Genetic Variation Within Populations of a Dietary Specialist, *Neotoma stephensi* (Stephen’s Woodrat), in Arizona

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**Abstract**

Seven microsatellite loci were used to develop multilocus genotypes for 49 individuals of *Neotoma stephensi* (Stephen’s woodrat) collected from nine sites in central and northern Arizona. Several statistical analyses were used to determine genetic structure, levels of genetic variability, and degree of relatedness. Structure analyses estimated two distinct groups within localities. The $F_{ST}$ value indicated moderate genetic differentiation among groups and sites. All populations displayed low to moderate levels of genetic diversity in terms of mean expected heterozygosity, and low to moderate levels of genetic diversity in terms of mean polymorphic information content. Mean relatedness values were low within groups and sites. Comparison of genetic diversity in *N. stephensi* to other species of *Neotoma* indicated that levels of genetic variation were comparable to other species of woodrats, including habitat specialists such as *N. magister* and wide ranging species such as *N. micropus*.

Key words: genetic variation, microsatellites, *Neotoma stephensi*, population structure

**Introduction**

*Neotoma stephensi* (Stephen’s woodrat) is restricted to northern Arizona, northwestern New Mexico, and southwestern Utah (Hall 1981; Hoffmeister 1986; Jones and Hildreth 1989). It lives in rock outcroppings in association with juniper and pinyon (Jones and Hildreth 1989). Vaughan (1982) described *N. stephensi* as “a relict species, mostly restricted to a series of isolated or semi-isolated remnants of xeric juniper woodland...” This species is unique among *Neotoma* in that it is a dietary specialist, feeding almost exclusively on junipers (Vaughan 1982; Dial 1988). Because this species has a restricted range and habitat, little is known about its biology; although, it has been widely studied from a physiological standpoint (Dearing et al. 2000, 2001, 2002; Boyle and Dearing 2003; Sorensen and Dearing 2003, 2004; Sorensen et al. 2004) and moderately examined from a systematic standpoint (Goldman 1910; Hoffmeister and de la Torre 1960; Hooper 1960; Planz et al. 1996; Edwards and Bradley 2002).

Only a few studies have examined genetic diversity at the population level within this genus (Castleberry et al. 2002; Matocq 2002, 2004; Monty et al. 2003; Méndez-Harclerode et al. 2005, 2007; Haynie et al. 2007). Castleberry et al. (2002) examined 357 *N. magister* (Allegheny woodrat) from nine populations throughout its range. Although the range of this species is moderate in size, occurring in the eastern...
United States in the Appalachian and Interior Highland regions (Hall 1981; Castleberry et al. 2002), it is considered to be endangered, threatened, or a species of concern throughout its range (Castleberry et al. 2002). Like A. stephensi, A. magister is a habitat specialist found in isolated rocky outcroppings in forested areas (Castleberry et al. 2002). Similarly, Haynie et al. (2007) and Matocq (2002, 2004) examined population level questions in A. macrotis (large-eared woodrat) and A. fuscipes (dusky-footed woodrat). Both of these species have moderately-sized ranges, with the distribution of N. macrotis encompassing southern portions of the Sierra Nevada Range, South Coast Ranges, southern California, and Baja California and the range of N. fuscipes extending from western Oregon to northern California, inner Coast Ranges, and northern portions of the Sierra Nevada Range (Matocq 2002). These species occur in a variety of habitats including chaparral, coastal sage-scrub, and densely wooded areas (Murray and Barnes 1969; Carraway and Verts 1991; Tietje and Vreeland 1997). Ménédez-Harc lerode et al. (2007) examined a single population (n = 549) of N. micropus (southern plains woodrat) from south Texas. This species is distributed in Kansas, Oklahoma, Texas, New Mexico, and northeastern Mexico (Hall 1981). Finally, Monty et al. (2003) examined four populations from a small portion of the range of N. floridana (eastern woodrat). This species has a wide range extending throughout large portions of the central and southeastern United States (Hall 1981). To date, no population genetic studies have been performed on N. stephensi. Of the five species examined from a population genetics context, none have a range that is as restricted as N. stephensi, nor are any dietary specialists. Based on ranges of the studied species, genetic diversity within N. stephensi would be expected to be lower than the more abundant species with a wider geographic range (i.e. N. floridana or N. micropus), but greater than that found in a potentially endangered species such as N. magister.

