

Goals

Our long-term goal is to understand and predict the spatial and temporal dynamics of chronic wasting disease. This goal includes three specific aims:

Aim 1: Describe mechanisms of CWD transmission between infected and susceptible individuals and determine if environmental sources of infectious prions (PrP^{CWD}) can contribute to disease transmission.

Aim 2: Describe spatial and temporal variation in disease prevalence.

Aim 3: Select the best approximating models of disease dynamics and use these models to investigate anthropogenic effects of habitat compression and fragmentation resulting from sustained changes in human land-use.

To achieve these aims, we are conducting laboratory, field, and modeling studies. Laboratory studies will reveal mechanisms of transmission and will provide a basis for formulating models. Field studies will assemble data for modeling spatial and temporal variation in the disease.

Here we report activities and findings relevant to each aim. We then describe opportunities for research and training offered by our project. We close with a section describing our investment in outreach activities.

Activities Relevant to Aim 1: Describe Mechanisms of Transmission

Investigations of Shedding of PrP^{res} and Mechanisms of Lateral Transmission

During summer 2001, 14 mule deer fawns were obtained as neonates from outside the CWD endemic area and hand raised in isolation facilities for use as “donor” ($n = 7$) and “recipient” ($n = 7$) animals for planned transmission studies. Another 10 mule deer and 2 white-tailed deer fawns from presumably unexposed herds were raised similarly during summer 2002 for use as recipients ($n = 5+2$) and controls ($n = 5$). Based on the original study plan, a third group of fawns ($n=10$; 7 mule deer and 3 white-tailed deer) were raised in 2003 for use as additional recipients ($n=4$; mule deer) and controls. Blood samples collected from these deer were used to determine their PrP genotype (see below for details of the techniques used).

In September 2001, seven of the 2001 cohort fawns were inoculated orally with 1 gram of CWD mule deer brain pool of known infectivity and housed at the Wyoming Game and Fish Department’s Sybille Wildlife Research Unit (Sybille). During April 2002, these donor fawns were immobilized and blood, saliva, feces, and urine were collected. Specimens were banked for use in assays for PrP and infectivity. In October 2002, three additional mule deer fawns were inoculated orally with 1 gram of the same CWD mule deer brain pool to bring the total number of “donor” deer to the proposed number of 10. All donor deer were sampled in October 2002, February 2003, October 2003, and May 2004 for blood, saliva, feces, and urine. Additional feces from donor deer were collected intermittently from February 2002 through May 2004.

Tonsil biopsies were collected from mule deer donors and control deer in February 2003 using techniques developed in association with this project (Wolfe et al. 2002). Disease associated PrP was detected in tonsils of all of the donor deer, but none of the control animals. From February through May 2004, seven deer of the donor group died and had evidence of CWD infection. Three deer remain alive, although they are in poor condition and we anticipate they will succumb to CWD within the next six months. Of the original cohort of seven deer, the only two animals that remain alive are heterozygotes at codon 225 as compared to the much more common homozygous genotype found in the deer that have died. The importance of this possible increase in incubation period in heterozygotes is not yet known (see below for a discussion of mule deer genetics).

In order to determine if CWD is transmitted by feces via the presumed natural route of oral exposure, recipient mule deer fawns are being exposed to feces collected from the orally inoculated donor deer housed at Sybille (beginning with feces collected in February 2002). Recipient deer are housed in isolation outdoor facilities at the University of Wyoming (UW) facility in Laramie; controls are housed in outdoor isolation at the UW's Red Buttes facility south of Laramie. The 2001 recipient deer were exposed over eight separate periods between June 2002 and January 2003 to feces (about 35 g/period) collected from donor deer 7-12 months after inoculation (PI). The 2002 recipient deer were exposed over twelve separate periods between November 2002 and January 2004 to feces (about 60 g/period) collected from donor deer 13-24 months PI. The 2003 recipient deer have been exposed over three separate periods between February 2004 and May 2004 to feces (about 45 g/period) collected from donor deer 12-18 months PI. Tonsil biopsy PrP positive deer have been found in the first two fecal recipient groups; the third group is still under study.

Transgenic mice expressing mule deer PrP are still under development at several institutions with which we have collaborative arrangements. Two of our collaborators at different institutions report that they now have test mice inoculated with CWD material and are in the process of validating this transgenic system. When adequate numbers of appropriately validated mice are available to use for bioassay, we hope to inoculate them with materials collected from inoculated mule deer. Results will be compared to data collected from direct deer challenges to assess the utility of such animal models for further study of CWD transmission.

Development of techniques for testing excreta from animals with CWD is ongoing. Currently assays used to detect disease associated PrP in tissues do not appear sensitive enough for use in excreta. We are evaluating modifications of western immunoblotting techniques with the goal of enhancing assay sensitivity. To that end frozen feces collected at necropsy of four elk with clinical signs of CWD were loosely pulverized and extracted with slow mixing on a rocker platform overnight at room temperature in Tris-NaCl buffer containing 0.5% NP40, 0.5% sodium deoxycholate, and 50 mM N-acetyl glucosamine. The extract was separated from residual solid material by centrifugation and aliquots of the supernatant treated with proteinase K, concentrated by trichloroacetic acid

precipitation and fractionated on a polyacrylamide gel. Western immunoblot detection did not reveal any PrP-specific bands.

Similar trials were made with deer as well as elk feces, replacing the non-ionic detergent NP-40/sodium deoxycholate combination in the first buffer with the detergent sodium sarkosyl which has the effect of aggregating PrP^{CWD} extracted from tissues, thus aiding in isolation of the disease associated isoform of the protein. Two additional protein precipitation techniques that have been reported to successfully concentrate PrP from tissues were tried as well—one utilizing phosphotungstic acid, another using methanol and chloroform. No PrP^{CWD} was detected in extracts from feces by any of these procedures. Samples were also assayed by a TSE-specific ELISA (Bio-Rad, CWDTsE Assay) routinely used for CWD tissue assays, with results not distinguishable from negative controls. At this time we have not detected CWD prion protein in feces of clinically ill cervids by western immunoblots or by ELISA.

Because our western blotting/detection results for PrP detection in tissue homogenates have improved markedly, we anticipate making additional trials to detect CWD prions in feces. We currently use the preformed NuPAGE Novex Bis-Tris gels in 4-12% polyacrylamide with MOPS buffer and blotting onto PVDF membranes in Tris-glycine-10% methanol transfer buffer, much as reported previously. Immunodetection by enhanced chemiluminescence utilizing F(ab₂)' secondary antibodies, either conjugated to horseradish peroxidase (HRP) or to alkaline phosphatase (AP), or a biotinylated secondary antibody and AP-conjugated streptavidin employed at much greater dilutions (1:50,000) than previously used, and with adjustments in the membrane washing regime, allows lengthy exposures (up to 18 hours) for greater sensitivity without unacceptable levels of non-specific background.

Investigations of Environmental Transmission

We completed an experiment comparing the relative contributions of live animals, contaminated environments, and infected carcasses to transmission of chronic wasting disease. Results were described in an article by Miller et al. published in June 2004 in *Emerging Infectious Diseases*.

