

Endemism and Population Relationships of the Black Hills *Oreohelix* Snails

Final Report

Tamara Anderson, Robert Guralnick, and Kathleen Weaver

February 2006



Figure 1. Example of the variation in shell size in Black Hills specimens of *Oreohelix cooperi*.
Photo copyright by T. Anderson.

TABLE OF CONTENTS

Table of Contents	2
List of Figures and Tables	3
Background	4
Objectives	4
Organization, Purpose, and Limitations of this Report	4
Methods	5
Genetic & Biogeography Results	11
Morphological Results	14
Discussion	26
Conclusion	31
Summary	32
Acknowledgements	32
Literature Cited	33
Author Information	36
Appendix 1: Sample Locality Information	37
Appendix 2: T-test tables of Morphological Analyses by Population	38

LIST OF TABLES AND FIGURES

Figure 1. Black Hills specimens of <i>Oreohelix cooperi</i>	1
Figure 2. Map of sampling locations	6-7
Figure 3. Phylogenetic tree showing relationships among <i>Oreohelix</i> samples	12
Figure 4. Map of biogeography model output	13
Table 1. Average shell and internal anatomy measurements for Black Hills populations	14
Figure 5. Shell diameter by population	16
Figure 6. Shell height by population	17
Figure 7. Whorl number by population	18
Figure 8. Height/diameter ratio by population	18
Figure 9. Total penis length by population	19
Figure 10. Penis length/shell diameter by population	19
Figure 11. Relationship of ribbed portion to total penis length	20
Figure 12. Percent ribbing by population	21
Table 2. Radula tooth formulas	21
Figure 13. Relationship between elevation and diameter	23
Table 3. Morphological measurements from populations outside the Black Hills	24
Table 4. Significant differences in morphology measurements between populations outside the Black Hills and those populations within the Black Hills	25
Table 5. Relationship to groups proposed by Frest & Johannes	29

BACKGROUND

Oreohelcid snails of the genus *Oreohelix* are found in the mountains of western North America from southern Canada to southern Arizona and New Mexico (Pilsbry 1939, Bequaert & Miller 1973). The latest edition of recognized names of mollusks lists 41 species of *Oreohelix* (Turegeon *et al.* 1998). In addition, countless subspecies and new species have been suggested (i.e. Frest & Johannes 1995).

In the Black Hills of South Dakota and Wyoming, three separate species were proposed after a survey by Frest & Johannes in 2002. One species, *Oreohelix cooperi* (occasionally referred to elsewhere as *Oreohelix strigosa cooperi*), is recognized as a state species of concern in South Dakota and is used as a management indicator species by the Black Hills National Forest. Frest and Johannes provide some description of their proposed species, but in-depth morphological or genetic examination has not been previously conducted. Frest & Johannes (2002) further consider all three to be endemic to the Black Hills, but genetic comparisons of *Oreohelix* snails from other areas had not been done previously. (Note: because the term "species" generally refers to a recognized taxonomic level, it is a source of confusion. In this report "species" refers to a distinct biological entity with a common phylogenetic history. Whether distinct groups are ultimately assigned to the rank of species or subspecies is a separate question that requires a thorough reworking of the taxonomy of the entire Oreohelcidae family, which is obviously beyond the scope of this work.)

Without a clear understanding of how many distinct biological entities exist in the Black Hills and how they relate to *Oreohelix* elsewhere, it is difficult for managers to identify appropriate strategies. Recent petitioning to have *O. cooperi* listed as a federally endangered or threatened species has further emphasized the need for more complete data.

OBJECTIVES

The three objectives of this study, as stated in the proposal, were as follows:

- 1) Determine if the *Oreohelix* snails in the Black Hills consist of one or more than one biological entity that can be defined by genetics, morphology, anatomy, and/or environment.
- 2) Determine if *Oreohelix* in the Black Hills represent an endemic group, unique from other *Oreohelix* in the geographical region.
- 3) Submit the results for publication in a peer-reviewed journal so data and conclusions are widely accessible and can be viewed and utilized by a variety of users from managers to malacologists.

ORGANIZATION, PURPOSE, AND LIMITATIONS OF THIS REPORT

This report is designed to provide results from more than two years of work focused on the *Oreohelix* snails in the Black Hills. The project included field work, genetics, morphology, and biogeography modeling to answer several questions about these animals. The report is

organized so genetic and morphological results are discussed separately, but overall conclusions are drawn from all data and are discussed in the final summary.

This work was funded by the South Dakota Department of Game, Fish & Parks. Therefore, this report serves to complete our final obligation for that funding.

As with any scientific study, some new questions arise when data is gathered, but we believe several important questions regarding the Black Hills *Oreohelix* snails are definitively answered in this study and we hope that the users of this report find it helpful. In fact, the questions addressed here were given high research priority in the species assessment prepared for the Rocky Mountain Region Forest Service (Anderson 2005). Although these results will assist managers and agency personnel by providing data and analyses previously unavailable, it is not the purpose of this report to decide if the species are endangered.

The results of this project will be submitted in multiple papers for peer-review and subsequent publication in scientific journals. The merits of this work will be fully evaluated during this scientific peer-review process.

METHODS

Sample Collection

During 2004 and 2005, Oreohelid snails were collected by hand from 22 sites in the Black Hills National Forest in South Dakota and Wyoming (Fig. 2, Appendix 1). Collection localities were selected to include sites across the known range of Oreohelids in the Black Hills National Forest (including both the Bear Lodge Mountains and the Black Hills proper) and on multiple drainages. Sites included representative locations for each of the three species previously proposed by Frest & Johannes (2002). Sampled locations were generally forested slopes with a variety of tree cover, usually predominated by ponderosa pine (*Pinus ponderosa*) and/or Black Hills spruce (*Picea glauca*), but occasionally including aspen (*Populus tremuloides*), birch (*Betula papyrifera*), or, rarely, bur oak (*Quercus macrocarpa*).

Only snails presumed to be adults, having at least 4 whorls (or the largest size class available at a site), were collected. After collection, snails were drowned in water until they did not respond. Tails were clipped for genetic study. The rest of the body was placed into 30% ethanol for 24 hours. After 24 hours, the sample was then placed in 90% ethanol for storage.

In order to evaluate the uniqueness of the Black Hills *Oreohelix*, specimens were also collected from 8 locations in the adjacent Eastern ranges of the Rocky Mountains in Wyoming, Montana, and Utah (Fig. 2, Appendix 1). The collections used are part of a larger set of collections to study the systematics of the whole *Oreohelix* clade. In order to decide which of the larger collection to use in this study, we generated initial phylogenetic trees for the whole sample to help determine which lineages were closely related to the ones in the Black Hills. For related clades that contained wide diversity throughout western North America, we included those lineages in the eastern Rockies closest to the Black Hills. We also included, as an outgroup, *O. haydeni hybrida* (located in Utah, not shown in Figure 2), which based on preliminary analyses of the larger *Oreohelix* dataset was basal to the ingroup populations. The specimens used in this study and location information are listed in Appendix 1.

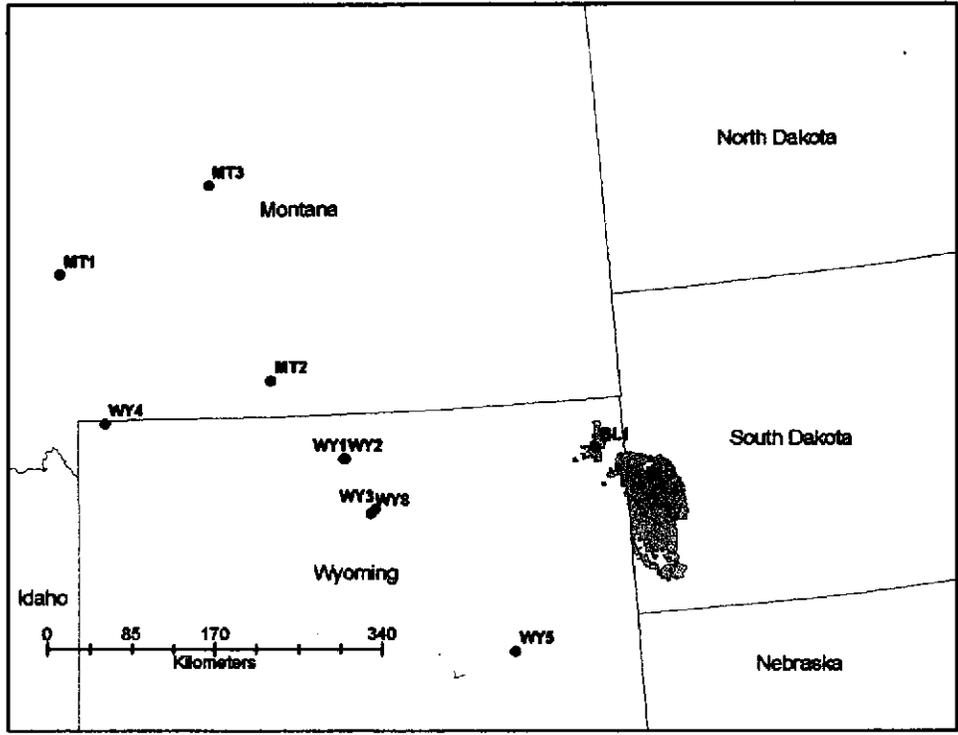
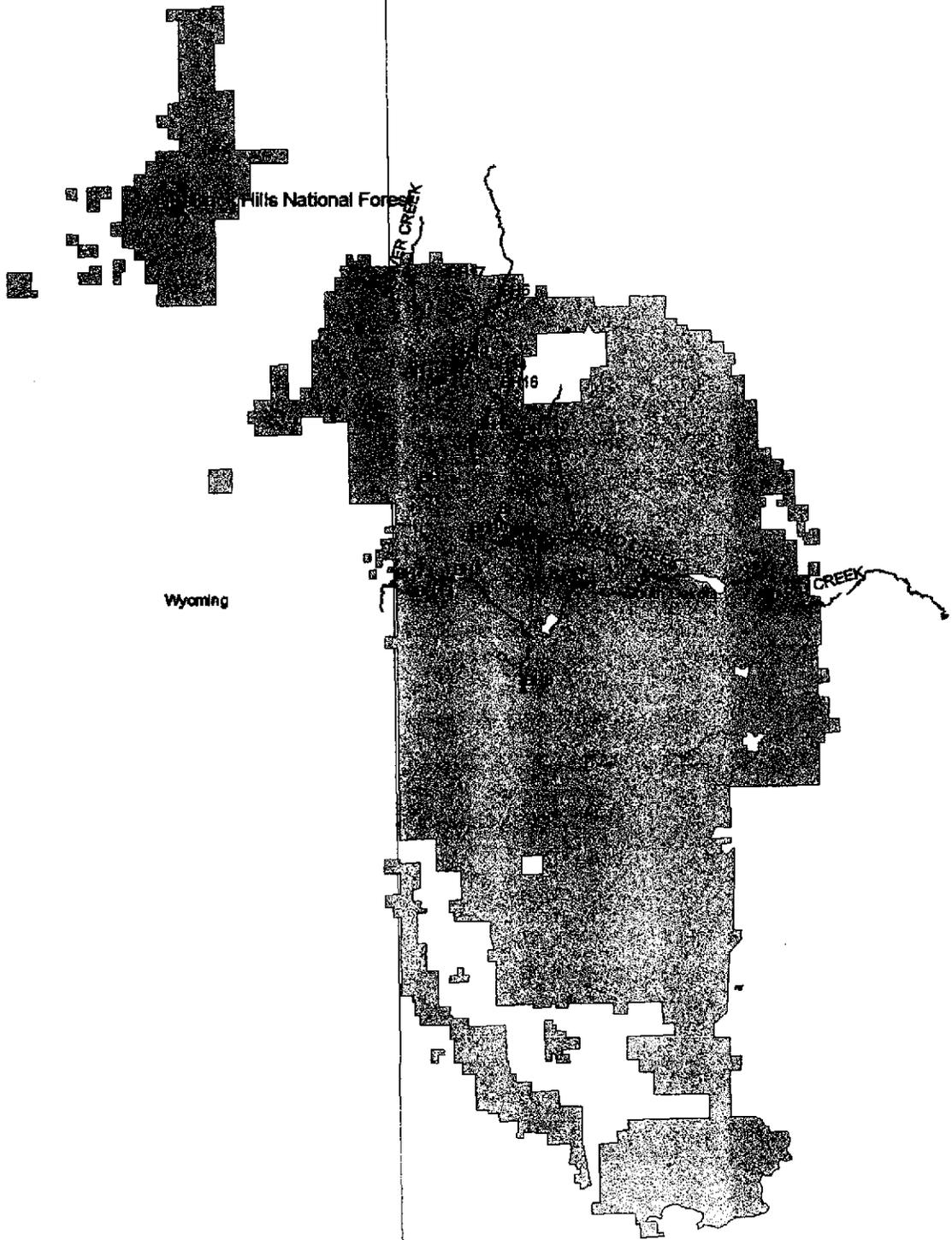


Figure 2. Map of sampling locations. Smaller map, above, shows regional sampling, with the shaded area (the Black Hills) enlarged in the main map, below. In the Black Hills populations, open circles indicate large-morph populations, while triangles indicate small-morph populations. Selected rivers are shown in the Black Hills.

0 5 10 20 Kilometers



Environmental Data Collection

At each Black Hills sampling location, soil characteristics were recorded. Soil moisture was recorded with a Quick Draw 2900FI Soil Moisture Probe (Soil Moisture Equipment Corp.). Moisture was measured as the soil suction in centibars. Soil temperature was recorded with a Weksler soil thermometer. Soil pH and calcium was measured using a LaMotte soil testing kit. Habitat variables measured included: % live ground cover, % dead litter cover, and % rock cover. The number of live snails and empty shells were also counted to assess density. At 15 of the collecting sites each of these measurements were obtained by marking out quadrats and averaging measurements from within 5 of them for habitat variables and soil temperature and for 3 quadrats for soil pH and calcium measurements. Measurements at the other 7 sites (BH19, BH18, BH15, BL2, BL3, BH13, BH12, BH11) consisted of only a single point for soil moisture and temperature measurements. General habitat descriptions by the collector were used to determine if hardwood species were present to contribute to the litter layer. Elevation was determined from GPS coordinates using the Graphical Locator program from the University of Montana (www.esg.montana.edu/gl/xy-data3.html).

DNA Sequencing and Analyses

Two mitochondrial loci were examined in 82 *Oreohelix* individuals: 55 individuals from 21 populations in the Black Hills; 24 individuals from 8 populations in the Bighorn, Gallatin, and Laramie Mountains in Wyoming and the Bighorn and Judith Mountains in Montana; and 3 individuals from an *O. haydeni hybrida* population in Western Utah.

Original plans included sequencing individuals for two additional loci, the mitochondrial locus 16S and the nuclear locus ITS. These plans were altered when these loci provided very little variation. For example, 32 individuals sequenced from 21 different populations showed a maximum of 0.008% sequence divergence for 16S (Weaver, unpublished data). Although 16S and ITS have been found to show variation in other mollusk species (i.e., Thomaz *et al.* 1996, Ross 1999, Pfenninger & Magnin 2001, Lee & O' Foighil 2003), projects must utilize loci that show variation at the level of the question they are asking, and that is what was done here.

The technical details of the genetic methods are outlined in detail below. In brief, DNA was extracted from each individual snail and multiple copies of the DNA at each locus of interest were made. The nucleotide sequence of each piece of DNA was determined and the results analyzed with standard computer software designed for that purpose. Trees are then constructed from these results that illustrate how similar the DNA is among the samples.

The manufacturer's protocol for the DNeasy Kit (Qiagen Inc., Valencia, CA) was followed to extract DNA from alcohol preserved foot tissue. The COI locus (673 bp) was amplified by polymerase chain reaction (PCR) using universal primers HCO2198 and LCO1490 (Folmer *et al.* 1994). Amplifications were performed in an Eppendorf Mastercycler (Eppendorf) under the following thermal conditions: 96°C for 2 minutes, followed by 28 cycles of 96°C for 40 seconds, 48°C for 20 seconds, and 72°C for 1 minute, and a final extension of 72°C for 7 minutes. The 12S rDNA locus (415 bp) was amplified by PCR with forward and reverse primers 12Sa-L and 12Sa-H (Kocher 1989). Amplifications were performed under the following thermal conditions: 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 20 seconds, and 72°C for 30 seconds, and a final extension of 72°C for 5 minutes. PCR products were cleaned using 2µl ExoSAP-IT (USB corp.). PCR products were then used as a template in 12µl cycle sequencing reaction using Big Dye chemistry v.3.1 (Applied Biosystems, Inc., Palo Alto, CA). Cycle sequencing reactions were cleaned using Sephadex dye terminator removal

protocol (Amersham Biosciences) and were visualized on an ABI 377XL (Applied Biosystems, Inc., Palo Alto, CA).

Nucleotide sequences were aligned using Clustal X (Thompson *et al.* 1997). COI sequences were converted to protein (Invertebrate Mitochondrial) and adjusted by eye in Se-Al v.2.0 (Rambaut 1996). The COI and 12S datasets were analyzed independently and as a combined dataset. Phylogenetic relationships were estimated using maximum-likelihood (ML) and the Bayesian method of phylogenetic inference (Felsenstein 1981; Ronquist & Huelsenbeck 2003).

The most likely model of molecular evolution was determined to be TVM+ Γ +I for COI, TVM+ Γ for 12S, and TVM+ Γ +I for the combined dataset using ModelTest (Posada & Crandall 1998). ModelTesting is a way of choosing the most likely model of evolution prior to doing a likelihood or Bayes based phylogenetic analysis. This is important because likelihood and Bayes approaches work by using the user-supplied model and deciding on the best (optimal) tree given the characters and the model of how they evolve. Prior to ModelTest, there was no objective way to pick which model of evolution best fit one's dataset, so researchers were left to pick models based on heuristics like bias in base composition of transition/transversion rate differences. ModelTest formalizes much of this process and is now extremely widely used. The TVM model selected in this analysis assumes variable base frequencies and variable transversion rates.