Abbott et al. (2004) examined 1,610 Neotoma from 51 localities in Arizona; 114 (7.1%) were N. stephensi compared to 1,250 (77.6%) N. albicula (white-throated woodrat). Eighty-five samples of N. stephensi were from a single locality and the 29 remaining samples were distributed among eight other localities. In another study (Kosoy et al. 1996), 756 Neotoma were collected; 26 (3.4%) were N. stephensi compared to 395 (52.3%) N. albicula. Lack of N. stephensi samples in these studies compared to other species of Neotoma (especially N. albicula) may be due to several factors including the fact that N. stephensi are habitat specialists with a restricted range; thus, little is known about the population biology of this species.

The objectives of this study were to: 1) examine genetic structure; 2) examine levels of genetic diversity within populations; and 3) determine degree of genetic relatedness within populations. To achieve these objectives, multilocus microsatellite genotypes were developed for individuals collected from throughout this species range in Arizona.

**Methods**

**Collecting localities and DNA extraction.**—One hundred fourteen individuals of N. stephensi were collected from nine sites throughout central and northern Arizona (Table 1; Fig. 1). These animals were collected to determine arenavirus prevalence in multiple woodrat species (Abbott et al. 2004). Eighty-five samples were from a single locality (Site 7; Fig. 1), and a random subsample of 20 was used in this study. The 29 other samples were distributed throughout the remaining eight localities. Genomic DNA was extracted from approximately 25 mg of liver using a DNeasy tissue extraction kit (Qiagen, Valencia, California).

**Microsatellite analysis.**—Castleberry et al. (2000) tested cross-species amplification of 13 loci developed for N. magister. Twelve of these primer pairs, known to amplify up to seven different species of Neotoma (N. magister, N. cinerea - bushy-tailed woodrat, N. floridana, N. fuscipes, N. lepida - desert woodrat, N. mexicana - Mexican woodrat, and N. micropus), were used in this study (Table 2) and were amplified via the polymerase chain reaction (PCR). PCR amplifications were conducted in 25 μl volumes containing 1 to 1.5 μl 50 ng genomic DNA, 0.6 μl 10 pM each primer, 2.5 μl 10 X PCR buffer, 0.75 to 2 μl 25 mM MgCl₂, 0.75
Table 1. Locality data for the 49 individuals of Neotoma stephensi collected from nine sites in central and northern Arizona. For each locality, site number (Site; corresponding to Fig. 1), specific locality name (Name), latitude/longitude (Lat/Long), number of individuals (N), and ID number (ID#) of individuals at each site (corresponding to Fig. 2) are provided.

<table>
<thead>
<tr>
<th>Site</th>
<th>Name</th>
<th>Lat/Long</th>
<th>N</th>
<th>ID#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AZ: Apache Co.; Three Turkey</td>
<td>36°14′44″/-109°24′46″</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>AZ: Apache Co.; Saint Johns</td>
<td>34°28′28″/-109°19′18″</td>
<td>2</td>
<td>27, 29</td>
</tr>
<tr>
<td>3</td>
<td>AZ: Apache Co.; Little Colorado River North</td>
<td>34°11′16″/-109°18′17″</td>
<td>3</td>
<td>31-33</td>
</tr>
<tr>
<td>4</td>
<td>AZ: Navajo Co.; Lone Pine Reservoir</td>
<td>34°20′42″/-110°4′53″</td>
<td>7</td>
<td>24, 28, 30, 34, 41-43</td>
</tr>
<tr>
<td>5</td>
<td>AZ: Navajo Co.; Trick Tank Draw</td>
<td>34°33′43″/-110°46′13″</td>
<td>13</td>
<td>25, 35-40, 44-49</td>
</tr>
<tr>
<td>6</td>
<td>AZ: Yavapai Co.; Granite Dells Ranch</td>
<td>34°36′55″/-112°23′44″</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>AZ: Yavapai Co.; Pine Flat</td>
<td>35°0′6″/-112°50′43″</td>
<td>20</td>
<td>3-22</td>
</tr>
<tr>
<td>8</td>
<td>AZ: Yavapai Co.; Sycamore Station</td>
<td>34°23′28″/-112°3′1″</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>AZ: Yavapai Co.; Sayer Spring</td>
<td>34°1′0″/-112°39′4″</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1. Map of Arizona showing nine collecting localities for Neotoma stephensi. Site numbers correspond to data in Table 1.


