

# Genetic analysis of the mating system and opportunity for sexual selection in northern water snakes (*Nerodia sipedon*)

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We used data collected over 3 years at two study sites to quantify the rates and consequences of multiple paternity and to determine the opportunity for selection on male and female northern water snakes (*Nerodia sipedon*). We sampled litters from 45 females that gave birth to 811 offspring. Using eight microsatellite DNA loci (probability of exclusion of nonparental males > 0.99), we assigned paternity to 93% of neonates from one study population and 69% of neonates from the other population. Observations of participation in mating aggregations predicted individual reproductive success poorly for two reasons. First, males regularly courted nonreproductive females. Second, more than half of all sexually mature males obtained no reproductive success each year, despite the fact that many of them participated in mating aggregations. The number of sires per litter ranged from one to five, with 58% of all litters sired by more than one male. Multiple paternity increased with female size, apparently both because bigger females mated with more males and because the larger litters of big females provide paternity opportunities to more males. Multiple paternity was also more prevalent in years with shorter mating seasons. We detected no advantage to multiple paternity in reducing either the number of unfertilized ovules or stillborn young. Despite the majority of males siring no young each year, some males fathered young with as many as three different females in one year. Male reproductive success increased by more than 10 offspring for each additional mate, whereas female success increased by fewer than 2 offspring for each additional mate. The opportunity for sexual selection was more than five times higher in males than females. *Key words*: mating system, microsatellites, multiple paternity, *Nerodia sipedon*, paternity analysis, sexual selection, snakes. [*Behav Ecol* 13: 800–807 (2002)]

Snakes are poorly represented as subjects of sexual selection research (Duvall et al., 1993; Olsson and Madsen, 1998) because their cryptic nature usually makes systematic observation of individually marked animals impossible. Molecular methods to determine parentage of offspring promise to greatly enhance the feasibility of studying sexual selection in snakes, as they have for other taxa (Gibbs and Weatherhead, 2001). Snakes possess a unique suite of traits that suggests they will be valuable model organisms for studying sexual selection. For example, multiple mating by females and multiple paternity of litters appear to be common in snakes (Olsson and Madsen, 1998). Unlike for many other vertebrates in which these phenomena occur (e.g., birds), however, identifying the proximate and ultimate factors influencing multiple mating in snakes should be simplified by the lack of social bonds between mating individuals and by the lack of parental care by either sex. Competition among male snakes for access to females is intense in many species (e.g., Devine, 1977; Madsen and Shine, 1993; Weatherhead et al., 1995). Unlike in most other taxa, however, strong competition among males is not always associated with male-biased size dimorphism in snakes

(Olsson and Madsen, 1998), and male snakes do not exhibit any elaborate ornamentation that is often associated with strong sexual selection. As a first step toward using DNA-based measures of paternity to investigate these phenomena in snakes, we used microsatellite DNA markers to describe the mating system of northern water snakes (*Nerodia sipedon*) and to quantify sexual selection (Arnold and Wade, 1984). We also began exploring the causes and consequences of sexual selection in this species.

Several attributes of the population of northern water snakes that we studied made them particularly appropriate for our purposes. First, the snakes we studied occurred in relatively small, isolated marsh populations (Prosser et al., 1999; Weatherhead et al., 1995), which allowed us to sample most of the adult population. This increases the potential success of determining parentage when using genetic techniques. Second, previous observational studies of this population suggested that sexual selection through competition between males was strong (Brown and Weatherhead, 1999b; Weatherhead et al., 1995). In addition to guiding the development of specific hypotheses to be tested (see below), this past research created the unique opportunity for us to determine how well behavioral indices of mating success agree with genetically determined measures of reproductive success, something not previously done for snakes. Finally, a previous study using the same population demonstrated that multiple paternity occurs frequently in this species (Barry et al., 1992), so *N. sipedon* was an appropriate subject for addressing questions about this phenomenon.

Our first goal was to address the technical issue of the extent to which the use of microsatellite DNA markers would alter previous estimates of reproductive patterns from the same water snake population. For male water snakes, previous estimates of reproductive success based on behavior could be

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unreliable for several reasons (e.g., disrupted copulations, unobserved copulations, sperm competition). Although female reproductive success can be documented accurately without genetic methods, the previous study of multiple paternity used allozyme-based genetic markers (Barry et al., 1992), which precluded determination of the exact number of sires per litter and the number of young sired by each male.

Our second goal was to identify the factors that contribute to variation in multiple paternity. Although there is no a priori reason to expect that the proclivity to mate with multiple males should vary with female attributes such as size, males might pursue larger females more vigorously because larger females are more fecund and thus provide males greater potential for reproductive success (Brown and Weatherhead, 1997; Duvall et al., 1993). If the number of males with which a female mates is related to the number of males that court her, then larger females should more often give birth to multiply sired litters. Alternatively, large females might be more likely to produce multiply sired litters because, for a given number of males mated, the females' larger litters provide opportunities for more of those males to sire young.

