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Genetic Variation and Structure in *Eurycea nana*, a Federally Threatened Salamander Endemic to the San Marcos Springs

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ABSTRACT.—**The San Marcos salamander (*Eurycea nana*), endemic to the headwaters of the San Marcos River, is federally threatened because of its restricted range and threats to its habitat. Captive propagation efforts for *E. nana* were initiated in 1996 to maintain a captive population for restocking in the event that *E. nana* becomes extinct. We surveyed DNA sequence data for a mitochondrial and a nuclear gene region (*ND4* and *rag1*) and 281 AFLP markers to obtain baseline data on the level of genetic diversity across the known range of *E. nana* and to test for population subdivision within its restricted range. Next, we compared levels of genetic variation between *E. nana* in the wild and captive-born individuals from the captive population to assess the efficacy of the captive breeding program. We investigated inbreeding in the captive population by testing for deviations from Hardy-Weinberg equilibrium at the *rag1* locus for captive-born individuals. The level of genetic diversity detected in wild *E. nana* was similar to that of other *Eurycea* populations in central Texas, and we found no evidence of population structure across the range of *E. nana*. The surveyed captive-born *E. nana* contained reduced genetic diversity at some loci, but similar genetic diversity at others relative to the wild, and there was no evidence of inbreeding within the wild-caught individuals that sired these captive-born individuals. The data we have obtained regarding the level of genetic variation in wild *E. nana* and in the captive population will provide baseline information for this species.**

The neotenic San Marcos salamander, *Eurycea nana*, is endemic to the underlying springs feeding Spring Lake, an impounded lake at the headwaters of the San Marcos River in Hays County, Texas. The salamander's range is restricted, including approximately 24 major spring vents and several springlike areas below the lake's dam, covering about 4.5-surface-hectares in total. *Eurycea nana* is dependent on the stable conditions (e.g., temperature, pH, and flow) of these springs (Nelson, 1993; Fries, 2002). The springs are a source of discharge of the Edwards Aquifer. Continued flow from the springs depends on sufficient regional rainfall and restricted groundwater withdrawal from the Edwards Aquifer. The Edwards Plateau region of Texas is experiencing high levels of human population growth, which will increase demand for water from the Edwards Aquifer (Brune, 1981). This problem may be exacerbated by local climate change (Chen et al., 2001; Loáiciga, 2003). As a result of its limited distribution and the threats of reduced spring flow, *E. nana* was listed as a federally threatened species (USFWS, 1980). Specific listed threats to *E. nana* are spring drying, urban storm runoff, urban pollutants, introduced exotic species, and recreational use of the San Marcos River (USFWS, 1984).

Management efforts for *E. nana* include the maintenance of a captive population, which was initiated in 1996. This serves as a source for reintroduction if the salamander becomes extinct in the wild. It is important that the captive population is managed to maximize the amount of genetic variation maintained and minimize inbreeding (Frankham et al., 2002). This increases the probability that a reintroduced population will have the capacity to adapt to a dynamic environment (Frankham et al., 2002).

The San Marcos National Fish Hatchery and Technology Center (NFHTC) houses approximately 150 wild-caught *E. nana* collected from three sites within Spring Lake: "Hotel Springs"—spring outflows in the upper boundary of Spring Lake; "Diversion Springs"—spring outflows about 100 m downstream of the Hotel Springs site; and "Below Dam"—a springlike area below Spring Lake (Fig. 1). Salamanders collected from these three sites are housed separately by collection site in captivity at the NFHTC. Additional salamanders from these three sites are added to the captive population parent tanks about every six months (average of 92 individuals/year) to maintain a parent population size of at least 150. In 2005, the NFHTC housed more than 200

