

Genetic variation in the Smooth Green Snake, *Opheodrys vernalis*, in South Dakota

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Brian E. Smith, Cynthia Anderson, Shane Sarver, and Laurelin R. Cottingham

Contact author: Brian E. Smith
Department of Biology
Black Hills State University
1200 University St. Unit 9044
Spearfish, SD 57709

Telephone: (605) 642-6879
E-mail: briansmith@bhsu.edu

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EXECUTIVE SUMMARY

The smooth green snake, *Opheodrys vernalis*, is a small enigmatic snake found throughout much of the northern and eastern United States and southeastern Canada. Isolated populations occur in the Great Plains, Rocky Mountains, and uplifts associated with the Rocky Mountains, such as the Black Hills and Bear Lodge Mountains in southwestern South Dakota and northeastern Wyoming. There has long been confusion over the taxonomic placement of the species and it has historically been placed in two genera, with three described subspecies, with different taxonomies used according to author. In the most recent analysis of morphological variation within the species, Grobman (1992a) concluded that Black Hills populations are distinct from Great Plains populations but are similar to populations found east of the Great Plains, a paradoxical conclusion. Of additional interest, anecdotal reports indicate that the species may be in decline rangewide, although the extent of this is difficult to determine. It appears to be quite rare in much of its range, although still relatively commonly encountered at our collection sites in the Black Hills, Bear Lodge Mountains, and Sugarite Canyon, in northern New Mexico. We concluded that DNA analysis might shed light on genetic variation across part of the range of this snake, especially within South Dakota, thereby clarifying some of the taxonomic issues. To manage any species effectively it is important to know which populations are distinctive enough to warrant special management consideration. Also important to management is knowledge of the distribution and abundance of the snake. Although the study was not designed to provide data on distribution and abundance, our collecting experiences were enlightening.

We collected snakes throughout the Black Hills (our largest collection) and Bear Lodge Mountains, as well as adjacent to the Ordway Prairie Preserve (near Leola, north-central South

Dakota), and a reference population from Sugarite Canyon, northern New Mexico. We removed small samples of the tail tips from these specimens for genetic analysis and released snakes at the sites of capture. In addition, colleagues sent us specimens from Utah, Montana, Nebraska, and New York. We analyzed variation in mitochondrial DNA (mtDNA), splitting the samples into four geographic locations for analysis: Black Hills, Bear Lodge Mountains, Ordway Prairie, and northern New Mexico. DNA was extracted from other specimens donated from around the country, but sample sizes were too low to include in the analysis of microsatellite variation.

Analyses of mtDNA sequence variation did not support the recognition of distinct sub-species for any of the populations included in the study. Thus, we concluded that smooth green snakes from the Black Hills of South Dakota and from Ordway Prairie, eastern South Dakota, were not separate sub-species. However, analysis of 8 microsatellite loci indicated population-level differences among these four smooth green snake populations. Statistically significant differences in allelic variation were shown in all analyses used to assess population structure, but there was some ambiguity in the exact nature of population structure amongst our samples. It was clear that the New Mexican population was genetically distinct from all other populations. We also concluded, based on STRUCTURE analysis and F_{st} comparisons, that the Black Hills and Bear Lodge Mountains populations likely represented a contiguous population, and also were distinct from samples collected at Ordway Prairie. Previous reports suggesting a transition of the western form (*O. v. blanchardi*) to the eastern form (*O. v. vernalis*) in Wisconsin and Minnesota appear to be inaccurate.

Further explanation of our statements on the abundance of the smooth green snake is warranted. It can commonly be found in suitable weather (most often in cooler and wetter weather during spring and fall) in Sugarite Canyon, New Mexico; the Black Hills; and the Bear

Lodge Mountains. It appears to be rare and localized in the Great Plains. Anecdotal reports indicate that the smooth green snake may be common in some parts of Maine, but uncommon in many areas east of South Dakota. Because the snake is rarely observed in the eastern United States we are missing critical data that would probably help resolve taxonomic issues. The smooth green snake is probably limited to mesic habitat, which may account for its scarcity in the Great Plains, where appropriate habitat is scarce. We cannot explain why it is rare across much of the northeast, but colleagues across its eastern range consistently commented that it was rarely or never found at various localities east of the eastern South Dakota border. Our comments on abundance should be taken with caution since we performed no population density estimates and many reports on abundance were anecdotal. However, we feel that the weight of the evidence suggests that the species is rare in many parts of its range, while spottily abundant in the Black Hills, Bear Lodge Mountains, and at Sugarite Canyon.

Analyses of the genetic data provide some evidence that the Ordway Prairie and Black Hills populations in South Dakota are genetically distinct and thus management strategies should take this into account. As a result, we recommend that resource managers treat the snake as a species worthy of special management. Additionally, the snake is rarely observed at many localities and we presume that it is generally rare in the Great Plains. Holocene climate data indicate that preferred habitat for the smooth green snake has been disappearing over the last 10,000 years in the Great Plains, and we conclude that populations there are relictual. Of importance, models of climate change in the northern Great Plains indicate a warmer and drier climate in the near-term (next 50 years), likely causing further population declines.

It is doubtful that this species disperses considerable distances and it is probably restricted to mesic conditions in which it lives and through which it moves. However, detailed

natural history studies have not been completed. Unfortunately, the ecology of small snakes is poorly known, so we cannot draw any conclusions about the biology of the smooth green snake based on similar model organisms. We suggest further studies of genetic variation within this species as well as detailed ecological studies to learn more about the natural history of the smooth green snake.

INTRODUCTION

The smooth green snake, *Opheodrys vernalis*, is a small, enigmatic snake found throughout the northeastern United States and southeastern Canada and in 24 isolated localities throughout the Midwest, northern Great Plains, and Rocky Mountain west (Conant and Collins 1998, Ernst and Ernst 2003, Stebbins 2003). It may have declined throughout much of its range and is now protected in Indiana, Missouri, Montana, North Carolina, and Wyoming (Ernst and Ernst 2003). Its status is poorly known in Idaho (Ernst and Ernst 2003). Smooth green snakes are thought to be secure in Colorado (Hammerson 1999) and New Mexico (Degenhardt et al. 1996), but records are sparse. Declines have likely occurred in Illinois (Phillips et al. 1999) and New York (Pete Ducey, State University of New York at Cortland, personal communication). Smooth green snakes are thought to be very rare in Utah (Daniel Mulcahy, Utah State University, personal communication) and Nebraska (Dan Fogell, personal communication), and very rare or perhaps extinct in northern Mexico (Jonathon Campbell, University of Texas at Arlington, personal communication). In contrast, smooth green snakes are relatively abundant in the Black Hills of South Dakota and Wyoming and in the Bear Lodge Mountains of Wyoming (Smith unpublished), though highly localized. Smooth green snakes also occur on the eastern plains of South Dakota but their status there is poorly known. Evidence of declines across the range is cause for concern. Declines may be connected to pesticide use (Minton 1972, Phillips et al. 1999). The smooth green snake is considered rare in South Dakota and is monitored by the South Dakota Natural Heritage Program.