At the population level, the occurrence of multiple mating, and thus multiple paternity, might depend on the number of males encountered by receptive females. We investigated three possible factors influencing encounter rates. First, the operational sex ratio (OSR) is male biased in northern water snakes but can vary substantially between years (Brown and Weatherhead, 1999b; Weatherhead et al., 1995). We tested the prediction that, as the OSR becomes more male biased, the incidence of multiple paternity increases. Second, we tested the prediction that multiple paternity would be more prevalent in years when the mating season is relatively protracted. Female water snakes can remain reproductively active throughout the mating season (Weatherhead et al., 1995), so we assumed that, with more time available, females should encounter more potential mates. Third, we tested the prediction that multiple paternity should be more common when the spatial distribution of reproductive females is both patchy and predictable. Previous research has shown that females are four times more clumped in one of our study populations than in the other, which results in females being courted by more males (Brown and Weatherhead, 1999b).

Our third goal was to determine, at least proximately, whether multiple mating has any adaptive consequence for females. We considered just two of the hypotheses that have been proposed to explain why females of many species mate with multiple males (Andersson, 1994). First, if a single mating is not sufficient to fertilize all a female's ova, then females that mate with more than one male should have fewer unfertilized ova than females that mate once (Gromko et al., 1984). Second, multiple mating may be a bet-hedging strategy to increase the genetic quality of offspring. In an inbred population of European adders (*Vipera berus*), Madsen et al. (1992) found that multiple mating reduced the proportion of young that were stillborn. They suggested that multiple mating could promote intrauterine sperm competition, whereby sperm of higher quality males are more likely to fertilize eggs. Although our northern water snake populations are not inbred, there is reduced gene flow between pond populations (Prosser et al., 1999). Therefore, we examined whether multiple paternity reduces the occurrence of stillborn young.

Our final goal was to use the data on reproductive success and mating patterns to determine the mating system, and hence the potential for sexual selection, in northern water snakes. Determination of the intensity of sexual selection requires measures of the true reproductive success of all individuals in the population (Duvall et al., 1993). We generated these estimates by documenting the mean number of mates

for males and females and then determining how reproductive success varies with the number of mates for both sexes (i.e., sexual selection gradients; Arnold and Duvall, 1994). Because individual males have been observed courting as many as eight different females in the same season (Brown and Weatherhead, 1999b), we predicted that the potential for sexual selection should be much greater in males than in females.

## METHODS

### Sampling individuals

We studied water snakes from 1994 to 1996 in two spring-fed beaver ponds (Barbs Marsh and Beaver Marsh) located approximately 1 km apart and 10 km from the Queen's University Biological Station in eastern Ontario, Canada (45°37' N, 76°13' W). Brown and Weatherhead (1999a) estimated mean population sizes as 101 and 84 for Barbs and Beaver Marshes, respectively. Beginning in late April when the snakes emerge from hibernation, and continuing through May (the mating season) until early September, we searched both marshes and attempted to capture all unmarked snakes (see below) by hand. We conducted searches twice each day during the mating season and at least twice a week thereafter.

We measured mass, total length and tail length, and determined the snout-vent length (SVL) of each snake by subtracting tail length from total length. We sexed each snake by probing its cloaca to detect the presence of hemipenes. We collected a sample of approximately 100  $\mu$ l of blood from the caudal vein in the tail and stored the sample in 800–1000  $\mu$ l of Queen's lysis buffer (Seutin et al., 1991). Snakes were permanently identified by implanting a passive integrated transponder (PIT tag, Anitech) subcutaneously. We also marked snakes captured for the first time each season with a unique three-color code using spots of nontoxic acrylic paint, which allowed us to identify individuals in the field with minimal disturbance (Barry et al., 1992). We occasionally recaptured marked individuals during the mating season to reapply color marks that were fading.

In late July and early August we attempted to capture all adult females in both marshes. Once these females gave birth or it became apparent that they were not gravid, we released them where they had been captured. We housed females individually or in pairs in fiberglass tanks with plastic carpeting covering the floor, in a room with an ambient temperature of 25–33°C. Heating rocks were rotated among the tanks so females could thermoregulate regularly. Females always had access to water and were fed minnows ad libitum twice a week. After parturition we collected approximately 50  $\mu$ l of blood from the caudal vein of each neonate. We removed the terminal 1 cm of the tail from young that were stillborn. We stored blood and tissue samples in lysis buffer (Seutin et al., 1991) in a freezer. We released all live neonates at their mother's marsh.

### Behavioral observations

While searching for snakes during the mating season, we also documented reproductive behavior. In Barbs Marsh the substrate appropriate for basking and mating occurs throughout the marsh, whereas the only appropriate substrate in Beaver Marsh occurs around the periphery of the marsh. This difference accounts for the much more clumped distribution of females in Beaver Marsh during the mating season (Brown and Weatherhead, 1999b). We modified our method of searching the two marshes to maximize the likelihood of finding mating aggregations.

