the meeting, I'll certainly do my best to try to get them to you. So that's just a commitment to try and help things along.

We will produce a report. I mentioned it's coming up depressingly soon, but that's delivered to the service and ultimately the release of that report will be governed by the service, and I'm sure they'll release it in good time and indicated that they won't keep everyone waiting.

A couple of other things. We -- one of our panelists has had a family emergency and is sitting, unfortunately, in a surgical hospital right now; and so he will be calling in at various points maybe to ask questions or to listen to testimony. We'll also be having at least one, perhaps other several scientists calling in on a speakerphone. So again, this isn't ideal, but remember we had -- you know, we have had this contract a month and the service has about a month left to make all its decisions, right.

So everyone's in a very tight time frame, and just bear with us, and I beg your indulgence for trying to do what it takes to make this work for everybody. So when we have the speakerphone up here, it would be great if, again, the folks who are speaking to the participants really are articulate and clear, and the rest of you can kind of keep the noise down once we're doing that.

The panel composition. Originally there were five panelists, and as you can see, there are only three sitting here. One of the panelists, as I mentioned, has a family emergency, Dr. Ron Van Den Bussche. I regard him as still a member of the panel, and he will participate in our discussions. We've been talking to him, exchanging ideas with him, and some of

the things we're going to be raising in our discussions this morning will have actually come from him. We'll be going back and forth with him during the course of this meeting. As I said, I hope that he'll be able to call in and ask his questions directly. He will participate in the write-up.

We had a fifth panelist, Dr. Eric Routman, who withdrew, and he withdrew at my request. I don't think it's any importance on the transparency issues to explain why. He had done nothing wrong, in my opinion, but there was an issue about whether he was perhaps perceived as being too close to one of the participants. I believe that what he -- he followed the practice -- and I discussed these issues with him, and he was following the normal code of scientific practice without saying, well, NSF allows you to still have contact with other scientists and to be, in some ways, associated with and provide you, are very clear about that.

And I agreed with that, but given the particular sensitivities of this issue, I decided just simply to avoid any appearance of lack of impartiality. And so Dr. Routman has withdrawn, he's not going to be taking part in the decisions. He's not going to be taking part in the write-up. We have four panelists,

and you'll get four votes on the status.

Let's see, recent panel activity. You should know that we've had two phone conversations with the panel, and we met briefly this morning just to talk about scope. I anticipate there will be a lot of to and fro amongst the panel. Some of it, you know, over beers in the evening. Some of it, you know, may be in the men's room, I don't know. Some of it won't be simply saying, you know, we really just need to focus in on some of these issues. Most of the discussions, though, will be here. The panel, you may see them having discussions among themselves up here, but bear with us because we're still, as I said, trying to figure out how to make sure we get all the information we need.

There have been changes to the schedule as we go forward. Some folks were more or less comfortable doing different things. And so be aware that, you know, we're trying to be responsive to that; make sure we get the information we need; and that the panel may take me to the side; take me out to the woodshed; say, hey, we need to focus in on these issues more. So be aware, again, as I said, we will perhaps take frequent breaks once we discuss what to do.

Other than that, I believe we're on to the last item, which is simply introductions. And,

Lisa, have I forgotten anything? Thank you. One of the things I should have mentioned about SEI is that we have a board of directors, and one of those directors is a gentleman called Barry Noon who's a professor of wildlife biology here at Colorado State, a long-term friend of the organization.

And Barry is the one who arranged for us to have this meeting room and, in fact, Colorado State has just bent over backwards to help us put this meeting together. I wanted, before we go any further, to acknowledge what a terrific job they've done and hope you find this is a nice place to have the meeting. So I know Barry's not here, but I'm certainly grateful for him for setting this up. Thank you. Anything else we need to talk about?

Then, perhaps, if you guys want to move up front. What I thought we might do is go around the room for a minute and just ask who you are, identify yourselves, don't go into long statements about what you want to do here or just a statement. Just say who you are so we can get a feel for who you are and what -- who you're representing. But before I do that, I thought I would just introduce the panel and maybe ask them to do exactly what I've asked you not to do, which is to talk a little bit about who they are, what they do, what

their interests are, and essentially spend a bit of time explaining to you what their skill sets that they might bring to this issue. So I don't know, Scott, you're first up.

DR. STEPPAN: Okay. Would you like me to use the microphones or can you hear me well enough? So I'm Scott Steppan. I'm an associate professor at Florida State University. My research -- and I've been there about eight years now. Previously I did a Ph.D. at the University of Chicago and postdoc at the Smithsonian.

My research has got several parts, but the largest part that I've been working on the last few years and the ones that most directly relate to this panel are -- deal with mammalian systematic phylogenetics, so I reconstruct evolutionary histories and relationships amongst organisms. Most of my focus is in rodents, not, however, including Zapus hudsonius; so I work with mice and rats and hamsters and gerbils and the muroid rodents. This is a very large group representing about a fourth of all mammal species.

And I use various DNA techniques, mostly DNA sequencing, mostly of nuclear genes -- which are slowly evolving because this is a fast-evolving group -- at molecular level and speciation level. And so I use

those techniques in fairly large scale in terms of large number of species and large amounts of data to reconstruct those evolutionary relationships.

The scope of my work ranges from fairly broad at the whole superfamily level, which includes about 1400 species, to within species phylogeography. And phylogeography is essentially phylogenetics within the species level, looking at geographic patterns and gene relationships, which is very much relevant to this topic. And so my expertise or the studies I've done in phylogeography involve both mitochondrial gene biogenetics as well as nuclear gene biogenetics. I have not used microsats which are used in some of these studies.

And most of my expertise is on some South American mice, and that's where I've done most of my work. I have not done a lot of work in North American -- on North American species, but a fair amount on South American as well as Philippines and some other groups.

I have done -- in addition to the molecular work, I also have background in what's called alpha taxonomy, which is identifying species and naming them. So I've named several species from both -- for molecular reasons and traditional morphological-based

taxon. A lot of museum work. I've measured thousands of individual animals for the South American groups that I work on. And I've also done some work with fossil animals and identifying -- I named some new species of extinct rodents.

And so that covers kind of a gamut of techniques, from fairly small scale traditional museum-based studies to fairly large scale molecular -- modern molecular approaches. Is there anything else, Steve, that you think should be covered?

DR. COURTNEY: No, I think that's good.

DR. DUMBACHER: Okay. I'm Jack Dumbacher. I'm curator and department chair in morphology and mammalogy at the California Academy of Sciences in San Francisco. I did my undergraduate work at Vanderbilt University in Nashville, Tennessee. I did some work on a master's degree at Clemson University and then transferred to University of Chicago where I got my master's and my Ph.D.

I worked at the Smithsonian for about seven years initially on a postdoc project in a different lab than Scott. Scott worked at the laboratory of molecular systematics, and I worked in Ron Fleischer's lab at the National Zoological Park and also at the Conservation Research Center in Front Royal,

Virginia. And while working with Rob, I did a lot of phylogenetics and also -- phylogenetics and phylogeography mostly with Rob there.

I'm perhaps better known for work that I've done on poisonous birds in New Guinea, extracting toxins and studying toxins, although that's not as relevant here, although a lot of the work we did there was studying population differences, population genetics, phylogeography to understand how those species were split and how the toxins evolved in the different groups. A lot of that was phylogeography.

And my interests are more in line with phylogeography and phylogenetics, and so most of the work that I'm doing now is more -- based on that, I have a grant from NSF right now, and we're studying phylogeography and phylogenetics of six very subdivided species that are spread throughout the lowlands of New Guinea, and that's an ongoing project. We have most of the data now, and we're hoping to get some papers out by the end of the year. And that's very much the same type of issue that we're dealing with here today with the Zapus mice.

And then I've also done a lot of phylogenetics of larger groups, so we worked on phylogeny for Aegothelidae, which are the

owlet-nightjars that are centered in southeast Asia. And also melanocharitidae, which is a group called the berrypeckers, which is an eminence in New Guinea. And we're working on a couple of other families as well, including pachycephalidae, which is also known as the the thickheads.

I should say too, these are all bird species that we're talking about here, not mammals. And so it's the genes that all these animals have in common that interest me most. And the thickheads include the toxic birds, so part of that project is trying to understand the evolution of toxicity in the group and mapping those characters on well-resolved phytological data.

I've done a little bit of mammal work, very little, and most of that was also genetics. And right now we're working on elephant shrews with Galen Rathbun, which is one of the world's elephant shrew experts, and we're working in Africa. We're trying to resolve some species level issues in that group. So that's most of my experience that's relevant to this matter.

DR. COURTNEY: Maybe you could just mention that -- the spotted owl work.

DR. DUMBACHER: Oh, yeah. And I have

been involved with SEI on two other projects. One of them is the spotted owl panel, so I sat on that panel with Rob Fleischer and Craig Moritz. Rob and I did most of the writing for that product that Steven had, and it was very much the same type of issue that we have here. So the northern spotted owl was under review, and it was -- it was currently protected. And the question was -- there were two different sorts of -- sources of data, some of which suggested, according to some interpretations, that the northern spotted owl was not a good subspecies and others that suggested that the northern spotted owl was a good subspecies.

And we have very much the same issues to deal with. What did the data say, what was the quality of the data, how do we analyze the data. And then once the data's all in hand, how do we actually interpret it, and what is the subspecies and where should one draw the line. And so this is very much the same issue that we have here.

And we also participated in a little workshop in Washington D.C. for the Department of Interior because Fish and Wildlife Services is engaged in these type of discussions a lot and they wanted to know a little bit, but they wanted to have a workshop and a discussion about how is genetics used for

taxonomic decisions. And that was one that I participated in as well as Keith Crandall and Bob Zink. So that's my experience with these issues.

DR. ARBOGAST: Hi, my name's Brian Arbogast. I'm an associate professor and curator of mammals at Humboldt State University in Arcata, California. I did my Ph.D. at Wake Forest University in North Carolina and had a postdoctoral fellowship at the University of Washington in Seattle.

My work mostly is at, sort of, the phylogeography level as well, so using genetic data to try to understand the biogeographic and evolutionary history of populations mostly within species or closely related species complexes. My work has focused primarily on mammals. I've worked on flying squirrels probably the most. I've also worked on tree squirrels and most recently on red tree voles, which are small rodents.

And most of my work has been the values of mitochondrial data, although of course I've used some nuclear markers, including amplified fragment polymorphism and some things like alzyme.

I've also done some work in Galapagos mockingbirds, but most of my work is on mammals and mostly on rodents. And I've done some theoretical work

on trying to infer different parameters from gene trees, like we're doing in much of this study, including when species and populations diverge from one another. And I think that's pretty much it for me.

DR. COURTNEY: Okay. My name's Ron Van Den Bussche. Ron is, of course, unable to tell you what he does, but I have his vitae here in my hand if you want to look at it. He's the -- interesting title, Curator of Frozen Tissues and Dean For Research at the College of Arts and Sciences at Oklahoma State. He's a full professor and has a long history and very eminent in his field. His -- his master's is from Memphis State, Ph.D. from Texas Tech and has a long publication record, it's -- as we're able to see here.

He works on a number of different issues, particularly mammals of various sorts. And his main expertise is in bats, though he has done some rodent work and also looked at a number of -- quite a number of endangered taxa, some of them for the Fish and Wildlife Service. I see he's done work on lesser prairie chickens and the whole issue of how many prairie chickens are there, and also on fish. And that's it. I mean, he has expertise in a number of genetic and morphological techniques. I have his paper here, just recently -- while I'm now talking about DNA,

chromosomal, and working gophers, a 1997 paper was on genetic integration between -- to fish tanks.

Anybody else want to weigh in on Ron's expertise for me? No.

DR. ARBOGAST: I'll also say moving into some coevolution between viruses and their hosts and the molecular issues involved with that.

DR. COURTNEY: So that kind of sets the scene for the panel -- panel expertise. I'm going to do a couple of quick things and then probably take a break. Is it hot in here or is it me? So we're going to try and fix that -- the temperature at least. So what I want to do is first alert you to the fact we've got a sign-up sheet somewhere in the back. And if you want to be -- if you'd please sign in so we have your materials and we can send you anything you want.

Secondly, I just want to invite you all to just say who you are so that we know -- we can put faces with the names. So we'll do that and then we'll take a short break. Keith.

DR. CRANDALL: Keith Crandall --

DR. COURTNEY: Maybe you might stand up so people can see you.

DR. CRANDALL: Keith Crandall. I'm a professor at Brigham Young University.

DR. RAMEY: Robert Ramey, I'm on the team to work on Preble's mouse systematic taxonomy issues, and currently I consult on some of the bigger species for DOI and Washington. I have to send my regrets to my other team members who couldn't make it today. They are in the field or in the laboratory right now.

MS. KOHLER: Judy Kohler with the Associated Press out of Denver.

MS. SZTUKOWSKI: Lisa Sztukowski with SEI.

DR. COURTNEY: If there's any problems, by the way, bring them to Lisa.

MR. MCDONALD: I'm Peter McDonald from the U.S. Forest Service in Denver.

MS. HOLTMAN: I'm Laura Holtman from the Denver Museum of Nature and Science.

DR. KING: Tim King with the U.S. Geological Survey in town science center.

MS. ASCHWANDEN: Christie Aschwanden with High Country News.

MR. MCCLEAN: Seth McClean with the Colorado Division of Wildlife out of Colorado Springs.

MR. NICHOLAS: Bob Nicholas, I'm with the Wyoming Attorney General's Office.

MS. LINNER: Susan Linner, Fish and

Wildlife Service in Colorado.

MR. BAKEMAN: Mark Bakeman, Ensight Technical Services.

MS. MEANEY: Carron Meaney, Meaney and Company.

MS. JENNINGS: Mary Jennings, Fish and Wildlife Services.

MS. ERWIN: Kathleen Erwin, Fish and Wildlife Services.

MS. MCCANN: Debby McCann with U.S. Senator Mike Lindsay's office in Cheyenne, Wyoming.

MS. LEGERSKI: Katie Legerski with Congresswoman Barbara Cubin's office in Wyoming.

MR. HANSEN: I am Craig Hansen, former grad student on the Preble's.

MR. BOHON: I'm Dennis Bohon with the U.S. Forest Service outside of Denver.

MR. MOLVER: I'm remember Erik Molver with Biodiversity Conservation Alliance in Laramie, Wyoming.

MS. ROBERTSON: I'm Erin Robertson with Center for Native Ecosystems.

MS. FALLON: I'm Sylvia Fallon, a science fellow at Natural Resources Defense Council.

MR. SLACK: Jay Slack, Fish and Wildlife

Service, Denver.

MR. WILLEY: I'm Seth Willey, Fish and Wildlife Service in Denver.

MR. PLAGE: I'm Pete Plage, Fish and Wildlife Service, Colorado field office.

MS. MICHAEL: Alison Michael, Fish and Wildlife, Colorado field office.

MR. ROSENLUND: Bruce Rosenlund, U.S. Fish and Wildlife Management Assistance and Preble's recovery team.

MR. BONAR: Mike Bonar with El Paso County Environmental Service.

MS. SCHERFF-NORRIS: Krista Scherff-Norris, Colorado Springs Utilities.

MS. BAYARD: Shelley Bayard, graduate student here at Colorado State.

MR. WURDER: Bruce Wurder, I'm a mammalogist here at CSU.

MR. SIEMERS: Jeremy Siemers, Colorado Natural Heritage Program.

MR. SHERMAN: Mike Sherman, Colorado Division of Wildlife out of Fort Collins.

MR. SCHORR: Rob Schorr, Colorado Natural Heritage Program.

MR. FAUX: Ken Faux, I'm a landowner and

that's highly affected by the issue and a former trusty in El Paso County, one of the areas that have the problem.

MR. MIHLBACHLER: Brian Mihlbachler, Fish and Wildlife Service and natural resource's manager at the Air Force Academy.

MR. CRIFASI: Bob Crifasi, I'm with the City of Boulder.

MR. POISTER: Paul Poister with Policy Communications in Boulder.

MS. PAXSON: I'm Mary Paxson, U.S. Senator Craig Thomas' office in Cheyenne.

MR. KUNZ: I'm John Kunz with the regional solicitors office in Denver.

MS. KOEHLER: I'm Amanda Koehler with the regional solicitors in Denver.

MR. COMER: Bob Comer, Interior Department.

MR. WILSON: Ken Wilson, Department of Fish and Wildlife, confidential team.

MS. JACKSON: Tina Jackson, I am with the Colorado Division of Wildlife in Colorado Springs.

MR. BRANDIS: Ben Brandis: Governor Freudenthal's office in Cheyenne, Wyoming.

MR. BUTLER: Steve Butler, ERO Resources.

MR. BLICKENSDERFER: I'm Tom Blickensderfer for the Colorado Department of Natural Resources.

DR. COURTNEY: Okay. So I hope you appreciate, you know, how much effort has gone into getting you all here, and I certainly welcome you-all, and you're aware of all the various interests that are represented. I want to assure you before we break that you-all now have an opportunity to have your issues raised if they're scientific for the panel, give you the opportunity to make sure you can come and talk to me. And so why don't we take, literally, 5 to 10 minutes -- 10 minutes, 10 minutes once we try and figure out the eating issues and, you know, talk amongst yourselves, and we'll discuss what we want to do with the time. Thank you.

(Recess taken from 11:02 a.m. to 11:16 a.m.)

DR. COURTNEY: All right. So here's what we'd like to do in this next section of the meeting, which is the panel and I talked over about maybe giving you a little bit of a roadmap of some of their issues and some of the things that they're going to be focusing on. So I know that this isn't on the agenda, but this is really to try to help you-all understand about what

are the things that the panel are identifying as some of the key issues they want to have addressed during the course of the meeting.

The agenda thus far kind of lays out broad areas. What they're going to do is tell you a little bit more about what they're focusing in on in some of those areas. Then after that -- after we've done that kind of introductory work, then I'm going to move ahead with the first kind of in-depth discussion and ask Dr. Ramey to come up. And we're going to talk a little bit about the history of the taxonomy of this group and invite him to kind of give his own view of the situation.

So let's begin, though, by asking the panel to talk about what they currently see as some of the questions they want to have addressed.

DR. DUMBACHER: Okay. I've been chosen to --

AUDIENCE MEMBER: Can we ask you to use the microphone? You kind of tail off.

DR. DUMBACHER: Is that okay? Can everybody hear me? Sounds like it's on. How's that? Is that better? And let me know if I start to move away from the microphone, and you can't hear me. Just, you know, put your hand up or say something; I'll do my

best.

So let me just preface this by saying thanks for so many people coming. It's really important for us to get a lot of the key players here in the room, and I know it was a big sacrifice for many of you to come; so thank you very much for all coming. This will be really important for us as we work through all this data.

I also want to say that we've read all these papers already, and we've read all the critiques of all these papers, some of them are quite in depth. And so this meeting is not necessarily -- so we've already formulated some of our opinions and we know what questions we need to ask and there will probably be some other questions that come up during this meeting that we realize that we need to ask.

And what I'd like to do now is say that we recognize that there is a fundamental disagreement, and that's one of the things that's caused riff in this community. And what we hope to do is try and understand where those disagreements stem from on some of the causes of these disagreements. And a lot of the questions that we're going to be asking will be focused on exactly that, those disagreements.

And so to preface this, I just want to

say to everyone in the room and to the people who are going to be asking these questions of, these are some of the issues that we think are going to be key. Now, some of these we already have a lot of this data, so we don't need to go into these; but some of these we will have substantive questions about. And this is in no particular order, and I'm going to -- since we haven't had a chance to go over all this material within the panel here, I might ask at various times for the panel members to weigh in and correct me and make sure that I've said anything that I need to say and not anything I didn't need to say.

So in no particular order, some of the disagreements stem from the morphological data and the morphological analyses. And some of the questions that are going to be key to us as panelists is that several key qualitative characters that were considered in the original description of preblei -- of Zapus hudsonius preblei were not considered by Ramey in his paper, and we believe that's probably largely because they're not easily quantified in the type of analysis that he did. So our questions are going to ask, well, how important are these characters for evolutionary significance for local adaptation for determining whether or not these are distinguishable.

And also in terms of the analysis, is random uniform measures of skull shape likely to recover the key differences among the taxa and are the sampling scheme in term of characters adequate for this work. We're also going to be looking at which statistical techniques were used and how appropriate those were for the work.

Another question that we've tossed back and forth amongst ourselves is burden of proof. I'm sorry, I tend to talk a little bit fast. Another issue for us -- and I'm not sure how we weigh in on this, to be honest -- is an issue of burden of proof. So when taxonomists sit down, we often work with a lot of information from the geographical distribution. We'll put, you know, sometimes hundreds of specimens on a table and begin sorting them into groups.

When we actually sit down to write our description, we usually try and focus on a few key characters that can easily be translated and used by others. And it usually, although we hope that it encompasses some of the key differences, it doesn't necessarily encompass all the differences that are found.

And so the question is, if this is a subspecies that's been recognized for a hundred years in

the field and we want to -- and we want to decide that it no longer deserves subspecies status, who has the burden of proof. Is it our burden to decide that there is no evidence and that these are -- that these should be synonymized; or if there's lacking evidence, do we decide to go with the original description and wait for more data that might be more definitive. And like I said, we haven't made a decision about whose burden of proof it is, but that is an important issue for us as panelists.

In addition to the morphology, there's an issue of contamination of samples for some of the genetic data; and these issues have been raised by both sides. For some of the work that's been done with study skins -- and the panelists here do have experience with the so-called ancient DNA or substandard sampling of DNA. Oftentimes, this is treated just like normal DNA in the laboratory and the results are fine, we get along, we publish these things.

But in cases where we get iconic classic results or it's a highly charged situation, we're often asked for additional proof. Oftentimes these data are replicated in a second laboratory or replicated in some ways in our own laboratory. And in most cases, we have separate laboratories for extraction and PCR setup.

And so one of the things that we're going to ask is a little bit about those data and what controls were made. I should also say that we're not doubting anyone's scientific integrity here, we're just looking at how these studies were done. And if we do come to the impasse where the data are disagreeing or that one set of data is unreplicable, we just have to make some decisions about how to proceed. And it's not a question about the integrity of any of the scientists.

This sort of thing happens all the time in the laboratories where data can't be replicated or one person gets one result. And we just want to get to the bottom of which data, if we have to choose -- and we may not have to choose because in some cases the data are agreeing -- but if we have to choose, we do want to make some decisions about which data are stronger than others.

DR. STEPPAN: And I just want to jump in that it may well be that there's absolutely no reason to us to doubt any of the data that comes through, and we will find absolutely no reason to have any specific concerns, and we may be left with some data that are not fully in agreement and we may not be able to understand why. We're just trying to explore why there might be differences in the two sets of results.

DR. DUMBACHER: And likewise, field collected data that tends to be of higher template quality can also be contaminated if the field methods allow the samples to mix. So there's been some questions about ear punches, those are very small ear punches. And if the tools used to take those samples have not been adequately cleaned or sterilized -- and there's a number of ways to do this in the field -- this can also cause contamination. So we'd like to ask some questions about some of the field techniques of both studies as well.

