Eleanor Steinberg and Dana Heller
Department of Zoology
University of Washington
Seattle, Washington 98195

USING DNA AND ROCKS TO INTERPRET THE TAXONOMY AND PATCHY DISTRIBUTION OF POCKET GOPHERS IN WESTERN WASHINGTON PRAIRIES

Eleanor Steinberg and Dana Heller

Abstract

The Mazama pocket gopher (*Thomomys mazama*) occurs in prairies, open fields, and subalpine meadows in northwestern California, western Oregon, southwestern Washington and in the Olympic mountains. With increasing development pressures in the southern Puget Sound area, *T. mazama* populations have been disappearing, thus several subspecies occurring in this region are considered to be species of conservation concern. These subspecies are patchily distributed in glacial outwash prairies. Based on molecular genetic analyses, we have found that the four extant prairie subspecies ranging from Fort Lewis to Olympia are not genetically differentiated, thus probably warrant taxonomic revision such that they will all be included as one subspecies. Examination of soil samples from sites with and without pocket gophers suggested that soil rockiness may influence the distribution of *T. mazama* in Puget Sound prairies.

Introduction

For well over half a century, biologists have been trying to make sense of the distribution of pocket gophers in the Pacific Northwest (i.e. Bailey 1915, Goldman 1939, Dalquest 1948, Johnson and Benson 1960, Steinberg 1995). Initially the interest was purely academic, arising in the golden age of "biogeography" which climaxed in the middle of the century, when a major focus of mammalogists throughout the world was the interpretation of faunal patterns in terms of glaciers, geology, and life zones. Today the interest in northwestern pocket gophers, particularly the populations of *Thomomys mazama* in glacial outwash prairies of western Washington, is more practical. Many subspecies in this range are thought to be threatened with extinction due to habitat loss and fragmentation, and an understanding of their patchy distribution is a prerequisite to effective conservation. But regardless of one's motivation, if we are to learn the "true story" of pocket gophers in Washington, we need to first untangle the various plot-lines that include glaciers, succession, land-use patterns, special habitat requirements, and presumed genetic differentiation. In this paper we use modern molecular genetic data and fine-scale soil sampling to offer a revision to an old story about the pocket gophers of Washington, and to suggest the next plot-lines that need to be pursued before we can be confident that we have deciphered pocket gopher distributions.

The "old story" about Washington pocket gophers proposed by Walter Dalquest and Victor Scheffer emphasized two observations: (i) the prairie habitat to which gophers in the southern Puget Sound region were limited was naturally fragmented by forests; and (ii) gophers were clearly variable from one population to the next in terms of coat color, body size and in some cranial characteristics (Goldman 1939, Dalquest and Scheffer 1942). Specifically, Dalquest and Scheffer (1942) suggested that after the retreat of the last continental glaciers in western Washington (approximately 10,000 years ago) much of the initially open "outwash apron" habitat left behind by the glaciers was invaded by pocket gophers migrating along outwash trains of valley glaciers from Mount Rainier. Then, with succession, forests grew and spread, dividing these outwash expanses into isolated prairies. Dalquest and Scheffer proposed that the range of the prairie and its pocket gopher inhabitants was first split by the Nisqually river into northern and southern units and then subsequently these units were further divided and reduced in area by invading forests. Such a fragmented population structure is known to promote evolutionary divergence, and the morphological variation seen was thought to represent meaningful evolutionary units -- subspecies. Altogether there were six subspecies of pocket gophers described from the prairies of the southern Puget Sound, four of which are extant (see Figure 1).

Until 1960, the pocket gophers of the southern Puget Sound area were thought to be subspecies of *T. talpoides*, which is broadly distributed in prairies, alpine meadows, brushy areas and open pine forests of northwestern North America and Canada (Burt and Grossenheider 1976). In 1960, Murray Johnson and Seth Benson published a paper that cast a new light on the history of Washington pocket gophers. Based on the size of the baculum (penis bone), Johnson and Benson (1960) found the western Washington gophers to be much more similar to *T. mazama*, which occurs in western Oregon and northwestern California, than to *T. talpoides*. Subsequently, the western Washington populations (excluding *T. talpoides douglasii* from the vicinity of Vancouver, WA) have been reclassified as subspecies of *T. mazama* (Hall 1981). While names may be just names, this taxonomic revision undermined a major part of Dalquest and Scheffer's explanation for the current distribution of western Washington pocket gophers, since these subspecies are clearly not descendants of *T. talpoides* from Mount Rainier.

