
**ROB ROY RAMEY, II - NEW PERSPECTIVES ON THE EVOLUTIONARY ORIGINS,
HISTORIC PHYLOGEOGRAPHY, AND POPULATION STRUCTURE OF NORTH
AMERICAN MOUNTAIN SHEEP**

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New perspectives in ecology and evolutionary biology are almost always preceded by the development of new analytical tools. These open new avenues of investigation and can produce paradigm shifts as previously untestable hypotheses become the subject of scientific investigation. In the field of mountain sheep evolution, the first revolutionary perspective occurred in 1940 when Ian McTaggart Cowan published the morphometric analysis of horn and skull variation using the recently invented, hand-crank adding machine (Cowan 1940). Cowan's work was also one of the first to overcome typological species concepts, recognize the variation present within species, and apply tests of statistical significance to taxonomy.

More recently, the advent of modern morphometric analysis, and the analysis of DNA sequence variation, has allowed us to refine Cowan's work and help us understand the evolution of mountain sheep in much greater depth: from the fine scale breeding structure of isolated populations to the evolutionary history of species. The refinement will no doubt accelerate as newer methods of analysis are developed.

I present here, several new perspectives on the evolutionary origins of wild sheep, the historic phylogeography of North American sheep, and aspects of bighorn population structure in the Southwest. Understanding the evolutionary history of organisms is important to their conservation. For example, the ultimate explanation for the susceptibility of bighorn sheep to a wide variety of domestic livestock diseases lies in the fact that there is a vast gulf of evolutionary time separating North American bighorn from the diseases that

coevolved with domestic livestock that originated in Eurasia (Ramey 1995).

Evolutionary history and phylogeography are also the basis of modern taxonomic classification, which is important for recognizing biogeographic barriers to movements between populations, for determining the most genetically similar sources for reintroduction stock, and for determining the allocation of scarce conservation resources.

Finally, understanding the population genetic structure of mountain sheep underlies knowing which demographic units should be the units of conservation. Is the traditional view of sheep "populations" as those animals that inhabit a mountain range really an accurate reflection of their biology? How can we conserve genetic diversity over the long-term?

Evolutionary origins: The traditional view on the evolutionary origins of wild sheep (Schaller 1977) is that they originated during the Pliocene in the Middle East and spread east across Asia and into North America in what has been termed "the great arc of the wild sheep" (Clark 1978). However, preliminary phylogenetic analysis of mitochondrial DNA sequence data from the ND5 gene suggests a very different scenario (Figure 1): there is a major split within the genus *Ovis*. One clade includes the mouflon (*O. musimon*), urial (*O. orientalis*), and argali (*O. ammon*) of west/central Asia, while the other clade includes the snow sheep (*O. nivicola*) of Siberia, and Dall (*O. dalli*) and bighorn sheep (*O. canadensis*) of North America. This split is well-supported using bootstrap resampling of the data, and is consistent with sequence data from the Cytochrome B gene (Hassanin et al. 1998, E. Randi, unpublished data).

Dall and bighorn sheep appear to share a most recent common ancestor with snow sheep, rather than with argali.

Within Asia, the argali appear to have evolved recently relative to other species of *Ovis*. Also, the DNA sequence divergence across the range of argali is less than that found between desert bighorn and Rocky Mountain bighorn in North America (Figure 1). It appears that argali have recently evolved and radiated into the open steppe habitat of central Asia. The 17 subspecies of argali that have been previously described (Nadler et al. 1973) will be reduced in the future as molecular analyses provide new information.

Within North America, Dall sheep (*Ovis dalli*) are paraphyletic with respect to bighorn sheep for mitochondrial DNA (Figure 1). In other words, they do not form a monophyletic group separate from Rocky Mountain and desert sheep. Rather, it appears that bighorn sheep mtDNAs were derived from one of the Dall sheep mtDNA lineages found in northern Alaska. That bighorn sheep are derived from Dall sheep makes sense from a biogeographic perspective. However, the presence of a second mtDNA lineage in Dall sheep that is not found in bighorn sheep suggests that either there was a second colonization into North America, or that there was a single colonization with multiple divergent mtDNAs which were subsequently lost due to founder events during the colonization of ranges south of Alaska.

