Behavioural correlates with hemipenis morphology in New World natricine snakes

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Copulatory organs (hemipenes) of male snakes vary markedly among species in shape and ornamentation. We suggest that sexual conflict over copulation duration may have shaped the evolution of hemipenis morphology, favouring more elaborate organs in species in which a long duration of copulation is especially beneficial to males, despite the associated costs to females. To test this proposition, we compare mating behaviour between two species of gartersnakes differing in hemipenis morphology. In addition, we review data on copulation duration and hemipenis morphology and relate hemipenis morphology to phylogeny among of New World natricines. As predicted, copulation duration was significantly shorter in the common gartersnake (*Thamnophis sirtalis*), a species with simple subcylindrical hemipenes, than in the plains gartersnake (*Thamnophis radix*), a species with more complex, bilobed organs. Furthermore, female *T. radix* frequently exhibited vigorous body rolls during copulation, a behaviour associated with copulation termination, whereas female *T. sirtalis* never exhibited this behaviour. Copulations were of shorter duration when female *T. radix* (but not *T. sirtalis*) more greatly exceeded males in body size, suggesting that females can more easily disengage from small males. Our review of New World natricines provides only weak evidence for an association between copulation duration and hemipenis morphology. Our mapping of hemipenis morphology onto the New World natricine phylogeny suggests that hemipenis morphology is evolutionarily plastic; both simple and bilobed hemipenes occur in all three major natricine clades, as well as in two of three gartersnake subclades and several sister-species pairs. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **98**, 110–120.

ADDITIONAL KEYWORDS: copulation duration – hemipenes – *Nerodia* – sexual conflict – *Thamnophis*.

INTRODUCTION

Morphological and behavioural traits associated with reproduction have fascinated evolutionary biologists ever since Darwin (1871) proposed sexual selection as a possible explanation for the elaboration of such traits. Historically, intrasexual selection and mate choice were seen as the primary mechanisms of sexual selection (Fisher, 1930; Anderson, 1994). More recently, biologists have recognized that sexual conflict may be an important factor in the evolution of sexual traits (Chapman *et al.*, 2003; Arnqvist & Rowe, 2005). Sexual conflict occurs when reproductive fitness is maximized in different ways in males and females and can produce antagonistic patterns of selection on male and female traits related to reproduction (Chapman *et al.*, 2003; Arnqvist & Rowe, 2005). Evolutionary outcomes of sexual conflict are sometimes unexpected and often only understood through detailed cost–benefit analyses. Such analyses have been conducted quite successfully in a number of taxa, especially those in which manipulative experiments or molecular analyses of paternity are feasible (Shine, O'Connor & Mason, 2000b; Royle, Hartley & Parker, 2002; Shine *et al.*, 2004b; Fitze & Le Galliard, 2008). In the present study, we suggest the possible occurrence of sexual conflict over snake copulation duration and describe how sexual conflict may have influenced the evolution of male snake copulatory organs.

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Male snake copulatory organs, or hemipenes, are paired, blind, tubular structures that are normally retracted within the base of the tail but are everted during copulation. Hemipenis morphology varies widely among snake taxa and includes cylindrical, bulbous, bilobed, and deeply divided structures ornamented with flounces, calyces, papillae, and spines (Dowling & Savage, 1960: figs 3–6) that may facilitate intromission, anchor males to females, or play a stimulatory role (Pisani, 1976; Murphy & Barker, 1980; Böhme, 1988; Olsson & Madsen, 1998). Consistent with this is the observation that hemipenial spines are widespread among snakes but are uncommon among lizards and, coincidentally, snakes exhibit markedly longer copulation duration than do lizards (Olsson & Madsen, 1998). Functional interpretations of intromittent organ morphology exist for many taxa (Eberhard, 1985; Birkhead & Moller, 1998) and suggest that variation in these structures often correlates with ecology.

