Dispersal of Microtus richardsoni in the Beartooth Mountains of Montana and Wyoming
by Marion Klaus

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences
Montana State University

Abstract:
The purpose of this study was to determine if Microtus richardsoni, the water vole, is geographically isolated within drainages or between four adjacent watersheds in the Beartooth Mountains of Montana and Wyoming. Water voles were named a sensitive species in the Rocky Mountain Region of the Forest Service, Region 2. Since local extinction is possible, their capacity to disperse becomes a question central to their management. Capture success, reproductive activity of males, the number of embryos found in trap-killed females, and the mean weight of Class I males (those weighing up to 49g) was significantly greater during the unusually wet summer of 1992 in comparison to the drier summers of 1990-91. In comparing water voles from grazed and nongrazed locations, capture success was significantly greater in ungrazed drainages and the mean weight of Class I females (those weighing up to 49g) was significantly greater in grazed than in ungrazed drainages. A relationship between an increase in population of water voles and precipitation is suggested by the data. Confirmation of any relationship between grazing of domestic livestock and impacts on water voles should be sought in other studies. Nuclear DNA obtained by nondestructive sampling of individuals representing each trapping location was examined at 31 enzyme or protein loci. Only ADH, EST-1 and SOD-1 were polymorphic. ADH and EST-1 were in Hardy Weinberg Equilibrium, but SOD-1 was not. Mitochondrial DNA, obtained from nondestructive sampling, was found to be polymorphic with 9 different restriction enzymes. The size of the fragments produced by each restriction enzyme was estimated and ordered into a site map. A total of 51 sites were found with the 9 restriction enzymes. This resulted in 29 different haplotypes from 142 individuals. Analysis of the allozyme and mitochondrial DNA variation suggests that the water voles are capable of dispersing across the four watersheds studied in the Beartooth Mountains, but that they are isolated from water voles found 150 km away at Togwotee Pass in the Absaroka Mountains of Wyoming. Their ability to disperse overland is also suggested by these data.

DISPERSAL OF *MICROTUS RICHARDSONI* IN THE BEARTOOTH MOUNTAINS OF MONTANA AND WYOMING

by

Marion Klaus

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Biological Sciences

MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana

April 1997

APPROVAL

of a thesis submitted by

Marion Klaus

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Robert E. Moore — Robert E Moore (Signature) — 14 April 1997 Date

Approved for the Department of Biology

Dr. Ernest R. Vyse — E R Vyse (Signature) — 4/14/97 Date

Approved for the College of Graduate Studies

Dr. Robert L. Brown — R L Brown (Signature) — 4/18/97 Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University-Bozeman, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the US Copyright Law. Requests for extensive copying or reproduction of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted "the exclusive right to reproduce and distribute my dissertation in and from microform along with the non-exclusive right to reproduce and distribute my abstract in any format in whole or in part."

Signature Marion Klaus

Date 14 April, 1997

ACKNOWLEDGMENTS

I would like to thank the chairman of my committee, Dr. Robert Moore, for his help in planning the project, organizing the field work, and securing partial funding for this dissertation. I thank Dr. Ernest Vyse and Dr. Matthew Lavin for their advice, encouragement, and patience during the laboratory and analysis phases of this work. I thank Dr. Katherine Hansen for helping locate climate data and partial funding and Dr. Harold Picton for his advice on analyzing the climate data. I would also like to thank the Center for High-Elevation Studies and NWCCD-Sheridan College for partial funding; Kent Houston, Ken Carver, Clare Jakee and the Beartooth Road Maintenance crew for providing and sharing facilities; Dr. Michele Girard and Carol Napoli for logistic support; Ronn Smith for developing formulas and evaluating the statistics; Jay Lance, Kelley Bonnet, Rebecca Leibinger, Cindi Phillips, Dr. J. R. Giurgevich, and Rod Carlson for technical assistance; the Sheridan College Library for help in obtaining resource materials; Dr. Roger Ferguson and Judy Carlson for administrative support and assistance; Dan and Karen Bilyeu for sharing their home and their constant support; and Valerie Burgess for traveling with me and for her encouragement. I thank my father, Sylvain Klaus, for his support and encouragement. I also thank my husband, Larry Mehlhaff, who managed our household during my long absences, who supported me both financially and emotionally, and who helped me find the strength and time to finish.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The purpose of this study was to determine if *Microtus richardsoni*, the water vole, is geographically isolated within drainages or between four adjacent watersheds in the Beartooth Mountains of Montana and Wyoming. Water voles were named a sensitive species in the Rocky Mountain Region of the Forest Service, Region 2. Since local extinction is possible, their capacity to disperse becomes a question central to their management. Capture success, reproductive activity of males, the number of embryos found in trap-killed females, and the mean weight of Class I males (those weighing up to 49g) was significantly greater during the unusually wet summer of 1992 in comparison to the drier summers of 1990-91. In comparing water voles from grazed and nongrazed locations, capture success was significantly greater in ungrazed drainages and the mean weight of Class I females (those weighing up to 49g) was significantly greater in grazed than in ungrazed drainages. A relationship between an increase in population of water voles and precipitation is suggested by the data. Confirmation of any relationship between grazing of domestic livestock and impacts on water voles should be sought in other studies. Nuclear DNA obtained by nondestructive sampling of individuals representing each trapping location was examined at 31 enzyme or protein loci. Only ADH, EST-1 and SOD-1 were polymorphic. ADH and EST-1 were in Hardy Weinberg Equilibrium, but SOD-1 was not. Mitochondrial DNA, obtained from nondestructive sampling, was found to be polymorphic with 9 different restriction enzymes. The size of the fragments produced by each restriction enzyme was estimated and ordered into a site map. A total of 51 sites were found with the 9 restriction enzymes. This resulted in 29 different haplotypes from 142 individuals. Analysis of the allozyme and mitochondrial DNA variation suggests that the water voles are capable of dispersing across the four watersheds studied in the Beartooth Mountains, but that they are isolated from water voles found 150 km away at Togwotee Pass in the Absaroka Mountains of Wyoming. Their ability to disperse overland is also suggested by these data.

INTRODUCTION AND NATURAL HISTORY OF *MICROTUS RICHARDSONI*

Microtus richardsoni (De Kay), the water or Richardson's vole, is a rodent in the family Muridae and subfamily Arvicolinae that has been present in North America since the mid-Pleistocene (Anderson, 1985; Burns, 1982; Hoffmann and Koeppl, 1985). It occupies a highly disjunct range within mountainous areas of Montana, Wyoming, Utah, Idaho, Oregon, Washington, Alberta and British Columbia (Hall, 1981; Hoffmann and Koeppl, 1985). Of the four subspecies recognized throughout its range only *M. r. macropus* (Merriam) is found in Montana and Wyoming (Hoffmann and Koeppl, 1985). Except for muskrats, water voles are the largest arvicoline rodents within their geographic range (Ludwig, 1984).

Water voles are found infrequently. Of the 4,500 individual small mammals captured during a 6 year study in the western Cascade Mountains of Oregon, only 1% were water voles (Hooven, 1973). They are typically found in small populations of 8-40 animals that are distributed linearly along alpine streams (Anderson et. al., 1976; Clark and Stromberg, 1987; Hollister, 1912; Hooven, 1973; Ludwig, 1988; Pattie, 1967; and Racey and Cowan, 1935). Water voles breed during June, July and August (Anderson et. al., 1976; Ludwig, 1984). Estimates of mean water vole litter size are 6.0 (Negus and Findley, 1959), 5.45 (Pattie, 1967), 7.85 (Brown, 1977) and 5.52-6.11 (Ludwig, 1981). Voles are well known for their three-to-four year population cycles (Taitt and Krebs,

1985), yet only one anecdotal record exists of water vole population irruptions. Racey (1960) described water vole population booms at Alta Lake in the Pemberton Valley in British Columbia during the summers of 1927, 1949, and 1958. During each of these years, precipitation exceeded the mean (Simpson, et al, 1932; Strauss and Reichelderfer, 1959; Connor and White, 1965).

Water voles are relatively slow to mature. Only 6.2-11.3% of the young females and 5.9-20.0% of the young males reproduce in their first year (Ludwig, 1984). Of the overwintered adult females, 62.5% were pregnant while 20% produced two litters in one breeding season (Ludwig, 1981). The majority of water voles overwinter only once and 88.9% of overwintered adults disappeared by the end of September (Ludwig, 1984). Ludwig (1981) radio-tracked one female and estimated her lifespan to be 16 months.

Adult water voles are big and can be distinguished from other voles by their total length (212-260 mm), weight (85-120 g.), and hind foot length (25-30 mm) (Clark and Stromberg, 1987). Male water voles are larger than females. Young water voles are more difficult to distinguish from other sympatric species of voles, but *M. r. macropus* have large hind feet that can help identify them. The length of the hind foot was the most reliable characteristic for identifying young water voles in this study. One young water vole that weighed only 18 g had hind feet measuring 23.5 mm. By comparison, *Microtus montanus* has an adult hind foot length of 17-21mm (Clark and Stromberg, 1987). The number of plantar tubercules on the hindfoot was suggested as a way to distinguish *Microtus richardsoni* from other species (Ludwig, 1984), but this was not found to be a

consistent feature. The number of tubercules varied between water voles and between the two hindfeet of one individual water vole.

Water voles were probably isolated in mountain ranges as they followed tundra-like vegetation into high elevations after the Pleistocene glaciations (Hoffmann and Koeppl, 1985). In Canada, they are found between 1,524 to 2,378 meters in elevation (Banfield, 1974) and between 914 to 3,201 meters in the United States (Ludwig, 1984). Water voles generally do not occur in isolated mountain ranges apart from the Cascades and northern Rocky Mountains. They are found in only two isolated mountain ranges in the northern great plains, the Big Belt mountains of Montana and the Bighorn mountains of Wyoming (Hoffmann and Jones, 1970). This suggests they are unable to disperse across lowland barriers to reach suitable subalpine or alpine habitat and have low colonization rates as well as high extinction rates. These characteristics are not unusual for montane mammals (Brown, 1971).

Water voles are nocturnal, semi-aquatic, and have highly specific riparian habitat requirements (Ludwig, 1981). Streams are used for escape and as transportation routes for daily movement and dispersal (Anderson et. al., 1976; Ludwig, 1981). Many of the smaller alpine streams used by these voles are ephemeral and dependent upon melting snow and precipitation for their existence. Water voles are found in linear colonies along alpine or subalpine streams with about 5° slope and narrow stream channels, a well-developed substratum of soil for burrowing next to the stream and mid-to-late seral stage stream-side vegetation consisting of willow, sedges, grasses and mesic forbs that provide

75% cover (Anderson et. al., 1976; Anthony, et. al., 1987; Blankenship, 1995; Getz, 1985; Ludwig, 1981; Pattie, 1967; Reichel, 1986). Preferred sites are often used by successive generations of water voles while similar habitat adjacent to a colony remains unoccupied (Ludwig, 1981). Preferred sites are well drained and have a deeply developed soil layer adjacent to the stream (Ludwig, 1981). During the summer, adults have a linear home range along a stream (Anderson, et. al., 1976; Pattie, 1967; Ludwig, 1981). Adult females stay within small, exclusive home areas while adult males move over longer portions of stream and overlap the seasonal ranges of adult females and adult males (Anderson et. al., 1976; Ludwig, 1981). The site loyalty and exclusive home range of adult females suggest that a stream-side home area with an underground nest site and adequate food are the keys to female reproductive success (Ludwig, 1981). The diet of water voles is known to include grasses, sedges, seeds, inner bark of willow twigs and forbs (Anderson et. al, 1976; Clark and Stromberg, 1987). The upper limit of water vole population size may be partially determined by the number of habitat sites available along streams.