When did *Ovis* first colonize North America? The oldest *Ovis* fossil was found in alluvial deposits in El Golfo de Sonora by Chris Shaw of the Los Angeles County Museum, and is approximately 500,000 to 750,000 years old (Shaw 1981, 1990, C. Shaw pers. commun.). The next *Ovis* fossils in North America are from Manix Lake, Mojave Desert and dated at 280,000 ybp (Geist 1985, G. Jefferson, pers. commun.). If one is willing to invoke a mtDNA molecular clock, sequence divergence between North American and snow sheep would indicate separation occurred approximately 750,000 years ago (Ramey 1993), a result corroborated by fossil evidence.

Historic phylogeography and subspecies:

Within bighorn sheep, there is a lack of concordance between traditionally accepted taxonomic categories. For example, California sheep from the Sierra Nevada, (*Ovis canadensis californiana*), appear to share a most recent common ancestor with desert bighorn sheep rather than with members of the California/Rocky Mountain bighorn sheep clade. Also, the phylo-genetic analysis does not support the hypothesis of Geist (1971) that Rocky Mountain bighorn sheep are more recently derived relative to desert bighorn sheep. If Rocky Mountain bighorn sheep were recently derived, they would appear as a branch of the desert bighorn lineage.

Cowan's (1940) original taxonomic designations were made with substantial zones of intergradation among subspecies, however, over the years these have hardened into taxonomic "boundaries" and in some cases these lines have been redrawn without any data or analysis whatsoever (Wehausen and Ramey 1993). The transplant history of North American sheep has largely depended on the subspecies categories of Cowan (1940) and much uncritical guesswork. This has resulted in several long-distance translocations of essentially exotic animals, such as the translocation of Rocky Mountain bighorn from Banff, Alberta into northern New Mexico (Figure 2).

A large part of the problem with the application of the term "subspecies", is that it has lacked sound conceptual basis until very recently. Ball and Avise (1992) were the first to present a realistic solution to the subspecies problem by proposing that subspecies should represent a major subdivision in the gene pool diversity of species, as indicated by the concordant distributions of multiple, independent, genetically-based traits. Ryder (1986) introduced the term Evolutionarily Significant Units (ESUs) to aid in prioritizing the conservation of biodiversity because traditional species/subspecies categories were poorly defined. Moritz (1994a,b) subsequently added that ESU's must show patterns of reciprocal monophyly for mitochondrial DNA, a pattern which would be expected to occur within approximately 4Ne

generations of initial population separation. Reciprocal monophyly refers to the pattern of mtDNA haplotypes found in each population, representing an evolutionarily distinct lineage. More recently, John Wehausen and I added the criteria of morphometric distinguishability based on discriminant analysis (Wehausen and Ramey 2000). Using this morphometric criteria and reciprocal monophyly, we considered ESU's and subspecies to be equivalent. Overall, these conceptual refinements require that subspecies/ESUs have both a quantitative and evolutionary basis. By treating traditional subspecies categories as testable evolutionary hypotheses, the taxonomy of subspecies becomes the subject of scientific inquiry.

Since Cowan's work in 1940 was the basis of our management and conservation of North American sheep for nearly 60 years, I decided to reexamine his conclusions using a more sophisticated statistical analysis of his original raw data. This was possible because Cowan had saved his original raw data sheets in his attic, thinking they might be of use someday, and he made these freely available to me. I found that his results were influenced by small sample sizes, age-related effects on size, and violation of statistical assumptions (Ramey 1993). I found no support for most of his subspecific designations.