Sexual conflict over copulation duration in snakes might be expected because of anticipated differences in the costs and benefits of increased copulation duration between males and females (Arnqvist & Rowe, 2005). For example, increased copulation duration may benefit males through greater insemination success; reduced opportunities for female remating, sperm competition, and cryptic female choice; and (in some species) more successful cloacal plug formation, but may also carry costs in terms of reduced mating opportunities, sperm depletion, or increased risk of predation (Olsson & Madsen, 1998; Shine *et al.*, 2000c, e). For females, increased copulation duration may result in higher fertility but reduce opportunities for multiple mating, sperm competition, and cryptic female choice; increase the risk of injury; or increase the risk of predation (Ross & Crews, 1978; Olsson & Madsen, 1998). Costs and benefits are likely to vary among populations and species. For example, predation risk may differ depending on the array of sympatric predators and the degree of exposure during copulation. Opportunities for multiple mating by males (and hence the cost to males of long copulation duration) may vary depending on male and female dispersion patterns, population density, operational sex ratio, and length of breeding season. Red-sided gartersnakes (*Thamnophis sirtalis parietalis* Say) in Manitoba may represent the extreme in this regard because males and females have an extremely clumped distribution, active males greatly outnumber females, multiple males court females simultaneously, and the breeding season is limited to few weeks each year (Gregory, 1977; Garstka, Camazine & Crews, 1982; Shine, Langkilde & Mason, 2003a). In this species, males exhibit short-duration copulation, thus potentially increasing their opportunity to mate

with multiple females (Shine *et al.*, 2000c, e). Males also deposit a gelatinous copulatory plug that reduces the likelihood that females remate (Devine, 1977; Shine, Olsson & Mason, 2000d). In other species (e.g. pit vipers), females are widely dispersed, male–male combat occurs, and victorious males guard females, thus limiting access to females by rival males (Duvall, Arnold & Schuett, 1992). Here, costs associated with long-duration copulation may be negligible to males because opportunities to mate with other females are rare.

In the present study, we take a comparative approach in testing for behavioural correlates with hemipenis morphology. First, we verify the occurrence of variation in copulation duration and copulatory behaviour using data from staged matings among captive snakes of two species. These species, the common gartersnake, *Thamnophis sirtalis* (Linnaeus), and the plains gartersnake, *Thamnophis radix* (Baird and Girard), differ markedly in hemipenis morphology, with the common gartersnake possessing a simple subcylindrical organ and the Plains gartersnake possessing a bilobed organ (Fig. 1A, E). Copulation duration differs between these species (significantly longer copulation duration in *T. radix*, the species with a bilobed organ; see Results), suggesting an association between copulation duration and hemipenis morphology. Therefore, we review data on copulation duration and hemipenis morphology among New World natricines more generally. Finally, we map hemipenis morphology on a phylogeny of New World natricine snakes to assess the degree to which hemipenis evolution may be driven by sexual conflict or constrained by phylogeny.

MATERIAL AND METHODS

STUDY ORGANISMS

We focus on New World natricine snakes (gartersnakes, watersnakes, and their allies) because behavioural and morphological data are available for many species and molecular data provide a strong phylogenetic framework. New World natricines include approximately 56 species divided into three well-resolved clades, the watersnakes (*Nerodia*, some *Regina*, *Tropidoclonion*), gartersnakes (*Adelophis*, *Thamnophis*), and semifossorial natricines (*Clonophis*, some *Regina*, *Seminatrix*, *Storeria*, *Virginia*) (Alfaro & Arnold, 2001). The gartersnake clade is further divided into Mexican, widespread, and ribbon snake subclades (de Queiroz, Lawson & Lemos-Espinal, 2002). New World natricines are live-bearing and females typically exceed males in body size (Rossman, Ford & Seigel, 1996; Gibbons & Dorcas, 2004). In general, reproduction occurs once per year.
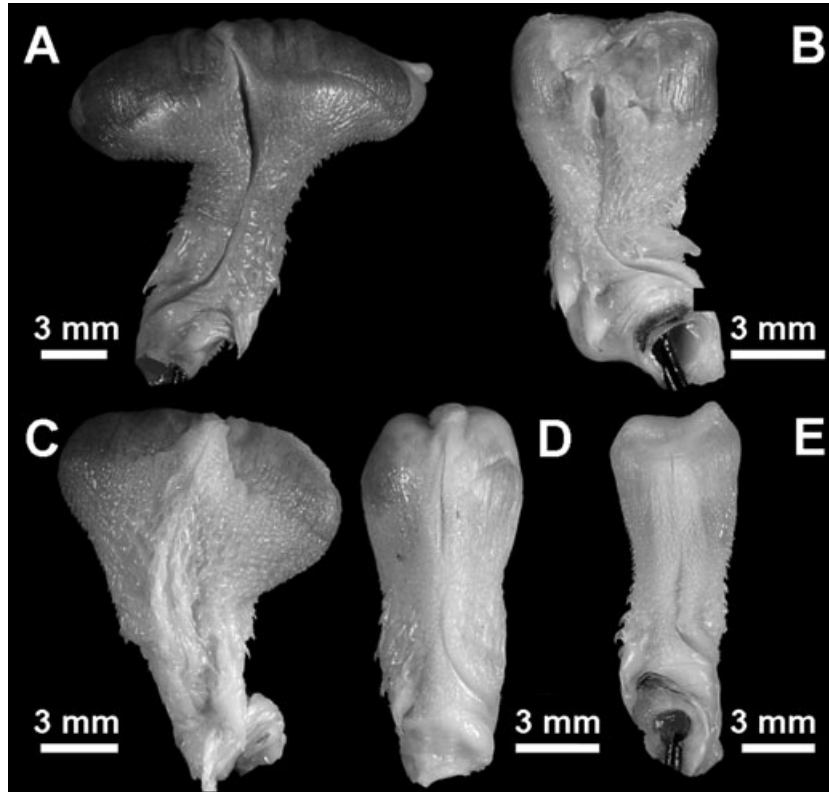
Figure 1. Everted hemipenes (sulcate view). A, *Thamnophis radix*, B, *Thamnophis butleri*, C, *Thamnophis eques*, D, *Thamnophis marcianus*, E, *Thamnophis sirtalis*. Hemipenes of *T. butleri*, *T. marcianus*, and *T. sirtalis* are simple and subcylindrical; those of *T. radix* and *T. eques* are bilobed.