Activities Relevant to Aim 2: Describe Variation in Prevalence

Sampling Infected Populations

Surveillance for CWD continued in Wyoming during 2003, with expanded state-wide collection of samples from hunter-harvested cervids, resulting in approximately 6,000 mule deer, white-tailed deer, and elk tested. These surveillance activities were led by the Wyoming Game and Fish Department, but we collaborated by confirming all positive ELISA tests with the “gold standard” immunohistochemistry. This collaboration allowed us to obtain CWD negative and positive samples that were used to evaluate genetic variability of mule deer across a wide geographic expanse. Prevalence of CWD in some Wyoming hunt areas has increased and a few new hunt areas, including several to the

north and west of the known CWD endemic areas, had CWD positive animals. For the first time CWD was detected west of the continental divide in Wyoming. Maps of locations of samples collected during CWD surveillance were done.

The Colorado Division of Wildlife sampled over 14,000 deer and elk harvested or culled in northeastern Colorado and other select locations. Prevalence data from these samples will be available to augment the existing database that provides the foundation for our analysis and modeling of temporal and spatial aspects of CWD epidemiology. This year's data will be useful in further exploration of local patterns of disease prevalence related to deer densities and land use patterns.

We also explored the utility of an alternative surveillance strategy for detecting CWD foci via sampling deer killed in vehicle collisions. Since 1996, tissue samples have been collected from deer (*Odocoileus* spp.) killed in vehicle collisions throughout Colorado as part of a monitoring program for detecting chronic wasting disease (CWD) in free-ranging populations. We estimated CWD prevalence among vehicle-killed mule deer (*O. hemionus*) statewide and compared this to estimated CWD prevalence among the surrounding mule deer population to determine if CWD-infected individuals were more vulnerable to vehicle collisions.

Development and Application of Techniques for Sampling Live Animals

In order to study CWD across landscapes where hunting and culling are not feasible sources of diagnostic samples, we have continued developing and evaluating techniques for sampling live animals. During the first two years of this project, we conducted a field study to evaluate tonsil biopsy immunohistochemistry (IHC) as a tool for diagnosing CWD in live, free-ranging mule deer (*Odocoileus hemionus*) and estimating prevalence. A small number of white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) have also been tested. Upon successful completion of our initial field study to refine and evaluate tonsil biopsy as a field tool for CWD work, we applied these techniques to gather data for new studies related to effects of land use patterns on CWD prevalence and its management, as described in the following sections.

We have also begun evaluating other candidate tests for diagnosing CWD in live animals, using tonsil biopsy IHC as a reference test. Unfortunately, none of the tests evaluated to date have shown sufficient reliability to replace our original approach.

Influence of Mule Deer Genotype on CWD Distribution

The primary objective of this activity has been to search the DNA sequence of the PrP encoding region in exon 3 of the *Prnp* gene of mule deer for genetic variation that might correlate with presence or absence of naturally acquired CWD. We analyzed the DNA sequence of the PrP coding region from 1,715 free-ranging mule deer including 1,186 from the CWD endemic area of Wyoming and 529 from four locations in Colorado. For 363 deer the complete PrP coding region sequence was determined; in the remaining 1,352 animals the genotype was assayed only for the presence or absence of the variant form of the gene encoding phenylalanine for residue 225. Development of the ability to

assay specifically for the residue at 225 greatly expanded our ability to screen large numbers of animals by decreasing costs and increasing speed.

Free-ranging mule deer from Wyoming and Colorado were genotyped: 1,156 from 13 contiguous herd units in southeastern Wyoming, 529 from four separated data analysis units in Colorado, and the balance (30) from other Wyoming herd units. Deer from Wyoming were taken during the Wyoming Game and Fish Department's 2001, 2002, and 2003 hunter surveys; we used frozen brain tissue (2001 and 2002), or homogenates of retropharyngeal lymph node (2003). Whole blood samples were obtained from live mule deer captured and released in Colorado during 2002-04 in the course of various Colorado Division of Wildlife research programs. A total of 326 of these were from the Estes Park herd (DAU10); 63 from DAU 18, 71 from DAU 9, and 69 from DAU 7. Information on the CWD status of all samples was obtained from Wyoming Game and Fish Department or Colorado Division of Wildlife with the exception of the last three DAU groups listed.

Total genomic DNA was extracted from each sample by various procedures appropriate to the tissue and the PrP coding region from each deer genome was amplified by polymerase chain reaction (PCR). Sequencing of the PCR amplified DNA was done commercially; sequencing results were received electronically and analyzed using Chromas v. 2.23 (Technelysium, AU) and the SeqMan module of Lasergene from DNASTar (Madison, Wisconsin).

In addition we genotyped 1,352 mule deer using a simple restriction enzyme digestion of the PCR amplified PrP gene. The polymorphism of interest in mule deer PrP, S225→F225 can be screened for by virtue of an EcoRI site that occurs when the TCC serine codon is replaced by a TTC phenylalanine codon. Because the preceding codon 224 in PrP is GAA glutamate, GAATCC becomes GAATTC, the recognition and cleavage site of the restriction endonuclease EcoRI. Results of the EcoRI assay can be viewed by standard DNA gel electrophoresis procedures common in most labs. Thus the survey of large numbers of animals became feasible using a faster, less expensive procedure to assay the presence or absence of a phenylalanine codon at 225 directly from PCR amplified DNA without sequencing. EcoRI genotyping produces cleavage fragments of known lengths, and a search of the PrP open reading frame DNA sequence verified that no other sites were potentially transformable to GAATTC with one base change, both of which observations support the use of this as a valid genotyping method.

Local Scale Study: Effects of Land Use on Prevalence

We have completed our current research aimed at understanding the effect of land use change on CWD prevalence in free-ranging mule deer (Farnsworth et al. 2004). For details on this research, refer to the 2003 annual report.

Landscape Scale Study: Spatial Heterogeneity of CWD Prevalence

To develop a dynamic, spatially explicit model of CWD requires an understanding of how features of the landscape contribute to the observed spatial heterogeneity in disease prevalence. Certain landscape attributes, such as private land where hunting is limited or forbidden, may

serve as “basins of attraction” for CWD, concentrating and exacerbating its effects over relatively small spatial scales. Conversely, other landscape characteristics, such as large gaps in habitat, may act as barriers to transmission, effectively shielding a portion of the population from the disease, allowing some deer to remain relatively free from infection in both time and space. The overall goal of this component of our research is to understand how biotic and abiotic features act to structure the heterogeneity in CWD prevalence that we have observed across Northern Colorado’s landscape. In turn, this should allow us to develop meaningful predictions about expected changes in the spatial and temporal transmission dynamics of CWD across the landscape. We foresee immediate application of this work in helping wildlife managers prioritize areas for control and surveillance.

During the past year, we continued and expanded our work using Bayesian hierarchical models that incorporate the spatial structure of the disease as a Markov Random Field (MRF) (Besag 1974, Künsch 1986, Besag et al. 1991, Cressie 1993, Bernardinelli et al. 1997). The MRF, which can be thought of as a grid imposed on the data to add structure, allows for the inclusion of spatial dependency in the model by assuming that prevalence of the disease in each grid-cell is determined, at least in part, by the spatial structure of CWD in surrounding grid cells, known as a neighborhood. Thus, the MRF is represented by a grid structure laid over a map indicating the locations of infected and uninfected deer.