Table 1

Summary of variation in length of mating season, female northern water snakes collected in the two study areas, the number that gave birth, and the numbers of live and dead (undeveloped follicles and stillborn young) offspring they produced each year

Study area	Year	Mating season (days)	No. of females		No. of offspring		
			Collected	Gave birth	Total	Live	Dead
Barbs	1994	25	12	6	111	108	3
	1995	11	7	1	17	17	0
	1996	23	19	15	260	256	4
Beaver	1994	29	12	7	138	135	3
	1995	19	19	8	159	159	0
	1996	19	19	8	126	123	3
	Total		79	45	811	789	13

We considered a female and male(s) to be in a mating aggregation when we observed at least one male courting the female. Courtship involves stereotypical behaviors by the male including chin-tapping, body jerking and alignment of the body to bring the male's cloaca into contact with the female's cloaca (Mushinsky, 1979; Perry-Richardson et al., 1990). We attempted to identify all the participants in aggregations, but to avoid disturbing the snakes, we did not approach closer than several meters. Because this made it impractical to determine whether intromission was occurring, we simply scored the number of times each individual was observed in mating aggregations, as has been done previously (Brown and Weatherhead, 1999a; Weatherhead et al., 1995).

#### Genetic analysis and paternity assignments

We used eight microsatellite DNA loci to assign paternity. Six of these loci (N $\mu$  2, 3, 4, 6, 9b, and 10) have been described previously (Prosser et al., 1999). The other two loci (N $\mu$  110 and 119) are reported in Prosser (1999). Locus-specific probabilities of correctly excluding males that were not the father of a given neonate (Westmeat et al., 1987) ranged from 0.21 to 0.86, with a combined probability across all eight loci of >0.99 in both marshes (Prosser, 1999; Prosser et al., 1999).

Detailed genotyping protocols are given in Prosser et al. (1999). For efficiency, we initially genotyped all adult males, mothers that gave birth, and all neonates at the four loci (N $\mu$  2, 3, 9b, and 10) with the highest probabilities of exclusion (all single locus  $P_E \geq 0.52$ ). We then genotyped offspring that could not be assigned to a single father, their mothers, and all other putative fathers (i.e., males not excluded in the initial screening) at the other four loci, using each locus as necessary until we excluded all but one male. However, for litters in which all but one or two neonates were assigned to a single male using the first four loci, we genotyped the entire litter using the remaining four loci to confirm assignments. Note that on the basis of an observed heterozygote deficiency, Prosser et al. (1999) suggested that N $\mu$  4 had a null allele(s), which was subsequently confirmed by analyzing patterns of segregation between mothers and their young (Prosser, 1999). Therefore, we only used genotypes at this locus for exclusions when both the parents and offspring being compared were identifiable heterozygotes and hence did not have null alleles. When assigning paternity we only considered males sampled within a study site as potential sires for neonates produced from that site because movement of snakes between the two sites was highly improbable. However, for neonates from a given year, we considered all males sampled at the study site across all years as potential sires, even if we had not observed some of those males that year.

#### Data analysis

For several reasons we decided that when pooling data across years, it was appropriate to treat individuals present in more than one year as independent observations. First, because water snakes continue to grow after sexual maturity, most attributes of interest (e.g., body size, fecundity) of individuals sampled twice change between samples. Second, our sample sizes were rather large and the numbers of individuals sampled more than once were relatively small (2 of 43 females, 55 of 150 males). Because the  $F$  distribution is rapidly asymptotic, the analytical danger of pseudoreplication (artificially increasing error degrees of freedom) is minimal (e.g., for females, a change in error df from 43 to 41 corresponds to a change in critical  $F$  value of approximately 0.03; Leger and Didrichsons, 1994). Third, among males, for which the proportion of resampled individuals is highest, the variance in reproductive success was higher within than among resampled individuals ( $F = 0.66$ ,  $df = 54,67$ ,  $p = .94$ ), suggesting that pooling data should not bias our results (Leger and Didrichsons, 1994).

We inspected plots and distributions of residuals from all parametric analyses and detected no significant violations of the assumptions of normality and homogeneity of variance.

#### RESULTS

Over the 3 years of the study we brought 81 females into captivity. One female died and one female gave birth to an entire litter of undeveloped follicles; we excluded both from subsequent analyses. Of the remaining 79 females, 45 (57%) gave birth to a total of 798 live (mean = 17.7, SD = 4.9, range 4–28) and 13 dead (i.e., stillborn or partially developed embryos) offspring (mean = 0.29, SD = 0.59, range = 0–2, Table 1). The overall mean litter size (i.e., live and dead combined) was 18.0 (SD = 4.7, range = 5–28) offspring. One additional female died in captivity before giving birth, and we dissected out 16 partially developed offspring from which we were able to extract DNA. We also found a gravid female dead in the field and were able to extract DNA from 19 of her 22 embryos. We used data from these two dead females only in our determination of levels of multiple paternity.