Environmental assessments required for reallotment of grazing permits in the Rocky Mountain Region of the Forest Service, Region 2, now include consideration of the water vole (USDA-Forest Service, 1994). The water vole was recently placed on the Region 2 sensitive species list because it is rare to uncommon in this region, and it requires specific riparian habitat that is declining and may be damaged by poor grazing practices (Friedlander, 1995). Continuous heavy grazing can alter the characteristics of

riparian habitat by widening the stream channel, compacting the soil, and moving the plant community to an earlier seral stage. These factors probably combine to make an area unsuitable for water voles (Blankenship, 1995; Clark and Stromberg, 1987). Because water voles are vulnerable to riparian changes, there is concern about the continued viability of water vole populations in the grazed areas of this region (Blankenship, 1995). The short alpine breeding season, narrow habitat range, and low population densities result in small populations that are not buffered against habitat degradation and local extinction. Since local extinction is a possibility, their capacity to disperse becomes a question central to their management as a sensitive species.

The purpose of this study was to determine if allozyme and mitochondrial DNA (mtDNA) variation suggest water voles are geographically isolated within drainages or between adjacent watersheds on the Beartooth Plateau of Montana and Wyoming.

Evidence concerning the capability of water voles to disperse is addressed by restriction fragment length polymorphism (RFLP) analysis of mtDNA with supporting evidence from allozyme analysis of nuclear DNA. Information from mtDNA can be used to study dispersal of females and population dynamics (Avise, 1986) while allozyme analysis of nuclear DNA can be used to study dispersal of both sexes. Dispersal can be reflected in the distribution of phenotypic frequencies (Birdsall, 1972). Avise et al. (1979a) compared mtDNA sequences and found heterogeneity within and among natural populations of three species of *Peromyscus*. This technique can be used to estimate

relatedness of mitochondrial genomes, and because mtDNA is maternally inherited, it is possible to follow intra- and interdemic movements of females. Since mtDNA evolves rapidly in terms of nucleotide substitutions, it can be used to distinguish between closely related organisms (Brown et al., 1979; Li and Graur, 1991). Kessler and Avise (1985) demonstrated that mtDNA heterogeneity in a population of *Sigmodon hispidis* was sufficient to describe spatial and temporal use of habitat. The mtDNA phylogeny suggests historic relationships among haplotypes that are inferred from shared and presumably derived mutations (synapomorphies) in a cladistic analysis. Geographic distribution can be coupled with phylogeny to describe the phylogeographic pattern for a species (Avise, 1989).

DEMOGRAPHICS OF *MICROTUS RICHARDSONI* IN THE BEARTOOTH MOUNTAINS

Water voles use streams as corridors for dispersal to preferred patches of habitat, but the glaciated mountain topography between watersheds may restrict movement. A study site with these features was selected in the Beartooth Mountains and straddles the Montana and Wyoming border (Figure 1). This area consists of ten drainages or parts of drainages within four adjacent watersheds that comprise the headwaters of the Clarks Fork of the Yellowstone River. All are managed by the Shoshone National Forest's Clarks Fork District office in Region 2 of the USDA-Forest Service except for Quad Creek, which is managed by the Custer National Forest's Beartooth District in Region 1. Elevations of the drainages where water voles were trapped range from 2,926 meters at Beartooth Creek to 3,188 meters on Wyoming Creek. Two watersheds, Beartooth Creek and Canyon Creek, are on the west side of Beartooth Pass. The other two watersheds, Rock Creek and Line Creek, are on the east side (Figure 2). The distances between trapping locations are in the Appendix, Table 12. The confluence of Beartooth Creek and the Clarks Fork to the confluence of the Clarks Fork and Rock Creek outside of Rockvale, Montana is approximately 160 km. It is another 94 km from the confluence of the Clarks Fork and Rock Creek to the headwaters of Rock Creek (Appendix, Table 12).

Only Wyoming Creek, in the Rock Creek watershed, and Line Creek are grazed by livestock. The Line Creek allotment is grazed by 91 head of cow/calves from May 16

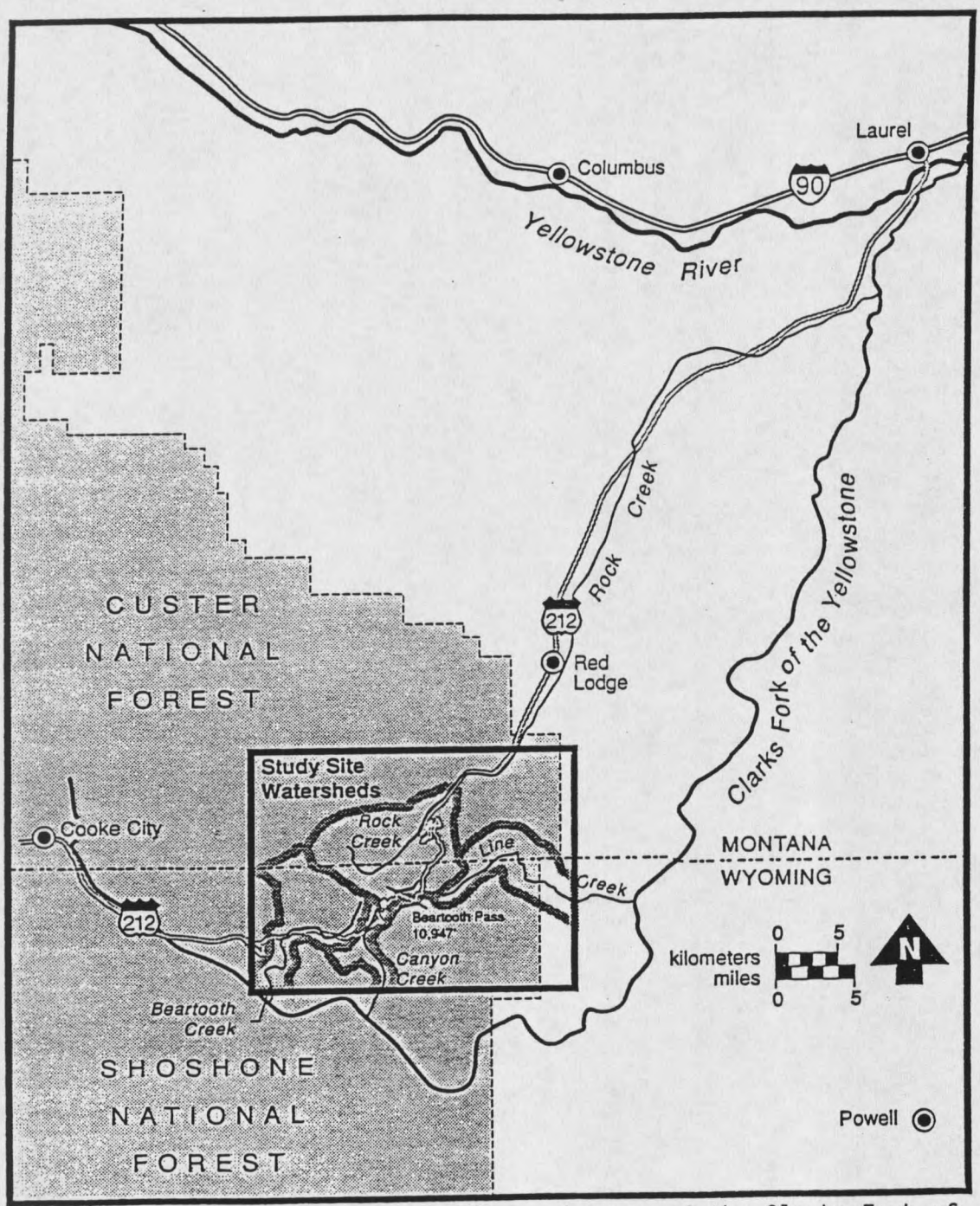

Figure 1. Regional map showing the headwaters of the Clarks Fork of the Yellowstone River.

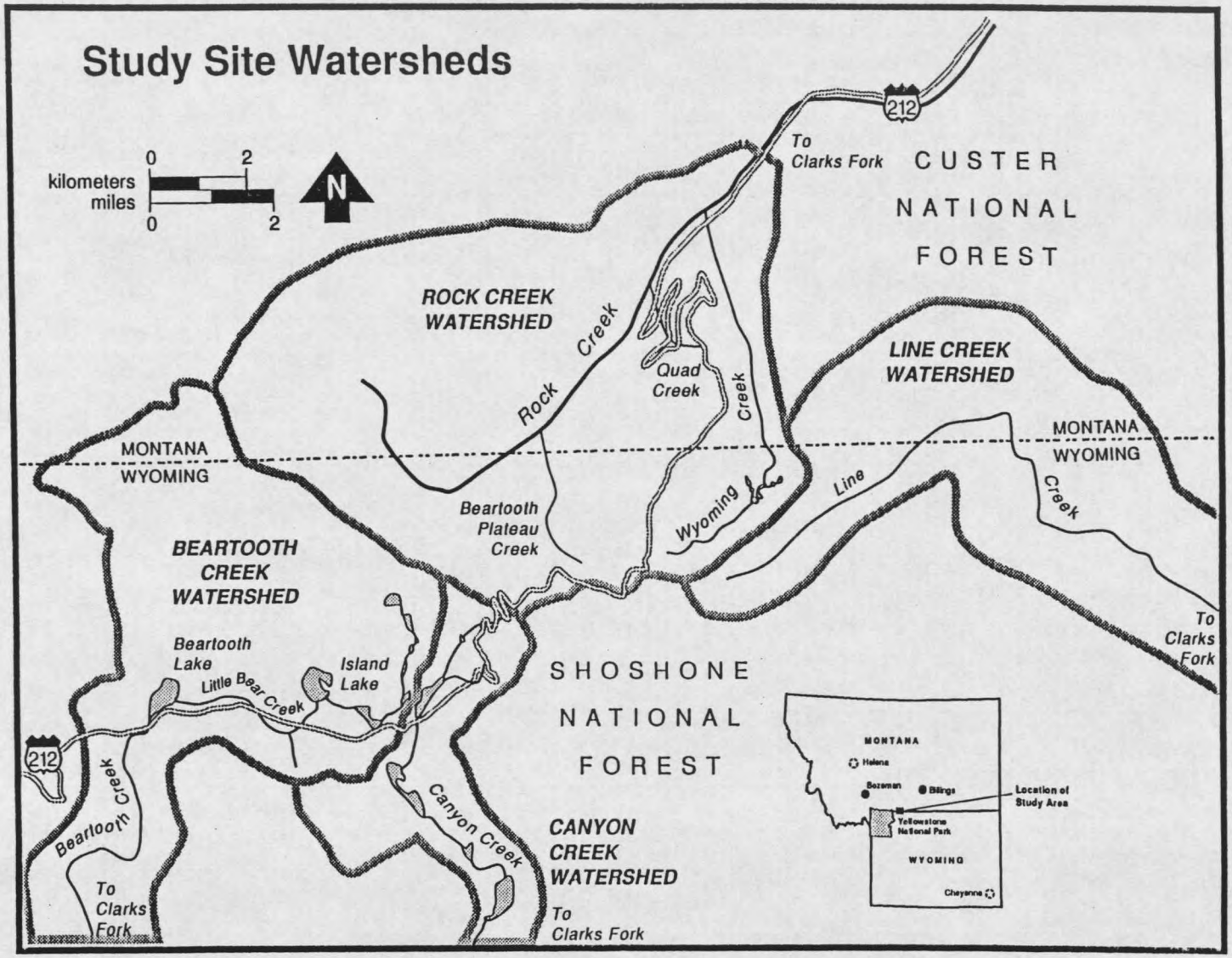

Figure 2. Area map of the study site watersheds.

to Oct. 31 and by 2,000 sheep from July 21 - Sept. 5 (Sanders, 1990). According to King (1997), the sheep graze Line and Wyoming creeks on a rotation schedule, but since these creeks fall within the middle of the allotment, they may often be grazed at about the same time each year. Sheep graze Line Creek about Aug. 8 - 14 and Wyoming Creek about Aug. 15-20. King (1997) reported 200 head of cow/calves grazed the allotment in 1991 and 1992 from July 21 - Sept. 29. The grazing season may have been slightly shorter in 1992. This area has been grazed by livestock since about 1900. Intensity of the grazing is determined by a range technician who measures the height of vegetation in the area once or twice per summer (Sanders, 1990).