ML analyses were performed in Phylml (Guindon & Gascuel 2003) based on the combined dataset model calculated using ModelTest and a calculated distance-based neighbor-joining (NJ) starting tree to begin the simultaneous adjustment of tree topologies and branch lengths. Bayesian method of phylogenetic inference was implemented in the MRBAYES program (Ronquist & Huelsenbeck 2003); separate evolutionary models for each locus were used in the combined analyses (see models above). For independent and combined analyses, 2,000,000 generations of six simultaneous Monte Carlo Markov chains were used, sampling every 1000 generations. Tree scores above the "burn-in" value were used to compute a 50% majority rule consensus tree in PAUP 4.0b (Altevec; Swofford 2002). Nodal support was evaluated by posterior probabilities values from the Bayes trees (Ronquist & Huelsenbeck 2003) and bootstrap support in Phylml (1000 replicates). ML and Bayesian reconstructions were rooted using the sequences of *O. haydeni* as outgroups.

Biogeography Modeling

In addition to the objectives described above, we were able to use sampling points in a biogeography modeling program to determine those areas where *Oreohelix* is likely not able to occur due to unfavorable environmental conditions. The modeling approach uses DesktopGARP (<http://www.lifemapper.org/desktopgarp/>; Stockwell & Peters 1999). Unlike other uses of GARP as a means to determine species or lineage distributions, we utilize GARP as a means to examine where it is likely, based on the environmental data layers used, that the Black Hills species could occur in favorable environmental conditions. The actual distribution of *O. cooperi* is likely limited by factors not included in the model, and therefore it is crucial that the model outputs not be considered an actual prediction of species distribution or range.

Based on the results from the phylogenetic analysis, we assembled a dataset of 24 species occurrences for the well-supported clade that we refer to as *Oreohelix cooperi* based on naming priority (see Discussion below). This clade contained all the Black Hills species occurrences as

well as populations located in the Judith Mountains of Montana and Tensleep Canyon in the Bighorn Mountains in Wyoming.

To construct a model of species distributions based on this presence data, we employed the program DesktopGARP (<http://www.lifemapper.org/desktopgarp/>). DesktopGARP provides species distribution predictions based on a set of environmental data layers and presence data only. It does so by creating subsets of the presence data, running the models using a genetic algorithm approach utilizing subset data and a set of environmental data layers, and then comparing predicted occurrence versus actual occurrence from the left-out samples. This provides a means to determine model accuracy. We ran 100 initial distribution models utilizing training datasets with a 0.01 convergence limit utilizing all available environmental data layers provided in the DesktopGARP distribution, (14 total), provided in the DesktopGARP distribution which are in 6 minute (0.1 degree) resolution and derived from the USGS and Intergovernmental Panel on Climate Change. These layers include information that likely shapes *Oreohelix* species' distribution: precipitation, temperature, elevation, slope and aspect layers.

Based on the starting subset of training data points and the genetic algorithm starting conditions, each run of DesktopGARP gives different model outputs. We refine our model choice in two ways. First we used Best Subsets methods in DesktopGARP to limit the models to those that have specified amount of commission and omission values (see Anderson *et al.* 2003 for discussion). The best subset approach works by selecting those models among the ones generated that reduce over- and underfitting models based on user selected parameters. We used default DesktopGARP best subset parameters. In order to refine the outputs even further, we uploaded each best subset model (25 total) into the Geographic Information System DIVA-GIS and created a stack based on each model. We then created a new grid based on a stack calculation where at least half of the 25 models predicted the species should occur. This grid output along with the elevation and stream environmental layers is shown in the results section.

In order to determine which layers were most crucial for limiting distributions, we also performed environmental jackknifing. In this case, we first removed layers and re-ran models to determine if outputs resembled the full dataset. We also ran DesktopGARP sequentially using only one layer at a time to determine individual layer contributions to the total model.

Internal Anatomy and Shell Morphology

Measurements were made with Mitutoyo digital calipers. Shell measurements of diameter, height, and whorl number follow Burch (1962). Height to diameter ratio was also evaluated as an indicator of shell shape. Each snail was then dissected under a Lomo MBC-10 stereo microscope to allow measurement of total penial length and length of ribbed portion of penis. Because the penis was often twisted or curved and intact specimens were desired, total penial length is the sum of measurements of all segments *in situ* with as little stretching as possible. A total of 205 individual snails from the Black Hills were dissected and measured for this study.

For comparison, Oreohelid shells from potential sister taxa as determined by the genetic data described above were also measured. These samples were collected from the Judith Mountains near Lewiston, Montana (MT3), the Bighorn Mountains near Shell (WY1) and Tensleep (WY3 and WY08), Wyoming, and from the Gallatin Mountains near Mammoth (WY4), Wyoming (see Fig. 2). The collections used from MT3, WY1, and WY4 are part of a larger set of collections to study the systematics of the whole *Oreohelix* genus (Weaver, unpublished data). These samples were not relaxed and preserved in the way described above,

so comparison of reproductive anatomy was not possible. Samples from WY3 and 8 were collected by other researchers (McDonald & Chak, unpublished data) and were relaxed to allow for dissection. Although *Oreohelid* snails are found elsewhere in Wyoming, genetic data placed those populations outside these clades (Weaver, unpublished data) so they are not included in these analyses. A total of 26 individual snails from 5 locations outside the Black Hills were measured for these analyses. All specimens are deposited at the University of Colorado Museum.

Radulae were also examined in a subset of the specimens. Radular tooth number is known to vary among species in a diagnostic manner (most recently shown in Ports, 2004) and, thus, provides another potentially useful dataset for discovering if morphological variation is consistent with known lineage information. Based on analyses of shell size morphs, we chose to examine 3 populations from the Black Hills representing small to large morphs. We also examined populations from adjacent areas in the eastern Rockies, including populations in the Bighorn Mountains in Wyoming and the Judith Mountains in Montana. Radula from two specimens per population were examined.

Radula dissections were performed under a Leica dissecting scope. The buccal mass containing the radula was isolated from the rest of the viscera and the tissue carefully removed from around the radula. A 5% bleach solution was used to help remove tissue from around the radula. After the radula had been cleaned, it was mounted onto a slide and visualized and photographed on a Fisher compound microscope with an attached Nikon 995 digital camera with ocular micrometer. For this study, a detailed morphological examination of the teeth was deemed unnecessary and the focus is on the tooth count per row, following Ports (2004) as number of lateral and marginal teeth on each side of the central tooth. Tooth counts were generated for each individual specimen across the broadest portion of the radula. Counts within populations may differ due to individual variation. However, it is difficult to get the very edge of the radular ribbons completely flat. The ribbon has a tendency to curl under at the very edge obscuring the smallest marginals. Some of the variance reflects this methodological issue.

Statistical analyses of morphological data were conducted using JMP Version 4.0.2 (SAS Institute, Inc.) software. Univariate analyses of each morphological measurement by population were conducted. Hierarchical cluster analyses using the Ward method was used to further test for distinct morphological groups. Regression analyses of mean diameter by soil temperature, soil pH, soil calcium, and elevation were also conducted.

RESULTS—GENETICS & BIOGEOGRAPHY MODEL

Phylogenetic Analysis

Maximum likelihood (ML) analyses provided well resolved phylogenies for COI (-ln = -2283.55), 12S (-ln = -1414.30), and combined (-ln = -3891.61) datasets. Tree topologies for ML and Bayes analyses were nearly identical for all datasets combined or no; therefore, only combined analyses will be presented. Using the combined dataset, corrected distances show between 0 % and 0.2% divergence between individuals within the Black Hills, 0.1% divergence to the nearest Montana locality in the Rocky Mountains (MT3), 0.6% to the nearest Wyoming locality (WY3), and approximately 2% divergence to other relatively closely related *Oreohelid* lineages in Wyoming (WY1, 2).

The maximum likelihood tree with nodal support from a ML Bootstrap analyses and Posterior Probabilities shows strong support (92, 0.93) for a monophyletic group in the Black Hills, *O. cooperi*, and the inclusion of the MT3 and WY3 populations within the Black Hills group (Figure 3).

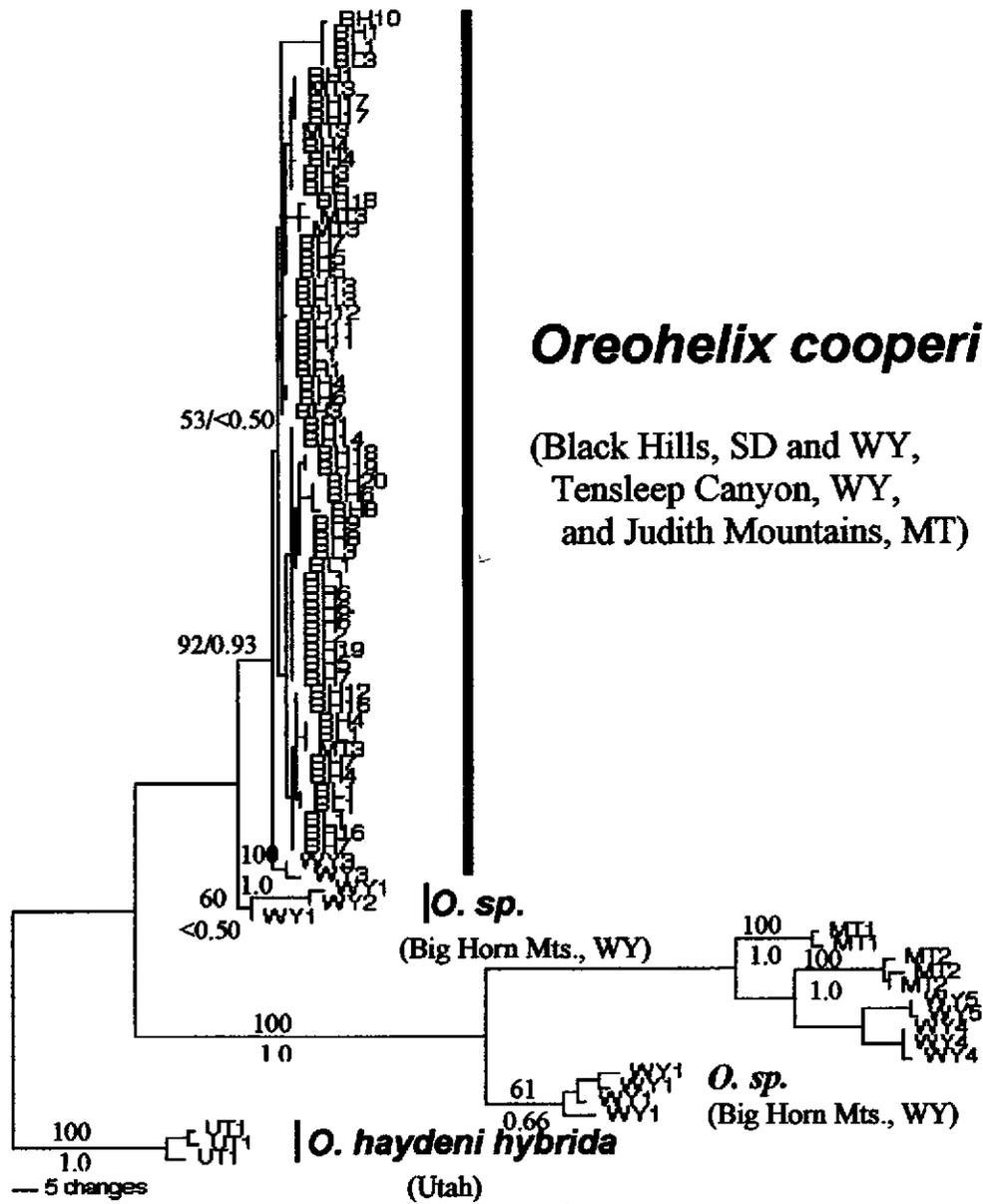


Figure 3. Maximum likelihood tree for the combined CO1 and 12S datasets for *Oreohelix cooperi* and related taxa. Scale below the tree refers to changes in nucleotide base-pairs between sequences. Branch lengths are shown proportional to the amount of change along the branches. Bootstrap values (top) are based on 1000 replicates within Phym1 (Guindon and Gascuel 2003) and Posterior probabilities (bottom) are based on 1,500,000 generations in a Bayesian analysis. *Oreohelix haydeni hybrida* is an outgroup for the Black Hills/Rocky Mountain groups.

Ecological Niche Model Results

Based on the included environmental data layers, Figure 4 shows where *O. cooperi* could occur according to the majority of the best subset model outputs from DesktopGARP. Color coding (from light to darker grays, representing fewer to greater models) shows how many models support the occurrence across the area. The models suggest that potentially suitable environments for *O. cooperi* exist outside the sampled areas in the Black Hills, eastern Montana, and Wyoming. In particular, models predict that areas in the Northern Great Plains, especially to the northwest and southwest, are within the same environmental range as that occupied by *O. cooperi* in the Black Hills and Rocky Mountains. However, in the northwest, there is no indication of a continuous connection of suitable habitat for migration between MT3 in the northeastern Rockies and the Black Hills populations. Towards the southwest, a nearly continuous stretch of potentially favorable environmental regions occurs between WY3 (on the west side of the Bighorns) and the Black Hills. This possible connection follows lowland and foothill routes.

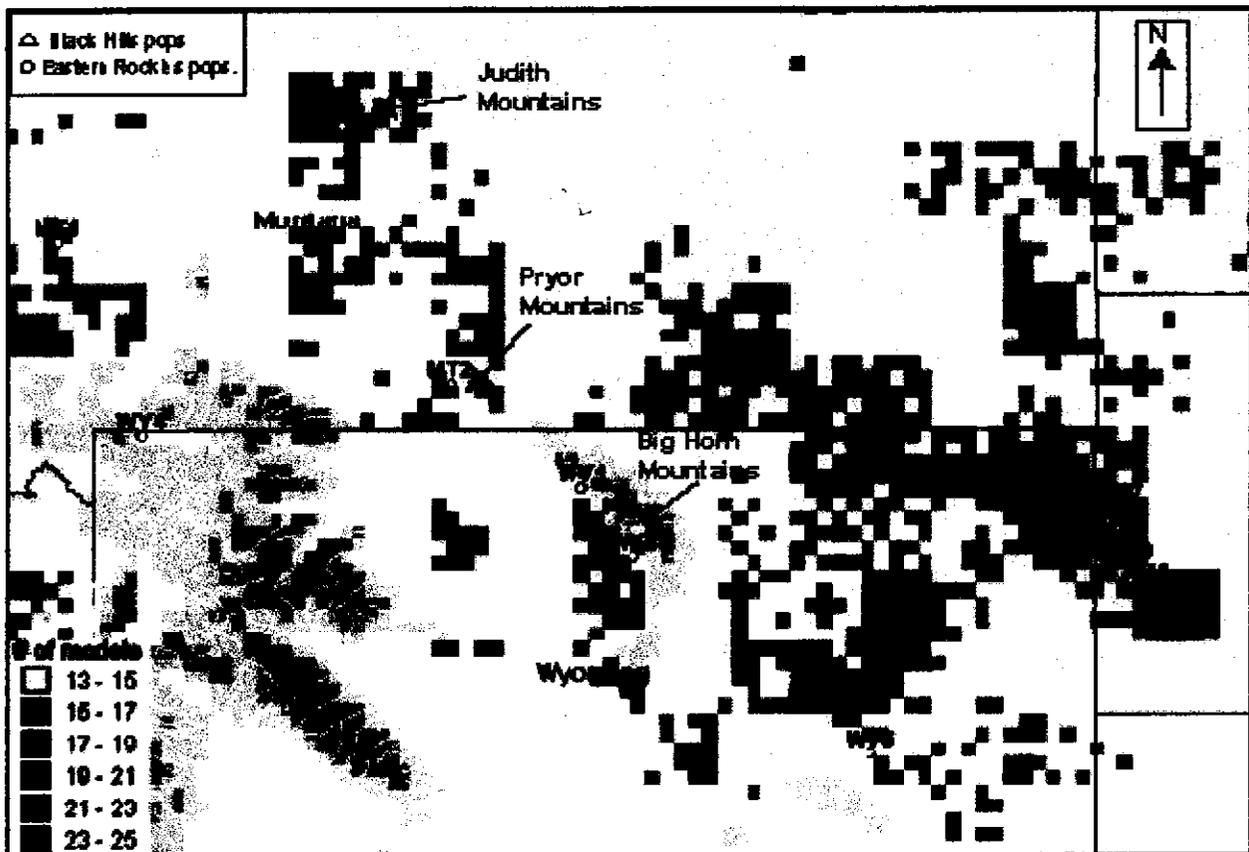


Figure 4. Results of biogeographical modeling by GARP. Those areas where *O. cooperi* are predicted by a majority of best subset models are shown in the blocks below on top of an elevation data layer as output from the computer program DIVA-GIS. The coloration of the predicted areas are shown in grayscale from lighter to darker, with lighter shading representing fewer models predicting environmental suitability and darker representing more models predicting suitability. Select mountain ranges referred to in the text are labeled.

Results of the environmental jackknifing indicate that no single environmental data layer is dominant in the models for limiting ecological niche. Instead it is the combination of environmental factors that limits the niche of *O. cooperi*. Temperature layers appear to constrain the latitudinal range of *Oreohelix* to areas in the northern United States and southern Canada. Precipitation, frost-free days, topographic index (a compound measure of wetness), and elevation constrains distributions in the basin and range areas in western North America, especially in mid-range elevation (lowest and highest elevations are excluded in the distribution models). Number of wet days appears to constrain distributions more toward eastern foothills and plains regions than the mountain west.