Litter sizes and numbers of live and dead offspring did not differ significantly among years in Barbs (all  $F < 0.53$ ,  $df = 1,19$ , all  $p > .48$ ) or Beaver Marsh (all  $F < 1.84$ ,  $df = 1,20$ , all  $p > .19$ ). Because only one female from Barbs Marsh gave birth in 1995, we excluded 1995 for the comparison of litter sizes across years. After pooling data for each marsh across years, we found no significant differences in litter sizes or numbers of live or dead offspring between the two sites (all  $F < 0.29$ , all  $p > .60$ ). Therefore, for analyses other than those specifically examining differences in rates of multiple pater-

nity in the two marshes, we pooled the data for all litters across years and sites. Using the first four loci (Ns $\mu$  2, 3, 9b, and 10), we were able to exclude all but one male for 290 of the 414 (70.0%) neonates from Barbs Marsh and 207 of the 432 (47.9%) neonates from Beaver Marsh. With an additional four loci (Ns $\mu$  4, 6, 100, and 119), we increased the percentage of neonates assigned to a single father to 92.8% in Barbs Marsh and 68.8% in Beaver Marsh. Less than 2% of these assignments involved cases in which two males matched all the paternal alleles and thus could not be distinguished. In these cases we assigned paternity to the male that had sired other offspring in the same litter, which always allowed us to exclude one of the two males. For all unassigned neonates we excluded all potential fathers we had sampled. For litters with unassigned neonates, we estimated the minimum number of sires for the litter from the number of paternal alleles at the locus with the most paternal alleles present.

Based on numbers of paternal alleles detected, we determined that at least five males from Barbs Marsh and 17 males from Beaver Marsh collectively sired the unassigned neonates. The mothers of those offspring provide clues regarding why those males may have avoided capture. Of the 10 females that produced offspring sired by unknown males at Beaver Marsh, 8 were captured for the first time the year they gave birth, and 7 of the 8 were captured at the end of, or after, the mating season. Thus, these females may have mated with males in one of the nearby wetlands before moving to Beaver Marsh. In contrast, at Barbs Marsh, which is more remote from other wetlands than Beaver Marsh, all three females that produced offspring with unknown sires were long-term residents of the marsh. The unknown sires for these three litters seem likely to represent cases where the males were present in the marsh but avoided capture.

We identified 46 males that sired offspring over the 3 years of the study. The smallest reproductively successful male had an SVL of 43.3 cm, which is similar to the estimated minimum size at sexual maturity of 42.5 cm based on mating behavior (Weatherhead et al., 1995). Therefore, we assumed that all males longer than 43.3 cm SVL were sexually mature and thus capable of siring offspring. Based on these criteria, the number of mature males in Barbs and Beaver Marshes from 1994 to 1996 ranged from 29 to 38 and from 33 to 47, respectively.

The 45 females who reproduced (Table 1) ranged in SVL from 63.3 to 83.1 cm. Our minimum size for sexual maturity for females is substantially larger than the minimum size estimated by Weatherhead et al. (1995) from mating behavior (63.3 vs. 55 cm). In contrast to males, female water snakes may not reproduce in consecutive years (Brown and Weatherhead, 1999a; Feaver, 1977). Of the 14 females that gave birth and that we brought into captivity the next year, none gave birth in the second year. Therefore, we estimated the number of sexually active females by the number of females seen mating in the field plus the number of females not seen mating but that gave birth. The number of reproductive females estimated this way ranged from 4 to 16 in Barbs Marsh and from 16 to 17 in Beaver Marsh over the 3 years.

#### Behavioral vs. genetic estimates of reproductive success

Pooling data from the two populations, we estimated that 85, 70, and 62 males were sexually active in 1994, 1995, and 1996, respectively. Of these 217 males, we only observed 92 (42%) courting or mating with a female in a total of 120 mating aggregations observed over the study. Of the 92 males observed attempting to mate, only 30 (32%) sired offspring. In total, 46 (21%) males achieved some reproductive success. Thus, 16 (35%) males that sired young were never seen in a mating aggregation. Furthermore, nearly half the mature

males (48.8%) known to be present were neither seen mating nor reproduced. Nonetheless, males observed in mating aggregations were more likely to sire offspring than males not seen in aggregations ( $\chi^2 = 9.11$ ,  $df = 216$ ,  $p < .0025$ ), so behavioral data had some value in predicting which males sired young. The number of offspring sired by individual males was not significantly correlated with the number of times individuals were seen in mating aggregations ( $F = 3.05$ ,  $df = 1,211$ ,  $r = -0.12$ ,  $p = .08$ ) or with the number of different females that males were seen with in aggregations ( $F = 0.13$ ,  $df = 1,91$ ,  $r = -.04$ ,  $p = .73$ ).