In our latest efforts, we have formulated our landscape model using a hierarchical structure that models CWD prevalence across three levels of spatial resolution; individual point locations where deer were harvested, a single grid-cell containing multiple deer (point locations), and a second-order neighborhood (i.e., eight nearest grid cells) component that captures the spatial dependency in prevalence among neighboring cells. At the first level in the hierarchy, the probability that an individual deer is infected with CWD is modeled as a combination of individual level effects, such as age and sex, and cell level effects, such as the proportion of private land and harvest pressure. Then, at the second level, prevalence within a grid cell is considered as arising from a cell-specific random effect that treats all deer harvested from within a grid cell as exchangeable. At the third level of the hierarchy, we use the MRF construction to model spatially structured dependencies that we observe in the disease among collections of neighboring grid cells.

We chose to structure the spatial component of the model as a MRF based on our current understanding of mule deer biology, CWD aetiology, and the accuracy of the data collected. As noted above, the spatial data on harvested deer are based on mapped point locations where deer were estimated to have been killed. These point locations are mapped by the hunters themselves. We know that these data are prone to location errors both due to the fact that deer move, and are thus unrealistically represented by a single point in space, as well as mapping errors introduced by the hunters. Because the MRF formulation is grid-based, we are able to aggregate individual deer samples into collections of deer at biologically relevant scales, thereby reducing location uncertainty while retaining biological realism. We have observed positive spatial autocorrelation in prevalence rates across the study area, which is precisely what one expects from a disease that is transmissible either via environmental or animal-to-animal pathways. The MRF is designed to capture the component of variability that is due to the spatially correlated nature of the sample. Specifically, the spatial component of the Bayesian model is cast in terms of a Conditional Autoregressive (CAR) framework (Besag et al. 1991, Besag and Kooperberg 1995, Mollie 1996), where the infection rate of the disease within a grid-cell is explicitly modeled as a function of the infection rates within the local neighborhood. Posterior distributions of all modeled parameters are being estimated using Markov chain Monte Carlo (Geman and Geman 1984).

The Bayesian modeling framework we have chosen provides several advantages over frequentist approaches. Using this approach, we are able to explicitly incorporate current knowledge about the biology and epidemiology of the system through the specification of prior distributions reflecting our understanding of the range of variability and functional form of model parameters, something that is impossible within a frequentist framework. In addition, posterior model predictions are used as prior information to update the model as new data become available, resulting in a continuous dialogue between data and the model. Finally, using a hierarchically structured model allows us to “borrow strength” across levels in the hierarchy when modeling disease occurrence, thereby incorporating the uncertainty from all levels in the hierarchy into our estimates of CWD prevalence.

Given our poor understanding of the dispersal range of this disease, i.e., the geographical range of influence exerted by disease cases on healthy individuals, our approach must necessarily model the spatial structure of CWD at several biologically relevant scales. One scale is the estimated home range size of wintering individuals. Based on a study of radio-collared deer that were tracked for five years, the estimated home range size of 9 km² (Conner and Miller 2004) is the finest scale of disease influence we have examined. A multi-scaled approach will allow us to determine which scale possesses the greatest likelihood for explaining the spatial dynamics of CWD. That is, by comparing the model’s predictions to the observed data we will be able to determine quantitatively which scale produces the best fitting model, and infer from this the scale of management activities that should be most effective in reducing the spread of CWD. This determination will potentially provide insights not only into the dispersal capabilities of infected individuals but also into the most likely mechanisms of long-range transmission of the disease (e.g., individual versus herd migration patterns).

We concluded that a Bayesian hierarchical model incorporating an autoregressive component is a natural approach to this problem given the ability of the model to simultaneously capture the spatial structure of this disease’s aetiology at biologically relevant spatial scales and model the contributions made by landscape features in shaping the observed spatial distribution of CWD. The model remains sufficiently flexible to be easily applied to our rapidly growing data set of spatial locations of infected and uninfected individuals.

Evaluation of an Urban CWD Management Strategy

We continued assisting the Colorado Division of Wildlife in a study to evaluate a “test and cull” strategy for managing CWD in urban habitats. Models exploring probable consequences of various management strategies identified selective removal of infected individuals as a potentially effective method for reducing CWD prevalence in mule deer populations, provided that infected deer were detected early and a large (>50%) proportion of the population could be sampled annually (Gross and Miller 2001). During winter–spring 2002–2004, we conducted a pilot field study to evaluate the feasibility of “test and cull” for managing CWD in urban mule deer populations. Based on initial success, we extended this work to examine the efficacy of this approach; our evaluation will continue over the next 5 years. In addition to the primary goal of assessing efficacy, data gathered in the course of this study also will be useful in improving our understanding and modeling of the influences of urban landscapes on CWD epidemiology.

Selective Predation by Mountain Lions on CWD-Infected Mule Deer

In addition to direct effects on the habitats used by the natural cervid hosts of CWD, urbanization could affect the ecology of systems where CWD may be introduced or has become established. Because land use patterns may alter the abundance and activity of large predators (e.g., mountain lions), we have been studying the potential role of selective predation in CWD ecology. Our specific objectives are to:

1. test for evidence of selective predation by mountain lions on CWD-infected mule deer;
2. collect data to help assess the broader ecological question of whether mountain lions selectively prey on debilitated or compromised animals rather than healthy ones;
3. continue refining and assessing the adequacy of field sampling techniques for studying selective predation on CWD-infected mule deer; and
4. evaluate and compare the performance of Lotek Wireless GPS4000 and GPS4400 collars and Tevevilt GPS-Simplex collars in a study of selective predation by mountain lions under field conditions.

To test for evidence of selective predation, we will compare prevalence of CWD among puma-killed mule deer to prevalence among mule deer harvested or randomly culled by humans within home ranges of collared mountain lions. A total of eight adult mountain lions have been collared, resulting in 39 collared cat months between 2001 and present. Sampling of predator-killed deer is ongoing.

Activities Relevant to Aim 3: Select Models of Disease Dynamics

The dynamics of CWD in a population can be considered from two perspectives: (1) the impact of the disease on the population, and (2) the spread of the disease through the population and its resulting prevalence.

Although CWD has an obvious effect on the survival rate of infected individuals, the resulting effect on the net vital rates at the population level has not been determined. Compensatory mechanisms and density dependent effects could result in less net mortality than rates of infection and death from CWD would suggest. Consequently, we have sought to characterize survival rates by age and sex class in mule deer from six mule deer populations in Colorado. This study has been structured to make spatial, temporal, and class (age & sex) comparisons among populations at various stages of CWD outbreak, from disease-free to the most highly infected population in Colorado. Fundamentally, we are asking whether and to what degree CWD reduces survival. To accomplish this, we have proposed a set of stage-structured models representing different hypotheses or factors controlling mule deer population dynamics, particularly the roles of population density and disease in shaping survival. The parameters of these models have been fit to data on population size, age and sex ratio, and harvest using both likelihood and Bayesian methods. Model comparisons have been made using Akaike's Information Criterion and Bayesian equivalents to estimate the strength of support for alternative models and, hence, each of the hypothesized effects.