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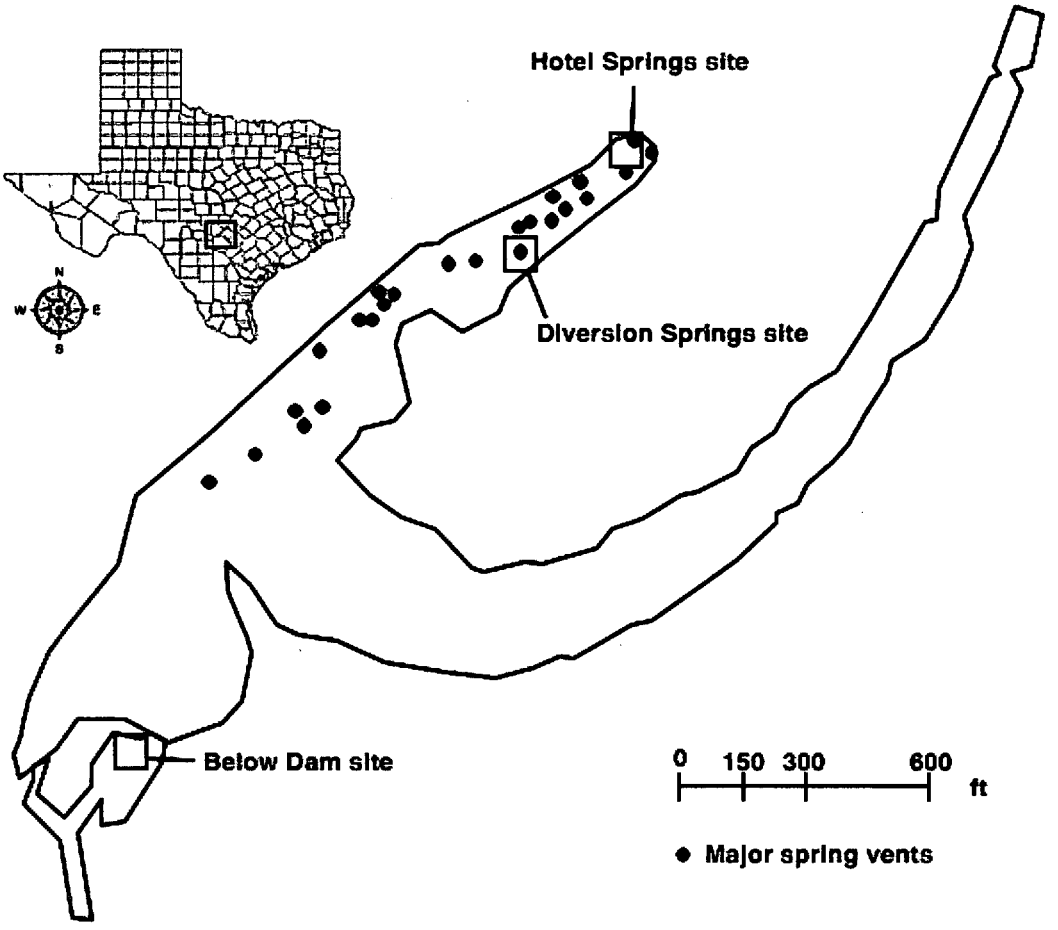

FIG. 1. Localities of the sample sites within Spring Lake are indicated with squares: Hotel Springs, Diversion Springs, and Below Dam. Black dots represent individual springs or closely spaced groups of springs.

first-generation offspring of wild-caught individuals and about 11 second-generation offspring. Offspring of each generation are pooled and housed separately. Little is known about the mating behavior of *E. nana*. A specific protocol for managing mating is not presently implemented at the NFHTC. Instead, mating occurs haphazardly within tanks housing 10 or more salamanders.

Given the conservation status of *E. nana* and threats to continued spring flows, baseline information regarding the level of genetic variation and structure across the range of *E. nana* is needed. Obtaining this information is a major aim of this study. With respect to captive propagation of *E. nana*, it is important to assess whether the genetic variation present in the captive population is representative of the variation in the wild and to test for evidence of inbreeding within the captive population. To address these questions, we examined genetic variation at mitochondrial and nuclear markers for individuals collected from three sites in the wild and first-generation captive-born individuals. The results of this study provide a critical test of the efficacy of the current management and captive propagation strategy for *E. nana*.