So those are some of the issues that we'd like to discuss a little bit having to do with contamination and reliability of data.

One of the things that we're not likely to talk as much about, just because the issues are very well outlined in the papers and seem to be very transparent, have to do with geographic sampling and which geographic areas have been sampled and also which genetic regions have been sampled. We think that some of the differences in the outcomes of the two studies have to do with the sheer amount of data available and the power involved in having different amounts of data that might have to do with the genetic sampling.

The geographic sampling is quite

different in the two studies. Both of them are very logical and they're very appropriate for the types of questions asked, but they do tend to lean to different types of outcomes; and so we'd like to -- this is something that we are going to be thinking about. And like I said, we probably won't raise too many questions because it's pretty clear to us the differences in the study designs and how this was done. But this will be very critical in our determination as a panel or how we think about these things as a panel.

And so the power to detect differences, how much data is enough, and if you don't see differences is the question that we -- do we have enough data, is it the right kind of data, or could we have done something -- or could we have found something different if we had more data or different data.

We also have a couple questions about the various analyses that were done and the use of various software, including structure, migrate, and TCS. So these are very complex software packages, and there's a variety of ways that you can set these software packages up.

In most cases, one hopes that the way that we set these up are -- that the outcome is fairly robustic against some of these different assumptions;

but in cases like ours with Preble's jumping mouse, the data is, you know, is so controversial; and we just want to make sure that we've got all those things set up correctly and that we're using the software appropriately.

And this is true for discriminate functional analysis and PCA-type analysis also for the morphological data, so the analysis -- we will have some questions about analyses.

One of the things that's come up quite a bit in the different authors' assessment and discussions of their own work as well as many of the critiques that we've read is that, to a large extent, it comes down to definition, what is our definition of subspecies and how do we work with that. And there are -- all I can say is as panel members, we have decided not to make a decision about what a subspecies is, but we've compiled a number of different definitions that are out there in the literature. And what we hope to do in our final report is to say that according to this definition, this is how we think the data fall. And according to this definition, this is how we think the data fall. And according to this definition, this is how the data fall.

And because the scientific community has not reached an agreement about definitions for things

like subspecies, we think it would be inappropriate for us to come to a decision on this panel about such things; but we will try our best to provide some sort of information for -- to Fish and Wildlife Service on these issues as well.

And if anyone has a favorite definition that they know of in the literature, feel free to bring that to us at some point and we'll make sure that we include it in our discussion. And this includes things like -- in many of these different reports, there's a discussion about whether there's reciprocal monophyly in these groups and how important is that.

Well, that may be important for some definitions of subspecies and it may not be important for other definitions of the subspecies. But yet we will try and look at what the different analyses say, whether or not these fall into clades, whether the clades are reciprocally monophyletic and such things, although we can't necessarily say that this says that they are subspecies or not, okay. But we will be trying to delve into some of those issues about rooting and clades and what these trees do show us.

And I think that's most of what is in our minds right now and most of what we, as a panel, are going to be focusing on. But like I said, there will be

other issues that come up during the discussions that we think are going to be appropriate too.

Are there any other things that I've left out?

DR. STEPPAN: I would add just sort of one general comment that I think Steve had already commented, that because of the hurried pace of this whole process, we haven't had a lot of time to discuss the material as a panel and so a lot of times we may actually understand the details fairly well individually from having read the papers, but we may want to discuss things amongst -- in the open but amongst ourselves. And so it's going to be kind of, I think --

DR. COURTNEY: Conversation.

DR. STEPPAN: -- conversation, and it's not always a straightforward agenda process, but we're still going to work through some of the things. And some of the points we will discuss, as I said, are in the literature and we would have read it, but we may want clarification and we want to just raise issues that are conceptually interesting even if we're not necessarily unsure about the facts.

DR. COURTNEY: Any other comments from the panel? Okay. So like I said, most of that conversation or discussion was to give you kind of a

heads-up of some of the things the panel is focusing on. I just want to emphasize one thing both of you mentioned, though, which was just because, you know, it may not be the topic for a lot of conversation doesn't mean it's not being addressed. Some of these issues perhaps the panel have already, you know, looked at -- and you mentioned the issue of sampling and it's fairly explicitly addressed in the papers -- and we may not address it in detail in this workshop; but you know, you can rest assured that those things will be addressed, or if you're not assured, you know, come and talk to us about it; and we will determine whether we are actually in good shape on that issue or not.

So with that, then I think we're ready to move on to the next topic. Yes? Okay. So I thought it would be useful -- you know, I've given all the scientists the opportunity to come and meet with the panel and talk about their work. Dr. Ramey, who's part of the reason we're all here, right, I think it's appropriate for him maybe to give a little bit of an overview of the history of the Preble's mouse issues and explain something about how this all came about. So maybe you could do that to begin with. And then we'll ask you to come up and set you up up here, and you can sit next to the panel --

DR. RAMEY: Okay. Thank you.

DR. COURTNEY: -- and that way you can both see the panel and see the audience. And like I said, this will probably be the only quasiformal presentation in the entire workshop. And as well, he's assured me it'll be very brief.

DR. RAMEY: Yes. You've already read the papers, so I don't need to go into a great deal of detail on those particular things. But first of all, in the interest of openness and talking about scientific issues, there's a certain offering of all branches I think that's appropriate here. So Tim King, I wanted to offer a personal apology to you for some comments that ended up in the press, I hope you accept that.

DR. KING: Certainly.

DR. RAMEY: Because this is really a debate about scientific issues and where one draws the line on what's considered to be a subspecies and also a distinct vertebrae population base under the ESA. I've had some previous experience in the prairie having done my dissertation work at Cornell on mountain sheep taxonomy and evolution, published a number of papers on that. Some of the tests that we used on the Preble's mouse date back to the discussions we had on those sorts of issues.

Here's the organism of interest today, the meadow jumping mouse. I'll visit with you and tell you about the taxonomic history a bit, why we asked the questions that we did, what kind of conceptual approach we used, and then what were our basic conclusions. And then I'll go into greater detail on other things under the morphology and genetics sections; but feel free to ask me questions at any point, please.

DR. COURTNEY: They do, trust me.

DR. RAMEY: Okay. Great. So the meadow jumping mouse, Zapus hudsonius, is a polytypic species that covers approximately half of North America. So from southeastern United States all the way to tree line in Alaska and Canada out to the Key Peninsula. This was -- the first real paper of significance was Preble in 1989, and relevant to the issues we're discussing today, the prairie jumping mouse, was described in this area by Preble.

There was a split -- a pallidus -- I've escaped the author right now that split that off, but the real significant work to come along next was by Phil Krutsch in 1954, Raymond Hall from the University of Kansas. And so Hall had promised the dying Preble that he would revise the taxonomy of Zapus in North America. There are actually three species, so the meadow jumping

mouse here has 12 subspecies within its species.

There's also the western jumping mouse, which is found in this area with some potential degree of overlap or potentially even hibernation in these areas. And there's also the trinotatus specific jumping mouse, which is found in this area of the northwest. So three species; and then each, of course, have been divided into various subspecies.

So Krutzch did his work in '54, split what was then the prairie jumping mouse, campestris, into three subspecies: preblei, campestris, and intermedius. And so that brings us forward to the consideration of the listing of Zapus hudsonius preblei as a subspecies here, a peripheral population at the western edge of its range as potentially endangered, and I think the first consideration was in 1992.

There were -- there was a morphometric study by Conner and Shenk which asked the question of can you distinguish between the two species around the zone of contact. And they measured skulls -- and this has some relevance to our morphometric work that I'll talk about later -- and he found that they could distinguish them with a high degree of separation using discriminate analysis.

There were a couple of genetic studies.

Actually, Bruce Wonder back here had developed some genetic markers to ask whether one can distinguish between the two species, and I realized the question was really an issue of subspecies. And so most of the effort and focus between the two -- go ahead.

DR. DUMBACHER: So how much variation was there between those two species, i.e., were those two species something that was all -- if I understand correctly, these were well accepted as two different species by the taxonomic community?

DR. RAMEY: They had -- that was actually the first real systematic quantitative examination of that. There was Jones 1981, which used a univarious statistical approach; and I believe that he considered them to be reasonable species, although there was some question. Jones' primary conclusion on hudsonius was he couldn't find recognition for any subspecies.

Now, the Conner and Shenk study, which utilized skull measurements similar to those taken by Krutzch, had used -- in fact, our same level of -- our same approach using discriminate analysis. And they said that, you know, a high degree of separation -- they actually didn't use any cut-off or posterior probability of individual samples. They just used whether it was greater than or less than .5, so some of the

classifications could not be better than flips of a coin.

I actually went back and looked at their original report which listed all the posterior probabilities and found that actually the two species using that method were well separated, so greater than 90 percent. I think it was in the high 90s using stepwise eliminated discriminate analysis and the posterior probability cut-off of .95 or better.

DR. DUMBACHER: Okay. But those were species?

DR. RAMEY: Species, right, exactly. In other words, a study that was put out as a report in 1997 by Larry Riggs and -- et al., and they asked whether Preble's was different from campestris using mitochondrial DNA. That report was never published, the data were never publically available. And they had a very large sampling of preblei and a very small sampling of campestris, a number of ear punches and skin used in that study.

Now, especially with the owl -- my involvement or King's involvement in this particular question --

DR. DUMBACHER: Can I ask you one more question about this map that you have up here? I notice

that there are two isolated subspecies as you've shown it here.

DR. RAMEY: Uh-huh.

DR. DUMBACHER: One of them is No. 4 down here and one of them is No. 1, which is Preble's jumping mouse. How much is this -- is this accurately drawn so that they are actually --

DR. RAMEY: Yes.

DR. DUMBACHER: Okay.

DR. RAMEY: Yes. I mean, there's been some trapping efforts out here to ask is there a gap; and for now it looks like there is a gap between Preble's and campestris. The question is: Not is there a gap, but how long has there been a gap there. And luteus, though, clearly has a gap in its distribution. Cherry Jones, the Museum of Nature and Science, department curator there, had discovered what she thought were luteus in southern Colorado and those are widely separated from pallidus. So there is Preble's, campestris, intermedius, pallidus, luteus.

Luteus was actually thought -- up to the time of Jones 1981 -- through Jones 1981 -- to be a Zapus princeps subspecies, and actually that's one of the reasons that Jones didn't publish his work is that Yates and Hafner came along and did an alzyme and

photometric study and considered luteus to be part of hudsonius; so there's a revision there. And Dr. Gwilym Jones, he's at Northeastern; and he said, you know, it was going to be a huge amount of work to revise that dissertation -- which you have, it's two inches thick -- and I just thought, you know, I just had to get on to other things. So anyway, that's basically what happened there.

DR. STEPPAN: I have a question, a followup on that.

DR. RAMEY: Please.

DR. STEPPAN: So these are the only subspecies that have gaps between them?

DR. RAMEY: To the best of my knowledge --

DR. STEPPAN: Aren't those gaps more significant? Are they large geographically than elsewhere throughout the range or all the other subspecies drawn as continuous?

DR. RAMEY: Well, let me point out, you know, a limitation to the studies you'll talk about today. We focused just on this group, about a quarter of the range of the species. So I'm not familiar enough with those to comment.

DR. STEPPAN: I'm just curious sort of

the general pattern of the species in terms of its natural habitat.

DR. RAMEY: I don't know the answer to that.

DR. STEPPAN: Was this just because this is where the detailed sampling has been because of the conservation issues? In fact, there could be just similar sorts of discontinuity throughout the species.

DR. RAMEY: Presently I think you're going to find discontinuity between some of these areas because of -- just simply agricultural development occurring in areas, so if you drive across Kansas and Iowa and extensive cornfields, so -- I suspect that would be the case.

You know, the important point to make here is that we all realize that this area was under an ice sheet 14,000 years ago, which it started to retreat, you know, basically about 12,000. And these are potentially all recolonizations. We don't know if they're from the south or from the region in the north, but there has been recent recolonization.

So this present gap is thought to be -- oh, I don't know what the current trapping information in Colorado did some work on this, but I believe it -- you know, upwards of 100 kilometers, maybe less than 60.

That could be addressed by actually asking questions of some of the other museum samples that are out there of individuals that have been trapped, maybe some -- they might be able to assign and tell you whether it's hudsonius or princeps or hybrid.

Now, this gap does seem to be very real and this is over, you know, several hundred kilometers across here.

DR. COURTNEY: Just a point of information, some of the critiques that have come in also talk about that gap across. So there are other comments we should look at.

DR. RAMEY: Yes. So let's fast-forward again to recent data, a fairly limited data analysis, just some strict consensus tree and majority rule tree for their phylogenetic analysis and said that they thought that Preble's was a good subspecies based on that in Krutzch '54.

So how did I get involved in our team? Cherry Jones was at the museum, and I had talked about doing a collaboration. She was a classic ecologist for small mammals. I worked on biogenetics, evolution, biogeography, conservation genetics; and I said let's think about a project. She suggested the Preble's mouse, so I read all the original papers cover to cover.

And so when I read Krutzch's '54 description, I saw a number of specimens examined, 11. And then, you know, knowing that this always has a table in the back, I flipped to the back and went uh-huh, this is based on three adult skull samples. That's the only quantitative basis was the measurement of three adult skulls. And he looked at the qualitative evaluation of four adult skins and seven juvenile skins in variation of halogen juvenile skins.

So I decided this was a reasonable question to ask as to whether this was a subspecies or -- so based on our previous work on mountain sheep, we realized that you can treat these taxonomic categories as test hypotheses and use some threshold that had been established in the literature to measure them against. We used that for mountain sheep taxonomy in evolution. We, in fact, split out the Sierra, Nevada paper, Syrian divini subspecies based on discriminate analysis, mitochondrial DNA analysis.

We synonymized the peninsular Bighorn sheep in southern California with desert Bighorn sheep down in here on the basis of morphometric analysis. We went back and retested the original basis. So I thought since most of the effort had focused previously on whether the two species are different, it would be

worthwhile to ask, in a very systematic way, whether the two subspecies were different -- the five subspecies were different.

Initially we only thought about comparing Preble's to campestris, pallidus, and intermedius. Subsequently, after some discussion with the service, we decided to include intermedius into that sampling. So you know, we're looking at a subset of the total range of these.

DR. COURTNEY: But you didn't look at the princeps?

DR. RAMEY: No, no, not yet. Joe Cook actually had a partial cytochrome B data set, but for the -- the three species and hudsonius; but as I recall, part of that data set had one section set of campestris, the other part of the data set had the other section of campestris. There wasn't a great deal of overlap in the middle, so obviously that's a limitation of all of this work.

Okay. So what could be listed on the ESA. You're familiar with this, that there's species, but some of these more difficult issues come in under subspecies because they are listable under the ESA and also distinct vertebrae population segments, which I don't know if you're charged with looking at.

DR. COURTNEY: Okay. No.

DR. RAMEY: Okay. Good enough.

DR. COURTNEY: In fact, you know, don't spend too much time on this because this is not really our charge.

DR. RAMEY: Well, subspecies, as you know, many have been arbitrarily defined using nonquantitative criteria and many of these taxonomists didn't really matter in the legal sense until the ESA came along in 1973 and suddenly they became very important. But we considered this to be important, and it's a part of our research to ask whether these would also fit into a distinct vertebrae population segment.

Most of you, I'm sure you realize, taxonomy is based on poorly defined traits, no quantitative basis for genetic uniqueness, small sample sizes, no hypotheses testing, genetic -- or presumed genetic differentiation could be slight, such as overlapping differences in size. There might be many subspecies within a species, and specimens are identified on the basis of geographic location alone.

And I think that's a pretty key point; that when I first asked my colleagues so how do you tell -- you know, looking at these tracings, the distinct vertebrae, well, how do you tell the subspecies

apart? And Jerry had said, well, you look at the immune tag, and it's the location because they're -- there is a great deal of overlap in them.

Okay. DPS policy, shall I not? Okay. Go past it. All right. Well, let me -- can I make the difference respectfully just once?

DR. COURTNEY: Fine. Go ahead.

DR. RAMEY: I want to say that the distinct vertebrae segment policy actually has some criteria out there for what could be recognized, and it might be sort of the lowest level of listing for populations. And they required discreteness markedly separated based on quantitative measures of genetic or morphological discontinuity and significance based on would the loss of that population result in a significant gap in the range of the species as a whole.

Okay. So we recognize there was this problem, that it's like a type 1, type 2 error in statistics. That if you're testing taxa -- we recognize this from our work on mountain sheet previously -- if you set the bar too high, some taxa may fail to be recognized and subsequently could go extinct. If you set the bar too low where you potentially will allow any population to qualify for a listing, there might be fewer resources. So this does have some policy

implications.

How do you try to minimize that error? So we try to sufficiently make sure that the criteria represented major discontinuities in the genetic diversity of species, in other words, long-term isolation or adaptation to unique environment, and this would allow us to distinguish between very recent genetic bottlenecks.

Some of the things, for example, we've recently published on over the last 50 years in the Mohave Desert affecting -- an interstate highway affect -- system has affected genetic diversity in the Bighorn sheep population. So it's important to distinguish between very recent and historic events, especially humans are a part of the very recent event.

So we decided the best way to test the taxonomic validity of the preblei was to ask if the original taxonomic description was statistically convincing and biologically meaningful, so look at the original basis of the description. If not, retest its quantitative basis where possible. That's where we did our morphometrics work.

We went back and did the same measurements that Krutzch did, realizing that there are some limitations here, but let's see how good it was,

also trying to see if we could quantify any of those qualitative characters. And then we wanted to see if the results were corroborated by multiple independent genetic data sets, so we included mitochondrial DNA and morphometric. And obviously there are caveats surrounding, you know, the distinguished genetic markers.

So the idea was to address that burden of proof issue to try and see if there was concordance amongst multiple data sets in a majority rule sort of situation, and we fit criteria to answer the data collection to make sure of activity. We actually started off with using -- setting very strict criteria, which we've stuck with our entire way through this.

Museum samples, so there are always limitations on these sort of samples you use; however, this was the first systematic study of the subspecific taxonomy of this group and this prairie region, and this allowed us to sample across the geographic range of the species. And we explicitly debated the different sampling schemes, large population sizes versus broad dispersion. And we decided, based on some of the public literature starting with Lynch and Crease in '86 that it was the most appropriate way. And voucher museum specimens are publicly accessible. You can look at them

for further study, and we all know that they're required for taxonomic description.

Okay. What were the criteria we used. We focused in on Ball and Avise, 1992, because they required a major subdivision into diversity of species. So we interpreted that as being greater diversity among putative groups than within. Mortiz also added the criteria of reciprocal monophyly to mitochondrial DNA. We used both of these.

We looked at concordant distributions and independent traits, so we thought morphology and molecules as well was what we had to do. And it must have some evolutionary basis; so in other words, this gap, for example, must have existed for a long time for there to be broad separation or there must be strong adaptation.

We utilized another test that's out there in the literature and has gained, I think, a pretty broad acceptance, and that's the Crandall, et al., 2000, to test for genetic and ecological exchangeability on recent and historic time scales.

So this is basically what we're looking at right now with the discussion on Preble's is that on -- if you used different time scales, are they genetically or ecologically exchangeable. And so I

think that a lot of the discussion today focuses in on this issue, are they genetically or exchangeable on a very recent time scale.

Crandall, et al., would require it had both, ecological and genetic data be unexchangeable; in other words, you would reciprocally translocate mice and they wouldn't survey. That would be, like, one test of ecological exchangeability.

DR. DUMBACHER: Had anyone done such a test?

DR. RAMEY: No, not yet. One could do such a test.

Okay. So what do we use as evidence? Morphometrics, some skull measurements, AMOVA, discriminate analysis, principle components, phylogenetic population genetic mitochondrial DNA, microsatellite DNA, a review of Krutzch's qualitative description, and a review of the literature on adaptive diversion. And we came to the conclusion that five lines of evidence refuted the original taxonomic conclusions of Krutzch.

And I've been -- I have been in touch with Krutzch, and he reviewed some of our original work, and he's been in communication since then, so . . .

DR. DUMBACHER: Would you say that your

results refuted the original work of Krutzch or did it fail to support the original work of Krutzch?

DR. RAMEY: Well, I think you could put it either way. It's basically the same thing that -- I think Krutzch -- and I could talk about this in the morphometrics, but I'll address it now. I mean, Krutzch said that with a quantitative basis -- he said that Preble's is smaller than most skull dimensions measured, didn't use any statistical test. And so there were nine measurements, and so he did a simple AMOVA test using a sample size of approximately 40 each Preble to the campestris. We also added intermedius into this later. And so they were, in fact, smaller for one interorbital breadth, larger for two, and then insignificant for six others. And then we decided, you know, that alone doesn't support the original quantitative basis.

DR. DUMBACHER: And what about the qualitative basis? There was some qualitative characters --

DR. RAMEY: And we agonized --

DR. COURTNEY: If you want to sit down, you can sit down.

DR. RAMEY: I don't mind standing. It keeps my blood going. Thank you.

We thought hard about this and looked at a lot

of specimens in the museum collection and concluded that we're going to have a very difficult time trying to do that. So how do you quantify less black tipped hair on the dorsal stripe. That's a -- you know, for example, more inflated bullae. It's -- it's a very difficult charge to do.

And then there's also the question of what do these characters mean in terms of the -- you know, the shape or adaptive diversion potentially of these organisms. So we decided that they were not correctly quantifiable.

I talked to one colleague -- actually Carron Meaney who's here -- and suggested, well, maybe we should take 50 specimens of each, cover up the tags, mix them up, and let the experts sort them out using Krutzch '54. And I think, Carron, your comment was that would be messy. And you know, having looked at a couple of hundreds of these in museum collections in Denver and Kansas, I come to that conclusion, although that's a qualitative one. But yet we just couldn't come up with a reasonable way to do that.

And people have tried using spectrometer readings and such, but the difficulty with that is the angle of the tack on the beam on the stuffed skin makes it really, really difficult. Bowen did this sort of

thing on beach mice where you have flat skins to be able to do that, can't do it here.

DR. DUMBACHER: Yeah, I'm familiar with the --

DR. RAMEY: So we decided it's just not -- it's not repeatable. But that's what we thought about this burden of proof issue and went down the road of let's get additional different data sets and see if we can find anything else that's different here and what degree of difference can we find.

DR. COURTNEY: We're going to talk about the quality in just a minute. Do you have any questions of Dr. Ramey on the overview, why things were done the way they were?

DR. ARBOGAST: Of the definitions that you sort of chose to represent the criteria in Ball and Avise, why did you choose that over -- you know, we were talking about there's a whole bunch of different definitions for subspecies in the ERCs and everything in between. And I was just curious as to why you felt that was the most appropriate one to equate with subspecies in this case?

