MOLECULAR EVIDENCE FOR PARTHENOGENESIS IN THE SIERRA GARTER SNAKE, *THAMNOPHIS COUCHII* (COLUBRIDAE)

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ABSTRACT—We used microsatellite analysis to investigate possible reproduction by parthenogenesis in the bisexual Sierra garter snake, *Thamnophis couchii*. The genotypes of four microsatellite loci were determined for each of four individuals, including three *T. couchii*, two of which were a female and her offspring, and *T. ordinoides*. The female *T. couchii* and her offspring were homozygous and identical for all four microsatellite loci; however, Ts3 and Ts4 are the only loci that supported reproduction by parthenogenesis as Ts1 and Ts2 were uninformative. These data along with absence of a unique paternal allele and known absence of an opportunity to mate prior to and during captivity is consistent with reproduction by parthenogenesis.

RESUMEN—Utilizamos el análisis de microsatélites para investigar la posible reproducción por partenogénesis de la culebra-listonada bisexual, *Thamnophis couchii*. Fueron determinados los genotipos de cuatro loci de microsatélites para cada uno de cuatro individuos, incluyendo tres *T. couchii*, dos de los cuales fueron una hembra y su cría, y *T. ordinoides*. La *T. couchii* hembra y su cría fueron homozigóticos e idénticos para los cuatro loci de microsatélites; sin embargo, Ts3 y Ts4 son los únicos loci que apoyaron la reproducción por partenogénesis puesto que Ts1 y Ts2 no rindieron ninguna información. Estos datos junto con la falta de un alelo paternal único y la conocida falta de oportunidad para aparear antes de y durante el cautiverio son consistentes con la reproducción por partenogénesis.

Most vertebrates reproduce sexually, although this is not always the case. Some reptiles are obligatory parthenogens (reproducing gynogenetically), including the Brahminy blind snake (*Ramphotyphlops brahminus*) and a variety of lizards in the families Teiidae, Lacertidae, Xantusiidae, Gekkonidae, Agamidae, and Chameleonidae (Vrijenhoek et al., 1989; Wright, 1993). Other parthenogenetic vertebrates occur among fishes in the families Poecilidae, Atherinidae, Cottidae, and Cyprinidae, and amphibians in the families Ambystomatidae and Ranidae (Vrijenhoek et al., 1989), but sperm from males of syntopic bisexual species are required to fuse with eggs from hybridogenetic females (Wright, 1993). There also are a number of examples of normally bisexual species that occasionally produce parthenogenetically. This facultative parthenogenesis (spontaneous development of zygotes from unfertilized eggs) is known from 10 orders of insects (Simon et al., 2003), and occurs occasionally in vertebrates. Facultative parthenogenetic reproduction in vertebrates has been presumed to have occurred in the common water snake *Nerodia sipedon* (Skalka and Voženílek, 1986), the Javan wart snake *Acrochordus javanicus* (Magnusson, 1979), the file snake *A. arafurae* (Dubach et al., 1997), the wandering garter snake *Thamnophis elegans*, the checkered garter snake *T. marcianus*, the timber rattlesnake *Crotalus horridus*, the Aruba Island rattlesnake *C. unicolor* (Schuett et al., 1997), the Burmese python *Python molurus* (Groot et al., 2003), the yellow-spotted monitor *Varanus panoptes* (Lenk et al., 2005), and the Komodo dragon *Varanus komodoensis* (Watts et al., 2006) based on isolation of females from males, analysis of genetic data, or both.

The bisexual Sierra garter snake (*Thamnophis couchii*) occurs in the Sierra Nevada and Tehachapi Mountains of California extending eastward into western Nevada (Rossman et al., 1996). It is highly aquatic and occurs along streams, rivers, ponds in meadows, and small lakes (Rossman et al., 1996). *Thamnophis couchii* is viviparous and produces 7–38 young in summer (Bartlett and Tennant, 2000). Here we report apparent parthenogenesis in this usually bisexual snake. A neonate was produced by a captive female *T. couchii* that had never been kept with a male. We

TABLE 1—Primers and results of microsatellite-fragment analysis for four loci in each of four individual garter snakes (*Thamnophis*), including a female *T. couchii* and her offspring.