RESULTS—MORPHOLOGY

Morphological groups within the Black Hills

Measurements for Black Hills populations are given in Table 1. Population was a significant factor in explaining the variation in Black Hills shells for diameter, height, number of whorls, total penis length, % ribbing, and height/width ratio (ANOVA, $P < 0.001$, except for % ribbing where $P = 0.0002$).

Table 1. Average shell and reproductive anatomy measurements for Black Hills populations. Note that the sample size (n) for all measurements is not equal due to sample conditions or dissection problems.

Population	Diameter, mm (n , SE)	# of Whorls (n , SE)	Height, mm (n , SE)	Penis length, mm (n , SE)	Ribbed portion, mm (n , SE)	H/D	Ribbed / total penis length	Assigned morph class
BH1	8.2 (10, 0.29)	4.05 (10, 0.074)	5.7 (10, 0.26)	8.5 (10, 0.69)	3.4 (10, 0.27)	0.70, (10, 0.013)	0.39 (10, 0.018)	small
BH3	9.1 (10, 0.29)	4.33 (10, 0.074)	6.2 (10, 0.26)	6.8 (10, 0.69)	2.9 (10, 0.27)	0.68 (10, 0.013)	0.43 (10, 0.018)	small
BH4	8.6 (10, 0.29)	4.30 (10, 0.074)	6.2 (10, 0.26)	9.7 (10, 0.69)	3.6 (10, 0.27)	0.72 (10, 0.013)	0.38 (10, 0.018)	small
BH5	9.1 (10, 0.29)	4.30 (10, 0.074)	6.3 (10, 0.26)	9.2 (10, 0.69)	3.9 (10, 0.27)	0.69 (10, 0.013)	0.43 (10, 0.018)	small
BH6	13.7 (10, 0.29)	4.85 (10, 0.074)	9.9 (10, 0.26)	13.2 (10, 0.69)	5.4 (10, 0.27)	0.73 (10, 0.013)	0.41 (10, 0.018)	large
BH7	9.3 (10, 0.29)	4.43 (10, 0.074)	6.7 (10, 0.26)	9.1 (9, 0.72)	3.6 (8, 0.30)	0.73 (10, 0.013)	0.37 (8, 0.021)	small
BH8	8.2 (11, 0.28)	4.05 (11, 0.071)	5.8 (11, 0.25)	8.0 (11, 0.65)	3.4 (11, 0.26)	0.71 (11, 0.012)	0.42 (11, 0.017)	small

BH9	9.0 (10, 0.29)	4.40 (10, 0.074)	6.5 (10, 0.26)	9.1 (10, 0.69)	3.6 (10, 0.27)	0.72 (10, 0.013)	0.41 (10, 0.018)	small
BH10	16.7 (9, 0.31)	5.17 (9, 0.078)	12.0 (9, 0.28)	16.7 (8, 0.77)	5.7 (8, 0.30)	0.71 (9, 0.013)	0.35 (8, 0.021)	large
BH11	10.8 (7, 0.35)	4.50 (7, 0.089)	7.7 (7, 0.32)	11.1 (7, 0.82)	4.7 (7, 0.32)	0.71 (7, 0.015)	0.42 (7, 0.022)	small
BH12	9.7 (7, 0.35)	4.39 (7, 0.089)	6.7 (7, 0.32)	10.5 (7, 0.82)	4.3 (7, 0.32)	0.69 (7, 0.015)	0.41 (7, 0.022)	small
BH13	8.9 (8, 0.32)	4.38 (8, 0.083)	6.5 (8, 0.29)	8.8 (8, 0.77)	3.8 (8, 0.30)	0.74 (8, 0.014)	0.43 (8, 0.021)	small
BH14	14.8 (10, 0.29)	5.03 (10, 0.074)	10.2 (10, 0.26)	14.2 (10, 0.69)	5.4 (10, 0.27)	0.69 (10, 0.013)	0.38 (10, 0.018)	large
BH15	9.1 (7, 0.35)	4.50 (7, 0.089)	6.3 (7, 0.32)	10.1 (7, 0.82)	3.8 (7, 0.32)	0.69 (7, 0.015)	0.37 (7, 0.022)	small
BH16	13.2 (13, 0.25)	4.85 (13, 0.065)	9.8 (13, 0.23)	13.5 (13, 0.60)	5.0 (13, 0.24)	0.74 (13, 0.011)	0.37 (13, 0.016)	large
BH17	12.6 (10, 0.29)	4.80 (10, 0.074)	8.7 (10, 0.26)	13.5 (10, 0.69)	4.8 (10, 0.27)	0.69 (10, 0.013)	0.35 (10, 0.018)	large
BH18	8.8 (7, 0.35)	4.46 (7, 0.089)	6.6 (7, 0.32)	10.3 (7, 0.82)	3.9 (7, 0.32)	0.74 (7, 0.015)	0.38 (7, 0.022)	small
BH19	14.3 (7, 0.35)	4.96 (7, 0.089)	10.7 (7, 0.32)	14.2 (7, 0.82)	4.7 (7, 0.32)	0.75 (7, 0.015)	0.33 (7, 0.022)	large
BH20	8.2 (11, 0.28)	4.23 (11, 0.071)	5.8 (11, 0.25)	8.9 (10, 0.69)	2.8 (10, 0.27)	0.71 (11, 0.012)	0.33 (10, 0.018)	small
BL1	10.9 (13, 0.25)	4.56 (13, 0.065)	7.7 (13, 0.23)	10.6 (11, 0.65)	4.4 (10, 0.27)	0.71 (13, 0.011)	0.41 (10, 0.018)	small
BL2	10.0 (6, 0.37)	4.58 (6, 0.096)	7.3 (6, 0.34)	11.7 (6, 0.88)	4.5 (6, 0.35)	0.73 (6, 0.017)	0.39 (6, 0.024)	small
BL3	10.2 (9, 0.31)	4.36 (9, 0.078)	7.2 (9, 0.28)	9.5 (9, 0.72)	4.3 (8, 0.30)	0.70 (9, 0.013)	0.42 (8, 0.021)	small

The diameters of all shells measured are shown in Figure 5. The overall mean diameter for Black Hills Oreohelicids was 10.6 mm (range 6.9 to 19.3 mm). Six populations (BH6, BH10, BH14, BH16, BH17, and BH19) have means which fall well above the overall mean. The remaining populations have means that fall very near or below the overall mean. Pairwise comparisons show significant differences between all six of the largest diameter populations and most of the rest of the populations (Tukey-Kramer paired t-test, $\alpha=0.05$).

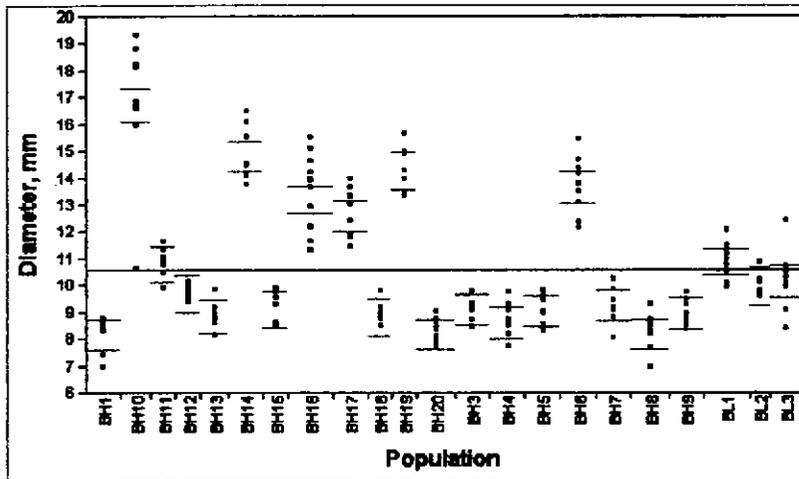


Figure 5. Shell diameter by population for Black Hills Oreohelicids. Lines shown are the 95% confidence intervals around the mean for each population. The long horizontal line shows the overall mean for all Black Hills samples.

Figure 6 shows height measurements for all Black Hills samples. Average height for all Black Hills samples measured is 7.6 mm (range 4.6 to 13.9 mm). Again 6 populations (BH6, BH10, BH14, BH16, BH17, and BH19) had means well above the overall mean. The five largest populations were again significantly different from all smaller populations (Tukey-Kramer paired t-tests, $\alpha=0.05$). BH17 was significantly different most of the smaller-sized populations, but could not be distinguished from three medium-sized populations (BH11, BL1, and BL2).

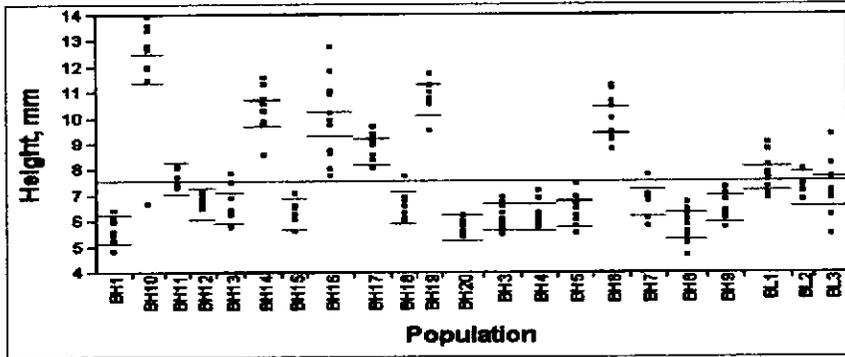


Figure 6. Shell height by population for Black Hills Oreohelicids. Lines shown are the 95% confidence intervals around the mean for each population. The long horizontal line shows the overall mean for all Black Hills samples.

The number of whorls for each Black Hills snail sampled is shown in Figure 7. Average number of whorls for all Black Hills samples measured is 4.5 (range 3.5 to 5.5). The same six populations (BH6, BH10, BH14, BH16, BH17, and BH19) again had means well above the overall mean. Five of those six populations had enough overlap with some of the medium sized populations that they could not be significantly distinguished (Tukey-Kramer paired t-tests, $\alpha=0.05$), but the smallest 9 populations were still significantly different from all six of the large populations. Whorl number is correlated to diameter ($r^2=0.71$), but it is not entirely dependent on diameter. For example, if all samples larger than 4.5 whorls are excluded, the correlation between whorl number and diameter drops dramatically ($r^2=0.26$). It also cannot be argued that these snails are not adults until they reach 5 whorls. Assuming that "adult" status is achieved when individuals can reproduce, Black Hills Oreohelicids can actually reach "adult" size at less than 4 whorls, at least in some populations, based on dissected individuals that have broods present (Anderson, in prep.).

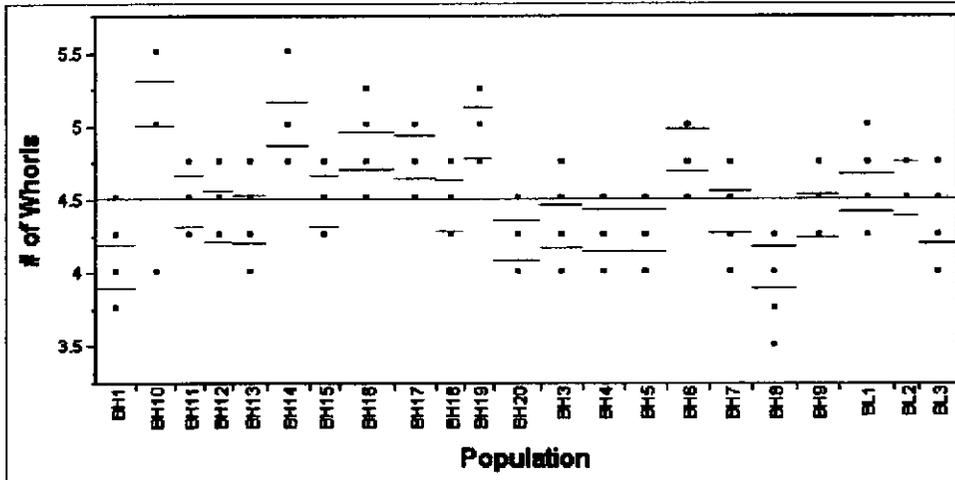


Figure 7. Whorl number by population for Black Hills Oreohelicids. Lines shown are the 95% confidence intervals around the mean for each population. The long horizontal line shows the overall mean for all Black Hills samples.

Height to diameter ratio (H/D) has also often been used to describe the shape of snail species. Average H/D for the Black Hills Oreohelicids measured is 0.71 (range 0.62 to 0.82). H/D is not related to the diameter of the shell ($r^2=0.013$, ANOVA $P=0.0992$, Fig. 8). None of the Black Hills populations are significantly different from each other based on H/D (Tukey-Kramer pairs comparison, $\alpha=0.05$, Fig. 8).

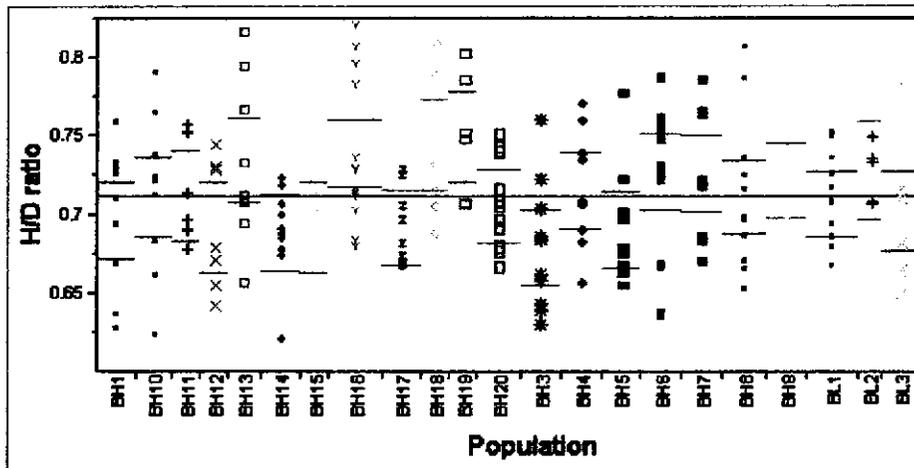


Figure 8. Height to diameter ratio by population.

Total penis length averaged 10.7mm (range 3.2 to 20.2 mm). The same 6 populations again had the highest means, but many more populations overlapped (Fig. 9). Only the largest population (BH10) was significantly different than all of the small-size populations (Tukey-Kramer pairs comparisons, $\alpha=0.05$). The next 5 populations could not be distinguished from between 3 and 6 of the medium sized populations. Total penis length was correlated to shell diameter ($r^2=0.62$, ANOVA $P<0.0001$). The ratio of penis length to shell diameter averages 1.0. BH3 has the smallest average ratio (0.74, SE=0.057) and is the only population that is significantly different from any of the other Black Hills populations (Fig. 10). BH3 is significantly different from 7 populations (BH4, BH12, BH15, BH17, BH18, BH20, BL2), but not the remaining 15 populations.

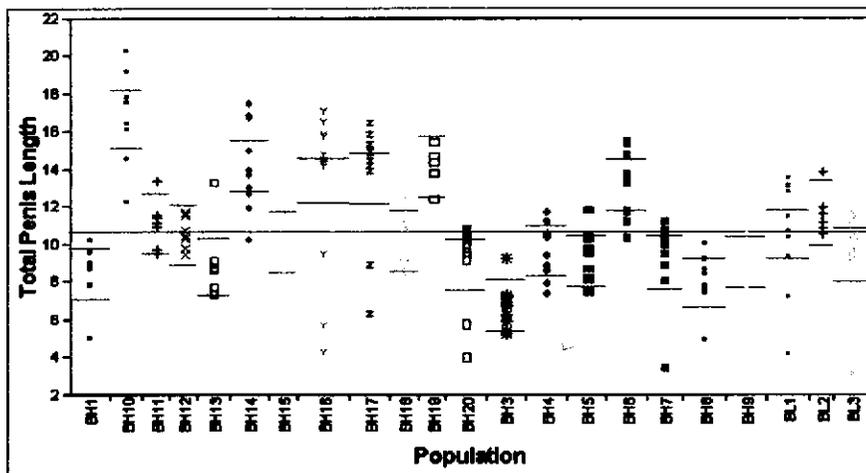


Figure 9. Total penis length by population.

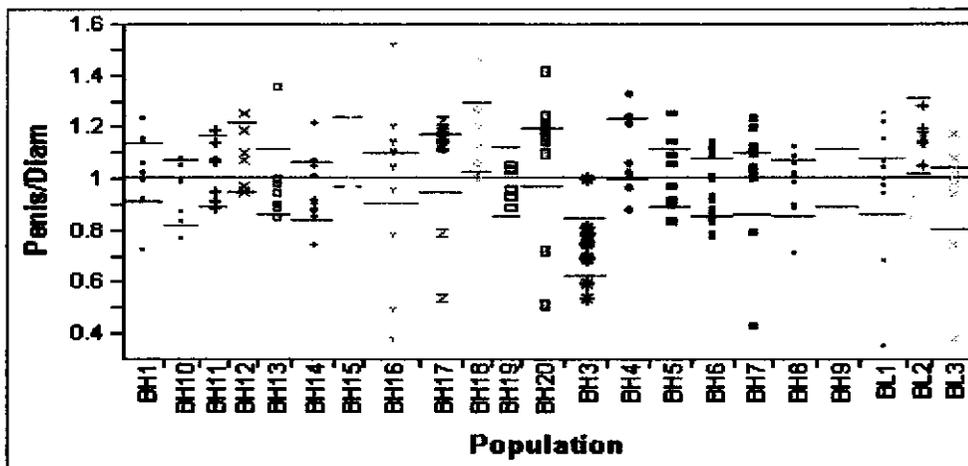


Figure 10. Penis length to shell diameter ratio by population.