Demographic data were collected at the same time tissue samples for genetic analysis were obtained. While this study was not designed for demographic analysis, the data collected are of interest to both USDA-Forest Service and Wyoming Game and Fish Department nongame biologists. Thus, they are included here.

Sherman live traps, baited with oats, were used to trap the water voles. Voles were trapped when drainages were mostly free of snow in July and August of 1990-92. Line Creek was not trapped in 1990 as no funds were available to pay for horses to pack in the traps. The south fork of Canyon Creek was not trapped in 1992 because bad weather shortened the total available trapping time. The sample already obtained from this creek was deemed sufficient. Traps were placed where evidence of water vole activity was observed, such as in runways, latrines, and by burrow entrances. The number of traps per location varied with the available habitat, the length of the drainage, and the amount of

vole sign. Consequently, the number of traps and the trap interval varied. If new areas of water vole activity were found, traps were added in those areas. This procedure was also used by Anderson et al. (1976) and Ludwig (1981). In 1990, each drainage was mapped, and the location of each trap was noted in relation to the topography and linear distance along the stream. Population density was determined by the mean number of water voles captured along the length of stream within a water vole colony. Traplines were checked early in the morning, when most captures were obtained, and late in the afternoon. Individuals accidentally killed in traps were frozen in liquid nitrogen. Dead females were examined for embryos and if embryos were found, they were individually frozen in liquid nitrogen.

Captured water voles were sexed, marked with an ear tag, and classified before release into one of three age categories by body weight. These categories were overwintered adults (70-125g), Class II (50-69g) and Class I (13-49g) (Ludwig, 1981). Reproductive condition of males was determined to be active if testes were scrotal or could be palpated. Females were considered reproductively active if their vaginal openings were perforate or their pubic symphyses were open (Ludwig, 1981). Approximately 2 cm of tail was collected from each water vole and frozen in liquid nitrogen for genetic analysis (Plante et. al., 1987). Specimens were transfered to an ultra-cold freezer at -80°C until they were used for either allozyme or mtDNA extraction.

In each comparison, mean percentages (p) were calculated and transformed to the angle whose sine is the $\sqrt{p}$ (arcsine transformed) prior to statistical analysis. Because

these data were percentages, arcsine transformation was needed to convert the discrete distribution of the data to a continuous distribution that would more accurately reflect statistical differences (Zar, 1996). This is especially important for small (0-30%) and large (70-100%) percentages since they deviate the most from a normal distribution (Zar, 1996). Then, each set of data was tested using one-way analysis of variance (ANOVA). If variances were homogeneous, a two-tailed t-test was used. If variances were heterogeneous, the Wilcoxon Mann-Whitney U-test was used. The impact of livestock grazing and precipitation on water voles was tested by examining differences in capture success (number captured per trap night), population density, age structure, reproductive indicators, and weight. These parameters were compared between grazed and ungrazed drainages and between years for precipitation variables. Data from 1990 and 1991 were analyzed by ANOVAs for each variable and the subsequent mean separation tests showed no differences. Therefore, data from the two drier summers, 1990 and 1991, were combined to increase total sample size and compared to data from the wetter summer of 1992. A 95% confidence interval was used to determine significance, and all significant differences are reported.

Once water vole colonies were identified and traps placed where evidence of their activity was observed, relatively few other species were captured. However, several small mammals including *Microtus montanus*, *Peromyscus maniculatus*, *Sorex pallustris*, *Zapus princeps*, *Eutamius minimus*, *Ochotona princeps*, and one bird, an American water pipit (*Anthus rubescens*), were also trapped.

Total precipitation during each of the three years of trapping exceeded the 30-year mean, with the most precipitation occurring in 1992 (Table 1). The summer reproductive season of 1990 and 1991 was drier than the 30 year mean, but 1992 had almost double the precipitation of the 30 year mean. Several statistically significant differences in demographic characteristics of water voles were found between the summers of 1990-91 and 1992. In 1992 significantly more embryos were found in trap-killed females ($t=2.67$, $df=5$, $p<0.05$;Table 2). Significantly more males showed signs of reproductive activity

Table 1. Precipitation (cm) recorded at the Beartooth Lake Weather Station, S40 (USDA-SCS, 1995)

Season	1990	1991	1992	30-yr. Mean
Winter (Dec.-Feb.)	29.21	20.58	17.27	24.56
Spring (Mar.-May)	24.89	32.51	25.40	24.07
Summer (June-Aug.)	13.47	11.69	30.23	16.51
Autumn (Sept.-Nov.)	17.27	22.86	24.64	16.34
TOTAL	84.84	87.64	97.54	81.48

Table 2. Comparison of demographics of water voles between the summers of 1990-91 and 1992.

Characteristics	Drier Summer (1990-1991 combined)	Wetter Summer (1992)
Capture Success (mean %)	0.04 (N=15 sites)	0.07 (N=7 sites)
Mean Population Density (voles/1000 m stream)	11.55 (N=15 sites)	15.14 (N=7 sites)
Young in Population Class I and II, (mean%)	0.45 (N=15 sites)	0.60 (N=7 sites)
Males Reproductively Active, (mean %)		
Class I	0.00 (N=5 sites)	0.51 (N=3 sites)
Class II	0.10 (N=5 sites)	1.00 (N=2 sites)
Adult	0.96 (N=5 sites)	1.00 (N=2 sites)
Female Reproductively Active, (mean %)		
Class I	0.00 (N=5 sites)	0.32 (N=3 sites)
Class II	0.40 (N=4 sites)	1.00 (N=2 sites)
Adult	0.78 (N=7 sites)	0.82 (N=3 sites)
Embryos/trap-killed female (mean±SEM)	5.75±0.54 (N=6)	7.75±0.63 (N=4)

$p<0.05$

in 1992 than in the two previous years ($t=2.49$, $df=6$, $p<0.05$; Table 2). This increase was primarily found in young, Class I and Class II, voles. None of the Class I individuals, male

or female, showed any sign of reproductive activity in 1990 or 1991. Weight of the water voles did not vary significantly between dry and wet summers, except for Class I males who weighed significantly more in the wet summer ($U=191.5$, $p<0.002$; Table 3). In 1992, capture success was significantly greater than in the drier summers ($U=110.5$, $p<0.04$; Table 2) and more water voles/1000 m stream were estimated within colonies. In 1992, Class I and Class II voles comprised a larger percentage of the population than in the drier years, but the differences were not significant. In 1990, male water voles ranged between 6.7 - 463.3 m and females ranged between 0 - 67.1 m, but the differences were not found to be significant.

The increase in precipitation in 1992 appeared to increase the availability of usable habitat. In 1992, runways, burrows, and latrines were observed along small, ephemeral creeks and side drainages that did not exist during the previous years. In 1992, the percentage of Class I males and females with indications of reproductive activity far exceeded the ranges reported by Ludwig (1981). On the average, trap-killed females carried more embryos in 1992 than in the two previous years, again with a mean exceeding that reported by Ludwig (1981), Negus and Findley (1959), and Pattie (1967) but lower than the mean reported by Brown (1977). Interestingly, the mean number of embryos reported for 1990-91 is close to the mean reported 30 years ago in the same general area by Pattie (1967). This suggests that female fecundity may be relatively stable in this area. A relationship between an increase in the water vole population and precipitation is suggested by the data, with anecdotal support from Racey (1960).

Table 3. Comparison of mean water vole weights between the summers of 1990-91 and 1992.

Weight by sex and age class	Drier Summers (1990-91 combined)	Wetter Summer (1992)
Male Weight (mean±SEM g)		
Class I	26.56 ± 1.93 (N=20)	39.89 ± 2.58 (N=9)
Class II	56.50 ± 1.68 (N=8)	53.00 ± 1.15 (N=11)
Adult	102.47 ± 2.45 (N=30)	100.50 ± 2.56 (N=12)
Female Weight mean±SEM g)		
Class I	31.24 ± 1.69 (N=17)	29.42 ± 1.79 (N=24)
Class II	60.73 ± 1.95 (N=11)	58.00 ± 2.00 (N=3)
Adult	90.32 ± (N=34)	88.18 ± 2.04 (N=17)

$p<0.05$

Significantly greater capture success was obtained in ungrazed drainages ($U=222.5$, $p<0.04$; Table 4). In 1992, captures more than doubled in all drainages except Quad, Wyoming, and Line creeks. Of the drainages without population increases in 1992, only Quad Creek was not grazed. No significant difference in the proportion of young to adult voles was observed in the population, but in both grazed and ungrazed areas, there were significantly more Class I than Class II females ($t=1.83$, $df=20$, $p<0.05$). While the mean number of embryos was slightly higher in grazed than in ungrazed areas, the

Table 4. Comparison of demographics of water voles between grazed and ungrazed drainages. Two drainages were grazed, Wyoming Creek and Line Creek. Wyoming Creek was trapped during three summers and Line Creek was trapped during two summers. The mean percents are means of means calculated for each sample site for each year trapped (N).

Characteristics	Grazed Drainages	Ungrazed Drainages
Capture success (mean %)	0.02 (N=5)	0.05 (N=17)
Population Density (voles/1000 m stream)	9.01 (N=5)	13.78 (N=17)
Young in Population (Class I and II, mean %)	0.80 (N=5)	0.86 (N=17)
Males Reproductively Active (mean %)		
Class I	0.25 (N=4)	0.25 (N=6)
Class II	0.50 (N=2)	0.50 (N=5)
Adult	1.00 (N=4)	0.97 (N=7)
Females Reproductively Active (mean %)		
Class I	0.25 (N=4)	0.08 (N=6)
Class II	1.00 (N=1)	0.52 (N=5)
Adult	0.88 (N=5)	0.74 (N=8)
Embryo/trap-killed Female (mean ± SEM)	7.0 ± 0.58 (N=3)	6.4 ±0.61 (N=7)

$p<0.05$

difference was not significant. More Class I females were reproductively active in grazed drainages, but all of these females were captured in 1992. The weight of Class I females on grazed drainages was significantly greater than on ungrazed sites ($U=557.5$, $p<0.002$; Table 5).

In a preliminary survey, Luce (1995) found that light to moderate grazing did not exclude water voles even though population density and viability may have been affected, but heavy grazing that affected the stream bank did preclude them from an area. Confirmation of any relationship between grazing of domestic livestock and negative impacts on water voles should be sought in other studies if the Rocky Mountain Region of the Forest Service continues to consider the water vole a sensitive species in their assessment of grazing allotments.

Table 5. Comparison of mean water vole weights between grazed and ungrazed drainages.

Weight	Grazed Drainages	Ungrazed Drainages
Male Weight (mean ± SEM g)		
Class I	25.00 ± 4.20 (N=4)	31.08 ± 2.12 (N=25)
Class II	57.00 ± 7.00 (N=2)	53.94 ± 0.91 (N=18)
Adult	105.11 ± 3.13 (N=9)	101.03 ± 2.24 (N=32)
Female Weight (mean ± SEM g)		
Class I	36.73 ± 6.65 (N=11)	27.84 ± 7.03 (N=31)
Class II	57.00 ± 3.00 (N=2)	60.67 ± 1.78 (N=12)
Adult	93.07 ± 3.08 (N=14)	88.30 ± 1.60 (N=36)

$p<0.05$

ALLOZYME ANALYSIS

Water vole populations are characterized by subdivision into small groups found in disjunct islands of favorable habitat along the length of alpine or subalpine streams (Anderson, et al., 1976; Ludwig, 1981; Getz, 1985). However, the extent to which these small groups are isolated along a stream or between adjacent watersheds has not been studied. Isolated populations of mammals generally have reduced levels of genic variability not observed when gene flow between groups is high. The genetic structure of water vole populations, as suggested by the relative amounts of diversity within and between individuals from four watersheds, is an indicator of gene flow and their capacity to disperse across rugged mountain topography or many kilometers of streams with plunging waterfalls and long stretches of unsuitable habitat. Genetic diversity was estimated using both allozymes and mtDNA RFLPs.