Further questions regarding classical interpretations of subspecies designations in pocket gophers have been raised by Jim Patton and his colleagues at U.C. Berkeley in their work on *Thomomys bottae* in California (synthesized in Patton and Smith, 1990). Patton has clearly demonstrated that there is a strong environmental component in much of the morphological variation that is the basis of subspecies distinctions in pocket gophers.

For example, pocket gopher body size depends on habitat quality, indicated by the fact that pocket gophers from recently established (i.e. less than 10 years old) alfalfa fields in Central California can be nearly twice the body weight of individuals of the same age and sex from the adjacent desert scrub habitat (Patton and Brylski, 1987). Furthermore, Smith and Patton's (1988) careful morphometric analyses indicate that cranial features in pocket gophers often correlate to overall body size. Finally, based on biogeographical analyses, Patton and Smith (1990) make a compelling argument for a strong environmental (i.e. rather than phylogenetic) influence on color variation in pocket gophers.

Based on their thorough analyses of

Based on their thorough analyses of environmental and genetic causes of variation in pocket gophers, Patton and Smith (1990) suggest that the 46 recognized subspecies of *T. bottae* in California be combined and reduced to 15 total subspecies.

Armed with this information, Steinberg (1995) sampled individuals spanning the current range of pocket gophers in southern Puget sound prairies with the goal of examining genetic differentiation among the extant subspecies. We will gloss over the details on the laboratory approaches applied in the genetic analyses; instead we recommend interested readers to Avise et al. (1987) and Hillis, Moritz, and Mable (1996). The general idea is that DNA is extracted from tissue samples from different individuals. Particular gene fragments are amplified (using the polymerase chain reaction, or "PCR") from each sample, and DNA sequences from these fragments from the different individuals are compared. Depending on the mutation rate of the genetic material examined (i.e. due to strong selection, DNA sequences from protein coding genes will be less susceptible to mutation than those from non-coding regions of the genome), differences between isolated populations arise and become fixed due to genetic drift.

Steinberg (1995) took advantage the fact that the mitochondrial gene "cytochrome b" has been used extensively on pocket gophers (i.e. Patton and Smith 1994), and thus it is known that at the subspecies level, pocket gophers generally show differences in 2-10% of the base pairs corresponding to this gene (Patton and Smith 1994). In contrast, Steinberg found that individuals from Roy (representing *T. mazama glacialis*) and Olympia (representing *T. mazama* *pugetensis*) were identical in 379 base pairs of sequence from cytochrome b, a significant similarity, given the expected difference of at least 7 base pairs if these were "typical" pocket gopher subspecies. In contrast, a sample from Shelton representing *T. mazama couchi* differed by seven nucleotides from the Roy and Olympia samples, which does reflect expected subspecies -level divergence (~2%). Another mitochondrial gene, NADH subunit III ("ND3"), showed a similar pattern. In particular, samples from Roy and Olympia were identical over 437 base pairs of nucleotide sequence, whereas the Shelton sample differed at 10 base pairs (~3%) from the Roy and Olympia samples. Table 2 shows the actual DNA sequences obtained for these two genes, with nucleotide differences between subspecies underlined.

Thus, based on mitochondrial sequences, the described subspecies spanning from Roy through Olympia do not appear to be genetically differentiated. This could be due either to current gene Table 2. DNA sequences flow between extant populations, or because isolation has been so recent that there has not been enough time for populations to diverge. Additional work is in progress expanding on this analysis to include the entire range of T. mazama to determine larger scale taxonomic associations, as well as using more variable genetic markers to determine whether there is genetic evidence in support of recent restrictions of gene flow.

Although genetic differentiation among many of the described subspecies of *T. mazama* in Western Washington now seems unlikely, the patchy and fragmented distributed distribution of the species still remains as an undisputed fact. We conducted thorough surveys of western Washington by visiting all sites known to have previously supported pocket gophers (based on museum records, published accounts, and collectors written and verbal records).