The species has a history of taxonomic confusion. It was placed in the genus *Opheodrys*, along with the rough green snake, *O. aestivus*, as far back as 1941 (Grobman 1941). Oldham and Smith (1991) presented evidence to establish the genus *Liochlorophis* for the smooth green

snake. Recently the smooth green snake was placed back in the genus *Opheodrys* (Committee on Standard English and Scientific Names 2000). Three subspecies have been named (Grobman 1941, 1992a, 1992b) but Collins (1992) rejected these subspecies. Following Collins (1992), Ernst and Ernst (2003) did not recognize subspecies. Grobman (1992a) described an unusual pattern of geographic variation in morphology that he used to support his interpretation of subspecific taxonomy in *O. vernalis*. Most importantly, he noted that snakes from the eastern United States (*O. v. vernalis*) were most closely related to those in the Black Hills and Bear Lodge Mountains (also *O. v. vernalis*), with a different geographic variant, *O. v. blanchardi*, in the Great Plains (figure 1). These conclusions require biologists to consider that smooth green snakes of the Black Hills are more similar to smooth green snakes found east of the eastern border of South Dakota, more than 1100 km east of the Black Hills, than they are to populations found in the Great Plains, ca. 500 km east. This pattern of morphologic variation seems nonsensical. However, in an unpublished multivariate reanalysis of Grobman's (1992a) data, David Chiszar (University of Colorado) reached the same conclusions.

The comparative genetic structure of the smooth green snake has not previously been studied. Additionally, work on the genetic structure of South Dakotan snakes will provide the South Dakota Department of Game, Fish, and Parks needed data to manage the species. If smooth green snakes in the Black Hills and in eastern South Dakota are genetically distinct, then smooth green snakes within the state consist of at least two distinctive genetic groups. In this case we would suggest that the state Department of Game, Fish, and Parks consider management of the snake in light of genetic differences amongst populations. If such variation exists it may be important to develop management plans that treat individual populations as separate groups. Our fundamental question was: Are there two genetically distinct populations of *Opheodrys*

vernalis found within the state of South Dakota? We approached this question by examining genetic variation within and among smooth green snakes collected in the Bear Lodge Mountains of Wyoming, the Black Hills, and adjacent to Ordway Prairie Preserve in north-central South Dakota (figure 2). We included snakes from the Bear Lodge Mountains because these mountains were formed at the same time as the Black Hills and are part of the same geological uplift. These four populations represent Grobman's (1992a) subspecies *O. v. vernalis* (Bear Lodge Mountains and Black Hills) and *O. v. blanchardi* (Ordway Prairie). A population of smooth green snakes found in northern New Mexico (Grobman's *O. v. blanchardi*) was also examined and included in our comparisons of smooth green snakes in South Dakota and far northeastern Wyoming.

METHODS

Smooth green snakes were hand collected within the Bear Lodge Mountains of Wyoming, the Black Hills, adjacent to Ordway Prairie, and Sugarite Canyon in northern New Mexico (Table 1). A small portion of the tail was removed from each specimen for genetic analyses and the snakes were released at the site of capture. Colleagues in New York, Montana, Utah, and Nebraska donated tissues, but because of small sample sizes these specimens were not included in the microsatellite analyses. Total DNA was isolated from tissue using a Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA).

Previously published primer sets for several mitochondrial genes were tested for their ability to amplify mtDNA from the smooth green snake (Arevalo et al. 1994). The primer sets included the mtDNA control region (*d-loop*), three nicotinamide adenine dinucleotide dehydrogenase genes (*ND2*, *ND4*, and *ND8*) and the cytochrome b gene (*cytb*). Of these, *ND2* provided the most consistent results and was used to obtain mtDNA sequence data on the available samples. A 1200 bp region of the *ND2* gene was amplified using primers L4437b (5'-

CAGCTAAAAAAGCTATCGGGCCCATACC-3') and tRNA-trpR (5'-GGCTTTGAAGGCTMCTAGTTT-3'). PCRs consisted of an initial denaturation of 92° C for 5 min, followed by 35 cycles of 1 min at 92° C, 1 min at 55° C and 1 min at 72° C and ended with a final extension of 5 min at 72° C. Sequencing reactions were done using BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems) and run on an ABI 3130 Genetic Analyzer (Applied Biosystems) at the Western South Dakota DNA Core Facility (WestCore) at Black Hills State University. The PCR amplicons were all sequenced in both directions and compared with Genbank (to verify target gene was sequenced) using the Basic Local Alignment Search Tool (BLAST), available at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/blast/>). Sequence alignments have been done using the software SEQUENCHER 4.5 (Gene Codes).

DNA was isolated for the construction of microsatellite-enriched libraries using a DNeasy extraction kit for animal tissues (QIAGEN) according to the manufacturer's recommendations. Genomic DNA from a single snake sample was digested with *Sau3AI* to yield fragments that range from 300bp to 1500bp in size. The digested DNA was size fractionated on a Chroma Spin+TE 400 column (Clontech) to retain fragments of size 400bp and greater. Linkers were added to the DNA fragments to facilitate PCR amplification. Size fractionated amplified genomic DNA was probed using 3'-biotinylated oligonucleotide probes (5'-(AG)₁₅TATAAGATA-/3bio/-3', 5'-(TG)₁₅TATAAGATA-/3bio/-3', 5'-(AAC)₈TATAAGATA-/3bio/-3', 5'-(AAG)₈TATAAGATA-/3bio/-3', 5'-(AAT)₈TATAAGATA-/3bio/-3', 5'-(ACT)₈TATAAGATA-/3bio/-3', and 5'-(ATC)₈TATAAGATA-/3bio/-3') and captured using Dynabeads MyOne Streptavidin C₁ (streptavidin coated paramagnetic beads) (Invitrogen). The microsatellite-impoverished supernatant was removed from the beads and re-probed to yield a

doubly enriched library. Captured fragments were released from the probes and PCR amplified. This microsatellite enriched fragment library was then TA cloned by ligation into the pCR2.1-TOPO vector (Invitrogen) according to the manufacturer's instructions. Following transformation, colony blots were done to confirm the presence of microsatellite-containing colonies. One hundred thirty-one colonies hybridized with the probes. These were grown in overnight cultures for plasmid isolation using the Qiaprep Spin Miniprep kit (QIAGEN). The plasmids were sequenced in both directions using M13 forward and reverse primers with a BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 3130 genetic analyzer (Applied Biosystems) by the Western South Dakota DNA Core Facility (WestCore) at Black Hills State University (BHSU).