Allozymes have been used as indicators of genetic subdivision in vole populations in many studies. Most allozyme studies of *Microtus* sp. have examined the relationship between allele frequencies and population fluctuations or dispersal behavior (Bowen, 1982; Bowen and Koford, 1987; Gaines et al., 1978; Gaines and Johnson, 1987; Gaines and Krebs, 1971; Gaines and Whittam, 1980; Kohn and Tamarin, 1978; LeDuc and Krebs, 1975; Lidicker, 1985; Lidicker and Patton, 1987; McGovern and Tracy, 1981; McGovern and Tracy, 1985; Mihok et al., 1983; Nygren, 1980; Nygren and Rasmuson, 1980; Plante, et al., 1989a; Rose and Gaines, 1981; Semeonoff and Robertson, 1968; Semeonoff, 1972; Tamarin and Krebs, 1969). Some have used allozymes to assess the role of isolation in

structuring populations (Anderson et al., 1976; Kilpatrick and Crowell, 1985; Petcoff, 1985) while others have discriminated between different species (Nadler et al., 1978). Based upon the apparent small size and isolation of water vole populations in these alpine watersheds, reduced levels of genic heterozygosity would be expected.

Comparisons were made of 31 enzyme or protein loci from individuals captured in each of four adjacent watersheds in the Beartooth mountains (Appendix, Table 13). Tissue samples included liver, kidney, cardiac muscle, and skeletal muscle from trap-killed water voles. The mixture of tissue types found in the tail was used for other individuals. All samples were homogenized in 0.2 ml cold buffer (0.1 M Tris, 0.001 M EDTA, and 5×10^{-5} M NADP; pH adjusted to 7.0), refrigerated for 20 minutes and centrifuged at 12,000 rpm for 5 minutes. Filter paper wicks were dipped in the supernatant and placed into a horizontal slice across 13% starch gels. Procedures for electrophoresis and staining followed May et al. (1979). Phenotypes observed on the gels were used to calculate genotype frequencies (Table 6). The inferred allozyme freqencies were calculated from the genotype frequencies.

Polymorphism, defined as the occurrence of the common allele at a freqency less than 99% (Selander et al., 1971; Berry and Peters, 1981), was found at three loci; ADH, EST-1, and SOD-1. Mean heterozygosity was determined by locus, and Hardy Weinberg Equilibrium (HWE) values were calculated and compared to the observed data in a X^2 test (Table 6). Both ADH and EST-1 are in HWE, but SOD-1 is not. There are several

Table 6. Allelic frequencies, mean heterozygosity and chi-square values at three variable loci

Locus and Watershed	Genotypes: BB	BC	CC	Allelic Frequency B	C	Mean Heterozygosity (Nei, 1975)*	Chi-Square (Hartl and Clark, 1989)
ADH							
Beartooth Crk	0	0	11		1.00		
Canyon Crk.	1	3	18	0.11	0.89		
Rock Creek	1	3	12	0.16	0.84		
Line Creek	0	2	0	0.50	0.50		
Subtotal (N=51)	2	8	41	0.12	0.88	0.21	1.58
EST-1							
Beartooth Crk	0	1	5	0.08	0.92		
Canyon Crk.	1	3	26	0.08	0.92		
Rock Creek	2	1	10	0.19	0.81		
Line Creek	0	2	0	0.50	0.50		
Subtotal (N=51)	3	7	41	0.13	0.87	0.23	4.43
SOD-1							
Beartooth Crk	0	0	3		1.00		
Canyon Crk.	0	0	5		1.00		
Rock Creek	3	0	9	0.25	0.75		
Line Creek	0	0	2		1.00		
Subtotal (N=22)	3	0	19	0.14	0.86	0.24	21.12

Chi-Square, 2 degrees of freedom (df), $p<0.05 = 5.99$ (Russel, 1996)

* $H = 1 - \sum_{j=1}^{i} (Pij)^2$

possible explanations why SOD-1 is not in HWE. This could result from the small sample size since, SOD-1 has about half the sample size of ADH and EST-1. Alternately, this difference may reflect a frequency change associated with increased population density and dispersal, a possibility reported by Gaines and Johnson (1987). Another possible

explanation is selection against heterozygotes. Individuals polymorphic for ADH and EST-1 came only from samples taken in the two dry years (1990/91) when population density was low whereas polymorphisms at the SOD-1 locus came only from individuals collected in the wet year, 1992, when population density was larger. It is also possible the SOD-1 locus was scored erroneously. More data are needed to differentiate between the possibile interpretations for the SOD-1 locus.

Nadler et al. (1978) reported that *Arvicola richardsoni macropus* (synonymous with *Microtus richardsoni macropus*) from the Wind River Mountains in Fremont County, Wyoming had a unique series of LAP alleles that distinquished it from other vole species. Anderson et al. (1976) reported the LAP locus to be polymorphic in *M. r. richardsoni* and Petcoff (1985) found it polymorphic in *M. r. arvicoloides* . Nadler et al.(1978) and Petcoff (1985) also reported 6-PGD to be polymorphic. Both of these loci were monomorphic in the Beartooth populations. Anderson et al.(1976) reported polymorphisms at EST- 2, 4, and 5 and Petcoff (1985) reported polymorphisms at EST-4, 5, 6. In this study, EST-1 was polymorphic, but EST-2 and 3 were monomorphic. Both Anderson et al. (1976) and Petcoff (1985) reported hemoglobin to be monomorphic and that was found in this study as well. The number of polymorphic loci is typically six or fewer in a vole population (Gaines et al., 1978; Gaines and Whittam, 1980; Kilpatrick and Crowell, 1985).

Data were grouped according to watershed from which the individual was sampled, mean heterozygosity across all loci, proportion of polymorphic loci, and F

statistics were calculated (Table 7). Nei (1987) recommends a sample size of 20 or 30 if the number of loci examined is 25. In this screening, 31 loci were examined, but the sample size varied.

Table 7. Sample size (n), mean heterozygosity ($\overline{H}$), proportion of polymorphic loci (P), fixation index (F_{ST}), and the estimate of the number of migrants per subpopulation per generation (N_m) for samples of water voles from four watersheds.

Watershed	n	$\overline{H}$	P	F_{ST}[1]	N_m[2]
Beartooth	18	0.03	0.03	0.17	1.22
Canyon	22	0.05	0.06	0.00	-
Rock	19	0.07	0.10	0.00	-
Line	2	0.03	0.06	0.25	0.75
Total/Mean	**61**	**0.05**	**0.06**	**0.11**	**2.02**[3]

1. $F_{ST} = \frac{H_T - \overline{H}_S}{H_T}$ (Hartl and Clark, 1989); 2. $F_{ST} = \frac{1}{4N_m + 1}$ (Nei, 1987)

3. Calculated from the mean F_{ST}, not the average of the column.

Sample sizes varied by watershed, with the Line Creek watershed represented by only two individuals. But, sample size is not as critical to heterozygosity estimates as the number of loci sampled (Gorman and Renzi, 1979; Nei, 1987). Only 8-12 individuals in a sample yield heterozygosity estimates within 1% of that calculated for larger samples, and 2 individuals yield heterozygosity estimates within 2.5% of larger samples (Gorman and Renzi, 1979). For all loci examined, Petcoff (1985) reported mean heterozygosity values of 0.07, 0.07 and 0.15 for three *M. r. arvicoloides* populations in the Cascades. Two of these means are the same as those reported for the Rock Creek watershed and are close to the overall mean heterozygosity across all four watersheds. Mean heterozygosity

values were not reported by Anderson et al. (1976) nor by Nadler et al. (1978) for this species. If only polymorphic loci are considered, the overall mean heterozygosity found in this study was 0.23. It is at the high end of the range (0.01 - 0.28) reported for *Microtus* species (Table 8).

Table 8. Comparison of mean heterozygosity ($\overline{H}$) and proportion of polymorphic loci (P) between some species of *Microtus*. NA indicates data were not available.

Species	Source	$\overline{H}$	P
M. richardsoni	This study	0.23*	0.06
M. pennsylvanicus	Kilpatrick and Crowell, 1985	0.13, 0.16	0.38, 0.42
M. pennsylvanicus	Kohn and Tamarin, 1978	NA	0.13
M. breweri	Kohn and Tamarin, 1978	0.10	0.07
M. californicus	Bowen, 1982	0.26	
M. chrotorrhinus	Kilpatrick and Crowell, 1985	0.01, 0.08, 0.06	0.28, 0.12, and 0.04
M. agrestis L.	Nygren and Rasmusen, 1980	0.22	0.5
M. miurus	Nadler et al., 1978	0.144	NA
M. ochrogaster	Gaines, et al., 1978	0.214	NA

*Only the three polymorphic loci were used to calculate this mean heterozygosity. Monomorphic loci were not included.

The average proportion of polymorphic loci across all four watersheds of this study was 0.06, low in comparison to values reported in other subspecies of water voles. It was 0.45 for M. *richardsoni richardsoni* (Anderson et al., 1976), and 0.43 for *M. richardsoni arvicoloides* (Petcoff, 1985). In comparison to other species of *Microtus*, the proportion of polymorphic loci is also low, except *M. breweri* which is endemic to Muskeget island, south of Cape Cod and one Essex Co. New York population of *M. chrotorrhinus* which is found in disjunct social groups inhabiting cold moist crevices of large rock fragments in Essex County, New York (Table 8).

If populations are isolated into subdivisions, homozygosity increases. This is measured as a decrease in heterozygosity (Hartl and Clark, 1989). The loss of heterozygosity due to genetic drift of an isolated subpopulation is described by the fixation index, F_{ST}, where zero implies no inbreeding and one implies complete inbreeding (Wright, 1978). Population subdivision is indirectly inferred from the heterogeneity of allozyme frequencies (Bowen, 1982). Bowen (1982), used the F_{ST} statistic to measure genetic differentiation of *Microtus californicus* during population cycles. It is used here to compare populations within each watershed. The two central and adjacent watersheds located on either side of Beartooth Pass, Canyon Creek and Rock Creek, have F_{ST} values of 0, indicating no loss of heterozygosity and little genetic differentiation (Wright, 1978; Table 7). The western-most watershed, Beartooth Creek, has an F_{ST} of 0.17 indicating moderate to large genetic differentiation and the eastern-most watershed, Line creek, has

an F_{ST} of 0.25 indicating a very large genetic differentiation (Wright, 1978). The overall mean F_{ST} of 0.11 indicates a moderate amount of genetic differentiation (Wright, 1978).

N_m is an estimate of the number of migrants/subpopulation/generation. A low N_m implies limited gene flow. When F_{ST} equals zero, N_m is sufficiently high to prevent genetic differentiation. Genetic drift is predicted to result in substantial local differentiation if N_m is less than one but not if N_m is greater than one (Hartl and Clark, 1989;Chakraborty and Leimar, 1987). Since Canyon Creek and Rock Creek watersheds have F_{ST} values of zero, dispersal is sufficiently high to prevent genetic differentiation. Beartooth Creek has an N_m value exceeding one so genetic drift should not cause local differentiation (Table 7). Water voles from the Line Creek watershed may be experiencing genetic drift since the N_m value is less than one, but more data are needed because the sample size from this watershed is so much smaller than the other three. The mean N_m is sufficiently large to suggest that, in general, no isolation has occurred.