MATERIALS AND METHODS

Sampling.—During 2004 and 2005, 103 *E. nana* were collected from three sites spanning the range of *E. nana*: "Hotel Springs," "Diversion Springs," and "Below Dam" (Fig. 1, U.S. Fish and Wildlife Service permit TE676811-0 and Texas Parks and Wildlife Department permit SPR-0390-045) and transported to the NFHTC to augment the captive population (hereafter referred to as "wild-caught" individuals). These three sites were chosen for this study because they are the sites used by the NFHTC to stock the captive population. Upon arrival, a maxi-

mum of 5 mm of tail was removed from each individual using sterile equipment. On a separate occasion, tissue samples from tails were collected from 26 first-generation captive-born individuals. These captive-born individuals were the offspring of wild-caught individuals sampled between 1996 and 2003, not the wild-caught adults used in this study. Tissue samples were stored in 95% ethanol at −80°C until DNA was extracted. Remaining tissue and DNA is housed in Michael Forstner's Tissue Catalogue at Texas State University, San Marcos.

Molecular Methods.—Total genomic DNA was extracted using the Wizard SV Genomic DNA purification kit (Promega). We sequenced a 817 bp fragment of the mitochondrial DNA (mtDNA) gene region referred to as *ND4* consisting of the 3′ portion of *NADH subunit 4*, *tRNA-His*, *tRNA-Ser*, and the 5′ portion of *tRNA-Leu* using the primers ND4F and LEUR (Arevalo et al., 1994). We also sequenced a 721 bp portion of nuclear gene *recombination activating gene 1* (*rag1*) using primers designed specifically for *Eurycea* found in central Texas: RAG1-F (5′ CAA CTG GAC GGC AGA TTT TC 3′), RAG1-IF2 (5′ TTG AAC TTG GGG GCA TAC TC 3′), and RAG1-R (5′ TCC AGA TTC GTT CCC TTC AC 3′). Fluorescently labeled dideoxy terminators were used for single-stranded sequencing reactions for both *ND4* and *rag1* according to Beckman Coulter, Inc. specifications. Labeled amplicons were separated and visualized using a capillary DNA sequencer (CEQ model 8800, Beckman Coulter, Inc.). Sequences were aligned using the program SEQUENCHER 4.5. Because heterozygosity was never complex (i.e., never involved more than one site), the alleleic composition of each heterozygote was easily determined by eye to produce fully phased genotypes. The number of individuals sequenced for *ND4* and *rag1* for each population is shown in Table 1A,B.

To compare overall genomic diversity and structure of wild and captive-born *E. nana*, AFLP marker profiles were produced for individuals at each site (for sample sizes, refer to Table 1C). Modifications of the original AFLP procedure were used to increase reliability of the multilocus banding patterns that were generated as a result of the large genome size of salamanders (following the precedent set by Voss and Shaffer, 1997). The restriction digestion step used *Eco*RI and *Mse*I and the ligation step followed standard methods (Vos et al., 1995). The primers *Eco*RI-A and *Mse*I-C were used during the first preselective PCR. Two additional PCR steps were used to increase repeatability of the banding patterns: a PCR step using primers *Eco*RI-AC and *Mse*I-CT followed by a PCR step using the following two selective primer pairs: *Eco*RI-ACA with *Mse*I-CAGA, and *Eco*RI-ACA with *Mse*I-CTGC. Amplicons were separated, and CEQ software was used to visualize AFLP bands, which were sized by comparison to a size standard ladder (DNA size standard-600, Beckman Coulter, Inc.). Similar-size bands were assigned to bins. Within each sample, bands with low peak height (less than 250 relative fluorescent units [rfu]) were not scored. To ensure only repeatable bands were analyzed, bands with average peak heights (across all individuals) of less than 1,000 rfu were not scored. Bands that were present in only one individual surveyed were not included for subsequent analysis. To ensure analysis of discrete bands, the ratio of mean distance between bins over distance of bins spanned was calculated. All bands with a ratio greater than or equal to 1.0 were kept for analyses.