Twenty-five sets of primers were designed for PCR amplification of microsatellite loci using OligoPerfect primer design software (Invitrogen). The forward primer of each set was designed with a 5'-M13(5'-CACGACGTTGTAAAACGAC) tail to make use of the universal dye-labeling technique described by Boutin-Ganache *et al.* (2001). The reverse primer was designed with a 5'-pigtail sequence (5'-GTTTCTT) to facilitate nontemplated polyadenylation of the PCR product. PCR products were run with the internal size standard GS350-ROX (Applied Biosystems) on an ABI 3130 Genetic Analyzer (Applied Biosystems) by WestCore, BHSU. Allele sizes were determined using the software *Genemapper*. Fifteen primer sets gave consistent amplification of specified target loci and were screened for polymorphism. Eight polymorphic loci were identified.

The 8 microsatellite markers were used to screen 60 smooth green snakes collected from the Black Hills to test for their utility as markers for assessing population differences. The number of alleles ranged from 3-25 and observed heterozygosities ranged from 0.036-0.867. The

microsatellite loci were tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENPOP 3.4 (Raymond & Rousset 1995). The loci 6-131, 2-261, 5-121, and 6-93 were found to not be in HWE ($P < 0.05$). Linkage disequilibrium (LD) was tested among all marker pairs and corrected for multiple comparisons using the Bonferroni method. No significant LD was detected for any of the marker pairs. These microsatellite markers were thus suitable for studies of population structure. Primer sequences, PCR conditions, repeat motif and summary statistics are given in Table 2.

Phylogenetic analysis of the mitochondrial DNA sequences was done using MRBAYES (Huelsenbeck and Ronquist 2001). The MCMC analysis was run for 2,000,000 generations with the phylogeny sampled every 1000 generations. The first 1000 trees sampled were discarded as burn-in to ensure the chain had reached stationarity and the final 1000 trees used to build the Bayesian consensus tree. The Genbank sequences correspond to the outgroup sequences *Masticophis flagellum* and *Coluber constrictor* (Serpentes, family Colubridae). A median-joining haplotype network estimated from the mtDNA data was done using the methods of (Bandelt *et al.* 1999).

Observed genotype frequencies were tested for Hardy-Weinberg and linkage equilibrium using randomization tests using GENEPOP 3.4 (Raymond M, Rousset F 1995). Pairwise F_{ST} estimates amongst populations were done using GENPOP 3.4 (Raymond M, Rousset F 1995) using the method of Weir and Cockerham (1984). The statistical significance of genetic differences between each pair of populations was tested using Fisher's exact method implemented in GENPOP. The microsatellite data were analyzed in several ways to assess genetic population structure. We used the model-based clustering method of Pritchard *et al.* (2000) to infer population structure and to assign individuals to uncovered clusters, i.e.,

populations (K). The structure analysis was run using the Admixture Model. Conditions for the MCMC analysis were 250,000 generations of burn-in followed by 500,000 generations of sampling. Analysis of molecular variance (AMOVA) was used to partition the genetic variation among populations in a fashion analogous to traditional ANOVA (Excoffier et al. 1992).

RESULTS

Genotypes for 8 microsatellite loci were determined for samples from 4 geographic locations (Black Hills, Bear Lodge Mountains, Ordway Prairie, and Sugarite Canyon) and 104 individual green snakes. Overall, 124 alleles were observed, indicating relatively high levels of genetic diversity. There was no evidence for linkage disequilibrium for any of the loci. The number of alleles ranged per locus from 0-25 and observed heterozygosities ranged from 0.036 – 0.867. A number of loci exhibited deviations from Hardy-Weinberg expectations due to deficiencies of heterozygous genotypes.

Pairwise population comparisons of F_{st} indicated low levels of allelic differentiation between the Black Hills and Bear Lodge populations and moderate to high levels amongst all other populations (Table 3). Highest F_{st} values were observed between the Sugarite Canyon population and the other populations. However, differences in allele frequencies were observed throughout the sampled area in this study. After application of the Bonferroni correction ($\alpha=0.05/28=0.0017$), comparisons of all 4 populations (i.e., Sugarite Canyon, Bear Lodge Mountains, the Black Hills, and Ordway Prairie) showed statistically significant differences amongst them ($P < 0.001$). AMOVA also indicated statistically significant differences amongst the sampled populations. A comparison of the 4 populations revealed that 13.5% of the genetic variation occurred among populations and 86.5% occurred within populations ($P < 0.001$).

Results from the STRUCTURE analysis indicated that it was most likely that two genetically distinctive populations existed within the dataset ($K=2$), but the likelihood that three populations ($K=3$) existed were similar to $K=2$, indicating some uncertainty in interpretation of STRUCTURE analyses. A $K=2$ model clusters the Sugarite Canyon specimens separately from those collected in the Black Hills, Bear Lodge Mountains, and Ordway Prairie. The $K=3$ model clusters the Black Hills and Bear Lodge Mountains specimens into a population, Ordway Prairie as a second population, and Sugarite Canyon as a third population. That is, at least two populations exist (Sugarite Canyon and all other populations), although it is likely that three populations exist (Sugarite Canyon, Bear Lodge Mountains/Black Hills, and Ordway Prairie).

Mitochondrial DNA sequence data from 88 individuals from 7 collections were analyzed for variation in the *ND2* gene. A median-joining haplotype network from the mtDNA data indicated no evidence of population structure of haplotypes (Figure 3b). The results of a phylogenetic analysis using a Bayesian maximum likelihood method (MRBAYES) found no significant clade structure among any of the samples from any of the 7 collections (Figure 3a).