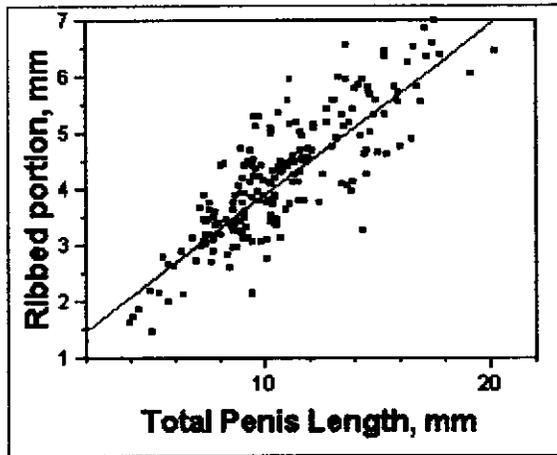


Figure 11. Relationship of the ribbed portion of the penis to the total penis length was significant for Black Hills samples

Because the length of the ribbed portion is dependent on the total penis length ($r^2=0.70$, ANOVA $P<0.0001$, Fig. 11), % ribbing was analyzed for population differences. The % ribbing averaged 39% (range 22 to 55%) for all Black Hills samples (Fig. 12). These measurements fall generally in line with Pilsbry's (1939) findings, where the ribbed portion of the penis was reported to be less than 50% in this species (only 11 of 197 individuals measured had ribbed portions of 50% or more of the penis length, with a range of 0.29 to 0.55; no one population contains more than 2 individuals with % ribbing greater than or equal to 50%). In this case the 6 "large" populations do not have the highest means for % ribbing (Fig. 12). Also, the only pairs that were significantly different than each other were BH5 and BH3 versus BH19 and BH20 (Tukey-Kramer all pairs comparisons, $\alpha=0.05$).

The ribbed portion of the penis may be somewhat influenced by the part of the reproductive cycle that the individual is in at the time of collection. The collection date (ANOVA, $P=0.0021$) and whether or not the individual had a brood (t-test, $P=0.0015$) both had significant influences on the % ribbing.

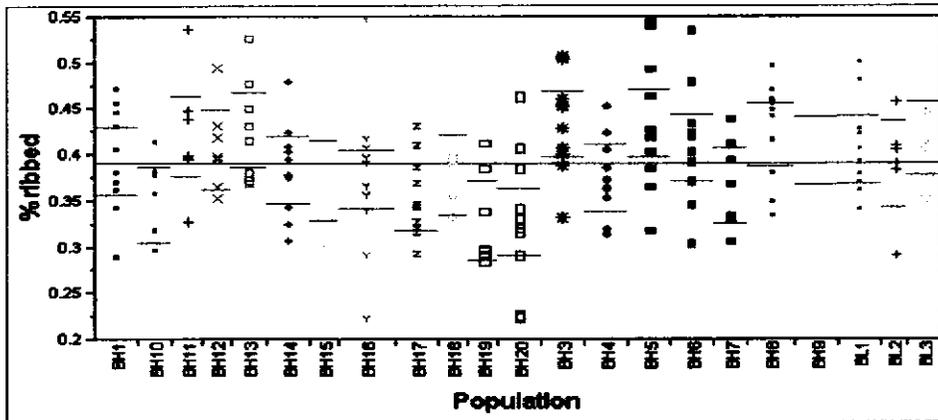


Figure 12. Percent ribbing by population.

Radula results for all individuals examined are summarized in Table 2. For the Black Hills specimens in particular, the radula formula varies from 23 laterals and marginals and one central tooth (23-1-23) to 26 laterals+marginals and one central tooth (26-1-26). The variance may be due to the edges of the ribbon curling slightly to obscure the most marginal teeth. Typically the Black Hills formula is 25-1-25 and is similar to the formula for those individuals from the Judith Mountains in Montana (MT3). Radula formula does not appear to be strongly influenced by body size; the populations chosen represent the full size range across the Black Hills and radula counts are relatively invariant.

Table 2. Radular tooth formulas for individual dissections by population. The putative genetic lineage based on mtDNA molecular analysis (described in the genetics section) is also indicated. Note that the genetic lineages listed are not related to the species names proposed by Frest & Johannes (2002) and described in Table 5.

Population#-specimen#	Genetic Lineage	Tooth Formula
BH1-05	<i>O. cooperi</i>	25-1-25
BH1-07	<i>O. cooperi</i>	23-1-23
BH10-02	<i>O. cooperi</i>	26-1-26
BH10-04	<i>O. cooperi</i>	25-1-25
BL1-02	<i>O. cooperi</i>	25-1-25
BL1-05	<i>O. cooperi</i>	25-1-25
MT1-01	" <i>O. sp. Lineage 2</i> "	30-1-30
MT1-02	" <i>O. sp. Lineage 2</i> "	31-1-31
MT3-01	<i>O. cooperi</i>	23-1-23
MT3-06	<i>O. cooperi</i>	25-1-25
MT2-01	" <i>O. sp. Lineage 1</i> "	32-1-32
MT2-02	" <i>O. sp. Lineage 1</i> "	30-1-30
WY1-04	" <i>O. sp. Lineage 3</i> "	21-1-21
WY1-05	" <i>O. sp. Lineage 3</i> "	23-1-23
WY2-08	" <i>O. sp. Lineage 3</i> "	23-1-23
WY3-08	<i>O. cooperi</i>	27-1-27
WY3-16	<i>O. cooperi</i>	28-1-28

Based on all of these results, we divide the Black Hills populations into two morphological groups, hereafter referred to as “large-morph” and “small-morph” (see Table 1). Hierarchical cluster analysis using the Ward method and including only external shell characteristics (diameter, height, and whorl number) showed a high level of agreement with two clusters. Assuming populations should all belong to the same morph, samples grouped in the correct cluster 94% of the time. Of the 205 samples, only 13 were incorrectly assigned with 3 incorrectly assigned to the large-morph cluster and 10 incorrectly assigned to the small-morph cluster.

Relationship of morphological groups to genetic lineages within the Black Hills

Morphological groups do not correspond to the genetic data. In fact, the genetic data suggest extensive gene flow among Black Hills populations, with the same haplotypes appearing in multiple populations that do not share the same size morph.

Relationship of morphological variation to geographic and environmental factors

Geographic distribution of the size morphs is shown in Figure 2. Size morphs are not distributed in distinct geographic regions. Several of the large-morph populations (BH17, BH16, BH14, and BH6) are clustered along one drainage, Spearfish Creek, but not all of the populations on that drainage exhibit the large-morphs. In addition, BH10 is on Rapid Creek and BH19 is on Little Spearfish Creek, with both drainages also supporting small-morph populations.

Because diameter was sufficient to separate populations, environmental factors were only tested against this variable. Average diameter per population was used because soil variables were not available for the precise location where each individual was collected.

Diameter showed no significant relationship to soil temperature, moisture, pH, or calcium (ANOVA, $P > 0.05$). Diameter was not related to the presence of hardwood species (t-test, $P > 0.05$). Habitat variables (% live ground cover, % litter layer cover, and % rock cover) also showed no significant relationships to diameter (ANOVA, $P > 0.05$). These variables were measured when the snails were collected and so were not all collected at the same part of the season or at the same time of day. Therefore, some relationships may actually exist that could not be detected with the limitations of the measurements.

Live snail density did not show a significant relationship to diameter (ANOVA, $P > 0.05$), but shell density showed a barely significant negative relationship (ANOVA, $P = 0.0531$) to diameter. It is unclear whether this is biologically relevant, but could be indicative of populations in different environmental conditions exhibiting a trade-off between individual size and population density.

Elevation did show a significant influence on the average diameter of a population (ANOVA, $P = 0.0118$; Fig. 13). Interestingly, although the overall relationship is negative (average diameter = $23.037 - 0.007$ elevation), when examining the large-morph populations separately, they have an insignificant positive relationship (ANOVA, $P = 0.2067$, average diameter = $6.013 + 0.005$ elevation). This further suggests something different in those habitats is influencing size.

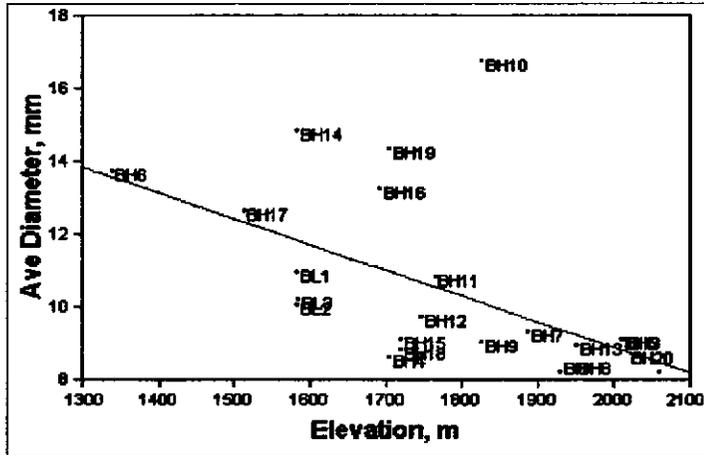


Figure 13. Relationship between elevation and the average diameter by population among Black Hills Oreohelicids.

Morphological variation among Black Hills and sister taxa

Shell and reproductive morphological measurements for the Oreohelicids from outside the Black Hills are given in Table 3. Snails from MT3 show no significant difference (Tukey-Kramer pairs comparison, $P=0.05$) from most of the small-morph populations in the Black Hills (Table 4) for diameter, whorl number, and height, which corresponds to the genetic data showing MT3 is not genetically distinct from the Black Hills snails. However, MT3 samples are significantly different than all of the large-morph populations in whorl number, height, and diameter. Populations from WY1, WY3, and WY4 are genetically distinct from the Black Hills, but have some morphological similarities (see Table 4).

Table 3. Morphological measurements of Oreohelicids from outside the Black Hills

Population	N	Mean Diameter, mm (standard error)	Mean Whorl Number (standard error)	Mean Height, mm (standard error)	Mean H/D (standard error)	Penis length, mm (standard error)	% ribbed (standard error)
MT3	8	8.85 (0.36)	4.09 (0.087)	5.81 (0.32)	0.65 (0.015)	---	---
WY1	4	12.04 (0.51)	4.81 (0.12)	9.02 (0.45)	0.77 (0.021)	---	---
WY3	4	12.00 (0.52)	5.00 (0.12)	9.94 (0.45)	0.83 (0.021)	14.91 (1.11)	0.33 (0.029)
WY8	4	15.43 (0.52)	5.06 (0.12)	11.94 (0.45)	0.78 (0.021)	10.77 (1.11)	0.47 (0.029)
WY4	6	13.78 (0.42)	4.83 (0.10)	10.10 (0.36)	0.73 (0.017)	---	---

Table 4. Significant differences between populations outside the Black Hills and the Black Hills populations for morphological measurements. Significance based on Tukey-Kramer all-pairs comparisons at the $P=0.05$ level.

Population	Black Hills populations with significantly different diameters	Black Hills populations with significantly different whorl number	Black Hills populations with significantly different height	Black Hills populations with significantly different peral length	Black Hills populations with significantly different % ribbing	Black Hills populations with significantly different H/D ratio
MT3 (8)	BH6,BH10, BH14,BH16, BH17,BH19, BL1	BH6,BH10, BH14,BH16, BH17,BH19	BH6,BH10, BH11,BH14, BH16,BH17, BH19,BL1	Not measured	Not measured	BH13,BH16, BH18,BH19
WY1	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH10, BH13,BH14, BH15,BH18, BH20	BH1,BH8, BH20	BH1,BH3, BH4,BH5, BH8,BH9, BH12,BH13, BH15,BH18, BH20	Not measured	Not measured	Not significantly different from any BH populations
WY3	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH10, BH13,BH14, BH15,BH18, BH20	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH12, BH13,BH20, BL3	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH11, BH12,BH13, BH15,BH18, BH20,BL1, BL2,BL3	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH13, BH20,BL3	Not significantly different from any BH populations	BH1,BH3, BH4,BH5, BH6,BH7, BH8,BH9, BH10,BH11, BH12,BH14, BH15,BH17, BH20,BL1, BL3
WY4	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH11, BH12,BH13, BH15,BH18, BH20,BL1, BL2,BL3	BH1,BH3, BH4,BH5, BH8,BH20	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH11, BH12,BH13, BH15,BH18, BH20,BL1, BL2,BL3	Not measured	Not measured	Not significantly different from any BH populations
WY8	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH11, BH12,BH13, BH15,BH17, BH18,BH20, BL1,BL2, BL3	BH1,BH3, BH4,BH5, BH7,BH8, BH9,BH12, BH13,BH18, BH20,BL3	BH1,BH3, BH4,BH5, BH6,BH7, BH8,BH9, BH11,BH12, BH13,BH15, BH16,BH17, BH18,BH20, BL1,BL2, BL3	BH10	BH19,BH20	BH3

Radula measurements appear to more closely follow the genetic lineages than shell morphology. The within-population variation appears small compared to major differences between some populations. The Judith Mountains (MT3) *Oreohelices* have a radula formula indistinguishable from the formula for the Black Hills populations (25 lateral and marginal, 1 central tooth); all the other populations outside the Black Hills appear to have different formulae. The Judith Mountain population is also genetically identical (based on mtDNA sequencing) to the Black Hills populations and is considered part of the *O. cooperi* clade. Populations located in the Pryor Mountains and in Western Montana that are closely related to one another but widely divergent from *O. cooperi* have a radula formula ranging from 30-1-30 to 32-1-32, similar to each other and clearly different from any other populations. Populations located in higher elevations in the Bighorn Mountains (WY1 and WY2) and relatively closely related to the *O. cooperi* clade appear to have slightly fewer teeth than that clade, ranging from 21-1-21 to 23-1-23. A seemingly genetically distinct population of *O. cooperi* also in the Bighorn Mountains (WY3) has slightly greater number of teeth compared to those from the Black Hills and the Judith Mountains (27-1-27).

DISCUSSION

Genetic distinctness and taxonomic identity of Black Hills Oreohelix

Phylogenetic results support the idea of a single genetic lineage within the Black Hills and allow us to resolve previous confusion over its taxonomic identity. In this study, we found a maximum of 0.2% divergence between different sampled populations in the Black Hills and no indication of population-level haplotype structure. In addition, strong bootstrap and Bayesian posterior probability values (92, 0.93, Figure 3) support one genetically distinct lineage in the Black Hills. From this data we conclude that there is only one genetic lineage within the Black Hills *Oreohelix*. We refer to this lineage as its own species, *O. cooperi*, given the strong bootstrap and Bayesian posterior probability values supporting the *O. cooperi* node, the relatively large amount of sequence divergence (>2%) from other lineages, and given that it is not closely related to other individuals that have been identified as *O. strigosa* (Weaver, in prep.).

The results from our analysis also strongly suggest that *O. cooperi* is not endemic to the Black Hills. Individuals from the Black Hills are genetically identical to a group of individuals located in the Judith Mountains of Montana (MT3 in 2) and closely related to another population in Tensleep Canyon of the Bighorn Mountains (WY3 in Figure 2). The Tensleep Canyon population appears to be a well-supported separate lineage from the Black Hills populations (bootstrap and Bayesian posterior probability values 100, 100, respectively, Figure 3); however, sequence divergence estimates (less than 1%) and strong support for the relationship between Tensleep and the Black Hills (bootstrap and Bayesian posterior probability values 92, 93, respectively) suggest that this lineage is likely an individual population of *O. cooperi* rather than a separate species. We base this conclusion on both observed divergence rates in the larger *Oreohelix* tree (Weaver, in prep.) and other studies which have examined population and species level divergences in pulmonates (Wethington & Guralnick 2004). Two other higher elevation *Oreohelix* populations in the Bighorn Mountains (WY1 & 2 in Figures 2 & 3) are also more closely related to *O. cooperi* than any other lineage (see ML tree, Figure 3), but are divergent enough (2 % seq. divergence and greater) to likely be another species.

Distinguishing among biogeographic hypotheses

Between the Black Hills and the Eastern Rocky Mountains lies approximately 100km of plains and desert grasslands and shrublands, a seemingly unusable and impassable habitat for *Oreohelix* mountainsnails. In order to explain the genetic similarities between groups in the Black Hills and Eastern Rocky Mountains and to examine the broader biogeographic patterns of *O. cooperi*, we use evidence from both the phylogenetic and DesktopGARP data. Because the larger clade of *O. cooperi* consists of three distinct geographic areas (Black Hills, Judith Mountains and Bighorn Mountains) and there are varying levels of divergence within this clade, we focus on discussing potential past or present connections between the Black Hills and either the Judith Mountains or Bighorn Mountains separately.

In the case of *O. cooperi* in the Black Hills and the Judith Mountains, the genetic data strongly support a very recent connection. There is no discernable genetic differentiation between the two groups, suggesting a very recent connection between populations. This is especially likely given the rapid rate of mitochondrial gene evolution in pulmonates (on the order of 6% divergence per 1 million years) based on full mitochondrial genome sequencing of multiple pulmonates (Yamazaki *et al.* 1997) and another biogeography study on land snails (Pfenniger and Posada 2002). As a result, we can discount both ancient vicariance and ancient dispersal events between the Black Hills and the Judith Mountains. Furthermore, because DesktopGARP models show no continuous connection of suitable habitat between the Judith Mountains and Black Hills and because *Oreohelix* dispersal rates are likely far too low for travel across the intervening distances (Henderson 1924), we do not believe that *O. cooperi* could actively disperse through the intervening habitat. Thus, it is more likely that passive long distance dispersal has occurred. Given that the Judith Mountain and Black Hills groups are genetically identical (0% sequence divergence), the separation appears to be recent; therefore, the long distance dispersal event may be either animal or human mediated. The actual vectors are unknown, but it has been documented that passive transport on large mammals or birds does occur in smaller pulmonates (Boag 1986). The individuals in the Judith Mountain population have a small body size, making passive transport more likely. Given the wide distribution of *Oreohelix* in the Black Hills and the isolated distribution in the Judith Mountains, it also seems likely that the polarity of transport has been from the Black Hills to the Judith Mountains and not vice versa. Dispersal into the Black Hills from the Judith Mountains would require rapid dispersal across the Black Hills region, which is unlikely in the relatively sedentary *Oreohelix*.