Subsequent to that study, John Wehausen and I examined Cowan's taxonomic conclusions with a more refined sampling design, larger sample sizes, modern methods of morphometric analysis, and the conceptual approaches outlined above. Our first paper (Wehausen and Ramey 1993) focused on testing the putative uniqueness of peninsular bighorn sheep (*O.c. cremnobates*) relative to desert bighorn sheep (*O.c. nelsoni*). We synonymized *cremnobates* with *nelsoni* because they were not morphometrically distinguishable and there were no convincing long term biogeographic barriers to suggest genetic isolation. Initially, this paper was not well received because it challenged the *status quo* and some long held beliefs.

That paper, however, was not the first to challenge the old taxonomy. Gonzales (1967) showed that *weemsi* and *cremnobates* were morphometrically indistinguishable, and Bradley and Baker (1967) found the same result for *mexicana* vs *nelsoni*, however, the authors of these papers used simple univariate methods and only suggested that these subspecies be synonymized.

I further tested subspecies categories and examined phylogeography in the Southwest using restriction site analysis of mtDNA (Ramey 1995). The evolutionary validity of subspecies was tested by asking if there was significantly greater mtDNA nucleotide diversity within or among putative subspecies. Based on that analysis and the lack of convincing biogeographic barriers separating putative subspecies, I concluded that all desert-dwelling bighorn sheep should be considered a single polytypic subspecies (*O. c. nelsoni*). This conclusion has also been corroborated by both microsatellite and MHC data (Gutierrez-Espeleta et al. in prep, Boyce et al. 1997).

One surprising result of the mtDNA restriction site study, was the discovery that California bighorn sheep from the Sierra Nevada (*O.c. californiana*) are genetically unique relative to populations of desert bighorn sheep in the mountains across the Owens Valley. The only plausible explanation for this genetic separation is the long term presence of Pleistocene pluvial lakes (e.g., Mono and Owens Lakes) and a riparian corridor in the bottom of the valley that acted as a barrier to dispersal.

In a similar study of mtDNA variation within the Rocky Mountains, Luikart (1992) found reduced levels of mtDNA variation in Alberta and British Columbia relative to populations in Montana. This result is consistent with the expected loss of genetic variation due to founder events as bighorn colonized previously glaciated habitat, in what is now Canada, at the opening of the Holocene, 12,000 years ago.

The next morphometric project that John Wehausen and I undertook was much more extensive because we examined the variation in the

northern regions of *O. canadensis* (Wehauseaen and Ramey 2000), including *nelsoni*, *canadensis*, *californiana*, and *auduboni*. In that study, we synonymized several subspecies and uncovered previously undescribed variation. *Auduboni* and the northern populations of *californiana* were synonymized with *canadensis*, and Sierra Nevada bighorn turned out to be unique relative to the northern populations of *californiana* and nearby populations of *nelsoni* from the Great Basin. These differences were apparent in skull shape and not simply skull size alone. Overall, these results were corroborated by mitochondrial DNA data from Ramey (1993), however no DNA samples were available for the extinct *auduboni*. Sierra Nevada bighorn, of which fewer than 100 remain, are the only remaining population of *californiana* and are now classified as endangered.

Morphometrically, there was substantial overlap between Rocky Mountain and California bighorn sheep in skull shape, making them indistinguishable using discriminant analysis. We suspect that the apparent size differences between Rocky Mountain bighorn and California bighorn may be because California bighorn in British Columbia have been living on river breaks and have a non-migratory life history. That means they are not growing as large because of differences in nutrition and not genetics. Our morphometric results were consistent with those obtained from analysis of mtDNA (Ramey 1993) and allozymes (Jessup and Ramey 1995) which show them to be virtually indistinguishable genetically.

When John Wehausen and I examined the variation in bighorn sheep within the larger context of variation that included Dall sheep, we found a north-south cline in the length of horn cores relative to horn volume. In other words, Dall sheep in the far north have substantially shorter horn cores than desert bighorn sheep in the hot deserts of the south, for the same horn volume. This pattern is even evident within the desert bighorn sheep from the cooler Great Basin south into the hot deserts. Because horn cores are vascularized and therefore potential sources of heat loss, there appears to be a selective constraint on horn core length in the

more northern ranges. The northern sheep have their horns on much shorter horn cores that lose less heat than the long horn cores found in the desert regions (Wehausen and Ramey 1993, 2000).