Table 2. Microsatellite loci examined for Neotoma stephensi. Castleberry et al. (2000) tested cross-species amplification in seven woodrat species for these 12 loci; however, no tests were performed on N. stephensi. Product length (PL), number of alleles (A), and sample size (N) for each locus for N. magister (Castleberry et al. 2000) and N. stephensi (this study) are shown. Loci Nma01, Nma02, Nma03, Nma08, and Nma12 were removed from this study due to amplification difficulties.

<table>
<thead>
<tr>
<th>Locus</th>
<th>PL</th>
<th>A</th>
<th>N</th>
<th>PL</th>
<th>A</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nma01</td>
<td>314–322</td>
<td>6</td>
<td>28</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nma02</td>
<td>197–205</td>
<td>4</td>
<td>33</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Nma03</td>
<td>180</td>
<td>1</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nma04</td>
<td>145–163</td>
<td>7</td>
<td>33</td>
<td>130–168</td>
<td>12</td>
<td>49</td>
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<tr>
<td>Nma05</td>
<td>227–232</td>
<td>4</td>
<td>38</td>
<td>204–231</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>Nma06</td>
<td>215–223</td>
<td>5</td>
<td>39</td>
<td>202–281</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>Nma08</td>
<td>125–127</td>
<td>7</td>
<td>38</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nma10</td>
<td>186–224</td>
<td>14</td>
<td>39</td>
<td>248–284</td>
<td>17</td>
<td>44</td>
</tr>
<tr>
<td>Nma11</td>
<td>150–160</td>
<td>8</td>
<td>8</td>
<td>144–207</td>
<td>27</td>
<td>49</td>
</tr>
<tr>
<td>Nma12</td>
<td>115–127</td>
<td>3</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>Nma14</td>
<td>144–160</td>
<td>7</td>
<td>7</td>
<td>135–203</td>
<td>16</td>
<td>49</td>
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<tr>
<td>Nma15</td>
<td>120–136</td>
<td>10</td>
<td>10</td>
<td>105–139</td>
<td>16</td>
<td>48</td>
</tr>
</tbody>
</table>

μl 10 mM dNTPs, and 0.25 μl 5U/μl Taq. The thermal profile was modified from Castleberry et al. (2000) and consisted of a denaturation and enzyme activation cycle at 94°C (2 min); 35 cycles of 94°C (30 s) denaturation, 55 to 58°C (30 s) annealing, 72°C (1 min) elongation; followed by a final elongation cycle at 72°C (10 min).

Variation at individual loci was examined using a 3100-Avant Genetic Analyzer (Applied Biosystems Inc., Foster City, California). Reactions included 13.5 to 14 μl Hi-Di Formamide (Applied Biosystems Inc.), 0.5 μl 400HD ROX size standard (Applied Biosystems Inc.), and 0.5 to 1 μl PCR product. Genotypes were scored using GeneMapper version 3.0 software (Applied Biosystems Inc.). Alleles that did not amplify above a predetermined peak height (signal strength), were difficult to score, or appeared aberrant were reamplified and rescored.

Statistical analyses.—The program Cervus 3.0.3 (Marshall et al. 1998) was used to compare alleles to bin files generated from GeneMapper software to identify typing errors that may have occurred during data entry. Micro-Checker version 2.2.1 software (Van Oosterhout et al. 2004) was used to test for presence of null alleles, large allele drop out, and error due to stutter. A random sample of at least four individuals per locus was genotyped twice without knowledge of previous scores. Using these samples, an error rate was calculated by dividing the number of erroneous allele scores at each locus by the total number of allele scores for all individuals for which at least two genotypes existed.

Structure version 2.2 software (Pritchard et al. 2000) was used to estimate the number of populations represented by collection localities. This was done in two ways. First, all individuals were grouped without prior information of collection site. This analysis was referred to as “no priors.” Parameters used to determine number of populations were: burn-in length = 90,000; Monte Carlo Markov Chains (MCMC) repetitions after the burn-in = 900,000; ancestry model = admixture; allele frequency model = allele frequencies correlated; k = 9 (k is the number of potential populations tested which, in this study, represented the number of collection localities); and iterations for each population test = 5. An individual was assigned to a population if it had at least an 80% posterior probability of being included in that population. The second approach used “prior knowledge” from the above analysis. Individuals were sorted into groups using results from the first
analysis and population assignment was analyzed. Parameters for the population assignment test were: burn-in length = 90,000; MCMC repetitions after the burn-in = 900,000; ancestry model = prior population information; allele frequency model = allele frequencies correlated; and G = 2. The G value estimates the probability of each individual having an ancestor that immigrated from another population. An individual was considered to be assigned correctly if it had at least an 80% posterior probability of being included in the population to which it originally was grouped based on geographic locality.