Statistical Analyses.—We estimated genetic variation for each sequenced locus for wild *E. nana* by estimating gene diversity (*h*, the probability that two randomly chosen haplotypes are different) and the mean number of pairwise nucleotide differences (π) and their 95% confidence intervals (95% CI) using the program ARLEQUIN 2.000 (S. Schneider, J. M. Kueffer, D. Roessli, and L. Excoffier, A software program for population genetic data analysis, Genetics and Biometry Laboratory, University of Geneva, 2000). For our AFLP data, we used the computer program AFLP-SURV v1.0 (X. Vekemans, Laboratoire de Genetique et Ecologie Vegetale, Universite Libre de Bruxelles, Belgium, 2002) to estimate the allele frequencies at each locus using a Bayesian method with a nonuniform prior distribution of allele frequencies (Zhivotovsky, 1999) for diploid species and assuming no deviation from Hardy-Weinberg genotypic proportions. Estimates of allele frequencies were used to calculate two measures of genetic variation, the percentage of polymorphic loci and unbiased expected heterozygosity (Nei, 1973), for the wild *E. nana* following the method described in Lynch and Milligan (1994).

To investigate population genetic structure among the three sites sampled within the wild, pairwise ϕ_{CT} values for *ND4* and *rag1* were estimated for each pair of populations under the Jukes and Cantor model as implemented in the program ARLEQUIN 2.000 (S. Schneider, J. M. Kueffer, D. Roessli, and L. Excoffier, A software program for population genetic data analysis, Genetics and Biometry Laboratory, University of Geneva, 2000). The Jukes and Cantor model was selected because levels of molecular variation were very low. Significance of pairwise ϕ_{CT} values was assessed using permutation tests with 1,000 permutations. We also used the

computer program AFLP-SURV v1.0 (X. Vekemans, Laboratoire de Genetique et Ecologie Vegetale, Universite Libre de Bruxelles, Belgium, 2002) to estimate the allele frequencies at each AFLP locus as described above. The significance of the genetic differentiation among salamanders sampled from the three sites in the wild was tested by comparison of the observed F_{ST}-value with a distribution of F_{ST}-values under the hypothesis of no genetic structure, obtained by means of 10,000 random permutations of individuals among sites. Furthermore, population subdivision among the three *E. nana* sampling sites was tested using the program STRUCTURE v2.2 (Pritchard et al., 2000; Falush et al., 2007). STRUCTURE v2.2 includes a new model for dominant molecular markers that explicitly incorporates uncertainty in genotype because of the presence of recessive alleles. This program employs a model-based Bayesian clustering algorithm to assign individuals probabilistically to clusters based on their AFLP banding profiles to minimize deviations from linkage equilibrium. Runs were performed using the admixture model with a Markov Chain Monte Carlo of 1,000,000 generations with an initial burn-in of 100,000 generations. Prior information regarding the sample site was ignored. STRUCTURE v2.2 was also used to estimate the number of clusters (k) that best explained the data. The likelihood of the STRUCTURE v2.2 model with k between 1 and 5 was estimated. Posterior probabilities were used to determine the number of clusters that best explained the data following the methods of Pritchard et al. (2000). Posterior probabilities were calculated assuming a uniform prior on k with a minimum of 1 and a maximum of 5. Two simulations were run for each k to verify convergence on the stationary distribution.

To compare the genetic diversity of each locus of the wild-caught individuals (sites pooled) and captive-born individuals, gene diversity (h) and the mean number of pairwise differences (π) and their 95% confidence intervals (95% CI) were calculated for the captive-born individuals as described above. Next, the 95% confidence intervals of h and π of the wild-caught population (sites pooled) and the captive-born individuals for *ND4* and *rag1* were compared to see whether they overlapped. The percentage of polymorphic loci and unbiased heterozygosity for the AFLP data from the captive-born individuals were calculated following the same methods described above. Next, the 95% confidence intervals of unbiased expected heterozygosity of the wild-caught population (sites pooled) and the captive-born individuals for *ND4* and *rag1* were compared to see whether they overlapped. Finally, population subdivision between the wild-caught (sites pooled) and captive-born individuals was tested using the program STRUCTURE v2.2 (Pritchard et al., 2000; Falush et al., 2007) in the same manner as was described above to test for structure among the three sampling sites in the wild.