Courtship and mating typically occur in spring and parturition in summer or fall. Multiple males simultaneously court individual females in what has been described as 'scramble competition', although detailed studies of red-sided gartersnakes suggest that the determinants of mating success are more subtle and complex than previously recognized (Shine *et al.*, 2000a, b, c, d, e, 2001, 2003a, c, d, 2004b, 2005; Shine, Langkilde & Mason, 2003b, 2004a; Shine & Mason, 2005).

Among natricine snakes, courting males align their bodies with that of the female, exhibit caudocephalic waves that may stimulate female receptivity, and attempt to oppose their cloaca with that of the female (Rossman *et al.*, 1996). In addition, males can take advantage of anatomical and physiological characteristics of females to force copulation (Shine *et al.*, 2003b). Intromission begins abruptly when the female gapes her cloaca and the male everts a hemipenis therein. At this point, copulating males become more passive and may be dragged, hemipenis first, as the female moves about. Intromission also ends abruptly, sometimes as a result of vigorous body rolls by females (Perry-Richardson, Schofield & Ford, 1990). In some natricines, including *T. radix* and *T. sirtalis*, males deposit a gelatinous mating plug at the end of copulation (Devine, 1977; Ross & Crews, 1977; Shine *et al.*, 2000d). This plug serves as a temporary barrier to subsequent mating by females but some females do mate multiple times (Shine *et al.*, 2000d) and individual litters are frequently sired by two or more males (Voris *et al.*, 2008).

DOES COPULATION DURATION AND BEHAVIOUR DIFFER BETWEEN *T. SIRTALIS* AND *T. RADIX*?

Captive matings involved descendents of wild-caught females from study sites in Ohio (*T. sirtalis*; King, 2003) and Illinois (*T. radix*; Stanford & King, 2004). Snakes were housed individually in cages containing paper substrate and a cover object. Fresh water was available continuously and food (earthworms, mice) was provided one to three times a week. The room in which snakes were housed was maintained at 24–26 °C under a 12 : 12 light/dark cycle. Heat tape under one end of cages provided a thermal gradient ranging from room temperature to approximately 30 °C for 12 h each day. All snakes were individually marked by clipping ventral scales. Prior to mating

trials, snakes underwent simulated hibernation by placing them in a reach-in environmental chamber at 8 °C with a 0 : 24 light/dark cycle for 8–12 weeks. Mating trials were conducted in a walk-in environmental chamber at 28–30 °C. A single female was placed in a 40-L glass aquarium with three to eight males. Males were marked with white latex paint for individual recognition and mating trials were videotaped so that the identity of mating males, copulation duration (in minutes), and the frequency with which females exhibited body rolls during copulation could be determined.

IS THERE AN ASSOCIATION BETWEEN COPULATION DURATION AND HEMIPENIS MORPHOLOGY IN NEW WORLD NATRICINE SNAKES?

Information on hemipenis morphology was obtained from published accounts and examination of museum material. We included only taxa for which hemipenis shape was clearly illustrated, specimens with fully everted hemipenes were available, or an unequivocal statement of hemipenis morphology exists. Specifically, we distinguished between simple (Dowling & Savage, 1960: fig. 3A–D) and bilobed organs (Dowling & Savage, 1960: fig. 3E). Furthermore, we distinguished between weakly bilobed and fully bilobed organs based on the depth to which lobes were divided *sensu* Keogh (1999) (i.e. our weakly bilobed classification corresponds to Keogh's shallowly forked category).