Interpreting the biogeographic connection between populations of *O. cooperi* in the Black Hills and Bighorn Mountains is more complex. One possible explanation is vicariance driven by late Pleistocene-Holocene warming, drying, and vegetation change that separated once contiguous populations across the Northern Plains from the Black Hills into Wyoming. However, we believe the data do not support ancient vicariance of a once continuous population for two reasons. First, one of the Bighorn populations is more closely related to Black Hills *O. cooperi* than it is to the other population located in the Bighorns. Under a vicariance model, we would expect Bighorn populations to be more closely related to each other than to the Black Hills populations. Second, since the majority of the DesktopGARP model outputs suggest environmentally suitable conditions are available between the Bighorns and the Black Hills along lowland routes (1000-2300 meters) and thus environmental temperature and precipitation changes (at least those used in the DesktopGARP models) are likely not driving these patterns. If those changes were limiting distribution, we would have expected that the models would not show potentially suitable environmental conditions across the Northern Great Plains.

Another possible explanation for the connection between *O. cooperi* in the Black Hills and the Bighorn Mountains is that dispersal is possible through "stepping stone" island habitats in the intervening Great Plains and that unsampled populations along that stretch remain. As discussed above, the DesktopGARP models do indicate that continuous suitable environments for *O. cooperi* may occur along lowland routes between the Black Hills and the Bighorns. However, no *Oreohelix* collection records in this area exist and we would argue for a low probability of multiple long-persistent intervening populations because of the lack of divergence across all sampled populations of *O. cooperi* regardless of distance. Given the low vagility of the snails (Henderson 1924; Anderson & Schmidt, in prep.), it is unlikely that we would observe so little genetic variation in long persistent populations separated by over a hundred kilometers.

Based on the available data, we argue that the most likely biogeographic scenario for the Black Hills and Bighorn Mountain connection is also passive long distance dispersal. Animal dispersal seems most likely in this case because the two groups, one in Tensleep Canyon (WY3, Figure 2) and the other in the Black Hills, are genetically divergent (0.6%) from each other, suggesting a longer separation than timeframes for human transport will allow (assuming approximately 6% sequence divergence per million years as in Pfenninger and Posada 2002). Determining the direction of potential dispersal events is difficult in this case. The wide occurrence of populations in the Black Hills and the limited occurrences in the Bighorns suggest that the polarity has been dispersal from the Black Hills back into the Bighorns. However, the phylogenetic tree suggests that the Bighorn Mountain groups (WY1, 2, 3) are basal to the Black Hills groups, supporting dispersal into the Black Hills from the Bighorns rather than vice versa. More sampling of haplotypes and other potential populations in the Bighorn Mountains and surrounding areas may be necessary to distinguish among alternate dispersal polarities between the Bighorns and the Black Hills populations.

Morphological groups within the Black Hills

Variation in shell morphology has long been used to differentiate among gastropod species. Glancing through Pilsbry (1939) or any other taxonomic key demonstrates how critical shell morphology is for defining snail species. However, plasticity in shell size and shape has also been observed within species, including those in the Oreohelicidae (Henderson 1924, Pilsbry 1916, 1939).

Observed phenotypes from non-Mendelian traits result from a combination of genetic, environmental, and gene by environment interactions. Goodfriend (1986) reviews research studying land snail shell variation, some of which has been correlated to specific environmental parameters. Goodfriend (1986) concludes, "...there are no overriding environmental factors to which shell form responds. Rather, shell form is responsive to a variety of environmental factors, whose relative importance may vary geographically or among taxa. Shell form may also relate to a snail's way of dealing with the environment (niche). [p.217-218]"

The morphological data clearly support distinct differences in size among populations of *O. cooperi* within the Black Hills. Size differences among populations are further supported by another study that tracked Black Hills *O. cooperi* populations throughout the summer (Anderson & Schmidt, in prep.). Average diameter of small-morph populations never approached the size of large-morph populations in that study.

On the whole, our shell measurements are slightly different than the descriptions of the three groups proposed by Frest & Johannes (2002). Their three proposed groups and the descriptions given are shown in Table 5.

Table 5. Groups proposed by Frest & Johannes (2002) and the descriptions given in that report. Sample size is unknown for their stated dimensions.

Group proposed by F&J	Diameter	Whorls	Height	H/W	Populations that correspond to F&J sampling points
<i>O. cooperi</i>	15 mm (from Binney, 1858)	“adults usually 5 to 5 ¾”	9 mm (from Binney, 1858)	Not given	BH6, BH12, BH14, BH17, BH19
<i>O. sp. 1</i>	9.1 to 9.7 mm	5	6.7 to 7.1 mm	0.74	BH1, BH3, BH4, BH5, BH7, BH8, BH9, BH10, BH11, BH13, BH15, BH18
<i>O. sp. 2</i>	maximum 9.3 mm	5 to 5 ¼	6.3 mm	0.67	BL1, BL2, BL3

Dimensions of additional samples collected by H. B. Baker (taken from Spearfish Creek drainage near Spearfish and Savoy, South Dakota), but measured and reported by Pilsbry (1939), include individuals that range in diameter from 9 mm (4.5 whorls) to 22 mm (5.5 whorls). None of our samples reached the size of Pilsbry’s measurements, with the largest diameter we measured at 19.3 mm.

Relationship of morphological groups to genetic lineages within the Black Hills

The morphological data presented here suggest multiple groups of Oreohelicids within the Black Hills. Due to the evident gene flow and similar radular tooth counts, however, it does not seem valid to suggest that these are separate species. These results contrast to a study of the morphologically variable snail species, *Ainohelix editha* in Japan, where genetic and morphological results were congruent (Teshima *et al.*, 2003).

Relationship of morphological variation to geographic and environmental factors

Genetic evidence for gene flow among populations suggests that the morphological variation is at least partially due to environmental factors. Unfortunately, we were unable to clearly identify any individual factor that was clearly related to size, except for an interesting relationship with elevation as discussed below. Lack of geographic structure to the variation does nothing to clarify the issue. The fact that individual size measurements (diameter, height, etc.) varied among populations, but not shape (as measured by H/D) lends more credence to a factor that influences growth equally in all dimensions. More standardized measurements over the entire year might still produce evidence supporting a single environmental factor as the cause for size differences.

Possible variation among years in size, if that is what explains the differences between these results and those reported by Frest & Johannes (2002), would be further evidence that environmental factors are important. For example, yearly variation in temperature and/or moisture levels could influence activity levels and, therefore, growth rates. Variation in weather has been related to differences in snail size in *Theba pisana*, where both spring humidity and winter population density was related to the rate of infantilism (Morna 1989).

An interesting case study involves populations BH9 and BH10, which are spaced only about 100 m apart on opposite sides of a creek and road. BH10 belongs to the large-morph group and has significantly larger average diameter, height, whorl number, and penial length than the BH9 population. The BH10 samples were collected on an east-facing slope that was drier than the northwest facing slope where the BH9 samples were collected. Anecdotal descriptions of the site also noted pine and spruce on the BH9 site, while only pine was noted at the BH10 site. Despite the close proximity of these populations, the environment and/or gene by environment interactions are producing different phenotypes. Genetic data do not show haplotypes from these two populations to be any more closely related to each other than to haplotypes from other Black Hills populations.

Unlike the habitat variables, elevation showed a negative relationship with average size, at least for the small-morph populations. This is the opposite relationship reported in the literature where several studies have shown decreasing shell size at *higher* elevations in the land snail, *Arianta arbustorum*, in Europe (i.e., Burla & Stahel 1983, Baur & Raboud 1988, Baur & Baur 1998). Burla & Stahel (1983) hypothesize that these differences are due to differential conditions at higher elevations. Life history characteristics such as clutch size, egg size, etc. are also intertwined in the observed patterns for *A. arbustorum* (Baur & Raboud 1988, Baur & Baur 1998). Density (Baur 1988) and competition (Baur & Baur 1990) have also influences adult size in snails through the apparent mechanism of slower juvenile growth rates (Baur 1988). Whether these life history factors play a different role in *O. cooperi* that can explain the opposite relationship may be able to be addressed when further data on clutch size and growth rates is fully analyzed (Anderson, unpublished data). These types of interactions might also be gene by environment influences.

Morphological variation among Black Hills and sister taxa

The shell, reproductive, and radula data are consistent with the genetic conclusion that the MT3 population is part of the same lineage as the Black Hills populations. Shell data for the other Wyoming populations measured do not distinguish these taxa from Oreohelicids in the Black Hills, despite genetic evidence to that effect. Radula data is consistent with the genetic data in differentiating between Black Hills and other Wyoming populations. Major differences in number of marginal+lateral teeth reflect large divergences of groups from each other, and more minor differences appear to reflect more recent divergences. Given the consistent results across the genetic and radula datasets and given previous literature about environmental induction of shell morphological response in pulmonates, the logical conclusion here is that shell morphology and body size are not fixed traits of lineages but likely vary within species depending on external conditions.

Counting number of radula teeth per row may be a very simple and fast way to at least distinguish between species or higher level clade groupings. However, Ports (2004) found different radular tooth counts in different populations of the same morphological species of *Oreohelix hemphilli* (Newcomb, 1869), so some care must be taken. Also, radula tooth number

is invariant to fixative methods and whether or not the animal is relaxed prior to fixation, unlike the soft-tissue reproductive anatomy. Therefore the radula is also likely more reliable for original identification or re-identification of material already collected and vouchered in natural history collections, or material collected under different field sampling methods:

CONCLUSIONS

The Black Hills lineage appears to be one genetically distinct lineage within the genus *Oreohelix*. Although there are other populations of *O. cooperi* outside of the Black Hills, it seems likely that these populations are the result human or animal dispersal events, are isolated to very small areas (i.e., Judith Mountains in Montana) outside the Black Hills, and are not likely to aide in the proliferation of this species.

The large and small morphs of *O. cooperi* found in the Black Hills do not represent distinct genetic lineages, but rather reflect some environmental influence(s) not detected as of yet. This suggests that all populations within the Black Hills should be considered to be *O. cooperi* during management decisions.

Whether or not the morphs represent invariable ecotypes with some heritable component cannot be determined from the available data. Common garden experiments would be required to determine whether the morphs are retained in other environments.

A surprising outcome of this study is the low genetic divergence between populations of *O. cooperi*. Assuming low vagilities and a long enough time span, we might have expected at least some differentiation between Bear Lodge Mountain populations (BL1-3) and Black Hills proper populations (Figure 2,3). Further analyses using the data collected here for population-level processes will potentially provide further insights into these questions. In this study, our sampling within the Black Hills' populations was not great enough to look more carefully at haplotype frequencies within and between populations, but future work will allow for such comparisons. The broader scale systematics of the genus *Oreohelix*, including the unknown species from this study, also need to be evaluated. This work is part of Weaver's dissertation research.

SUMMARY

In summary, we return to the original objectives.

- 1) The *Oreohelix* snails found in the Black Hills consist of only one group genetically. Differences in shell morphology among groups do exist, presumably due to variations in habitat in different areas of the Black Hills.
- 2) *Oreohelix cooperi* in the Black Hills cannot be distinguished from *Oreohelix* specimens from one site in Montana and are very similar to specimens in one site in the Bighorn Mountains (the Tensleep Canyon population). However, they are distinct from other *Oreohelix* in Wyoming and elsewhere in Montana.
- 3) The results of this work are being prepared for submission to scientific journals for publication. We anticipate submitting two manuscripts within the next month, one on the phylogenetic and biogeography results to the Journal of Biogeography and a second on the morphological variation to the Journal of Molluscan Studies. South Dakota Department of Game, Fish & Parks will be acknowledged in each manuscript for their financial support. Upon publication, copies will be supplied to South Dakota Department of Game, Fish & Parks.

ACKNOWLEDGEMENTS

Collections in South Dakota were made under Scientific Collection Permits #37 (2004) and #26 (2005) to T. Anderson. Collections elsewhere were made with appropriate permits. Funding for this project was provided through the South Dakota Department of Game, Fish & Parks. Special thanks to Christina Schmidt for her excellent work in the field. Doug Backlund of the South Dakota Department of Game, Fish & Parks was very helpful. Patti Lynch of the Black Hills National Forest also provided helpful insights on locations. Thanks also to David McDonald and Solomon Tin Chi Chak of the University of Wyoming for the loan of their samples from Wyoming locations outside the Black Hills (populations WY3 and WY08).

LITERATURE CITED

- Anderson, R. P., D. Lew, and A. T. Peterson. 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. *Ecological Modeling* 162:211-232.
- Anderson, T. K. 2005. *Oreohelix strigosa cooperi* (Cooper's Rocky Mountain snail): A Technical Conservation Assessment. USDA Forest Service, Rocky Mountain Region. Available: www.fs.fed.us/r2/projects/scp/assessments
- Anderson, T. K. In prep. Reproductive strategies of the live-bearing *Oreohelix cooperi*: brood size, adult size, and habitat. To be submitted to *Journal of Molluscan Studies*.
- Anderson, T. and C. Schmidt. In prep. Notes on the life history of *Oreohelix cooperi* of the Black Hills of South Dakota as obtained from a mark-recapture study. To be submitted to the *Canadian Journal of Zoology*.
- Baur, B. 1988. Population regulation in the land snail *Arianta arbustorum*: density effects on adult size, clutch size and incidence of egg cannibalism. *Oecologia* 77: 390-394.
- Baur, A. & Baur, B. 1998. Altitudinal variation in size and composition of eggs in the land snail *Arianta arbustorum*. *Canadian Journal of Zoology* 76: 2067-2074.
- Baur, B. & Baur, A. 1990. Experimental evidence for intra- and interspecific competition in two species of rock-dwelling land snails. *Journal of Animal Ecology* 59: 301-315.
- Baur, B. & Raboud, C. 1988. Life history of the land snail *Arianta arbustorum* along an altitudinal gradient. *Journal of Animal Ecology* 57: 71-87.
- Bequaert, J. C. and W. B. Miller. 1973. *The Mollusks of the Arid Southwest*. University of Arizona Press, Tucson, AZ.
- Boag, D. A. 1986. Dispersal in pond snails: potential role for waterfowl. *Can. J. Zool.* 64: 904-909.
- Burch, J. B. 1962. *How to Know the Eastern Land Snails*. Wm. C. Brown Co. Publishers, Dubuque, Iowa.
- Burla, H. & W. Stahel 1983. Altitudinal variation in *Arianta arbustorum* (Mollusca, Pulmonata) in the Swiss Alps. *Genetica* 62: 95-108.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17: 368-376.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the Bootstrap. *Evolution* 39(4): 783-791
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.
- Frest, T. J. and E. J. Johannes. 2002. Land snail survey of the Black Hills National Forest, South Dakota and Wyoming, Summary Report, 1991-2001. Final Report Contract #43-67TO-8-1085. USDA Forest Service, Black Hills National Forest.
- Frest, T. J. and E. J. Johannes. 1995. Interior Columbia Basin Mollusk Species of Special Concern. Final Report to Interior Columbia Basin Ecosystem Management Project. Deixis Consultants, Seattle, Washington.
- Goodfriend, G. A. 1986. Variation in land-snail shell form and size and its causes: a review. *Systematic Zoology* 35: 204-223.
- Guinden, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52(5): 696-704.

- Henderson, J. 1924. Mollusca of Colorado, Utah, Montana, Idaho, and Wyoming. University of Colorado Studies 13.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, A.M., Paabo, S., Villablanca, F.X., and Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*, 86(16): 6196-6200.
- Lee, T. and D. O Foighil 2003. Phylogenetic structure of the Sphaeriinae, a global clade of freshwater bivalve mollusks, inferred from nuclear (ITS-1) and mitochondrial (16S) ribosomal gene sequences. *Zoological Journal of the Linnean Society* 137: 245-260.
- Moran, S. 1989. Weather- and population density-induced infantilism in the landsnail *Theba pisana* in a semi-arid climate. *International Journal of Biometeorology* 33: 101-108.
- Pfenninger, M. and F. Magnin. 2001. Phenotypic evolution and hidden speciation in *Candidula unifasciata* ssp. (Helicellinae, Gastropoda) inferred by 16S variation and quantitative shell traits. *Molecular Ecology* 10: 2541-2554.
- Pfenninger, M. and D. Posada. 2002. Phylogeographic history of the land snail *Candidula unifasciata* (Helicellinae, Stylommatophora): fragmentation, corridor migration, and secondary contact. *Evolution* 56(9):1776-88.
- Pilsbry, H.A. 1916. Notes on the anatomy of *Oreohelix*, with a catalogue of the species. *Proceedings of the Academy of Natural Sciences of Philadelphia* 1916: 340-362.
- Pilsbry, H.A. 1939. Land Mollusca of North America (North of Mexico). *Academy of Natural Sciences of Philadelphia Monographs* 3: volume 1, part 1.
- Ports, M.A. 2004. Biogeographic and taxonomic relationships among the mountain snails (Gastropoda: Oreohelicidae) of the central Great Basin. *Western North American Naturalist* 64:145-154.
- Posada, D. and Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14(9): 817-818.
- Rambaut, A. E. 1996. Se-Al: sequence alignment editor. Available: evolve.zoo.ox.ac.uk/software/Se-Al
- Ronquist, F. and Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572-1574.
- Ross, T. K. 1999. Phylogeography and conservation genetics of the Iowa Pleistocene snail. *Molecular Ecology* 8: 1363-1373.
- Stockwell, D. R. B., and D. P. Peters. 1999. The GARP modelling system: Problems and solutions to automated spatial prediction. *International Journal of Geographic Information Systems* 13:143-158.
- Swofford, D.L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony. Version 4. Sinauer Associates, Sunderland, MA.
- Teshima, H., Davison, A., Kuwahara, Y., Yokoyama, J., Chiba, S., Fukuda, T., Ogimura, H. & Kawata, M. 2003. The evolution of extreme shell shape variation in the land snail *Ainohelix editha*: a phylogeny and hybrid zone analysis. *Molecular Ecology* 12: 1869-1878.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analyses tools. *Nucleic Acid Research* 25(24): 4876-4882.