DISCUSSION

We believe that there are three factors that are of importance in interpreting our results. The smooth green snake is a small snake (adult snout-vent length ranging from 30.3 – 51 cm, Conant and Collins 1998). Although its movement patterns have not been studied, since the smooth green snake is a small snake we do not expect that adults can travel far during their lifetimes. Additionally, the species is found most commonly in mesic habitats within evergreen forests and we suspect that they need mesic corridors through which to disperse. Finally, fossil data from the Pleistocene epoch indicate that the range of the smooth green snake has changed

according to advances and retreats of boreal forests, extending as far south as modern-day Florida during glacial advances (Holman 2000).

The results of the mtDNA analyses are in conflict with previous morphology-based studies of *Ophedrys* that concluded geographic differences among smooth green snake populations. Mitochondrial DNA data do not support previous suggestions of sub-specific classification, if the same geographic distributions are assumed. Furthermore, mtDNA data do not support the conclusions of previous studies that hypothesized sub-specific differences between eastern South Dakotan specimens and snakes from the Black Hills of South Dakota and Wyoming. It is possible that genetically distinct forms of *O. vernalis* exist in other geographic regions, but samples from these regions were not included in the present study. Results from mitochondrial DNA sequence data in the present study indicate that previously described patterns of morphological variation are not congruent with DNA sequence data.

The analyses of microsatellite data indicate evidence of significant differences among populations of *O. vernalis* that we studied. Considering these results, the fossil data, and the natural history of the species, we conclude that there is significant genetic differentiation among populations, but previous suggestions of distinct subspecies in our sampling range are not warranted. Analyses of population structure suggest the existence of 3 genetically distinct groups in the data, which correspond to the Sugarite Canyon, Black Hills/Bear Lodge Mountains, and Ordway Prairie populations. Collection data indicated that specimens were captured in very localized areas within the four groups we defined for this study (Sugarite Canyon, Bear Lodge Mountains, Black Hills, and Ordway Prairie), and these snakes are restricted to specific habitats with limited dispersal. Thus, the fact that significant genetic differences exist among geographic regions is not surprising.

A number of loci exhibited deviations from Hardy-Weinberg expectations due to deficiencies of heterozygous genotypes, but it is difficult to assign biological significance to such findings because there is a tendency for microsatellite markers (in general) to exhibit deficiencies of heterozygotes. Deficiencies of heterozygotes could be due to population sub-structure (i.e. the Wahlund effect) or null alleles or a combination of factors. Interestingly, the greatest tendency towards heterozygote deficiencies was observed in the Black Hills sample. The number of samples analyzed for the Black Hills was greater than any of the other populations and all samples were pooled for analyses. This leaves open the possibility that there might be further sub-structure of populations in the Black Hills. Further sampling, so statistically meaningful comparisons can be made, will be required to assess the possibility of population substructure.

Bearing on the generally isolated nature of smooth green snake populations, we offer the following anecdotal evidence. In the Bear Lodge Mountains and Black Hills, snakes were frequently taken in small patches of suitable habitat. For example, several snakes were collected at a single inlet at Deerfield Lake, but not at nearby inlets. Several specimens were taken at a riparian improvement area in the Bear Lodge Mountains, but nowhere adjacent to this area along the same creek. According to various correspondents, they are very rare in Nebraska and in Utah. However, refuge staff reported that they are somewhat common at the Medicine Lake National Wildlife Refuge in Montana, an area from which they have never been reported. Despite their wide range across the eastern United States, correspondents in Illinois (Chris Phillips, Natural History Survey of Illinois, personal communication) and New York (Pete Ducey, State University of New York, Cortland, personal communication) reported that they are very rare in these states. Yet, one correspondent (John Willson, University of Georgia, personal communication) reported that they were locally common on certain islands off the coast of

Maine, yet nowhere on the mainland. In addition, drought appears to have significant effects on the species. At Ordway Prairie, drought conditions had persisted for ca. 8 years and smooth green snakes had not been found in 8 years, according to preserve staff. During this time, >75% of permanent and semi-permanent wetlands on the preserve had dried completely. It was adjacent to the preserve that we found two dilapidated farmhouses, beneath which we found all nine of the specimens we analyzed from this area (seven snakes were collected from a single farmhouse).

Further discussion of Great Plains populations is warranted. We found the snakes to be fairly common (though highly localized) in boreal forest, but extremely rare in the Great Plains and very localized, even more so than in boreal forest. Considering that fossil data shows that the snake's range advances and retreats in concert with the advance and retreat of boreal forest (due to the influence of glaciation), we infer that the species is associated with boreal forests and that Great Plains populations are living at the edges of their tolerance.

Regarding the presence of boreal forest in the Great Plains, several studies have examined the Holocene drying trend in the northern Great Plains (Fritz et al. 1991, Kennedy 1994, Laird et al. 1996). Of special note, Laird et al. (1996) found that the vegetation surrounding Moon Lake, in east-central North Dakota, changed from a boreal (spruce-dominated) flora to a plains flora during the period from ~10,000 to ~7,000 years before the present. In addition, over the last 10,000 years several long-term droughts have been identified that were more extreme than historical droughts (Forman et al. 2001). Considering recent (historical) drought, and long-term drought and drying of the Great Plains, we conclude that Great Plains smooth green snake populations are remnant populations. Of related interest, Sorenson et al. (1998) found that most (11 of 12) climate change models they studied predict

increasing drought in the northern Great Plains in the near future (modeled from 2000 to 2060). Given these factors, especially climate change, we conclude that smooth green snakes in the Great Plains are deserving of further monitoring and conservation efforts. It may be that certain localities in the Great Plains are important refuges for remaining populations in the region.

MANAGEMENT RECOMMENDATIONS

Given the evidence for genetic population structure in *O. vernalis*, management units should be defined based on geographic populations. In South Dakota, the Black Hills population should be considered distinct from eastern South Dakotan populations. Overall genetic variation appears high, suggesting that populations have not undergone a bottleneck. These results fit the little data we have on the natural history of the smooth green snake, which is that it is a snake found in localized populations at suitable times of the year. In addition, the species' size probably precludes it from moving great distances during its lifetime.

Fossil literature and our collection experiences indicate that the species is primarily restricted to boreal forest and is most likely rare in the Great Plains. Holocene climate and vegetation studies show a recent (from ~10,000 to ~7,000 years before the present) change from boreal forest to grasslands in the northern Great Plains, thus indicating that Great Plains populations are probably remnant populations. On the other hand, habitat in the Black Hills and Bear Lodge Mountains is generally suitable and the snakes appear to be reasonably abundant there but localized. Great Plains populations may be particularly important to management, as suitable habitat within the Great Plains is probably not extensive and is patchily distributed. In addition, many climate change scenarios suggest additional drying in the Great Plains in the near future (i.e., from 2000 to 2060).