Genetic distances and coancestry identities were calculated for the three polymorphic loci using GDA1.D5 (Lewis and Zaykin, 1996). A distance matrix based on the set of differences between each of the four watersheds was calculated (Table 9) and an unweighted pair-group method using an arithmetic mean (UPGMA) was used to make a phenogram (Figure 3). Water voles from the Beartooth Creek watershed were most similar to those from Canyon Creek and most divergent from those in Line Creek. This reflects the geographic arrangement of the watersheds from west to east. The same relationship is true between Line Creek and Rock Creek. In the UPGMA phenogram,

Beartooth Creek and Canyon Creek, both on the west side of Beartooth Pass clustered together as did Rock Creek and Line Creek which are both on the east side of Beartooth Pass.

Table 9. Distance matrix based on three loci for the four watersheds

Watershed	**Beartooth Creek**	**Canyon Creek**	**Rock Creek**
Canyon Creek	0.0000		
Rock Creek	0.1411	0.0748	
Line Creek	0.5372	0.3780	0.0000

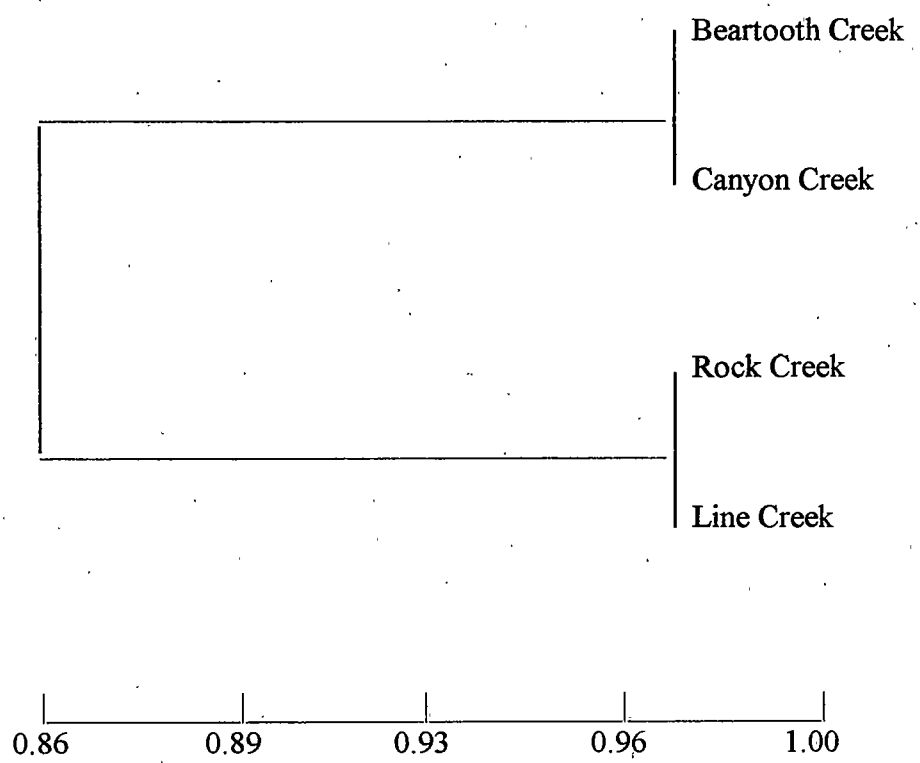

Figure 3. UPGMA phenogram based on three loci for the four watersheds

MITOCHONDRIAL DNA ANALYSIS

The use of allozymes to describe population structure of voles has been limited by the low number of polymorphic loci and because allozyme expression can be environmentally determined, hence subject to natural selection (Gaines et al., 1971;Gaines and Whittam, 1980; Kohn and Tamarin, 1978; McGovern and Tracy, 1981). The usefulness of allozymes to establish relationships between local subpopulations has also been limited (Avise et al., 1979b).

RFLP assays have been important for detecting mtDNA heterogeneity in a number of *Microtus* sp. (Debry, 1989; Jaarola and Tegelström, 1995; Plante et al., 1989a; Plante et al., 1989b; Thomas and Beckenbach, 1986). The rapid evolution of the mitchondrial genome (Brown et al., 1979, Olivo et al., 1983; Upholt and Dawid, 1977), the extensive mtDNA sequence polymorphism among and within species (Lansman, et al., 1983), the small amount of heterogeneity of mtDNA in one individual (Upholt and Dawid, 1977), and the apparent selective neutrality of restriction sites make mtDNA RFLP analysis a good approach for studying population substructure (Avise, 1986).

Total cellular DNA was extracted from the individual tail segments by homogenizing the tissue in 650µl 0.1 M EDTA and 0.2M Tris, 100µl 10% SDS, and 20µl Proteinase K. The homogenate was incubated at 65°C for 1 - 4 hours then at 35°C for 3 - 24 hours. The tissue was mashed with a glass pestle and 400µl of 5M potassium acetate was added. The homogenate was iced and then centrifuged before the supernatant was

decanted. DNA was extracted using a phenol-chloroform-isoamyl alcohol (25:24:1) mixture. The aqueous layer was extracted in a chloroform-isoamyl alcohol (24:1) mixture to remove the phenol and complete the protein extraction before centrifugation. The aqueous layer was mixed with an equal volume of cold isopropanol to precipitate the DNA and placed in a -20°C freezer for at least 2 hours. After centrifugation, the pellet was resuspended in 70% ethanol and centrifuged again. The pellet was air dried and resuspended in 300µl sterile water. RNA was removed with 1µl of standard 10mg/ml RNAase.

Each individual sample of 100-500 ng DNA was digested with a single restriction enzyme according to the manufacturer's directions (New England Biolabs or Sigma). From a total of 15 restriction enzymes, all 6-base cutters, 9 were polymorphic: BamHI, BclI, BstEII, DraI, EcoRI, EcoRV, HindIII, PstI, and PvuII. Fragments were separated electrophoretically on 1% agarose gels to which 1µl of 0.05 µg/ml per 50 ml of 1% agarose was added. Restriction fragments from HindIII cut λ DNA were used as molecular weight markers. Gels were photographed in shortwave UV light, blotted with the Southern technique (Southern, 1975) and hybridized to radioactive nick-translated mule deer mtDNA (Sambrook, et al., 1989). The Zetabind membranes were washed and autoradiographed with one intensifying screen on Kodak X-ray film (Sambrook, et al., 1989). The film was developed using Kodak GBX developer. Membranes were washed and stored in 50ml hybridization buffer in sealed seal-a-meal bags in the refrigerator (Sambrook, et al., 1989).

The size of fragments produced by each restriction enzyme was estimated and ordered into a site map. A mtDNA genome size of about 16,600 bp has been observed in other *Microtus* species and was assumed in development of the site map (Thomas and Beckenbach, 1986; Plante et al. 1989a). A data matrix with presence-absence (1,0) information for each individual and each restriction enzyme was compiled. From this data matrix, the number of restriction fragments and the number of unique patterns were summarized for each location sampled (Appendix, Table 14). Each unique pattern was assigned a letter (Appendix, Table 15). The haplotype patterns found in each watershed were designated by 9 letters that correspond to the restriction patterns obtained from each of the 9 enzymes. The frequency of each haplotype occurrence was estimated by watershed and each unique haplotype was assigned a number (Appendix, Table 16).

A total of 51 sites were found with the 9 restriction enzymes. Nineteen of these sites were polymorphic for the Beartooth water voles and resulted in 29 different haplotypes from 142 individuals. The number of unique haplotypes and their frequencies are similar to those reported by Plante et al. (1989a) for *Microtus pennsylvanicus*. Debry (1989) found 2 different haplotypes that differed at 3 sites for 4 individual water voles from Red Cliff campground near Big Sky, Gallatin Co., Montana.

Most mtDNA haplotypes were localized geographically. Shared haplotypes were geographically contiguous except for haplotype #1 which was not found in the Canyon Creek sample, but was shared among samples from the other watersheds. A similar

geographic pattern of haplotypes was also found to occur in *Geomys pinetus*, the pocket gopher (Avise et al., 1979b).

The total number of haplotypes, the frequency of unique haplotypes localized in each watershed and the estimated haplotype diversity is summarized in Table 10. The estimate of haplotype diversity ($\hat{h}$) is analogous to heterozygosity computations for allozymes (Nei and Tajima, 1981). High diversity values are close to 1.0 and low diversity values are close to 0. Estimated haplotype diversity ranged from 0.81 in the Rock Creek watershed to 0.28 in the Canyon Creek watershed and was within the range reported for *Microtus pennsylvanicus* (Plante, et al., 1989a).

Table 10. Total number of haplotypes, the frequency of unique haplotypes, and the estimated haplotype diversity by watershed

Watershed	N	Total # Haplotypes	Unique haplotype freq.	Haplotype Diversity [1]
Beartooth Creek	34	10	0.60	0.79
Canyon Creek	26	4	0.25	0.28
Rock Creek	68	19	0.74	0.81
Line Creek	13	4	0.50	0.65

1. $\hat{h} = \frac{n\left(1 - \sum_{i=1}^{l} x_i^2\right)}{n-1}$ (Nei and Tajima, 1981)

Except for Line Creek, three sites from each watershed were sampled. Rock Creek watershed has the most haplotype diversity as well as the largest number of unique haplotypes. The Rock Creek watershed is different because it is composed of 3 distinct geographically separate drainages; Beartooth Plateau Creek, Quad Creek and

Wyoming Creek (Figure 2). However, the Beartooth Creek watershed also has high haplotype diversity and the sampling sites were located along one drainage, as they were in the other watersheds (Figure 2). Voles from Beartooth Creek and Rock Creek watersheds share three haplotypes represented by a total frequency of 0.41 in the Beartooth Creek watershed and 0.34 in the Rock Creek watershed. Line Creek voles share one haplotype with the voles from Beartooth Creek watershed with a frequency of 0.31 and one haplotype, at a frequency of 0.13, with the voles from the Rock Creek watershed.

The RESTSITE program was used to compute the number of nucleotide substitutions per site (genetic distance) by the Jukes-Cantor algorithm between water voles from each sampling location for all years combined (Miller, 1990;Table 11). Standard errors were derived by jackknifing (Nei and Miller, 1990).

The water voles sampled from the Canyon Creek watershed were very similar and had low genetic distances. The haplotype diversity was also considerably lower for this watershed (Table 11). The voles of Canyon Creek and voles from Beartooth Creek below Island Lake were very similar and their genetic distance was low. This suggests dispersal is occurring between these watersheds. They are adjacent to each other on the west side of Beartooth Pass. A small 12.2 meter hill separates Little Bear Lake (Beartooth Creek watershed) from Long Lake (Canyon Creek watershed) by about 3 km (Appendix, Table 12). By the nearest water route, they are separated by about 39 km (Appendix, Table 12).

Table 11. Jukes-Cantor genetic distances between water voles from each site are in the lower matrix and standard errors by jackknifing are in the upper matrix (Miller, 1990). Sites are arranged in order from west to east over Beartooth Pass.

