

Male reproductive success and sexual selection in northern water snakes determined by microsatellite DNA analysis

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Male northern water snakes (*Nerodia sipedon*) have high variance in reproductive success relative to females. We used DNA-based paternity analyses from a 3-year study of two marsh populations of water snakes to investigate the factors that contribute to variation in male success. Male traits investigated included body size, condition, tail length, home range size, activity during the mating season, and genetic profile (genetic similarity to females, heterozygosity, and genetic variability [d^2]). We successfully assigned > 80% of offspring to sires from a sample of 811 offspring from 45 litters. Male reproductive success did not vary significantly with body size, tail length, condition, home range size, or the number of microsatellite loci at which males were heterozygous, nor with other features of their genetic profiles. However, we found evidence of positive assortative mating by size in the marsh in which receptive females were not spatially clumped. Also, males that were most active during the mating season were more successful, particularly where females were not clumped. We failed to find evidence of selection acting on male size through variance in reproductive success, indicating that sexual selection does not have an important influence on sexual size dimorphism in this species (males are smaller than females). We propose that males are smaller than females because the lack of advantage to large size allows males to adopt a low-energy, low-growth strategy that reduces their risk of predation outside the mating season. *Key words*: assortative mating, DNA loci, heterozygosity, microsatellites, *Nerodia sipedon*, northern water snake, paternity analysis, sexual selection, sexual size dimorphism. [*Behav Ecol* 13:808–815 (2002)]

Snakes are underrepresented as subjects of sexual selection research, despite their rich variation in mating behavior and sexual size dimorphism, which suggests there is much to be learned from their study (Duvall et al., 1993; Olsson and Madsen, 1998; Shine, 1993). Sexual size dimorphism in snakes is of particular interest because available evidence is poorly explained by current theory. In many species of snakes males are smaller than females, despite intense mating competition among males (e.g., Madsen and Shine, 1993b; Weatherhead et al., 1995). Furthermore, in these same species, larger males appear to have a mating advantage. These patterns are contrary to the conventional view that intense mating competition among males is associated with male-biased sexual size dimorphism (Darwin, 1871), particularly given evidence that large males have an advantage. DNA-based analyses of paternity have the potential to contribute fundamentally to our understanding of sexual selection in snakes (Duvall et al., 1993; Gibbs and Weatherhead, 2001). Snakes are often difficult to observe systematically in the wild, and even when observations are possible, they may be unreliable predictors of parentage (Prosser et al., 2002). We used genetic methods to identify the males that sire offspring, and thus also to identify those males that do not, in a population of northern water

snakes (*Nerodia sipedon*). We used this approach to identify the factors that contribute to variation in male reproductive success and that therefore may be subject to sexual selection, toward our general goal of understanding why male water snakes are smaller than females (Brown and Weatherhead, 1999c).

Male northern water snakes compete for access to females through scramble competition. Two previous studies of the population we report on here identified factors affecting male reproductive success by quantifying observations of mating behavior in the field. Weatherhead et al. (1995) found that large males had a mating advantage in 1 of 2 years, whereas relative tail length appeared unrelated to mating success. Brown and Weatherhead (1999b) investigated the effect of both behavioral and morphological attributes of males on mating success, as well as how the spatial distribution of females affected males. Where females were dispersed, males increased the size of their home ranges, and males with larger home ranges encountered more females. The opposite patterns occurred where females were spatially clumped. Where females were dispersed, larger males and males in better condition appeared to have a mating advantage, but neither factor appeared to affect male success when females were clumped. Evidence that behavioral data poorly predict reproductive success in northern water snakes (Prosser et al., 2002) renders the results of these previous studies suspect. Thus, we reexamined all of these results using genetic measures of paternity.

Understanding why male water snakes are smaller than females requires knowing how body size affects male reproductive success. In addition to the evidence for water snakes, there is behavioral evidence of a large-male mating advantage in other snake species in which males compete for matings by scramble competition (Madsen and Shine, 1993a; Shine et al., 2000). It is possible that genetic evidence will confirm that larger males have a reproductive advantage because of superior competitive ability. If so, the question arises as to what

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selection pressure prevents males from increasing in size to become more similar to females (Weatherhead et al., 1995). Alternatively, it is possible the genetic evidence will show that small males have a reproductive advantage perhaps because smaller males are more mobile (Shine, 1978). We consider various alternative explanations for sexual size dimorphism in northern water snakes in light of what our results reveal about the relationship between male body size and reproductive success.

