Title
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Journal
Journal of Heredity, 102(3)

ISSN
0022-1503

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Publication Date
2011-05-01

DOI
10.1093/jhered/esr017

Journal of Heredity 2011:102(3):347–351
doi:10.1093/jhered/esr017
Advance Access publication March 30, 2011

Genetic Monandry in 6 Viviparous Species of True Sea Snakes

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Using a suite of 10 highly variable microsatelite loci, we conducted genetic paternity analyses for 76 embryos in the broods of 12 pregnant females representing 6 viviparous species of true sea snakes (*Hydrophis* clade) in the family Elapidae. To our surprise, we uncovered no evidence for multiple paternity within any of the clutches despite the fact that the genetic markers showed high intraspecific heterozygosities and as many as 20 conspecific alleles per locus. This outcome stands in sharp contrast to the rather high (but also variable) frequency of multiple paternity previously reported in many other reptilian species. However, because our study appears to be the first assessment of genetic parentage for any sea snake species (and indeed for any member of the elapid clade), we can only speculate as to whether this apparent monandry by females is a broader phylogenetic characteristic of elapid snakes or whether it might relate somehow to the sea snakes' peculiar lifestyle that uniquely combines viviparity with a marine existence.

Key words: *Elapidae, genetic parentage, Hydrophiinae, mating systems, microsatellite, multiple paternity*

As recently reviewed by Uller and Olsson (2008), multiple paternity has been genetically documented within the clutches of numerous reptilian species including snakes (McCracken et al. 1999; Gibbs and Weatherhead 2001; Voris et al. 2008; Ursenbacher et al. 2009), lizards (Olsson and Madsen 2001), and turtles (Pearse and Avise 2001). For species with internal fertilization, a brood is likely to have multiple sires when its dam has copulated repeatedly during the mating season and especially when she can store viable sperm. Sperm-storage tubules have been documented in the oviducts of many reptile groups (Birkhead and Moller 1993) including numerous snake species (Sever and Ryan 1999). Thus, perhaps it is not too surprising that multiple paternity has been genetically confirmed in several groups of advanced snakes including viperids (adders) (Ursenbacher et al. 2009), homalopsids (mud snakes) (Voris et al. 2008), colubrids (Blouin-Demers et al. 2005), and natricids (summary in Uller and Olsson 2008). To date, however, there seems to be no published genetic evidence for (or against) polyandrous mating behaviors by the dams of clutches in any of the ~300 extant species of snakes in the large family Elapidae.

Australian terrestrial elapids have invaded the marine environment on at least 2 separate occasions, thereby producing 2 extant lineages: the sea kraits (*Laticauda*, with ~8 species) and the "true" sea snakes (with ~60 extant species in tropical waters of the Indo-West Pacific, thus making them by far the world's most species-rich group of marine reptiles). In contrast to the egg-laying sea kraits, all "true" sea snakes are viviparous and give birth at sea, so they constitute the only reptilian group that has completely freed itself from the terrestrial environment. Although a marine existence makes sea snakes interesting biologically, it has also complicated the logistics of long-term observational studies on their reproductive tactics. Indeed, to our knowledge, no such studies have been published for these animals.

All sea snake species exhibit, to greater or lesser extents, sexual dimorphism: Males invariably are smaller than females. Casual field observations indicate that several males simultaneously pursue individual females during the mating season and actively compete for matings (Lukoschek V, personal observation). Although polyandrous copulatory behavior by a female has not been recorded in the field, it seems likely that sea snake dams have taken multiple mates during a breeding season (Heatwole 1999). No one has reported observing sea snake births in the wild and small juveniles are rarely seen, thus making it difficult to sample families for genetic analysis. To gain insights into the mating system of sea snakes, we developed hypervariable microsatellites (Lukoschek and Avise, forthcoming) to investigate genetic paternity in broods of deceased pregnant females obtained opportunistically as inadvertent trawl bycatch. True sea snakes comprise 2 distinct clades (Lukoschek and Keogh 2006) and the larger *Hydrophis* group, which includes >40 closely related species widely distributed from Australia to the Arabian Gulf, is the focus of our study.