There is a similar north-south cline in lambing period described by Bunnell (1982). In that paper he describes a selective constraint on lambing periods in the northern ranges, starting at approximately 37 degrees north. In the northern ranges, there is a narrow optimal window for lambs to be born in the late spring. If lambs are born too early, they are at risk for perishing in late season storms. If they are born too late in the summer, they will not have grown large enough to survive the coming winter. In the hot deserts of the south there is no such selective constraint because winters are mild.

Population structure: Traditionally, wildlife researchers and managers have considered a "population" of bighorn sheep to be those animals that occupy a mountain range. This view has changed over time, however, as metapopulation theory and a long-term conservation perspective have been applied to bighorn sheep (Schwartz et al. 1986, Bleich et al. 1990). Observations of philopatry by ewes and long-distance dispersal by rams, along with analysis of genetic data (Ough and deVos 1984, Bleich et al. 1996, Ramey 1995, Boyce et al. 1999) reveal a clearer picture of metapopulation structure in bighorn sheep. It now appears that bighorn sheep in the Southwest can be viewed at three different scales: female subpopulations, populations, and metapopulations (Figure 3).

The value that this new perspective brings to conservation and management is that: 1) female subpopulations, which appear to be stable over time, are the most basic demographic units that should be identified and monitored in the wild, and 2) for long term conservation of genetic variation and population persistence, movement corridors must be maintained between female subpopulations that provide an opportunity for male-mediated gene flow, and the potential for recolonization of female subpopulations. Due to

the rapidly spreading urbanization across the desert valleys of southern California, Nevada, and Arizona, identifying and prioritizing the conservation of movement corridors between female bighorn subpopulations is urgently needed.

The future: New non-invasive sampling techniques that use fecal DNA, rather than the physical capture of animals, will become increasingly important for understanding the fine scale breeding structure of small populations of bighorn, and will contribute greatly to our understanding of phylogeography, especially for endangered populations (Taberlet et al. 1996, 1997). Additionally, "neutrality tests" for genetic data will be used to infer aspects of a population's demographic history and/or intensity of natural selection (Rand 1996, Cornuet and Luikart, 1996, Ramey et al. in press). These new analytical methods, that are based on neutral models of molecular evolution, can be used to test for genetic signatures of population bottlenecks, rapid population expansion, and natural selection (Luikart et al., 1998a,b; Rand, 1996). Like other advances in methodology that I have discussed earlier, we can expect these methods to yield new insights into the evolution, phylogeography, and population structure of mountain sheep.