Variation in body size could also affect reproductive success through size-assortative mating. Larger female water snakes are more fecund (Brown and Weatherhead, 1997; Weatherhead et al., 1999) and therefore should be preferred by males. If large males have a competitive advantage, large males should tend to produce offspring with large females. Shine et al. (2001) reported this pattern in red-sided garter snakes. Thus, in addition to testing the prediction that larger males will be more successful, we also tested the prediction that there will be a positive correlation between the size of males and females that produce offspring together.

Although our central interest is body size, we also considered other morphological attributes of males that could affect reproductive success. Relative to body size, male snakes have longer tails than females (King, 1989). Males “wrestle” with their tails when courting females, and the length of a tail may affect success in this competition. Shine et al. (1999) reported a mating advantage to male red-sided garter snakes with intact tails relative to individuals that had lost part of their tail. Among all males, Shine et al. (1999) found stabilizing selection acting to maintain sexual dimorphism in relative tail length. Thus, we tested the prediction that males with tails that are either longer or of intermediate length should be more successful. We also tested the prediction that males in better condition (mass corrected for body size; Weatherhead and Brown, 1996) at the start of the mating season will be more successful. Locating and competing for mates seems likely to be energetically costly, so males in better condition should be able to devote more energy to mating.

We studied two populations of water snakes that differed substantially in the extent to which females were spatially clumped (Brown and Weatherhead, 1999b) and predicted that male attributes should affect reproductive success differently in the two populations. Where females are clumped, and thus competition among males is higher, larger males and males with relatively longer tails should have a greater reproductive advantage. Where females are more dispersed, we predicted that the most successful males should be those that were more active, in better condition, and with larger home ranges because these attributes should enhance mate-searching ability.

A final question we addressed is whether genetic differences affect male reproductive success directly. If females choose among potential mates, they may prefer genetically dissimilar males to avoid the costs of inbreeding (Charlesworth and Charlesworth, 1987; Partridge, 1983). Females might also prefer the most heterozygous males to increase the heterozygosity, and thus the fitness, of their offspring (Brown, 1997). Recently, Pemberton et al. (1999) showed that offspring fitness was positively related to measures of individual genetic variation in two species of mammals. Even without active female choice, multiple mating by females in combination with sperm competition could result in some males having a reproductive advantage. For example, Olsson et al. (1996) found that although female sand lizards (*Lacerta agilis*) appeared to mate randomly, unrelated males sired more offspring. We tested two predictions. First, male(s) that sire a female's offspring will be more dissimilar genetically from the female than expected by chance. Second, male reproductive

success will be positively correlated with individual male heterozygosity.

METHODS

The methods we used to collect blood for DNA analysis and to assign paternity are explained in detail in a companion paper from the same study (Prosser et al., 2002). We collected DNA samples from 811 offspring from 45 litters that were collectively sired by 48 males. Overall, 92.8% of offspring were assigned to sires in one study population (Barbs Marsh) and 68.8% of offspring to sires in the other study population (Beaver Marsh). Across both marshes 58% of litters were sired by more than one male. All unassigned neonates were attributed to unsampled males.

The study populations and the individual snakes we studied here were also the same as those used by Brown and Weatherhead (1999b), so field methods for the two studies are identical. Therefore, we only provide a brief overview of those methods here. We conducted this study from 1994 to 1996 at two beaver ponds (Barbs Marsh and Beaver Marsh) near the Queen's University Biological Station in eastern Ontario. We captured snakes by hand in the two marshes, marked them individually, and collected blood for paternity analysis. We weighed each individual and measured total length, tail length (cloaca to tail tip), and determined snout-to-vent length (SVL) by subtracting these two measures. To estimate condition we used Weatherhead and Brown's (1996) equation to estimate the lean mass of each male from its SVL. By subtracting lean mass from total mass, we estimated total fat. Condition is total fat expressed as a percentage of total body mass. To control for growth during the active season, we used the equation in Brown and Weatherhead (1999c) to adjust all SVL measures to the median capture date for that year. No formula was available to adjust tail length, but we assumed that any growth during the season would be less than our measurement error.

In late July we searched both marshes intensively to capture all adult females (Weatherhead et al., 1995) and brought them into the lab until parturition. We returned females to the marshes after they gave birth or it became apparent they were not gravid. To obtain DNA from neonates we collected 50 μ l of blood from the caudal vein of each live young and removed the terminal 1 cm of the tail from young that were stillborn. After sampling we released neonates at their mother's marsh. When assigning paternity for a given litter, we screened all males sampled in the marsh from which that litter came, including males sampled in years other than that in which the litter was produced. We used eight highly variable microsatellite DNA loci for assigning paternity that had a combined probability of exclusion across all loci of > 0.99 in both marshes (Prosser et al., 1999, 2002).