Figure 1 is our resulting map of current and historic locations of *T. mazama* in Washington. Details of the locations mapped can be found in Steinberg (1996). One thing that was immediately obvious from our surveys is that gophers are even more patchy in their distribution than are prairies, as there are many seemingly high-quality prairies within the range of *T. mazama* that lack pocket gophers. While human habitat modification has clearly influenced the distribution of pocket gophers in Puget Sound prairies (i.e. the paving of Tacoma!) reading back over Walter Dalquest's and Victor Scheffer's field notes from the 1940's, it is clear that populations have always been somewhat patchy. Physical characteristics of soil are obvious factors that can influence distributions of burrowing anmals. Thus, we decided to explore a simple idea – the hypothesis that microvariable soil attributes of Puget Sound prairies can explain gopher distributions.

Table 1. Puget Sound Pocket Gopher Nucleotide Sequences

CYTOCHROME B

Roy	TATTGTTAGA	AATAAGAGTA	GGATACCAAT	ATTTCATGTT	TCTTTATATA	GGTAAGAGCC
Olympia	TATTGTTAGA	AATAAGAGTA	GGATACCAAT	ATTTCATGTT	TCTTTATATA	GGTAAGAGCC
Shelton	TATTGTTAAA	AATAAGAGTA	GGATACCAAT	ATTTCATGTT	TCTTTATATA	GGTAAGAGCC
Roy	ATAGTAAATT	CCTCGTCCAA	TATGGATATA	TAAGCAAATA	AAGAAAAGAG	AGGCCCCATT
Olympia	ATAGTAAATT	CCTCGTCCAA	TATGGATATA	TAAGCAAATA	AAGAAAAGAG	AGGCCCCATT
Shelton	ATAATAAATT	CCTCGTCCAA	TATGGATATA	TAAGCAAATA	AAGAAAAGAG	AGGCCCCATT
Roy	AGCATGAATC	AGTCATATAT	ATCGACCGTA	GTTTACGTCT	CGGCAAATGT	GGGTTACTGA
Olympia	AGCATGAATC	AGTCATATAT	ATCGACCGTA	GTTTACGTCT	CGGCAAATGT	GGGTTACTGA

Shelton	AGCATGAATT	AGTCATATAT	ATCGACCGTA	GTTTACGTCT	CGGCAAATGT	GAGTTACTGA
Roy	TGAGAAAGCT	GTAAGTGTAT	CCGATGTATA	GTGTATAGCT	AGGAATAGCC	CTGTAAAGAT
Olympia	TGAGAAAGCT	GTAAGTGTAT	CCGATGTATA	GTGTATAGCT	AGGAATAGCC	CTGTAAAGAT
Shelton	TGAGAAAGCT	GTAAGTGTAT	CCGATGTATA	GTGTATAGCT	AGGAATAGCC	CTGTAAAGAT
Roy	TTGTAAGACT	AAGCATATTC	CAAGTAGAGA	TCCAAAGTTT	CATAAACCTG	AAATGTTAGG
Olympia	TTGTAAGACT	AAGCATATTC	CAAGTAGAGA	TCCAAAGTTT	CATAAACCTG	AAATGTTAGG
Shelton	TTGTAAGACT	AAGCATATTC	CAAGTAGAGA	CCCAAAGTTT	CATAAACCTG	AAATGTTAGG
Roy	TGGGGTTGGT	AAATCAATAA	ATGCGTGGTT	AACAATTTTG	AATAATGGAT	GCGATTTACG
Olympia	TGGGGTTGGT	AAATCAATAA	ATGCGTGGTT	AACAATTTTG	AATAATGGAT	GCGATTTACG
Shelton	TGGGGTTGGT	AGATCAATAA	ATGCGTGGTT	AACAATTTTG	AATAATGGAT	GCGATTTGCG
Roy	TATGATTGTC	ATTAGTTTC				
Olympia	TATGATTGTC	ATTAGTTTC				
Shelton	TATGATTGTC	ATTAGTTTC				