Information on copulation duration was obtained from our observations (*T. siratlis*, *T radix*) and published accounts. Many of these data were based on observations of captive animals and consisted of only a single observation. Our interpretations of patterns of association between hemipenis morphology and copulation duration is necessarily qualitative. Data are too few to conduct formal phylogenetically-based analyses (Martins, 1996).

HOW DOES HEMIPENIS MORPHOLOGY MAP ONTO NEW WORLD NATRICINE PHYLOGENY?

To take the analysis of snake hemipenis morphology one step further, we mapped hemipenis morphology (single, weakly bilobed, bilobed) onto a molecular phylogeny of New World natricine snakes. Phylogenetic relationships were based on combined analyses of de Queiroz *et al.* (2002) (i.e. for *Thamnophis* and *Adelophis*) and Alfaro & Arnold (2001) (for other genera) using strict consensus (de Queiroz *et al.*, 2002: fig. 1) and maximum likelihood (Alfaro & Arnold, 2001: fig. 5) with poorly supported nodes (bootstrap proportions < 65%) collapsed.

RESULTS

DOES COPULATION DURATION AND BEHAVIOUR DIFFER BETWEEN *T. SIRTALIS* AND *T. RADIX*?

Copulation duration was determined for 15 matings involving 13 female and five male *T. sirtalis* and averaged 17 min (range = 7–25 min). Copulation duration was determined for 13 matings involving ten female and seven male *T. radix* and averaged 98 min (range = 43–201 min). Variance in copulation duration was significantly lower in *T. sirtalis* than in *T. radix* (*T. sirtalis* = 20 min^2; *T. radix* = 2214 min^2; Levene's test for equality of variances, $F = 23.40$, $P < 0.001$). However, coefficients of variation in copulation duration were similar between species (*T. sirtalis* = 27%; *T. radix* = 48%, $F_{14,12}$ = 2.52, $P > 0.10$) (Zar, 1999: 141). Using Welch's approximate t to accommodate differences in variance (Zar, 1999: 128), copulation duration was significantly shorter in *T. sirtalis* than in *T. radix* ($t = 6.18$, d.f. = 12.19, $P < 0.001$). Because some individuals participated in more than one mating, the assumption of independence may be violated in these analyses. However, the magnitude of the difference in copulation duration suggests that conclusions were little affected by this problem: the shortest copulation observed in *T. radix* (43 min) was almost two-fold greater than the longest copulation observed in *T. sirtalis* (25 min).

To determine whether copulation duration was influenced by the degree to which males and females differed in body size, we calculated the ratio of male mass to female mass for each mating pair. Copulation duration increased significantly with increasing male mass/female mass in *T. radix* (Spearman rank correlation, $R_s = 0.59$, $N = 13$, $P = 0.034$) (Fig. 2). No such pattern was evident in *T. sirtalis* ($R_s = 0.05$, $N = 12$, $P = 0.878$), although there was a narrower range of variation in male mass/female mass in this species (Fig. 2), Copulation duration was uncorrelated with male mass (independent of female mass) in both *T. radix* ($R_s = 0.11$, $N = 13$, $P = 0.729$) and *T. sirtalis* ($R_s = -0.34$, $N = 12$, $P = 0.239$).

Females initiated body rolls in ten of 12 matings in *T. radix* (the occurrence of body rolls could not be observed in one mating), with an average of 60 body rolls per copulation (range = 0–246). By contrast, body rolls were never observed in 15 matings in *T. sirtalis*. Body rolls occurred throughout copulation in *T. radix* and, in four cases, termination of copulation was associated with body rolls. Copulation duration was significantly shorter in these four matings than in eight matings in which body rolls were not exhibited or were not associated with the termination of copulation, averaging 57 versus 123 min ($t = 2.88$, d.f. = 10, $P = 0.016$).
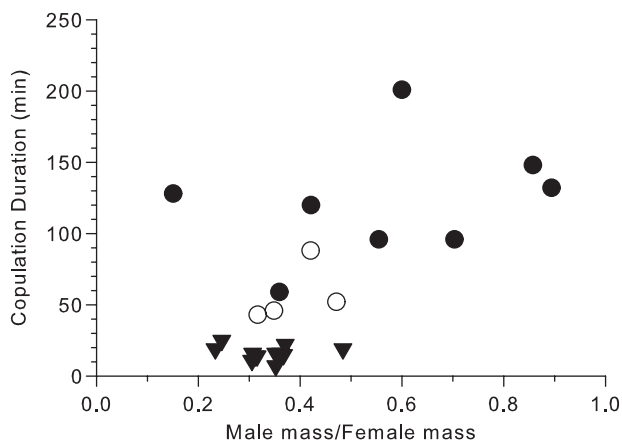
Figure 2. Relationship between copulation duration and male mass/female mass in *Thamnophis radix* (circles) and *Thamnophis sirtalis* (inverted triangles). In *T. radix*, matings that ended with body rolls are indicated by open circles, whereas matings in which the termination of copulation was unassociated with body rolls are indicated by closed circles.