Over the 3 years, 45 (57%) of 79 females brought into captivity gave birth (excluding the female that died and the female that produced entirely undeveloped follicles). Only 22 of these 45 reproductive females (49%) were observed in mating aggregations, so 23 females that produced offspring were never observed mating. Similarly, 15 females that we had seen in mating aggregations did not give birth. Thus, unlike males, females observed in mating aggregations were no more likely to give birth than females not seen in aggregations ( $\chi^2 = 0.18$ ,  $df = 78$ ,  $p = .67$ ). In addition, the number of offspring a female produced was not significantly correlated with either the number of times she was seen in mating aggregations ( $F = 0.53$ ,  $df = 1,43$ ,  $r = .11$ ,  $p = .47$ ) or with the number of different males seen courting her ( $F = 2.21$ ,  $df = 1,43$ ,  $r = .22$ ,  $p = .15$ ).

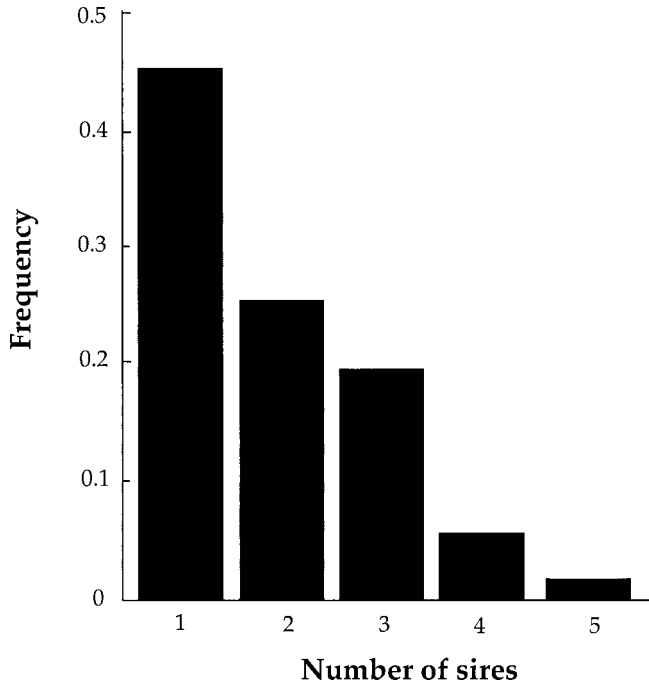
Finally, we considered the identities of males and females seen together in mating aggregations and determined the frequency with which those individuals produced offspring together. We observed 156 dyads of known males and females in the field. Of these, only seven (4.5%) produced offspring together.

#### Patterns of multiple paternity

Over all years, 58% of litters were sired by more than one male. Although one litter had five different sires, nearly 80% of all litters were sired by either one or two males (Figure 1). The proportion of offspring sired by each male in multiply sired litters ranged from 0.05 to 0.95.

We predicted that multiple paternity would be higher in litters of larger females. Consistent with our prediction, the number of sires per litter increased significantly with maternal size ( $F = 13.90$ ,  $df = 1,43$ ,  $r = .49$ ,  $p = .0006$ ; Figure 2). This pattern could be a consequence of bigger females mating with more males. Alternatively, because bigger females produce larger litters (Weatherhead et al., 1999), multiple paternity might increase with female size because larger litters provide opportunities for more males to sire young. When both litter size and maternal SVL were included in an ANOVA model, using data for all females ( $N = 45$ ), only maternal size explained significant variation in the number of sires (SVL:  $F = 6.51$ ,  $df = 1,42$ ,  $p = .014$ ; litter size:  $F = 0.60$ ,  $p = .44$ ). When the same analysis was restricted to the 26 females that produced multiply sired litters, however, only litter size explained significant variation in the number of sires (SVL:  $F = 2.56$ ,  $p = .12$ ; litter size:  $F = 5.78$ ,  $df = 1,23$ ,  $p = .028$ ). These results suggest that, relative to smaller females, bigger females copulated with more males, but thereafter, chance tended to determine which of the males that mated sired offspring.

We predicted that multiple paternity should have been more prevalent in longer mating seasons and when the OSR was more male biased. The length of the mating season (the interval between the first and last day we observed mating aggregations) ranged from 11 to 29 days (Table 1). We estimated the OSR by dividing the number of sexually active females (females seen in mating aggregations and/or known to have reproduced) by the number of sexually active males (all



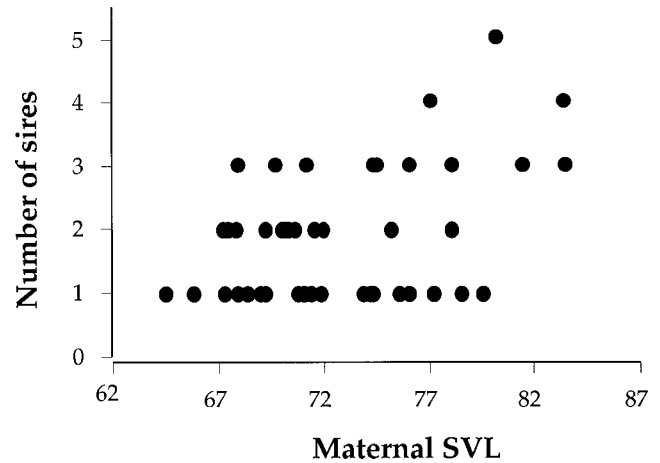
**Figure 1**  
Frequency distribution of northern water snake litters with different numbers of sires. All years and populations are pooled ( $N = 45$ ).