Male spatial patterns

We collected data on the spatial patterns of males using observations of marked individuals collected during the mating seasons. We searched both marshes intensively for several hours at least once each day beginning in mid-April before the snakes emerged from hibernation and continuing until mid-June after all mating activity had ceased. Snakes captured during this period were individually marked with spots of non-toxic, acrylic paint, in addition to being given PIT (passive integrated transponder) tags. Paint marks allowed us to identify snakes at a distance without disturbing them. Most adults were measured and marked soon after emergence from hibernation, before mating began. We also used radiotelemetry to monitor the activity of a sample of snakes throughout the

active season (Brown and Weatherhead, 1999b). Transmitters (Holohil Systems Ltd., Ottawa, Ontario) were surgically implanted in the snakes' coelom. Transmitter mass was < 7.5% of body mass of each snake. We tracked 19 males during the mating season, during which time we located each individual at least once a day.

Scale maps of both study sites were overlain with a 20 m × 20 m grid. Each time we saw a painted snake or located a snake with a transmitter, we recorded its grid location. We estimated home ranges using the number of grid squares in which an individual was seen, using only individuals seen a minimum of five times. This method has the advantage of simplicity, and home range estimates derived using this method were highly correlated with estimates from more conventional methods (e.g., correlation with concave polygon method: $r = 0.81$, $p < .0001$). Because home range size was correlated with the number of times a male was observed (Brown and Weatherhead, 1999b), we included the number of observations as a factor in multiple regressions investigating the effects of home range area on reproductive success. We also used the number of times we observed males without transmitters as an index of their activity. Males that were more actively searching for females should have been easier for us to see and should also have been more likely to find females. Brown and Weatherhead (1999b) found that males observed attempting to mate were seen more than twice as often overall during the mating season as males never seen courting a female.

We restricted analysis of spatial patterns (and all other patterns) to adult males (individuals potentially capable of siring offspring). We assumed all males longer than 43.2 cm SVL were sexually mature, based on observations of mating behavior (Weatherhead et al., 1995) and on the smallest reproductively successful male (Prosser et al., 2002). The two methods were consistent to within < 1 cm SVL.

Data analysis

We conducted analyses for each year separately and pooled across years for each marsh. We considered pooling across years justified for several reasons (Prosser et al., 2002). Particularly relevant here is that water snakes continue to grow after sexual maturity, so most attributes of interest (e.g., body size) of individuals sampled twice change between samples. Also, among males, where the proportion of resampled individuals is highest, the variance in reproductive success was as high within as among resampled individuals ($F = 0.67$, $df = 36,42$, $p = .89$), suggesting that pooling data should not bias our results (Leger and Didrichsons, 1994). We inspected plots and distributions of residuals from all parametric analyses to determine whether our data met assumptions of normality and homogeneity of variance. We used transformations where necessary to meet those assumptions, and when transformations were unsuccessful we used nonparametric analysis.

To assess the effects of male traits on reproductive success, we calculated selection gradients using the standardized partial regression coefficients of each character from a multiple regression of relative reproductive success (Lande and Arnold, 1983). Relative reproductive success (w'_i) was estimated by dividing an individual's success by the mean success of the sample. All morphological traits were standardized (z_i) to have a mean of zero and standard deviation of 1 for pooling across years. We estimated directional selection from the partial regression coefficients from a model containing standardized linear terms for tail length, SVL, and condition:

$$w'_i = \text{intercept} + \sum \beta_1 z_i. \quad (1)$$

We estimated curvilinear selection from the coefficients after adding quadratic terms for each male trait to the model:

$$w'_i = \text{intercept} + \beta'_1 z_i + \beta_2 z_i^2. \quad (2)$$

The coefficient β_1 represents the intensity of directional selection on each trait after the effects of correlated traits have been removed. A positive coefficient indicates that directional selection is acting to increase that trait. The coefficients of the quadratic terms (β_2) represent stabilizing or disruptive selection. Stabilizing selection is inferred to be acting on traits if the sign (positive or negative) of the quadratic term is opposite to that of the corresponding linear term.

We performed three variations of these analyses. First, we excluded males with obviously damaged (and shortened) tails. Second, we included only males that sired offspring in case unsuccessful males included some individuals that had not attempted to mate, as opposed to having been unsuccessful. Third, for the analyses involving data pooled across years, we used a mean trait and reproductive success value for each male present in multiple years. Because none of these analyses produced results qualitatively different from the original analyses, we do not present results from these alternative analyses.