IS THERE AN ASSOCIATION BETWEEN COPULATION DURATION AND HEMIPENIS MORPHOLOGY IN NEW WORLD NATRICINE SNAKES?

Information on copulation duration and hemipenis morphology was obtained for 11 species of New World natricines (Table 1). Across species, copulation duration varied from as little as six minutes (*Thamnophis marcianus* Baird and Girard) to more than 390 min (*Tropidoclonion lineatum* Hollowell). By contrast, copulation duration within species was much less variable. Among four species in which five or more matings were observed (*T. marcianus*, *Thamnophis melanogaster* Peters, *T. radix*, *T. sirtalis*), copulation duration varied by less than a factor of five (e.g. from 43–201 min in *T. radix*) and coefficients of variation in copulation duration were similar, ranging from 0.21 (*T. melanogaster*) to 0.56 (*T. sirtalis*) (Table 1).

Differences in copulation duration between species with simple versus bilobed hemipenes only approached statistical significance and ranges overlapped broadly. In five species with simple hemipenes, median duration = 27 min, range = 6–88 min; in six species with bilobed hemipenes, median duration = 60 min, range = 14–390 min (one-tailed Mann–Whitney U-test, $P = 0.056$). Furthermore, the sister taxa *T. radix* and *T. butleri* (Cope) had apparently similar copulation duration despite having different hemipenis morphology (Fig. 1A, B).

HOW DOES HEMIPENIS MORPHOLOGY MAP ONTO NEW WORLD NATRICINE PHYLOGENY?

We combined phylogenetic information from Alfaro & Arnold (2001) and de Queiroz *et al.* (2002) to generate

a single phylogeny that included most species of New World natricine snakes to which we added available information on hemipenis morphology (Fig. 3). Although hemipenis morphology is not known for all species, it is apparent that this character is evolutionarily plastic within New World natricines, having changed between simple and bilobed seven or more times: (1) members of the semifossorial clade all have simple or weakly bilobed hemipenes; (2) with the exception of *Regina septemvittata* (Say), and *Regina grahamii* Baird and Girard), members of the watersnake clade all have bilobed hemipenes; and (3) within the gartersnake clade, all three members of the ribbon snake subclade have simple hemipenes, whereas members of the Mexican subclade and the widespread subclade exhibit both simple and bilobed hemipenes.

DISCUSSION

Sexual conflict theory suggests that the evolution of snake hemipenis morphology may have been driven by differing costs and benefits of longer copulation duration in males versus females. In particular, a greater benefit to males of longer copulation duration would favour the evolution of more complex hemipenis morphology, allowing males to better resist female termination of copulation. Our two-species comparison provides evidence consistent with this expectation. Female *T. radix* typically move about during copulation and, although males attempt to keep their body aligned with that of the female, they are often dragged along hemipenis first. Furthermore, female *T. radix* frequently exhibit repeated body rolls during copulation, requiring males to roll in response and sometimes resulting in males' bodies encircling that of the females multiple times. Such body rolls may function in disengaging a copulating male, as has been suggested in the checkered gartersnake, *T. marcianus* (Perry-Richardson *et al.*, 1990). Body rolls also occur during copulation in the Mexican gartersnake, *T. melanogaster* (Ball, 1978). The incidence of body rolls appears similar in *T. radix* and *T. marcianus*, occurring in ten of 12 *T. radix* matings and eight of 12 *T. marcianus* matings (range = 1–206 versus 2–109 complete rolls per mating in *T. radix* and *T. marcianus*, respectively), although copulation duration is markedly shorter in *T. marcianus* (Table 1). Furthermore, we observed that copulation duration was shorter in matings in which the termination of copulation was associated with such body rolls. We also observed that copulation duration in *T. radix* (but not *T. sirtalis*) was briefer when females greatly exceeded males in body size, suggesting that perhaps females can more easily disengage from small males (with proportionately smaller hemipenes). By contrast,