Modeling of the disease dynamics has focused on extending last year's work that fit alternative models to data from studies of CWD outbreaks in captive populations. Additional models have been developed that extend the set of hypothesized transmission mechanisms. For example, environmental transmission may occur through multiple routes, including concentrated point sources such as carcasses of CWD victims or dispersed sources such as feces and urine. These alternative mechanisms are expected to produce different rates of infection at different times during the course of an epidemic. Data from the field for fitting and comparing these alternative models still covers a relatively short period and spatial area, making definitive analysis difficult. However, we have explored the range of possible dynamics that each model can produce and compared these to available information. This process has led to new hypotheses about the dynamics that would be expected under each alternative, which suggests future data collection enhancements that will enable us to more clearly distinguish the one actually operating.

These two approaches to modeling and understanding the dynamics of CWD, both from the perspective of the infected population and from the perspective of the disease, are leading toward an integrated model of the disease-population interaction. Such a model is a prerequisite to applying formal adaptive management methods that will identify optimal or near-optimal management strategies for controlling or eradicating CWD.

Findings Relevant to Aim 1: Describe Mechanisms of Transmission

Investigations of Shedding of PrP^{Pres} and Mechanisms of Lateral Transmission

Studies of the shedding of PrP^{CWD}, focusing on the potential for feces to be involved in transmission, are still in progress. We have not yet been able to develop a laboratory assay with adequate sensitivity to detect PrP^{CWD} in excreta; however additional avenues for increasing sensitivity are being investigated.

Investigations of Environmental Transmission

Our experiment revealed that CWD can be transmitted indirectly, from environments contaminated by excreta or decomposed carcasses to susceptible animals. Under experimental conditions, mule deer became infected in 2 of 3 paddocks containing naturally infected deer, in 2 of 3 paddocks where infected deer carcasses had decomposed *in situ* ~1.8 years earlier, and in 1 of 3 paddocks where infected deer had last resided 2.2 years earlier. Our data suggest that indirect transmission and environmental persistence of infectious prions will complicate efforts to control CWD, and perhaps other animal prion diseases.

Our findings were published in the online version of *Emerging Infectious Diseases* in April 2004, and the print version became available in May (Miller et al. 2004).

Findings Relevant to Aim 2: Describe Variation in Prevalence

Sampling Infected Populations

Our analysis of data from vehicle-killed mule deer suggested that CWD-infected individuals are more susceptible to vehicle collisions than uninfected deer. Overall prevalence was 66% higher in the vehicle-kill population; prevalence for vehicle-killed deer was 0.101 (95% confidence interval [CI] = 0.064–0.139) compared to 0.061 (95% CI = 0.051–0.072) for mule deer harvested or culled in the vicinity of vehicle-killed deer. The probability of detecting a CWD-infected, vehicle-killed deer, given that at least one other CWD-infected deer had been detected within a 3 km radius of the vehicle-kill site, was 16.67%. It follows that using vehicle-killed mule deer may be exploited in designing surveillance programs for detecting new foci of infection, but that this differential vulnerability also may bias estimates of CWD prevalence in natural populations when data from vehicle-killed deer are included in calculating such estimates. Evidence of increased susceptibility to vehicle collisions may aid in understanding vulnerability of CWD-infected individuals to other forms of death, particularly predation.

We summarized our findings in a draft manuscript submitted to the *Journal of Wildlife Diseases*; that paper is currently in review.

Development and Application of Techniques for Sampling Live Animals

Tonsil biopsy has proven to be a useful tool for estimating CWD prevalence in non-hunted mule deer populations, and the techniques we developed are being used in at least six other field studies or investigations of CWD epidemiology (WY, NM, WI, SD, CO, NE).

Influence of Mule Deer Genotype on CWD Distribution

Compiling DNA sequences of 363 mule deer PrP genes, we found that only four codons in the open reading frame exhibit variation, and only one of the four results in a change in the final version of the protein. This is a change from the amino acid serine (S), the high frequency allele, to phenylalanine (F) at codon 225.

Calculating estimated frequency of the F allele in gene pools by herd unit (HU) (WY) or Data Analysis Unit (DAU) (CO) showed a range from 0.111 (n=126) and 0.095 (n=652) in DAU18 and Estes Park, respectively, to 0.005 (n=200) in HU427. The Wyoming frequencies were calculated from stratified random samples of the 2001 and 2002 samples, maintaining the same proportions of CWD positives and negatives taken in the hunter survey for each unit. The Estes Park data were obtained from all samples collected and represented greater than 50% of the herd. Seven herd units in the Wyoming endemic area did not differ in gene frequency and were pooled. The estimated F allele frequency was 0.043 (n=932) (CI=.030 to .056) for these combined units. Another group of three HU's with comparable F allele frequencies was also pooled; it had a combined frequency of 0.019 (n=580).

The frequency of F225 in the genomes of all CWD positive animals taken from each herd unit for the years 2001-2003 were then compared to the unit's overall frequency. For the

two groups of pooled data from Wyoming and for the Estes Park herd, the comparisons are as follows:

seven HU pool: overall frequency 0.043 (n=932); frequency in CWD positives 0.00 (n=288).

Three HU pool: overall frequency 0.019 (n=580); frequency in CWD positives 0.005 (n=190);

Estes Park herd: overall frequency 0.095 (n=652); positives in CWD positives 0.00 (n=50).

The F225 allele is present among the CWD positive mule deer taken from these areas at a lower frequency than expected if it is a neutral mutation. We are interested in knowing if the CWD prevalence among wild mule deer with a F225 allele is significantly lower than among deer without F225, and are analyzing the data to see if recombining into pools of like CWD prevalence will support this type of analysis. We observed a clear drop in F allele frequency moving north from Colorado into Wyoming, and west across the Continental Divide. It will be of interest to survey genotypes further south in Colorado to locate the center of highest frequency, and to derive some estimate, if possible, of time since the mutation arose and became fixed in the mule deer genome. We did not observe a clear cut inverse relationship between F225 gene frequency and CWD prevalence, suggesting that if there is some type of restraining effect on CWD infection by this genetic variation, it has not had a large enough effect to be easily discerned at this gross scale. Using herd units as data groups, or even their constituent hunt areas, is possibly too large of a scale to see any effects or interactions between genotype and CWD. We know that experimentally exposed captive animals of the S/F 225 genotype can become infected with CWD, so it is clearly not a CWD resistance gene. It is possible that mule deer expressing the variant PrP may exhibit altered CWD pathogenesis than deer with serine at position 225, perhaps through a gene dosage effect and a prolonged incubation time. Hints of this effect have been observed in differences in incubation times in the inoculated donor deer. What effect this has on CWD transmission in mule deer remains to be determined.

Local Scale Study: Effects of Land Use on Prevalence

The methods and results are described in the 2003 Annual Report. We provide a summary of our findings here. The primary insight gained from this study was that human alteration of the landscape appears to differentially affect CWD prevalence rates between the sexes. Specifically, the potential for development to alter the demographic composition of the deer population and subsequent prevalence rates suggests that urban areas may act to increase infection rates in male deer. Among adult males, it appears as if urbanization positively influences CWD prevalence rates. Concentrating older males in urban locations where there is little to no harvest pressure and where supplemental feeding is available throughout the winter may play a role in increasing prevalence rates in these areas. Unfortunately, we do not have data that allow us to compare the age structure of deer in urban and non-urban areas. Females may respond differently than males, for a variety of reasons, to the human footprint. Sex effects were considered *apriori* based on recent findings (Miller and Conner 2004) and our results support the

observation that males have higher prevalence rates. However, not only did this analysis support those findings, but also the differential response to human development by the two sexes, with urban areas appearing to increase CWD prevalence in male and having little or no effect on female prevalence rates.