To determine whether parentage was assortative with respect to body size, we regressed the SVL of successful males on the SVL of the female for each litter in which all fathers were identified. Regressions were weighted by the proportion of the litter sired by each father. Thus, males siring more offspring in a given litter were given a higher weighting than males siring fewer offspring in that litter. We excluded data from Barb's Marsh in 1995 because we only obtained one litter.

To determine whether parentage of offspring was nonrandom with regard to the similarity of the parents' genetic profiles, we generated relatedness scores (i.e., r values; Queller and Goodnight, 1989) using Kinship 1.2 (Goodnight and Queller, 1999) for every male and female pairwise combination in each population, pooled across years. Therefore, individuals were included in each year they were present. We generated distributions of r values, pooled into intervals that differed by 0.1 units of r , for pairs that produced offspring and for those that did not. We then assessed the significance of the differences in these two distributions of r values using contingency table analysis based on a randomization technique (STRUC subroutine of GENETOP; Raymond and Rousset, 1995).

To test the prediction that levels of individual genetic variation should increase male reproductive success, we used only males that had been scored at four or five of the same loci (Ns2, 4, 6, 9, and 10). We assumed that variation in allele sizes at those loci was due to mutations causing the gain or loss of one or a few repeats, and hence consistent with a stepwise mutation model (cf. Valdes et al., 1993). This assumption is supported by the roughly continuous frequency distributions shown by alleles of different sizes in these populations (Prosser et al., unpublished data). We estimated levels of individual variation in two ways. First, we simply determined the percentage of microsatellite loci at which each male was heterozygous. Second, we used the method of Pemberton et al. (1999) to estimate a measure of individual internal genetic distance, d^2 , from the difference in allele sizes at each locus. Formally, this measure of variation (mean d^2) is calculated as:

$$\text{mean } d^2 = \left(\frac{1}{n}\right) \sum (i^a - i^b)^2 \quad (3)$$

where i^a and i^b are the length of alleles a and b at locus i , and n is the total number of loci at which the individual was scored. This measure requires that a stepwise mutation model applies to the loci used because it assumes that the difference

Table 1
Directional (β_1) and curvilinear (β_2) selection gradients on morphological traits of male water snakes

Site	Year	<i>N</i>	Trait	β_1	<i>p</i>	β_2	<i>p</i>
Barbs	1994	38	Fat	-0.23	.53	-0.24	.49
			SVL	0.21	.38	-0.01	.96
			Tail	0.33	.56	-0.07	.77
Barbs	1996	30	Fat	0.27	.42	-0.29	.22
			SVL	0.2	.53	-0.52	.05
			Tail	-0.22	.54	0.76	.25
Barbs	1994–96	68	Fat	0.09	.72	-0.18	.38
			SVL	0.36	.24	-0.25	.16
			Tail	-0.33	.27	-0.01	.98
Beaver	1994	44	Fat	0.03	.95	-0.08	.81
			SVL	0.17	.73	-0.16	.59
			Tail	0.02	.96	-0.1	.77
Beaver	1995	37	Fat	-0.36	.28	-0.18	.55
			SVL	-0.22	.58	-0.05	.88
			Tail	0.21	.61	-0.28	.44
Beaver	1996	29	Fat	-0.38	.32	0.63	.02
			SVL	-0.65	.13	-0.35	.31
			Tail	0.44	.29	0.19	.5
Beaver	1994–96	110	Fat	-0.09	.72	0.22	.22
			SVL	-0.23	.42	-0.08	.71
			Tail	0.22	.43	-0.05	.79
Both	1994–96	178	Fat	-0.07	.69	-0.02	.88
			SVL	-0.05	.81	-0.18	.18
			Tail	0.04	.83	0.02	.86

SVL, snout-vent length.

in the number of repeats between two alleles is roughly proportional to the amount of time since the divergence of the alleles. These two measures emphasize different ends of the inbreeding–outbreeding continuum in terms of measuring the effects of individual variation on fitness (Pemberton et al., 1999). Individual heterozygosity should reflect recent matings between relatives and hence be a more sensitive measure of any inbreeding effects that are present. In contrast, d^2 should be more affected by events deeper in a pedigree that affect allele length variation, and hence should be more sensitive to outbreeding effects, in that it assumes that individuals with high d^2 scores are more likely descended from matings between parents from genetically divergent populations.