DR. RAMEY: I think that it was the first one that really put forward an evolutionary basis in a substantial level of divergence. I mean, it requires

that they be distinguishable and that there be an evolutionary basis and that there be multiple lines of evidence. And so epistemologically, we thought that that was the strongest inference one can do. Now, one can set different criteria within those broader categories. We found that to be the most logically consistent approach there.

But you know, we were very attracted to the distinct population approach in testing genetic and ecological exchangeability in Crandall, et al. I mean, you could really -- and we argue this in our paper as you had seen. You could really just set one criteria distinct population, which would probably be more quantitative than any subspecies concept out there.

DR. DUMBACHER: Could you just repeat for me real quickly what those three things were that you thought were important? Were they distinguishability?

DR. RAMEY: Distinguishability, have an evolutionary basis, and concordance of multiple data sets. I mean, the systematic decisions require distinguishability, I mean, that's what Lanay's charge was in the 1700s.

DR. COURTNEY: Maybe I'm out of line here, but I'm just asking for clarification. Why multiple character sets? Why is it not okay to have a

key character set?

DR. RAMEY: Well, there are caveats with using single -- you know, any single data set. So, for example, with mitochondrial DNA, there can be different levels of disbursals, males versus females, and that alone will cause a discordance of the pattern potentially between nuclear and mitochondrial DNA. It might make things look more different than they, in fact, are. There might be strong selection on a particular trait, whether it's morphometric, biochemical, physiological, or even just the single nuclear gene. And so it's by having multiple independent lines of evidence that I think you reach the strongest inference.

I mean, there are a number of cautionary cases -- for example, Gordon Lukehart found on the systematics of goats in Eurasia that there's a substantial intercorrection of mitochondrial DNA; and if you just let mitochondrial DNA phylogeny, you wouldn't recover the real history of the species. So acknowledging that and acknowledging that there are limitations of various data sets, we thought that would produce the strongest inference.

DR. COURTNEY: Maybe I'd invite the panel to comment on that.

DR. DUMBACHER: I would agree that usually one hopes for multiple levels of evidence, but does multiple levels -- or multiple types of evidence can be all sorts of things, a distinct geographic range, plus genetic differences, morphological.

DR. COURTNEY: You might want to talk into the mic.

DR. DUMBACHER: So yeah, I think that most of us do agree that these are biological or evolutionary significant entities that we should be able to find more than one. And there's a lot written about problems with mitochondrial DNA, and sometimes you'll get a signature from mitochondrial DNA but no signature from any other marker; and one feels uncomfortable with that. Or likewise, there might be a lot of signatures in the morphology and even nuclear DNA, but the mitochondrial has been able to spread or intergress from one, and many of us are very reluctant to throw away the species simply because the mitochondria are not reciprocally monophyletic.

So there are a lot of these sort of criteria that have been thrown out there; but for -- for every one of the criteria, you can find in the literature exceptions to those so no one has to think on their toes.

DR. STEPPAN: Yeah. It seems to me, though, there are circumstances where you can have -- in the subspecies concept, one is looking for some history of evolutionary independence and that -- the record of that, if it's -- particularly if it's a recent history, the independence might only be reflected in one or a small number of character systems or it's a small number, but you only have the resources to access one of those character systems.

And, you know, chromosomal rearrangements is one of them that may reflect real species' boundaries, for example, a much higher level; but all the other data sets you might be looking at, including geography and mitochondria nuclear, they show essentially no evidence of separation. So it's not clear to me that that might not be a rather high standard in terms of requiring multiple lines before something is recognized.

DR. RAMEY: Well, it was important to us to -- you know, for our comfort level to require a higher standard to achieve this, and also we received a number of peer reviews. I mean, this has been fantastic in terms of getting peer-review feedback and so --

DR. COURTNEY: I don't think there's any shortage of feedback.

DR. RAMEY: No shortage of feedback. And so, you know, we listened to a lot of those and that's where, you know, the microsatellites came in. So for example, our first round of submission to -- of our paper to the animal conservation group rejected but encouraged us to resubmit, you know, because we had a nuclear gene. And we've been in discussions with the service about doing that, including intermedius into the analysis. And so we extended, you know, as a result of that sort of thing.

I mean, the ideal scenario, which is, I think, difficult to achieve given resources, is one would look across the entire species range and, in fact, across all of North America at the group. But that's a fairly, you know, expensive proposition and we had a fairly limited budget. So traveling to museums to get skins was a very efficient way to recover this information.

DR. DUMBACHER: And I think, especially since, as you said, there were no other published studies that did this, I think it's a very good first -- it's very good contribution. I would add, though, that just for the audience, I mean, we all know this, in fact, there are also things known as cryptic species and these are basically things that you find

genetically that these are quite distinct; so morphologically we can't find any evidence or any way to split them off.

When we worked on the spotted owl panel, we went back to the original descriptions of each subspecies, and we found that they were likewise described -- in just a very small number, I think it was three -- individuals, and none of the characters that were used in that original description held. And we actually contracted a student from Berkeley to go back and use many specimens to try and find some characters that he could use, and he was unable to find any as well, and yet the genetics were fairly strong.

So one has to be careful because even though we want multiple levels, any one level that one might choose may or may not support the species status. So we do look for multiple levels, but they can come from a variety of different sources.

DR. COURTNEY: I have a question here that's been handed to me, and I actually think it'll be addressed by the panel in the next section when we go on to talk about morphology. But is there anything else you want to raise with Dr. Ramey about the general overview?

DR. STEPPAN: Not at this point.

DR. DUMBACHER: No.

DR. COURTNEY: So we're going to have, you know, lots of opportunities to talk about these things. We're actually scheduled to go straight into the morphological issues now, and since we've got Dr. Ramey up here, are we comfortable just going straight into that?

DR. STEPPAN: Sure.

DR. RAMEY: Save a bit of time.

DR. STEPPAN: Jack had raised a couple of questions on morphology that I had wanted to follow up on anyway, but I was going to wait for this section. And just, first off, for clarification, the proper pronunciation is Preble's? Is there a consensus on that?

DR. COURTNEY: Preble's or Preble's.

DR. STEPPAN: Preble's.

DR. RAMEY: The common vernacular is Preble's.

DR. STEPPAN: It's not always said, but I often know that it's -- one of my specimen organisms that's being mispronounced the way I'm used to hearing it, it kind of sounds odd.

So a couple of questions on morphology, and some of this is just -- I mean, clarification points

that are already out there and some of these I just want to get into the discussion. And so how -- I know some of the reasons why you said some of the -- Krutzch's original characters you did not include because of difficulties that you perceived in trying to quantify them, but how were the characters that you did include chosen or how were they chosen?

DR. RAMEY: We debated that and thought about using geometric morphometric analysis; and then we decided, you know, we should really retest the original basis of the subspecies. And then, you know, if it is supported, then this is a good subspecies, we're done. If it's not supported, then there are issues, and we need to go a bit further. So that was the reason for doing that.

We explicitly went after the same nine measurements that Krutzch did, recognizing that there is a correlation between some of these variables, but they do reflect the original taxonomic basis and you can recover information about size and shape variation.

DR. STEPPAN: Great. So a couple of the characters that Krutzch had used, like bullae inflation. I'm not sure why that was -- I mean, there's clearly difficulties in that kind of shape of getting exact, precise; but you know, I've done it in several

circumstances where there are ways to get a fairly reasonable estimate of volume. And I was wondering what was it -- is there something about either the orientation of the bullae that might change, such as it's really hard to find landmarks or orientations that made that particularly difficult because that's a character that has -- in fact, I'll just give one example. In the South American Muridae mice I work on, there's an isolated population that seemed to have small bullae and quantitatively that popped out before I actually saw it qualitatively. That's since been confirmed by molecular evidence this is distinct, but the only morphological feature is, in fact, bullae inflation.

So -- and I was successful in identifying this clade, be it subspecies or species, so -- but bullae take different forms and different lineages, so I was wondering if there's a particular problem in --

DR. RAMEY: Well, the issue with that was trying to find homologous landmarks in order to take the measurements from, and there's different ways you can measure these because the volume is going to differ by shape. And so that was the difficulty there, and that's why we decided let's stick with those things that have reasonable landmarks you can consistently measure.

And I should also point out when we're talking about morphometrics, Lance Carpenter did all the measurements, and he took four measurements of each specimen. He did two, and go through the entire series of specimens, come back, do two more measurements, and then we took a mean of those four measurements, recognizing there's always potential measurement error in use of the calipers. And then utilized Grubb's and Dixon's tests to remove any statistical outliers before proceeding with the analysis.

DR. STEPPAN: And so you said that the nine measurements that you chose are the ones highlighted by Krutzch with the exception -- so you looked at all the ones he highlighted and you measured all -- all the ones you choose, are the ones you highlighted?

DR. RAMEY: Yeah.

DR. STEPPAN: With the exception of ones you felt uncomfortable quantifying?

DR. RAMEY: And then they had no -- they had no -- he had mentioned that there might have been a difference in volume, but he didn't measure how much of a difference in volume, for example.

DR. COURTNEY: It may be worth, if you can remember, Rob, just talking a little bit more about

the characters you rejected and why. Just give a little bit more detail.

DR. RAMEY: Well, that's actually the -- I can I go through it all really fast. What was -- Krutzch's original basis description utilized skull measurements. There were pelage characteristics that figured prominently in this description. If you look at very few specimens -- so, for example, he had Zapus princeps on the right two specimens; Zapus hudsonius luteus in the middle, slightly more orangish-reddish; and Zapus hudsonius probably on the left -- you might see what look like to be obvious differences. And we think this is what may have happened with Krutzch's limited sampling of specimens.

Note here on the left, Zapus hudsonius campestris is crossed out and it says preblei, and so that was added after Krutzch's work. However, if you look at many samples, many specimens in using traits, we have many of these. These are from the Museum of Nature and Science in Denver. The -- what appear to be a difference may suddenly fall within the range of variation from others, and so this addresses that question of pelage of how do we try to find differences in that.

I've actually put two intermediate

specimens here on the trap, they're on the yellow tag just so you can tell the range of variation we're looking at. I've also put two Preble samples in with the western jumping mouse specimens. It's a bit difficult at this level to tell them apart, but morphometrically you can tell them apart, size and shape differences. So Krutzch said that Preble's was smaller in most skull dimensions measured, you know, like -- I'm not going to repeat that result.

DR. STEPPAN: Can I interrupt here for one question?

DR. RAMEY: Yeah. Go ahead, please.

DR. STEPPAN: So what was -- I haven't had a chance to go through Krutzch in detail. So what was he comparing preblei to?

DR. RAMEY: He compares --

DR. STEPPAN: So he weights it as smaller in most nations. Smaller than what?

DR. RAMEY: Campestris. And I believe he might have mentioned that -- and I have Krutzch's manuscript here with me, I can check that, but it was campestris for which he was splitting it off from. And then he compared intermedius to campestris and claimed that intermedius was smaller; and that conclusion, I think, is borne out. It is a little bit smaller with

some overlap in variation.

DR. STEPPAN: So his differential diagnosis was limited to those two to three subspecies; is that correct?

DR. RAMEY: Exactly. Exactly. Yeah. So interorbital breadth -- sorry, my old laptop died with my graphics program, so I had to do this in a cell, but here's the distribution of measurements for interorbital breadth for Preble and campestris with the outliers polluted. Preble's was a little bit smaller, but I think we'd have a hard time arguing it's particularly diagnostic.

So a great deal of range of overlap, but the distributions are slightly different, but you know.

DR. ARBOGAST: Could you put that back, please?

DR. RAMEY: Yeah, go ahead.

DR. ARBOGAST: I was wondering --

DR. RAMEY: Millimeters in interorbital breadth.

DR. ARBOGAST: Because it's smaller in its interorbital breadth was one of the original --

DR. RAMEY: Yeah, and that's the one out of the nine characters; but he said it was -- for most

of the characters, it was smaller.

DR. ARBOGAST: And I'm reading from one of the reviews by Wayne Spencer where he said -- at least in one of the drafts, and I'm not sure if this was in the final published paper of yours -- but that it was also significantly larger for both zygomatic and mastoid breadth.

DR. RAMEY: Uh-huh, correct.

DR. ARBOGAST: And so he had also suggested that those could have important functional roles in terms of feeding. Those three characters combined, that might play into the ecological exchangeability idea. Do you have any thoughts on that before we jump --

DR. RAMEY: Yeah. It's important, whenever you find any statistically significant differences worthwhile, to ask what's the basis of that, you know. I'm going to argue here that, first of all, the range of difference between these is nearly that of measurement error in the skulls themselves; but second of all, what does it mean from an evolutionary basis.

So we can classify taxa on the basis of hypothetical uniqueness, but I don't think that's the primary goal of systematics. You need to have some reasonable evolutionary basis for that. So if we had,

for example, comparative studies of others Zapus that indicated that that was important for feeding or we had some other reasons to think that there was strong adaptive differences in diet, for example, then I think we might come to that conclusion.

What's interesting with Zapus hudsonius is that they're generalists in their food habits, solely vegetation and vertebrates and fungi; so they're not -- we don't have any evidence of specific adaptive differences.

DR. STEPPAN: We have a question from the audience or the participants that I think is relevant at this point. So citing Vignieri, et al., quote, The sole unitary character cited by Krutzch that -- REA, which is Ramey, et al., did examine interorbital breadth was found to be narrower in preblei than campestris as described in the definitive findings Krutzch. Thus, the small fraction of Krutzch's morphotaxonomic hypothesis actually tested by REA confirmed Krutzch's initial findings and distinctiveness for preblei.

And so it seems like you've already personally addressed that here; but when you say it's smaller, is that a statistically significant difference?

DR. RAMEY: Yes.

DR. STEPPAN: Is it the means or

significantly different?

DR. RAMEY: And Vignieri, et al., had argued this is diagnostic; and so, you know, I leave it up to you to decide if that's the case.

DR. ARBOGAST: I had one follow-up question. In our data --

DR. RAMEY: And -- excuse me.

DR. ARBOGAST: Go ahead.

DR. RAMEY: Our data set's available on the web if you want to look at it.

DR. ARBOGAST: In Wayne Spencer's review, he also noted that in your original 2000 premanuscript, that you had reported the upper tooth row to be significantly larger in Preble's than in campestris, but this was not in later ones. Is that -- do they have a significant larger tooth row?

DR. RAMEY: No, that might have been a consequence of how we removed outliers originally, and we decided to have a -- you know, quantitative basis for outlier removal. We didn't want to have any, you know, that one looks like it's way out there by itself, we need to remove it. So we decided to rerun everything, excluding using Grubbs and Dixon tests. We reran all the analyses, and so what you see in the manuscript is what we did there.

DR. ARBOGAST: So you think that would --

DR. RAMEY: That would be a correction, an artifact of --

DR. ARBOGAST: Of outliers.

DR. RAMEY: -- how we did outliers initially.

DR. STEPPAN: So another question here, and I'll just read it directly, "So how does Dr. Ramey explain the fact that Vignieri, et al., charge did not examine the same quantitative morphological characters?"

DR. RAMEY: So I think we already addressed that.

DR. STEPPAN: They just want to know. If you want to just restate it.

DR. RAMEY: I think we're done.

DR. STEPPAN: I think the question came after the point which you had previously brought it up, so there may be some --

DR. COURTNEY: It goes to my question, which was, what are the other characters that you rejected and why?

DR. STEPPAN: Which I think we were still working towards, correct?

DR. RAMEY: Yeah, we're still working towards that. So let's keep going there a little bit.

And so we utilized discriminate analysis and skull measurements, and we used the criteria we previously published on mountain sheep. It is used, as I recall, to some extent, in the systematic literature. I also found it being used in geologic research in, for example, the treatment of particular types of cancers or a situation of where you want to assign an individual to a treatment group and you want to make that assignment a high degree of probability because the outcome is incredibly assigned they die, they die; so it's the logic behind this.

And we utilized the criteria that greater than the 90 percent of the skulls be correctly classified subspecies at jackknifed posterior probabilities of .95 or better. Conner and Shenk, for example, just used criteria better than or less than 55, I'm pretty sure.

DR. COURTNEY: Thanks.

DR. RAMEY: Thanks. I appreciate that. Anyway, the point here is that we wanted to make sure that these are reasonably good classifications, were made with confidence, the different groups, and not those that are potentially just slightly better than chance. For example, if you had a posterior probability of .52, you could make the assignment of an individual

to a particular group, but it would be the very low degree of confidence.

So when you apply this to Preble's versus campestris and it falls far short of 50 percent. I can't remember right off the top of my head, it's 42 percent or so. Anyway, slight -- not that much better than flips of a coin using discriminate analysis, which recovers, you know, shape variation and some size variation.

We decided to go a little bit further -- oh, there it is, 42 percent -- and we used a forward and reverse stepwise discriminate analysis and FSTAT, which asked which variables were most important, and I have those listed in the paper. And at the urging of reviewers, we decided to do principal components analysis, PCA --

DR. STEPPAN: Before you get to that, did you do that? Did you do a discriminate function? Did you actually plot -- do a discriminate function plot in addition to a PCA plot?

DR. RAMEY: Yeah, I did, and we just didn't include those. I could probably dig those up somewhere.

DR. DUMBACHER: Do those show any more separation, because principal component typically will

tend to blur a lot of things, especially when there's a large amount of size variation? And did you limit -- and related to that, did you limit this to clear type adults?

DR. RAMEY: Yes. Yeah, based on two eruption.

DR. COURTNEY: So it sounds like there was a suggestion there that you might want to see the, perhaps, more pathoanalysis, PCA.

DR. STEPPAN: Well, yeah, I would personally be curious to see what the plot of discriminate functions would look like, if that were possible.

DR. RAMEY: Well, the main point, though, is we test relative to criteria in advance, that it was a pretty clear-cut result, we thought, the 42 percent classified correctly. For PC1, intermedius was slightly smaller, but there's a great deal of overlap between Preble's and campestris. Campestris is a substantive variation with preblei.

We also did plots for PC2 versus PC3 and there was, you know, even more overlap. So PC1 primarily is a size component, so . . .

DR. STEPPAN: On the other characters that you chose not to -- I think you mentioned before

that the other characters you choose not to measure included -- was it the shape of the interorbital or the isoforamen.

DR. RAMEY: Yes, exactly. You know, it was -- we thought it difficult to try and quantify those. You know, perhaps it could be done. One can criticize on the basis of not absolutely everything being quantified; but it's, once again, the burden of proof sort of evidence of where does this thing fall relative to the data that you have and is repeatable.

DR. STEPPAN: Does anyone have any other questions?

DR. DUMBACHER: I have sort of a general question about mammalogy because I'm not really a mammalogist. So when I look at these things in a drawer, I find them hard to tell apart, but it might just be my unfamiliarity with the taxon. And I'm curious to what extent are well-supported molecular species in rodents difficult to distinguish using morphological data or a subspecies genetically distinguishable or well-accepted subspecies within the rodent groups? How often are they difficult to distinguish morphologically with these same types of techniques?

DR. RAMEY: As I recall, I think it was

Baker 2000, Journal of Mammalogy, did a paper on looking at genetic diversions of various rodent groups between species and -- below the level of species, subspecies, and between populations for -- for example, cytochrome B. It's very similar to the things you get from molecular markers.

I think as I've looked further at this at other taxonomic groups, I think that we found a situation of -- for example, with Peromyscus species and a very, very large number of subspecies, I think it's arguable that a number of -- some of these are going to be very good, are going to be readily distinguishable on the basis of geographic range and clear separation in terms of shape or other characteristics, pelage. Coloration is one, but I think you can get local adaptations.

So it really comes down to a question of degree and what's the basis of that. So, for example, Nachman found in rock mice -- Nachman Hockstra found color variations within a subspecies that are very distinctly different, but is that enough to draw the line and call them different subspecies, I wonder. So that's where the multiple data sets provides a stronger inference.

Ultimately, I think, we're going to the

place of doing a very systematic survey of some of these major taxonomic groups to inquire at what depth is the differentiation for genetic markers and compare that to morphology. I don't think we have all that information yet.

DR. STEPPAN: If I can, I assume that was a general question. It varies, and so there are cases where it can be very difficult, even for very distantly related members of the same genus. And speaking from my own personal experience, there's several distant-related members of muroid mice that are genetically just far apart from each other, separated by probably 4 or 5 million years, where some of the best morphological taxonomists could not tell them apart using a whole suite of characters that they had developed to distinguish them, but there are populations where you just can't tell them apart. And I've gone in, and I can't tell. There are just a handful of things you can't tell apart.

Now, that's a low -- that's not necessarily doing a discriminate function analysis, but that's a low frequency of misidentifications, but those are very different animals genetically. So it can be very difficult to find the right characters, we'll succeed in distinguishing them.

DR. RAMEY: I think it is important to recognize that it is a question of where one draws the line and what definition one uses as subspecies, what is the depth.

DR. ARBOGAST: Maybe to give some context, it seems like there's been some misidentification of the different species of jumping mouse, which, based on your genetic data, are very, very different. Like the princeps -- I forget what the amount was, but it's very large, in essence, and they can have a hard time sometimes discriminating certain characters, right?

DR. RAMEY: No. Actually, for discriminate analysis, they are --

DR. ARBOGAST: I think the field -- you know, common field analysis and stuff like that.

DR. RAMEY: Qualitatively most people can tell them apart. There's a question about hybrid. So that's, you know, a reasonable separation one can make. I wouldn't call them a particularly cryptic species. It might make, you know, the question of what would be the level of misidentification.

DR. ARBOGAST: Well, wasn't luteus thought to have been a princeps?

DR. RAMEY: Yes, exactly.

DR. ARBOGAST: So that seems to me that's a case where they're genetically quite different from the rest of the princeps, but were thought to be princeps. That was sort of my point.

DR. RAMEY: And, you know, to jump ahead, the molecular data suggests that luteus is something out there that's quite different, you know, relative to the range we looked at, talked about something else.

DR. STEPPAN: Could you clarify a point for me?

DR. RAMEY: Go ahead.

DR. STEPPAN: Which is did you actually measure the original types specimen --

DR. RAMEY: No.

DR. STEPPAN: -- the topo types?

DR. RAMEY: No. We decided to try and get a dispersion of samples across the range of the subspecies in order to get the range of variation. We thought that was more important.

I focused on this issue previously on our work on mountain sheep and that, you know, while it can be important to utilize topo types, there's sometimes limitations with the topo types themselves, for example, if they're immature specimens or broken or something like that. We thought it was more important to sample

across the range of variation within subspecies with the samples available.

DR. STEPPAN: So just to be clear, you did not actually examine the original --

DR. RAMEY: No.

DR. STEPPAN: -- Krutzch's type and where it falls in the variations?

DR. RAMEY: And I believe that might be -- I mean, it could be done.

DR. COURTNEY: It's a morphology question, Scott gets it.

DR. STEPPAN: Or morphology comment. And perhaps you might want to comment on and respond. So Jones 1981 suggested synonymy of the Pacific and western jumping mice -- I think that's what JM refers to -- based on extensive morphology, but that was never accepted 25 years later. Could you perhaps comment on the basis of that?