Based on our findings, it appears that mule deer wintering in developed locations need to be included in control efforts intended to reduce overall CWD prevalence in north-central Colorado. Modification of land use practices and other human activities that foster congregation or sedentary behavior in urban mule deer populations could have beneficial effects on reducing opportunities for CWD transmission. Because urban areas may serve as refugia from hunting, alternative management strategies like the “test-and-cull” program under evaluation by the Colorado Division of Wildlife (Wolfe et al. 2004) may be necessary adjuncts to more traditional population management approaches in such areas. A better understanding of the specific features of urban landscapes that have the greatest potential influence on CWD transmission among mule deer should aid in further refining landscape-level control strategies.

Landscape Scale Study: Spatial Heterogeneity of CWD Prevalence

We are currently fitting spatially structured Bayesian hierarchical models, relating biotic and abiotic factors to CWD prevalence at the scale of the individual and individual home range (i.e., 9 km² grid cells) using BUGS (Bayesian inference using Gibbs sampling) software (Spiegelhalter et al. 2003). Using biotic and abiotic predictors (Table 1) within spatial and non-spatial models, each having a different level of population connectivity, our preliminary model selection results (Table 2), based on an information criterion known as Deviance Information Criteria (DIC) (Spiegelhalter et al. 2002), suggest that models which include the spatial structure of CWD provide a better representation of the observed distribution of prevalence rates than do models that only consider covariates and not CWD spatial structure. The effect of including spatial structure on the fit of the model can be seen by examining Figures 1-3. Spatial models do a better job of replicating the “patchy” distribution of CWD heterogeneity across Northern Colorado’s landscape than do non-spatial models. Statistically speaking, spatial models had much greater support in the data than did non-spatial models, with the proportion of male deer being the only significant non-spatial covariate in all three spatial models. These findings support the sex-effect that has been seen in previous research conducted at various other scales (Miller and Conner 2004, Conner and Miller 2004, Farnsworth et al. 2004). Additionally, the superiority of the spatial models in capturing the observed heterogeneity in prevalence rates demonstrates the importance of explicitly incorporating spatial dependencies in landscape models.

These results suggest that local-scale processes, such as increased transmission rates with increasing deer density, may act as key drivers of CWD heterogeneity in North-central Colorado. The consistent pattern of increased prevalence rates with increasing proportion of male deer lead to the following hypotheses: 1) Behavioral differences between the sexes may result in increased exposure to PrP⁺ in males relative to females. 2) Because male deer face greater harvest pressure, they may be driven to seek out refugia, i.e.,

private land with restricted or forbidden hunting, which could skew the population's age structure, leading to an older male cohort with a higher likelihood of coming into contact with PrP⁺. Future work will explore the relationship between deer density and CWD prevalence. We will develop models that explore how the relationships between CWD and the covariates used in this analysis change as a function of the scale of observation (grid size).

We are encouraged by these findings, our goals for the next year of the project include; 1) Gaining further understanding of the relative roles played by the aetiology of the disease and the landscape covariates in structuring the observed heterogeneity in prevalence rates, 2) Determining how these relationships change as the scale of analysis is varied.

Evaluation of an Urban CWD Management Strategy

Data from our pilot trial showed that testing and culling mule deer appears to be a viable approach for managing CWD in Estes Park; results of our feasibility study (Wolfe et al. 2004) will be published in the July issue of the *Wildlife Society Bulletin*.

Based on the success of pilot testing, the Colorado Division of Wildlife committed to a 5-year management experiment to evaluate the efficacy of test and cull in lowering CWD prevalence in an urban mule deer population. In year 2, over 50% of the adult mule deer wintering in Estes Park and neighboring Rocky Mountain National Park were again tested for CWD. This year, testing was accomplished under a cooperative program operated by the Colorado Division of Wildlife and the National Park Service. Effects on prevalence will be evaluated at the end of this study.

Selective Predation by Mountain Lions on CWD-Infected Mule Deer

Three collar styles have been deployed, and we are continuing to test and evaluate this new technology; aside from our main objective of data gathering related to CWD ecology, evaluation of this technology should be a substantial contribution to future studies of predator-prey relationships.

We have detected and examined over 85 kill sites from radio-collared mountain lions and successfully sampled tissues from 28 sites where adult mule deer were present; we also have collected 17 samples opportunistically from mule deer killed by mountain lions that were not radio-collared. We will continue capturing mountain lions to reach the objective of six to nine collared cat years, and will continue sampling carcasses of lion-killed mule deer to reach our target sample size (n = 157).

Future Research

While our findings will not allow us to determine causal factors driving the observed spatial variations in CWD prevalence, they will suggest landscape attributes, and possibly biological mechanisms, that play an important role in mediating the spread of CWD. As an example, we will be able to infer the scale at which human-based alteration of the landscape bears the greatest influence on the distribution of CWD heterogeneity. Additionally, predictions made from these

models will allow wildlife managers to develop prescriptions reflecting the intensity of harvest and culling efforts that are needed within a specific area to reduce prevalence rates.

Findings Relevant to Aim 3: Select Models of Disease Dynamics

Modeling of population dynamics of mule deer has led to several notable conclusions. Compensation for CWD mortality is insufficient to prevent a net decline in survival of adults. That is, it appears that declines in population density caused by CWD have not caused equal or larger compensatory decreases in other sources of mortality. Adult survival is lower in the CWD endemic areas (Figure 4) and has declined over time as the epidemic has progressed through a population. Survival rates of adults are more severely impacted than those of juveniles, a result that is consistent with the protracted clinical course of the disease. Population growth is highly sensitive to survival of adults because juveniles do not reproduce. The magnitude of the reduction in adult survival is sufficient to produce a decline in the population. In the absence of effective management intervention, we currently project local extinct of populations over periods of several decades based on this analysis (Figure 5).

Conversely, our models of disease dynamics include the possibility that CWD could produce oscillations in both disease prevalence and deer populations. These oscillations have periods on the order of decades, so field data do not cover a sufficient period to conclusively distinguish between models that predict local extinction versus those that predict periodic disease dynamics.

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Contributions to the Development of the Principal Discipline of Project

To date, we have developed a reliable, non-lethal method for detecting chronic wasting disease (CWD) in mule deer that represented a significant breakthrough in improving the ability to monitor prevalence of the disease over time and space. We also have demonstrated that CWD can be transmitted indirectly. Moreover, we have developed epidemic models for CWD in captive mule deer that align well with the independent empirical findings on transmission processes, underscoring the likely importance of indirect transmission in the ecology of CWD in natural populations. We are the first to show evidence that CWD will likely lead to local extinctions of mule populations.

Contributions to the Development of Human Resources

We have provided training for a graduate student (Matt Farnsworth), a post-doc (Vicky Dreitz), and two early career research associates (Bruce Lubow and Mary Conner).

Contributions to Public Welfare beyond Science and Engineering

Chronic wasting disease threatens recreation based economies throughout Western North America. Our work provides a scientific foundation for actions needed to abate this threat.