Table 1. Copulation duration and hemipenis morphology of New World natricine snakes

| Species	Duration (min)	N	Reference	Hemipenis shape	Reference
Nerodia fasciata (Linnaeus)	> 60	–	Ernst & Ernst (2003)	Bilobed	Present study
Nerodia sipedon (Linnaeus)	23	2	Brown (1940)	Bilobed	Mittleman (1947); Dowling & Savage (1960)
Storeria dekayi (Hollbrook)	24 ~30	1 –	Noble (1937) Ernst & Ernst (2003)	Simple	Trapido (1944); present study
Thamnophis brachystoma (Cope)	> 40	1	Pisani (1967)	Simple	Present study
Thamnophis butleri (Cope)	135 40	1 1	Ruthven (1912) Noble (1937)	Simple	Present study
Thamnophis elegans (Baird and Girard)	105	1	Riches (1967)	Bilobed	Present study
Thamnophis marcianus (Baird and Girard)	6, CV = 0.29	12	Perry-Richardson, Schofield & Ford (1990)	Simple	Present study
Thamnophis melanogaster (Peters)	13 (10–18), CV = 0.21	6	Ball (1978)	Bilobed	Present study
Thamnophis radix (Baird and Girard)	98 (43–201), CV = 0.48	13	Present study	Bilobed	Dowling & Savage (1960); this study
Thamnophis sirtalis (Linnaeus)	14, CV = 0.36 20, CV = 0.56 19 (8–50), CV = 0.53 17 (7–25), CV = 0.27	113 131 ~100 15	Shine et al. (2000b) Shine et al. (2000c) Present study	Simple	Pfrender et al. (2001); this study
Tropidoclonion lineatum (Hallowell)	> 390	1	Ramsey (1946)	Bilobed	Dowling & Savage (1960)

When sample size (N) is ≤ 3, the duration of each copulation is given if known. Otherwise mean, lower limit (indicated by >), or approximate duration is reported. Ranges are indicated in parentheses. Coefficients of variation (CV) are reported for N ≥ 5. The material examined in this study is reported in the Suporting information (Appendix S1).

copulation duration was uncorrelated with male body size, suggesting that early disengagement does not result solely from a preference by females for larger males. Body rolls were not observed in *T. sirtalis* and termination of copulation appeared more deliberate (as described in *Lampropeltis getulus* Linnaeus; Secor, 1987), suggesting that sexual conflict over copulation duration may be reduced in this species. However, there may also be among-population variation in this behaviour. *Thamnophis sirtalis* from Manitoba, Canada frequently exhibited body rolls ('axial rotations') during staged mating trials involving four to 24 males (Shine et al., 2003b).

By limiting copulation duration, females may increase their opportunity for multiple mating and hence sperm competition and cryptic female choice. Accumulating evidence suggests that multiple paternity is taxonomically widespread in snakes generally and in natricines in particular (Voris et al., 2008). However, direct evidence of sperm competition and

cryptic female choice in reptiles remains scant (Olsson & Madsen, 1998; Uller & Olsson, 2008). Multiple paternity occurs in both *T. sirtalis* (Schwartz, McCracken & Burghardt, 1989; McCracken, Burghardt & Houts, 1999; King et al., 2001; Garner et al., 2002) and *T. radix* (T. Wusterbarth, pers. comm.), although whether this is attributable to multiple within-season matings, fall and spring matings, or sperm-carry over between years, remains unknown.