To determine whether inbreeding has occurred in the wild-caught individuals that gave rise to the captive-born individuals, deviations from Hardy-Weinberg equilibrium using the diploid nuclear marker *rag1* were evaluated using a modified version of Fisher's exact test (Guo and Thompson, 1992), as implemented in ARLEQUIN 2.000 (S. Schneider, J. M. Kueffer, D. Roessli, and L. Excoffier, A software program for population genetic data analysis, Genetics and Biometry Laboratory, University of Geneva, 2000).

RESULTS

Two and three haplotypes were identified when genetic variation was surveyed in the sampled wild-caught *E. nana* at *ND4* and *rag1*, respectively (Genbank accession numbers EF443117, EF443123, EF443108, EF443109, EF443113; Table 1A,B). Nucleotide diversity in the wild-caught *E. nana* was 0.1927 (95% CI, 0.1453–0.2401) for *ND4* and 0.4785 for *rag1* (95% CI, 0.3847–0.5830) (Table 1A,B). As most haplotypes differed by a single nucleotide, estimates of gene diversity (h) were nearly identical to nucleotide diversity (π) (Table 1A,B). The use of two AFLP primer combinations yielded 281 molecular markers that could be reliably scored, of which 79.7% were polymorphic within the wild-caught *E. nana* (Table 1C). Mean expected heterozygosity for the AFLP data in the wild-caught individuals was 0.1724 (0.1612–0.1836 95% CI).

We found no evidence of subdivision among the three sampling sites in the wild. For all pairs of sampling sites, pairwise ϕ_{CT}-values based on *ND4* and *rag1* were not significantly different from zero ($P > 0.05$ for all pairs of sampling sites). F_{ST}-values based on AFLPs ($F_{ST} = -0.0138$, $P = 0.8845$) for the three sites in the wild were not significantly different from zero. The Bayesian clustering analysis performed with STRUCTURE revealed that the AFLP data for the three sites in the wild were best explained by two clusters ($k = 2$). However, although individuals varied slightly in their genomic composition, this variation was not associated with sample locality, and all individuals were shown to have the majority of their genome derived from the same cluster (Fig. 2). This indicates a lack of population structure, despite $k = 2$ having the highest posterior probability (Pritchard et al., 2000). Based on

TABLE 1. Sample sizes (*N*) and genetic diversity data for *ND4* (A) and *rag1* (B) sequence data, and AFLP (C) data for each sample site. Values for *h* represent gene diversity, and values for π represent the mean number of pairwise differences among haplotypes.

A	*N*			
Sample site		ND4 haplotypes (N)	ND4 h (95% CI)	ND4 π (95% CI)
Hotel Springs	33	nC (29), nI (4)	0.2197 (0.1902-0.2492)	0.2197 (0.1280-0.3113)
Diversion Springs	37	nC (32), nI (5)	0.2402 (0.2136-0.2668)	0.2402 (0.1494-0.3311)
Below Dam	33	nC (31), nI (2)	0.1174 (0.0924-0.1424)	0.1174 (0.0524-0.1824)
Total wild	103	nC (92), nI (11)	0.1927 (0.1834-0.2020)	0.1927 (0.1453-0.2401)
Captive-born	26	nC (26)	0	0
B				
Sample site		rag1 haplotypes (N)	rag1 h (95% CI)	rag1 π (95% CI)
Hotel Springs	17	rA (10), rB (24)	0.4278 (0.3961-0.4595)	0.4278 (0.2393-0.6164)
Diversion Springs	24	rA (20), rB (27), rF (1)	0.5204 (0.5076-0.5332)	0.5381 (0.3564-0.7198)
Below Dam	28	rA (21), rB (35)	0.4773 (0.4645-0.4901)	0.4773 (0.3216-0.6330)
Total wild	69	rA (51), rB (86), rF (1)	0.4785 (0.4732-0.4838)	0.4839 (0.3847-0.5830)
Captive-born	26	rA (10), rB (42)	0.3167 (0.2906-0.3428)	0.3167 (0.1904-0.4430)
C				
Sample site		% polymorphic loci	Heterozygosity (95% CI)	
Hotel Springs	8	67.6	0.1784 (0.1653-0.1915)	
Diversion Springs	14	84.3	0.1803 (0.1691-0.1915)	
Below Dam	11	72.2	0.1745 (0.1622-0.1868)	
Total wild	33	79.7	0.1724 (0.1612-0.1836)	
Captive-born	19	77.2	0.1613 (0.1513-0.1713)	

these results, data from the three Spring Lake sites were pooled for comparisons to the captive-born individuals.