ND III

Roy	TTTTTTAGTA	TATAAGTACA	TTTGACTTCC	AATCAAGGAG	ATTTGGTAAT	GAATCCAAAA
Olympia	TTTTTTAGTA	TATAAGTACA	TTTGACTTCC	AATCAAGGAG	ATTTGGTAAT	GAATCCAAAA
Shelton	TTTTTTAGTA	TATAAGTACA	TTTGACTTCC	AATCAAAGAG	ATTTGGTAAT	GAATCCAAAA
Roy	AAAAGTAATT	AATCTAGCAT	TATCATTATT	ATTAAACTTT	ACTTTATCAA	CAATCTTAAC
Olympia	AAAAGTAATT	AATCTAGCAT	TATCATTATT	ATTAAACTTT	ACTTTATCAA	CAATCTTAAC
Shelton	AAAAGTAATT	AACCTAGCAT	TATCATTATT	ATTAAACTTT	ACTTTATCAA	TAATCTTAGC
Roy	TACAATTGCT	TTCTGAATTC	CACAAATAAA	TATTTACTCA	GAAAAAGTAA	ATCCATATGA
Olympia	TACAATTGCT	TTCTGAATTC	CACAAATAAA	TATTTACTCA	GAAAAAGTAA	ATCCATATGA
Shelton	TACAATTGCT	TTCTGAATCC	CGCAAATAAA	TATTTACTCA	GAAAAAGTAA	ATCCATATGA
Roy	ATGTGGGTTT	GATCCAATAA	ATTCTGCACA	TCTTCCCTTT	TCTATAAAAT	TTTTCTTAGT
Olympia	ATGTGGGTTT	GATCCAATAA	ATTCTGCACA	TCTTCCCTTT	TCTATAAAAT	TTTTCTTAGT
Shelton	ATGTGGATTT	GATCCAATAT	ATTCTGCACA	TCTTCCCTTT	TCTATAAAAT	TTTTCTTAGT
Roy	GGCAATTACT	TTCCTTCTAT	TTGATTTAGA	AATCGCCTTA	CTACTTCCAC	TTCCATGAGC
Olympia	GGCAATTACT	TTCCTTCTAT	TTGATTTAGA	AATCGCCTTA	CTACTTCCAC	TTCCATGAGC
Shelton	GGCAATTACT	TTCCTTCTAT	TTGATCTAGA	AATCGCCTTA	CTACTTCCAC	TTCCATGAGC
Roy	TTCCCAGTTC	CAAAATATTA	AATCAATAAT	CATTTTGTCA	CTAGCCCTGA	TTGTTATTTT
Olympia	TTCCCAGTTC	CAAAATATTA	AATCAATAAT	CATTTTGTCA	CTAGCCCTGA	TTGTTATTTT
Shelton	TTCCCAGTTC	CAAAGTATTA	AATCAATAAT	AATTTTGTCA	CTAGCCCTGA	TTGTTATTTT
Roy	AGCTTTAGGC	TTAGCATACG	AGTGAATAAA	TAAAGGCCTC	GAATGAGATG	AGTAATAGTG
Olympia	AGCTTTAGGC	TTAGCATACG	AGTGAATAAA	TAAAGGCCTC	GAATGAGATG	AGTAATAGTG
Shelton	AGCTTTAGGG	TTAGCATACG	AGTGAATAAA	TAAAGGCCTC	GAATGAGATG	AGTAATAGTG
Roy	GTTAGTTTAA	AAAAAATGA				
Olympia	GTTAGTTTAA	AAAAAATGA				
Shelton	GTTAGTTTAA	AAAAAATGA				

Gophers make their living by burrowing both with their teeth and their claws through the soil. Obviously, extremely rocky soils must make it difficult for them to dig. To explore how much of the distribution of *T. mazama* in Puget Sound prairies could be explained by soil rockiness, we conducted soil sampling in nine different prairies, five with pocket gophers and four without. At each prairie we sampled between one and four transects, collecting data from five soil samples (spaced 50 meters apart) for each transect. For each sample, we first removed all above-ground vegetation, and then dug a cylindrical hole approximately 50 centimeters wide and 60 centimeters deep in the ground, placing the soil and rocks from the hole into a bucket. We weighed the entire sample with a 50 Kg pesola spring scale and then divided the rocks in the sample into three size classes by sifting through two inch, then 1 inch and finally 1/2 inch wire mesh. We refer to the three size classes of rock separated by this method as: small, medium, and large. We weighed each of these size classes of rocks separately and then combined their weights and subtracted the sum from the total sample weight to determine the weight of soil in each sample. In addition to these data, we recorded presence or absence of fresh pocket gopher mounds within five meters of each soil sample collection site.