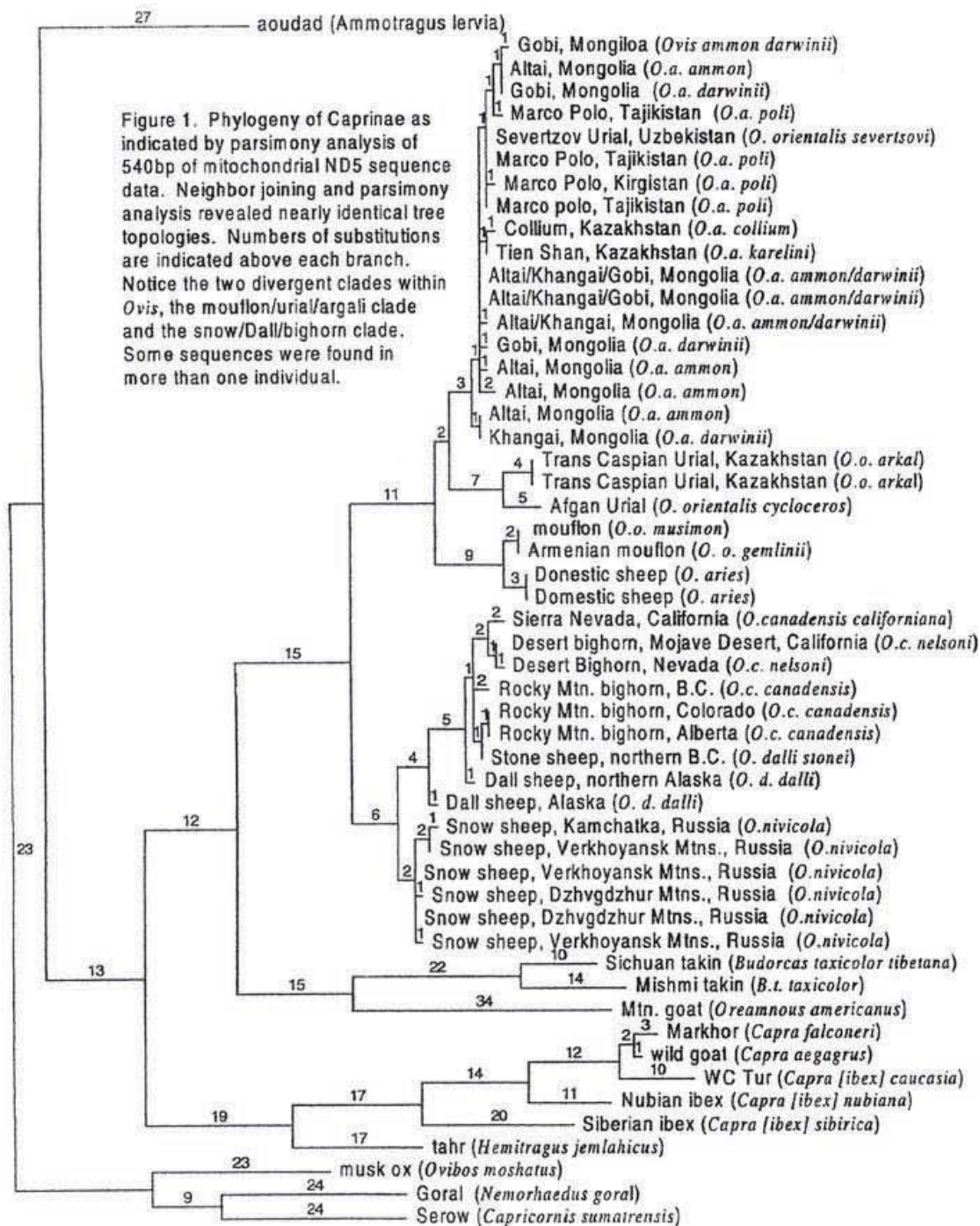
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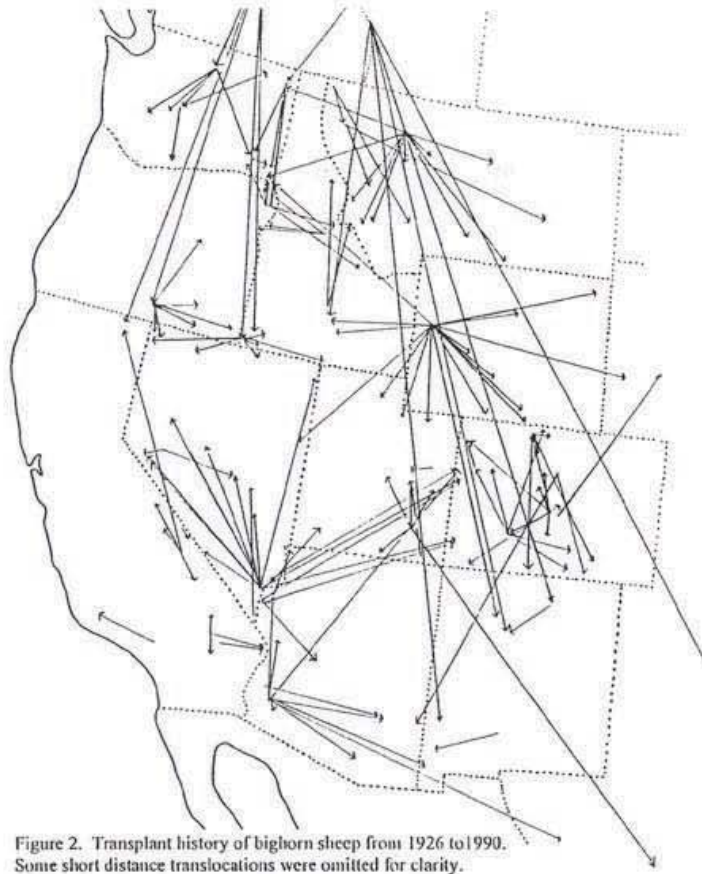