DR. RAMEY: I think it's outside the range of the discussion here, but --

DR. COURTNEY: The issue is really that --

DR. RAMEY: The species.

DR. COURTNEY: No, the issue is about how --

DR. STEPPAN: How diagnostic --

DR. RAMEY: How diagnostic morphology characters.

DR. STEPPAN: Which I think is some of the comments about, for example, luteus.

DR. RAMEY: You know, I don't know enough about that. Jones, I know, didn't go into publish since '91 -- I mean, since '81, the transcript of this and for the reasons he told me, which I conveyed to you; so I don't know if that's particularly the case or not. There might be other reasons to question the basic level taxonomy of beyond that of what was revised were luteus.

DR. DUMBACHER: Usually for change to be accepted, it at least has to be published; isn't that right? So if that was never published, then that might be why.

DR. RAMEY: Well, under the international rules of zoological nomenclature, it has to be published. It doesn't necessarily have to be peer reviewed and published, it has to appear in a minimal of four libraries, and it has to reference some sort of type or specimen. Bob Timmon actually wrote a letter to Science recently about this IBM description, need to have a body or parts thereof, and the rules require that data be in libraries, so . . .

DR. DUMBACHER: So the thesis should qualify for that?

DR. RAMEY: Yeah. I guess that's true, yeah.

DR. ARBOGAST: But correct me if I'm wrong, but it sounds to me the problem there was that the luteus things were included the wrong way, and that's why they thought maybe it caused enough problems that they decided not to go forward with that; is that correct?

DR. RAMEY: To revise the whole -- that's what Gwilym told me because I called him and asked, so what happened here, why didn't you publish this. Said it's a pretty substantial piece of work. He traveled extensively in order to sample specimens, so he has probably handled more of these than anyone or a broader range than anyone. He's reachable at Northeastern.

DR. COURTNEY: Are those all the questions to the panel? Thanks. You finish going through what you need to do?

DR. RAMEY: Preble's versus campestris, upper parts generally dull, averaging lighter between Preble and campestris. The sides are duller, less black-tipped hair. And once again, we thought about ways we could potentially quantify this, you know, in

comparison to campestris to intermedius utilizing color variation. We concluded these were not quantifiable. One could, like I said, use the spectrometer reading, but I think it would be extremely difficult to get -- to make that.

Adapted differences, so we're talking still morphology here, potential physiology or behavior. And 106, now 107 years of study since Preble 1899, there hasn't been any published quantitative evidence to support the hypothesis or potentially that there are any adaptations that would distinguish campestris -- Preble from campestris or any other nearby subspecies.

So we relied on Krutzch, Jones, Quimby, Whitaker, a number of other papers. Also Cryan had an excellent review of literature on Zapus hudsonius. And so, you know, while an absence of evidence doesn't necessarily mean there's evidence of absence, at some point it does; and so it's always a possibility out there but nobody's noticed it yet.

So I think it's reasonable to conclude that it's not at a depth that would be recognizable as an adaptation that would bring you to a conclusion that they're a different subspecies, particularly given the other evidence.

DR. DUMBACHER: So how many of those

studies were actually specifically designed to study ecological differences between preblei and other subspecies?

DR. RAMEY: Well, the Krutzch, for example, is a description of a -- you know, of morphology of adaptation. One could go and do something, for example, like, ecological niche modeling but -- and ask the difference in the ecology, but you -- asking that question, you have to ask what is the range of food habits or other life history traits of the organism itself. So for example, I've pointed out that, you know, they feed on vegetation, vertebrates, and fungi; so it's pretty broad dietary characteristics there.

In Colorado, they found them generally along stream areas, riparian areas, and a little bit out in the meadows or along drainage ditches, however, you find them on reclaimed mine sites. According to Krutzch and Quimby, beaches, you know, for hudsonius, in forests, a wide range of habitat. So there's no evidence to suggest that there's an adaptive difference, particularly one that would rise to the level of subspecies status, particularly independent of the other information.

DR. COURTNEY: I guess part of my role in

this is to press things even beyond reasonableness just to make sure that everything is clear. That's part of my job. I'm still not sure I heard the answer to my question, what of all the characters you rejected and why did you reject them? You gave us a couple of examples, but are there other characters you rejected and should we hear about those?

DR. RAMEY: I think we've covered those.

DR. COURTNEY: Okay. All right. So then questions from the panel or comments?

DR. DUMBACHER: No, it all seems pretty clear. I mean, I think that we're still -- I think one of the key things that still weighs on my mind is the burden of proof because, you know, not finding a difference is different than finding that there's no difference.

In so many of these studies, ecological studies especially, unless you're specifically testing, you know, and specifically looking for differences -- I mean, they might not be -- they might all be eating fungi pods and fungus and arthropods and fungus, but they might be very different fungi pods and very different funguses and in different places; so your point is well taken.

And I do agree that at some point after

106 years of study, the question is, you know, if there were differences, would we not have found them and at what point do we move on and change the taxonomy. But at the same time, you know, how many studies have specifically been seeking that. And there have been a lot of studies on these mice, but it's not clear to me how many of these are specifically designed to test the taxonomic distinctness.

So one of the things that continues to weigh on my mind -- and it sounds like it weighs on yours as well -- is how do we do this. You know, at what point do we decide to synonymize and at what point and what evidence do we need to do that. So that's something that still weighs heavily on me.

DR. RAMEY: I think it comes down to do you base your -- I mean, do you base your systematic decisions on the basis of hypothetical uniqueness or potential variation or something that you may not have found yet, or do you base it on something that you can find distinguishable and at a reasonable depth. And so there's an absence of evidence to support that latter conclusion.

DR. DUMBACHER: Well, let me ask you one question along those lines because I think it was in the Vignieri critique that mentioned that there were these

different echo types or habitat types that had been defined, and so from that point of view, how different are the habitats in the -- I'm thinking of what they call this now. A lot of GIS people, GIS experts will look at the range of a particular species, and then using 17 or 25, or however many are available, climatic factors that include rainfall and humidity and days of rain, and average mean temperature and, you know, all these -- seasonality, things like that, they can map what a particular species or subspecies' niche is.

And we did that with the owls and it was incredibly interesting because we found that the northern spotted owl was predicted to be right where it is and that there are very nice variables that distinguish the northern spotted owl from the California spotted owl and make sharp predictions about where those two species would be.

And, you know, so -- so from that point of view, one might be able to define or examine whether there are actual differences in the niches, if you will. Now, looking at whether or not they're adaptations is another thing that's -- you know, is another step further. But for a lot of people in our field, that's -- you know, looking for those correlates of range are pretty important and can be very telling.

So I'm curious, has anything like that been done? In looking at the critique that Vignieri had in saying that these are well distinguished and recognized differences in habitat, has anyone done an examination, like a GIS-based bioclime analysis or something like that to ask how different are these habitats and how different are the climates in the climological database?

DR. RAMEY: Checking on literature, Armstrong's lab did some work on some vegetation associations found around these, but that's my -- best of my knowledge, all beyond descriptive work other people had done on trying to measure differences in the animal to try to find larger systematic samples, but it didn't specifically address that issue.

Now, let me address the Vignieri, et al., and you already know the -- you already read the response article. It's been accepted now. Very, very minor edits in there. Kuchler's -- Kuchler's natural potential vegetation is a hypothetical association of species, of plants in a particular area given a stable climate at its -- at its succession level where there's been no apparent change; so it's really a potential vegetation. It could exist at a point in time in a completely stable state environment; and, therefore, we

consider it to be hypothetical in nature.

In nature, there's a great deal of variation in successional states, great deal of variation in habitats and soil types, which are not captured in that kind of potential natural vegetation. So the Steffan paper we cite, you know, cites cautionary notes on that.

DR. DUMBACHER: If I recall your answer to that, you were basically saying that there may be differences in the vegetation, but that doesn't -- you know, just because the mice are found in two different places doesn't necessarily mean there are adaptations to those different climates or variables or vegetation types, so . . .

DR. STEPPAN: I was just going to -- one sort of last concern, which you probably already have guessed based on the questions I've had dealing with the characters you chose not to include. And while certainly sympathetic to the need to be as objective in the measurements to have things that are reproducible and not subjective -- because you can easily have one person, oh, well, that looked bigger to me or that had a different shape and I classified it this way, you know, there's an element of nonreproducability in that. But I also know that a lot of the characters that may be

difficult to quantify, nonetheless, the human mind is actually excellent at pattern recognition for complex shapes that are very difficult to quantify.

So -- and this is not to completely question it, but I still do have the concern that there were, perhaps, in fact, diagnostic or at least very characteristic features that didn't get into the analysis because of that difficulty of trying to fit them to a certain measurement criterion. So it's not really a question because I know you've already answered that, but it's still a statement of concern, that while the objective to be objective is plausible, there are times when it may actually -- there's variation there that sometimes doesn't get captured.

And I'm certainly -- whether the choice is campestris that I'm going with did, in fact, capture the relevant -- may have missed the relevant differences that actually show a concordant pattern with geography, may be indicative of the history of separation.

DR. RAMEY: Although the majority of measurements that Krutzch had used, there was no support for them as being -- so the majority of evidence is, in fact, against Krutzch's classification. And Krutzch himself said that this is something that's no longer supported; and when the original author says that, I

think it carries some degree of weight.

You know, you talk about diagnosability or distinguishability, there's a great deal of overlap on all of these amongst all the specimens, and I think you would see that as well as I would in these specimens, and that's why we came to the conclusion. Campestris and this huge range of variation.

DR. STEPPAN: I think for the characters that you measured and presented, I would -- I think that's pretty clear. Statistically detectable difference is not the same thing as biological, meaning for a certain diagnosable difference.

DR. DUMBACHER: I'm just going to read this, this is another question from the audience.

DR. RAMEY: Yeah.

DR. DUMBACHER: It just said that Krutzch identified six skull characters, qualitative ones, as discriminatory between preblei and campestris. And the person asks is it true that you measured only one in your treatment.

DR. RAMEY: I have to go back and see which ones specifically, you know.

DR. DUMBACHER: Interorbital breadth they're referring to here.

DR. RAMEY: Oh, no. We did interorbital,

zygomatic breadth, mastoidal breadth. You know, a variety. Upper tooth row, basal length, that sort of stuff, so that's like six or eight.

DR. DUMBACHER: And then that's --

DR. RAMEY: Yeah. Nine -- nine skull measurements that he had to use that you could repeat, so . . .

DR. ARBOGAST: But I thought also -- in the Vignieri, et al., paper they also -- one of their points was that they suggest that of the characters you measured, only one was in the original description.

DR. RAMEY: No.

DR. ARBOGAST: Is that a --

DR. RAMEY: No, no. One was significantly smaller, and that was interorbital breadth. And I showed you the distribution of that.

DR. ARBOGAST: But there were actually several of those included?

DR. RAMEY: Oh, yes. Nine -- nine of the original measurement variables. And we also incorporated this morphometric information, you know, although it has its limitations, the PCA in our paper in asking what about ecological exchangeability. We put all the caveats around that, so there's no evidence of any adaptations. Utilizing the skull measurements that

we have, is there any shape differences that would bring up some different conclusion; and so we concluded no.

So that was within the context of the Crandall, et al., discussion. The Crandall, et al., specific test for genetic and ecological exchangeability.

DR. COURTNEY: This seems like a comment you guys might want to read and ask Dr. Ramey. Let me read this to the group. It's not a question, it's just a comment, which is the CDW does have a GIS model for Preble's habitat in Colorado which can predict the occurrence of Preble's based on riparian habitat types. And based on aerial photos, the CDW is not aware of any other models for any -- from subspecies Aphis, but the modeling process write-up is available if you want to look at it.

And the statement here is that the model from Preble's showed a statistically significant difference in Preble's occurrence based on riparian habitat differences or characteristics.

DR. DUMBACHER: Is this the climate analysis that somebody has done?

DR. COURTNEY: Yeah.

DR. DUMBACHER: And where is this available?

DR. COURTNEY: California Department of Wildlife.

DR. RAMEY: Colorado, Colorado.

DR. STEPPAN: Can I just ask a clarification? So can you -- the last sentence, statistically?

DR. COURTNEY: The model for Preble's showed a statistically significant difference in Preble's occurrence based on riparian habitat characteristics. So it's like an ecological model.

DR. STEPPAN: Can I ask actually what that means, that it's not -- in fact, it could be found in other places where --

DR. COURTNEY: I think it's okay to ask the person who handled this.

MR. MCCLEAN: Seth McClean, Division of Wildlife. The characteristics were based on -- whether it was the riparian shrub community with willows or riparian herbaceous with where it was just a grass -- grassy riparian characteristics or whether it was primarily a riparian tree or cottonwood. And what -- it was basically just occurrence or nonoccurrence, and it showed that Preble's were highly associated with riparian communities that have willows as part of their component.

It was just for Preble's, it was just for Colorado. It was not done across Preble's entire range in Colorado, and we're not aware of any other studies. But at least within riparian habitat used within Colorado, we're seeing a difference in -- at least within the riparian habitat, how they were using it.

DR. STEPPAN: So it basically said that it doesn't use all riparian habitat, but certainly a subset of riparian habitats.

DR. DUMBACHER: And you didn't explore what other areas in the US might have suitable habitat, like in Wyoming or other states?

MR. MCCLEAN: No, because the riparian mapping we were using is very expensive.

DR. DUMBACHER: Okay.

MR. MCCLEAN: If we had the data, we would have applied the model broader, but . . .

DR. RAMEY: Can I talk about that? I've seen this, you know, applied on the spotted owl and also discussed on specific mice, for example. And so once again, it's a -- if one can find a quantifiable difference in the habitat but you don't know how that reflects on the organism, should that be the basis of a systematic decision.

MR. MCCLEAN: No, no.

DR. RAMEY: Now you're getting into hypothetical scenarios for classification. I don't think taxonomy as a science has ever gone there.

DR. STEPPAN: First, I don't think that was actually quite the full criterion for the basis as much as providing information that may suggest other -- either -- either an actual ecological difference, which can be followed up with or suggested other differences.

DR. RAMEY: Just make one more point that any statistical difference -- the question is what's the biological relevance of that, so if you did find a statistically significant difference in some, you know, willow height, what's the biological relevance of that to the question at hand, and I think that's something we always have to ask ourselves. And we'll certainly be talking about that later today, statistical significance versus biological significance, at what depth is the difference.

DR. DUMBACHER: I just had one comment to make. There was another question or basically comment talking about the differences in the ecologies of the different regions, and so let me just say that we'll make note of this and we'll be -- we will discuss this in our final write-up. I'm not sure that we need to talk about it here, and we need to look at more of this

preliminary data or more of this type of data before we can really comment on it. But I would say that this is an area that -- I wouldn't say it's totally unprecedented that this sort of analysis is done.

And one of the ways that it is done is trying to understand if there are close associations of haplotypes or genetic types or other things that are distinguishable, and we're not sure we have that yet, but if there are close associations with distinguishable types and the habitat that they use, it's often been used to figure out where the genes from that group might be likely to spread. And it's a fairly common use for understanding range of -- potential ranges of species, especially in light of global warming.

So I do think it might be an appropriate thing for us to look at in light of looking at multiple independent lines of evidence. It may not be the strongest, and we would certainly not want to rely on it singularly, but it is a line of evidence that I think is relevant to looking after.

DR. RAMEY: Could I just add one point there? Along these lines, you might want to look at Gary Duvay's work at the University of Wyoming on -- I think there's been sort of parallel efforts, Colorado versus Wyoming, on mapping habitat. But Duvay's work is

fascinating relative to what's potential habitat out there and where connectivity might either presently occur or occurred in the recent past.

DR. DUMBACHER: Yeah, that's a very good point too. And since we're sort of on the topic of ecological exchangeability --

DR. STEPPAN: See it as part of the morphology discussion.

DR. DUMBACHER: And since you brought it up in your presentation, I wonder if maybe we can ask Keith who's actually published a bit about this and using it in conservation frame works if he could tell us a little bit about what is ecological exchangeability. And from what you know about the habitats that we're talking about, what sort of things would qualify as ecological exchangeability.

DR. STEPPAN: If I could add just one more question, whether this type of information about the distribution and association with habitats provides -- what kind of information does it provide about ecological exchangeability?

DR. COURTNEY: You might want to come up and use a mic.

DR. STEPPAN: Since I'll say that I think for now we've probably covered the morphological

questions unless anyone else has anything further at this time.

DR. RAMEY: Thank you very much.

DR. CRANDALL: The general idea of ecological exchangeability is that you can take a mouse from one location and put it in another location, and it will not just survive, but will serve the same fundamental ecological role in that location, right. And I think ecological niche modeling that you've been discussing here is an excellent way of looking at ecological exchangeability, and I think it is becoming more used
in -- especially species limitation discussions, in fact, in the evolutionary meetings --

DR. COURTNEY: Hold on a second, Keith, we've got the drill going on. Why don't you use the microphone.

DR. CRANDALL: At the -- we had our evolution meetings just last week in New York, and they had a symposium that was run by John Weems on species to limitations, and Leslie Wriskler from the University of Alabama gave her talk all on ecological modeling and showed how, in fact, it can be very effectively used in species to limitation questions. And I think it's very germane to the idea of ecological exchangeabilities.

It's an excellent way to look at that. Unfortunately, you can't just look at, you know, the small part of half of one subspecies range, but you have to do it across the relevant taxa across the distribution so that they're all taxa.

So you know, whether those data are available to do that sort of broader scale -- to do that broader scale -- to do those broader scale niche modeling is -- I don't know, you'd probably know better than I. Apparently it's not readily available limited amounts.

MR. MCCLEAN: Well, it was very high detailed mapping, and you can't just map the riparian areas. It costs lots of money to do that, and so that's why it wasn't available over the entire range.

DR. CRANDALL: So there certainly was this broader spectrum of the 12 subspecies and certainly the 5 that are kind of germane to this particular discussion, presumably there's great data on temperature and precipitation and those sorts of general variables that you could use to do at least a reasonable first pass of whether -- of niche modeling and see if the defined niches map to the corresponding distributions and to the genetic distinctions that you see.

DR. COURTNEY: Again, I want to press

things as far as I can because that's my role. The -- it was raised -- you know, a number of the critiques have raised the issue of, well, do we even have any real evidence on those exchangeability criteria. And the question that was raised earlier by Jack, you know, well, has anybody ever really managed to do that. Yeah, I know you've got this nice paper; but you know, isn't that kind of, like, a high standard that no one's ever really been able to address?

DR. CRANDALL: No. I think the morphometric data is exactly the kind of data that you collect. That's perfectly reasonable kind of data to collect for ecological exchangeability. And when you do the morphometric discriminate function analysis on the skull morphology, which was the basis of the -- of the species -- subspecies designations in the first place, you find you can't discriminate them; and to me that suggests they're ecologically exchangeable given those data, right. That's -- that's one of the kinds of pieces of data that you'd collect for measuring ecological exchangeability is morphometric data because that speaks to adaptability, adaptive differences in those species.

And your point earlier, those adaptive morphological characters showing up with large genetic

differences or not depends very much on the taxon -- on this particular taxon, and you can get large genetic differences with very little morphological difference. But here you do not see large genetic difference and here you don't see much morphometric differences either.

DR. STEPPAN: So I wonder how predictive -- well, I'd say two different aspects, but staying with the morphology for the moment, whether any particular set of morphological features, for example, might capture exchangeability if the key adductive responses had been physiological, let's say response to water stress or something like that. So how -- you know, how predictive are morphological models in terms of morphology as a surrogate for adaptive differences in organisms.

DR. CRANDALL: Right. So you have to -- you have to base it on the organism at hand, right. And here the subspecies designation are based on morphometric differences and skull morphology, so that's what you measure because you presume that that's the adaptive difference. If the taxonomic description said that the differences in these things is actually physiological, then you'd measure that, right, as your measure of ecological exchangeability.

If the differences were behavioral, you'd

measure that as your evidence for ecological exchangeability. If it describes subspecies were based on differences in life history evolution, you'd measure that. So -- but here the differences are based on skull morphology, so that's what they measure.

DR. STEPPAN: Would you agree that the lowest with all taxonomy is based on a combination of geography and a fairly standard set of morphological features because those are things that are easy to access and measure as opposed to the much harder to access behavioral characteristics or physiological characteristics?

DR. CRANDALL: Yeah. Certainly there are --

DR. STEPPAN: Is that more considered artifact than, you know, a research program?

DR. CRANDALL: Yeah, I think that's right. And there are certainly nice examples, like some of the California salamanders that David Wake works on, that have very nice behavioral differences that have led to hypotheses about taxonomic differentiation, but certainly the standard in taxonomy is -- and the default is to look for morphological characteristics as differentiating the taxon.

MR. STEPPAN: And then my similarly

related question is how predictive are the niche modeling approaches to actually predicting true ecological exchangeability? I guess there hasn't been any true test of that, is that correct, that you actually have done the exchange, not in obviously the Zapus, but other organisms that there have been exchange noncarbon -- noncarbon -- common garden approaches but exchanges?

DR. CRANDALL: No, and we certainly don't advocate doing those sort of studies. I mean, it's a bad idea in general moving organisms around just to test a hypotheses on exchangeability, but we do advocate taking these measurements of whatever seems relevant, whether it's behavioral differences, like history, differences, other kinds of ecological variables, niche modeling to look at ecological exchangeability. So -- and people have done those sorts of studies and they have done life history differences and fish studies and they have done -- even genetic data for ecological exchangeability, right.

If you have candidate genes that are associated with adaptive differences, you can measure the genetic differences and use that to look at differences in -- whether things are ecologically exchangeable or not. In fishes, you know, the great

system is because they know so much about everything, although you'd think that we'd be just as well off in mammals, but apparently not.

DR. COURTNEY: Are we -- are you guys done with Keith?

DR. STEPPAN: Thanks, Keith.

DR. COURTNEY: Okay. So we're kind of at a natural break point at this point. We're going to stop to take a lunch break, to have lunch, lots of options downstairs.

The -- I just wanted to comment on the process that you see so far. I don't know how many of you want to volunteer to be up here in front of the panel, but I've often described this sort of process to folks as being pretty much like being put in front of Anthelia spiders. And I think you'll see that the level of questioning, the depth of questioning that the panel subject the scientists to, it's serious; and I think that should be seen as a measure of the seriousness of our intent and of the professionalism of the process with which we've enacted here. You may think that you are more -- getting more or less grilled, it's just like being in front of Mr. Sciliary and his buddies. You actually don't know, from the way the questioning is going, exactly how it's all going to show up.

So I just wanted to thank you all for -- you know, this has been a good meeting so far. I think things have been going well. I think we've really grilled a couple of the participants well on significant issues. If you have other questions you want to raise about these issues, morphological issues, we're not done with it yet, although we're at a break point. I'm hoping to contact Dr. Patton soon and have him call in to comment on some of this stuff, and Dr. Vignieri may also choose to comment too. So we may revisit this, and we -- you should feel like you can submit questions or comments as necessary.

Okay. With that, we're going to reconvene in an hour. I warn you that when we do come back, we may be in a different room, so . . .