- Thomaz, D., Guiller, A., and B. Clarke. 1996. Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proceedings of the Royal Society, London B* 263: 363-368.
- Turgeon, D. D., J. F. Quinn, Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: Mollusks, 2nd edition. American Fisheries Society, Special Publication 26, Bethesda, Maryland.
- Wethington, A. R. and R. Guralnick. 2004. Are populations of physids from different hot springs distinctive lineages? *American Malacological Bulletin* 19: 135-144.
- Yamazaki N, Ueshima R, Terrett JA, Yokobori S, Kaifu M, Segawa R, Kobayashi T, Numachi K, Ueda T, Nishikawa K, Watanabe K, Thomas RH. 1997. Evolution of pulmonate gastropod mitochondrial genomes: comparisons of gene organizations of *Euhadra*, *Cepaea* and *Albinaria* and implications of unusual tRNA secondary structures. *Genetics* 145(3):749-58.

AUTHOR INFORMATION

Dr. Tamara Anderson is an Adjunct Curator at the University of Colorado Museum. She specializes in mollusks and has been actively involved in many Black Hills gastropod studies. Her previous mollusk work has included laboratory growth studies of mussels, population genetic analyses of endangered snails, gastropod community surveys, and mark-recapture field studies.

Dr. Robert Guralnick is an Assistant Professor in the Department of Ecology and Evolutionary Biology and Zoology Curator at the University of Colorado Museum. His broad interests include mapping and analysis of biodiversity data utilizing in part past and present species occurrence datasets. Recent work has ranged from analysis of morphological and distributional responses of animals to past climate changes, to using molecular and morphological approaches to document current regional lineage diversity. His regional focus is western North America and his taxonomic focus is on mollusks and mammals.

Mrs. Kathleen Weaver is a Ph.D. student in the Ecology and Evolutionary Biology Department at the University of Colorado. She is currently in the process of completing her dissertation on the biogeography and systematics of the mountain snail, *Oreohelix*. She conducted the genetic work for this project as well as taking a lead role in the biogeographic hypothesis testing.

APPENDIX 1

Locality Information

Population	Location Description	GPS	location
BH1	South Fork near Trebor Draw, Black Hills	13T 589567	4890336
BH3	Castle Creek tributary, Black Hills	13T 583343	4881947
BH4	Little Spearfish Canyon, Black Hills	13T 582014	4909155
BH5	Grand Canyon, Black Hills	13T 578727	4894632
BH6	Botany Bay Picnic Area, Spearfish Canyon, Black Hills	13T 589218	4919562
BH7	Near Mallo Camp, Black Hills	13T 575440	4881406
BH8	Ditch Creek South of Porcupine Draw, Black Hills	13T 593074	4866839
BH9	Along Rapid Creek, Black Hills	13T 590957	4887096
BH10	Along Rapid Creek, Black Hills	13T 590898	4887111
BH11	Near Hanna, Black Hills	13T 592834	4901093
BH12	Dead Ox Picnic Area, Black Hills	13T 586473	4901818
BH13	SE of Rd 109.3, just before junction with Rd 111, Black Hills	13T 578133	4879991
BH14	Near McKinley Gulch, Black Hills	13T 587659	4909367
BH15	Near Timon Campground, Black Hills	13T 580916	4908916
BH16	1.3 Miles N. of Cheyenne Crossing, Black Hills	13T 589227	4907379
BH17	2 miles S of Cook Peak Trailhead, Higgins Gulch, Black Hills	13T 582010	4922025
BH18	E of Rd 222.1, S of junction with Rd 223, Black Hills	13T 582309	4909574
BH19	Little Spearfish Canyon, SW of Roughlock Falls Picnic area, Black Hills	13T 584050	4910943
BH20	Castle Creek drainage, Black Hills	13T 592783	4886573
BL1	Togus Spring area, Bear Lodge	13T 546667	4932412
BL2	Off Togus Creek Rd, Bear Lodge	13T 546312	4932305
BL3	Off Togus Creek Rd, 0.7 miles up from Beaver Creek, Bear Lodge	13T 545774	4932086
WY1	Shell, Bighorn Mountains, Big Horn County, WY	13T 291250	4940245
WY2	Shell, Bighorn Mountains, Big Horn County, WY	13T 290417	4940284
WY3	Ten Sleep Canyon, Big Horn Mountains, Big Horn County, WY	13T 318583	4886373
WY4	Mammoth, Park County, WY	12T 522725	4978217
WY5	Ayres Natural Bridge, Converse County, WY	13T 450013	4731330
WY8	TenSleep Canyon, Bighorn Mtns	13T 0313406	4882774
MT1	Mtns	12T 477284	5130167
MT2	Pryor Mtns, MT	12T 691913	5021992
MT3	Lewistown, Judith Mountains, Fergus County, MT	12T 628472	5221422
UT1, <i>Oreohelix haydeni hybrida</i>	Temple Fork and Logan River, Cache County, Utah	---	---

Appendix 2. T-tables

The following pages show the t-test comparisons output from JMP for the morphological measurements of the Black Hills populations. Positive values show significantly different populations.

Oneway Analysis of Diameter By Population

Means Comparisons

Dif=Mean(I)-Mean(J)

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

3.64781

Abs(Dif)-LSD

	BH10	BH14	BH19	BH6	BH16	BH17	BL1	BH11	BL3	BL2	BH12	BH7	BH3
BH10	-1.57634	0.39135	0.75688	1.53035	2.07545	2.59435	4.40314	4.25117	4.99477	4.98704	5.33688	5.92535	6.08035
BH14	0.39135	-1.49545	-1.13362	-0.35645	0.19116	0.70755	2.51886	2.36067	3.10691	3.09487	3.44638	4.03855	4.19355
BH19	0.75688	-1.13362	-1.78740	-1.02319	-0.48425	0.04081	1.84344	1.70688	2.44387	2.44699	2.79260	3.37181	3.52681
BH6	1.53035	-0.35645	-1.02319	-1.49545	-0.94784	-0.43145	1.37986	1.22167	1.96791	1.95587	2.30738	2.89955	3.05455
BH16	2.07545	0.19116	-0.48425	-0.94784	-1.31160	-0.80122	1.01610	0.84322	1.59562	1.57359	1.92894	2.52978	2.68478
BH17	2.59435	0.70755	0.04081	-0.43145	-0.80122	-1.49545	0.31586	0.15767	0.90391	0.89187	1.24338	1.83555	1.99055
BL1	4.40314	2.51886	1.84344	1.37986	1.01610	0.31586	-1.31160	-1.48447	-0.73207	-0.75411	-0.39876	0.20209	0.35709
BH11	4.25117	2.36067	1.70688	1.22167	0.84322	0.15767	-1.48447	-1.78740	-1.05042	-1.04729	-0.70169	-0.12248	0.03252
BL3	4.99477	3.10691	2.44387	1.96791	1.59562	0.90391	-0.73207	-1.05042	-1.57634	-1.58407	-1.23423	-0.64576	-0.49076
BL2	4.98704	3.09487	2.44699	1.95587	1.57359	0.89187	-0.75411	-1.04729	-1.58407	-1.93062	-1.58777	-1.01446	-0.85946
BH12	5.33688	3.44638	2.79260	2.30738	1.92894	1.24338	-0.39876	-0.70169	-1.23423	-1.58777	-1.78740	-1.20819	-0.85946
BH7	5.92535	4.03855	3.37181	2.89955	2.52978	1.83555	0.20209	-0.12248	-0.64576	-1.01446	-1.20819	-1.49545	-1.34045
BH3	6.08035	4.19355	3.52681	3.05455	2.68478	1.99055	0.35709	0.03252	-0.49076	-0.85946	-1.05319	-1.34045	-1.49545
BH15	5.94280	4.05210	3.39831	2.91310	2.53465	1.84910	0.20696	-0.09597	-0.62851	-0.98206	-1.18169	-1.48190	-1.63690
BH5	6.12135	4.23455	3.56781	3.09555	2.72578	2.03155	0.39809	0.07352	-0.44976	-0.81846	-1.01219	-1.29945	-1.45445
BH9	6.21135	4.32455	3.65781	3.18555	2.81578	2.12155	0.48809	0.16352	-0.35976	-0.72846	-0.92219	-1.20945	-1.36445
BH13	6.25292	4.36384	3.70507	3.22484	2.84969	2.16084	0.52199	0.21078	-0.31819	-0.67759	-0.87493	-1.17016	-1.32516
BH18	6.23403	4.34352	3.68974	3.20452	2.82608	2.14052	0.49839	0.19545	-0.33708	-0.69063	-0.89026	-1.19048	-1.34548
BH4	6.57335	4.68655	4.01981	3.54755	3.17778	2.48355	0.85009	0.52552	0.00224	-0.36646	-0.56019	-0.84745	-1.00245
BH8	7.04752	5.16166	4.49167	4.02266	3.65512	2.95866	1.32743	0.99739	0.47641	0.10395	-0.08833	-0.37234	-0.52734
BH1	7.01435	5.12755	4.46081	3.98855	3.61878	2.92455	1.29109	0.96652	0.44324	0.07454	-0.11919	-0.40845	-0.56145
BH20	7.05207	5.16621	4.49622	4.02721	3.65966	2.96321	1.33197	1.00193	0.48096	0.10850	-0.08378	-0.36779	-0.52279

Positive values show pairs of means that are significantly different.

Oneway Analysis of Height By Population

Means Comparisons

Dif=Mean[i]-Mean[j]

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

3.64781

Abs(Dif)-LSD

	BH10	BH19	BH14	BH6	BH16	BH17	BL1	BH11	BL2	BL3	BH7	BH12	BH18
BH10	-1.43473	-0.27775	0.36549	0.63249	0.84452	1.85449	2.95375	2.75796	3.08648	3.36416	3.83549	3.71796	3.86653
BH19	-0.27775	-1.62683	-0.99200	-0.72500	-0.51858	0.49700	1.59065	1.40889	1.74127	2.00907	2.47800	2.36889	2.51746
BH14	0.36549	-0.99200	-1.36110	-1.09410	-0.87978	0.12790	1.22945	1.02800	1.35500	1.63660	2.10890	1.98800	2.13657
BH6	0.63249	-0.72500	-1.09410	-1.36110	-1.14678	-0.13910	0.96245	0.76100	1.08800	1.36960	1.84190	1.72100	1.86957
BH16	0.84452	-0.51858	-0.87978	-1.14678	-1.19376	-0.19155	0.91547	0.70065	1.02416	1.31486	1.78945	1.66065	1.80922
BH17	1.85449	0.49700	0.12790	-0.13910	-0.19155	-1.36110	-0.25955	-0.46100	-0.13400	0.14760	0.61990	0.49900	0.64757
BL1	2.95375	1.59065	1.22945	0.96245	0.91547	-0.25955	-1.19376	-1.40858	-1.08507	-0.79437	-0.31978	-0.44858	-0.30001
BH11	2.75796	1.40889	1.02800	0.76100	0.70065	-0.46100	-1.40858	-1.62683	-1.29445	-1.02664	-0.55772	-0.66683	-0.51825
BL2	3.08648	1.74127	1.35500	1.08800	1.02416	-0.13400	-1.08507	-1.29445	-1.75717	-1.49574	-1.02683	-1.13206	-0.98349
BL3	3.36416	2.00907	1.63660	1.36960	1.31486	0.14760	-0.79437	-1.02664	-1.49574	-1.43473	-0.96340	-1.08093	-0.93236
BH7	3.83549	2.47800	2.10890	1.84190	1.78945	0.61990	-0.31978	-0.55772	-1.02833	-0.96340	-1.36110	-1.48200	-1.33343
BH12	3.71796	2.36889	1.98800	1.72100	1.66065	0.49900	-0.44858	-0.66683	-1.13206	-1.08093	-1.48200	-1.62683	-1.47825
BH18	3.86653	2.51746	2.13657	1.86957	1.80922	0.64757	-0.30001	-0.51825	-0.98349	-0.93236	-1.33343	-1.47825	-1.62683
BH13	3.96251	2.61019	2.23383	1.96683	1.90949	0.74483	-0.19974	-0.42552	-0.89285	-0.83638	-1.23617	-1.38552	-1.53410
BH9	4.07349	2.71600	2.34690	2.07990	2.02745	0.85790	-0.08178	-0.31972	-0.79033	-0.72540	-1.12310	-1.27972	-1.42829
BH15	4.12653	2.77746	2.39657	2.12957	2.06922	0.90757	-0.04001	-0.25825	-0.72349	-0.67236	-1.07343	-1.21825	-1.36683
BH5	4.29549	2.93800	2.56890	2.30190	2.24945	1.07990	0.14022	-0.09772	-0.56833	-0.50340	-0.90110	-1.05772	-1.20629
BH3	4.37549	3.01800	2.64890	2.38190	2.32945	1.15990	0.22022	-0.01772	-0.48833	-0.42340	-0.82110	-0.97772	-1.12629
BH4	4.40149	3.04400	2.67490	2.40790	2.35545	1.18590	0.24622	0.00828	-0.46233	-0.39740	-0.79510	-0.95172	-1.10029
BH8	4.76911	3.40952	3.04338	2.77638	2.72595	1.55438	0.61672	0.37380	-0.09813	-0.02978	-0.42662	-0.58620	-0.73477
BH20	4.83275	3.47316	3.10701	2.84001	2.78959	1.61801	0.68036	0.43744	-0.03449	0.03386	-0.36299	-0.52256	-0.67113
BH1	4.86949	3.51200	3.14290	2.87590	2.82345	1.65390	0.71422	0.47628	0.00567	0.07060	-0.32710	-0.48372	-0.63229

Positive values show pairs of means that are significantly different.

3H13	BH9	BH15	BH5	BH3	BH4	BH8	BH20	BH1
5251	4.07349	4.12653	4.29549	4.37549	4.40149	4.76911	4.83275	4.86949
1019	2.71600	2.77746	2.93800	3.01800	3.04400	3.40952	3.47316	3.51200
3383	2.34690	2.39657	2.56890	2.64890	2.67490	3.04338	3.10701	3.14290
6683	2.07990	2.12957	2.30190	2.38190	2.40790	2.77638	2.84001	2.87590
0949	2.02745	2.06922	2.24945	2.32945	2.35545	2.72595	2.78959	2.82345
4483	0.85790	0.90757	1.07990	1.15990	1.18590	1.55438	1.61801	1.65390
9974	-0.08178	-0.04001	0.14022	0.22022	0.24622	0.61672	0.68036	0.71422
2552	-0.31972	-0.25825	-0.09772	-0.01772	0.00828	0.37380	0.43744	0.47628
9285	-0.79033	-0.72349	-0.56833	-0.48833	-0.46233	-0.09813	-0.03449	0.00567
3638	-0.72540	-0.67236	-0.50340	-0.42340	-0.39740	-0.02978	0.03386	0.07060
3617	-1.12310	-1.07343	-0.90110	-0.82110	-0.79510	-0.42662	-0.36299	-0.32710
8552	-1.27972	-1.21825	-1.05772	-0.97772	-0.95172	-0.58620	-0.52256	-0.48372
3410	-1.42829	-1.36683	-1.20629	-1.12629	-1.10029	-0.73477	-0.67113	-0.63229
2176	-1.41317	-1.35624	-1.19117	-1.11117	-1.08517	-0.71852	-0.65488	-0.61717
1317	-1.36110	-1.31143	-1.13910	-1.05910	-1.03310	-0.66462	-0.60099	-0.56510
5624	-1.31143	-1.62683	-1.46629	-1.38629	-1.36029	-0.99477	-0.93113	-0.89229
9117	-1.13910	-1.46629	-1.36110	-1.28110	-1.25510	-0.88662	-0.82299	-0.78710
1117	-1.05910	-1.38629	-1.28110	-1.36110	-1.33510	-0.96662	-0.90299	-0.86710
8517	-1.03310	-1.36029	-1.25510	-1.33510	-1.36110	-0.99262	-0.92899	-0.89310
1852	-0.66462	-0.99477	-0.88662	-0.96662	-0.99262	-1.29776	-1.23412	-1.19899
5488	-0.60099	-0.93113	-0.82299	-0.90299	-0.92899	-1.23412	-1.29776	-1.26262
1717	-0.56510	-0.89229	-0.78710	-0.86710	-0.89310	-1.19899	-1.26262	-1.36110

Oneway Analysis of Whorls By Population

Means Comparisons

Dif=Mean[i]-Mean[j]