To analyze these data we applied logistic regression analysis (see Wilkenson, 1989). This statistical method is designed to assess models that use continuous independent variables (single or multiple variables) to predict presence/absence or yes/no data. The logistic function has the general form:

$$Y = \frac{\exp^{(a_0 + a_1X_1 + a_2X_2 + \ldots a_nX_n)}}{1 + \exp^{(a_0 + a_1X_1 + a_2X_2 + \ldots a_nX_n)}}$$

Equation 1

where Y is the dependent ("predicted") variable, X_i's are the independent variables, a_i's are the fitted constant parameters, and n is the number if independent variables in the model. This function allows for a "step" or threshold prediction, so that, for example, a Table 2. DNA sequences species absence might be predicted across some threshold. Logistic regression is ideally applied to predictions about singular events (was the species present or absent?) or the frequency of binary events (what fraction of times was a species present or absent?). Often logistic analysis is used to test if "indicator variables" are effective. It is a very flexible approach because it can describe anything from a linear response to a sharp "step function". Like any regression model, goodness-of-fit is determined by some statistically defensible criteria. We used the most familiar "least squares" method, which minimizes the summed squared differences between predicted and observed values.

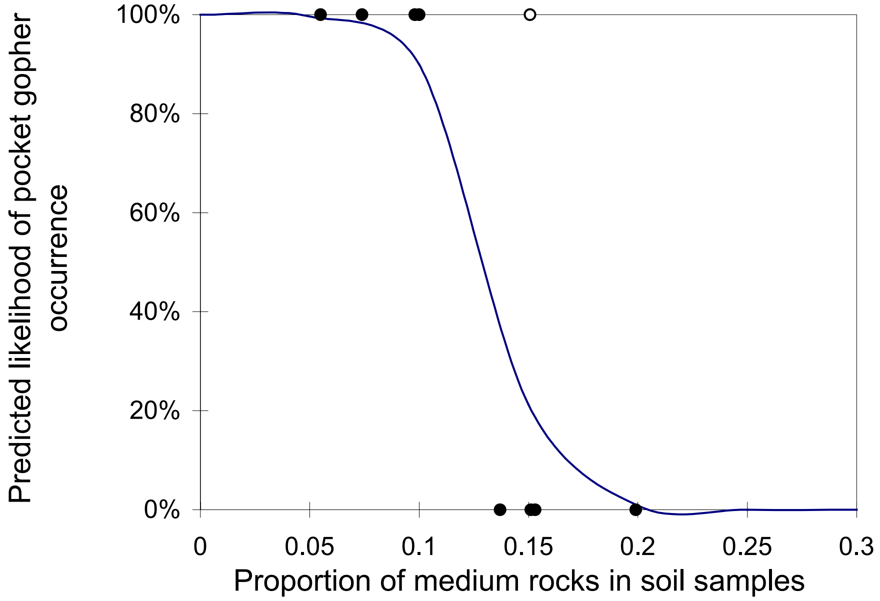
Soil data are summarized in Table 2. Using the presence or absence of gophers as our dependent variable, we performed four distinct logistic regressions (see equation 1). Each regression corresponded to a model using one of four different independent variables: fraction of weight due to soil, and fraction of weight represented by each of the three size-classes of rocks.

Although all of these models predicted gopher occurrences on prairies relatively well, the model using medium rocks was the best predictor. Indeed, the proportion of weight due to medium rocks alone correctly predicted presence or absence of pocket gophers in eight of the nine prairies we sampled (Figure 2). Only Weir Prairie, which had a high proportion of medium rocks in soil samples, would have been misclassified as not supporting pocket gophers, when gophers are in fact present at this site (Table 2. and Figure 2).

Table 2. Presence or absence of pocket gophers and average proportion of dirt and rocks of varying sizes in soil samples

SITE	GOPHERS PRESENT	SMALL ROCKS	MEDIUM ROCKS	LARGE ROCKS	DIRT
Scatter Creek	yes	.085	.055	0	.86
Rock Prairie	yes	.106	.098	.209	.586
Upper Weir	yes	.214	.148	.066	.573
Marion Prairie	yes	.079	.074	.046	.893
Johnson Prairie	yes	.144	.1	.041	.715
13th Division Prairie	no	.22	.199	.052	.529
Fort Lewis Training Area # 7S	no	.228	.137	.016	.619
Fort Lewis Training Area # 8	no	.196	.153	.032	.619
Wolf Haven	no	.094	.151	.248	.506

Figure 2. Presence (100%) or absence (0%) of pocket gophers in prairies relative to average proportion of medium rocks in soil samples (the open circle indicates the one point "misclassified" by the model).