RESULTS

Male morphology

Selection gradients revealed little evidence of selection acting on male morphology (Table 1). There was weak evidence of curvilinear selection on SVL in Barbs Marsh in 1996 and on condition at Beaver Marsh in 1996. However, given that the probabilities presented in Table 1 are not corrected for multiple comparisons, and that the overall models at each marsh were not significant, the simple inference from these analyses is that variation in male morphology did not affect variation in male reproductive success. It is possible that low statistical power may have limited our ability to detect selection. The mean absolute value of all our estimates of selection was 0.22 (Table 1). Power calculations indicate that the mean least-significant values of our regressions was 0.62, so selection on the traits we measured would have had to have been about three times greater, on average, before we could have detected it with a power of 50%. Thus, although we can be confident that no strong selection was acting on the traits we measured, we are less certain regarding more modest levels of selection. However, we replicated our analyses both spatially

and temporally (which is rare among selection studies; Kingsolver et al., 2001) and drew the same conclusions from them all, which strengthens the inference that selection on the male traits we considered was unimportant.

It is possible that male morphology does not affect mating success but does affect sperm competition after mating. To assess this possibility we compared traits of males that sired young in the same litter. The simplest approach was to restrict the analysis to litters with only two sires, which were also the most common among multiply sired litters. We had 11 litters for which both sires were known and current morphological measurements were available. Within litters the most successful male sired significantly more offspring than the other male (74% vs. 26%, $t = 8.9$, $df = 20$, $p < .0001$). We used logistic regression to determine whether male attributes (SVL, tail, mass, condition) affected which male was more successful. None of the attributes had a significant effect (all $\chi^2 < 2.04$, all $p > .15$). A regression analysis of the difference in reproductive success relative to the difference in morphology of sires within litters also showed no significant effects. The result that was closest to being significant was that the discrepancy in male success tended to increase as the more successful male became larger relative to the less successful male ($F_{1,9} = 2.99$, $r = .50$, $p = .11$).

Despite the lack of evidence that male morphology affected male reproductive success, we did find some evidence that mating was nonrandom with regard to male morphology. In Barb's Marsh, weighted regression indicated the SVL of females was significantly correlated with that of their mates ($F_{1,23} = 5.99$, $p = .02$, Figure 1). However, we found no evidence of this pattern in Beaver Marsh ($F_{1,18} = 0.22$, $p = .65$, Figure 1). In both these analyses we included each female–male pair that produced offspring, so a female with multiple mates was included once for each mate that we identified. Repeating the analysis using the mean size of each female's mates weakened the results. The positive assortative mating by size in Barbs Marsh became nonsignificant ($F_{1,14} = 3.21$, $p = .10$), while mating in Beaver Marsh remained random with respect to male and female size ($F_{1,11} = 0.18$, $p = .65$).

Male activity and home range

We used logistic regression to determine whether males observed more often during the mating season, and thus assumed to be more active, were more likely to achieve reproductive success. In Barbs Marsh, males that were seen more often were more likely to be successful ($\chi^2 = 6.36$, $N = 67$, $p = .012$). The same was true in Beaver Marsh, but the effect was not significant ($\chi^2 = 2.34$, $N = 114$, $p = .13$). In the two marshes combined over all years, successful males were seen about twice as often as unsuccessful males ($t = -3.18$, $df = 179$, $p = .002$, Figure 2).

In Barbs Marsh, males seen in more grid squares were more likely to be reproductively successful ($\chi^2 = 5.26$, $N = 57$, $p = .022$). In Beaver Marsh, the number of different grids a male occupied did not affect the likelihood of being successful ($\chi^2 = 0.35$, $N = 81$, $p = .55$). A potential problem with this analysis is that the estimated size of home ranges increased with the number of times a male was observed (Brown and Weatherhead, 1999b). We used multiple logistic regression to examine the relation between male reproductive success and home range size, controlling for the number of times a male was observed. In both marshes home range size did not contribute significantly to the model in any year or overall (Barbs Marsh overall: $\chi^2 = 0.88$, $N = 57$, $p = .35$; Beaver Marsh overall: $\chi^2 = 0.05$, $N = 81$, $p = .82$).