Table 1 Summary information about pregnant females and clutch sizes (mean and SE) for 8 species of hydrophiine sea snakes obtained from trawl bycatch studies

		Clutch size		Clutches genotyped	
Species	No. of dams	Mean	SE	No. of clutches	Clutch size (*n* genotyped)
Acalyptophis peronii	3	2.0	0.6	0	n/a
Hydrophis elegans	4	10.5	1.0	2	18 (16), 6 (4)
Hydrophis kingii	2	7.0	1.0	1	8 (6)
Hydrophis mcdowelli	8	3.5	0.2	3	4 (4), 4 (4), 4 (3)
Hydrophis major	6	4.7	0.2	0	n/a
Hydrophis ocellatus	4	4.3	1.0	1	6 (4)
Hydrophis pacificus	1	10.0	n/a	1	10 (10)
Lapemis curtus	4	6.3	1.1	4	9 (9), 7 (7), 5 (5), 4 (4)

A subset of 12 clutches with the largest clutch sizes for each species, were genotyped. Number of clutches per species, clutch sizes, and the number of embryos per clutch successfully genotyped for at least 8 of the 10 microsatellite loci (in brackets) are also given.

Materials and Methods

All true sea snake species are protected under Australia's *Environment Protection and Biodiversity Conservation (1999) Act* that prohibits the catch and euthanization of live specimens, and stringent permitting requirements limit the potential to collect and hold pregnant females. To overcome these logistical constraints, we took advantage of the sad fact that sea snakes comprise a nontrivial component of the bycatch of trawl fisheries in Australian waters (Stobutzki et al. 2000; Fry et al. 2001; Courtney et al. 2009). Many of these snakes are landed dead and pregnant females were obtained from this source.

Female snakes were dissected and their reproductive condition noted. The yolked oviducal eggs of each pregnant female were carefully removed and the numbers of yolked eggs and yolked eggs with embryos were counted. Each embryo was dissected from its yolk sac and assigned an alpha-numeric code indicating whether it came from the right (R) or left (L) oviduct and its ranked position along the oviduct (starting with the most anterior embryo). This labeling scheme was intended to reveal whether embryos from different sires might have different locations in the oviducts. Maternal and embryonic tissues were preserved in 80% ethanol. DNA was extracted from maternal and embryonic tissues using a standard phenol/chloroform protocol and genotypic profiles of each dam and her clutch were obtained for 10 microsatellite loci developed for the "true" sea snake, *Hydrophis elegans* (Lukoschek and Avise, forthcoming). Maternal genotypes were observed directly and paternal alleles were deduced by subtracting maternal alleles from each offspring's genotype. Broods with more than 2 paternal alleles at one or more marker loci suggest multiple sires.

Numbers of alleles per locus and observed and expected heterozygosities were obtained for 2 sea snake species (*H. elegans* and *Lapemis curtus*) for which larger population sample sizes were available (for more details, see Lukoschek and Avise, forthcoming). To assess whether the multilocus genotypes would detect the presence of multiple sires in a clutch, we calculated the probability of identity (PI) for each species. Specifically, we were interested in the genetic probability that 2 males (potential sires) in the population had the same or similar genotypes and thus could have contributed identical-by-state (nondistinguishable) alleles to the brood. We also calculated the DNA profile probability (DPP) of each sire's reconstructed genotype, which estimates the probability that a second unrelated male in the population had the same multilocus genotype as the apparent sire. PI and DPP values were calculated in GenAlEx v. 6.4 (Peakall and Smouse 2006).

Results

A total of 32 pregnant female sea snakes were obtained from 8 species (Table 1). Mean clutch size per species ranged from 2.0 to 10.5 (Table 1). In 28 of the 32 clutches examined, all yolked eggs contained embryos. Of the remaining 4 clutches, the ratios of yolked eggs with embryos to yolked eggs without embryos were as follows: 3:1, 4:6, 6:7, 12:5. Because some samples had obviously become severely degraded by the time we acquired them, we focused our genetic analyses on the 12 dams (from 6 species) with the largest broods whose members could be successfully genotyped (and showed consistent Mendelian inheritance) at all loci (Table 1). In all 12 of these cases, subtracting the known dam's alleles from the offspring genotypes left either 1 or 2 paternal alleles per brood at each locus, thus implying monandry in each case. In addition, we uncovered no evidence for multiple sires in any of the other broods for which only partial genetic data were collected.

For *H. elegans* (for which the microsatellite loci were developed), 9 of the 10 microsatellite loci had moderate to high numbers of alleles (7–20) per locus (mean 11.3 ± 1.6 standard error [SE], across all 10 loci), whereas allelic diversity was somewhat lower for *L. curtus* (3–16 alleles per locus, mean 7.1 ± 1.4 SE). Expected heterozygosities (H_e) for *H. elegans* were typically greater than 0.80 (mean 0.71 ± 0.07 SE), whereas H_e values in *L. curtus* were lower (mean 0.45 ± 0.10 SE). The probability that 2 unrelated individuals shared the same genotype across all 10 loci (PI) varied among the 6 species, but in all cases was very low (ranging from 1.5×10^{-10} to 4.2×10^{-5} for the locations (populations) from which dams were sampled; Table 2). The probabilities