The program Cervus 3.0.3 (Marshall et al. 1998) was used to estimate allele frequencies, observed and expected heterozygosities, null allele frequencies, and polymorphic information content (PIC; index of variability associated with expected heterozygosity). Probability of identity (PI) was estimated with IDENTITY 1.0 software (Wagner and Sefc 1999), using equations reported by Paetkau et al. (1995). This program also was used to identify identical genotypes among samples and indicate parent-offspring combinations. Pairwise and mean relatedness values were estimated with Relatedness 5.0 software (Queller and Goodnight 1989).

The program Fstat 2.9.3.2 (Goudet 2001) was used to estimate deviations from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, F-statistics (Weir and Cockerham 1984), R_{ST} (Slatkin 1995; Rouset 1996; Goodman 1997), pairwise tests of differentiation, and relatedness values (Hamilton 1971; Queller and Goodnight 1989). Deviations from HWE were estimated by evaluating whether F_{IS} values within each sample were significantly different from zero. Tests of disequilibrium were performed between all pairs of loci over all samples and between all pairs of loci within each sample. The G-statistic, used for pairwise tests of differentiation, was corrected for comparisons over multiple loci, unlike traditional F-statistics. Hamilton’s (1971) relatedness was estimated using an equation that is comparable to Queller and Goodnight (1989). This estimator compares average relatedness within groups compared to all other samples. Sequential Bonferroni corrections (Holm 1979; Rice 1989) were performed on all analyses. The indicative adjusted nominal level was set at 5%, following traditional tests for significance at the 95% level. For all tests, 1,000 permutations were performed.

Results

Five loci were removed from this study due to an inability to amplify samples (Table 2). The remaining seven loci (Table 2) were used for all further analyses. No data entry errors or genotype scoring errors were detected for any locus. Additionally, no evidence for scoring error due to stutter or large allele drop was detected at any locus using Micro-Checker software. The putative presence of null alleles was found at all loci except Nma10, Nma11, and Nma14.

Using the program Structure and assuming no knowledge of geographic localities, two groups were detected (Fig. 2). Group I consisted of 20 individuals (4, 7, 8, 19, 22, 26-32, 36, 37, 39, 40, 44, 45, 47, and 49; Fig. 2). Six of these individuals had posterior probabilities <0.800 (range = 0.725-0.790), but were assigned to group I as they consistently clustered with this group. Group II consisted of 19 individuals (2, 3, 5, 9-12, 15, 17, 18, 20, 21, 23, 24, 33, 41-43, and 46; Fig. 2). Five of these individuals had posterior probabilities <0.800 (range = 0.742-0.793), but were assigned to group II as they consistently clustered with this group. Ten individuals (1, 6, 13, 14, 16, 25, 34, 35, 38, and 48; Fig. 2) could not be assigned to either group with any confidence. When “prior knowledge” was considered (i.e. samples were assigned as a member of group I or group II, or were left unassigned, based on results for the “no priors” analysis) the same results were obtained (data not shown).

Mean number of alleles was 8.29 within group I, 13.43 within group II, and 17.1 over all samples (Table 3). Allele frequencies and null allele frequencies are reported by Haynie (2006) or are available from the senior author upon request. Mean observed heterozygosity was 0.687 in group I, 0.753 in group II, and 0.709 over all samples (Table 3). Mean expected heterozygosity was 0.769 in group I, 0.887 in group II,
Figure 2. Bar graph of Structure analysis showing placement of all 49 samples collected in this study in either group I or group II. Dark gray bars represent group I and light gray bars represent group II. Ten individuals (1, 6, 13, 14, 16, 25, 34, 35, 38, 48) could not be placed in either group with confidence. A list of corresponding sites for each individual can be found in Table 1.