Our observation that copulation duration was correlated with the difference in male and female body size in *T. radix* but not *T. sirtalis* may have implications regarding the evolution of snake body size dimorphism. Specifically, males might be expected to be small relative to females in species in which males gain little from longer copulation duration (e.g. species in which polyandry is rare). By contrast, males might be expected to be larger relative to females in species in which males benefit from longer
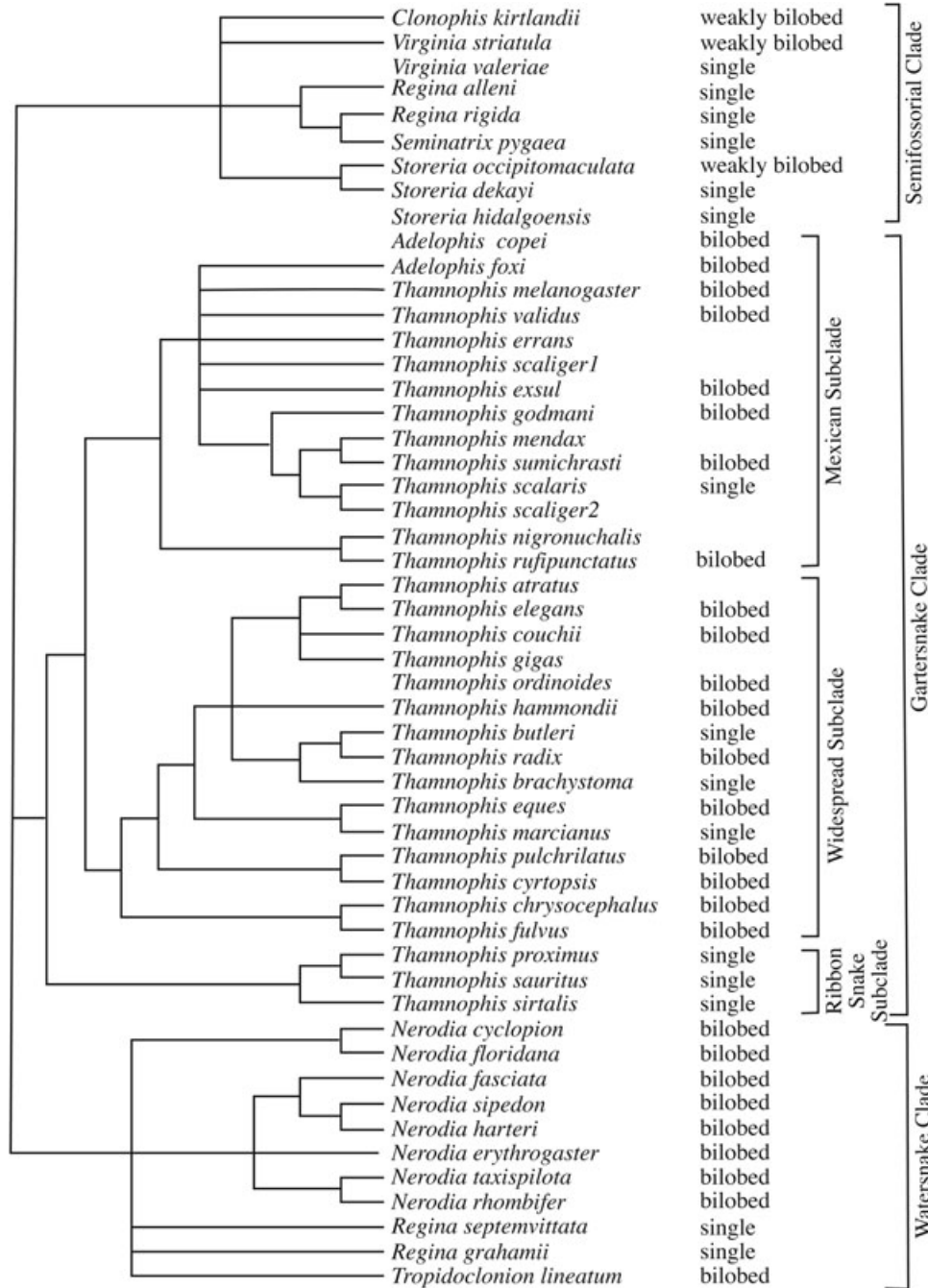
Figure 3. Phylogenetic relationships and hemipenis morphology of New World natricine snakes. Phylogenetic relationships are based on combined molecular analyses of de Queiroz *et al.* (2002) (for (*Thamnophis* and *Adelophis*) and Alfaro & Arnold (2001) (for other genera). Tree shown is based on strict consensus (Queiroz *et al.*, 2002) and maximum likelihood (Alfaro & Arnold, 2001) with poorly supported nodes (bootstrap proportions < 65%) collapsed. New World natricines not included are *Nerodia paucimaculata* (Tinkle and Conant) and *Storeria victa* (Hay). Species for which molecular phylogenetic data are lacking but for which hemipenial morphology is known (*Adelophis copei* Dugès, *Nerodia clarkia* Baird and Girard, *Storeria hidalgoensis* Taylor, *Storeria storeroides* Cope, *Virginia valeriae* Baird and Girard) have been placed next to congenerics. Sources for information on hemipenis morphology not included in Table 1, but are available in the Supporting information (Appendix S2).

copulation duration (e.g. species in which polyandry is more common). Male mating success increases with increasing body size, even in species in which females are the larger sex (Madsen & Shine, 1993; Luiselli, 1996; Shine *et al.*, 2000c, 2001; Kissner, Weatherhead & Gibbs, 2005). Furthermore, larger males sire more offspring within multiply sired litters (Blouin-Demers, Gibbs & Weatherhead, 2005; Ursenbacher, Erny, & Fumagalli, 2008; Dubey *et al.*, 2009). Possibly, larger males achieve longer duration copulations and, as a consequence, transfer larger quantities of sperm and achieve greater success in sperm competition.