Table 2 PI for 6 true sea snake species for which clutches were genotyped and DPP for each sire

Species	Dam's location	PI (*n*)	Australian locations sampled	PI (*n*)	DPP
Hydrophis elegans	Mornington Island, GoC	1.5×10^{-10} (16)	GBR, GoC, WA	1.9×10^{-1} (71)	1.9×10^{-10}
	Weipa, GoC	1.9×10^{-8} (7)		1.4×10^{-10}	
Hydrophis kingii	GBR	4.2×10^{-5} (4)	GBR, GoC	3.1×10^{-6} (9)	1.1×10^{-7}
Hydrophis mcdowelli	GBR	3.0×10^{-6} (8)	GBR, GoC, WA	9.9×10^{-7} (10)	1.5×10^{-5} to 1.6×10^{-6}
Hydrophis occellatus	GBR	5.6×10^{-6} (3)	GBR, GoC	1.2×10^{-8} (14)	2.0×10^{-8}
Hydrophis pacificus	GoC	4.3×10^{-5} (6)	GoC	4.3×10^{-5} (6)	2.8×10^{-7}
Lapemis curtus	GoC	3.5×10^{-7} (34)	GBR, GoC	8.7×10^{-7} (76)	4.1×10^{-8} to 7.4×10^{-9}

PI was calculated for the location from which the dam was sampled and also for all Australian samples collected for each species: (*n*) is the number of individuals used to calculate PI. DPP was calculated for the reconstructed genotype of the sire of each clutch (see text for more details). GBR, Great Barrier Reef; GoC, Gulf of Carpentaria; WA, Western Australia.

that a second unrelated male had the same genotype as the focal sire (DPP) were also very low (ranging from 1.4×10^{-10} to 1.5×10^{-5} across the 12 sires; Table 2). PI and DPP values for *H. elegans* were by far the lowest (Table 2), typically on the order of 10^{-10}, but sires in most of the other species also had DPPs generally in the range of 10^{-7} to 10^{-8} (Table 2). The only partial exception involved *H. mcdowelli*, where 4 focal sires had higher DPPs (10^{-5} to 10^{-6}), primarily because the reconstructed paternal genotypes in each instance were homozygous at 5 of the 10 microsatellite loci (unlike the situation for any of the focal sires in the 5 other species). Nonetheless, even DPPs that are as high as 10^{-5} suggest that there was less than a one-in-one-hundred-thousand chance that another undetected sire shared the same multilocus genotype as the deduced focal sire of a brood.

Discussion

Despite the documented prevalence of multiple paternity across all major taxonomic groups of nonavian reptiles (Uller and Olsson 2008), we did not find any genetic evidence for multiple paternity in the 6 species of "true" sea snakes examined in the current study. This result was completely unexpected, given that nearly all previous analyses of genetic paternity in snakes had documented moderate to high incidences of polyandrous matings by dams (13 studies summarized in Table 1 of Uller and Olsson 2008 and 17 studies in Table 1 of Voris et al. 2008). These earlier reports had focused on 7 species from 3 terrestrial groups of advanced snakes (Natricidae, Colubridae, and Viperidae) plus the water python (Pythonidae), and in 5 of those species, more than 50% of the surveyed clutches had 2 or more sires. In addition, Voris et al. (2008) recently documented multiple paternity in 2 mud snake species (Homalopsidae), as did Wusterbath et al. (2010) for several phylogenetic lineages of New World natricids.

Methodological Reasons for Failing to Demonstrate Multiple Paternity

Highly polymorphic genetic markers are essential for ensuring that cases of multiple paternity, if present, can be detected with high assurance. We used 10 moderately to highly variable microsatellite loci (Lukoschek and Avise, forthcoming) to screen 12 broods from 6 species of sea snakes (Table 1), yet we uncovered no evidence for genetic polyandry by the pregnant dams. Both the PI for each species and the DPP of each sire's reconstructed genotype were very small in each case (Table 2); thus, multiple paternity within the surveyed broods, if present, almost certainly would have been detected by these markers. Indeed, previous genetic reports of multiple paternity in snakes typically used fewer microsatellite loci (see Voris et al. 2008, Table 1 for summary of previous studies) than we have employed in the current study. For example, Wusterbath et al. (2010) employed only 1 or 2 microsatellite loci (see their Table 1) to deduce multiple paternity in 6 of the 7 natricid snake species they examined.