Table 3. Summary statistics for Neotoma stephensi for each group, site, and samples combined. Allele frequencies are reported by Haynie (2006) or are available from the senior author upon request. Site numbers correspond to localities in Fig. 1 and Table 1. Four localities (Sites 1, 6, 8, 9) contained a single individual and were not included in any population level analyses. Number of individuals (N), mean number of alleles (A), mean observed ($H_o$) and expected ($H_e$) heterozygosity, $F_{ST}$, HWE P-values over all loci (P), mean polymorphic information content (PIC), and mean relatedness (R) values are shown below. Bonferroni corrected $\alpha$ for tests of HWE was 0.004 for comparisons among groups and 0.001 for comparisons among sites.

<table>
<thead>
<tr>
<th>Group/Site</th>
<th>N</th>
<th>A</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$F_{IS}$</th>
<th>P</th>
<th>PIC</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>20</td>
<td>8.3</td>
<td>0.687</td>
<td>0.769</td>
<td>0.174</td>
<td>0.004</td>
<td>0.713</td>
<td>0.004</td>
</tr>
<tr>
<td>Group II</td>
<td>19</td>
<td>13.4</td>
<td>0.753</td>
<td>0.887</td>
<td>0.110</td>
<td>0.011</td>
<td>0.849</td>
<td>0.108</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2.3</td>
<td>0.571</td>
<td>0.619</td>
<td>0.143</td>
<td>0.405</td>
<td>0.366</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>4.9</td>
<td>0.714</td>
<td>0.924</td>
<td>0.268</td>
<td>0.005</td>
<td>0.732</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>7.4</td>
<td>0.735</td>
<td>0.811</td>
<td>0.101</td>
<td>0.041</td>
<td>0.728</td>
<td>NA</td>
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<tr>
<td>5</td>
<td>13</td>
<td>8.1</td>
<td>0.723</td>
<td>0.765</td>
<td>0.058</td>
<td>0.135</td>
<td>0.706</td>
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</tr>
<tr>
<td>7</td>
<td>20</td>
<td>12.0</td>
<td>0.695</td>
<td>0.840</td>
<td>0.177</td>
<td>0.001</td>
<td>0.796</td>
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<tr>
<td>Total</td>
<td>49</td>
<td>17.1</td>
<td>0.709</td>
<td>0.854</td>
<td>NA</td>
<td>NA</td>
<td>0.827</td>
<td>-0.022</td>
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</tbody>
</table>

and 0.854 over all samples (Table 3). PIC was 0.713 in group I, 0.849 in group II, and 0.827 over all samples (Table 3). No identical genotypes were detected at any site. Additionally, no parent-offspring groupings were found. PI was 1.3e-9 (1 chance in 800 million of randomly selecting two individuals with the same genotype). Mean relatedness values, estimated using Relatedness software, were 0.004 in group I, 0.108 in group II, and 0.067 over all samples (Table 3). Relatedness, estimated using FSTAT software, within groups compared to all other samples, with 95% confidence intervals in parentheses, was 0.127 (0.069, 0.194).

$F_{IS}$ was 0.174 within group I and 0.110 within group II. Both group I (Bonferroni corrected $\alpha = 0.004$, $P = 0.004$) and group II (Bonferroni corrected $\alpha = 0.004$, $P = 0.011$) were in HWE (Table 3). When tests of genotypic disequilibrium were performed over all samples, no evidence of genotypic disequilibrium was detected for any locus among regions (Bonferroni corrected $\alpha = 0.002$, $P > 0.107$ for all pairwise comparisons). When comparisons were made between loci within groups, no evidence for genotypic disequilibrium was detected within either group (Bonferroni corrected $\alpha = 0.001$, $P \geq 0.169$ for all pairwise comparisons within group I, $P \geq 0.104$ for all pairwise comparisons within group II, and $P \geq 0.105$ for all pairwise comparisons among loci over all groups).

$F_{IT}$, $F_{ST}$, and $F_{IS}$ between the two groups, with 95% confidence intervals in parentheses, were as follows: $F_{IT} = 0.210$ (0.096, 0.357), $F_{ST} = 0.077$ (0.039, 0.129), and $F_{IS} = 0.144$ (0.050, 0.268). Pairwise differentiation values (i.e. G-statistics), estimated using the program FSTAT, indicated that the two groups were significantly different from one another (Bonferroni corrected $\alpha =$...
Population genetic variables also were estimated for each site. Sites 1, 6, 8, and 9 contained a single individual (Table 1) and were not considered in any site-level analyses. Mean number of alleles within sites ranged from 2.3 (Site 2) to 12.0 (Site 7) (Table 3). Allele frequencies and null allele frequencies for individual localities are reported by Haynie (2006) or are available from the senior author upon request. Mean observed heterozygosity ranged from 0.571 (Site 2) to 0.735 (Site 4), mean expected heterozygosity ranged from 0.619 (Site 2) to 0.924 (Site 3), and mean PIC values ranged from 0.366 (Site 2) to 0.796 (Site 7) (Table 3). Mean relatedness and pairwise relatedness values were not estimated for sites. However, relatedness, estimated using FSTAT software, within sites compared to all other samples, with 95% confidence intervals in parentheses, was 0.096 (0.057, 0.136).