males large enough to be sexually mature) in each marsh each year. OSR estimates ranged from 0.13 to 0.55. We found no correlation between the proportion of multiply sired litters and OSR (Spearman rank correlation,  $r_s = .49$ ,  $p = .32$ ), but multiply sired litters appeared to have been more prevalent when the mating season was shorter (Spearman rank correlation,  $r_s = -.81$ ,  $p = .05$ ). The latter relationship was affected by the very brief mating season for Barbs Marsh in 1995 (11 days), when only one female gave birth (to a multiply sired litter). When we excluded this value, the correlation between the proportion of multiply sired litters and length of the mating season remained negative but was no longer significant (Spearman rank correlation,  $r_s = -.67$ ,  $p = .22$ ).

Finally, we predicted that multiple paternity should be higher in Beaver Marsh than in Barbs Marsh because females in Beaver Marsh are more predictably located by males and are courted by more males, on average, than females in Barbs Marsh (Brown and Weatherhead, 1999b). In total, 16 of the 23 (70%) litters from Beaver Marsh and 10 of the 22 (46.7%) litters from Barbs Marsh were sired by more than one male ( $\chi^2 = 2.70$ ,  $df = 44$ ,  $p = .10$ ). In Beaver Marsh, litters were sired by a mean of 2.04 (SD = 0.88) males compared to a mean of 1.77 (SD = 1.11) males in Barbs Marsh ( $t = -0.91$ ,  $df = 43$ ,  $p = .37$ ). Although the difference was in the predicted direction, it was not close to significant.

### Consequences of multiple paternity

Pooling data across marshes and years, there was no significant difference in the proportion (or absolute number) of unfertilized ova between singly and multiply sired litters (both  $t < 1.13$ , both  $p > .26$ ). There was also no significant difference in the mean proportion of stillborn young (including partially developed embryos) between singly and multiply sired litters ( $t = 0.65$ ,  $df = 43$ ,  $p = .52$ ).

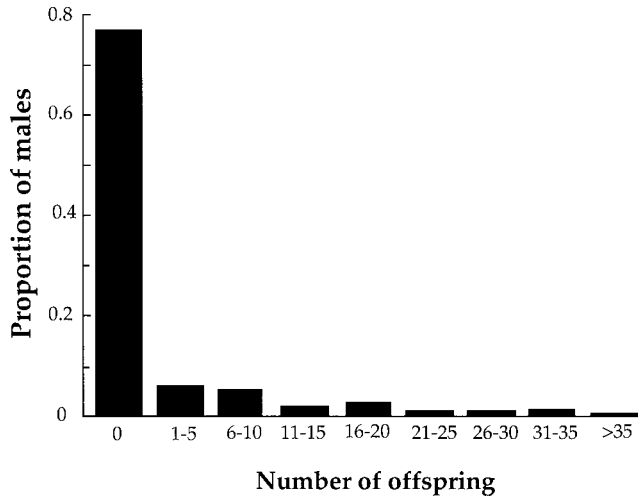


**Figure 2**  
Relation between maternal size (snout-vent length; SVL) of northern water snakes and the number of males that sired their litters for all years and populations pooled.

### Mating system and sexual selection

In quantifying the mating system, we assumed that no sexually mature female failed to reproduce because she could not find a mate (i.e., failure to reproduce is a life-history decision for females rather than a sexual selection issue). We consider this a reasonable assumption for several reasons. The mobility of northern water snakes would allow the snakes to circumnavigate either study marsh in a matter of hours, so in a mating season of several weeks a female should have no problem finding a mate. The male-biased OSR indicates that there are always excess males available, and in both marshes, mating locations were often quite predictable, further increasing a female's ability to find males. In contrast, we assume that sexually mature males should not willingly forgo reproduction in any given year. Indeed, males that sired offspring in one year were no less likely than males unsuccessful in the same year to be successful ( $\chi^2 = 2.05$ ,  $df = 66$ ,  $p = .15$ ) or to be seen in mating aggregations ( $\chi^2 = 1.69$ ,  $df = 66$ ,  $p = .19$ ) the following year. Males sired young with a mean of 0.32 and 0.26 females at Barbs Marsh and Beaver Marsh, respectively. However, male reproductive success was highly skewed (Figure 3), with the proportion of males achieving no reproductive success ranging from 59% to 97% among marshes and years. Considering only successful males, in Barbs Marsh males sired young with a mean of 1.46 females, whereas in Beaver Marsh males sired young with a mean of 1.15 females. The maximum number of mates for any male was three.