Null (i.e., nonamplifying) alleles can also cause genuine cases of multiple paternity to go undetected, and null alleles at microsatellite loci can be especially problematic in cross-species amplifications via the polymerase chain reaction (Dakin and Avise 2004). However, tests of HWE for *H. elegans* and *L. curtus*, for which reasonably large sample sizes of adults were available (Lukoschek and Avise, forthcoming), suggest that null alleles were not a serious issue. Perhaps more relevant is the fact that the microsatellite genotypes with "non-null" (i.e., detected) alleles were fully consistent with Mendelian inheritance in all broods, further supporting the conclusion that only one male sired each clutch.

The third possible reason for failing to detect multiple paternity is screening a small number of clutches. Our study focused on 12 clutches from 6 species but also obtained genetic information for 20 additional partial clutches spanning a total of 8 *Hydrophis* group species (Table 1). In no case was there evidence of more than 1 sire per clutch at any of the 10 microsatellite loci screened. Nonetheless, reviews of previous studies of multiple paternity in reptiles (Pearse and Avise 2001; Avise 2007; Voris et al. 2008; Uller and Olsson 2008) highlight one salient fact: Estimated rates of multiple paternity can vary dramatically (from 0% to 100% of assayed clutches) within and among the species

investigated. Thus, any future studies of genetic paternity in sea snakes or the closely related Australian terrestrial elapids might yield outcomes that are very different from what we have reported here.

Biological Reasons for Paucity of Multiple Paternity in Sea Snakes

Arguably, the possibility that multiple sires will have contributed to a clutch generally tends to increase with larger clutch sizes. True sea snakes give birth to live young and many species have small broods (Fry et al. 2001). In our study, 4 of the 8 surveyed species carried fewer than 5 embryos per clutch, on average, and only 2 species had mean clutch sizes of 10 or more (Table 1). Thus, viviparity coupled with a relatively small clutch size might itself predispose sea snakes to monandry. On the other hand, most of the terrestrial snake species in which polyandry previously has been documented are also live-bearers, albeit typically with somewhat larger clutch sizes (7–23 embryos on average; Uller and Olsson 2008). For example, Voris et al. (2008) documented a minimum of 3–5 fathers for each of 5 clutches containing 18–36 embryos in 2 viviparous mud-snake species, and Wusterbath et al. (2010) documented multiple paternity in 6 of 7 clutches from several live-bearing natricid species with a mean of 18 progeny per brood. Moreover, the 2 oviparous snake species previously shown to have multiple paternity do not have particularly large clutch sizes (Madsen et al. 2005), and high levels of multiple paternity have been demonstrated in many lizard species with extremely small clutches (<5 offspring in most cases; Uller and Olsson 2008). Thus, overall, the mode of parturition (live-bearing vs. egg-laying) and any associated differences in mean clutch size clearly are inadequate predictors, by themselves, of monandrous versus polyandrous genetic outcomes in reptiles.

For the sea snakes, we focused our paternity analyses on the largest available clutches for each species and on the 2 species in which the pregnant females carried broods with 10 or more progeny (Table 1). Nevertheless, highly polymorphic microsatellite markers clearly indicated that only 1 sire was present in each case. Although much larger genetic surveys are needed, the current evidence for monandry in the sea snakes cannot easily be dismissed as some overt sampling bias in the particular genetic markers or broods analyzed.

Phylogenetic Reasons for the Paucity of Multiple Paternity in Sea Snakes

Although multiple paternity is known to be common in snakes, Voris et al. (2008) suggested broadening the taxonomic scope of genetic paternity analyses to better understand the evolution of snake mating systems. To the best of our knowledge, the current study is the first to investigate multiple paternity in any of the ~300 species of Elapidae, so it is unclear whether monandry is typical of elapids or whether it is limited to the highly specialized and relatively recent phylogenetic radiation of sea snakes. Multiple paternity has been genetically documented in 4 major groups of advanced snakes (viperids, homalopsids, colubrids, and natricids), as well as one primitive python species. The viperids and homalopsids are basal to the Elapidae, whereas the colubrids plus natricids are reciprocally monophyletic with respect to the elapid clade (Vidal et al. 2007). As such, it seems parsimonious to assume that multiple paternity is ancestral in advanced snakes and perhaps has been lost somewhere in the elapid radiation, either before or after the evolutionary emergence of true sea snakes. Only further analyses will tell.

Funding

Sea World Research and Rescue Foundation; the University of California, Irvine; and the ARC Centre of Excellence for Coral Reef Studies.