Figure 2. Transplant history of bighorn sheep from 1926 to 1990. Some short distance translocations were omitted for clarity.

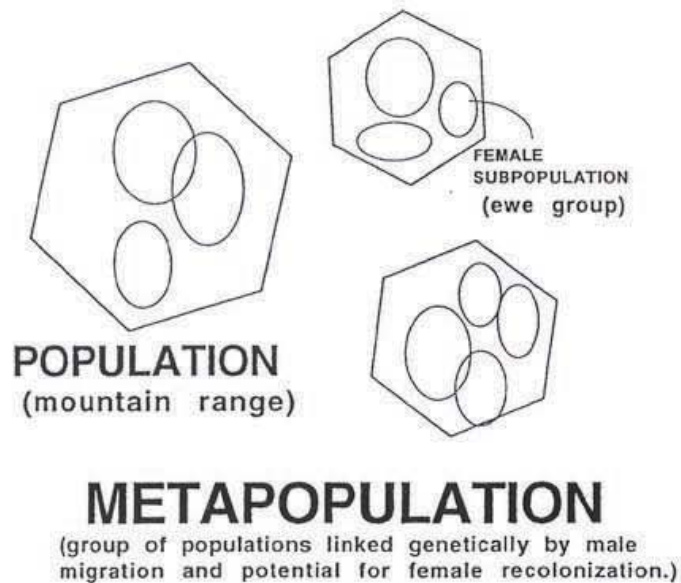


Figure 3. The metapopulation model of bighorn sheep population structure.

QUESTIONS, ANSWERS AND COMMENTS - ROB RAMEY PRESENTATION

STACEY OSTERMANN, CALIFORNIA: How would you describe the size of the difference that you found between Sierra Nevada sheep and other California sheep compared to the differences you found between peninsula sheep and nearby *nelsoni*.

ROB RAMEY: In the mitochondrial control region there were 27 base pair differences between those supposed California sheep from British Columbia and Sierra Nevada sheep. The difference between Sierra Nevada sheep and those in the desert to the east, was on the order of between 12 to 15 mutational steps. That's actually slightly higher than we found between, for example, those in the peninsula ranges and those in the Mojave Desert. There's quite a bit of difference there. I think the largest mutational step we have is about 12 mutations out of the 1200.

Once again, Sierra sheep appear to be on separate lineage, they're different morphometrically. Morphometrics is a combination of both genes and environment. There are shape differences that are not related to size.

I'm going to venture to guess that that's reasonable evidence for understanding their nuclear differences. We haven't gotten that data just yet.

RAY DEMARCHI, BRITISH COLUMBIA: I came to the same conclusion about Seton's estimates as you have, but from a different direction, and I actually gave a paper back, I don't know, probably 20, 25 years ago. I figure that Seton was about ten times too high in all of his estimates. He estimated between 16 and 20 million barren ground caribou. His estimates of bison were way out to lunch, and certainly his North American bighorn sheep estimates were not substantiated.

I came at it from a different direction from you and it's interesting to see that your genetic work supports the estimates. My estimates were based on habitat. My brother Dennis was at that workshop 25 years ago, and he's with me today. We're doing a lot of work on habitat, habitat mapping and so on, and we've got some stuff and hopefully somewhere along the way we can fit it in. It's interesting stuff.