F$_{IS}$ ranged from 0.058 (Site 5) to 0.268 (Site 3) within sites (Table 3). Across all loci, sites were in HWE (Table 3), although Site 7 showed marginal significance (Bonferroni corrected $a = 0.001$, $P = 0.001$). When tests of genotypic disequilibrium were performed over all samples, no evidence of genotypic disequilibrium was detected for any locus among sites (Bonferroni corrected $a = 0.002$, $P > 0.038$ for all pairwise comparisons). When comparisons were made between loci within sites, no evidence for genotypic disequilibrium was detected within any site (Bonferroni corrected $a = 0.0003$, $P > 0.026$ for all pairwise comparisons among loci over all sites). Three estimators of $R_{ST}$ were calculated among sites: weighted = 0.012, Goodman = 0.024, and unweighted = 0.021.

**Discussion**

**Population assignment.**—Structure analyses estimated two distinct groups within the *N. stephensi* samples. There appeared to be a general north-south break among groups; however, individuals collected at the same sample site often were assigned to different groups (see Fig. 2 and corresponding information in Table 1). Additionally, there appeared to be no recognizable geographic or ecological barrier between the two groups. Currently, there are two described subspecies within *N. stephensi* (Hoffmeister 1986). The split between these two subspecies is located in the northeastern part of the state. Based on information provided by Hoffmeister (1986), Site 1 (Fig. 1) should contain a different subspecies compared to the rest of the collection sites. Results of this study indicate that the line between the two subspecies as currently drawn may not be accurate. The two groups detected using Structure software may correspond to two different subspecies, although there are not sufficient samples to address this possibility. Additional studies need to be performed to reassess the demarcation of the subspecies.

All sites and groups were in HWE, although Site 7 did show marginal significance (Table 3). Several localities (Sites 1, 6, 8, and 9) contained a single individual (Table 1) and were not useful for population level analyses. Collection of additional samples may aid in defining population boundaries.

**Genetic structure.**—The positive $F_{IS}$ value estimated over all sites suggested heterozygote deficiency within sites. Comparison of the $F_{ST}$ value among groups (0.077) to guidelines provided by Wright (1978) indicated moderate genetic differentiation between groups. The same was true for differentiation among sites ($F_{ST} = 0.057$). Based on examination of pairwise differentiation values that were corrected for comparisons over multiple loci, Site 4-Site 7 and Site 5-Site 7 showed significant levels of differentiation (Bonferroni corrected $a = 0.001$; $P = 0.001$). Overall, there was little genetic structure among the samples. Lack of structure within the samples could be the result of small sample sizes available for most of the sites. Only sites 5 and
7 contained >10 individuals. All other sites contained ≤7 individuals.

**Genetic variation.**—Estimators of variability (H\textsubscript{0}, H\textsubscript{e}, and PIC) suggested moderate variation within groups and collection sites (Table 3), with the exception of site 2. Site 2 contained two samples, both of which were homozygous at three of seven loci, thus reducing heterozygosity and PIC values. However, each site had a unique allele for at least one locus. Additionally, no identical genotypes existed among samples. PI, which was estimated over all samples, suggested that these loci were highly variable and that individual genotypes were unique. Small samples sizes for most of the populations were reflected in the moderate levels of variation.