Locus	Primers (5′-3′)[a]	Genotype/size of fragment (bp)			
		T. couchii (female)	*T. couchii* (offspring)	*T. couchii* (outgroup)	*T. ordinoides* (outgroup)
Ts1	CGGCATAAATCTTATCTAGC ACTTTTTCAGGCTGATGTTC	190/190	190/190	123/123	190/190
Ts2	GGCTAGCCCCTGTGTCCTT CACAACTCCAAATATTGAAGATT	150/150	150/150	150/150	151/175
Ts3	CAACTGGCSGCTGTGATACAA GTGTTAATGTGTTGGACAGGGC	126/126	126/126	118/121	110/110
Ts4	ACTGAACAAGTTGGGTGTAG GCAAGAAGATGGCTATCTTG	149/149	149/149	148/148	152/152

[a] Primers are from McCracken et al. (1999).

used microsatellite analysis to determine the genetic relationship of the offspring to its mother.

We collected tissues from four individual *Thamnophis*. Two samples were obtained in 2006 from an adult female *T. couchii* and her offspring, both of which were housed in an indoor reptile facility at the California Living Museum, Bakersfield, Kern County, California. The female had been in captivity since 20 September 2000 when she was put on display with another female *T. couchii*. She weighed 38.3 g in February 2001. She produced her first clutch of young (not counted) 5 July 2005 when she weighed 181 g post laying, but young were still-born. She produced a second clutch (not counted) 20 April 2006 (adult weight was 211 g) and one neonate was born alive. This female never was housed with a male garter snake and was a juvenile when she was brought into captivity. An additional female *T. couchii* (also a captive from the California Living Museum, tissue taken May 2008) was included as a within-species outgroup. The fourth sample was obtained from an adult male northwestern garter snake (*T. ordinoides*) that was a captive, and served as an unrelated outgroup. Tissues consisted of tail clippings that were placed in 95% ethanol.

DNA was extracted from each of the four samples using the DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California). Voucher specimens and surplus genomic extracts were stored at −70°C in the laboratory of PTS. PCR-primer sequences developed specifically to investigate paternity in *T. sirtalis* (Ts1, Ts2, Ts3 and Ts4; Table 1) by McCracken et al. (1999) were synthesized and labeled with Fam6 or Hex (Integrated DNA Technologies, Coralville, Iowa) for fragment analysis. PCR-amplification conditions were described by McCracken et al. (1999) and consisted of 2 min at 95°C, followed by 27 cycles of 1 min at 95°C and 1 min at 56°C (Ts1 and Ts4), 54°C (Ts2), or 57°C (Ts3), and 1 min at 72°C on a T-Gradient thermocycler (Biometra, Labrepco Inc., Horsham, Pennsylvania). The PCR products were submitted to the University of Florida Interdisciplinary Center for Biotechnology Research Genetic Analysis Laboratory for processing on a MegaBACE 1000 (Amersham Biosciences, Piscataway, New Jersey; Ts3) or ABI 3730 (Applied Biosystems, Foster City, California; Ts1, Ts2, and Ts4) and were analyzed using GeneMarker (Version 1.50, Soft Genetics, State College, Pennsylvania).

Locus Ts1 was not informative as the female *T. couchii*, her offspring, and *T. ordinoides* were homozygous for the same-sized allele (190/190), but the outgroup *T. couchii* was homozygous for a different-sized allele (123/123; Table 1). Similarly, Ts2 was not informative as this locus was invariant among the three *T. couchii*, but was genetically different from the heterozygote *T. ordinoides* (Table 1). The female and offspring *T. couchii* were homozygous and invariant for locus Ts3, but were different from both outgroups. The outgroup *T. couchii* was heterozygous for Ts3 with both alleles being different in size compared to the female and offspring (Table 1). Finally, all four individuals were homozygous for

locus Ts4 with the female and her offspring being identical, but different from both outgroups. Because the outgroup *T. couchii* was homozygous for a different allele compared to the female and her offspring, Ts4 has potential for heterozygosity within this species.

In summary, the female and her offspring were identical for all four microsatellite loci that were analyzed with no unique paternal allele detected; however, only Ts3 and Ts4 were informative with respect to supporting reproduction by parthenogenesis. Locus Ts2 was homozygous and identical for the three *T. couchii*, but differed from *T. ordinoides* indicating that this locus may be useful only for determination of species. Data presented here, along with absence of a unique paternal allele in the offspring and known absence of an opportunity to mate prior to and during captivity, is consistent with and expected for reproduction by parthenogenesis in this usually bisexual species.

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