A quantitative method for defining mating systems involves comparing sexual selection gradients (the relationships between fecundity and the number of mates) for males and females (Arnold and Duvall, 1994). Reproductive success of male water snakes increased significantly with the number of females with which a male sired young ( $F = 615.38$ ,  $df = 1,215$ ,  $r = .68$ ,  $p < .0001$ ; Figure 4). We estimate that for each additional female mated, male success increased by an average of 11.41 offspring. If we include males that achieved no reproductive success, then success increased by 10.08 offspring for each additional mate. For female water snakes, reproductive success also increased with the number of males that sired young in their litters ( $F = 7.29$ ,  $df = 1,43$ ,  $r = .38$ ,  $p = .01$ ). However, if we control for female size, the effect of number of sires becomes nonsignificant ( $F = 0.59$ ,  $df = 1,42$ ,  $p = .37$ ). Thus, the apparent increase in litter size with number of sires



**Figure 3**  
Frequency distribution of annual male northern water snake reproductive success (number of offspring sired) for both study sites and all years combined ( $N = 217$ ).

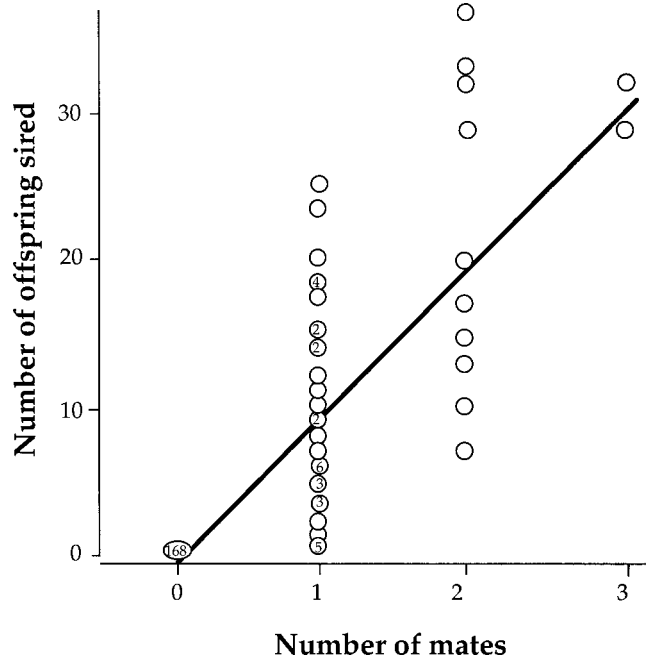
is a consequence of larger females producing larger litters that are sired by more males (Figure 2).

The potential for sexual selection to act on males relative to females is estimated from their respective relative variances in reproductive success ( $I_s = \text{variance in success divided by the square of mean success}$ ). Relative variance in success provides an upper limit to the strength of directional selection and has been termed the “opportunity for selection” (Wade, 1979; Wade and Arnold, 1980). Following the same reasoning as above, we included mature males that achieved no reproductive success in our calculation of  $I_m$  and excluded females that failed to reproduce from our estimate of  $I_f$ . Values of both  $I_m$  and  $I_f$  varied between years and marshes, with the former ranging from 2 to 9 times higher than the latter. Overall, the relative opportunity for selection ( $I_m$  divided by  $I_f$ ) was 5.21 times higher for males than for females.

**DISCUSSION**

Our results demonstrated the value of using genetic markers to study sexual selection in snakes. Observations of mating behavior did not reliably predict reproductive success for either males or females. Previous research on this population of water snakes assumed that males only courted females that were going to reproduce that year (Barry et al., 1992). Males sometimes ignore sexually mature females that are later seen in aggregations, suggesting that the females advertised their receptivity (presumably using pheromones) and thus are courted only when receptive. However, we found that males regularly courted females that subsequently failed to reproduce. Females may have copulated with males but stored the sperm; sperm storage between years has been documented in many species of snakes (e.g., Fox, 1956; Gist and Jones, 1987; Halpert et al., 1982; Schuett, 1982), although not for water snakes. If females stored sperm, then some males present in one year but not the next should have sired offspring that second year. However, none of the 46 males identified as sires involved this circumstance. Thus, misdirected male behavior seems a more likely interpretation and is an important reason that male mating behavior poorly predicted male reproductive success.

Two other factors also seem likely to have contributed to the unreliability of behavioral estimates of male reproductive



**Figure 4**  
Relationship between the number of mates and number of offspring sired for male northern water snakes. All years and populations are pooled.

success. First, the facts that some females that gave birth were never seen mating and that some males that sired young were never captured means that our mating observations and population samples were not as thorough as we had assumed. Capture records for mothers of offspring with unknown sires suggested that part of the sampling problem involved movements of snakes between wetlands, particularly at Beaver Marsh. Second, even though females in this population regularly mate with multiple males (Barry et al., 1992; this study), assuming that all males in a mating aggregation achieve some success is erroneous. Documenting intromission may have improved our behavioral observations, but because little is known about factors affecting sperm competition in snakes (e.g., timing and order of mating), even observations of intromission success may be imperfect predictors of reproductive success.