The results obtained in the present study may also have implications regarding the evolution of snake hemipenis allometry (Bernstein & Bernstein, 2002; Hosken & Stockley, 2004; Ramos *et al.*, 2005). Specifically, males might be expected to have smaller hemipenes that vary isometrically with body size in species in which males gain little from longer copulation duration and larger hemipenes that show negative allometry (i.e. small males have relatively larger hemipenes) in species in which males benefit from longer copulation duration (Eberhard, Rodriguez, & Polihronakis, 2009: fig. 2). Hemipenis volume increases with increasing snout–vent length, with a slope not differing significantly from three in *T. sirtalis* (Shine *et al.*, 2000c: table 1), suggesting isometric variation in hemipenis size in this species. However, formal allometric analyses have not been conducted.

Our compilation of copulation duration (Table 1) emphasizes the variability of this trait across New World natricines. As in snakes, copulation duration is highly variable in mammals but, contrary to predictions based on predation risk, time/energy budgets, and mating system, copulation duration is longer (not shorter) in small-bodied mammals and does not vary with degree of polyandry (i.e. as measured by relative testis size) (Stallman & Harcourt, 2006). One possibility is that reduced agility of large animals results in the negative correlation between body size and copulation duration in mammals (Stallman & Harcourt, 2006). The observation that copulation duration is long in some large-bodied snake species (e.g. 7–32 h in some Boidae: Huff, 1980; Gillingham & Chambers, 1982; > 3 h in *Drymarchon corais* Fitzinger: Gillingham & Chambers, 1980) suggests that this pattern may not hold in snakes.

Our mapping of hemipenis shape onto a phylogeny of New World natricine snakes (Fig. 3) demonstrates that at least one aspect of hemipenis morphology, whether the hemipenes are simple or bilobed, is evolutionarily plastic. Both morphologies appear in all three clades of New World natricines, as well as in two of three gartersnake subclades and several sister taxa pairs (e.g. *T. radix* and *T. butleri*; *T. marcianus* and *Thamnophis eques* Reuss) (Fig. 1A–D). Unfortunately, the ancestral condition of the hemipenes of New World natricines (simple, bilobed) is uncertain. Only bilobed organs are found in the Old World genus *Natrix* (Branch & Wade, 1976; Rossman & Eberle, 1977; Dowling & Duellman, 1978; Schleich, Kästle & Kabisch, 1996), the sister taxon to New World natricines (Lawson *et al.*, 2005). However, both simple and bilobed organs are found among other Old World genera (e.g. *Afronatrix*, *Nariciteres*, *Atretium*, *Hydraethiops*; Zaher 1999: figs 17–19), which, together with *Natrix* and New World natricines, constitute the subfamily Natricinae (Lawson *et al.*, 2005). Regardless, it is clear that hemipenis morphology has changed state multiple times within the New World natricines. Nor is this plasticity limited to the Natricinae. Both morphologies occur among members of the genera *Arrhyton* (Xenodontinae) and *Lampropeltis* (Colubrinae) (Zaher, 1999) and among Old and New World ratsnakes (Utiger *et al.*, 2002: fig. 5; for the distribution of bilobed versus simple hemipenes at higher taxonomic levels, see Pinou *et al.*, 2004: fig. 1).

Our suggestion that the evolution of snake hemipenis morphology may be driven by sexual conflict should stimulate more detailed research. In particular, documentation of costs and benefits of increased copulation duration in males versus females is needed to distinguish sexual conflict from mate-choice on the part of females. Possibly, hemipenis elaboration is the result of selection imposed by females for males that can resist disengagement and both males and females benefit. Future research might also profitably focus on other aspects of snake hemipenis morphology, including size, allometry, and ornamentation.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Material examined (institutional abbreviations: FMNH, Field Museum of Natural History; HDW NIU, Harlan D. Walley Northern Illinois University; INHS, Illinois Natural History Survey; KUMNH, University of Kansas Museum of Natural History; UIMNH, University of Illinois Museum of Natural History; UMMZ, University of Michigan Museum of Zoology; UTA, Amphibian and Reptile Diversity Research Center, University of Texas at Arlington).

Appendix S2. Sources of information on hemipenis morphology of North American natricine snakes (Fig. 3).