RAMEY: You should write a paper about it.

DEMARCHI: I read the one and a half million to two million figure and know that people are still using it. I saw it the other day in a publication in which one esteemed sheep biologist quoting there were one and a half to two million bighorn sheep in North America. I don't believe it. It's closer to 150,000 to 200,000, maximum. All you have to do is look at Dall sheep and Stone sheep where the habitat hasn't changed. The numbers haven't changed compared to the habitat. We just didn't have it.

RAMEY: Those are just guesses as to how many animals Seton thought there could have been. These old traditions die hard. Similarly, the problem with sheep taxonomy. People are using subspecies names that have essentially died and gone by the wayside. Instead of adopting a new evolutionary perspective and more mature understanding of population structure, they tend to hold on to those old names and such. And I think it's much to the detriment of the sheep overall that those stay. Not to say all these are right, but this is what the data supports right now, and my interpretation of it.

DEMARCHI: The same applies to the numbers. I agree with you totally on that. If you have the idea that there were one and a half to two million bighorn sheep in North America and you're trying to recover the species, you're never going to get there. If it's 150,000 to 200,000, a more reasonable number, maybe you could get there and it's a more achievable number.

RAMEY: Yes.

DEMARCHI: Same thing as using outdated taxonomic classifications and so on. This frustrates you when you're trying to do a conservation plan.

RAMEY: I think John Wehausen looked at the demographics of trying to get 10,000 to 20,000 sheep in the desert area in Southern California, and what rate of increase we would need to achieve that goal and it was totally unrealistic.

JIM BAILEY, NEW MEXICO: I wonder if you'd comment on the accepted practice of using California bighorn for transplants into what was supposedly *auduboni* habitat, why California bighorn?

RAMEY: Presumably, the reason people have done that is these animals live on river breaks. They're going to put a similar kind of animal that has a similar kind of habitat memory out into those river breaks. I think we really need to ask a hard question, what is our goal in trying to restock historic ranges? Is it to put back what was originally there?

In terms of genetics, are we trying to use the most closely related thing that was originally there, trying to reconstruct the animal's evolutionary history as best we can. Or is it to provide hunting opportunities, bringing in essentially exotic animals from distant locations to provide an opportunity for revenue and support?

Those are potentially conflicting goals. My personal ethic, and this is a personal one, is that we should try to put back what was originally there first and that should be our major emphasis, and the conservation effort should go to the local populations rather than bringing something from a very distant location. I think bringing the animals from a very distant location flies in the face of what we know of sheep evolution.

It might be out of convenience that these sort of things happen, too. That's certainly true of a lot of the early transplants.

WAYNE HEIMER, ALASKA: Playing off what Jim Bailey had to say, the question that occurs to me is, you mentioned, I think an ND5 gene; do we know what it does? You talk about cytochrome B variability in a base, and you look at mitochondrial DNA. Is there any measureable, demonstrable significance in the variability that we see? If there isn't, does it really matter? If there is, restocking becomes a philosophical choice.

RAMEY: For understanding evolutionary history of groups, you're better off looking at neutral molecular markers. It's true that the ND5 cytochrome genes have a high degree of function and some of the differences that we see amongst the groups can be the result of selection.

So the more modern methods of analysis use information on trying to determine which base pairs are under selective constraint versus which are neutral.

When you look at microsatellite data, which largely happens to be neutral, they are good indicators of gene flow amongst populations and of understanding the evolutionary history of groups, independent of selection.

So that's why we are trying to look at those presumably neutral markers. They give us a good estimate and we don't know where the selection is occurring. We can determine that at some particular bases in a sequence, but overall we prefer these neutral markers to try to understand the genealogy of these animals, and my interest in working with morphometrics is to try to understand where there's been some sort of selective constraints on some inheritable feature of the organism.

