

Sex-Linked Inheritance of Fumarate Hydratase Alleles in Natricine Snakes

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Fumarate hydratase genotypic frequencies in natricine snakes deviate significantly from Hardy-Weinberg expectations in that heterozygotes are lacking among females. This is consistent with sex-linked inheritance in organisms having ZW sex chromosomes and suggests that FUMH alleles in natricine snakes are carried on the Z chromosome.

Allozymes have been used extensively as genetic markers in studies of population and species differentiation (Murphy et al. 1990). Allozymes generally show patterns consistent with simple Mendelian inheritance, but in few organisms other than humans (McKusick 1992) have specific allozymes been associated with individual

chromosomes or chromosome regions. Here we report evidence of sex-linked inheritance of fumarate hydratase alleles (FUMH, E.C. 4.2.1.2) in natricine snakes. Because sex chromosomes are variable in snakes (some species lack heteromorphic sex chromosomes, others have ZW sex chromosomes), sex-linked markers may be useful in understanding chromosomal evolution in this group (Beçek et al. 1990; Ohno 1967; Olmo 1986).

As part of an investigation of gene flow among Lake Erie island and mainland locations (King and Lawson 1995; Lawson and King, in press), we analyzed allozymic variation in seven populations of water snakes, *Nerodia sipedon*, and ten populations of garter snakes, *Thamnophis sirtalis*. Two FUMH alleles (designated A and B) were detected, but initial analyses showed that heterozygotes were underrepresented within populations of both species ($n = 413$ water snakes, 21 to 100 per population, and 674 garter snakes, 21 to 122 per population). Because such a pattern is consistent with sex-linked inheritance, we considered males and females separately in subsequent analyses. Genotypic frequencies fit Hardy-Weinberg expectations for males [exact P values from BIOSYS-1 (Swofford and Selander 1981) $> .05$ for 7 of 7 water snake populations and 10 of 10 garter snake populations] but not for females (exact $P < .05$ in 5 of 7 water snake populations and 10 of 10 garter snake populations). With only a few exceptions (see below), apparent heterozygotes were absent among females as expected with sex-linked inheritance in species in which females are heterogametic. When females were assumed to be hemizygous for FUMH (and apparently heterozygous females were omitted), allelic frequencies were similar between males and females [two-tailed P values calculated as in Neter et al. (1978, p. 323) $> .05$ in 7 of 7 water snake populations and 8 of 10 garter snake populations]. A similar pattern was seen in *N. clarkii* \times *N. fasciata* hybrids from a site in Volusia County, Florida ($n = 36$; Lawson et al. 1991): heterozygotes were absent among females ($P < .001$), but allelic frequencies were similar between males and females ($P = .63$) when females were assumed to be hemizygous for FUMH. FUMH heterozygotes were present among males but absent among females in *N. sipedon* ($n = 30$), *N. clarkii* ($n = 26$), *N. clarkii* \times *N. fasciata* hybrids ($n = 20$), and (with one exception) *T. radix* ($n = 29$) analyzed in other studies (De Queiroz and Lawson 1994; Lawson et al. 1991). However, be-

cause these data were pooled across localities, formal statistical tests were not carried out.

These results suggest that FUMH alleles are carried on the Z chromosome and inherited in sex-linked fashion. We attribute those few cases of apparently heterozygous females (7 of 413 *N. sipedon*, 12 of 674 *T. sirtalis*, 1 of 29 *T. radix*) to errors in classifying snakes by sex or in scoring FUMH genotypes. *N. sipedon* and *T. sirtalis* from Lake Erie were classified by sex in the field based on tail shape or probing (Laszlo 1973), and some males may have been misclassified as females (snakes were released following tissue sampling, precluding definitive sex determination). FUMH genotypes were visualized using an agar overlay staining technique that sometimes results in blurred banding patterns, hence some homozygotes may have been misclassified as heterozygotes.

The presence of ZZ/ZW sex chromosomes has been confirmed karyologically in *N. sipedon* (Cundall 1970; Kilpatrick and Zimmerman 1973; but see Eberle 1972) and in some other members of the genera *Nerodia* and *Thamnophis* (Cundall 1970; De Smet 1978; Kilpatrick and Zimmerman 1973; Mengden 1981; Mengden and Stock 1980; Olmo 1986). Because these genera show close evolutionary relationships (Colubridae: Natricinae; De Queiroz and Lawson 1994; Lawson 1987; Lawson et al. 1991), it is possible that FUMH polymorphism was present in ancestral taxa as well. Information on patterns of FUMH variation in other snakes would be useful in testing this possibility. Unfortunately, aside from the leafnose snake (*Phyllorhynchus arenicolus*), a nonnatricine colubrid in which FUMH is apparently monomorphic (Murphy and Ottley 1980), we are unaware of other snakes for which FUMH genotypes have been determined. Tests of the specific activity of FUMH would also be of interest to determine whether dosage compensation occurs. We noted no difference in the staining intensity of FUMH in males and females, suggesting that dosage compensation does occur, contrasting with other taxa having ZW sex chromosomes (e.g., birds, butterflies) in which dosage compensation is apparently lacking (Baverstock et al. 1982; Johnson and Turner, 1979).

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