Comparison of genetic variation in the pooled wild-caught individuals to that of the sampled captive-born individuals indicates that, although the captive-born individuals have significantly reduced genetic diversity at some loci, they contain a fair representation of the standing variation in the wild at other loci. Specifically, the 95% confidence intervals of *h* and π for the wild-caught individuals (sites pooled) and the captive-born individuals for *ND4* do not overlap (Table 1A). However for the *rag1* data, the 95% confidence interval of the estimates of π overlap for the wild-caught and captive-born individuals, but this is not true regarding *h* (Table 1B). Estimates of mean expected heterozygosity for the AFLP data set are similar for the wild-caught and captive-born individuals, moreover, the 95% confidence intervals of these estimates overlap (Table 1C). However, for both our DNA sequence data and AFLP markers, even in the cases where the 95% confidence intervals for our estimated diversity indices overlap, the point estimates are lower for the captive-born individuals than the wild-caught individuals.

The captive-born individuals contain many of the same alleles sampled from the wild-caught individuals, with the exception of the rare alleles. Of the two *ND4* alleles sampled from the wild-caught individuals, the captive-born individuals harbor the common haplotype nC but do not contain the much rarer haplotype nI (Table 1A). Of the three *rag1* haplotypes sampled from the wild-caught individuals, only the rare rF allele was not sampled from the captive-born individuals (Table 1B). Of the 281 AFLP markers that were sampled from the wild-caught individuals, only 33 were not sampled from the captive-born individuals; conversely, there were seven AFLP markers sampled from the captive-born individuals that were not sampled from the wild-caught individuals. At present, an unknown subset of wild-caught individuals has sired first-generation captive-born offspring; thus, genetic diversity in the NFHTC captive population may increase over time when and if additional wild-caught individuals sire offspring.

The Bayesian clustering analysis performed with STRUCTURE revealed that the AFLP data for the wild-caught and captive-born individuals were best explained by two clusters ($k = 2$). However, individual variation was not partitioned between the wild and captive-born individuals (Fig. 2), indicating a lack of population structure. The wild-caught and captive-born individuals are not significantly differentiated.

We found no evidence of inbreeding within the wild-caught individuals that sired the first-generation captive-born individuals. Observed heterozygosity for the *rag1* locus in the captive-