We suggest continued monitoring of this snake throughout its range in South Dakota, especially within the Great Plains, but we also recognize that we still know little about the species across its range. Studies of distribution and basic natural history are needed to further refine the distribution of smooth green snakes within South Dakota and to study habitat use and movements. These studies should be partnered with further genetic studies to uncover patterns of genetic substructure of the populations that we have identified. Discovery of new populations in the plains would be of particular importance since this may indicate the presence of refugia. For this reason, as well as the likelihood of decline as a result of climate change, we emphasize long-term monitoring of abundance in the plains. Like most small snakes, little is known about this species and we lack good examples of other species that have been well studied that exhibit a similar natural history. Therefore, we cannot make inferences from model species that would help in managing the smooth green snake.

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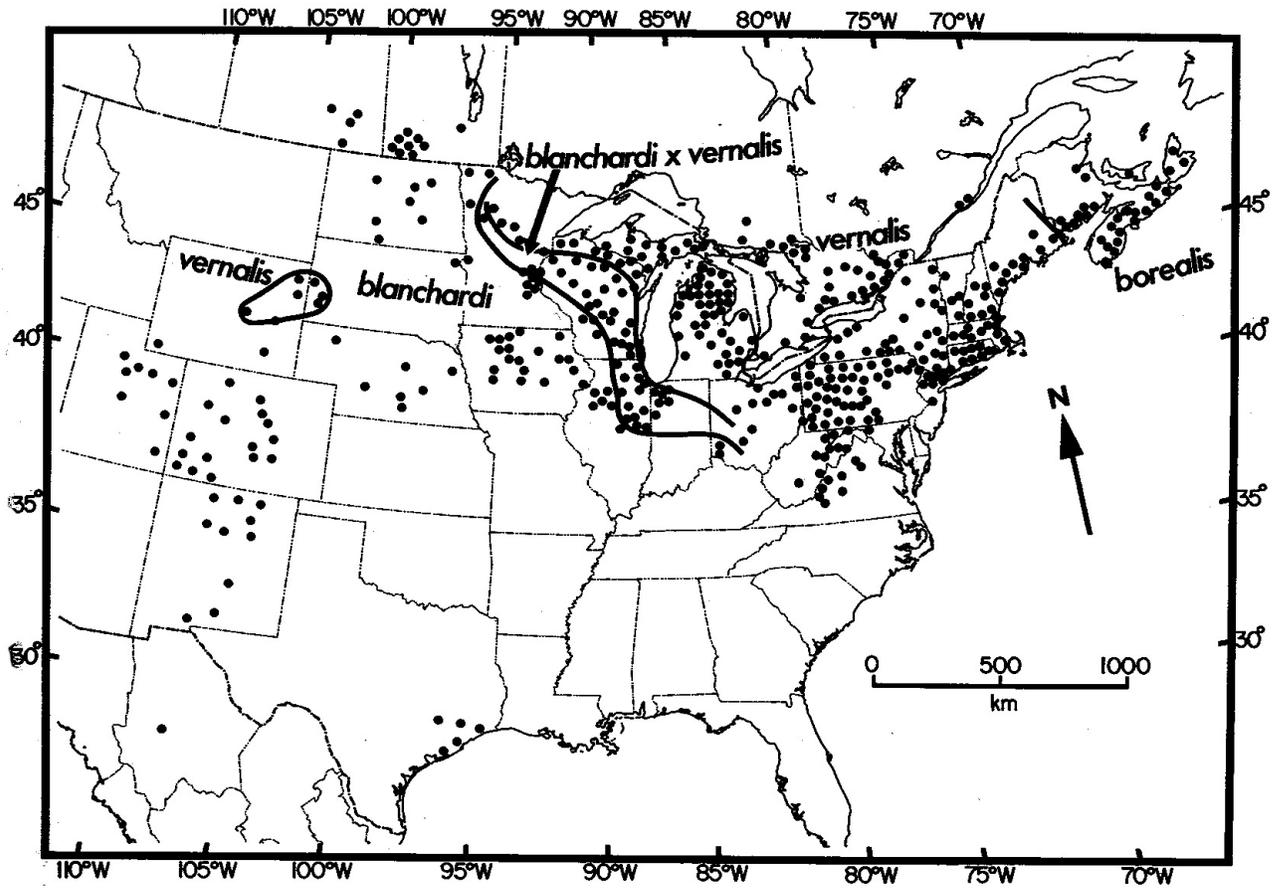


Figure 1: Grobman's (1992a) taxonomic arrangement of subspecies of *Opheodrys vernalis*, showing *O. v. vernalis* in the Black Hills, Bear Lodge Mountains, and the eastern United States; and *O. v. blanchardi* in the Great Plains, with a hybrid zone between the two running through Minnesota, Wisconsin, Illinois, Indiana, and Ohio. *Opheodrys v. borealis* occurs in Newfoundland, Canada.



Figure 2: Locations from which samples were obtained for this study, including Sugarite Canyon in northern New Mexico, the Bear Lodge Mountains in northeastern Wyoming, the Black Hills in southwestern South Dakota, and Ordway Prairie in north-central South Dakota.

Figure 3. Phylogenetic analysis of *Ophedryx vernalis* based on ND2 mitochondrial DNA sequences and inferred haplotype network: (A) Bayesian maximum likelihood phylogeny with significant posterior probability values given above branches. The MCMC analysis was run for 2000000 generations with the phylogeny sampled every 1000 generations. The first 1000 trees sampled were discarded as burn-in to ensure the chain had reached stationarity and the final 1000 trees used to build the Bayesian consensus tree. The genbank sequences correspond to the outgroup sequences *Masticophis flagellum* and *Coluber constrictor* from the family Colubridae (B) Median-joining haplotype network estimated from the mtDNA data. The circles represent haplotypes and the size of the circles is proportional to the number of individuals sampled that possessed that haplotype. The colors show the location of individual samples. The numbers on the branches connecting haplotypes correspond to the position on the ND2 that is polymorphic between haplotypes.