Site	LBL	AIL	BIL	AFL	ALL	SFC	BTP	QC	WC	LC	TPE	TPW
LBL		.0005	.0002	.0013	.0013	.0012	.0018	.0003	.0020	.0016	.0129	.0132
AIL	.0004		.0004	.0018	.0007	.0007	.0004	.0010	.0012	.0004	.0128	.0125
BIL	.0001	.0003		.0001	.0002	.0000	.0010	.0009	.0021	.0010	.0128	.0142
AFL	.0022	.0018	.0000		.0001	.0000	.0036	.0019	.0024	.0037	.0127	.0153
ALL	.0023	.0017	.0002	.0001		.0000	.0036	.0019	.0018	.0037	.0127	.0153
SFC	.0018	.0017	.0000	.0000	.0001		.0037	.0015	.0025	.0037	.0126	.0153
BTP	.0025	.0010	.0024	.0037	.0038	.0036		.0013	.0019	.0002	.0125	.0111
QC	.0004	.0015	.0015	.0034	.0032	.0029	.0022		.0010	.0008	.0129	.0124
WC	.0022	.0017	.0025	.0042	.0032	.0040	.0027	.0012		.0008	.0130	.0120
LC	.0018	.0009	.0024	.0042	.0040	.0040	.0001	.0012	.0010		.0127	.0112
TPE	.0174	.0172	.0184	.0192	.0194	.0185	.0166	.0166	.0186	.0161		.0002
TPW	.0235	.0221	.0257	.0275	.0277	.0268	.0189	.0215	.0234	.0185	.0001	

Beartooth Creek Watershed:**LBL**=Little Bear Lake, **AIL**=Above Island Lake, **BIL**=Below Island Lake; Canyon Creek Watershed: **AFL**=Above Frozen Lake, **ALL**=Above Long Lake, **SFC**=South Fork Canyon Creek; Rock Creek Watershed: **BTP**=Beartooth Plateau Creek, **QC**=Quad Creek, **WC**=Wyoming Creek; Line Creek Watershed=**LC**; **TPE**= Togwotee Pass East side, and **TPW**=Togwotee Pass West side.

The voles from Beartooth Plateau, Quad and Wyoming Creeks within the Rock Creek watershed exhibit genetic distances an order of magnitude greater than the Canyon Creek voles. This is consistent with its high haplotype diversity and its high number of unique, geographically localized haplotypes. Voles from Beartooth Plateau Creek are more similar to voles from Line Creek, by an order of magnitude, than they are to voles from the other creeks within the Rock Creek watershed. Beartooth Plateau Creek is

about 7 km from Line Creek by land, but about 262 km by water (Appendix, Table 12). Voles from Quad Creek were more distant, by an order of magnitude, from voles in both Beartooth Plateau Creek and Wyoming Creek, all within the Rock Creek watershed, than from voles at Little Bear Lake. Quad Creek is 13 km by land and 290 km by water from Little Bear Lake (Appendix, Table 12). Matrix correlation of the genetic distance matrix was made with the air and water distance matrices using the Mantel test and cophenetic correlation (Rohlf, 1993). No significant correlations were found. Genetic structuring of the Beartooth water voles was not found. This supports the RESTSITE data.

Voles from the east and west sides of Togwotee Pass were similar to each other genetically, but differed by an order of magnitude from voles at any of the Beartooth sites. The average genetic distance between water voles from Togwotee Pass in the Absaroka Mountains and the water voles from all four watersheds in the Beartooth mountains was estimated to be 0.02. If mtDNA diverges at a rate of 2 - 4 % every million years (Brown et al., 1979), these two groups of water voles have been separated an estimated 0.5 to 1.0 million years ($D = 1 - e^{-\alpha t}$;Smith, 1996). Geographically, they are separated by a straight line overland distance of about 150 km. Togwotee Pass is on the continental divide. Creeks on the west side drain into the Columbia River basin and have no point of confluence with the drainages from the Beartooth Mountain sites while the east side ultimately drains into the Yellowstone River east of Billings, Montana. The distance between the Togwotee Pass voles and the Beartooth voles is the only significant branch length in the tree, because its variance was less than the branch length (Figure 4).

The Togwotee Pass voles were the outgroup that rooted the neighbor-joining tree near the Line Creek voles. The other sites cluster by watershed from east to west on the tree (Figure 4). Branch lengths and nodes for Figure 4 are in the Appendix, Table 17. This is consistent with the allozyme results (Figure 3).

Significant differences were not found in the distance values between voles captured during the dry summers of 1990 and 1991 with those from the wet summer of 1992.

A distance comparison was also made between two sympatric vole species, *Microtus richardsoni* and *Microtus montanus*. The estimated genetic distance between them was 0.12, indicating these species diverged approximately 3.25 to 6.5 million years ago (Brown et al., 1979; Smith, 1996).

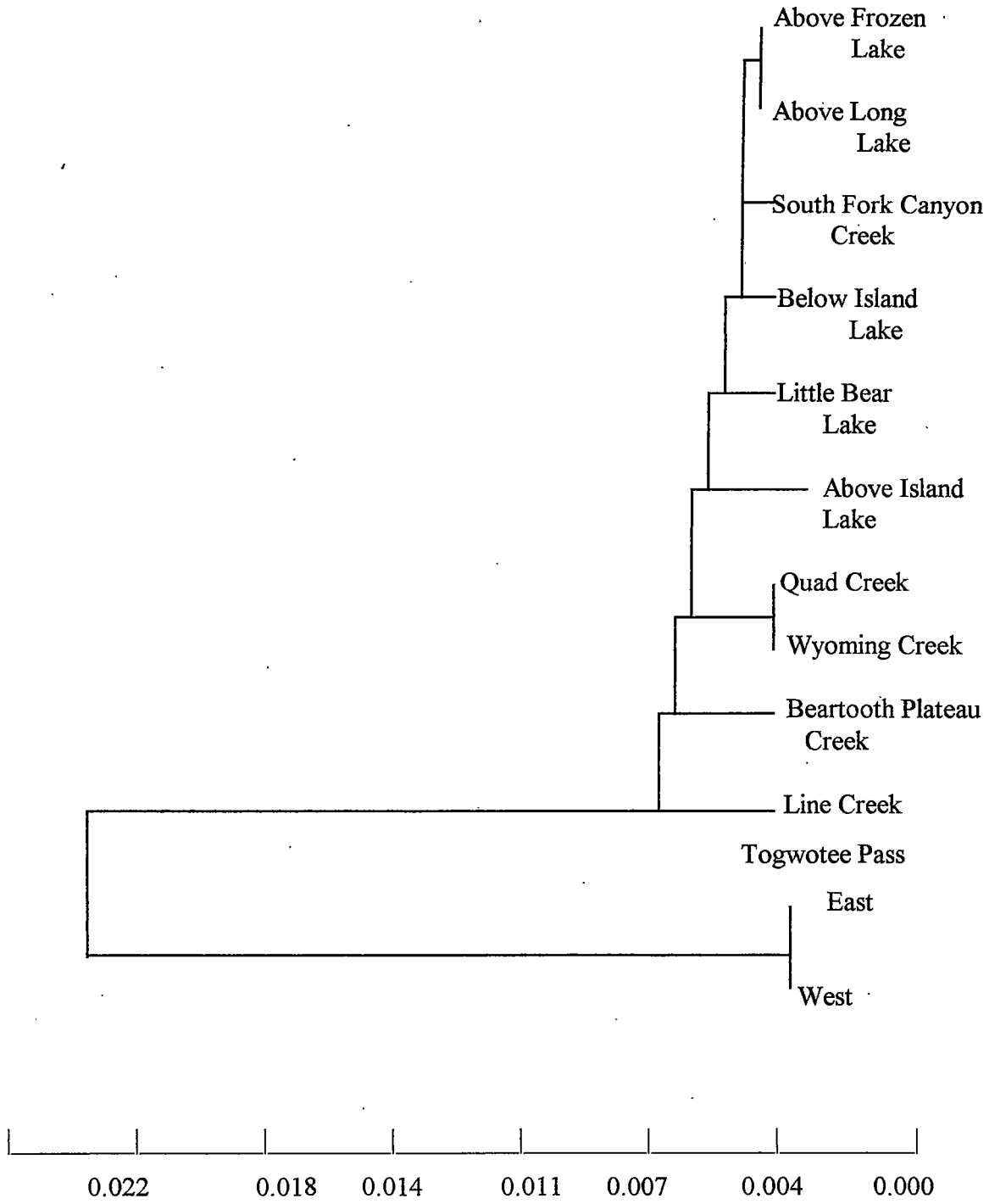

Figure 4. Neighbor-joining clustering based on Jukes-Cantor distances between water voles from each sampling site.

CONCLUSIONS

There is no evidence for geographic isolation of water voles from the four adjacent watersheds in the Beartooth Mountains. Using RESTSITE analysis of the mtDNA data, only the outgroup from Togwotee Pass showed isolation. The mtDNA data were the most robust in terms of sample size and polymorphisms. Also, no significant differences were found using the Mantel test to compare the genetic distance matrix with the air and water distance matrices. The allozyme data supported this too, with the possible exception of voles from Line Creek where the N_m value indicated genetic drift may be occurring. However, this is doubtful since the allozyme sample size was small and the mtDNA data did not support it.

While water voles are rarely captured away from water, their ability to disperse overland is suggested. The low genetic distance between voles from the Beartooth watersheds indicate they are not isolated and that gene flow is occurring between them. The direct land distance between sampling locations is small in comparison to some of the water routes between sites (Appendix, Table 12). Furthermore, these water routes include large waterfalls, swift currents, and many kilometers of lower elevation stream that lack the specific habitat requirements of water voles. These lines of evidence, coupled with their relatively short 16 month lifespan and the severity of winters throughout the region, make it likely that water voles can disperse relatively short overland distances from one watershed to an adjacent one.

Should they become locally extinct, they do appear to have the capacity to recolonize a site from another, close location. However, they do not readily disperse the 150 km straight line distance to Togwotee Pass, even though the Absaroka and the Beartooth Mountains are adjacent. This is consistent with Brown (1971) who found that low elevation areas were barriers to small boreal mammals who colonized mountains during the Pleistocene and that " a few thousand feet of elevation, with the associated differences in climate and habitat, constitute a nearly absolute barrier to dispersal by small mammals (with the exception of bats)." He noted that there have been extinctions but no colonizations since that time and that immigration to isolated mountains was very restricted.

Given the number of unique haplotypes found among the Beartooth voles, the genetic distance between the Beartooth and Togwotee Pass voles, and the disjunct nature of their range, it is likely that unique genetic types would be found at more distant as well as more isolated sites. This should be confirmed in other studies, particularly in the areas where water voles are listed as sensitive species.

LITERATURE CITED

Anderson, P. K., P.H. Whitney, and J. Huang, 1976. *Arvicola richarsoni*: Ecology and biochemical polymorphism in the front ranges of southern alberta. Acta Ther. 21:425-468.

Anderson, S. 1985. Taxonomy and Systematics. Pages 52-83, in R.H. Tamarin, editor, Biology of New World *Microtus*, Special Publication #8, American Society of Mammalogists.

Anthony, R.G., E.D. Forsman, G.A. Green, G. Witmer, and S.K. Nelson. 1987. Small mammal populations in riparian zones of different-aged coniferous forests. The Murrelet. 68:94-102.

Avise, J.C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. R. Soc. Lond. B 312:325-342.

Avise, J.C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43(6):1192-1193.

Avise, J.C., R.A. Lansman, and R.O. Shade. 1979a. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. Genetics, 92:279-295.

Avise, J.C., C. Giblin-Davidson, J. Laerm, J.C. Patton and R.A. Lansman. 1979b. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proc.Natl. Acad. Sci. USA. 76:6694-6698.

Banfield, A. W. F. 1974. The Mammals of Canada. Univ. of Toronto Press, Toronto, 438pp.

Berry, R. J. and J. Peters. 1981. Allozymic Variation in House Mouse Populations. Pages 242-253 in M.H.Smith and J. Joule, eds. Mammalian Population Genetics, Univ. of Georgia Press, Athens, GA.

Birdsall, D. A. 1972. Allele and phenotype frequency variation of three loci in natural populations of deer mice (*Peromyscus maniculatus*). Edmonton, Alberta:Univ. of Alberta. Dissertation. p94-95.

Blankenship, A. 1995. Grazing permit reissuance, USDA-Forest Service, Region 2, Rocky Mountain Region, biological evaluation for the water vole, *Microtus richardsoni*. Denver, Colorado.

Bowen, B.S., 1982. Temporal dynamics of microgeographic structure of genetic variation in *Microtus californicus*. Journal of Mammalogy, 63:625-638.