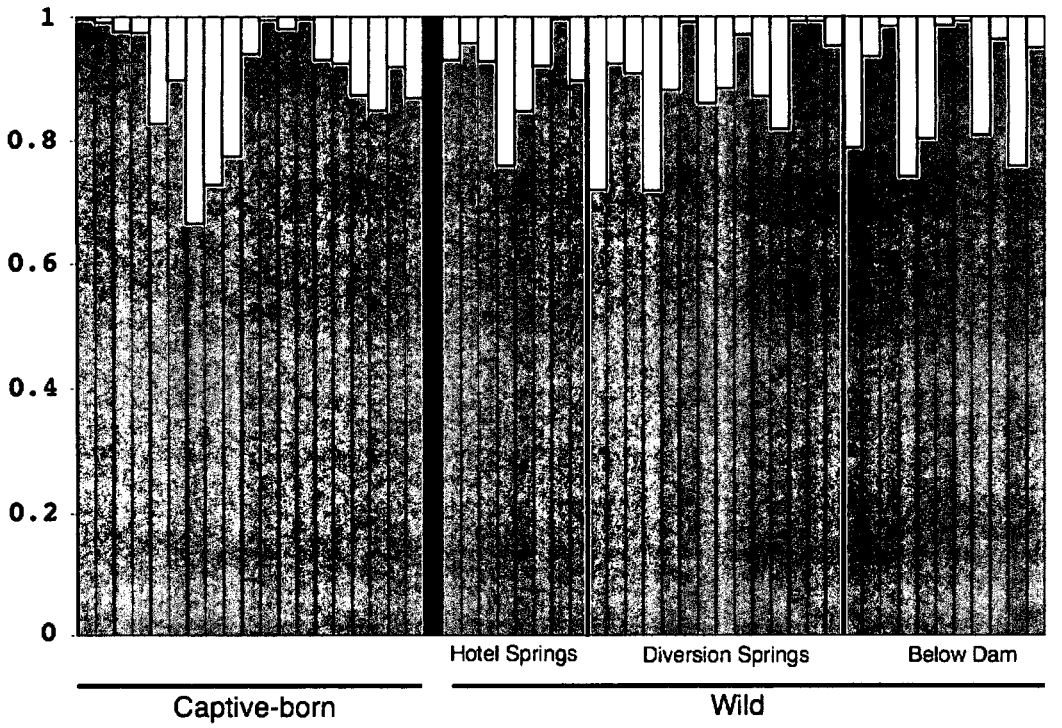

FIG. 2. Bayesian assignment probabilities for $k = 2$. Each bar corresponds to one individual. The proportion of each bar that is dark grey represents an individual's assignment probability to cluster 1; the proportion of each bar that is white represents an individual's assignment probability to cluster 2.

born individuals (0.23) was similar to expected heterozygosity (0.35). We did not detect deviation from Hardy-Weinberg equilibrium at the *rag1* locus ($P = 0.19$). Furthermore, estimates of average expected heterozygosity based on the AFLP data were similar for the wild-caught and captive-born individuals; 95% CIs of these estimates overlapped (Table 1C).

DISCUSSION

Estimates of genetic diversity at the *ND4* and *rag1* loci for the federally threatened *E. nana* are similar to estimates of genetic diversity for populations of other *Eurycea* species found in the Edwards Plateau region of central Texas (e.g., *E. pterophila* and *E. neotenes*; L. K. Lucas, Z. Gompert, J. R. Ott, and C. C. Nice, unpubl. data). These individual populations cover geographic areas of similar sizes to *E. nana*. However, migration cannot reintroduce genetic variation into *E. nana* of Spring Lake because *E. nana* does not likely occur outside of Spring Lake and does not experience gene flow with other *Eurycea* populations or species (L. K. Lucas, Z. Gompert, J. R. Ott, and C. C. Nice, unpubl. data). Thus, although *E. nana* harbors a similar level of genetic diversity to other *Eurycea* populations, this diversity will not recover naturally if it is lost.

We found no evidence of genetic structure within wild *E. nana*. Although this finding is not surprising based on the small geographic range of *E. nana*, it is interesting because one of the sampled sites (Below Dam) is separated from the other two sites by a spillway. Thus, it is unlikely that migration occurs from the Below Dam site to *E. nana* in Spring Lake proper. The lack of genetic differentiation between the Below Dam site and *E. nana* in Spring Lake is likely the result of unidirectional migration from Spring Lake to the Below Dam site. In fact, the Below Dam site may represent a sink subpopulation that is maintained by regular migration from Spring Lake.

Given the lack of structure and the limited geographic range of *E. nana* in the wild, there is no genetic reason that individuals caught from different sites in the wild need to be housed separately in captivity. Additionally, the habitat within Spring Lake does not exhibit sufficient variation to indicate that differential local adaptation of *E. nana* from different sites should be expected. In fact, pooling collected individuals may facilitate long-term maintenance of genetic diversity in the captive population by

increasing the size of the breeding population. However, there are potentially alternative reasons for maintaining separate populations in the lab including, for example, the prevention of disease transmission throughout the entire captive population.