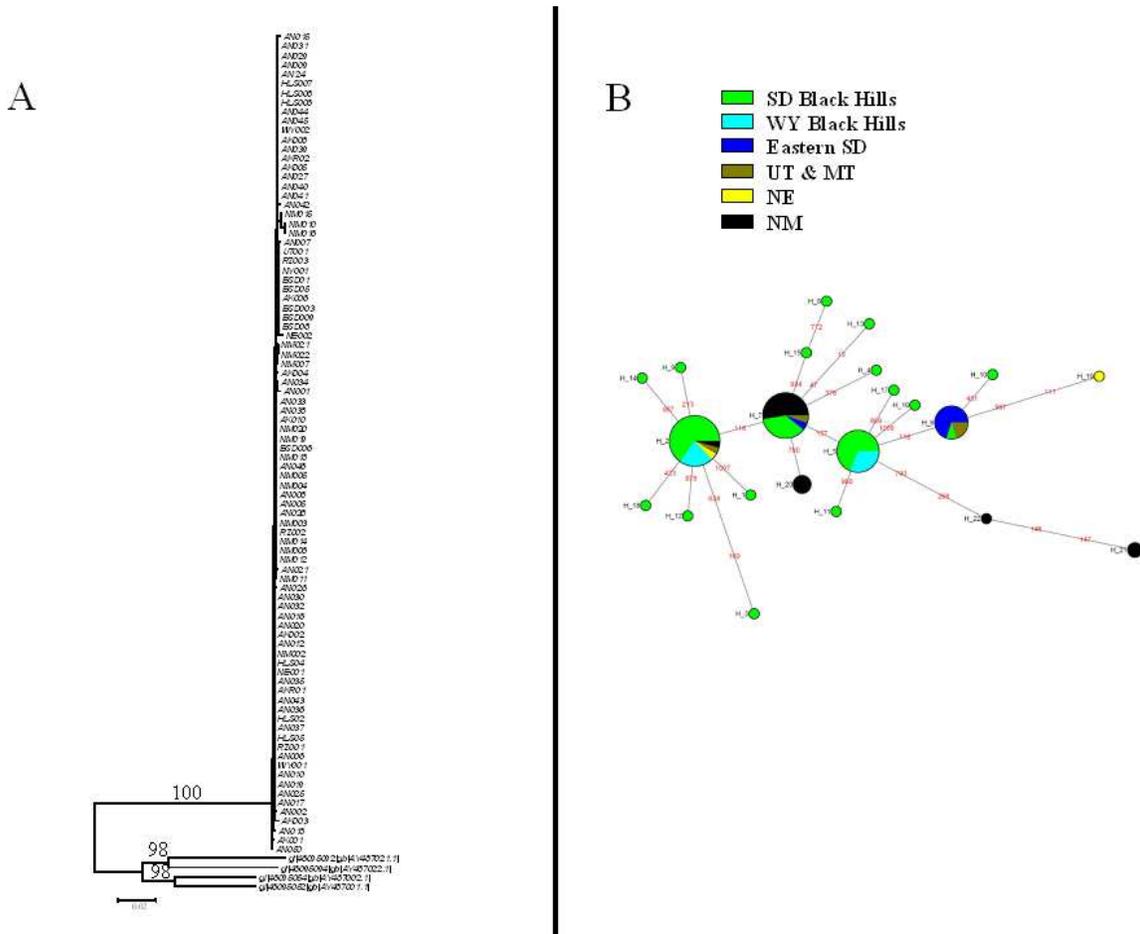


Figure 4. Summary plots of q estimates generated by sequential cluster analysis of the program STRUCTURE performed on the multilocus microsatellite genotypes. The number of K and likelihood are shown above the figures. The structure analysis was run using the Admixture Model. Conditions for the MCMC analysis were 250000 generations of burn-in followed by 500000 generations of sampling.

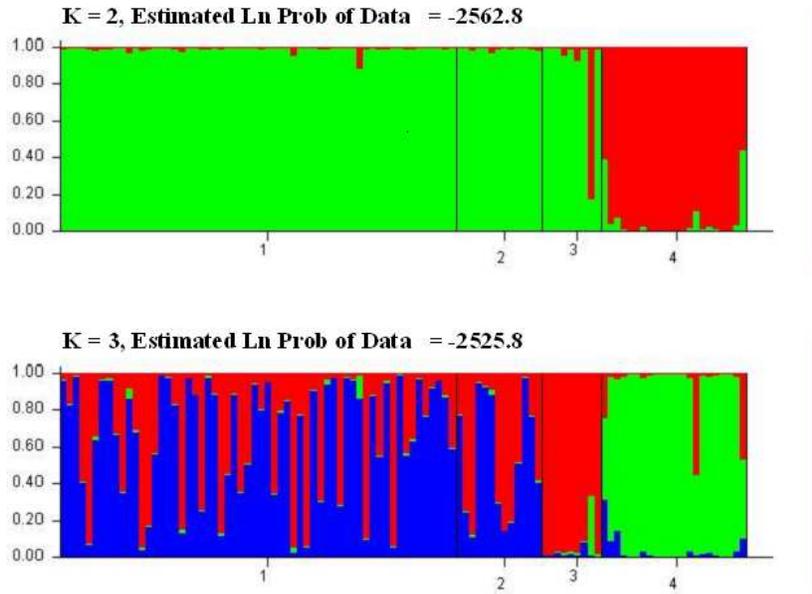


Table 1. Collection data for smooth green snake (*Ophedrys vernalis*) specimens collected or donated for this study.

Date	ID #	Locality (state: county; habitat description)	UTM	Notes
	LA001	SD: Deerfield Lake N shore		
	LA002	SD: Deerfield Lake N shore		
	LA003	SD: Deerfield Lake N shore		
	LA004	SD: Deerfield Lake N shore		
	LA005	SD: Deerfield Lake N shore		
	LA006	SD: Deerfield Lake N shore		
	LA007	SD: Deerfield Lake N shore		
	LA008	SD: Deerfield Lake N shore		
	LA009	SD: Deerfield Lake N shore		
	LA010	SD: Deerfield Lake N shore		
25-May-05	AK001	SD: Iron Creek Lake #1		E:(30-Jun-05)
25-May-05	AK002	SD: Iron Creek Lake #2		E:(30-Jun-05)
25-May-05	AK003	SD: Iron Creek Lake #3		E:(30-Jun-05)
26-May-05	AK004	SD: Deerfield inflow #1		E:(30-Jun-05)
26-May-05	AK005	SD: Deerfield inflow #2		E:(30-Jun-05)
29-May-05	AK006	SD: Deerfield outflow #1		E:(30-Jun-05)
26-May-05	AK007	SD: Newton Picnic area #1		E:(30-Jun-05)
26-May-05	AK008	SD: Newton Picnic area #2		E:(30-Jun-05)
26-May-05	AK009	SD: Newton Picnic area #3		E:(30-Jun-05)
26-May-05	AK010	SD: Newton Picnic area #4		E:(30-Jun-05)
24-May-05	AN001	SD: Mystic Rochford Jac		E:(17-Jun-05)
24-May-05	AN002	SD: Mystic Rochford Jac		E:(17-Jun-05)
24-May-05	AN003	SD: Mystic Rochford Jac		E:(17-Jun-05)
24-May-05	AN004	SD: Mystic Rochford Jac		E:(17-Jun-05)
24-May-05	AN005	SD: Mystic Rochford Jac		E:(17-Jun-05)

Table 1. cont.