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

3.64781

Abs(Dif)-LSD

	BH10	BH14	BH19	BH6	BH16	BH17	BL2	BL1	BH15	BH11	BH18	BH7	BH9
BH10	-0.4035	-0.25162	-0.22898	-0.07662	-0.05065	-0.02662	0.132208	0.237809	0.235308	0.235308	0.271022	0.348384	0.373384
BH14	-0.25162	-0.38279	-0.3611	-0.20779	-0.18119	-0.15779	-0.00034	0.107276	0.103183	0.103183	0.138897	0.217207	0.242207
BH19	-0.22898	-0.3611	-0.45752	-0.30753	-0.28314	-0.25753	-0.09525	0.005318	0.006761	0.006761	0.042475	0.117469	0.142469
BH6	-0.07662	-0.20779	-0.30753	-0.38279	-0.35619	-0.33279	-0.17534	-0.06772	-0.07182	-0.07182	-0.0361	0.042207	0.067207
BH16	-0.05065	-0.18119	-0.28314	-0.35619	-0.33573	-0.31388	-0.15963	-0.04727	-0.05512	-0.05512	-0.01941	0.061122	0.086122
BH17	-0.02662	-0.15779	-0.25753	-0.33279	-0.31388	-0.38279	-0.22534	-0.11772	-0.12182	-0.12182	-0.0861	-0.00779	0.017207
BL2	0.132208	-0.00034	-0.09525	-0.17534	-0.15963	-0.22534	-0.49418	-0.39681	-0.39287	-0.39287	-0.35716	-0.28368	-0.25868
BL1	0.237809	0.107276	0.005318	-0.06772	-0.04727	-0.11772	-0.39681	-0.33573	-0.34358	-0.34358	-0.30787	-0.22734	-0.20234
BH15	0.235308	0.103183	0.006761	-0.07182	-0.05512	-0.12182	-0.39287	-0.34358	-0.45752	-0.45752	-0.42181	-0.34682	-0.32182
BH11	0.235308	0.103183	0.006761	-0.07182	-0.05512	-0.12182	-0.39287	-0.34358	-0.45752	-0.45752	-0.42181	-0.34682	-0.32182
BH18	0.271022	0.138897	0.042475	-0.0361	-0.01941	-0.0861	-0.35716	-0.30787	-0.42181	-0.42181	-0.45752	-0.38253	-0.35753
BH7	0.348384	0.217207	0.117469	0.042207	0.061122	-0.00779	-0.28368	-0.22734	-0.34682	-0.34682	-0.38253	-0.38279	-0.35779
BH9	0.373384	0.242207	0.142469	0.067207	0.086122	0.017207	-0.25868	-0.20234	-0.32182	-0.32182	-0.35753	-0.35779	-0.38279
BH12	0.342451	0.210326	0.113904	0.035326	0.052021	-0.01467	-0.28573	-0.23644	-0.35038	-0.35038	-0.3861	-0.38967	-0.41467
BH13	0.375749	0.243987	0.146289	0.068987	0.086525	0.018987	-0.25393	-0.20194	-0.318	-0.318	-0.35371	-0.35601	-0.38101
BL3	0.402057	0.270607	0.171816	0.095607	0.113878	0.045607	-0.2289	-0.17458	-0.29247	-0.29247	-0.32818	-0.32939	-0.35439
BH3	0.448384	0.317207	0.217469	0.142207	0.161122	0.092207	-0.18368	-0.12734	-0.24682	-0.24682	-0.28253	-0.28279	-0.30779
BH5	0.473384	0.342207	0.242469	0.167207	0.186122	0.117207	-0.15868	-0.10234	-0.22182	-0.22182	-0.25753	-0.25779	-0.28279
BH4	0.473384	0.342207	0.242469	0.167207	0.186122	0.117207	-0.15868	-0.10234	-0.22182	-0.22182	-0.25753	-0.25779	-0.28279
BH20	0.554673	0.423736	0.323166	0.248736	0.268221	0.198736	-0.07835	-0.02024	-0.14112	-0.14112	-0.17683	-0.17626	-0.20126
BH1	0.723384	0.592207	0.492469	0.417207	0.436122	0.367207	0.091322	0.147660	0.028183	0.028183	-0.00753	-0.00779	-0.03279
BH8	0.736491	0.605554	0.504984	0.430554	0.450039	0.380554	0.103467	0.161578	0.040699	0.040699	0.004984	0.005554	-0.01945

Positive values show pairs of means that are significantly different.

IH12	BH13	BL3	BH3	BH5	BH4	BH20	BH1	BH8
2451	0.375749	0.402057	0.448384	0.473384	0.473384	0.554673	0.723384	0.736491
3326	0.243987	0.270607	0.317207	0.342207	0.342207	0.423736	0.592207	0.605554
3904	0.146289	0.171816	0.217469	0.242469	0.242469	0.323166	0.492469	0.504984
5326	0.068987	0.095607	0.142207	0.167207	0.167207	0.248736	0.417207	0.430554
2021	0.086525	0.113878	0.161122	0.186122	0.186122	0.268221	0.436122	0.450039
1467	0.018987	0.045607	0.092207	0.117207	0.117207	0.198736	0.367207	0.380554
3573	-0.25393	-0.2289	-0.18368	-0.15868	-0.15868	-0.07835	0.091322	0.103467
3644	-0.20194	-0.17458	-0.12734	-0.10234	-0.10234	-0.02024	0.147660	0.161578
5038	-0.318	-0.29247	-0.24682	-0.22182	-0.22182	-0.14112	0.028183	0.040699
5038	-0.318	-0.29247	-0.24682	-0.22182	-0.22182	-0.14112	0.028183	0.040699
3861	-0.35371	-0.32818	-0.28253	-0.25753	-0.25753	-0.17683	-0.00753	0.004984
3967	-0.35601	-0.32939	-0.28279	-0.25779	-0.25779	-0.17626	-0.00779	0.005554
1467	-0.38101	-0.35439	-0.30779	-0.28279	-0.28279	-0.20126	-0.03279	-0.01945
5752	-0.42514	-0.39961	-0.35396	-0.32896	-0.32896	-0.24826	-0.07896	-0.06644
2514	-0.42798	-0.40203	-0.35601	-0.33101	-0.33101	-0.25	-0.08101	-0.06818
3961	-0.40203	-0.4035	-0.35717	-0.33217	-0.33217	-0.25088	-0.08217	-0.06906
5396	-0.35601	-0.35717	-0.38279	-0.35779	-0.35779	-0.27626	-0.10779	-0.09445
2896	-0.33101	-0.33217	-0.35779	-0.38279	-0.38279	-0.30126	-0.13279	-0.11945
2896	-0.33101	-0.33217	-0.35779	-0.38279	-0.38279	-0.30126	-0.13279	-0.11945
4826	-0.25	-0.25088	-0.27626	-0.30126	-0.30126	-0.36498	-0.19672	-0.18316
7896	-0.08101	-0.08217	-0.10779	-0.13279	-0.13279	-0.19672	-0.38279	-0.36945
3644	-0.06818	-0.06906	-0.09445	-0.11945	-0.11945	-0.18316	-0.36945	-0.36498

Oneway Analysis of H/D ratio By Population

Means Comparisons

Dif=Mean[i]-Mean[j]

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

3.64781

Abs(Dif)-LSD

	BH19	BH18	BH16	BH13	BL2	BH6	BH7	BH9	BH4	BH10	BH11	BH8	BL1
BH19	-0.07553	-0.07012	-0.05593	-0.05844	-0.05725	-0.04741	-0.04633	-0.0416	-0.03514	-0.03316	-0.03721	-0.02984	-0.02314
BH18	-0.07012	-0.07553	-0.06134	-0.06385	-0.06266	-0.05282	-0.05174	-0.047	-0.04055	-0.03857	-0.04262	-0.03525	-0.02855
BH16	-0.05593	-0.06134	-0.05542	-0.05912	-0.05869	-0.04752	-0.04644	-0.04171	-0.03525	-0.03353	-0.03824	-0.02972	-0.02263
BH13	-0.05844	-0.06385	-0.05912	-0.07065	-0.06964	-0.05949	-0.05841	-0.05367	-0.04721	-0.0453	-0.0495	-0.04187	-0.03508
BL2	-0.05725	-0.06266	-0.05869	-0.06964	-0.08158	-0.07211	-0.07102	-0.06629	-0.05983	-0.05778	-0.06166	-0.0546	-0.048
BH6	-0.04741	-0.05282	-0.04752	-0.05949	-0.07211	-0.06319	-0.06211	-0.05738	-0.05092	-0.0491	-0.05354	-0.04549	-0.03855
BH7	-0.04633	-0.05174	-0.04644	-0.05841	-0.07102	-0.06211	-0.06319	-0.05846	-0.052	-0.05018	-0.05463	-0.04657	-0.03964
BH9	-0.0416	-0.047	-0.04171	-0.05367	-0.06629	-0.05738	-0.05846	-0.06319	-0.05673	-0.05491	-0.05936	-0.0513	-0.04437
BH4	-0.03514	-0.04055	-0.03525	-0.04721	-0.05983	-0.05092	-0.052	-0.05673	-0.06319	-0.06137	-0.06582	-0.05776	-0.05083
BH10	-0.03316	-0.03857	-0.03353	-0.0453	-0.05778	-0.0491	-0.05018	-0.05491	-0.06137	-0.06661	-0.07094	-0.06308	-0.05622
BH11	-0.03721	-0.04262	-0.03824	-0.0495	-0.06166	-0.05354	-0.05463	-0.05936	-0.06582	-0.07094	-0.07553	-0.06816	-0.06145
BH8	-0.02984	-0.03525	-0.02972	-0.04187	-0.0546	-0.04549	-0.04657	-0.0513	-0.05776	-0.06308	-0.06816	-0.06025	-0.05326
BL1	-0.02314	-0.02855	-0.02263	-0.03508	-0.048	-0.03855	-0.03964	-0.04437	-0.05083	-0.05622	-0.06145	-0.05326	-0.05542
BH20	-0.02399	-0.0294	-0.02387	-0.03601	-0.04874	-0.03963	-0.04072	-0.04545	-0.05191	-0.05723	-0.0623	-0.0544	-0.05666
BL3	-0.02397	-0.02937	-0.02434	-0.0361	-0.04859	-0.0399	-0.04099	-0.04572	-0.05218	-0.05742	-0.06228	-0.05474	-0.05713
BH1	-0.01632	-0.02173	-0.01643	-0.0284	-0.04102	-0.0321	-0.03319	-0.03792	-0.04438	-0.04966	-0.05464	-0.0469	-0.04923
BH15	-0.01806	-0.02347	-0.01908	-0.03035	-0.0425	-0.03439	-0.03547	-0.0402	-0.04666	-0.05179	-0.05637	-0.04932	-0.05188
BH12	-0.01781	-0.02322	-0.01883	-0.0301	-0.04225	-0.03414	-0.03522	-0.03995	-0.04641	-0.05154	-0.05612	-0.04907	-0.05163
BH17	-0.01167	-0.01708	-0.01178	-0.02375	-0.03636	-0.02745	-0.02853	-0.03326	-0.03972	-0.04501	-0.04998	-0.04225	-0.04457
BH5	-0.01052	-0.01593	-0.01063	-0.0226	-0.03522	-0.0263	-0.02738	-0.03212	-0.03858	-0.04386	-0.04884	-0.0411	-0.04343
BH14	-0.00837	-0.01378	-0.00848	-0.02045	-0.03307	-0.02415	-0.02524	-0.02997	-0.03643	-0.04171	-0.04669	-0.03895	-0.04128
BH3	0.001121	-0.00429	0.001010	-0.01096	-0.02357	-0.01466	-0.01574	-0.02048	-0.02694	-0.03222	-0.03719	-0.02946	-0.03178

Positive values show pairs of means that are significantly different.

	BL3	BH1	BH15	BH12	BH17	BH5	BH14	BH3
3H20								
2399	-0.02397	-0.01632	-0.01806	-0.01781	-0.01167	-0.01052	-0.00837	0.001121
0294	-0.02937	-0.02173	-0.02347	-0.02322	-0.01708	-0.01593	-0.01378	-0.00429
2387	-0.02434	-0.01643	-0.01908	-0.01883	-0.01178	-0.01063	-0.00848	0.001010
3601	-0.0361	-0.0284	-0.03035	-0.0301	-0.02375	-0.0226	-0.02045	-0.01096
4874	-0.04859	-0.04102	-0.0425	-0.04225	-0.03636	-0.03522	-0.03307	-0.02357
3963	-0.0399	-0.0321	-0.03439	-0.03414	-0.02745	-0.0263	-0.02415	-0.01466
4072	-0.04099	-0.03319	-0.03547	-0.03522	-0.02853	-0.02738	-0.02524	-0.01574
4545	-0.04572	-0.03792	-0.0402	-0.03995	-0.03326	-0.03212	-0.02997	-0.02048
5191	-0.05218	-0.04438	-0.04666	-0.04641	-0.03972	-0.03858	-0.03643	-0.02894
5723	-0.05742	-0.04966	-0.05179	-0.05154	-0.04501	-0.04386	-0.04171	-0.03222
0623	-0.06228	-0.05464	-0.05637	-0.05612	-0.04998	-0.04884	-0.04669	-0.03719
0544	-0.05474	-0.0469	-0.04932	-0.04907	-0.04225	-0.0411	-0.03895	-0.02946
5866	-0.05713	-0.04923	-0.05188	-0.05163	-0.04457	-0.04343	-0.04128	-0.03178
6025	-0.0606	-0.05276	-0.05518	-0.05493	-0.0481	-0.04696	-0.04481	-0.03531
0606	-0.06661	-0.05886	-0.06098	-0.06073	-0.0542	-0.05305	-0.05091	-0.04141
5276	-0.05886	-0.06319	-0.06548	-0.06523	-0.05854	-0.05739	-0.05524	-0.04575
5518	-0.06098	-0.06548	-0.07553	-0.07528	-0.06914	-0.06799	-0.06584	-0.05635
5493	-0.06073	-0.06523	-0.07528	-0.07553	-0.06939	-0.06824	-0.06609	-0.0566
0481	-0.0542	-0.05854	-0.06914	-0.06839	-0.06319	-0.06205	-0.0599	-0.0504
4696	-0.05305	-0.05739	-0.06799	-0.06824	-0.06205	-0.06319	-0.06104	-0.05155
4481	-0.05091	-0.05524	-0.06584	-0.06609	-0.0599	-0.06104	-0.06319	-0.0537
3531	-0.04141	-0.04575	-0.05635	-0.0566	-0.0504	-0.05155	-0.0537	-0.06319

Oneway Analysis of Total Penis Length By Population

Means Comparisons

Dif=Mean[i]-Mean[j]

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*		Abs(Dif)-LSD																
		BH10	BH14	BH19	BH17	BH16	BH6	BL2	BH11	BL1	BH12	BH18	BH15	BH4				
BH10	-3.95643	-1.26990	-1.56636	-0.59290	-0.30052	-0.27590	0.72574	1.46507	2.42253	2.06792	2.35507	2.46364	3.28710	3.				
BH14	-1.26990	-3.53874	-3.85407	-2.86174	-2.55663	-2.54474	-1.57051	-0.82264	0.15844	-0.21978	0.06736	0.17593	1.01826	1.				
BH19	-1.56636	-3.85407	-4.22960	-3.26792	-2.98334	-2.95092	-1.93207	-1.19817	-0.25543	-0.59531	-0.30817	-0.19960	0.61208	0.				
BH17	-0.59290	-2.86174	-3.26792	-3.53874	-3.23363	-3.22174	-2.24751	-1.49964	-0.51856	-0.89678	-0.60964	-0.50107	0.34126	0.				
BH16	-0.30052	-2.55663	-2.98334	-3.23363	-3.10368	-3.10601	-2.16140	-1.40444	-0.39756	-0.80158	-0.51444	-0.40587	0.45699	0.				
BH6	-0.27590	-2.54474	-2.95092	-3.22174	-3.10601	-3.53874	-2.56451	-1.81664	-0.83556	-1.21378	-0.92864	-0.81807	0.02426	0.				
BL2	0.72574	-1.57051	-1.93207	-2.24751	-2.16140	-2.56451	-4.56849	-3.84111	-2.91577	-3.23826	-2.95111	-2.84254	-2.04485	-1.				
BH11	1.46507	-0.82264	-1.19817	-1.49964	-1.40444	-1.81664	-3.84111	-4.22960	-3.28685	-3.62674	-3.33960	-3.23103	-2.41935	-2.				
BL1	2.42253	0.15844	-0.25543	-0.51856	-0.39756	-0.83556	-2.91577	-3.28685	-3.37405	-3.76192	-3.47478	-3.36620	-2.51619	-2.				
BH12	2.06792	-0.21978	-0.59531	-0.89678	-0.80158	-1.21378	-3.23826	-3.62674	-3.76192	-4.22960	-3.94245	-3.83388	-3.02221	-2.				
BH18	2.35507	0.06736	-0.30817	-0.60964	-0.51444	-0.92864	-2.95111	-3.33960	-3.47478	-3.94245	-4.22960	-4.12103	-3.30935	-3.				
BH15	2.46364	0.17593	-0.19960	-0.50107	-0.40587	-0.81807	-2.84254	-3.23103	-3.36620	-3.83388	-4.12103	-4.22960	-3.41792	-3.				
BH4	3.28710	1.01826	0.61208	0.34126	0.45699	0.02426	-2.04485	-2.41935	-2.51619	-3.02221	-3.30935	-3.41792	-3.53874	-3.				
BL3	3.38588	1.11163	0.71420	0.43463	0.54440	0.11763	-1.93877	-2.31723	-2.42505	-2.92008	-3.20723	-3.31580	-3.44537	-3.				
BH5	3.80410	1.53526	1.12908	0.85826	0.97399	0.54126	-1.52785	-1.90235	-1.99919	-2.50521	-2.79235	-2.90092	-3.02174	-3.				
BH7	3.74699	1.47274	1.07531	0.79574	0.90551	0.47874	-1.57766	-1.95612	-2.06394	-2.55897	-2.84612	-2.95469	-3.08426	-3.				
BH9	3.86410	1.59526	1.18908	0.91826	1.03399	0.60126	-1.46785	-1.84235	-1.93919	-2.44521	-2.73235	-2.84092	-2.96174	-3.				
BH20	4.01110	1.74226	1.33608	1.06526	1.18099	0.74826	-1.32085	-1.69535	-1.79219	-2.29821	-2.58535	-2.69392	-2.81474	-3.				
BH13	3.90607	1.62560	1.23828	0.94860	1.05160	0.63160	-1.41010	-1.79315	-1.91361	-2.39600	-2.68315	-2.79172	-2.93140	-3.				
BH1	4.46610	2.19726	1.79108	1.52026	1.63599	1.20326	-0.86585	-1.24035	-1.33719	-1.84321	-2.13035	-2.23892	-2.35974	-2.				
BH8	5.05889	2.79481	2.38094	2.11781	2.23881	1.80081	-0.27941	-0.65049	-0.73769	-1.25335	-1.54049	-1.64906	-1.76219	-2.				
BH3	6.17910	3.91026	3.50408	3.23326	3.34899	2.91626	0.84715	0.47265	0.37581	-0.13021	-0.41735	-0.52592	-0.64674	-0.				