(Noon recess taken from 11:57 a.m. to 1:19 p.m.)

DR. COURTNEY: Okay. Let's reconvene. I always find it funny, we have these science meetings and they're pretty pointy headed; and you've got a big audience in the first section, and then the audience size shrinks progressively as the meeting goes on.

We're going to switch focus this afternoon and talk about what may be considered the central issues to some of these papers, which is the

issues of genetics. I think you've understood and seen the process now. I hope none of you have nightmares about being put in front of the panel. You can see that it's an intense scientific process, and I thank you all for following that. And you know, I think it's working well in terms of the written question thing. So let's just keep it like that for now.

You've been alerted to the fact that some of the questions we're going to be asking about the genetics issues were probably asked not just of these two first participants, Doctors Ramey and King, but probably other folks who might be brought on the telephone today or tomorrow. So we're going to be asking these questions. They may be repetitive, just be warned.

So first off is Dr. Ramey, and he's volunteered to continue being grilled by the supreme court here.

DR. DUMBACHER: Okay. So I'll just -- so I'll start the discussion here on some of the molecular data. And the things I think we're going to be talking about mostly are issues of data quality, and we'll talk a little bit about how you got the data, how you got the samples from the museum skins, which samples were from museum skins, and then we'll talk a little bit about the

genetic regions that you looked at and how much data there was and go over some of your findings as well.

So let's start first on -- let's talk a little bit about some of the museum specimens and how you -- where you got the DNA from those and what controls you had in the laboratory.

DR. RAMEY: Museum specimens were obtained by traveling to the museums instead of requesting them. I -- we -- all the Preble's specimens came from -- tissue samples, virtually all, I'm sure, came from tissue samples that were taken of specimens that are now at Texas Tech archives. The rest of them were skin samples that were two-year punches actually of preblei that I got from Pioneer Environmental. They're up in northern Larimer County. And so those actually came into the museum because they wanted a test of whether they were -- they sent us five samples as unknowns, and they wanted to know if these were Preble's.

DR. DUMBACHER: So these are all fresh tissue samples with --

DR. RAMEY: No. They're all Preble's, yeah. Fresh or ear punch.

DR. DUMBACHER: Okay. So the ear punch ones were from?

DR. RAMEY: Just two, just two. Those were from northern Larimer County, and those -- we designated where they came from in here. This is Pioneer, the specimen.

DR. DUMBACHER: And that was from fresh specimens that you also vouchered and there's vouchers --

DR. RAMEY: No, there's no vouchers to those. Those are the only two we don't have vouchers for. We tried to -- let me back up here.

DR. DUMBACHER: Okay. Sure.

DR. RAMEY: When we decided to do the study, we looked at two sampling schemes; and we asked, well, should we get many samples from two locations. And we looked at where those might possibly be and looked at the literature a bit more and thought, you know, we should probably try and do this across the range of the subspecies and that way we can capture the total range of variation that's out there. Obviously it limits some of the analysis you can do, but you're likely to catch things, particularly near zones of contact.

So we contacted museums, obtained lists of specimens, and then went through with maps and noted where all these specimens were located. And then we

just tried to select locations to give us a nice even distribution across the range of these subspecies all the way across, and I think you can see that in our figure in our paper. Then we -- so the Preble's samples, you know, we had in-house in the freezer there at the museum, got a couple from Pioneer Environmental. Then I traveled to KU, University of Kansas, Museum of Natural History and snipped skin specimens there.

DR. DUMBACHER: So that the snips, were they from center lines or were they toe pad or were they --

DR. RAMEY: They were generally along center lines.

DR. DUMBACHER: Okay.

DR. RAMEY: And so generally, I think this is a qualitative assessment, I would say, oh, a patch about like yea. Probably, you know, just a few milligrams. Probably wet, it would probably be, you know, 20 or so, maybe 30 milligrams even. The scissors that I used, I would lease before I went; and when I was there, I would actually reuse. But what I would do is to spray them down with alcohol, wipe them, spray them down again, flame them for about 30 seconds with a lighter in order to burn off any residual DNA on them, cool them, and then I would, you know, use a set of

those. Fresh gloves for every sample, separate tubes so, you know, wouldn't have any carryover there in any of those specimens.

Norm Clippinger took the ones from intermedius -- some of the intermedius specimens that came from the Nebraska State Museum. We got the ones from New Mexico on loan from the Museum of Southwestern Biology --

DR. ARBOGAST: The --

DR. RAMEY: -- and pallidus also came from KU also.

DR. ARBOGAST: The ones from Texas Tech, those are --

DR. RAMEY: Those are sitting --

DR. ARBOGAST: -- frozen tissues --

DR. RAMEY: Yeah.

DR. ARBOGAST: -- specimens?

DR. RAMEY: Yeah, exactly. And most of the Preble's specimens in the Museum Nature of Science in Denver are all 1990s onward. And so the problem with these sort of questions is sometimes they're local, so DMH has all of the Zapus hudsonius preblei specimens; whereas KU has, you know, campestris, pallidus, and intermedius. Museum of Southwestern Biology has -- and I can't remember if we took it from the University of

New Mexico, I'd have to check -- were all from down there.

We had ten luteus samples at the Museum of Nature and Science that were taken at the very southern part of Colorado, Las Animas County; and so I snipped skins on those, so . . .

DR. DUMBACHER: And when you brought these back to the laboratory, were they handled in the same lab as all of your other work or did you have a separate facility for your --

DR. RAMEY: No. Hsiu-Ping did all the DNA extractions on these; and so she did those at the University of Denver. And so, apparently, she did the extractions -- I asked her about this -- in different batches. So the Preble's was separate from the campestris.

DR. DUMBACHER: Okay. But they were all done in the same lab as the PCR machine and the other?

DR. RAMEY: I think you should specifically ask her that.

DR. DUMBACHER: Okay.

DR. RAMEY: But my understanding is that it was done at very different times in a lab shared with many other people, so . . .

DR. DUMBACHER: And do you know what

techniques she used for extracting the DNA?

DR. RAMEY: Qiagen, Qiagen.

DR. DUMBACHER: Or DNAs adhesion or whatever they call it? Okay.

DR. STEPPAN: What amplification -- what size fragment were you amplifying then from the skin samples?

DR. RAMEY: I think it was around 380 bases or so, but of course you have primer sequences on each end so you may see more than that, which she had done. And then trim the ends down, you get 346 mitochondrial.

We went after a control region because there was some preliminary data out there from Norm Clippinger -- actually went out when he was a student at CU on a project to ask about, you know, genetic relatedness of various Preble's to other populations and subsequently decided not to continue with the project; but I had some experience with working with this from back then.

DR. STEPPAN: So what is the success rate on those amplifications?

DR. RAMEY: My understanding is that the -- you're going to have to ask Joe King specifically about that; but my understanding was it was not a

hundred percent; but it was, you know, high, so --

DR. STEPPAN: And so --

DR. RAMEY: And --

DR. STEPPAN: -- were all the ancient samples then done in one large fragment? Did Hsiu-Ping ever have to go to two smaller fragments or three?

DR. RAMEY: Yeah. Well, as we detail in our paper, things were done both in single amplifications and also with nested PCR. So you know, we talked about this extensively. When she did the nested PCR, she always had negative controls in the initial reaction and then carried those through to the second reaction. And so, you know, that's the standard operating procedure with nested PCR. And most of the campestris samples, I think, as we indicated in the email, were obtained by nested PCR in some of the Intermedius.

DR. DUMBACHER: Did you try to replicate these results internally in the lab? For example, many labs will require that they get at least two amplified sequences to agree with each other to make sure that they believe the sequences. Was anything like that done?

DR. RAMEY: To the best of my knowledge, I don't believe the replicates were run. Outside if

there was any ambiguity, it was rerun.

DR. DUMBACHER: And what was your -- we haven't been able to open up the sequencer file so we haven't looked at the chromatograms ourselves, but what was your take? Did it look like the chromatograms were very clear and unambiguous in most cases?

DR. RAMEY: I saw some of these early on, but Hsiu-Ping had handled the nested and mitochondrial DNA. So Lance did all the skull measurements. I helped him with the analysis. Hsiu-Ping did the mitochondrial -- the DNA extractions, PCR amplifications; and I ran microsatellites.

DR. STEPPAN: So on the nested PCRs, what was the relative position of the --

DR. RAMEY: I have got to --

DR. STEPPAN: You never had that happen?

DR. RAMEY: I have to refresh my memory.

DR. STEPPAN: I'm just trying --

DR. RAMEY: Excuse me, because I helped to design all these tests.

DR. STEPPAN: Are nested primers sort of inside the original primers?

DR. RAMEY: As I recall, that's the case. And it's easy enough to put these sequences into the context to see that. Also of significance, we used

ammonium sulphate-based buffers for the DNA. And previous experience showed when we had amplifications of collusion or products, sometimes you get cleaner product using ammonium sulphate-based buffers.

DR. DUMBACHER: And the microsatellite data was all worked up from the same extracts; is that correct?

DR. RAMEY: Yes. We split those, and then I had a set for running that.

DR. DUMBACHER: Okay. Okay. And there were four microsatellite loci?

DR. RAMEY: There were six, and we dropped one from the analysis because of -- it was -- we had a Hardy-Weinberg proportion.

DR. DUMBACHER: Did you have any indication of dropouts of alleles or things like that?

DR. RAMEY: There were a few cases of dropout. I did run some replicates, particularly early on for a bunch of these samples, but we also ran into some time constraints and so we -- and also DNA constraints because we had to use a lot of templates for some of these microsatellite reactions, so I was concerned we were going to run out of some of these things.

For the Preble's/campestris comparison, I

had initially -- I believe it was, like, 24 of each, and I was running replicates of those to compare and that was all during the optimization period. During the optimization, based on previous work with the microsatellites, it's important to run replicates than to look and see if you have allele dropouts, potentially false alleles, any consistent scoring on those. And you also have to sometimes modify the amount of templates you add in order to get good amplification, diluted in some cases.

DR. DUMBACHER: Have you ever seen cases of, like, a third allele, anything like that that would indicate contamination?

DR. RAMEY: No, not on the ones we had used. We had -- I initially screened -- I believe it was, like, ten loci, and I had a number of those that were -- I think it was amplifying more than one locus. I had multiple peaks on those, and we dropped those out of the analyses from use early on and then settled in on these six because you can get clean results.

DR. STEPPAN: So you said a lot of these questions we should talk to Hsiu-Ping, is there a way which we can direct questions to her?

DR. RAMEY: Yeah. He needs to bring her up, or pop her an email.

DR. STEPPAN: Do we have that on our --
DR. RAMEY: I can help you out.
DR. COURTNEY: Do you happen to know whether she's available?
DR. RAMEY: I asked her if she'd be around today, she said yes. She'd be good.
DR. COURTNEY: We might need to do that.
DR. DUMBACHER: That's most of my questions about the lab technique.
DR. ARBOGAST: No.
DR. COURTNEY: Do you want to talk about other issues because we have Dr. Ramey here, sampling regimen?
DR. DUMBACHER: Well, those would definitely be very important to us.
DR. COURTNEY: I assume that we may bring scientists up and down as we need to answer questions. And we may take a step out for a minute or two or a half an hour, whatever you feel like you need to do. So I'm making sure that you run this the way you want. So if you want to move on and ask some questions of Dr. Ramey on other issues, that's okay.
DR. DUMBACHER: That's all I have about these issues for now. I don't know if you guys have any others. If you wanted to -- I don't know if in your

PowerPoint you had a couple slides on your analysis that you would want to go over real quick.

DR. RAMEY: You have all that. You already read all that. I have a few other things I'll talk about later, hopefully; but yeah, let's just cut to the chase.

DR. STEPPAN: Sounds like most of my questions would be to Hsiu-Ping. That's what I was interested in.

DR. COURTNEY: Sounds like we should do that immediately rather than try to come back to it, don't you agree?

DR. DUMBACHER: Sure. Can we get her on the phone now?

(Brief interruption in proceedings.)

DR. COURTNEY: This is obviously not going to work just yet, so we've got the rest of the afternoon and tomorrow to make sure this stuff gets done. So Dr. Ramey's going to perhaps just give a quick call and leave a message, and we'll get her to call in and do what's necessary.

So in which case then, I think we're ready to ask Dr. King to step up for the first time and take the hot seat. So I guess it's up to you guys to ask questions again.

DR. DUMBACHER: Okay. Thanks a lot for coming. Along the same lines, I wonder if you could just describe some of the field hole punch techniques that were used for collecting the DNA samples in the field.

DR. KING: The samples were collected -- the samples that we collected from the field were collected by Paul Cryan from the --

DR. COURTNEY: Tim, you have a soft voice and maybe --

DR. KING: I also have a frog or something in my throat.

The samples that were collected from the field were collected by Paul Cryan from the Fort Collins Science Center as part of the USGS. Paul, I believe, has submitted a statement as to what his methodology was; but in short, between individuals, he took the hole punch and submerged it approximately 1 inch in bleach solution between each individual and used a fresh pair of gloves with each specimen as well.

DR. DUMBACHER: Okay.

DR. STEPPAN: Do you know how long it was submerged? I mean, was it a dip or was it just lay it in there for -- until the next sample?

DR. KING: That I don't know.

DR. DUMBACHER: And these were all collected as animals were caught from traps?

DR. KING: Yes.

DR. DUMBACHER: So probably along the trap line.

DR. KING: Yes.

DR. DUMBACHER: So there was some time between sampling from one individual to sampling from the next.

DR. KING: That's my understanding, yes.

DR. COURTNEY: Perhaps I could just stop for a second because I've just been handed something from the Fish and Wildlife which addresses this. And so I don't know whether maybe you can just read it.

DR. KING: This is an email addressed to Seth Willey of the Fish and Wildlife Service by -- from Paul Cryan. It says, "I've received your message about the meeting later this week and unfortunately previous commitments will prevent me from being able to attend. However, I heard that there were questions about our sampling techniques; so I wanted to write and give you a clear picture of how we collected the samples." I'll provide this to you if that'll help. "All of the ear punch samples that we collected were taken using a 2-millimeter diameter scissor-type ear punch tool from

World Precision Instruments in Sarasota, Florida, and stainless steel forceps. Before taking samples from each mouse, the punch tool and the forceps were emerged in a 10 percent bleach solution to a depth of at least 1 inch for a minimum of 30 seconds, but usually several minutes, then rinsed in clean water and shaken dry.

We never deviated from this protocol. Clean rubber gloves were worn while handling the instruments and mice. In addition, blood was blotted from the punch wounds of mice on to Whatman FTA cards, which were also handled with clean rubber gloves.

DR. DUMBACHER: Those samples were then frozen or were they put in ethanol or some sort of a tissue buffer?

DR. KING: Those were submerged in ethanol, the tissue samples. The FTA cards were just maintained at ambient temp.

DR. DUMBACHER: And what technique was used for extracting DNA back at the laboratory?

DR. KING: We used the PUREGENE, basically a salt-based solution for the extractions for those tissues. It's the -- Gentra Systems is the name of the company that produces the PUREGENE kit. And that extraction was used on all samples, whether it was the tissue or whether it was the blood -- the ear tissue or

the blood. When we choose to use the blood sample, the sample was -- a circle was cut out of the FTA card and then that piece of FTA card was treated as one piece of tissue subjected to pro K and other enzymatic digestions.

DR. DUMBACHER: Any other questions about that?

DR. ARBOGAST: I just want to followup on the methods in the lab. Did you guys run multiple samples to see if you got multiple sequence from the same sample or any of the things that Jack asked about?

DR. KING: Are we specifically talking about the samples that were used in the primary study or are we talking about the museum specimens?

DR. ARBOGAST: I was just talking in general. I guess more specifically for the museum specimens.

DR. KING: Well, we probably should clarify then what was actually done with the museum specimens. After our initial results were obtained, we found some inconsistencies between our data and the data that were published in the Ramey, et al., 2005.

DR. DUMBACHER: Can you describe what you mean by "inconsistencies"?

DR. KING: Well, we -- we had looked at

61 individuals, 61 campestris individuals. 30 of those were from the exact same collection site as a series of samples that Ramey, et al., had reported as having preblei-type haplotypes.

DR. DUMBACHER: Were any of these the same individual or just the same collecting locality?

DR. KING: These were from the same collecting location. These were fresh caught specimens.

DR. DUMBACHER: So these were fresh caught. And do you know -- and, Dr. Ramey, and do you -- do you recall how old the specimens were that you sampled in museums? I'm just curious about the chronological difference between his collection and the collection that you were using.

DR. RAMEY: Checked into that, 67, 68, and 70.

DR. DUMBACHER: So it's about 40 years difference?

DR. KING: 30. But the ones in question, but the range goes all the way up until just very recently, then, you know, 2000. Yeah, so those are the old.

DR. KING: Most of the specimens in question are approximately 40 years old.

DR. DUMBACHER: Okay. But the same

location?

DR. KING: Yes, according to the specimen tag and using the record.

DR. DUMBACHER: Yeah, please continue. Okay.

DR. KING: So as a result of these inconsistencies, we offered to request tissue from the same museum specimens, and there were seven of them in question originally that the Ramey, et al., manuscript suggested had preblei haplotypes even though the individuals were collected within the campestris range. Those specimens were provided to us by the KU Museum. Dr. Robert Timmon provided the samples at our request.

The samples were, according to Dr. Timmon, were sampled using standard tissue sample protocols. The specimens -- only one specimen was working at a time, gloves were used, photographs were taken of the skins, the tag was legible. The tissue sample was placed into a vial dry and wrapped with paraffin, labeled, and cataloged and then sent to you also by FedEx. Then the Qiagen DNeasy kit was used for extractions.

And I need to distinguish these seven specimens from the other eight specimens that are provided in our manuscript. Those seven specimens were

of interest to us because they were the only specimens in either study that suggested that there might be some gene exchange between preblei and campestris. So what we did with those samples when they arrived, we signed for them, we took them to a new laboratory at our building that was not occupied, and never had any DNA extracted or amplified.

We took that tissue there, we separated it into two samples. Each -- each tissue sample was cut in half, one sample was given to one technician, another sample was given to another technician. They went off in separate directions, amplified the work in different locations at different times, provided us. We went through the amplification, the sequencing reactions, the cleanup, provided the sequence results, and we compared them. As a result, six of the seven specimens, we obtained matching sequences from the two technicians. The seventh specimen, the technician was unable to get any amplification, and we exhausted that template.

DR. DUMBACHER: Both technicians were unable to get any?

DR. KING: No, one technician was. The other was not.

DR. STEPPAN: Just to back up and clarify. So you went to an extraction lab that had not

been used for any?

DR. KING: Right. It was a brand-new genetics lab that was --

DR. STEPPAN: And you split the skin sample in two parts and then had the two technicians do independent extractions?

DR. KING: Independent extractions.

DR. STEPPAN: In that same lab?

DR. KING: Same lab.

DR. STEPPAN: But they handled them independently?

DR. KING: Yes.

DR. STEPPAN: And the PCR amplifications were done separately?

DR. KING: The PCRs reactions were done separately in separate hoods. The PCR reactions were run in the same lab as all the others as far as the thermocyclers, they were placed in the same thermocyclers as all the other samples, but the reactions were set up independently under laminar flow.

DR. STEPPAN: And of the six that worked where you had matching sequences from the two technicians, did those haplotypes match other haplotypes from other individuals?

DR. KING: Yes. Other than campestris

individuals.

DR. STEPPAN: But not necessarily each other?

DR. KING: No, they --

DR. STEPPAN: So six haplos had -- amongst the six haplos, how many different haplotypes were represented, do you recall offhand?

DR. KING: I can tell you. We have the table and the manuscript. We observed -- of those seven specimens, we observed two haplotypes. Two of the common campestris haplotypes.

DR. DUMBACHER: So these six samples -- I just want to repeat to make sure I have this straight -- they were taken from the campestris range?

DR. KING: Yes.

DR. DUMBACHER: And --

DR. KING: The seven -- seven species.

DR. DUMBACHER: Seven of them, okay. And they all returned campestris haplotypes?

DR. KING: Yes.

DR. DUMBACHER: That's using the control region sequences. We didn't have sufficient template -- or probably didn't have sufficient quality or quantity of template to do the control region sequences.

DR. STEPPAN: On the --

DR. KING: I'm sorry, the cytochrome B, excuse me, right. And part of the reason that we didn't have enough template is that we also ran microsatellite DNA analyses on these for two reasons. One, we wanted to perform an assignment test to see if the individuals were also assigned campestris using microsatellite, but also to look for any sign of contamination. Microsatellite using micromarkers are ideal for testing for contamination given these are discotic markers.

DR. DUMBACHER: And how did these perform in the assignment test, were they assigned?

DR. KING: All seven individuals were assigned to campestris. And on average, using the gene class assignment that we used, they were on average twice as likely to be campestris than to be preblei.

DR. STEPPAN: And did you find any evidence for additional balance?

DR. KING: No.

DR. STEPPAN: Any evidence for possible contamination in those?

DR. KING: No, nothing more than a heterozygous condition.

DR. STEPPAN: And what was -- for the six that didn't work, what was the amplification success rate? Did it require -- I forget whether you used

nested PCR, are they weak or strong amplifications? Did it take multiple tries?

DR. KING: It did take multiple tries, and it did take -- take nested primers. We were not able to amplify them with the primers that were -- that were published in the Ramey, et al. We were not able to -- for the museum skins for the other samples, we were, but not for the museum skins.

DR. DUMBACHER: Did you ever try to amplify any of those using cytochrome B primers, just different mitochondrial, you get from PCR?

DR. KING: We did not try that. Again, there was not a lot of template to begin with, and we exhausted most of it in trying to get the mitochondrial results so that we could have a direct comparison. But we were able to get as many as 15 of the 21 microsatellites to amplify in those museum skins. In table B-1 of the appendix will tell you the number of microsatellites that we were able to get to amplify.

But the template was scarce and, you know, we -- we strongly considered -- seriously considered going back and asking the museum for additional samples -- for additional tissue samples, but I finally decided to lay that ball in the lap of Fish and Wildlife Service; because regardless of what we

generated, it was going to be contentious. And it was my opinion that a third lab or a fourth lab should be called on to verify our findings. So I recommended to the Fish and Wildlife Service that they -- that they do that, they find someone to reanalyze those. And I don't know if that -- if that's been done or not.

DR. STEPPAN: Does anyone know if a decision's been made on that point?

MR. WILLEY: Fish and Wildlife has not followed up on that.

DR. STEPPAN: And so the control region amplifications you got were four similar size fragments that Ramey, et al., did, right?

DR. KING: Yes, yes. We were able to piece together a control region to have basically the same fragment.

DR. STEPPAN: But when you did your initial amplifications was for the 380 approximately, 340 fragments and then --

DR. KING: And as you'll see in the sequence, we --

DR. STEPPAN: Yeah, I haven't had a chance to look at it yet.

DR. KING: We had to piece that together in different-sized fragments. Basically it was to -- in

essence, it was really two fragments that were pieced together.