		Ponderosa pines; NE slope	4860166N	E:(30-Jun-05)
27-Jun-05	AN021	SD: Custer Co.;	614916E	found under limestone
		W slope	4905558N	E:(8-Jul-05)
27-Jun-05	AN022	SD: Custer Co.;	614916E	found under limestone
		W slope	4905558N	E:(8-Jul-05)
28-Jun-05	AN023	SD: Custer Co.;	624928E	found under board
		level ground	4841514N	E:(8-Jul-05)
29-Jun-05	AN024	SD: Pennington Co.;	609502E	found under stone
		S slope	4876695N	E:(8-Jul-05)
29-Jun-05	AN025	SD: Pennington Co.;	609563E	found under stone
		S slope	4876671N	E:(8-Jul-05)
6-Jul-05	AN026	SD: Lawrence Co.;	581807E	found under log
		S slope	4913961N	E:(8-Jul-05)
7-Jul-05	AN027	SD: Pennington Co.;	597271E	found under slate
		E slope	4887093N	E:(7-Sep-05)
7-Jul-05	AN028	SD: Pennington Co.;	597757E	found under slate
		S slope	4884854N	E:(7-Sep-05)
11-Jul-05	AN029	SD: Pennington Co.;	611686E	found under slate
		S slope	4868844N	E:(7-Sep-05)
11-Jul-05	AN030	SD: Pennington Co.;	606338E	found under slate
		W slope	4868910N	E:(7-Sep-05)
12-Jul-05	AN031	SD: Custer Co.;	601144E	found under wood
		W slope	4850063E	E:(7-Sep-05)
13-Jul-05	AN032	SD: Pennington Co.;	599375E	found under board
		S slope	4883697N	E:(7-Sep-05)
13-Jul-05	AN033	SD: Pennington Co.;	599375E	found under board
		S slope	4883697N	E:(7-Sep-05)
15-Jul-05	AN034	SD: Pennington Co.;	606114E	found under wood
		N slope	4889613N	E:(7-Sep-05)
15-Jul-05	AN035	SD: Pennington Co.;	606377E	found under wood
		N slope	4889285N	E:(7-Sep-05)
19-Jul-05	AN036	SD: Pennington Co.;	615255E	found under wood
		E slope	4884234N	E:(7-Sep-05)
19-Jul-05	AN037	SD: Pennington Co.;	615232E	found under wood
		E slope	4884320N	E:(7-Sep-05)
19-Jul-05	AN038	SD: Pennington Co.;	615222E	found under wood
		E slope	4884320N	E:(7-Sep-05)
19-Jul-05	AN039	SD: Pennington Co.;	618106E	found under slate
		W slope	4881287N	E:(7-Sep-05)
21-Jul-05	AN040	SD: Pennington Co.;	N43°53.909	found under wood
		E slope	W103°43.565	E:(7-Sep-05)
26-Jul-05	AN041	SD: Pennington Co.;	N43°53.951	found under slate
		E slope	W103°40.946	
26-Jul-05	AN042	SD: Pennington Co.;	N43°53.855	found under wood
		E slope	W103°41.171	
26-Jul-05	AN043	SD: Pennington Co.;	N43°53.862	found under quartz
		E slope	W103°41.296	
26-Jul-05	AN044	SD: Pennington Co.;	N43°53.862	found under limestone
		E slope	W103°41.262	
26-Jul-05	AN045	SD: Pennington Co.;	N43°59.086	found under slate
		W slope	W103°46.356	
26-Jul-05	AN046	SD: Pennington Co.;	N43°59.251	found under slate
		W slope	W103°46.594	
26-Jul-05	AN047	SD: Pennington Co.;	N43°59.251	found under slate
		W slope	W103°46.594	
2-Aug-05	AN048	SD: Pennington Co.;	N44°08.142	found under wood

Table 1. cont.