We did not detect genetic differentiation between wild-caught and captive-born individuals. Furthermore, although estimates of genetic diversity for the wild-caught and captive-born individuals are similar at some loci, there is a general trend toward reduced diversity in the first-generation captive-born individuals relative to the sampled wild-caught individuals. This reduced diversity is partially the product of the loss of some rare haplotypes within the captive-born individuals; however, this loss of rare haplotypes is potentially an artifact of our lower sample size for the captive-born individuals relative to the wild-caught individuals. It is likely that the periodic infusion of new founders from the wild has helped retain genetic diversity within the captive population. It is unclear whether the present number of salamanders collected annually to restock the captive population negatively impacts wild *E. nana*. Based on a recent census estimate (Nelson, 1993), approximately 50,000 *E. nana* occur in Spring Lake, which would suggest that the collection of 92 individuals per year to augment the captive population should not impact the probability of extinction in the wild.

There are several ways in which the effectiveness of the captive breeding program may be improved. We suggest that individuals be exchanged among groups of salamanders in different tanks on a regular basis. This is important because inbreeding can be minimized when pedigrees are unavailable, and species breed in small groups because of various reasons such as constraints on the space available for captive propagation, as is the case with *E. nana* (Princée, 1995; Wang, 2004). Furthermore, to avoid a loss of genetic variation in the captive population, captive-born individuals could be mated back to wild-caught individuals. It would be ideal to pair individuals that possess novel genetic variation; however, the genetic composition of individual salamanders is not available at this time (logistical constraints precluded us from physically marking or tracking individuals, which would have allowed us to match our genetic data with individual salamanders).

The data regarding the level of genetic variation in wild and captive *E. nana* will provide baseline data for this species. Monitoring of genetic variation in the captive *E. nana* population should be conducted every few years to verify that random mating in the captive population is maintained. Maintenance of the current level of genetic variation in the captive population is crucial for enhancing the probability of survival of *E. nana* if reintroduction becomes necessary.

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Literature Cited

Arevalo, E., S. K. Davis, and J. W. Sites. 1994. Mitochondrial-DNA sequence divergence and phylogenetic-relationships among 8 chromosome races of the *Sceloporus-Grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology 43:387–418.

Brune, G. 1981. Springs of Texas. Texas A&M University Press, College Station.

Chen, C. C., D. Gillig, and B. A. McCarl. 2001. Effects of climatic change on a water dependent regional economy: a study of the Texas Edwards Aquifer. Climatic Change 49:397–409.

Falush, D., M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574–578.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Fries, J. N. 2002. Upwelling flow velocity preferences of captive adult San Marcos salamanders. North American Journal of Aquaculture 64:113–116.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Loáiciga, H. A. 2003. Climate change and ground water. Annual Association of American Geographers 3:30–41.

Lynch, M., and B. G. Milligan. 1994. Analysis of population genetic-structure with RAPD markers. Molecular Ecology 3:91–99.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences USA 70:3321–3323.

NELSON, J. M. 1993. Population Size, Distribution, and Life History of *Eurycea nana* in the San Marcos River. Unpubl. master's thesis, Southwest Texas State University, San Marcos, Texas.

PRINCÉE, F. P. G. 1995. Overcoming the constraints of social structure and incomplete pedigree data through low-intensity genetic management. *In* J. D. Ballou, M. Gilpin, and T. J. Foose (eds.), Population Management for Survival and Recovery: Analytical Methods and Strategies in Small Population Conservation, pp. 124–154. Columbia University Press, New York.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

USFWS. 1980. Endangered and threatened wildlife and plants; listing of the San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild rice, San Marcos salamander, San Marcos gambusia, and fountain darter. Federal Register 45(136): 47355–47364.

———. 1984. San Marcos River Recovery Plan, p. 109, Albuquerque, New Mexico.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRIJTERS, J. POT, J. PELEMAN, M. KUIPER, AND M. ZABEAU. 1995. AFLP—a new technique for DNA-Fingerprinting. Nucleic Acids Research 23:4407–4414.

VOSS, S. R., AND H. B. SHAFFER. 1997. Adaptive evolution via a major gene effect: Paedomorphosis in the Mexican axolotl. Proceedings of the National Academy of Sciences USA 94:14185–14189.

WANG, J. L. 2004. Monitoring and managing genetic variation in group breeding populations without individual pedigrees. Conservation Genetics 5:813–825.

ZHIVOTOVSKY, L. A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Molecular Ecology 8:907–913.

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