DR. DUMBACHER: And I noticed you have multiple mitochondrial lined up. Do you know, Dr. Ramey, were most of your control region sequences from a single amplicon from the entire region? You mentioned just the two end primers and nested PCRs, but it sounded like you never had to do that in multiple pieces?

DR. RAMEY: Anything that was amplified, basically Hsiu-Ping went and tried nested; and some she wasn't able to get successful amplifications, but she did do nested on those using the primers that we designated in our paper. And also the amplification conditions, once again, utilizing -- you know, based on previous experience, you have to optimize reactions. So that's why she went down the road using ammonium sulfate-based buffers, and I don't know what conditions were used here.

DR. KING: And I guess I should also say we limited this comparison to 15 individuals because of timing. We started discussions with the Fish and Wildlife Service in the spring of 2005 to do this research project. As of July, we were still negotiating with folks from the Department of Interior who were

trying to -- trying to squash the study and to keep it from going forward. So we had a very limited amount of time to work on the samples, and it takes a long time to get samples from the museums. They like -- they request a lot of information and, you know, they verify a lot of that information, and we just -- we just didn't have the time to look at additional samples. We are looking at additional samples now, but at the time that this report -- when we were working on this study report, we didn't have the time to do more.

DR. DUMBACHER: So you also added data from cytochrome B for your fresh tissues?

DR. KING: Yes.

DR. DUMBACHER: So let me ask you a question because I think one -- one big difference between the two studies has to do with the genes chosen for the study and also the sheer number of data, and I'm curious when you look at just the control region sequences, was your resolution of the haplotypes similar to what Dr. Ramey got; or just looking at control regions, were your control region phylogenies pretty similar in resolution to what he published?

DR. KING: They were similar with the exception of the haplotype sharing. In our data we saw no haplotype sharing among the subspecies, each

subspecies had a unique suite of haplotypes, control region haplotypes.

DR. DUMBACHER: Okay. And in your paper, you also have a cytochrome B haplotype network published. Did you, at any point, combine the control region with cytochrome B?

DR. KING: Yes. In the phylogenetic portions of the study and the analysis, yes. The tree that's presented in -- I think it's figure 5, is a combination of the two data sets.

DR. DUMBACHER: Okay. So I have some questions about that network and then that next phylogeny, but do we have any other questions about laboratory procedures before we move on? Okay.

DR. KING: Are we going to go to the genetic analyses now, or should Dr. Ramey come up, or how do we want to --

DR. DUMBACHER: I'm flexible.

DR. COURTNEY: He just stepped out. There's just a question of technique which Dr. Ramey wants to make a point, and I think he should be allowed to.

DR. DUMBACHER: Sure, yeah.

DR. RAMEY: Because I was a curator of the Museum of Nature of Science, I had some familiarity

with the ear punch specimens that were taken for preblei; and those specimens, to the best of my knowledge -- and I have a communication here to share with you, it's from Renee Taylor who took many of the southeastern Wyoming Preble's samples. And it was my knowledge, though I haven't seen the actual protocol, that the ear punch specimens were taken and then the punches were wiped down with alcohol, no gloves were used, and that was consistent across the Preble's samples taken.

In Renee Taylor's communication -- I had called her to say, you know, I can't lay my hands on that protocol. I've looked around really hard, but I do recall you contributed a large number of these ear punch specimens. And so she had provided me with this communication, which indicates that it's contrary to the protocol that Cryan had used.

I looked in Cryan's paper -- and I think that's a worthwhile thing to look at it -- it says it uses the Division of Wildlife protocol. To my knowledge, that Division of Wildlife protocol used alcohol and not bleaching or flaming specimens.

DR. COURTNEY: Seth?

MR. WILLEY: This is -- this is the protocol.

DR. COURTNEY: Oh, good. Thanks.

DR. STEPPAN: So this is which protocol?

DR. COURTNEY: It's Colorado's.

MR. PLAGE: I'm Pete Plage. That was given to me by Tanya Shenk. The little cover email there indicates it's the protocol DOW has used all along, and that was when Dr. Cryan was looking for the protocol. So based on that -- I mean, I can only go with what Tanya Shenk who's a researcher for DOW said. That was the DOW protocol.

DR. STEPPAN: So this was the DOW protocol that Paul Cryan had access to?

MR. PLAGE: Yeah.

DR. STEPPAN: But --

MR. PLAGE: Also if you see her cover letter, goes back to Riggs. And DOW who is -- my understanding was doing the kits, were taking the ear punch samples with various researchers in the field. They were preparing the kits. And I assume that that -- you know, based on her comment with the email and the cover, it's the same protocol they distribute to folks. I don't know specifically Renee Taylor, whether she got it. Is Tanya here? Sorry, I didn't know. Tanya Shank's here who's the DOW person who did a lot of that work.

DR. KING: One point that I failed to mention is that throughout this study, all the specimens -- not only the specimens that were provided by the Denver Museum, but all of the specimens, every individual that we looked at in the study had a unique multilocus genotype; so that tells me that there was no contamination at any point in the study. If there had been some contamination or crosscontamination where one specimen's DNA had swapped the other, we would have seen -- we would have seen individuals that had the same multilocus genotype, but we did not.

The 21 locus -- multilocus genotype was very robust at discriminating individuals, and there was no indication whatsoever of any two individuals that had the same multilocus genotype.

DR. DUMBACHER: Did you ever see cases of third allele in your microsatellite?

DR. KING: No. If we would have seen that, we would have tossed the sample or retracted it from the original tissue and started over, but we did not see any of that.

DR. ARBOGAST: And just to clarify, you used the same extractions for both the microsatellite and the nested PCR?

DR. KING: Yes.

DR. ARBOGAST: Great. Thank you.

DR. COURTNEY: I've asked the panel if they wanted -- because we're getting a lot of things thrown at us quickly and there were some folks in the audience who we may want to talk to, so we're going to take, like, a five-minute, ten-minute break. Just mill around outside. We'll just talk things over, decide what we want to do, and then we'll call you back in.

(Recess taken from 3:07 p.m. to 3:20 p.m.)

DR. DUMBACHER: So what we thought we might do, for the benefit of everyone else in the room, because probably many of you have read a bit of these comments back and forth, we wanted to talk a little bit about the two different types of contamination that we often get in genetics labs and that we all see in our labs, and we do everything that we can to try to cut these off or recognize them when they start to happen.

One type of contamination is when your field sample or initial sample gets switched with something else, and that can happen for a variety of reasons. It can happen because your scissors in the museum collection or your hole punch in the field has not been cleaned, and you've actually mixed a little bit of sample from one to the other.

We also -- it occurs, you know, not infrequently, that we'll mix up the numbers on a vial, things like that. And so there is this type of contamination that we do see regularly in the lab. And we hope that we can catch that and cut it off and use a variety of techniques that the two PIs have both mentioned here to try and recognize when there is contamination. And we oftentimes will throw out specimens that seem especially perplexing in our analysis because they may be contaminated for one reason or another, and sometimes it's just easier to look at the other specimens.

So there's a variety of things that we try and do to cut that off. So that's one type of contamination that happens when we're collecting our samples, and we want to make sure that nothing gets mixed up in those samples.

There's another type of contamination that happens, and it happens especially in laboratories that use ancient DNA and most of us had some occasion to do some ancient DNA work. The laboratory that Scott worked in at the Smithsonian has a designated facility that's closed off from the rest of the laboratory for their ancient DNA work. And when I worked at the Smithsonian with Rob Fleischer and in my laboratory now,

we have a separated room that has a different air handling system, and it's separated physically from the other lab. You can never go from the post-PCR lab back to the extraction lab.

And the reason is this: Is once you do a PCR reaction, you're creating millions and millions and millions of copies of DNA that's very low molecular weight that can become volatile in those tubes. It can get on your clothing, it can get on your hands and your hair. And when you go to set up another PCR reaction, it can get in that tube.

Now, oftentimes in a normal laboratory, that's not a problem because we have so much DNA template in the bottom of our tubes that the good quality DNA template gets preferentially amplified, and we don't normally see much problem from that sort of thing, although it does occur in laboratories.

But when we're working with substandard DNA and ancient DNA, oftentimes there's no template in the bottom of our tube; and we'll get amplification of other things, like our own human DNA from hair, from our own tissue. Sometimes we'll see things like cow, chicken, and pig, which we just assume is probably from our last meal; so that's -- that's how sensitive some of these reactions are. And we often -- in Rob's lab, we'd

get things like crickets and cockroach, mosquito DNA, things like that.

So when we're working in the laboratories, what we're doing is very, very sensitive. And so the second type of contamination that sometimes occurs is this amplification or contamination of PCR product, okay. So once you've amplified DNA in the laboratory and you've got PCR product around, it can also get into the next PCR reaction.

And so we've already talked to both PIs here and we've talked a lot about what they've each done to try to minimize contamination or the probability of contamination, and so I think we've kind of exhausted the questions there.

But we would like to talk to Hsiu-Ping when we can get her on the phone just to find out what some of her results were and what she found will tell us whether or not, you know, there was -- whether there was a significant possibility of contamination. That doesn't mean that there is contamination.

And I should say, too, nobody's done anything wrong here. What both of these labs have done is commonly done in a variety of different labs. Some labs have very high stringencies. And we have to remember too, King is working with having had all this

knowledge of what's gone on before and can compare and say, well, wait a second; and you know, take extra steps break; whereas in Ramey's lab, it was the first pass-through.

And, you know, so everything that was done here was appropriate. We're not criticizing anyone, but we are trying to just figure out, you know, what steps were taken, and so we will probably have some more questions.

We did have one question real quickly about this email that you had given us. Who is Renee Taylor again?

DR. RAMEY: She was a consultant that worked a lot on Preble's mice, and she collected a bunch of Preble's ear punch specimens back in the '90s. And so I looked around in my files and couldn't find the ear punch protocol, so I just rang her up one day and said so do you happen to have a copy of this.

DR. DUMBACHER: And these are some of the same ear punches that were then used in your lab; is that correct, or no?

DR. KING: I don't know.

DR. RAMEY: Yes, those are.

DR. DUMBACHER: Okay. Thanks a lot for that clarification.

DR. KING: Which specimen?

DR. RAMEY: I believe southeastern Wyoming and potentially other Preble's specimens, I mean, there was a number of people who collected.

DR. DUMBACHER: But these were all Preble's and not campestris?

DR. RAMEY: Good to ask that question.

MS. JENNINGS: I'm Mary Jennings with Fish and Wildlife Service, and I believe of the specimens you looked at, they were -- they would have all been Preble's or from the range of Preble's, none of them thought to be campestris. Some of them may have turned out to be princeps. Actually, she collected for True Ranches. They own several properties along the front range and southeastern Wyoming; and she collected from many, many drainages. And so several of your specimens came from her collection.

DR. KING: Okay.

DR. DUMBACHER: That's informative too.

DR. ARBOGAST: Some of them would have been from other people like Cryan as well?

DR. KING: No, no. The Wyoming samples -- the southwestern -- the southern Wyoming samples that we used were -- many of them were just identified in the museum as being Zapus. They weren't identified to

species. We used control region cyt B to determine the species and the subspecies as far as hudsonius is concerned, and those that were preblei were used as the preblei collection from southern Wyoming.

DR. DUMBACHER: Okay. But none of these were the campestris where there was preblei?

DR. KING: (Shakes head back and forth.)

DR. DUMBACHER: Okay. Great. Then I think that we've exhausted that line of inquiry. And I think what we'd like to do next would be to look at the genetic data and some of the analyses that were done, and we'd like to proceed pretty much the way we did this morning with the molecular questions. So we wonder if we could ask you --

DR. RAMEY: Sure.

DR. DUMBACHER: So your control region sequences, how did you choose that region and tell us a little bit about your analyses and what you did there.

DR. RAMEY: Well, one thing that made me competitive, you know, in putting a program together on this question was that I worked with this grad student at CU. We together developed primers for the control region, north specific, and so we were able to potentially make rapid progress on this. And the control region obviously is one of the more faster

evolving regions, and so we chose that to run with on this initially and realized there was some limitations potentially on some specimens for how long an amplification we could get.

And we did have some preliminary cytochrome B data, but we realized that was going to be very, very expensive long road to get that data. So we decided we're not going to run with this part, the number of specimens, I don't know, 20 or 50 specimens we ran. 20 or 30, I think.

DR. STEPPAN: Which part of the control region was that again?

DR. RAMEY: I'll have to check. I don't recall.

DR. DUMBACHER: Was it part of a highly variable region or was it one of the --

DR. RAMEY: Just ask Hsiu-Ping that. She has that.

DR. DUMBACHER: With your mitochondrial analysis on your tree, figure 3 on your published paper, the neighbor-joining phylogram, and this was based on distance data; is that correct?

DR. RAMEY: Yes, based on distance.

DR. DUMBACHER: And you choose your --

DR. RAMEY: Primary 2.

DR. DUMBACHER: -- primary 2. And how did you choose that model?

DR. RAMEY: We ran a series of models and decided on -- you know, we did a series of phylogenetic analyses with different models and we had strong congruence on those; and so we, you know, produced this tree just as one of many that we could have produced with the data set. And so we had strong concordance between these, was that there was two lineages of mitochondrial DNA that was found. One, essentially, a pallidus, luteus lineage and another preblei, campestris, intermedius lineage.

So really the distance you use and such are probably less relevant than the fact you have congruence across many models and many genetic analyses. You know, not shown in there is Parsonian analysis, networking, split decomposition. Thanks, just ask.

Same basic structure, low bootstrap support for the nodes beyond this major split in the lineages. And I think that, you know, when we compare our study with King's, you find, I think, remarkable concordance between these outside of the shared haplotypes. Types that we found between preblei and campestris between the campestris and intermedius, so it's the only real difference.

DR. DUMBACHER: And the Preble's haplotypes are here in blue; is that right?

DR. RAMEY: Uh-huh, uh-huh.

DR. DUMBACHER: So these were the ones that were primarily found within the Preble's range?

DR. RAMEY: Yes.

DR. DUMBACHER: And they're found pretty close in the tree here, but that portion of the tree really has no bootstrap supported?

DR. RAMEY: Correct.

DR. DUMBACHER: No particular phylogeny in that region can really be supported or argued for?

DR. RAMEY: Correct.

DR. DUMBACHER: And so there's no evidence for monophyly here?

DR. RAMEY: Correct.

DR. DUMBACHER: But no evidence against monophyly either?

DR. RAMEY: No evidence -- the question is there is resolution -- sufficient resolution to detect monophyly if it exists.

DR. DUMBACHER: Yeah, I guess that's kind of what I'm getting at.

DR. RAMEY: Yeah.

DR. DUMBACHER: Okay. But there's no

alternative phylogeny that it's nonmonophyletic that is supported by these data either?

DR. RAMEY: No.

DR. DUMBACHER: Okay. Okay. Is there anything else that you'd like to say about these data that you think are especially relevant or telling?

DR. RAMEY: Well, once again, there's a remarkable concordance between these two mitochondrial DNA trees that were obtained. Bootstrap support for both of these for any of the nodes beyond those clades, and so I think there's some uncertainty as to the phylogenetic resolution there; however, it's obviously at a depth that is very shallow.

DR. DUMBACHER: Right, and very difficult to resolve with the data. Okay. Any other questions about the mitochondrial?

DR. STEPPAN: No.

DR. DUMBACHER: But you didn't use Modeltest?

DR. RAMEY: Yes, we did use Modeltest.

DR. DUMBACHER: And you recommended having the 2 parameter or something else?

DR. RAMEY: I believe so.

DR. DUMBACHER: Okay. Then you were asked to look at more data by a review panel, is that

correct, and that was looking at some nuclear markers and that was while you went back into some microsatellite work?

DR. RAMEY: Well, it was -- we had a number of reviews that suggested, okay, let's look at microsatellites; however, none of those reviews specified any kind of threshold with which to apply to the microsatellite data, just to gather microsatellite data was the essential suggestion.

And similarly, we submitted our paper and then we were asked to revise, resubmit. It was -- you know, the reviewer suggested getting microsatellite or some other nuclear marker. Now, ideally we'd go after nuclear genes on these things. And microsatellites are relatively easy to gather the data for, and so we pursued that into the question because that's relevant to the splitting of the prairie jumping mouse into these other entities by Krutzch '54.

DR. ARBOGAST: Can I ask a followup question?

DR. RAMEY: Yes, yes, yeah.

DR. ARBOGAST: It says in the paper that the Modeltest selected the TVM model with a discrete gamma distribution, yet you were saying you used the 2 parameter --

DR. RAMEY: Uh-huh.

DR. ARBOGAST: -- for the gene tree?

DR. RAMEY: Uh-huh.

DR. ARBOGAST: So I'm interested in why the Modeltest chose one model and you used a different one for the tree.

DR. RAMEY: You know, let's just go ahead and ask Hsiu-Ping that because we worked together on this, and she was really the ace on the analysis on this. But we worked very close on this.

DR. ARBOGAST: It sounds here like actually you used the TVM + I + G model for the tree.

DR. RAMEY: All this stuff was done certainly years ago, so I'd have --

DR. ARBOGAST: I think there is, yeah.

DR. RAMEY: -- to reach back.

DR. STEPPAN: Backing up just a little bit because I was just trying to figure something out. I don't think this is actually terribly important one way or the other, but just comparing the animal conservation paper to the technical report in December of '04 and the two trees are -- both adjoining trees are similar coloring schemes. And I notice the branch lengths are different between two of them for many of the clades, and on the technical report there are

various branches that don't have haplotype labels associated with them. And from what I can tell, were they same haplotype labels that are here?

DR. RAMEY: Well, the paper represents a refinement on that.

DR. STEPPAN: Right. Was that just collapsing -- finding haplotypes that were identical and further collapsing them?

DR. RAMEY: I believe so, but the thing to rely upon is the published paper.

DR. STEPPAN: But they should be the same two data sets, correct?

DR. RAMEY: Yeah, same data sets.

DR. STEPPAN: And both neighbor-joining with the same model?

DR. CRANDALL: I don't think so. I think the technical report is the 2 parameter and then that's why the branch links are denying.

DR. RAMEY: You know, you're reaching back pretty far now. This is only my fourth summer on this.

DR. STEPPAN: I just noticed a few little things, I'm just --

DR. RAMEY: Fine.

DR. DUMBACHER: Just for everybody in the

audience, I'd hate to be asked a bunch of questions about papers I published four years ago, so we appreciate your --

DR. RAMEY: That's okay.

DR. STEPPAN: My studies -- my analyses go through a few permutations too, so --

DR. RAMEY: One thing I would like to point out here is on these trees is that, you know, there was -- actually in our initial work, you know, we used a microanalysis number, which is a lab identifier number, and we have a voucher number. And so, you know, our first trees that we produced had the isolated numbers instead of the voucher number.

And relative to -- I want to go back, if I could, and revisit this discussion of contamination and such, that, you know, there's been some discussion as to whether there's been a mix-up of samples and there's -- I would like to take issue with some minor points there in Tim's analysis that there was inconsistent numbering of samples.

And I think I communicated that with you earlier that, in fact, Genbank had -- we had sent the subsequent files in and had the voucher number and the isolated number and that that just hadn't been updated. And so, you know, communicating with them, we actually

have the final updated files but items just an oversight by their group in doing that.

So anyway, you could look back -- I think it was our first report we used those KU numbers and, you know, instead of the voucher numbers because it was just easier to move along. But we were very consistent by having all that together and checked out multiple times.

DR. COURTNEY: Are we done with Dr. Ramey for a moment?

DR. DUMBACHER: I think so. Are there any questions about the microsatellite analysis?

DR. ARBOGAST: Not right now.

DR. DUMBACHER: So I wonder if we could ask you some of the same questions, just how did you choose your loci, what did you find, what do you see is the critical differences in the two studies or at least in terms of the data that were used?

DR. KING: Do you want to stick with the mitochondrial?

DR. DUMBACHER: Yeah, let's stick with the mitochondrial first.

DR. KING: Well, we ran the mitochondrial in the control region obviously because the Ramey, et al., publication reported control regions, so we tried

to -- or we did amplify and sequence and analyze the same region. That's the reason why we did control region.

We choose to do an additional region, which was cytochrome B because, you know, we felt that 346 base pairs of DNA sequence was not sufficient. And again, I don't know if I've said this or not, but when we started this work for -- planning this work, the Ramey, et al., 2005 had not been published and there was no microsatellite data available. So we thought that facing this type of question on 346 base pairs of control region was insufficient, so we added a commonly used fragment of cytochrome B to the study to see if we got congruent results.

If that answers your question as to why we chose a separate region, but we just felt that one gene tree, control region control tree, the short fragment like that was not sufficient with which to make these types of decisions.

DR. DUMBACHER: One other significant -- or at least to me looking at the tree, one of the things that I see in your tree, having more data in it, you're able to get better resolution. There may not be strong bootstrap support from these groups, but you're getting partitions for many of the subspecies campestris, which

is -- appears to be monophyletic in your tree there. And intermedius is Zhi?

DR. KING: Yes.

DR. DUMBACHER: It's intermedius. So it actually breaks out into two clusters where the campestris is in between. You also ran a nested clade analysis?

DR. KING: No, we did not.

DR. DUMBACHER: This is just the network?

DR. KING: That's the TCS network.

DR. DUMBACHER: TCS network, which is a nonrouted partitioning of the data, so it doesn't necessarily place a root. So one of the things that struck me about this that I wanted to ask you a couple of questions about is that you did find a clustering of the Preble's haplotypes in many of the other subspecies, so you found pretty tight clustering?

DR. KING: Yeah. There was -- the haplotype were diagnostic among the subspecies.

DR. DUMBACHER: Right. And being diagnostic could be predicted. If you got a new haplotype, you could make predictions about where it fell and see whether or not it --

DR. KING: With the limitations of the data that we have, yes.

DR. DUMBACHER: Now, in terms of the rooting of this tree -- so one of the things is that Preble's is not monophyletic, which is one of the things that a lot of taxonomists look for, hope for, and conservation biologists too. And just looking at the length of branches on this tree --

DR. KING: Are you looking at the tree from the report or are you looking at the tree from the manuscript? I believe you have the report.

DR. ARBOGAST: Is this the most recent?

DR. CRANDALL: None of these subspecies are monophyletic.

DR. KING: Pardon?

DR. CRANDALL: None of the subspecies are monophyletic.

DR. RAMEY: They're paraphyletic.

DR. DUMBACHER: It says campestris is monophyletic.

DR. COURTNEY: I know that we're behaving like a normal scientific meeting, and I want to remind you that we're not a normal scientific meeting, so let's let the panel ask you questions. If you have a question or comment, you know the process.

DR. DUMBACHER: But it's okay with me if Tim --

DR. COURTNEY: If you want to ask him a question.

DR. DUMBACHER: Okay. I guess one of the things that I've -- been looking at in trying to understand about this is the rooting in this tree is an outgroup rooting, right, so you've just put all your taxa in and you let the computer figure out where the root is for this group?

DR. KING: Yes.