Positive values show pairs of means that are significantly different.

BL3	BH5	BH7	BH9	BH20	BH13	BH1	BH8	BH3
8588	3.80410	3.74699	3.86410	4.01110	3.90607	4.46610	5.05889	6.17910
1163	1.53526	1.47274	1.59526	1.74226	1.62560	2.19726	2.79481	3.91026
1420	1.12908	1.07531	1.18908	1.33608	1.23828	1.79108	2.38094	3.50408
3463	0.85826	0.79574	0.91826	1.06526	0.94860	1.52026	2.11781	3.23326
4440	0.97399	0.90551	1.03399	1.18099	1.05160	1.63599	2.23881	3.34899
1763	0.54126	0.47874	0.60126	0.74826	0.63160	1.20326	1.80081	2.91626
3877	-1.52785	-1.57766	-1.46785	-1.32085	-1.41010	-0.86585	-0.27941	0.84715
1723	-1.90235	-1.95612	-1.84235	-1.69535	-1.79315	-1.24035	-0.65049	0.47265
2505	-1.99919	-2.06394	-1.93919	-1.79219	-1.91361	-1.33719	-0.73769	0.37581
2008	-2.50521	-2.55897	-2.44521	-2.29821	-2.39600	-1.84321	-1.25335	-0.13021
0723	-2.79235	-2.84612	-2.73235	-2.58535	-2.68315	-2.13035	-1.54049	-0.41735
1580	-2.90092	-2.95469	-2.84092	-2.69392	-2.79172	-2.23892	-1.64906	-0.52592
4537	-3.02174	-3.08426	-2.96174	-2.81474	-2.93140	-2.35974	-1.76219	-0.64674
3015	-3.30904	-3.36904	-3.24904	-3.10204	-3.21329	-2.64704	-2.05171	-0.93404
0904	-3.53874	-3.60126	-3.47874	-3.33174	-3.44840	-2.87674	-2.27919	-1.16374
6904	-3.60126	-3.73015	-3.61015	-3.46315	-3.57440	-3.00815	-2.41283	-1.29515
4904	-3.47874	-3.61015	-3.53874	-3.39174	-3.50840	-2.93674	-2.33919	-1.22374
0204	-3.33174	-3.46315	-3.39174	-3.53874	-3.65540	-3.08374	-2.48619	-1.37074
1329	-3.44840	-3.57440	-3.50840	-3.65540	-3.95643	-3.39640	-2.80361	-1.68340
4704	-2.87674	-3.00815	-2.93674	-3.08374	-3.39640	-3.53874	-2.94119	-1.82574
5171	-2.27919	-2.41283	-2.33919	-2.48619	-2.80361	-2.94119	-3.37405	-2.26056
3404	-1.16374	-1.29515	-1.22374	-1.37074	-1.68340	-1.82574	-2.26056	-3.53874

Oneway Analysis of Penis/Diam By Population

Means Comparisons

Dif=Mean[j]-Mean[i]

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

3.64936

Abs(Dif)-LSD

	BL2	BH18	BH4	BH15	BH12	BH20	BH17	BH11	BH1	BH16	BH9	BH5	BH19
BL2	-0.37876	-0.35839	-0.28812	-0.30482	-0.28182	-0.25307	-0.23578	-0.22759	-0.20037	-0.16237	-0.17638	-0.17577	-0.18777
BH18	-0.35839	-0.35066	-0.27924	-0.29709	-0.27409	-0.24419	-0.22689	-0.21987	-0.19148	-0.15273	-0.16749	-0.16888	-0.18005
BH4	-0.28812	-0.27924	-0.29338	-0.31378	-0.29078	-0.25833	-0.24104	-0.23655	-0.20563	-0.16517	-0.18163	-0.18103	-0.19673
BH15	-0.30482	-0.29709	-0.31378	-0.35066	-0.32766	-0.29776	-0.28046	-0.27344	-0.24505	-0.2063	-0.22106	-0.22045	-0.23362
BH12	-0.28182	-0.27409	-0.29078	-0.32766	-0.35066	-0.32075	-0.30346	-0.29643	-0.26805	-0.22929	-0.24405	-0.24345	-0.25661
BH20	-0.25307	-0.24419	-0.25833	-0.29776	-0.32075	-0.29338	-0.27609	-0.2716	-0.24068	-0.20022	-0.21669	-0.21608	-0.23178
BH17	-0.23578	-0.22689	-0.24104	-0.28046	-0.30346	-0.27609	-0.29338	-0.2889	-0.25798	-0.21752	-0.23398	-0.23337	-0.24908
BH11	-0.22759	-0.21987	-0.23655	-0.27344	-0.29643	-0.2716	-0.2889	-0.35066	-0.32228	-0.28352	-0.29828	-0.29768	-0.31084
BH1	-0.20037	-0.19148	-0.20563	-0.24505	-0.26805	-0.24068	-0.25798	-0.32228	-0.29338	-0.25292	-0.26939	-0.26878	-0.28449
BH16	-0.16237	-0.15273	-0.16749	-0.2063	-0.22929	-0.20022	-0.21752	-0.28352	-0.25292	-0.25731	-0.27496	-0.27435	-0.29176
BH9	-0.17638	-0.16749	-0.18163	-0.22106	-0.24405	-0.21669	-0.23398	-0.29828	-0.26939	-0.27496	-0.29338	-0.29278	-0.30848
BH5	-0.17577	-0.16888	-0.18103	-0.22045	-0.24345	-0.21608	-0.23337	-0.29768	-0.26878	-0.27435	-0.29278	-0.29338	-0.30909
BH19	-0.18777	-0.18005	-0.19673	-0.23362	-0.25661	-0.23178	-0.24908	-0.31084	-0.28449	-0.29176	-0.30848	-0.30909	-0.35066
BH13	-0.17572	-0.16754	-0.18325	-0.22111	-0.2441	-0.2183	-0.23559	-0.29833	-0.271	-0.27763	-0.295	-0.2956	-0.33815
BH7	-0.16304	-0.15448	-0.16935	-0.20805	-0.23104	-0.2044	-0.22169	-0.28527	-0.2571	-0.26317	-0.2811	-0.2817	-0.32509
BL1	-0.13646	-0.12729	-0.14079	-0.18086	-0.20385	-0.17585	-0.19314	-0.25808	-0.22855	-0.23368	-0.25254	-0.25315	-0.2979
BH6	-0.13915	-0.13026	-0.14441	-0.18383	-0.20683	-0.17946	-0.19675	-0.26106	-0.23216	-0.23773	-0.25615	-0.25676	-0.30087
BH8	-0.13163	-0.12246	-0.13597	-0.17603	-0.19902	-0.17102	-0.18831	-0.25325	-0.22372	-0.22885	-0.24771	-0.24832	-0.29307
BH14	-0.12674	-0.11786	-0.132	-0.17143	-0.19442	-0.16705	-0.18435	-0.24865	-0.21976	-0.22533	-0.24375	-0.24436	-0.28847
BH10	-0.13624	-0.12807	-0.14377	-0.18164	-0.20463	-0.17883	-0.19612	-0.25886	-0.23153	-0.23815	-0.25552	-0.25613	-0.29868
BL3	-0.10137	-0.09281	-0.10768	-0.14638	-0.16938	-0.14274	-0.16003	-0.22361	-0.19544	-0.2015	-0.21943	-0.22004	-0.26343
BH3	0.087844	0.096731	0.082586	0.043161	0.020166	0.047535	0.030241	-0.03406	-0.00517	-0.01074	-0.02916	-0.02977	-0.07388

Positive values show pairs of means that are significantly different.

	BH7	BL1	BH6	BH8	BH14	BH10	BL3	BH3
3H13								
7572	-0.16304	-0.13646	-0.13915	-0.13163	-0.12674	-0.13624	-0.10137	0.087844
6754	-0.15448	-0.12729	-0.13026	-0.12246	-0.11786	-0.12807	-0.09281	0.096731
8325	-0.16935	-0.14079	-0.14441	-0.13597	-0.132	-0.14377	-0.10768	0.082586
2111	-0.20805	-0.18086	-0.18383	-0.17603	-0.17143	-0.18164	-0.14638	0.043161
2441	-0.23104	-0.20385	-0.20683	-0.19902	-0.19442	-0.20463	-0.16938	0.020166
2183	-0.2044	-0.17585	-0.17946	-0.17102	-0.16705	-0.17883	-0.14274	0.047535
3559	-0.22169	-0.19314	-0.19675	-0.18831	-0.18435	-0.19612	-0.16003	0.030241
9833	-0.28527	-0.25808	-0.26106	-0.25325	-0.24865	-0.25886	-0.22361	-0.03406
0.271	-0.2571	-0.22855	-0.23216	-0.22372	-0.21976	-0.23153	-0.19544	-0.00517
7763	-0.26317	-0.23368	-0.23773	-0.22885	-0.22533	-0.23815	-0.2015	-0.01074
0.295	-0.2811	-0.25254	-0.25615	-0.24771	-0.24375	-0.25552	-0.21943	-0.02916
2956	-0.2817	-0.25315	-0.25676	-0.24832	-0.24436	-0.25613	-0.22004	-0.02977
3815	-0.32509	-0.2979	-0.30087	-0.29307	-0.28847	-0.29868	-0.26343	-0.07388
2801	-0.31463	-0.28692	-0.29013	-0.28209	-0.27773	-0.28854	-0.25296	-0.06314
1463	-0.30925	-0.28109	-0.28452	-0.27626	-0.27211	-0.28344	-0.24759	-0.05753
8692	-0.28109	-0.27973	-0.2835	-0.2749	-0.2711	-0.28327	-0.24697	-0.05651
9013	-0.28452	-0.2835	-0.29338	-0.28494	-0.28098	-0.29275	-0.25666	-0.06639
8209	-0.27626	-0.2749	-0.28494	-0.27973	-0.27593	-0.2881	-0.2518	-0.06134
7773	-0.27211	-0.2711	-0.28098	-0.27593	-0.29338	-0.30516	-0.26907	-0.07879
9854	-0.28344	-0.28327	-0.29275	-0.2881	-0.30516	-0.32801	-0.29244	-0.10261
5296	-0.24759	-0.24697	-0.25666	-0.2518	-0.26907	-0.29244	-0.30925	-0.11919
16314	-0.05753	-0.05651	-0.06839	-0.06134	-0.07879	-0.10261	-0.11919	-0.29338

Oneway Analysis of % ribbed By Population

Means Comparisons

Dif=Mean(i)-Mean(j)

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q*

3.65033

Abs(Dif)-LSD

	BH5	BH3	BH13	BH8	BH11	BL3	BH6	BH12	BL1	BH9	BH1	BL2	BH14
BH5	-0.09471	-0.09366	-0.0926	-0.08016	-0.08988	-0.08402	-0.06834	-0.07624	-0.06657	-0.06524	-0.05366	-0.06519	-0.04403
BH3	-0.09366	-0.09471	-0.09365	-0.08121	-0.09093	-0.08507	-0.0694	-0.07729	-0.06763	-0.06629	-0.05472	-0.06624	-0.04509
BH13	-0.0926	-0.09365	-0.10589	-0.09389	-0.10298	-0.09731	-0.08195	-0.08934	-0.08017	-0.07884	-0.06726	-0.07806	-0.05763
BH8	-0.08016	-0.08121	-0.09389	-0.0903	-0.10028	-0.09434	-0.07854	-0.08664	-0.07677	-0.07544	-0.06386	-0.07568	-0.05423
BH11	-0.08988	-0.09093	-0.10298	-0.10028	-0.1132	-0.10765	-0.09249	-0.09956	-0.09071	-0.08938	-0.07781	-0.08814	-0.06817
BL3	-0.08402	-0.08507	-0.09731	-0.09434	-0.10765	-0.10589	-0.09053	-0.09792	-0.08875	-0.08742	-0.07585	-0.08664	-0.06621
BH6	-0.06834	-0.0694	-0.08195	-0.07854	-0.09249	-0.08053	-0.09471	-0.10261	-0.09294	-0.09161	-0.08003	-0.09155	-0.0704
BH12	-0.07624	-0.07729	-0.08934	-0.08664	-0.09956	-0.09792	-0.10261	-0.1132	-0.10435	-0.10302	-0.09144	-0.10177	-0.08181
BL1	-0.06657	-0.06763	-0.08017	-0.07677	-0.09071	-0.08875	-0.09294	-0.10435	-0.09471	-0.09338	-0.0818	-0.09333	-0.07217
BH9	-0.06524	-0.06629	-0.07884	-0.07544	-0.08938	-0.08742	-0.09161	-0.10302	-0.09338	-0.09471	-0.08313	-0.09466	-0.0735
BH1	-0.05366	-0.05472	-0.06726	-0.06386	-0.07781	-0.07585	-0.08003	-0.09144	-0.0818	-0.08313	-0.09471	-0.10623	-0.08508
BL2	-0.06519	-0.06624	-0.07806	-0.07568	-0.08814	-0.08664	-0.09155	-0.10177	-0.09333	-0.09466	-0.10623	-0.12227	-0.10286
BH14	-0.04403	-0.04509	-0.05763	-0.05423	-0.06817	-0.06621	-0.0704	-0.08181	-0.07217	-0.0735	-0.08508	-0.10286	-0.09471
BH18	-0.0483	-0.04935	-0.0614	-0.0587	-0.07162	-0.06998	-0.07467	-0.08526	-0.07644	-0.07777	-0.08935	-0.10593	-0.09898
BH4	-0.03508	-0.03614	-0.04868	-0.04528	-0.05922	-0.05726	-0.06145	-0.07286	-0.06322	-0.06455	-0.07613	-0.09391	-0.08576
BH16	-0.02807	-0.02912	-0.04201	-0.03812	-0.05276	-0.05059	-0.05443	-0.06639	-0.0562	-0.05753	-0.06911	-0.08768	-0.07874
BH15	-0.04273	-0.04378	-0.05582	-0.05313	-0.06605	-0.06441	-0.06909	-0.07969	-0.07087	-0.0722	-0.08377	-0.10036	-0.09341
BH7	-0.03319	-0.03425	-0.04648	-0.04351	-0.05683	-0.05506	-0.05956	-0.07047	-0.06133	-0.06266	-0.07424	-0.09128	-0.08387
BH17	-0.01467	-0.01572	-0.02827	-0.02486	-0.03881	-0.03685	-0.04103	-0.05245	-0.04281	-0.04414	-0.05571	-0.07349	-0.06535
BH10	-0.01148	-0.01253	-0.02477	-0.0218	-0.03512	-0.03335	-0.03785	-0.04876	-0.03962	-0.04095	-0.05253	-0.06957	-0.06216
BH19	0.001219	0.000166	-0.01188	-0.00918	-0.0221	-0.02046	-0.02515	-0.03574	-0.02692	-0.02825	-0.03983	-0.05641	-0.04946
BH20	0.011684	0.010631	-0.00192	0.001491	-0.01246	-0.0105	-0.01468	-0.02609	-0.01645	-0.01778	-0.02936	-0.04714	-0.03899

Positive values show pairs of means that are significantly different.

BH18	BH4	BH16	BH15	BH7	BH17	BH10	BH19	BH20
1.0483	-0.03508	-0.02807	-0.04273	-0.03319	-0.01467	-0.01148	0.001219	0.011684
24935	-0.03614	-0.02912	-0.04378	-0.03425	-0.01572	-0.01253	0.000166	0.010631
1.0614	-0.04868	-0.04201	-0.05582	-0.04648	-0.02827	-0.02477	-0.01188	-0.00192
1.0587	-0.04528	-0.03812	-0.05313	-0.04351	-0.02486	-0.0218	-0.00918	0.001491
27162	-0.05922	-0.05276	-0.06605	-0.05683	-0.03881	-0.03512	-0.0221	-0.01246
26998	-0.05726	-0.05059	-0.06441	-0.05506	-0.03685	-0.03335	-0.02046	-0.0105
27467	-0.06145	-0.05443	-0.06909	-0.05956	-0.04103	-0.03785	-0.02515	-0.01468
28526	-0.07286	-0.06639	-0.07969	-0.07047	-0.05245	-0.04876	-0.03574	-0.02609
27644	-0.06322	-0.0562	-0.07087	-0.06133	-0.04281	-0.03962	-0.02692	-0.01645
27777	-0.06455	-0.05753	-0.0722	-0.06266	-0.04414	-0.04095	-0.02825	-0.01778
28935	-0.07613	-0.06911	-0.08377	-0.07424	-0.05571	-0.05253	-0.03983	-0.02936
10593	-0.09391	-0.08768	-0.10036	-0.09128	-0.07349	-0.06957	-0.05641	-0.04714
29898	-0.08576	-0.07874	-0.09341	-0.08387	-0.06535	-0.06216	-0.04946	-0.03899
1.1132	-0.1008	-0.09433	-0.10763	-0.09841	-0.08039	-0.07669	-0.06368	-0.05403
1.1008	-0.09471	-0.08769	-0.10236	-0.09282	-0.07429	-0.07111	-0.05841	-0.04794
29433	-0.08769	-0.08307	-0.09866	-0.08891	-0.07005	-0.0672	-0.05471	-0.0437
10763	-0.10236	-0.09866	-0.1132	-0.10398	-0.08596	-0.08227	-0.06925	-0.05961
29841	-0.09282	-0.08891	-0.10398	-0.10589	-0.08768	-0.08418	-0.07128	-0.06132
28039	-0.07429	-0.07005	-0.08596	-0.08768	-0.09471	-0.09152	-0.07882	-0.06836
27669	-0.07111	-0.0672	-0.08227	-0.08418	-0.09152	-0.10589	-0.09299	-0.08303
26368	-0.05841	-0.05471	-0.06925	-0.07128	-0.07882	-0.09299	-0.1132	-0.10356
25403	-0.04794	-0.0437	-0.05961	-0.06132	-0.06836	-0.08303	-0.10356	-0.09471