Outreach Activities

Miller traveled to an international workshop on chronic wasting disease, providing an overview of CWD epidemiology and management.

Wolfe provided training to over 30 wildlife veterinarians and biologists in tonsil biopsy techniques and field evaluation of chronic wasting disease suspects, and oversaw a field investigation of a new CWD focus in an urban mule deer herd in southern Colorado.

Williams participated in many outreach activities during the past year including serving as a consultant to the Institutes of Medicine committee on assessing prion science and on a national Task Force to develop a CWD management plan with the USDA and the US Department of the Interior. She gave numerous presentations in scientific meetings and symposia including an international symposium on TSEs Fort Collins, Colorado; several presentations about CWD for CJD conferences in New York and Washington, D.C.; a presentation on CWD for the American College of Veterinary Pathologists, Banff, Alberta; and a presentation on CWD for the Michigan Governor's CWD Task Force. In addition, she participated in numerous interviews for local and national radio and print media.

Jewell presented results of her work on mule deer genetics at the annual meeting of the Wildlife Disease Association, Saskatoon, Saskatchewan.

Hobbs gave interviews to Colorado Public Radio and National Public Radio. He gave seminars on our work at the National Science Foundation, the University of Colorado, and the University of Washington.

Training and Education

Williams uses material from our project in the classes and the lectures she teaches at the University of Wyoming (especially Diseases of Wildlife, PATB 4120 and 5120) and in graduate and undergraduate seminars (Department of Animal Sciences Departmental Seminar Series, Emerging Issues in Agriculture, Environmental Policy). Five undergraduate students (including one honors student and one McNair scholar) are working with Jewell and Williams on projects supporting or related to our project.

Hobbs used examples from the CWD project in teaching a graduate course in Systems Ecology. The project is one of the major research areas in an IGERT Project at Colorado State University, the Program in Mathematics, Ecology, and Statistics.

Other Products

Farnsworth, M. L. Poster Presentation: Spatial Epidemiology of Chronic Wasting Disease in Colorado Mule Deer Ecology and Evolution of Infectious Disease. Atlanta, Georgia. May 2004.

Publications

Journal Publications:

Conner, M. M., and M. W. Miller. 2004. Movement patterns and spatial epidemiology of a prion disease in mule deer population units. *Ecological Applications* 14: in press.

Farnsworth, M. L., L. L. Wolfe, N. T. Hobbs, K. P. Burnham, E. W. Williams, D. M. Theobald, M. M. Conner, and M. W. Miller. 2004. Human land use influences chronic wasting disease prevalence in mule deer. *Ecological Applications* (Accepted, in re-review).

Krumm, C. E., M. M. Conner, and M. W. Miller. Assessing relative vulnerability of chronic wasting disease infected mule deer to vehicle collisions. *Journal of Wildlife Diseases*: in review.

Miller, M. W., and M. M. Conner. Epidemiology of chronic wasting disease in free-ranging mule deer: Spatial, temporal, and demographic influences on observed prevalence patterns. *Journal of Wildlife Diseases*: in review.

Miller, M. W., E. S. Williams, N. T. Hobbs, and L. L. Wolfe. 2004. Environmental sources of prion transmission in mule deer. *Emerging Infectious Diseases* 10(6): 1003–1006.

Wolfe, L. L., M. W. Miller, and E. S. Williams. 2004. Feasibility of "test and cull" as a strategy for managing chronic wasting disease in urban mule deer populations. *Wildlife Society Bulletin* 32(3): in press.

Tables and Figures

Table 1. Model variables. Population “connectivity” (Con_i) varied among models

Variable	Name	Description
n	n_i	Number of deer sampled in cell i
Y	Y_i	Number of cwd infected deer in cell i
x_1	Xdir _i	X direction coordinate in cell i
x_2	Ydir _i	Y direction coordinate in cell i
x_3	%adult _i	Proportion of adult deer in cell i
x_4	%male _i	Proportion of male deer in cell i
x_5	%priv _i	Proportion of privately owned land in cell i
x_6	%urb _i	Proportion of urban land in cell i
x_7	move _i	Number of radio-collared deer relocated in cell i
x_k	Con _i	Indicator variable representing “connectivity” among sub-populations
γ	γ_i	Spatial Covariate in cell i

Table 2. DIC values and significant covariates for spatial and non-spatial models fit to CWD prevalence data .

Model	Connectivity	Significant predictors *	DIC
spatial	50%	Xdir, %male	768.0
spatial	100%	%male	770.7
spatial	0%	Xdir, %male	772.0
non-spatial	50%	Xdir, Ydir, %male, %priv, move, Con ₁₋₂	799.0
non-spatial	0%	Xdir, Ydir, %male, %priv, Con ₁₋₃	799.0
non-spatial	100%	Xdir, Ydir, %male, %priv, move	806.5

* Significance is defined as parameter estimates with 95% Credible Intervals that do not include 0.

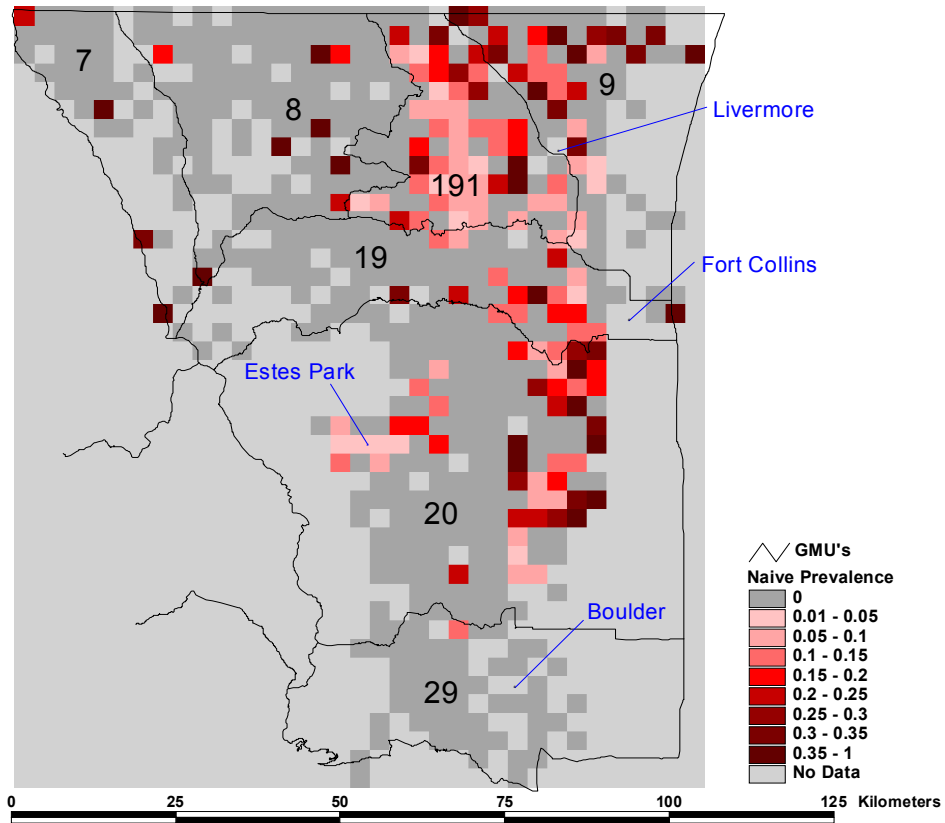


Figure 1. Map of naïve prevalence estimates in North-central Colorado is comprised of 610, 3km X 3km grid cells. Prevalence is estimated as y_i/n_i (See Table 1).