DR. DUMBACHER: But if you looked at an unrooted phylogeny or unrooted network for just hudsonius, then you would get what's pretty much in this figure before it?

DR. KING: Yes.

DR. DUMBACHER: Okay. And what support was there for the placement of that root, because one of the things that also strikes me on these sorts of things is that -- I guess, how do I phrase this? The next outgroup -- oh, I guess it's the Zapus hudsonius, is that what it is?

DR. ARBOGAST: That should be luteus.

DR. KING: Luteus and pallidus.

DR. DUMBACHER: So luteus is what you mean nested hudsonius in this?

DR. STEPPAN: To everyone in the

audience who can't look at what we're looking at, we apologize.

DR. DUMBACHER: Yeah, we were talking about this a little bit in the car coming over yesterday, which is why it's kind of fresh in my mind.

DR. ARBOGAST: I think the point that jumped out is that the morphology is fairly odd looking for a typical habitat tree in that a lot of haplotypes are clustered around down at the base, close to the outgroup instead of being out sort of, you know, more of a shape of a tree like in the Ramey, et al., paper. Those are based on similar data. And regardless of the samples in question that the inconsistent results are in, the shapes of those two trees are really very different to me. And I was just wondering if you -- my personal question is could you comment on the effect of the outgroup rooting.

DR. COURTNEY: By the way, Tim, if this is coming at you faster and you wanted to take a few minutes to think about it, you can do that.

DR. KING: I'm not sure what -- that I understand the question.

DR. DUMBACHER: I guess if we look at this haplotype network, we get nice clusters of many of the different groups. And the thing that tells us that

this is not a good cluster is that the root happens to fall right inside of this cluster. What support do we have that the root belongs here as opposed to any other place? And placement of that root may be a very critical point for whether or not this is monophyletic, and I guess that's one of the things that I was just -- we were just curious about.

DR. KING: Well, I think what we should do is maybe back up a little bit and try to understand why the haplotype network is there. And you may remember from reading in the manuscript that we felt like this question that we're trying to address is not a species level question, which is exactly what this analysis is trying to force it into. We provided this analysis simply as a comparison with Ramey's.

DR. ARBOGAST: Are you talking about the tree?

DR. KING: Yes.

DR. ARBOGAST: Yeah, thank you.

DR. KING: Yes. We felt -- I felt strongly that this is an intraspecific comparison, and we're trying to force it here into a higher level analysis, phylogenetic analysis. And the haplotype network, which gives us some information from an ancestral standpoint, is more important and more

revealing in these taxas that are relatively recently diverged.

And that's why we provided the haplotype network; because it shows not only that the haplotypes for each subspecies are most related to other haplotypes within that subspecies, but it also takes the information from the haplotype network and combines it with the -- the haplotype appendices shows there's no haplotype sharing between and among the subspecies.

What the analysis is that you're proposing now -- what that analysis is telling us is what we already knew going into that based on the Ramey, et al., manuscript is that whatever -- whatever differentiation there is that exists there, it's relatively shallow and it's relatively recent. It doesn't mean it isn't important, but addressing questions about monophyly on this data are inappropriate in my opinion. It's not the question. I don't want to know whether it's species or not. I want to know whether it's a subspecies.

DR. ARBOGAST: I think there are a couple issues. One is that, yes, there's a whole issue of whether we would even expect that, which I think is a good point. The other point, though, is that -- maybe this is my direct question, is I wonder how good

princeps is as an outgroup in this at all because of how distant it is. And if you could comment just on maybe the -- if you know, the approximate level of sequence divergence between princeps and some ingroup versus outgroup amounts which are pretty low.

DR. KING: Right. Well, I believe the sequence divergence for control region between princeps and hudsonius on the average is about 10 percent for control region. It's approximately 20 percent using cytochrome B.

DR. ARBOGAST: Right. So maybe tenfold or more?

DR. KING: Right. And again, you know, your point is well taken, it may not be the appropriate outgroup, but I would again contend that this analysis is inappropriate for this question and that's why we put emphasis on that in the manuscript. But the genealogical concordance is an important issue in this study, I believe.

DR. ARBOGAST: I think that, you know, we would ideally root these trees, but in some cases with these intraspecific-type questions, the next closest group is very divergent and they can, in some ways, lead to some problems. And I'm not sure if that's the case here; but given the large amount of divergence of

princeps, it makes me suspicious that it could be; so I think that's something that we need to consider.

DR. KING: And since we've mentioned princeps, I think it's important that I tell you that we've -- we have sequenced some tissue samples from the Denver Museum -- that were provided by the Denver Museum but requested for analysis by the Fish and Wildlife Service, and these were specimens that were either identified as Zapus general or identified as hudsonius princeps.

And we've looked at 50 individuals most recently, and I think approximately 100 individuals total. And we see, again, very large sequence diversity, differences or sequenced -- sequence divergence differences between princeps and hudsonius. And we were using control region, cyt B, and what we believe to be a diagnostic microsatellite.

We assumed no indication of hybernization between princeps and hudsonius even in southern Wyoming where we have obtained samples from the same drainage -- different locations within this drainage, but from the same drainage. We see no indication out of a hundred specimens of hybernization. So again, whether it's a good outgroup or not, that's a very valid question.

DR. STEPPAN: Can you think of anything

that would? There's only one other species in the genes, correct?

DR. KING: Yes, trinotatus.

DR. STEPPAN: Is there any expectation it would be closer?

DR. KING: Well, geographically it's not so in theory. We didn't even go there, to be quite honest.

DR. COURTNEY: I think as part of that, it turned out geographically is not taxonomically closest.

DR. DUMBACHER: So your point is well taken that this -- these type of analyses are the kinds of things that a lot of taxonomists would consider valid for species level, and I appreciate that point. And I guess one of the questions is, depending upon how this rooting goes, if the rooting goes in a different place, you could actually get phylogeny that would suggest preblei would be a good species. We haven't gotten that in this -- in these analyses, but it's unclear to me that any rooting here is well supported and that it's hard to look at this phylogeny and read too much into it.

DR. KING: That's exactly my point. I mean, you've made the point better than I had in the

manuscript. That's exactly the point why that analysis is inappropriate in this to address this question.

DR. DUMBACHER: Well, are there any other questions about this?

DR. ARBOGAST: I don't think about the mitochondrial data.

DR. DUMBACHER: I'm curious about your -- let's see, the tree that you did show there. What kind of tree is that again, can you remind me?

DR. KING: It was a parsimonious.

DR. DUMBACHER: That's parsimonious.

DR. KING: Then there's a basic analysis right on the back.

DR. DUMBACHER: Okay. Which model of evolution did you use?

DR. KING: Well, for the control region, it was HKY + I + G.

DR. DUMBACHER: And did you explore site-specific rate models for that?

DR. KING: No, I do not -- I don't believe that we did.

DR. ARBOGAST: The partition Bayesian analysis for the --

DR. KING: Yes.

DR. ARBOGAST: So partition by control

agent and by cytochrome B?

DR. KING: Yes.

DR. ARBOGAST: And there are a number of -- there are a number of parameters which you can vary or not vary that when you do the Bayesian analysis.

DR. KING: Yes.

DR. ARBOGAST: And do you recall which -- in the manuscript it doesn't clearly state that -- which ones were allowed to vary and which ones weren't?

DR. KING: I don't recall, but I think that we could probably talk to John, John Switzer who did the analysis if you'd like to -- if you'd like to do that.

DR. ARBOGAST: It's more out of curiosity than anything.

DR. COURTNEY: Okay. Are we done with Dr. King for a little bit?

DR. DUMBACHER: Yep.

DR. COURTNEY: Do you want to address the same sort of tree issues with Dr. Ramey or Dr. Crandall?

DR. DUMBACHER: I don't know if you guys have any more to add to that.

DR. CRANDALL: In our report, if you look at figure 6, we did use cytochrome B data from --

DR. COURTNEY: Keith, we can't all hear

you. You need to come up.

DR. CRANDALL: So this is the tree. So in our report that we did for the State of Wyoming, we did -- in figure 6 it shows cytochrome B data from the subset of the species in question with a number of representatives from the other species in that genus, the other two. And the one -- what's the one with the TA?

DR. ARBOGAST: Trinotatus.

DR. CRANDALL: That one comes in between the other two, although the bootstrap valley is very low for that particular location. There's only one haplotype of that type.

DR. ARBOGAST: Is it much more closely related to terms of sequence divergences, or do you recall?

DR. CRANDALL: I don't recall what the sequence divergences are in there.

DR. DUMBACHER: And Preble's is in black in this one, right?

DR. CRANDALL: Right.

DR. COURTNEY: Maybe I, plus the panel, are thinking about what they want to ask. Maybe I'll just ask Keith to comment on the whole issue of rootiness of these trees and appropriateness of using

this sort of approach at a subspecific level.

DR. CRANDALL: Well, I've made a living telling people what Tim just told you, which is, you know, it's -- the phylogeny is good for asking questions about if you -- I mean, the first thing you want to know when you do a study like this is is the species monophyletic, right, so the phylogeny is appropriate, and that's the question you were all asking. And phylogeny is a great tool to look at that, is this species monophyletic.

When you get below the species level, there are some ESU criteria, like Moritz' that do require reciprocal monophyly as one of their criterion; so in that case, you have to use a phylogeny at least as part of your analysis to address that criterion of what is an evolutionary significant unit. Some subspecies definitions aren't based on reciprocal monophyly, so phylogeny is an appropriate thing to use in that case.

If you want to look at population dynamics and partitioning history from current goings-on in the population structure, then the network approach, in my opinion, is a far better approach. It gives you statistical support for those relationships that, basically, when you do the phylogeny, you get two groups, right. I mean, both data sets show you get two

groups. You get the three subspecies clustering together in one clade and the other two clustering together in the other. And they basically give you no information about what's going on within those two clades.

So then that's an appropriate point to say if we want to designate ESUs on reciprocal monophyly, there are two groups. If we want to do something else, then we have to go to a different technique, like the nesting or the network approaches to look at what's going on within those groups. And when you look at the network of Tim's, you get, in fact, at least two, if not three distinct networks. Yeah, so three distinct networks, right. One with luteus, one with pallidus, and one with the three other subspecies. Although it's not true that they cluster exclusively by subspecies, he's drawn it that way, but this one is actually connected to campestris but boxed in the intermedius because it's an intermediate.

DR. STEPPAN: Right, but it's not a haplotype shared by individuals of two different subspecies; is that correct?

DR. KING: That's correct.

DR. CRANDALL: Right, but it's an intermedius haplotype that's boxed.

DR. STEPPAN: And that that's most closely linked to a haplotype.

DR. KING: But the next step would be a campestris, yes, but it is not.

DR. CRANDALL: And the other point to realize is that those boxes don't have anything to do with the nested clade analysis, right? That's not -- neither group did that sort of analysis?

DR. DUMBACHER: So what do you make of Preble's jumping mouse being in it's own box? I mean, there's three specific --

DR. CRANDALL: Well, Tim just drew his box. So when you do the nested clade analysis, then you look at the historical population structure that's going on there. What you see is that you have the inferences across multiple nesting levels, which means the inferences throughout the evolutionary history of this -- these three subspecies that are in this particular clade is a history of isolation by distance, right. And that's reflected here in that you get the preblei haplotypes as each other's closest relatives, and for the most part, the intermedius haplotypes as each other's relatives except for this one, and then the same with these.

You've got some isolation by distance and

with the isolation by distance you have to -- you have to worry a lot about the geographic spread of the sampling, right.

DR. ARBOGAST: So has anyone actually looked to see -- to test that in any of these data? We were talking about to see whether, you know, sampling made a big difference was to actually test to see if you're doing the nested test or something like that?

DR. CRANDALL: I haven't done that, but Tim actually tested a little bit in that when he submitted his report, he didn't have this southeast Wyoming population in there. And then in the manuscript -- accepted manuscript for Molecular Ecology, he puts that in. And if it's truly isolation by distance structure, you wouldn't expect to see that -- and this is with the microsatellite data -- that population falling in between. And that's -- if you look at the other -- that one, you see that.

DR. STEPPAN: Is it figure 3, which is the --

DR. CRANDALL: This one doesn't -- this is the wrong one. That's the one without the southwest population. And what happens with the southwest population is it comes in intermediate between preblei and campestris, which is where you'd expect it if you

have isolation by distance.

DR. ARBOGAST: This one?

DR. CRANDALL: So that one. So here's all the preblei and now here's this one south Wyoming that comes in in between campestris and all the preblei, which is exactly where you'd expect it with isolation by distance masquerading as population structure because you've done -- you haven't sampled throughout the range of the thing.

DR. KING: Can I make point of clarification?

DR. COURTNEY: I think you should.

DR. KING: First, what we should say is that because of differences in coalescence time, we see different patterns of the microsatellites than we see in the mtDNA. What we see with the haplotype network is that there is no -- there isn't isolation by distance when we're talking about preblei, campestris, and intermedius because the haplotypes for intermedius are somewhat intermediate as you might expect between preblei and campestris.

Those haplotypes -- those intermedius haplotypes come into the network before the campestris do. And if you look at the tree that's published in Ramey, et al., 2005, if you look at where those

individuals are, those four control region haplotypes, you'll see that they are actually rested inside the intermedius. So our haplotype network is consistent with the tree in that respect.

And the last point that Dr. Crandall made was that we see isolation by distance. We do see a little bit of that, at least between -- from the nuclear standpoint between preblei and campestris. And the point that he made was if we had had additional sampling, we would have seen -- seen a tighter relationship. Well, in fact, that's not the case because the sample from southeastern Wyoming is the northern extent of the range for preblei, at least of the samples that we have and that we know of. So that is the northern extent of the range.

And that collection, while it appears to be intermediate between preblei and campestris, we get 98 percent bootstrap support for the intermedius -- excuse me, for the preblei collections to be differentiated from campestris and intermedius.

DR. DUMBACHER: So that's a STRUCTURE analysis, is that what?

DR. KING: That's the STRUCTURE analysis of the DNA -- Nei's DNA distance.

DR. ARBOGAST: So although this --

DR. KING: But it's consistent with the STRUCTURE analysis with -- all the microsatellite analyses are very consistent.

DR. ARBOGAST: So although this tree is not rooted here under your figure 3, the point is is that the bootstrap joining the SOWY haplotypes with the other Preble's haplotypes is 98?

DR. KING: It's 98 percent bootstrap support. When you look at the rooted tree, that's the way that it counted out as well. 98, 9 -- 98 or 99 percent bootstrap support. But you know, so we do see isolation by distance on certain scales, but not all scales.

DR. ARBOGAST: I think the question would be it's not an issue of the isolation by distance within a subspecies, but if you see it across multiple subspecies, then it sort of makes you think it's really just one big group that you are creating it?

DR. KING: Right, right. But the other thing to take into consideration is that -- I hope I've made this point clear -- that we're contending that the differentiation is relatively recent. It's significant, it's diagnostic, but it's relatively recent. And because the nuclear DNA has a four-time longer coalescence time than the mtDNA is we might expect to

see the microsatellite data to be somewhat behind on the evolutionary trail, expect to see it somewhat behind the mtDNA.

DR. ARBOGAST: Given that, were you surprised to see the structure analysis and some of these other analyses of the microsatellite data and the nuclear DNA to basically be congruent with the mitochondrial DNA given the mitochondrial DNA is pretty shallow?

DR. KING: Not -- not really. We see the same trends when you compare -- when you compare the tree, the neighbor-joining tree of the DNA distance with the haplotype network. I mean, we see similar trends, it's just not -- it's just not as strong, not as diagnostic. And that's -- to me, that's what we would expect under coalescence theory.

No, I wasn't surprised. And I think, you know, as we learn more and more about these statistical analyses that help define populations, I think what we find is that those analyses underestimate the structure. And in fact, there's a paper that's just come out in Molecular Ecology, Robin Waffles is the senior author on that paper, and he mentions STRUCTURE and BAPS by name saying that they understood the structure, the population structure that exists.

So this analysis didn't -- you know, would say that we've underestimated the structure that exists, and I think we probably have. I think that you could probably make a case for preblei to be divided into three distinct groups based on haplotype or -- excuse me, allele frequency heterogenetic test, the STRUCTURE analysis, the cluster analysis, the heterogeneity analysis. Whether or not they're distinct DPSs or not, that's another question, but clearly there's population structures there that's detectable and it's statistically significant.

DR. DUMBACHER: I wonder, it might not be a bad idea to take a short break.

DR. COURTNEY: Yeah, request to share more information. What I'd like to suggest is take a five-minute break so I can convene with the panel, talk to the panel for just a minute, and then reconvene and we may take a longer break after that. So why don't you all just take five minutes, take a stretch, and don't go far.

(Recess was taken from 4:10 p.m. to 4:35 p.m.)

DR. COURTNEY: I warned you we would chop as we chose -- as the panel chose, and so the panel has chosen to do the following, which is we'd like to ask

Dr. Crandall a few questions or the panel would like to ask Dr. Crandall a few questions about his report. Then we've got -- we're going to give Dr. King, Dr. Ramey the opportunity to just comment on our proceedings, whether we've covered the things they want to have covered. There may be emphasis they'd like to see or issues they'd like to see raised we haven't really dealt with yet.

So we're going to do the following, which is bring Dr. Crandall up to the torture chair and talk about his report, then talk to Dr. King and Ramey about the process and -- maybe if there were things that they need to have addressed or what they'd like to see addressed.

And then the key thing for us that we want to really attempt to do today is we still don't have the chromatograms from Ramey group yet, not for any reason other than just transfer issues, I think. So we really want to get ahold of those because the panel is going to be working on those tonight and Dr. Ping's stuff too. So we're going to focus our efforts on that.

Tomorrow, then, there are a few things that would still need to be addressed. We still have to raise some data quality questions with your group, we can get on the telephone. We want to talk to

Dr. Vignieri who will be calling in hopefully. We want to listen to some of the -- or discuss some of the issues about nuclear genes. And then shift to the remaining large topic, which is what constitutes a subspecies, what does this all mean in terms of what are the standards that apply. So that's going to be all shifted till tomorrow.

Don't -- don't worry that we've forgotten those are important issues, we haven't. Those are key issues we're going to be focusing on. As I said, Dr. Vignieri and -- Vignieri and Patton will be hopefully on the line to help us with that point if that's okay. Comments from the panel? In which case, if Dr. Crandall could step up to the podium.

DR. CRANDALL: Sure.

DR. ARBOGAST: So one of the questions that had come up was the use of the computer program MIGRATE for the migration rates and the data sets. The combined data sets that you had examined, you were able to use migrate on the microsatellite data; is that correct?

DR. CRANDALL: Right.

DR. ARBOGAST: I had more of a methological question in that in researching this, I have also seen you had done a -- coauthored a paper

where you evaluated migration performance along with fluctuate. And I think one of the things that you had found was that migration rates and the confident intervals associated with them were poorly estimated using MIGRATE. And so I just would like to get your expertise or comments on how well -- how much faith you put in these MIGRATE estimates and this computer program to actually be able to say something meaningful about migration?

DR. CRANDALL: Well, we put as much faith in the estimates as we get the same answer back after a few times running it. Often with -- especially deeper divergences, you get very different answers every time you run the software. That's when we scratch our heads and that's kind of problematic, and those are some of the simulation runs that we did.

And in fact, that was one of the criticisms by Burley, the author of MIGRATE, was that our divergences were too much -- you know, we're too high for MIGRATE to give you actually reasonable answers. Yet we used divergence as typical for population genetic studies, at least a lot of them. Here the divergence are quite low, so we anticipate that MIGRATE will actually give you reasonable estimates; and when we run it multiple times, we get the same

estimates.

But you know, it's a coalescent-based approach which brings in a whole lot of assumptions, one of which is a constant affected population size, another is a large affected population size, another is no selection in the markers under consideration, so there's silent markers. So you know, you have to always be aware of the assumptions of the methods that you're using, but we thought it was important that somebody estimate gene flow because that's a pretty critical component of specificity of taxa and nobody had estimated gene flow yet, so . . .

DR. ARBOGAST: And do you think, just your general feelings, is that the assumptions of large affected population size is constant that those would be problematic or not?

DR. CRANDALL: I think that the -- at least for the microsatellite data, the fairly recent history, the nested clade analysis suggests that most of the action going on is isolation by distance, so there's not range expansion or things like that. So it doesn't look like they were large fluctuations in population size.

Certainly when you get into the deeper coalescence events -- I mean, the whole species has

clearly moved over the last 10,000, 15,000 years because most of it is up in central Canada where it was covered with ice. So presumably that's a big population expansion up into that whole area.

But for the group of concentration here, you know, I don't know if that's a reasonable assumption or not. From the data that we can estimate, it seems to be a reasonable assumption. And the microsatellite data -- you guys did the Hardy-Weinberg test and all that looked good, so it looks like they're reasonably neutral loci, right.

DR. ARBOGAST: Thank you.

DR. STEPPAN: And so your concerns about MIGRATE are not necessarily unique to MIGRATE, correct, or --

DR. CRANDALL: No, they are --

DR. STEPPAN: -- would you characterize -- I guess, your -- you chose to use MIGRATE, so would you characterize to --

DR. CRANDALL: -- mine.

DR. STEPPAN: -- migration estimates as being the best available, do you think?

DR. CRANDALL: I think so. I think when you can meet the assumptions of the method, MIGRATE fluctuate are the best -- it's the mark -- it's the

package -- it's the best package out there at the moment. Others make -- the standard way to do estimates of migration rates is to take your S statistic, then do some algebra and solve for effective number of migrates, and that is clearly not a very good thing to do or a very reasonable approach.

There are some new approaches, one by Jody Hey, called IM that estimates migration rates, but it does it in pairwise sorts of things, so you have to know what you're populations are ahead of time and set those up in an appropriate way.

It so long depends on how much you buy into these assumptions. We've used both MIGRATE and IM with some of our data that we've been analyzing from our lab, and it just kind of depends on the relative amount of divergence which one we use.

DR. ARBOGAST: And so you could have also -- one could also examine the mitochondrial data Fluctuate and MIGRATE as well, right?

DR. CRANDALL: Right.

DR. ARBOGAST: And you didn't do that, right, in the report? I think it was just for microsatellite, if I'm not mistaken. So as far as we know, no one has done MIGRATE for the mitochondrial data?

DR. KING: We did not, but again, we found no haplotype sharing so there was no point.

DR. ARBOGAST: Okay.

DR. KING: There is no gene flow among the same species.

DR. DUMBACHER: Yeah, MIGRATE would have returned no migration. Keith, I had a real quick question about --

DR. CRANDALL: Well, the other thing is that the MIGRATE -- lots of studies, simulation studies have shown the power of MIGRATE is with multilocus data, not with single locus data, so . . .

DR. ARBOGAST: Right.

DR. CRANDALL: So it makes sense to do it with multiple locus data set. In fact, when you do that, we got positive migration rates between preblei and some of the other subspecies that were on the order of population level migration rates for squirrels and other small mammal studies that we cited in there.