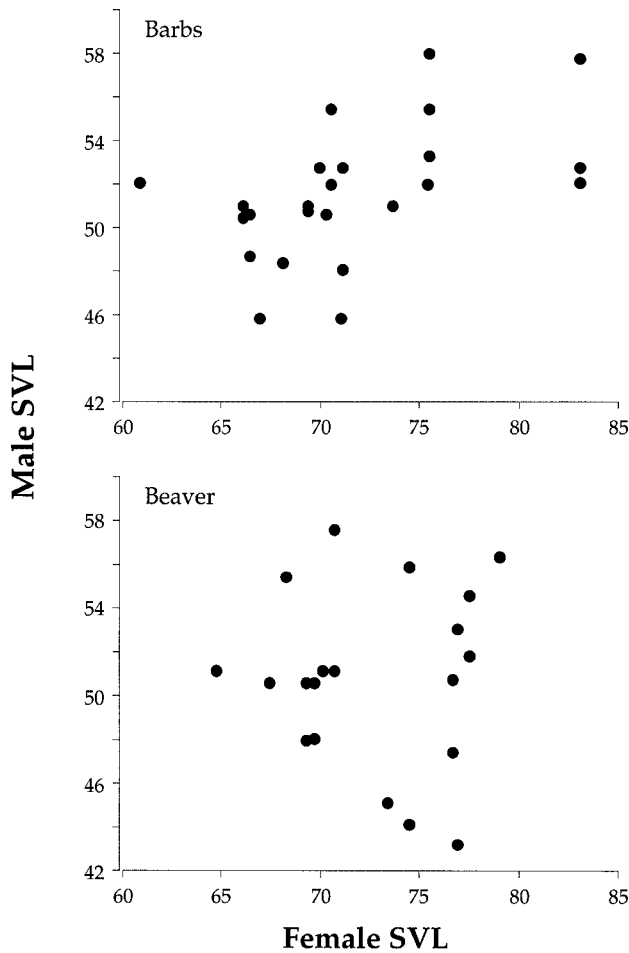


Figure 1

The size (snout-vent length [SVL] in centimeters) of male water snakes and of the females with which they sired young in Barbs and Beaver Marshes (data combined across years). Females with multiply sired litters are shown once for each male.

Genetic similarity and variability

Mean relatedness scores (r values; Queller and Goodnight, 1989) for pairs that produced offspring together were similar to those for all pairs of males and females from the same marsh that did not produce offspring together in both Barbs (0.014 and 0.073) and Beaver Marsh (-0.039 and 0.005). A randomization analysis (STRUC subroutine of GENPOPOP; Raymond and Rousset, 1995) indicated that the distribution of r values for reproductive and non-reproductive pairs of snakes were not significantly different in either marsh (Barbs: $p = .64$; Beaver $p = .16$). Thus, water snakes appear to mate at random with respect to genetic similarity.

Our first test of the prediction that individual variation should increase male reproductive success simply involved determining whether male reproductive success varied with the percentage of microsatellite loci at which males were heterozygous. Male success did not vary significantly with heterozygosity at Barbs Marsh in either year (1994: Spearman $r = .19$, $N = 36$, $p = .26$; 1996: Spearman $r = .17$, $N = 19$, $p = .49$) or overall (Spearman $r = .10$, $N = 55$, $p = .47$). The same was also true at Beaver Marsh (1994: Spearman $r = .15$, $N = 40$, $p = .36$; 1995: Spearman $r = -.26$, $N = 36$, $p = .13$; 1996: Spearman $r = .17$, $N = 21$, $p = .46$; overall: Spearman $r = -.02$, $N = 97$, $p = .87$). Our second method substituted natural log-transformed mean d^2 (Pemberton et al., 1999) as our

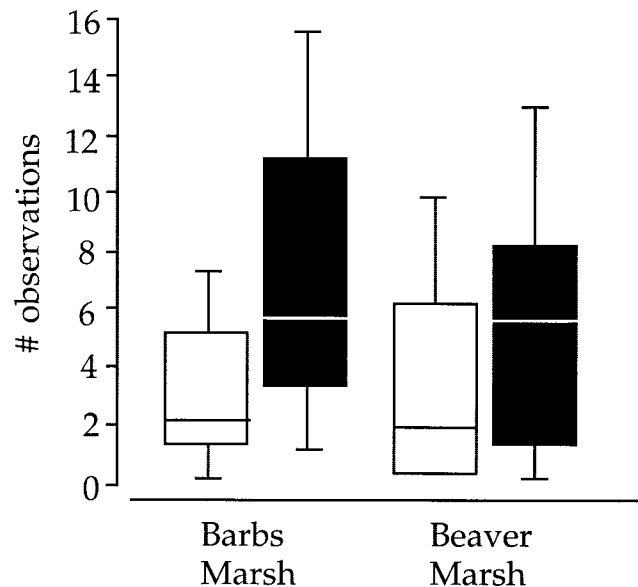


Figure 2

Box plot of the number of times male water snakes were observed during the mating season for individuals that sired offspring (shaded boxes) and those that did not (open boxes) at each marsh. Data are combined within marshes across years. Whiskers show 95th percentiles, boxes indicate 50th percentiles, and horizontal lines are medians.

measure of individual variability. In Barbs Marsh male success did not vary significantly with mean d^2 in either year (1994: Pearson $r = -.15$, $N = 36$, $p = .37$; 1996: Pearson $r = -.17$, $N = 19$, $p = .48$) or overall (Pearson $r = -.16$, $N = 55$, $p = .23$). Similarly, in Beaver Marsh male success did not vary with mean d^2 in any year (1994: Pearson $r = .24$, $N = 40$, $p = .15$; 1995: Pearson $r = .09$, $N = 36$, $p = .61$; 1996: Pearson $r = -.08$, $N = 21$, $p = .74$) or overall (Pearson $r = .13$, $N = 97$, $p = .21$).