Acknowledgments

We thank Andrei Tatarenkov, Felipe Barreto, and Rosemary Byrne for practical advice and lively discussions about the development of microsatellites and cross-species amplification. We thank Dee White for enthusiastically applying for DEH permits to collect dead snakes from trawlers and depositing specimens at the Museum and Art Gallery of the Northern Territory (MAGNT). We thank Paul Horner and Dane Trembath from the MAGNT, and Andrew Amey and Patrick Couper from the Queensland Museum for assistance with accessing collections and obtaining samples from gravid females and embryos.

References

Avise JC. 2007. Conservation genetics of marine turtles—ten years later. In: Hewitt D, Fulbright T, editors. Frontiers in wildlife science: linking ecological theory and management applications. Boca Raton (FL): CRC Press. p. 295–314.

Birkhead TR, Moller AP. 1993. Sexual selection and the temporal separation of reproductive events: sperm storage data from reptiles, birds and mammals. Biol J Linn Soc. 50:295–311.

Blouin-Demers G, Gibbs HL, Weatherhead PJ. 2005. Genetic evidence for sexual selection in black ratsnakes. Anim Behav. 69:225–234.

Courtney AJ, Schemel BL, Wallace R, Campbell MJ, Mayer DG, Young B. 2009. Reducing the impact of Queensland's trawl fisheries on protected sea snakes. Brisbane (Australia): Queensland Primary Industries and Fisheries.

Dakin EE, Avise JC. 2004. Microsatellite alleles in parentage. Heredity. 93:504–509.

Fry GC, Milton DA, Wassenberg TJ. 2001. The reproductive biology and diet of sea snake bycatch of prawn trawling in northern Australia: characteristics important for assessing the impacts on populations. Pac Conserv Biol. 7:55–73.

Gibbs HL, Weatherhead PJ. 2001. Insights into population ecology and sexual selection in snakes through the application of DNA-based genetic markers. J Hered. 92:173–179.

Heatwole H. 1999. Sea snakes. Sydney (Australia): University of New South Wales Press.

Lukoschek V, Avise JC. 2011. Development of ten polymorphic microsatellite loci for the sea snake Hydrophis elegans (Elapidae: Hydrophiinae) and cross-species amplification for fifteen marine hydrophine species. Cons Gen Res. doi:10.1007/s12686-011-9388-5

Lukoschek V, Keogh JS. 2006. Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation. Biol J Linn Soc. 89:523–539.

Madsen T, Ujvari B, Olsson M, Shine R. 2005. Paternal alleles enhance female reproductive success in tropical pythons. Mol Ecol. 14:1783–1787.

McCracken GF, Burghardt GM, Houts SE. 1999. Microsatellite markers and multiple paternity in the garter snakes *Thamnophis sirtalis*. Mol Ecol. 8:1475–1479.

Olsson M, Madsen T. 2001. Promiscuity in sand lizards (*Lacerta agilis*) and adder snakes (*Vipera berus*): causes and consequences. J Hered. 92:190–197.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 6:288–295.

Pearse DE, Avise JC. 2001. Turtle mating systems: behaviour, sperm storage, and genetic paternity. J Hered. 92:206–211.

Sever DM, Ryan TJ. 1999. Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*): Part 1. Evidence for oviducal sperm storage. J Morphol. 241:1–18.

Stobutzki I, Blaber S, Brewer D, Fry GC, Heales D, Miller M, Milton DA, Salini JP, Van der Velde T, Wassenberg TJ, et al. 2000. Ecological sustainability of bycatch and biodiversity in prawn trawl fisheries. FRDC Final Report 96/257. Cleveland (OH): CSIRO Marine Research.

Uller T, Olsson M. 2008. Multiple paternity in reptiles: patterns and processes. Mol Ecol. 17:2566–2580.

Ursenbacher S, Erny C, Fumagalli L. 2009. Male reproductive success and multiple paternity in wild, low-density populations of the adder (*Vipera berus*). J Hered. 100:365–370.

Vidal N, Delmas AS, David P, Cruaud C, Couloux A, Hedges SB. 2007. The phylogeny and classification of caenophidian snakes inferred from seven nuclear protein-coding genes. C R Biol. 330:182–187.

Voris HK, Karns DR, Feldheim KA, Kechavarzi B, Rinehart M. 2008. Multiple paternity in the Oriental-Australian rear-fanged watersnakes (Homalopsidae). Herp Conserv Biol. 3:88–102.

Wusterbath TL, King RB, Duvall MR, Grayburn WS, Burghardt GM. 2010. Phylogenetically widespread multiple paternity in New World natricine snakes. Herp Conserv Biol. 5:86–93.

Received December 8, 2010; Revised January 25, 2011; Accepted February 22, 2011

Corresponding Editor: Stephen Karl