Synthesizing what we have learned from our genetic analysis, our prairie surveys, and our soil analysis, we can begin to construct a different story for pocket gophers in Puget Sound prairies. As long as pocket gophers have resided on these glacial outwash prairies, their distribution has probably been highly patchy, with that patchiness due in part to the distribution of prairies, but also to an even patchier distribution of soil rockiness within the prairie expanses. The Puget Sound prairie pocket gophers are clearly not substantially genetically differentiated, thus nomenclature should probably be altered to recognize only two prairie subspecies; with couchi continuing to represent the southern (Shelton) form, and *yelmensis* representing the other Puget Sound prairie forms (currently named *glacialis*, *pugetensis*, *tumuli*, *tacomensis*, and *yelmensis*).

However, formal taxonomic revision will not take place until all *T. mazama* subspecies have been examined for both morphological and genetic differences. In addition, determining phylogenetic relationships of *T. mazama* subspecies throughout the entire range of the species will allow us assess whether the pocket gophers in Puget Sound prairies more likely spread to this post-glacial habitat from refugial populations in Olympic National Park or from the other side of the Columbia River in Oregon.

Finally, it is important to discuss our findings in light of management. First, the fact that five of Washington's subspecies will probably be combined into one taxonomic unit does not mean those populations are no longer of conservation concern. The entire prairie subspecies complex of pocket gophers may be threatened with extinction, because all populations are small and isolated, and the geographic distribution of the entire group clearly appears to be shrinking. Secondly, the discovery that soil rockiness is a good predictor of presence or absence of pocket gophers in prairies does not mean that the story is "all soils", and that habitat destruction and degradation is trivial. Indeed, it is just the opposite. Because soil rockiness can be an ultimate filter on whether or not pocket gophers can persist in an area, our conservation challenge is even greater. To protect pocket gophers, we not only have to preserve and restore prairies, we must preserve and restore prairies that do not have overly rocky soils. Sadly, if we do not act quickly to protect the right prairies, we may never figure out the story of the gophers of Puget Sound prairies, because there will no longer be any gophers to study.

Literature Cited

Avise, J.C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, NY.

Bailey, V. 1915. Revision of the pocket gophers of the genus *Thomomys*. North American Fauna 39, 1-136.

Burt, W.H., and R.P. Grossenheider. 1976. A field guide to the mammals. Houghton Mifflin Company, Boston.

Dalquest, W.W. 1948. Mammals of Washington. University of Kansas Publications, L

Dalquest, W. W. and V.B. Scheffer. 1942. Three new pocket gophers (Genus *Thomomys*) from western Washington. Proceedings of the Biological Society of Washington, 55:95-98.

Goldman, E.A. 1939. Remarks on pocket gophers, with special reference to *Thomomys talpoides*. Journal of Mammalogy, 20: 231-244.

Hall, E.R. 1981. The mammals of North America. Second ed. John Wiley & Sons, NY.

Hillis, D.M., C. Moritz, and B.K. Mable. 1996. Molecular Systematics. Sinauer Associates, Sunderland, MA.

Johnson, M.L., and S.B. Benson. 1960. Relationships of the pocket gophers of the *Thomomys mazama-talpoides* complex in the Pacific Northwest. The Murrelet, 41(2): 17-22

Patton, J.L., and P.V. Brylski. 1987. Pocket gophers in alfalfa fields: causes and consequences of habitat-related body size variation. American Naturalist ,130(4): 493-506.

Patton, J.L., and M.F. Smith. 1990. The evolutionary dynamics of the pocket gopher *Thomomys bottae*, with emphasis on California populations. University of California Publications in Zoology, 123:1-161.

Smith, M.F.,and J.L. Patton. 1988. Subspecies of pocket gophers: causal bases for geographic differentiation in *Thomomys bottae*. Systematic Zoology, 37:163-178.

Steinberg, E.K. 1995. A study of genetic differentiation and variation in the Mazama pocket gopher (*Thomomys mazama*) with an emphasis on Fort Lewis populations. Final report to The Nature Conservancy on contract # WAFO-100193.

Steinberg, E.K. 1996. Population studies and management of the threatened Mazama pocket gopher: a regional perspective.