DISCUSSION

Analysis of the relative variance in reproductive success for males and females in our study population of northern water snakes revealed that the potential for sexual selection was approximately five times greater for males (Prosser et al., 2002). Despite this potential, however, paternity analyses revealed no evidence that any male traits were consistently or strongly targeted by sexual selection. Male reproductive success did not vary significantly with body size, tail length, or condition. Even within litters sired by two males, the relative success of the two males was unrelated to their absolute or relative sizes, tail lengths, or condition. Male success also did not vary significantly with home range size, even though males responded to the highly clumped distribution of reproductive females in Beaver Marsh by having much smaller home ranges than those of males in Barbs Marsh (Brown and Weatherhead, 1999b). Finally, reproductive success did not vary with the number of microsatellite loci at which males were heterozygous, with individual genetic variability (d^2), or with their genetic similarity to females.

We did find some evidence that mating was not completely random. First, we found evidence of positive assortative mating by size in Barbs Marsh, although we found no evidence of assortative mating in Beaver Marsh. Second, males that were more active during the mating season were more successful, particularly in Barbs Marsh. We first discuss possible

reasons that no male morphological features correlated with reproductive success and then discuss the implications of the few factors we did find that were correlated with male success. We conclude by considering the implications of all these results for the central goal of explaining sexual size dimorphism in water snakes.

In any study that involves assignment of paternity, unassigned young could affect the results. Overall, we successfully assigned just > 80% of offspring. At Barbs Marsh our assignment success was 93%, with only three females producing young sired by unknown males. A 3-year telemetry study indicated that both males and females rarely moved out of Barbs Marsh other than to hibernate (Brown, unpublished data). Thus, the unassigned offspring in Barbs seem likely to have been sired by males we failed to capture. It also seems likely that snakes we considered resident in Barbs were not leaving to mate with snakes elsewhere. Thus, our assessment of patterns of reproductive success in Barbs is probably an accurate reflection of the true patterns. At Beaver Marsh just fewer than one-third of all offspring were unassigned. The capture patterns of females producing those young suggested there may have been some mating activity by Beaver snakes elsewhere (Prosser et al., 2002), although telemetry again indicated strong site fidelity by snakes in Beaver Marsh. For the mating activity outside the marsh to alter our results would require that mating patterns outside Beaver Marsh were strongly and consistently different from patterns within Beaver Marsh. We can envision no plausible scenario for this happening, particularly given the overall similarity of patterns we documented in Barbs and Beaver Marshes.

Sampling error could also have occurred if we failed to capture some gravid females and thus did not attribute any reproductive success to the males that sired their offspring. We have no way to assess this possibility directly. However, it seems unlikely to have been a problem because of the behavior of gravid females. Thermoregulatory demands cause females to bask more when gravid (Brown and Weatherhead, 2000), making them much easier to find than other snakes. They also are probably easier to capture because they are gravid. Because we searched marshes intensively for gravid females, and because we caught many females that were not gravid, we think it unlikely that we missed any gravid females. Thus, it is reasonable to conclude that we have documented an accurate picture of mating patterns in the water snake populations we studied.

We predicted that more active males (i.e., those we saw more often) should be more successful based on Brown and Weatherhead's (1999b) observation that male water snakes seen courting females were observed more than males not seen courting. It is possible that courting males are more visible than noncourting males or, alternatively, that courting males were more active than noncourting males. The increase in reproductive success with the number of times males were seen supports the latter interpretation. Observations of courtship did not reliably predict with which male(s) a female mated (Prosser et al., 2002), so males we saw courting more must also have courted more when we did not observe them. Madsen et al. (1993) found that the distance traveled by male adders during the mating season was a significant predictor of the number of females mated. In other taxa with scramble mating competition among males (e.g., voles: Gaulin and Fitzgerald, 1989; ground squirrels: Schwagmeyer, 1988), searching ability of males also appears to be important to male success (Andersson, 1994).