Our estimate that 58% of all northern water snake litters were multiply sired is substantially less than the 86% estimated by Barry et al. (1992) for the same population. We found that multiple paternity declined in longer breeding seasons. In 1991, the year Barry et al. (1992) collected their data, the mating season in Barbs Marsh was relatively long (Weatherhead et al., 1995), so it seems unlikely that differences in length of breeding seasons explain the difference between studies. We also found that multiple paternity increased with litter size, which also was the case in Barry et al.’s (1992) study (the 2 litters out of 14 that only had a single sire were the second and third smallest in their sample). The mean litter size in our study was 18.0 compared 22.7 in Barry et al.’s (1992) study. Thus, the smaller litters in our study might have contributed to the lower prevalence of multiple paternity we documented. Indirect evidence suggested that the increase in multiple paternity with litter size that we documented was a consequence of two factors. First, bigger females appeared to have copulated with more males relative to smaller females. Second, larger litters provide more opportunities for pater-

nity, so multiple paternity also occurred more often in larger litters by chance.

We predicted that longer mating seasons should provide more mating opportunities for females, thereby increasing multiple paternity. Although our analyses lacked power (involving only five or six values), the results indicated that longer mating seasons did not promote multiple paternity and may, in fact, even have acted to decrease its incidence. Multiple paternity could be more common in shorter seasons if the time between matings is also shorter and if temporal proximity of inseminations somehow makes the outcome of sperm competition among males more equitable. However, evidence from sand lizards (*Lacerta agilis*; Olsson et al., 1996) and European adders (Stille et al., 1986, 1987) suggests that longer intervals between inseminations increases multiple paternity. Timing of ovulation relative to insemination could influence how the interval between inseminations affects sperm competition (Olsson and Madsen, 1998). However, both adders and water snakes ovulate approximately 1 month after mating (Aldridge, 1982; Bauman and Metter, 1977; Nilson, 1981), so on that basis the relationship between the timing of inseminations and sperm competition should be similar in the two species. An experimental approach may be the most productive way to investigate this phenomenon.

We addressed two possibilities for why female water snakes mate with multiple males. We found no support for the hypothesis that a single male does not produce adequate sperm to fertilize all of a female's litter (Gromko et al., 1984); unfertilized ova were rare and no more prevalent in singly sired than in multiply sired litters. We also found no support for the hypothesis that females mate with multiple males to reduce the costs of inbreeding (Madsen et al., 1992). The small number of undeveloped follicles and stillbirths rendered these weak tests of the hypotheses (power to detect a significant difference at  $\alpha = 0.05$  given observed effect and sample size = 0.10–0.27). Given the small differences we found between singly and multiply sired litters, much larger sample sizes would have been required to find significant differences. Nonetheless, because the incidence of unfertilized follicles and stillborn young was so small, we can conclude that any potential benefit from multiple mating in reducing this incidence would necessarily also have to be small. Furthermore, the occurrence of stillborn young is a blunt tool for detecting possible genetic benefits of multiple mating. Olsson et al. (1994) showed that multiple mating can produce offspring that are more viable after they are born, a possibility we could not explore with our data. Thus, it would be premature to conclude that female water snakes do not realize a genetic benefit from multiple mating.

Genetic evidence confirmed the suggestion from research on mating behavior of northern water snakes that competition among males is intense (Barry et al., 1992; Brown and Weatherhead, 1999b; Weatherhead et al., 1995). Standardized variances in reproductive success (Wade, 1979; Wade and Arnold, 1980) indicated that the opportunity for selection was more than five times greater for males than for females. More than half of all sexually mature males never sired young, and males that were successful regularly sired young with more than one female. Thus, multiple mating by females was far from sufficient to allow most males any reproductive success.

Our estimates of competition among males might change if seasonal estimates of success poorly reflect lifetime success. However, there are several reasons to expect this not to be the case. Over the 3 years of our study, only two males sired young in more than one year. This was a consequence of some males that were successful one year being unsuccessful in the other years, as well as many other males only being present for one year as a mature individual. Year-to-year survival of

male water snakes is less than 50% (Brown and Weatherhead, 1999a), so many males only have one or two potential breeding seasons. Thus, estimates of lifetime reproductive success seem unlikely to change our general conclusion that competition among males is intense.

Our estimate that the opportunity for sexual selection due to variance in reproductive success was approximately five times greater for males than females is similar to the value for European adders, the only other snake species for which this has been estimated. Madsen and Shine (1994) calculated an  $I_m$  of 0.96 and an  $I_f$  of 0.17, giving a relative value of 5.65. These values were based on estimates of lifetime reproductive success. However, the value for males has the potentially serious shortcoming of relying on mating success rather than on reproductive success. Nonetheless, the opportunity for sexual selection in their study appeared to be much greater for males than for females. Demonstrating variance in reproductive success is not adequate to imply that selection occurs on phenotypic traits (Fincke, 1988; McVey, 1988; Sutherland, 1985). The challenge now is to determine how the variance in true reproductive success of male snakes differs among species, the ecological reasons for those differences, and whether those differences explain variation among species in traits such as sexual size dimorphism.

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