**Relatedness.**—No family groups were detected within any site, using Identity software. Relatedness within groups compared to all other samples was 0.127, indicative of a third-order (e.g. cousin) relationship among individuals. Relatedness within sites compared to all other samples was 0.096, indicative of a fourth-order (e.g. distant cousin) relationship. Relatedness over all samples, as estimated using Relatedness software, was 0.067, again indicative of a fourth-order relationship among all samples. Relatedness within group I was 0.004, indicative of a very low level of relatedness among samples, and relatedness within group II was 0.108, indicative of a third-order relationship among samples. These results suggest that individuals within groups and sites are more closely related to other individuals within the group or site than to individuals outside the group or site. Pairwise comparisons ranged from negative to highly positive, indicating that some individuals were closely related to others. Interestingly, the pairwise relatedness value between the sample from site 8 and the sample from site 9 was 0.562 (indicative of a parent-offspring or full-sibling relationship), despite the geographic distance between the two sites (~100 km). This was indicative of the potential for dispersal and gene flow between these localities. The relatedness values estimated for *N. stephensi* were similar to what has been recorded for other species of *Neotoma* (Matocq and Lacey 2004; Haynie et al. 2007). Relatedness values in this study may reflect small sample sizes and sampling strategy.

**Comparison to other Neotoma species.**—Despite low to moderate levels of genetic variation, based on loci characterized by Castleberry et al. (2000), within these sites, number of alleles, expected heterozygosity values, and PIC values present in this study fell within the range reported for other species of *Neotoma* (Table 4). For example, number of alleles ranged from eight (Nma05) to 27 (Nma11) with a mean of 17.1 over all sites for this study (Table 2). Number of alleles for *N. magister* (Castleberry et al. 2000) ranged from one

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>NP</th>
<th>NL</th>
<th>AR</th>
<th>MA</th>
<th>H\textsubscript{e}</th>
<th>PIC</th>
<th>F\textsubscript{ST}</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. stephensi</em></td>
<td>49</td>
<td>7</td>
<td>7</td>
<td>8–27</td>
<td>17.1</td>
<td>0.854</td>
<td>0.827</td>
<td>0.052</td>
</tr>
<tr>
<td><em>N. macrotis-1</em></td>
<td>127</td>
<td>4</td>
<td>5</td>
<td>21–36</td>
<td>25.6</td>
<td>0.934</td>
<td>0.930</td>
<td>0.028</td>
</tr>
<tr>
<td><em>N. fuscipes-1</em></td>
<td>29</td>
<td>5</td>
<td>5</td>
<td>9–21</td>
<td>13.4</td>
<td>0.804</td>
<td>0.761</td>
<td>0.249</td>
</tr>
<tr>
<td><em>N. magister</em></td>
<td>357</td>
<td>9</td>
<td>11</td>
<td>5–19</td>
<td>10.4</td>
<td>0.618</td>
<td>NA</td>
<td>0.170</td>
</tr>
<tr>
<td><em>N. floridana</em></td>
<td>84</td>
<td>5</td>
<td>6</td>
<td>1–9</td>
<td>2.8</td>
<td>0.365</td>
<td>0.666</td>
<td>0.522</td>
</tr>
<tr>
<td><em>N. micropus</em></td>
<td>549</td>
<td>1</td>
<td>5</td>
<td>16–47</td>
<td>26.0</td>
<td>0.845</td>
<td>0.829</td>
<td>NA</td>
</tr>
<tr>
<td><em>N. macrotis-2</em></td>
<td>195</td>
<td>1</td>
<td>5</td>
<td>11–19</td>
<td>15.0</td>
<td>0.850</td>
<td>0.840</td>
<td>NA</td>
</tr>
<tr>
<td><em>N. fuscipes-2</em></td>
<td>81</td>
<td>8</td>
<td>3</td>
<td>16–21</td>
<td>18.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Comparing levels of genetic differentiation ($F_{ST}$) among populations among the species of Neotoma, the value reported for *N. stephensi* (0.057) was lowest among the species (Table 4). This $F_{ST}$ value indicated moderate levels of genetic differentiation among sites in this study, even though sites were widely distributed. Lack of structure may have been an artifact of small sample sizes. $F_{ST}$ values suggested moderate levels of genetic differentiation among populations of *N. macrotis* and high levels among populations of *N. fuscipes* (Table 4; Haynie et al. 2007). High levels of genetic differentiation were found among populations of *N. magister* (Castleberry et al. 2002) collected from a large portion of this species range (Table 4). Finally, Monty et al. (2003) reported great degrees of genetic differentiation among five populations of *N. floridana*, despite studying a limited portion of this species range (Table 4). Even though *N. stephensi* is a dietary and habitat specialist with a restricted range, levels of genetic diversity within sites examined in this study were comparable to levels found in other species of Neotoma.

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