DR. DUMBACHER: Quick question about your migration rates. Are any that you calculated asymmetrical between your Preble's -- your Preble's jumping mouse group and any other groups that you recovered in your structure analysis. And you know,

when you get any much less than one, we usually sort of assume that the populations are evolving in isolation and independently, and we like to think of them as different units. And when it's much greater than one, we usually think that there's a lot of gene flow there and that you almost like to treat them as cladistic populations, and I think around one is when you get to that confusing region.

And what was interesting to me was that, if I got the directionality right, the migration out from Preble's to campestris and intermedius is 2.14 and the migration rate back into Preble's from the nearest group campestris and intermedius is .46 and from the other group was .47, so slightly under one but still sort in that gray zone.

But it was interesting to me that -- I mean, there's a couple different ways to interpret this. It might be that Preble's is a source population and is contributing alleles to the neighboring populations, but it's more or less evolving independently from input from the other populations; so in that sense, one might think of it as being more independent.

I'm curious how you would interpret that and if there's anything significant there that you would -- that you would take home from those numbers?

DR. CRANDALL: Well, the significance is that there's clearly an asymmetry going on, right, in terms of the relative amounts of migration. I mean, this is the problem, right, is that your -- the magic number of one, it's like, you know, you're exactly on both sides of it and around it, which is why we're all here because this is -- this is the problematic beast.

And you know, I really don't know what to make of that. I'd make that same conclusion that you just did that it seems to be fairly -- there seems to be some movement, although highly limited, into the preblei population, but a reasonable amount of movement out of it, so . . .

DR. KING: Would now be a good time?

DR. COURTNEY: Did you have a comment to a question back here?

DR. KING: Well, it is more of a comment.

DR. COURTNEY: Is that okay?

DR. DUMBACHER: Sure.

DR. KING: A couple of things we need to keep in mind when we consider this.

DR. COURTNEY: Maybe you should use the mic.

DR. KING: Sorry, go ahead and stay. A couple of things that I think we need to keep in mind,

one is that the nature of microsatellites and the way they evolve, there's a great deal of homoplasy in microsatellites, and I know of no one who has said anything other than the fact that microsatellites underestimate the amount of structure that exists because of this homoplasy.

The other thing to take into consideration is that the sample size for preblei, in general, is large. It's as large as all the other subspecies combined, so there are more reels there represented in that population, and I think that's why you see the pattern that you see, that there's nothing coming in and there seems to be things going that way. It's an artifact. It's an artifact that the sample size for the preblei is so much larger, and you take it -- take all of the three major populations or the seven populations in our study into consideration, that's why you see the pattern that you see.

One last point is that I think we should be very careful about using the one migrant per generation rule. There are multiple publications, but there's one that's led by Fred Allendorf that suggests that that number could be somewhere between five and ten. I mean, the populations can diverge even when the data tells us that -- or suggests there are low levels

of migration between -- between one and five individuals; in some cases, one and ten. So we need to be very careful with throwing around that one migrant per generation rule.

DR. CRANDALL: And I just echo those sentiments that it really is -- the sampling design is problematic, especially for estimating gene flow and these sorts of things because it's so uneven across the different subspecies that are being looked at. And the MP of one rule, you know, is problematic in the other direction as well.

You have -- we're all looking at neutral -- neutrally evolving loci, which can be off on their own evolutionary trajectory and then you get one selected allele go through and then everybody's the same. So it's -- you know, it goes both ways, the problem with the golden MP one rule.

DR. COURTNEY: Okay. Are we done?

DR. DUMBACHER: Just one other thing because I think one of the things that was really nice about your paper, if I read it correctly, was that you looked at the microsatellite data from both studies, and putting together and analyzing them separately, pretty much said that there was a lot of congruence and that you were able to recover some of the same data

partitions; am I reading that correctly?

DR. CRANDALL: We didn't put them together, we analyzed them independently. We tried to put them together, but we couldn't because they didn't use common standard standardization. So we couldn't tell which piece from which on the different data sets. And Tim had the same problem in trying to combine his microsatellite.

So we did put the control region data together and then combined that -- combined control region data set with the cyt B data set for mitochondrial analysis, but just basically redid the structure analyses on the two microsatellite data sets independently just so we could: One, see for ourselves what was going on; two, then move on to the estimate of gene flow, which neither group had done with those multilocus data.

DR. COURTNEY: Okay.

DR. DUMBACHER: Thanks very much.

DR. STEPPAN: Thank you.

DR. KING: The 500-pound gorilla in the room is what does -- what does the fact that you've used data, which we've said suggests that there has been no recent gene exchange between preblei and campestris. You used those haplotypes in your analysis, and what

affect do you think those have had on the analysis as far as -- well, for all the analysis. What do you think that those -- those data, if they're incorrect, what effect those data may have had on the analyses and the interpretations.

DR. DUMBACHER: Actually, I should just say that was a question that we did have, and because it's late in the day, we forgot to ask it. But that is a question that we've been talking about too, and we wanted to just ask if you had excluded those data, would it have affected your analysis or your conclusions?

DR. CRANDALL: Well, there are two things going on. One is that between the report that was published and the manuscript that was accepted, there was a question of some of these samples; but in addition, there were additional samples added right from the Wyoming population, so -- of preblei. So we wanted to do that analysis and asked for the data set from Dr. King and from Seth Willey and other people at the Fish and Wildlife Service.

So far we haven't -- nobody's allowed us to access those data to do those analyses, so I don't know what the effect is because you haven't allowed us to look at the data.

DR. KING: No, that's not true.

DR. CRANDALL: It is true.

DR. KING: No, no, no. The samples that were identified as being incorrect in my laboratory, that information was made available in January.

DR. CRANDALL: I emailed you last week for a data file that included your data set that you used in the Molecular Ecology paper so that we could do an analysis on the same date set, and I haven't gotten a reply.

DR. DUMBACHER: If we could, I think there are two different questions here. One would be what would happen if we included the data that you had questioned, the other is what if we include the additional data that you have added? And perhaps those are two separate questions, and let's just try and make -- maybe it's not a good idea for me to ask you to tell me what you think you would have gotten.

DR. CRANDALL: Well, I think Rob has actually done those analyses excluding those questionable samples; is that right?

DR. RAMEY: I asked Hsiu-Ping last night to rerun our analyses, both biogenetic analysis and also AMOVA, utilizing -- which she didn't do, Lance did -- excluding sample. So here's what we did, first of all, we said okay, what about these seven samples that shared

haplotypes. Let's just go ahead and rerun without those and see what happens. Actually did that about a week and a half ago. And AMOVA value had gone from about .37 to .52.

And the structure of the tree doesn't change because all you're doing is pruning off those specific individuals off the tree, so it's the same phylogenetic conclusions -- phylogenetic conclusions outside of there being shared haplotypes.

Next I suggested yesterday that, well, let's do this. Let's take out the 13 samples that King takes issue with and rerun the analysis on those. And so -- I'll just -- I could report that to you. It doesn't change things that much. Actually it just increased the value of the AMOVA up a little bit to around .36 in one case.

We also reran taking out all of the nested PCR samples and taking -- that meant taking out all of the campestris samples, and then we stuck King's data in place for campestris for the control region, reran the AMOVA, and reran the phylogenies. I haven't looked at the phylogenies yet, but Hsiu-Ping just sent me a quick summary. But basically, the basic result does not change that you do not have reciprocal monophyly. You have paraphyletic relationship. You

have low bootstraps in support of this Preble's group for mitochondrial DNA. And then -- so I think that that's, you know, very worthwhile to go through that exercise regardless of whether there's a real issue there or not.

DR. CRANDALL: And clearly in terms of the Fist and the Fst, they're going to elevate, right?

DR. RAMEY: Although the intermedius, the campestris one actually dropped a little bit more, so . . .

DR. KING: But when the Fst or what should have been used, the Fist go up. What that means --

DR. RAMEY: Actually --

DR. KING: I'm sorry?

DR. DUMBACHER: But as they go up --

DR. KING: In the new analysis. When you use the new analysis, if the Fst value or the Fist is whatever used is above .5, then that then meets the threshold that you've established in the manuscript for them to be discrete.

DR. RAMEY: No. Actually what's really important to realize here, that our conclusions did not rise exclusively on the mitochondrial DNA data set and also it didn't rely specifically just on that test.

That we had used five different lines of evidence.

DR. KING: Right, right, but --

DR. DUMBACHER: If we could -- if we could interpret. If we're going to talk about specific data sets --

DR. RAMEY: Please.

DR. DUMBACHER: -- we'll just address one data set at a time and confine it to the questions that we have for those; and we, as a panel, will put those together later in our analysis. So I think we've visited that question as much as we're going to today.

And the other question is: What -- do you think that having added those other southwest Wyoming populations, how do you think that those might have affected your migration analysis, your structure analysis? And I know without having them it's really hard to say, so maybe it's inappropriate for me to ask, but --

DR. CRANDALL: Those might actually -- because they -- in Tim's -- I don't know what you call it, a neighbor-joining distance thing for the microsatellite.

DR. DUMBACHER: The network of DNA.

DR. CRANDALL: That -- no, not the network, the microsatellite distance tree, right. Those

southwest things fall out in between preblei -- the rest of preblei and intermedius, so those can actually decrease statistics, to a certain degree, because they're falling out in between those two.

DR. DUMBACHER: So if they're forced to fit in one --

DR. CRANDALL: And increase the relative amount of gene flow -- certainly will increase the relative amount of gene flow between preblei and intermedius because now you've got -- you've got a whole population that's on what was a much longer branch isolating preblei from intermedius. You've broken it up with that one location.

DR. DUMBACHER: Okay. Just one other question about this and maybe this is more of a philosophical one because we had a very similar problem with the northern spotted owl and California spotted owl and that different researchers had sampled -- and some of them had sampled quite extensively right in the putative hybrid zone. And if southwest Wyoming is closer to the hybrid zone or more likely to have experienced some gene flow, does -- I guess if you're forcing them to be just two populations and you've got some individuals or some populations that are notably in the middle, is it appropriate to try and put those in

the analysis or is it appropriate to exclude them? What do we do with those?

DR. CRANDALL: I think it's essential to put them in. It's highly inappropriate to exclude them because then you're going to get isolation by sampling design, right, which is what I perceive is the problem. And so you really need to include those. And you know, we all have this dilemma with sampling, right. We all get a limited budget and sit down and scratch our heads and think what am I going to do now, am I going to do the broad sampling that Ramey did or the dense sampling that King did.

And you know, in the ideal world, you do both, right. You do broad, dense sampling. And the next best thing is to do exactly what both of them have done, which is start with the broad sampling, figure out where the action is, and then do go back and do more dense sampling around the boundaries, around where you're finding very different kinds of haplotypes and discontinuities and things like that and, you know, keep going. Add those data to your data set, do an analysis, figure out where you need to go back to. I mean, it's an iterative process, and we've had two iterations.

And let me just say that I think both groups have done a great job. I mean, the data

collected by Ramey, the breadth of it in terms of the morphometric and the microsatellites and the mitochondrial DNA is a fantastic data set. Tim's lab did an exceptional job under conditions I would not want to have, right. I mean, really time-constrained stuff to do this kind of work and to produce the volume of data that his lab produced is really phenomenal.

And you know, it's a shame that we have to come here, set up as adversaries when we're all trying to get to the same point, which is to get solid science behind a conservation issue. And you know, both groups have done some very nice science, collected some exceptional data sets. And you know, there are -- there are always problems with these studies, always limitations with the sampling, both from the geographic sampling and the molecular sampling; and that's just, you know, part of their reality of doing this work.

DR. DUMBACHER: Great. Well, thank you very much. It's about 4 o'clock and we were thinking maybe doing one more thing, am I right?

DR. COURTNEY: We were thinking about not try to deal with issues raised by Doctors King and Ramey, but simply to ask them if there are issues they feel should be addressed. We will listen to that, decide whether we want to address them; and if we do,

it'll help us design how we're going to spend our time.

DR. DUMBACHER: Yeah, just say that we're going to have one day tomorrow, and we've got a lot to cover in the day tomorrow. Because a lot of things that we were hoping to touch on today and other people that we were hoping to talk with today, we're not going to have time to do that. And so what we'd like to try and do would be to have both PIs talk a little bit about which issues they think we should be asking about or focusing on that we haven't already focused on, and it's their opportunity for them to both tell us what they -- you know, other things that they think we might be missing or that might be germane to the issue that's in front of us.

And so what we'd like to do, just one at a time -- maybe we'll start with Dr. Ramey and then with Dr. King. And if you could just -- and we won't address those now and there won't be any opportunity right now for rebuttal or for questions, but we'll make note of all those for the record, and we'll try and deal with as many of those tomorrow as we can, if that's a fair way to do it.

DR. RAMEY: Thank you. I'll just work through a list of things, some small, some that are of larger significance. Actually it probably may not be a

bad idea to put up the -- wait, the figure representative -- I guess I talked to you about that.

DR. COURTNEY: Right, you don't --

DR. RAMEY: Good enough.

DR. STEPPAN: -- we're just looking for key points that need to be addressed.

DR. RAMEY: Actually, King's study does have a shared mitochondrial DNA haplotype frequency and in our work presently. And look at King's, look at table B-1 and you'll find that there's a shared campestris/intermedius haplotype that occurs outside of the range of their sampling. And let me go fetch that right now.

That's table B-1, sample No. KU-115730 from Walworth County, South Dakota. And I've already given you the plot of geographic distance versus genetic distance from microsatellite markers, and so I think this is just another example of how sampling design can influence the perception of discreteness of populations based on microsatellite data or mitochondrial DNA.

Another point I'd like to make along those lines and a 900-pound gorilla in the corner is the fact that none of us have yet talked about the difference between male and female dispersal rates and that influence on the discordance of mitochondrial DNA

versus microsatellite results. And so I have just talked to Tom Ryan in the back of the room who I had heard this before, I don't remember the source, that there's a difference in the dispersal of male versus female Zapus hudsonius in terms of distance and frequency. I mean, females are the higher investment sex, so they're probably going to be more phylopathic than males.

And I heard this before, but I think this is probably something worthwhile for the panel to look into because that also can explain some of the discordance between mitochondrial DNA and microsatellite data sets.

There's a number of inaccuracies in table B-1, I'll provide you with a list of these. For example, KU 112357597 is listed as haplotype CP-1, it really should be CP-3, KU 123592. You got all that? It's listed as CP-1, but it should be CP-3. Anyways, there's a number of these that I think are worthwhile to point out. King, et al., line 183, the author states that the control region of interest could not be amplified from these KU museum specimens with primers L15926 and H16498 as described in REA. However, we didn't use those primers that are listed. And the sequence for primer H16498 is actually different than we

used. We used 15320 and Zap 5P1R.

And the nested PCR used on some of our samples was using nested sequence and as described in our paper, but King apparently, it looks like, had used a different set of primers for this. So anyway, worthwhile just to look into that.

There's also a different primer sequence for H16498, one that we had modified from the Kuchler primer. And King, et al., used the actual Kuchler primer in that one.

So do mitochondrial DNA results rest on these, for example, shared haplotypes between campestris and preblei? No, they don't. I mean, total results. We looked at the multiple lines of evidence. So we reran the samples without our analysis, the AMOVA without the 7 samples in question shared mitochondrial DNA. It increased the AMOVA from .37 to .52. If we exclude all 13 samples, that increases it to .6 -- 66 percent, .66, between intermedius and campestris to 9.17 percent between intermedius and preblei to 46 percent.

We also reran the analysis without any of the nested samples, and I'll provide you with those results. Anyway, here's the basic point. That when we set out our critical test in our proposal for the

mitochondrial DNA, we used a threshold of where there's AMOVA greater than or less than .5 for reciprocal monophyly. Of course we set it up, we ran the test, so it would now pass the test as we set it up for just the mitochondrial DNA.

But the fact is we set up the whole sampling design so that first it was testing the original basis. Next it was using the molecular data and ecological exchangeability to ask whether there is a difference between these putative subspecies, and the mitochondrial DNA was just one data set in that. We used the majority rule for that. It would be the majority of data sets that show using our critical path differences. The microsatellite results don't change. The morphometric results don't change. And also the ecological exchangeability does not change as well.

So I think that a -- the larger issue, I think we'll tackle it tomorrow, is, you know, what thresholds are and discuss the appropriateness of those; so I won't go too far into that. Now, would we change some of these criteria? Certainly. And -- but, you know, the way we set up the design was to run the results through that and not change the, you know, the criteria as we went along.

But I think that's an important point to

make that, yes, these criteria can be debated, but the first step in making reasonable thresholds is to just state them explicitly and be consistent in their application.

So I'd like to also point out in the King, et al., paper, I do hear something, I'd very much like to hear King address this maybe tomorrow or later today, but a very major difference in these two studies is where that bar is -- where that threshold is. And so in looking at our data to King's, you can see that he used mitochondrial DNA analysis and microsatellite, but the bar there that is set is such that there is no hypotheses that is tested, it's that there's, essentially, that these are homogeneous entities.

And so as we -- as I showed you in one of my opening slides, the idea is that you want reasonable thresholds such that if it's set too high, you don't want things to go -- you know, you don't want the results ending in extinction in some populations. You set the bar too low, essentially any population might be considered to be a subspecies EPS or listable entity under the DNA.

In this particular case, I think it's reasonable to argue that these criteria are a level or sampling design that have a major effect, and also at a

level such that local populations would be recognized. So that, I think, is a very key difference despite many other alleged differences amongst the studies. Thanks so much.

DR. COURTNEY: I'm sure we're going to be talking about the last issue at great length tomorrow. So, Dr. King.

DR. KING: I guess I should start probably by addressing some of Dr. Ramey's concerns. The primer issue was identified in the manuscript -- the revised manuscript that was submitted to the Fish and Wildlife Service a week or so ago. I think it was provided to the panel.

MS. SZTUKOWSKI: Yes, we got it.

DR. KING: Revisions in the primer --

DR. DUMBACHER: If we could, though, I'd like to try and stick to larger issues and --

DR. KING: Yeah, I'm not sure what --

DR. COURTNEY: If there's small issues like this, we can just deal with those by email or even by passing.

DR. KING: Yeah, I'm not sure what changed about picking a niche, but I do want to say one thing, that, you know, I don't care whether a male preblei weighs 800 or 900 pounds, it's not going to move

60 to a hundred miles to exchange genes with a campestris. We're dealing with the sex-biased dispersal question.

All in all, I think -- I think that most of the issues that I had have been addressed. I am sure that tomorrow we'll get into the subspecies designation issues and whether or not the criteria that have been established previously deal with more a species level than at a subspecies level. And I think that's -- you know, that's an issue that we need to discuss tomorrow, but we're not going to resolve that tomorrow.

I think we all realize that that there's a fundamental division within the genetic community that some folks just don't believe subspecies should exist. Others set the criterion so high that some subspecies -- or some species concepts would declare those species rather than subspecies. So again, it'll be interesting to discuss these issues, but I don't know that we're going to generate anything more than heat probably, probably a lot more heat than light.

DR. COURTNEY: If I could just interject on that. You know, the panel have talked about the -- amongst themselves about the issues of -- you know, that there are different concepts out there; and it's not our job -- fundamentally is not our job to resolve what

anybody should apply in terms of should we apply phylogenetic species says concept, should we apply, you know, a very high bar or low bar.

It is our job to say these are the sorts of things that apply that other people have considered are relevant in this situation and to look at what the data -- how the data match up.

DR. DUMBACHER: Right. And what's been applied in other species, things like that, what definitions have been used.

DR. COURTNEY: So I'm hopeful that we're not generating, because once we recognized that that's the case and it's not our job, then we can move on.

DR. KING: And one other point of clarification, I was able to pull the data together and provide that to the Fish and Wildlife Service yesterday before leaving. And I don't know if they've had a chance to post that on their website or not, but that was submitted to them yesterday before I left.

DR. DUMBACHER: Okay. Well, one of the things that we're going to be trying to do tonight is we're going to be reanalyzing and playing with a lot of this data tonight and so if we could get that from you or them --

DR. COURTNEY: Well, we have it.

DR. DUMBACHER: We have the chromatograms.

DR. KING: You have the KU samples.

DR. DUMBACHER: Right. And likewise, we'll be trying to get some of this data from Hsiu-Ping too.

DR. COURTNEY: Okay. I hope you find this is intense, as I find it, up-front. And at the same time, I want to extend my appreciation for the fact that I think this meeting has gone well. It's difficult stuff. It's emotionally charged. And I hope that you recognize that, understand the difficulties that we're all going through; and yet we're doing this in, I think, a very professional manner. And I think you'll see that the panel are aware of these issues and they're really doing their best to keep this nice and clean and tidy, and I appreciate the fact the rest of you are too.

Here's what we're going to do. We are trying to get some more data tonight. We're going to reconvene at 8:30 tomorrow morning. We'll just have some opening materials and tell you about what we've done overnight. At 9 o'clock, Dr. Vignieri is going to call in from England. She's sitting at John Maynard Smith's desk, if you know who that is. And she's going to be calling in, and she'll just essentially raise

issues. She has sent the documents, which I've got on email, and so I haven't had time to print it out. Essentially reiterating a large number of the points that they've made up previously, but she'll be given basically the chance to make some points to the panel and the panel will be asked, you know, the questions they want to raise or even just ask some opinions about some of the things we've talked about today.

We're going to probably then move to a few other things and start talking about this -- what I think, obviously, is the big gorilla actually, which is what does the subspecies constitutes. You know, that's where the rubber hits the road on this issue. And at that point, we'll probably get ahold of Dr. Patton to bring in his opinions. So that's kind of our plan.

If any of you have issues -- and I keep getting notes and my mailbox is full, so if there are issues that you need to bring forward to me, this is an opportunity to do that. And you know, if you have my email, those of you who want to go away and think about things and email me things or email the SEI account, that's cool. Other than that, you know, it's an intense day. And we've got another one coming up. So I hope --

MS. SZTUKOWSKI: If your mailbox bounces, send it to Lisa at SEI.

DR. COURTNEY: I think they're talking about the telephone there. So that's it and reconvene at 8:30.

WHEREUPON, the within proceedings were adjourned at the approximate hour of 4:28 p.m. on the 6th day of July, 2006.

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REPORTER'S CERTIFICATE

STATE OF COLORADO)
) ss.
CITY AND COUNTY OF DENVER)

I, LYNNETTE L. COPENHAVER, Certified Shorthand Reporter and Notary Public, State of Colorado, do hereby certify that the said proceedings were taken in machine shorthand by me at the time and place aforesaid and was thereafter reduced to typewritten form; that the foregoing is a true transcript of the questions asked, testimony given, and proceedings had.

I further certify that I am not employed by, related to, nor of counsel for any of the parties herein, nor otherwise interested in the outcome of this litigation.

IN WITNESS WHEREOF, I have affixed my signature this 21st day of July, 2006.

My commission expires April 26, 2010.

_____ Reading and Signing was requested.

_____ Reading and Signing was waived.

__X__ Reading and Signing is not required.