Bowen, B.S. and Koford, R.R., 1987. Dispersal, population size, and genetic structure of *Microtus californicus*: empirical findings and computer simulation. Pages 180-192 in B.D. Chepko-Sade and Z.T. Halpin, editors. Mammalian dispersal patterns - the effects of social structure on population genetics. Univ. Chicago Press, Chicago, Il.

Britten, H.B. 1991. The conservation biology of the uncompahgre fritillary and the related northern dingy fritillary, Bozeman, Montana:Montana State Univ. Dissertation.

Brown, J.H., 1971. Mammals on mountaintops: nonequilibrium insular biogeography. The American Naturalist, 105:467-477.

Brown, L. N. 1977. Litter size and notes on reprodution in the giant water vole, (*Arvicola richardsoni*). Southwestern Naturalist, 22:281-282.

Brown, W.M., M. George, Jr., and A.C. Wilson, 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 76:1967-1971.

Burns, J.A. 1982. Water vole *Microtus richardsoni* (Mammalia, Rodentia) from the Late Pleistocene of Alberta. Canadian Journal of Earth Science. 19:628-631.

Chakraborty, R. and O. Leimar. 1987. Genetic variation within a subdivided population. Pages 89-120 in N. Ryman and F.Utter, editors. Population Genetics and Fishery Management. Univ. of Washington Press, Seattle, WA.

Clark, T.W. and M.R. Stromberg, 1987. Mammals in Wyoming. University of Kansas Museum of Natural History, Public Education Series No. 10. p. 173-175.

Clayton, J.W. and D.N. Tretiak. 1972. Aminecitrate Buffers for pH Control in Starch Gel Electrophoresis. J.Fish. Res. Board, Can. 29:1169-1172.

Connor, J.T. and R.M. White. 1965. World Weather Records 1951-1960. Vol. 1, North America. published by U.S. Dept. of Commerce and Weather Bureau Washington, D.C. p141-142.

Debry, R. W., 1989. Molecular systematics and biogeography of some new world *Microtus* (Mammalia:Arvicolidae). Lansing, Michigan, Michigan State Univ., dissertation.

Friedlander, J. 1995. R-2 Sensitive species evaluation sheet. USDA-Forest Service, Rocky Mountain Region, Denver, Colorado.

Gaines, M.S. and M.L. Johnson, 1987. Phenotypic and genotypic mechanisms for dispersal in *Microtus* populations and the role of dispersal in population regulation. Pages 162-179 in B.D. Chepko-Sade and Z.T. Halpin, editors. Mammalian Dispersal Patterns - the effects of social structure on population genetics. Univ. Chicago Press, Chicago, Il.

Gaines, M.S. and C.J. Krebs, 1971. Genetic changes in fluctuating vole populations. Evolution, 25:702-723.

Gaines, M.S., L.R. McClenaghan, Jr. and R.K. Rose. 1978. Temporal patterns of allozymic variation in fluctuating populations of *Microtus ochrogaster*. Evolution: 32: 723-739.

Gaines, M.S., J. H. Myers, and C.J. Krebs, 1971. Experimental Analysis of relative fitness in transferrin genotypes of *Microtus ochrogaster*. Evolution, 25:443-453.

Gaines, M.S. and T.S. Whittam, 1980. Genetic Changes in fluctuating vole populations: selective vs. nonselective forces. Genetics, 96:767-778.

Getz, L.L. 1985. Habitats. Pages 286-309 in R.H. Tamarin, editor, Biology of New World *Microtus*. Special Publication #8, American Society of Mammalogists..

Gorman, G.C. and J.Renzi, Jr., 1979. Genetic distance and heterozygosity estimates in electrophoretic studies:effects of sample size. Copeia, 2:242-249.

Hall, E.R. 1981. The Mammals of North America. Second ed. John Wiley, New York, 2:601-1181pp.

Hartl, D.L. and A.G. Clark. 1989. Principles of Population Genetics, Second ed. Sinauer Assoc., Inc. Sunderland, Mass. p38.

Hoffmann, R.S. and J. K. Jones, Jr. 1970. Influence of late-glacial and post-glacial events on the distribution of recent mammals on the northern great plains. pages 356-394, Dort, W. and Jones, J.K. editors. Pleistocene and Recent Environments of the Central Great Plains. Publication 3, Dept. of Geology, Univ. of Kansas, Lawrence.

Hoffmann, R.S. and J. W. Koeppl. 1985. Zoogeography. Pages 84-115 in R.H. Tamarin, editor, Biology of New World *Microtus*. Special Publication #8, American Society of Mammalogists.

Hollister, N. 1912. Mammals of the Alpine Club expedition to Mount Robson Region. Can. Alp. J., Sp. No. 1912:1-75.

Hooven, E.F. 1973. Notes on the water vole in Oregon. J. Mammalogy, 54:751-752.

Jaarola, M. and H. Tegelström, 1995. Colonization history of north European field voles (*Microtus agrestis*) revealed by mitochondrial DNA. Molecular Ecology, 4:299-310.

Kessler, L.G. and J.C. Avise, 1985. Microgeographic lineage analysis by mitochondrial genotype: variation in the cotton rat (*Sigmodon hispidis*). Evolution 39:831-837.

Kilpatrick, C.W. and K.L. Crowell, 1985. Genic variation of the rock vole, *Microtus chrotorrhinus*. Journal of Mammalogy, 66:94-101.

King, C. 1997. Wildlife Biologist, Clarks Fork District of the Shoshone National Forest, personal communication.

Kohn, P.H. and R.H. Tamarin, 1978. Selection and electrophoretic loci for reproductive parameters in island and mainland voles. Evolution, 32:15-28.

Lansman, R.A., J.C. Avise, C.F. Aquadro, J.F. Shapira and S.W. Daniel, 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Peromyscus maniculatus*. Evolution, 37:1-16.

LeDuc, J. and C.J. Krebs. 1975. Demographic consequences of artificial selection at the LAP locus in voles (*Microtus townsendii*). Canadian Journal of Zoology, 53:1825-1840.

Lewis, P.O. and D. Zaykin, 1996. Genetic Data Analysis version 1.0d5 (16-bit) for Windows 3.1.

Li, W.H. and D. Graur. 1991. Fundamentals of Molecular Evolution. Sinauer Associates, Inc., Sunderland, Mass. 86-96

Lidicker, W.Z. 1985. Dispersal. Pages 420-454 in R.H. Tamarin, editor. Biology of New World *Microtus*. Special publication No. 8. The American Society of Mammalogists.

Lidicker, W.Z. and J.L. Patton, 1987. Patterns of dispersal and genetic structure in populations of small rodents. Pages 144-161. B.D. Chepko-Sade and Z.T. Halpin, editors. Mammalian dispersal patterns - the effects of social structure on population genetics. Univ. Chicago Press, Chicago, IL.

Luce, B. 1995. Interim completion report, sensitive species inventory, Challenge Cost-Share Agreement #110214109410, Wyoming Game and Fish Department and Shoshone National Forest.

Ludwig, D. R. 1981. The population biology and life history of the water vole, *Microtus richardsoni*. Calgary, Alberta: University of Calgary; Dissertation.

Ludwig, D.R. 1984. *Microtus richardsoni* Microhabitat and Life History. Winter Ecology of Small Mammals. Special Publication, Carnegie Museum of Natural History. No. 10, Pittsburgh, Pa. 380pp.

Ludwig, D.R. 1988. Reproduction and population dynamics of the water vole, *Microtus richardsoni*, Journal of Mammalogy, 69:532-541.

May, B.J., J.E. Wright, and M. Stoneking. 1979. Joint Segregation of Biochemical Loci in Salmonidae: Results from Experiments with *Salvinus* and a Review of the Literature of Other Species. J. Fish. Res. Board Can. 36:1114-1128.

McGovern, M. and C.R. Tracy, 1981. Phenotypic variation in electromorphs previously considered to be genetic markers in *Microtus ochrogaster*. Oecologia, 51:276-280.

McGovern, M. and C.R. Tracy, 1985. Physiological plasticity in electromorphs of blood proteins in free-ranging *Microtus ochrogaster*. Ecology, 66:396-403.

Mihok, S., W.A. Fuller, R.P. Canham, and E.C. McPhee, 1983. Genetic changes at the transferrin locus in the red-backed vole (*Clethrionomys gapperi*). Evolution, 37(2):332-340.

Miller, J. 1990. RESTSITE v1.1 for dos. Whitehead Institute, 9 Cambridge Center, Cambridge, Mass.

Nadler, C.F., N.M. Zhurkevich, R.S. Hoffman, A.I. Kozlovskii, L. Deutsch, and C.F. Nadler, Jr. 1978. Biochemical relationships of the Holarctic vole genera (*Clethrionomys, Microtus, and Arvicola* (Rodentia: Arvicolinae). Canadian Journal of Zoology, 56:1564-1575.

Negus, N.C., and J. W. Findley. 1959. Mammals of Jackson Hole, Wyoming. Journal of Mammalogy, 40:371-381.

Nei, M. 1975. Molecular Population Genetics and Evolution. North-Holland Publishing Co., Amsterdam, p. 288.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, NY. p287-386

Nei, M. and Tajima, F. 1981. DNA polymorphism detectable by restricion endonucleases. Genetics, 97:145-163.

Nei, M. and J.C. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. Genetics, 125:873-879.

Nygren, J. 1980. Allozyme variation in natural populations of field vole (*Microtus agrestis* L.) III. Survey of a cyclically density-varying population. Hereditas, 93:125-136.

Nygren, J. and M. Rasmuson, 1980. Allozyme variation in natural populations of field vole (*Microtus agrestis* L.) I. Survey of the 'semi-stable' population in southern Sweden. Hereditas, 92:65-72.

Olivo, P.D., J.J. Van de Walle, P.J. Laipis and W. W. Hauswirth, 1983. Nucleotide sequence evidence for rapid genotypic shifts in the bovine mitochondrial DNA D-Loop. Nature, 306:400-402.

Pattie, D.L. 1967. Dynamics of Alpine Small Mammal Populations. Helena, Montana: University of Montana. Dissertation. p63.

Petcoff, D.W. 1985. Biochemical Polymorphism in the water vole, *Microtus richardsoni*, of the Cascades. Central Washington University,WA. p.17.

Plante, Y., P. T. Boag and B. N. White. 1987. Nondestructive sampling of mitochondrial DNA from voles (*Microtus*). Canadian Journal of Zoology, 65:175-180.

Plante, Y., P.T. Boag, and B.N. White, 1989a. Macrogeographic variation in mitochondrial DNA of meadow voles (*Microtus pennsylvanicus*). Canadian Journal of Zoology, 67:158-167.

Plante, Y., P.T. Boag, and B.N. White, 1989b. Microgeographic variation in mitochondrial DNA of Meadow voles (*Microtus pennsylvanicus*) in relation to population density. Evolution, 43:1522-1537.

Racey, K., and I.M. Cowan. 1935. Mammals of the Alta Lake Region of Southwestern British Columbia. Report of Provincial Museum of British Columbia for the year 1935. 15-29

Racey, K. 1960. Notes Relative to Fluctuations in numbers of *Microtus richardsoni* about Alta Lake and Pemberton Valley, B.C. The Murrrelet. 41:13-14.

Reichel, J.D. 1986. Habitat use by alpine mammals in the Pacific Northwest, USA. Arctic and Alpine Research, 18:111-119.

Rohlf, F.J. 1993. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, version 1.80. Exeter Software, New York.

Rose, R.K. and M.S. Gaines, 1981. Relationships of genotype, reproduction and wounding in Kansas prairie voles. Pages 160-179 in M.H. Smith and J. Joule, editors. Mammalian population genetics, Univ. Georgia Press, Athens, GA.

Russel, P.J. 1996. Genetics, 4th edition. Harper Collins College Publishers, New York, NY.

Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989. Molecular cloning- a laboratory manual, 2nd. ed. Cold Spring Harbor Laboratory Press, Plainview, N.Y.