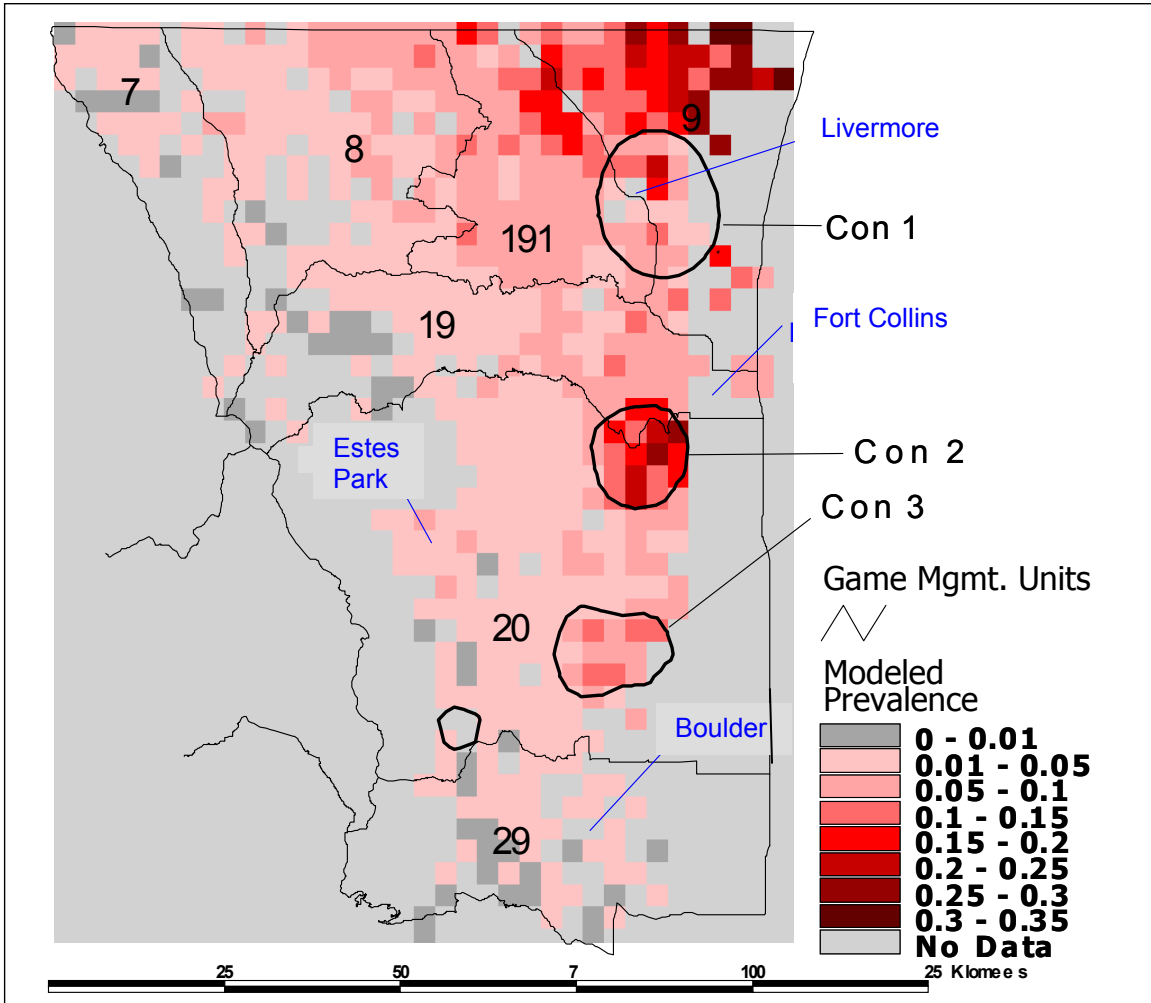
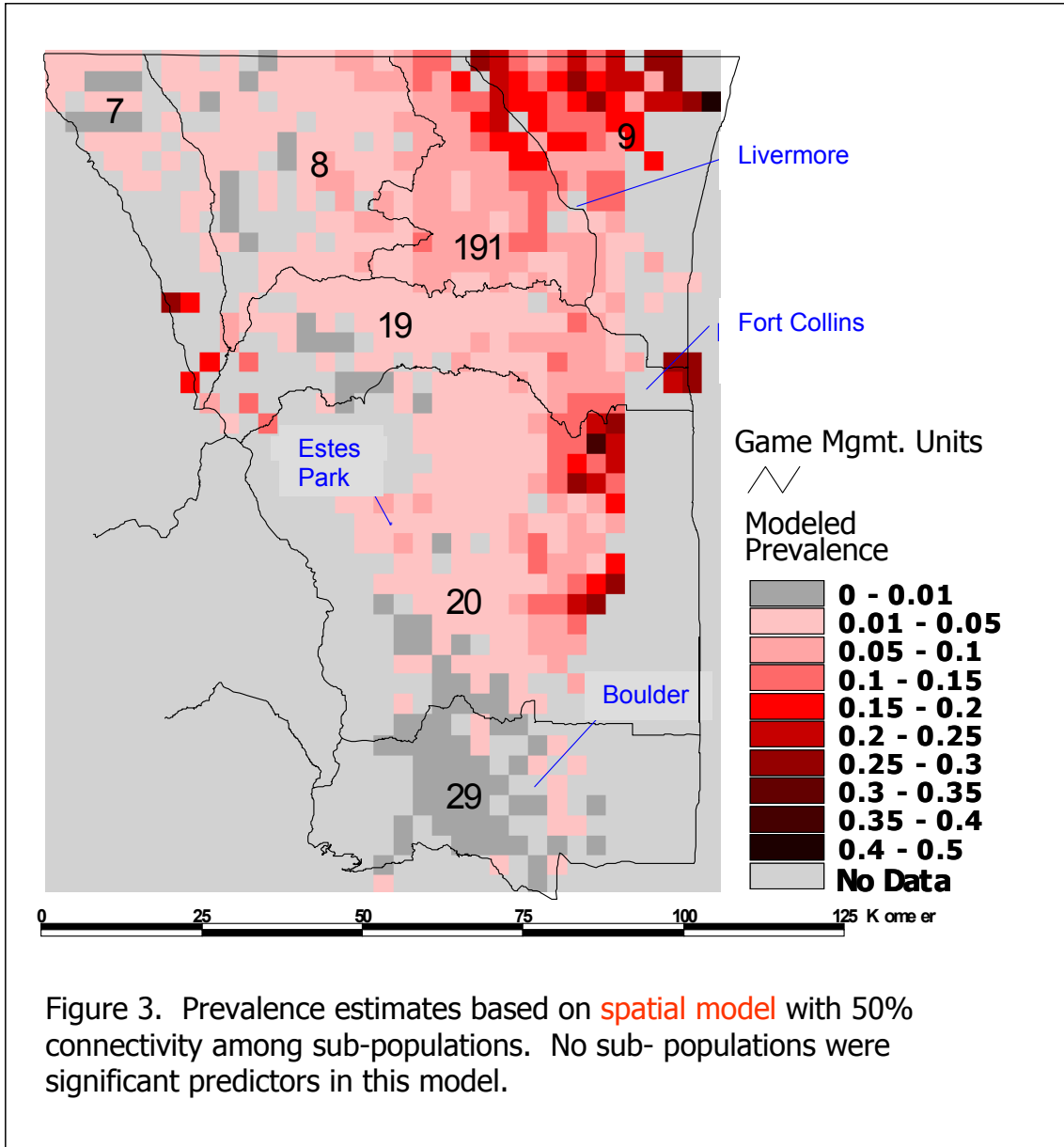


Figure 2. Prevalence estimates based on **non-spatial model** with 50% connectivity among sub-populations. Con_i polygons show locations of sub-populations that were significant predictors in the model.