21-Jul-05	AN040	SD: Pennington Co.;	N43°53.909	found under wood
		E slope	W103°43.565	E:(7-Sep-05)
26-Jul-05	AN041	SD: Pennington Co.;	N43°53.951	found under slate
		E slope	W103°40.946	
26-Jul-05	AN042	SD: Pennington Co.;	N43°53.855	found under wood
		E slope	W103°41.171	
26-Jul-05	AN043	SD: Pennington Co.;	N43°53.862	found under quartz
		E slope	W103°41.296	
26-Jul-05	AN044	SD: Pennington Co.;	N43°53.862	found under limestone
		E slope	W103°41.262	
26-Jul-05	AN045	SD: Pennington Co.;	N43°59.086	found under slate
		W slope	W103°46.356	
26-Jul-05	AN046	SD: Pennington Co.;	N43°59.251	found under slate
		W slope	W103°46.594	
26-Jul-05	AN047	SD: Pennington Co.;	N43°59.251	found under slate
		W slope	W103°46.594	
2-Aug-05	AN048	SD: Pennington Co.;	N44°08.142	found under wood
		W slope	W103°39.196	
3-Aug-05	AN049	SD: Pennington Co.;	N44°08.990	found under slate
		W slope	W103°50.165	
3-Aug-05	AN050	SD: Pennington Co.;	N43°53.909	found under slate
		W slope	W103°43.565	
30-Jul-05	HLS01	WY: Crook Co.;	N44°30.217	grassy meadow; beaver ponds
		Whitelaw Creek;	W104°26.351	cattle excluded
30-Jul-05	HLS02	WY: Crook Co.;	N44°30.917	grassy meadow; beaver ponds
		Whitelaw Creek;	W104°26.333	cattle excluded
31-Jul-05	HLS04	WY: Crook Co.;	N44°30.214	Riparian improvement area
		Whitelaw Creek;	W104°26.205	
31-Jul-05	HLS05	WY: Crook Co.;	N44°30.217	Riparian improvement area
		Whitelaw Creek;	W104°26.201	
31-Jul-05	HLS06	WY: Crook Co.;	N44°30.217	Riparian improvement area
		Whitelaw Creek;	W104°26.201	
31-Jul-05	HLS07	WY: Crook Co.;	N44°30.217	Riparian improvement area
		Whitelaw Creek;	W104°26.201	
31-Jul-05	HLS08	WY: Crook Co.;	N44°34.267	near Black Hills
		Blacktail Creek	W104°29.018	flowing stream was intermittent
31-Jul-05	HLS10	WY: Crook Co.;	N44°34.249	near Black Hills
		Blacktail Creek	W104°28.976	flowing stream was intermittent; E:(9-Sep-05)
3-Aug-05	RZ001	MT: Sheridan Co.;	T 32N R55E S 33	SVL: 7"; Headquarters in front of 5-stall
		Medicine Lake National Wildlife Refuge		
4-Aug-05	RZ002	MT: Sheridan Co.;	T 32N R55E S 33	SVL: 9"; dead alongside bunkhouse at headquarters; head was smashed
		Medicine Lake National Wildlife Refuge		
24-Aug-05	RZ003	MT: Sheridan Co.;	T 32N R55E S 33	SVL: 13"; DOR in parking lot
		Medicine Lake National Wildlife Refuge		
24-Jul-04	UT001	UT: Utah Co.;	40.036967	ca. 1 mi NE of Hwy 6; elev. 5000 ft
21-Aug-02	MVZ#24	Diamond Fork Canyon Rd	-111.491633	
N/A	NE001	NE: Kearney Co.;		no other data
N/A	NE002	NE: Washington Co.;		no other data
29-Jul-05	AKR001	WY: Crook Co.;	N44°50.386	SVL: 16"; by beaver dam in rocks
		Bear Lodge	W104°44.131	observed 2 more greensnakes
29-Jul-05	AKR002	WY: Crook Co.;	N44°50.386	
		Bear Lodge	W104°44.131	
19-May-05	NY01	NY: Cortland Co.;		Collectors: Matthew McCormick and Peter K. Ducey
		Township: Cincinnatus		
22-May-06	ESD01	SD: McPherson Co.;	N 45.70931	juvenile--see data sheet
		Ordway Prairie	W 99.15677	
23-May-06	ESD02	SD: McPherson Co.;	N 45.10910	juvenile--see data sheet
		Bieber Ranch	W 99.15763	

Table 1. cont.

23-May-06	ESD03	SD: McPherson Co.;	N 45.70977	9 inches
		Bieber Ranch	W 99.15652	
24-May-06	ESD04	SD: McPherson Co.;	N 45.70881	
		Bieber Ranch	W 99.15753	
24-May-06	ESD05	SD: McPherson Co.;	N 45.70881	
		Bieber Ranch	W 99.15753	
24-May-06	ESD06	SD: McPherson Co.;	N 45.70895	
		Bieber Ranch	W 99.15768	
24-May-06	ESD07	SD: McPherson Co.;	N 45.70882	
		Bieber Ranch	W 99.15695	
25-May-06	ESD08	SD: McPherson Co.;	N 45.70918	under object
		Bieber Ranch	W 99.15759	
25-May-06	ESD09	SD: McPherson Co.;	N 45.73660	in grass
		Heupel Ranch	W 99.11555	
30-Jun-06	NM001	NM: Colfax Co.,	N 36.98596	oak forest at top of flaky sandstone cliff
		Sugarite Canyon S. P.	W 104.37693	
	NM002	NM: Colfax Co.,		caught by cat near visitor center
		Sugarite Canyon S. P.		
	NM003	NM: Colfax Co.,		caught by cat near visitor center
		Sugarite Canyon S. P.		

Table 2 Characterization of 8 polymorphic microsatellite loci for *Opheodrys vernalis*.

Locus	Genbank Accession Number	Repeat motif	Primer (5'-3')	T _a (°C)	Product size (bp)	Number of alleles	H _E	H _O
6-131	EF666126	A ₁₄ (GA) ₁₇	F: M-13-CTCAAGTTTCTGGCTTTGG R: Pigtail-TGGGCTGTCCTTGATAGATCC	60	234-285	12	0.616	0.462
6-261	EF666127	A ₁₃ ...(GA) ₂ CA(GAGAGAGG) ₂ (GA) ₁₈	F: M-13-AGCACTCAGCAGAGCATGAA R: Pigtail-CTTAATGCCTGGACCCTGAA	55	253-278	8	0.733	0.339
10-09	EF666130	(TG) ₂ ...(TG) ₆ (AGTG) ₂ (AG) ₃ G ₆ ...G ₅ (AG) ₆	F: M-13-GCGGGAAGAAAGAAAGATCC R: Pigtail-CCTGAAAATGGCCTGAAAAA	60	198-212	3	0.036	0.036
8-51	EF666128	(GT) ₂₂	F: M-13-TAGCCAAGCTAAGCCAAAGG R: Pigtail-CAGACACGAAGCCAAAGACA	60	212-253	15	0.86	0.86
9-271	EF666129	(TC) ₁₀ (AC) ₁₃	F: M-13-GGCAAAACAGTTTCACAGCA R: Pigtail-AAATGCAGCTGGGTTTGTGT	60	115-172	25	0.885	0.867
5-121	EF666124	(GA) ₁₀ AAT(GA) ₈ ...(GA) ₇	F: M-13-GCTTCTCCTAAAGCGGGTGT R: Pigtail-TCCCTCCAGCTCACTCTCAT	60	282-311	7	0.457	0.271
5-111	EF666123	(CT) ₂₀	F: M-13-AGGCCTTGCCGTTTAAAAAT R: Pigtail-GAAGCCTGCAGAGCAGAGAT	46	247-309	20	0.901	0.865
6-93	EF666125	(GT) ₉ ...G ₈ AGG(GA) ₅	F: M-13-CATTAACGATGGGGTTGCTT R: Pigtail-AACGTCCAAACCTTCAATGG	60	224-233	7	0.679	0.25

T_a, optimal annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity.

Table 3. Pairwise table of F_{st} estimates. BH=Black Hills of South Dakota, BL=Bear Lodge Mountains of Wyoming, ESD=eastern South Dakota, NM=New Mexico.

Population	BH	BL	ESD	NM
BH	-			
BL	0.027	-		
ESD	0.130	0.113	-	
NM	0.168	0.144	0.181	-