Sanders, J. 1990. Range Manager, Clarks Fork District of the Shoshone National Forest, personal communication.

Selander, R.K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry, 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse *Peromyscus polionotus*. Studies in Genetics VI. Univ. Texas Publ. 7103:49-90.

Semeonoff, R. 1972. Esterase polymorphism in *Microtus ochrogaster*: Interaction and Linkage. Biochemical Genetics, 6:125-138.

Semeonoff, R. and F.W. Robertson, 1968. A biochemical and ecological study of plasma esterase polymorphism in natural populations of the field vole, *Microtus agrestis* L. Biochemical Genetics, 1:205-227.

Simpson, G.C., G. Walker, R.C. Mossman, F. L. Clayton. 1932. World Weather Records 1921-1930, Vol. 90, North America. publication of U.S. Department of Commerce and Weather Bureau,Washington, D.C. p 302.

Smith, R. 1996. P.E., TSP Engineering Consultants, Sheridan, WY. Personal communication.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology, 98:503-517.

Strauss, L.L. and F.W. Reichelderfer. 1959. World Weather Records 1941-1950. publication of U.S. Department of Commerce and Weather Bureau, Washington, D.C. p. 910.

Taitt, M.J. and C. J. Krebs. 1985. Population Dynamics and Cycles. Pages 567-620 in R. H. Tamarin, editor. Biology of New World *Microtus*. Special Publication #8, American Society of Mammalogists.

Tamarin, R.H. and C.J. Krebs. 1969. *Microtus* population biology. II. Genetic changes at the transferrin locus in fluctuating populations of two vole species. Evolution, 23:183-211

Thomas, W.K. and A.T. Beckenbach, 1986. Mitochondrial DNA restriction site variation in the Townsend's vole, *Microtus townsendii*. Canadian Journal of Zoology, 64:2750-2756.

Upholt, W.B. and I.B. Dawid, 1977. Mapping of mitochondrial DNA of individual sheep and goats:rapid evoluiton in the D Loop region. Cell, 11:571-583.

USDA-Forest Service. 1994. Sensitive Species. USDA-Forest Service Manual 2670.22. Denver, Colorado. p14

USDA-Soil Conservation Service Snow Survey Group, 1995. USDA-Soil Conservation Service. Bozeman, Montana.

Wright, S. 1978. Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. Univ. Chicago Press, Chicago, Il.

Zar, J.H. 1996. *Biostatistical Analysis*, Third edition. Prentice Hall, Upper Saddle River, New Jersey. p282-283.

APPENDIX

Table 12. The bottom matrix is the estimated straight line overland distance between drainages at their closest approach in km. The top matrix is the estimated distance between sample sites by the closest water route in km.

Site	LBL	AIL	BIL	AFL	ALL	SFC	BTP	QC	WC	LC
LBL		2	5	40	39	37	298	290	296	130
AIL	2		2	38	37	35	296	288	294	129
BIL	4	2		36	35	33	294	286	292	126
AFL	4	5	6		1	3	282	274	280	115
ALL	3	4	5	1		2	281	273	279	114
SFC	3	4	6	1	1		283	278	281	115
BTP	6	6	8	2	3	3		11	21	262
QC	13	14	15	9	10	10	8		14	254
WC	10	11	12	6	7	7	4	6		260
LC	11	12	13	8	9	8	7	7	2	

Beartooth Creek Watershed:**LBL**=Little Bear Lake, **AIL**=Above Island Lake, **BIL**=Below Island Lake; Canyon Creek Watershed: **AFL**=Above Frozen Lake, **ALL**=Above Long Lake, **SFC**=South Fork Canyon Creek; Rock Creek Watershed: **BTP**=Beartooth Plateau Creek, **QC**=Quad Creek, **WC**=Wyoming Creek: Line Creek Watershed=**LC**.

Table13. Loci and conditions used for electrophoresis of allozymes

Locus	Enzyme or Protein	Buffer
AAT-1, 2	Aspartate aminotransferase	C[1]
*ADH	Alcohol dehydrogenase	R[2]
DIA	NADH diaphorase	R
EST-*1,2,3	Esterase	4[3]
GAPDH	Glyceraldehyde-3-phospahte dehydrogenase	C
GP	General protein	R
G6PDH	Glucose-6-phosphate dehydrogenase	C
GPI	Glucosephosphate isomerase	4
HBDH	Hydroxybuteric dehydrogenase	C
Hb	Hemoglobin	C
IDH-1	Isocitrate dehydrogenase	C
LAP	Leucine aminopeptidase	C
LDH-1,2	Lactate dehydrogenase	4
ME-1,2,3	Malic enzyme	C
MDH-1,2	Malate dehydrogenase	C
MPI	Mannosephosphate isomerase	R
PEP-A	Peptidase-A (leucyl-alanine or L-valyl-1-leucine substrate)	R
6-PGD	Phosphogluconate dehydrogenase	4
PGM-2,3	Phosphoglucomutase	4 or C
SDH-1	Sorbitol dehydrogenase	4
SOD-*1,2	Superoxide dismutase	C
XDA	Xanthine dehydrogenase	R

*Loci found to be polymorphic in this study

[1]Clayton and Tretiak, 1972; [2]Selander et al., 1971; [3]Britten, 1991

Table 14. The number of mtDNA restriction fragments obtained from each enzyme at each locality and the number of unique fragment patterns (in parentheses)

Locality	n	EcoRI	PvuII	BclI	BamHI	HindII	DraI	EcoRV	BstEI	PstI
Beartooth Ck										
Little Bear L	18	5(3)	3(2)	3(2)	3(1)	7(2)	6(1)	3(1)	4(1)	3(2)
Above Island L	10	5(4)	3(2)	3(2)	5(2)	7(2)	6(1)	3(1)	4(1)	3(2)
Below Island L	7	4(2)	3(2)	3(2)	3(1)	8(2)	6(1)	3(1)	4(1)	2(1)
Canyon Ck										
Above Frozen	5	3(1)	3(1)	3(1)	3(1)	6(1)	6(1)	3(1)	4(1)	2(1)
Above Long L	15	3(1)	3(1)	3(1)	5(2)	6(1)	6(1)	3(1)	4(1)	2(1)
SF Canyon Ck	12	5(2)	3(2)	3(2)	3(1)	6(1)	6(1)	3(1)	4(1)	2(1)
Rock Creek										
BT Plateau Ck	32	5(3)	3(2)	3(2)	3(1)	6(1)	6(2)	3(1)	4(1)	3(2)
Quad Creek	20	6(3)	3(2)	3(2)	5(2)	6(1)	6(2)	3(1)	4(1)	3(2)
Wyoming	22	6(3)	3(2)	3(2)	5(3)	6(1)	6(1)	3(1)	4(1)	2(1)
Line Creek	13	5(2)	3(2)	3(2)	5(2)	6(1)	6(1)	3(1)	4(1)	2(1)
Togwotee Pass										
East side	2	7(2)	4(1)	3(2)	3(1)	6(1)	6(1)	5(1)	4(1)	2(1)
West side	5	5(1)	4(2)	2(1)	3(1)	6(1)	7(2)	5(1)	5(2)	3(2)
M. montanus	3	3(1)	4(1)	3(1)	3(1)	2(1)	ns*	5(1)	ns	2(1)

*not scorable

Table 15. Letter designations for each unique restriction pattern by enzyme

Enzyme	A	B	C	D	E	F	G
EcoRI	1	1	1	1	1	1	1
	1	1	1	1	1	0	1
	1	1	0	0	0	1	0
	0	0	0	1	1	0	1
	0	0	1	1	0	0	1
	0	1	0	0	0	1	0
	0	0	0	0	0	0	0
PvuII	1	1	1	1			
	0	1	1	1			
	1	1	1	1			
	0	0	0	1			
	0	0	1	1			
BclI	1	1	1				
	1	1	1				
	0	1	0				
	0	0	1				
BamHI	1	1	0				
	1	1	1				
	1	0	0				
	0	1	1				
	0	1	1				
HindIII	1	0	0	1			
	1	1	1	0			
	1	0	0	0			
	1	1	1	0			
	1	0	0	0			
	1	1	0	0			
	0	1	0	0			
	0	0	1	0			
	0	0	1	1			
DraI	1	1	1				
	1	1	0				
	1	0	1				
	1	1	1				
	1	0	1				
	1	1	1				
EcoRV	1	1	1				
	1	1	0				
	1	0	0				
	0	1	0				
	0	1	0				
	1	1	0				

	A	B	C	D	E	F	G
BstEII	1	1	1				
	1	1	1				
	1	0	0				
	1	1	1				
	0	1	0				
PstI	1	1					
	1	1					
	0	1					

Table 16. MtDNA composite phenotypes found in each watershed including the outgroup from Togwotee Pass, WY and *Microtus montanus*

Location	n	Frequency(1)	Phenotype (2)	Haplotype #
Beartooth Creek	34	0.26	AAAAAAAAA	1
		0.06	AABAAAAAA	2
		0.03	BABABAAAA	3
		0.03	AAAAAAAAB	4
		0.38	CBBAAAAAA	5
		0.06	ABAAAAAAA	6
		0.09	ABBAAAAAA	7
		0.03	AABBBAAAB	8
		0.03	BABAAAAAA	9
		0.03	ABBACAAAA	10
Canyon Creek	26	0.04	AABAAAAAA	2
		0.04	ABAAAAAAA	6
		0.85	ABBAAAAAA	7
		0.08	ABBBAAAAA	11
Rock Creek	68	0.21	AAAAAAAAA	1
		0.09	AABAAAAAA	6
		0.04	ABBAAAAAA	7
		0.01	EBBAABAAA	12
		0.28	DBBAAAAAA	13
		0.01	ABAAABAAA	14
		0.03	DBAAAAAAB	15
		0.07	DBBAAAAAB	16
		0.01	AABAAAAAB	17
		0.01	DAAAAAAAB	18
		0.07	DBBBAAAAA	19
		0.01	ABBBAAAAB	20
		0.01	DBAAAAAAA	21
		0.01	AAAAABAAA	22
		0.04	AAABAAAAA	23
		0.01	DBBAABAAB	24
		0.01	BBBAAAAAA	25
		0.01	BBBBAAAAA	26
		0.01	ABBBAAAAA	27
Line Creek	13	0.31	AAAAAAAAA	1
		0.13	DBBAAAAAA	13
		0.08	DBABAAAAA	28
		0.08	DABBAAAAA	29

Togwotee Pass	7	0.14	FCCAAABDA	30	
		0.43	GBAAAABBA	31	
		0.14	GDAAACBBA	32	
		0.14	GBAAAABAA	33	
		0.14	GDAAAABBBB	34	
					0.86
M. montanus	3	1.00	FCCCD?C?A	35	0.00

1. Frequency of mtDNA composite phenotype within each watershed; 2. Enzyme sequence: EcoRI, PvuII, BclI, BamHI, HIndIII, Dra I, EcoRV, BstEII, and PstI.

Table 17. Branch lengths and nodes for Figure 4, the neighbor joining tree

NODE: OTU1	**(branch length)**	**& OTU2**	**(branch length)**
1 East	(-0.0029)	Togwotee Pass, West	(0.0029)
2 Node 1	(0.0181)	Line Creek	(-0.0008)
3 Beartooth Plateau Ck	(-0.0001)	Node 2	(0.0004)
4 Above Frozen Lk	(0.0001)	Above Long Lk	(0.0000)
5 Node 4	(0.0001)	South Fork Canyon Ck	(-0.0001)
6 Node 5	(0.0008)	Below Island Lk	(-0.0008)
7 Node 6	(0.0011)	Little Bear Lk	(0.0000)
8 Node 7	(0.0003)	AbIslandLk	(-0.0001)
9 Node 8	(0.0005)	Node 3	(0.0011)
Node 10 joins:			
Node 9	(0.0003),		
Quad	(0.0002),	and Wyoming Creek	(0.0010).