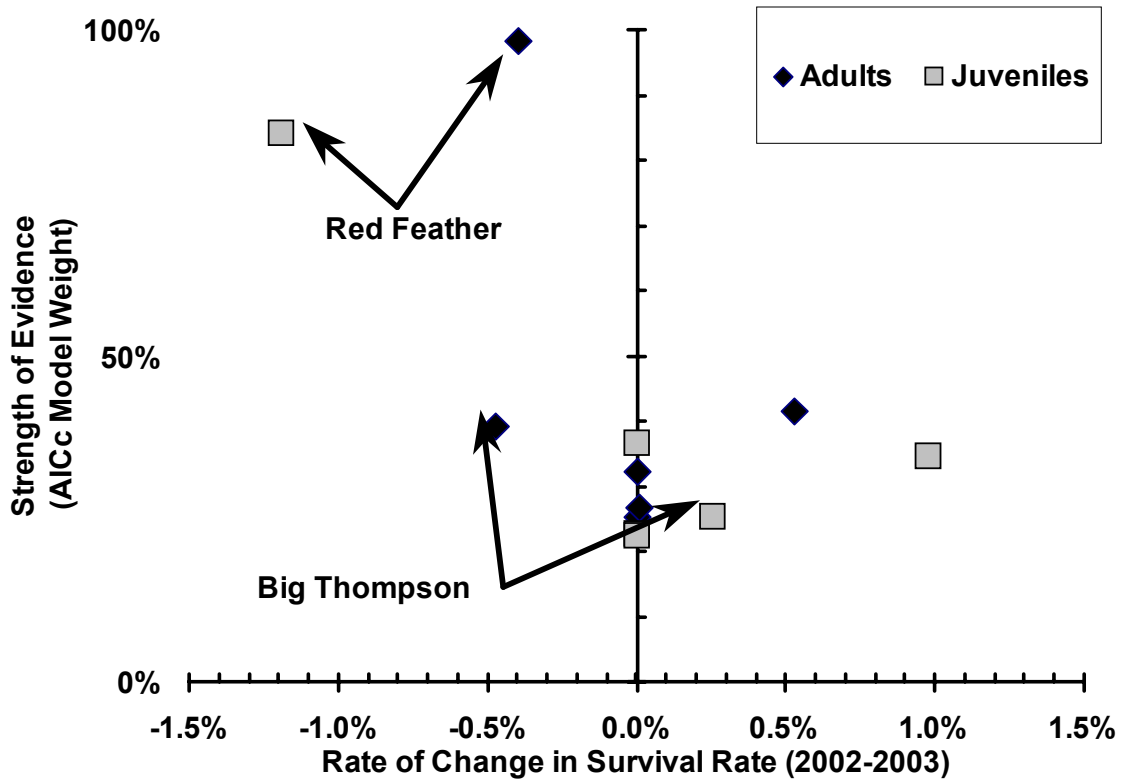


Figure 4. Results of comparison among six Data Analysis Units for mule populations in Colorado. Figure presents the annual rate of change survival rates estimated from stage-structured population models fitted to data on population, age and sex ratio, and harvest recorded by the Colorado Division of Wildlife. Vertical axis indicates the strength of support for models containing a non-zero rate of change in survival. The Red Feather population has the most advanced CWD endemic, Big Thompson also has a well established epidemic. The remaining four populations have few or no known cases of CWD.

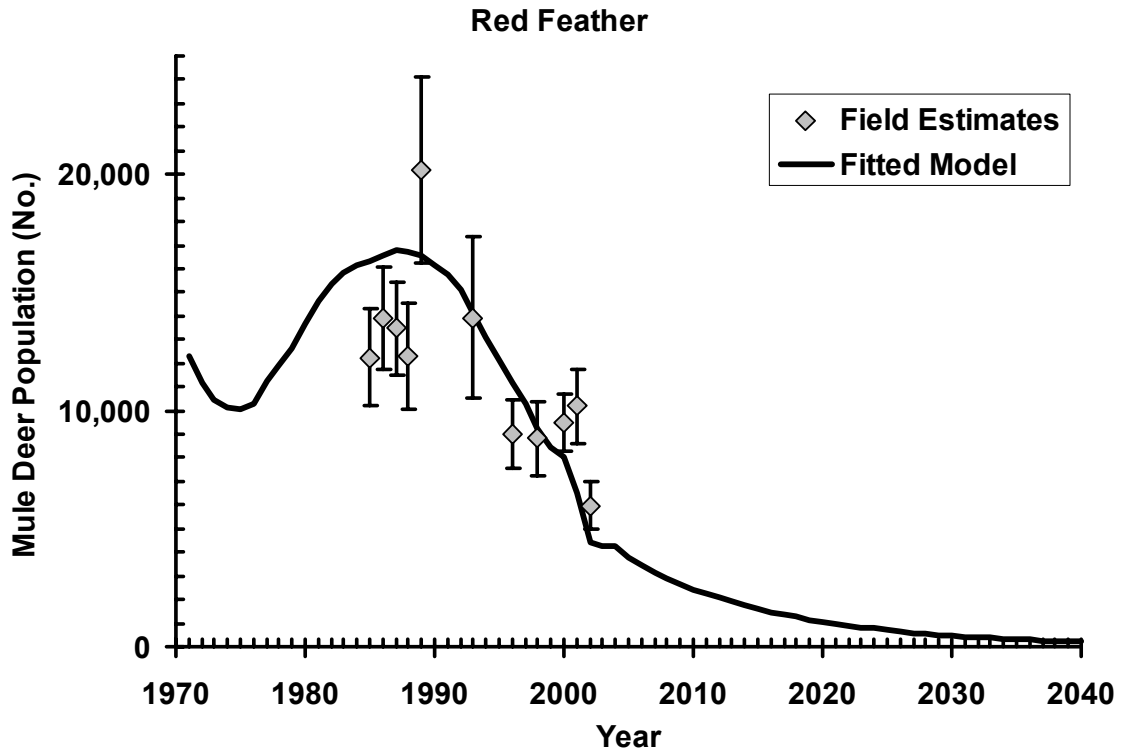


Figure 5. Stage-structured model of mule deer population for the Red Feather Data Analysis Unit in Colorado, which contains the most highly CWD infected population in the state. Projections based on vital rates estimated in fitting the model to field data suggest local extinction in approximately 35 years.