HEIMER: As a manager, I hope you can understand why it might be difficult for some of us who sort of think that adaptive significance is what determines speciation, and to have trouble following why things that have nothing discernible to do with adaptation would be a better mark than something which determines whether you survive or not.

RAMEY: You've highlighted a difference between the schools of selectionists thinking. That's what drives speciation, realizing that there's a lot of selection which can drive adaptation, but a lot of the differentiation we see is the result of random stochastic changes and in gene frequencies in evolution over time.

I'd say we can learn a lot about the pattern of evolution by looking at the neutral markers and that it is very interesting when we find nonneutral evolution that has gone on. And we can go in and test in the sequence data bases and see whether there's some sort of adaptation going on.

To try to strictly base all of your taxonomic classification on sort of a selectionist view I think really ignores most of the development and evolutionary thought over the last century that, yes, adaptation has certainly occurred, but, animals came into North America and they split on the different evolutionary pathways, perhaps by chance, and there's been some local adaptation following so it's not as if selection has driven the change all the way along.

BILL DUNN, NEW MEXICO: I was looking at your map. You've got the north as north and south as south and neither the twain shall meet. How do you reconcile a subspeciation in large mammals that we know are linked by mountain ranges that are within distances we know bighorn sheep can move? New Mexico is not quite as simple as that. We've got mountain ranges that are linked from southern New Mexico to Colorado, from Wyoming on to Banff. How do you reconcile clearly stating subspeciation on a large mammal capable of moving between all the mountains?

RAMEY: I think of subspecies as having these major fuzzy differentiations, not even boundaries between the subspecies. I don't believe in this notation of subspecies boundaries for the most part.

I think what you're trying to get at is there probably was some conductivity between the southern Rocky Mountains through New Mexico sometime in the past. I'll agree, I think that's highly likely, and that if we went back in sequence, both nuclear genes and mitochondrial for across that area, we'll find some gradation in the nuclear gene of those animals.

Our morphometric data suggests that those northern New Mexico to southern New Mexico sheep are more aligned from the desert than they are from the Rocky Mountains. I think it's a reasonable working hypothesis. There's gradation that occurs. There's no major separation. There's no line between desert and Rocky Mountain sheep in that area.

PHIL HEDRICK, ARIZONA: I'm going to answer Heimer's question. These neutral markers give you an idea for the potential of adaptive differences between groups, and you may have them or you may not. You don't know how closely these neutral markers trace adaptive differences, but if there are no differences in neutral markers, you suggest there may be little difference in adaptive differences, although that's not true in all cases either.

I was going to say that the other system that we're looking at besides the neutral markers are genes, a major histocompatibility process which is involved with pathogen resistance in humans and other organisms.

Our idea is to look at different populations for variation of these genes, which we think has an adaptive significance and see if we can correlate that with resistance or susceptibility to different diseases.

There's a gene that we're looking at specifically to determine if there are adaptive differences, and what we found so far is a lot of variation and difference, say, desert bighorn groups have different variations. I'm not going to say that it's similar to our microsatellite data, but it seems to be following the same pattern of how different California populations and Arizona populations are differentiated.

I think you potentially can look at genes that you know have some adaptive significance. I think that's one thing we can go to in the future.

RAMEY: That's the candidate gene approach. If you know some physiological trait in the animals that you think is under selection, there is enough understanding of what genes control what characteristic, we can go to a particular gene and ask the question, is this under selection for this particular group of animals?

DUNN: Rob, did you find any variation in the Rocky Mountain sheep that you sampled throughout the range?

RAMEY: Yes. In fact, the best work is Luikart's thesis from Montana that for mitochondrial DNA, he found fairly substantial differences among groups, north/south in the Rocky Mountains. And that's just for mitochondrial DNA. The only other data sets are fragmentary and not enough to make a major conclusion. John Wehausen and I found some differentiation between Colorado and New Mexico and southern and northern Alberta. There's variation out there. It seems to be mostly, as you would expect, sort of isolation by distance. I think the way you think of subspecies is groups flowing across the landscape and integrating in areas.