Given that males that were more active during the mating season were more successful, it seems surprising that male condition at the start of the mating season was not correlated with reproductive success. However, Brown and Weatherhead

(1999b) found that male water snakes do not lose weight over the breeding season. Furthermore, males have been observed feeding during the mating season (Brown and Weatherhead, personal observations), so, at least in this population, mating does not rely on the use of stored fat. Because male mortality increases after sexual maturity, and mortality is highest during the mating season (Brown and Weatherhead, 1999a), the cost of greater activity by males appears to be in the form of increased risk of predation rather than increased risk of starvation.

We predicted that males that were genetically more variable or genetically different from particular females would be more successful, either through direct female preference or through sperm competition. Madsen et al. (1999) recently demonstrated experimentally that male European adders introduced into an inbred population from elsewhere were disproportionately successful. We found no evidence that any aspect of male genotypes we considered affected mating success. Multiple mating by females, as indicated by the frequency of multiply sired litters, should have provided adequate opportunity for sperm competition. Furthermore, although there may be limited gene flow between water snake populations, they do not appear to be highly inbred (Prosser et al., 1999). It may be that genetic factors such as heterozygosity or specific differences among mating partners only affect reproductive success when inbreeding is more pronounced.

Although male success did not vary with body size, male body size did affect paternity patterns, and it did so differently in the two marshes. In Barbs Marsh, the sizes of males and females that produced young were positively correlated, but the pattern was random in Beaver Marsh. Brown and Weatherhead (1999b) found that larger males courted more females in Barbs Marsh, but in Beaver Marsh no size advantage existed. Thus, in Barbs Marsh (where females are more dispersed), large males appear to have an advantage in finding and mating with larger females. Furthermore, because larger females produce more offspring (Weatherhead et al., 1999), large males should realize a reproductive advantage from mating with those females. A factor that might eliminate that advantage is that the ratio of females to males appears to decline with size (Brown and Weatherhead, 1999c; Weatherhead et al., 1995), so there could be proportionately more males competing for larger females. Consistent with that interpretation is the observation that multiple paternity increases with female size in water snakes (Prosser et al., 2002). In Beaver Marsh (where females are clumped), male size does not affect success at finding females (Brown and Weatherhead, 1999b), and paternity is random with respect to male size. Thus, for apparently different reasons, sexual selection does not favor males of a particular size in either marsh.

Our central goal was to determine how male reproductive success varied with male body size and thus to determine whether sexual selection is responsible for maintaining male water snakes at a substantially smaller size than females (Brown and Weatherhead, 1999c). We found no evidence that sexual selection favored smaller males, or indeed that body size affected male success in any way. Given that sexual selection does not explain sexual size dimorphism in northern water snakes, what does explain why males are so much smaller than females? Based on our previous work with this population, we propose the following hypothesis. First, female size is a trade-off between survival and fecundity. Fecundity and survival both increase with female size, although survival declines once females become reproductive (Brown and Weatherhead, 1997, 1999a). Even though females survive better as they grow, mortality is still high, so delaying maturation too long to maximize fecundity makes the probability of dying before repro-

ducing too high. Thus, females mature at the size that maximizes lifetime fecundity (Brown and Weatherhead, 1999a).

Male survival also increases with size up to the point of sexual maturity (Brown and Weatherhead, 1999a). However, because male reproductive success does not increase with size, selection favors maturation at a smaller size in males than in females. Furthermore, the lack of reproductive advantage to larger size means that males do not need to forage extensively to promote growth, which allows them to adopt a low-energy strategy outside the breeding season (Brown and Weatherhead, 2000). One aspect of water snakes' natural history that does not appear to fit this scenario is that males grow more slowly than females beginning at birth (Brown and Weatherhead, 1999c). The optimal strategy for males would seem to be to follow the same growth trajectory as females up to the size that males mature sexually and then to adopt the low-energy/low-growth strategy thereafter. The fact that they do not follow this pattern may indicate that the size at sexual maturation imposes some constraint on the growth trajectory that can be followed. Comparative analyses of patterns of growth in snakes will be necessary to assess this possibility.

We have ruled out an important role for body size in affecting the outcome of sexual competition among the male northern water snakes we studied. However, the fact that body size did influence patterns of mating in one of the marshes suggests the possibility that in different ecological circumstances body size could be important. Brown and Weatherhead (1999c) compared the water snake population that we studied with two other populations and showed that female growth patterns were quite similar among populations. However, male growth patterns differed substantially among populations, particularly with respect to asymptotic sizes. Thus, comparative studies of sexual selection on male water snakes in different ecological circumstances both within and among populations may yet reveal situations under which male size is subject to sexual selection.

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