Insights into Population Ecology and Sexual Selection in Snakes Through the Application of DNA-Based Genetic Markers

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Hypervariable genetic markers have revolutionized studies of kinship, behavioral ecology, and population biology in vertebrate groups such as birds, but their use in snakes remains limited. To illustrate the value of such markers in snakes, we review studies that have used microsatellite DNA loci to analyze local population differentiation and parentage in snakes. Four ecologically distinct species of snakes all show evidence for differentiation at small spatial scales (2–15 km), but with substantial differences among species. This result highlights how genetic analysis can reveal hidden aspects of the natural history of difficult-to-observe taxa, and it raises important questions about the ecological factors that may contribute to restricted gene flow. A 3-year study of genetic parentage in marked populations of the northern water snake showed that (1) participation in mating aggregations was a poor predictor of genetic-based measures of reproductive success; (2) multiple paternity was high, yet there was no detectable fitness advantage to multiple mating by females; and (3) the opportunity for selection was far higher in males than in females due to a larger variance in male reproductive success, and yet this resulted in no detectable selection on morphological variation in males. Thus genetic markers have provided accurate measures of individual reproductive success in this species, an important step toward resolving the adaptive significance of key features including multiple paternity and reversed sexual size dimorphism. Overall these studies illustrate how genetic analyses of snakes provide previously unobtainable information of long-standing interest to behavioral ecologists.

Studies of genetic relationships from the level of the individual to the population have been revolutionized by hypervariable DNA-based genetic markers (Avise 1994). Genetic analyses have been especially useful for inferring patterns of demographic connectedness of populations (Koenig et al. 1996) and in providing accurate measures of individual reproductive success through genetic parentage analyses (Westneat and Webster 1994). Such work has provided new information on patterns of intergroup dispersal in relation to the age and sex of individuals, and has emphasized an often striking disparity between "direct" and "indirect" measures of dispersal (Dobson 1994; Edwards 1993; Waser and Elliott 1991; see review in Koenig et al. 1996). For many species (notably birds; e.g., Gibbs et al. 1990), DNA-based analyses of parentage have also revealed substantial differences between the "genetic" and "behavioral" mating systems, attributable to reproductive phenomena such as extra-pair fertilizations, that can only be detected using genetic methods. This has lead to the recognition that behavioral measures of reproductive success (e.g., mating activity) may be inadequate for assessing sexual and natural selection (Westneat and Webster 1994).

Application of DNA-based techniques has been uneven among vertebrate taxa. Most studies have been carried out on birds and mammals, which in many cases are comparatively easy to observe with respect to reproductive and dispersal activities. However, genetic approaches may be even more valuable for difficult-to-observe or cryptic taxa in which systematic observation of individual behavior is extremely difficult. Snakes represent such a group whose secretive behavior has hindered attempts to uncover important features of their behavior and ecology (Parker and Plummer 1987).

Previously DNA-based markers have not been widely used for analyses of kinship in snakes (Duvall et al. 1993). Studies of population structure have focused on levels of differentiation or phylogeographic

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relationships among populations at broad geographic scales (Dessauer et al. 1987; Parkinson et al. 2000; Rodríguez-Robles et al. 1999) and few have used detailed analyses of local kinship to examine dispersal and mating behavior (but see Bushar et al. 1998; Gibbs et al. 1997; 1998; Lougheed et al. 1999; Prior et al. 1997). In addition, whereas attempts to assess patterns of sexual and natural selection in snakes have increased (reviews in Duvall et al. 1993; Olsson and Madsen 1998), all studies to date have been confined to behavioral measures of individual reproductive success such as number of matings (Madsen and Shine 1994) or mating attempts (Weatherhead et al. 1995; Brown and Weatherhead 1999b). There are several reasons why such behavioral estimates of reproductive success may be inaccurate, including the possibility of incomplete and/or misleading observations of mating behavior and the effects of sperm competition (e.g., Devine 1984).

In this article we review the results from studies that have used microsatellite DNA markers (Quellar et al. 1993) to conduct population and parentage studies in snakes. We argue that information from such markers can make significant contributions to behavioral, ecological, and evolutionary studies of snake biology by (1) helping to identify the patterns of dispersal and/or mating behavior that must underlie observed patterns of relatedness; and (2) improving the accuracy of estimates of reproductive success of individuals (particularly males) relative to estimates based solely on behavior. Accurate estimates of individual reproductive success are essential for elucidating mating systems and for determining how natural and sexual selection act on individual variation to produce patterns of long-standing interest such as differences in the direction and degree of size dimorphism observed among species (e.g., Shine 1993).

We illustrate these points in two ways. First, we review studies that have documented population structure at small ($<$20 km) geographic scales for four snake species, and we discuss the implications of these studies for mechanisms of gene flow. Second, for one of these species [northern water snake (*Nerodia sipedon*)], we briefly review new results from a long-term study of sexual selection in a marked population (Weatherhead et al. 1995). This latter study allowed us to address questions about the relationship between behavioral and genetic measures of reproductive success, advantages of multiple paternity for females, and patterns of sexual selection in males and females.

Microgeographic Genetic Structure in Snake Populations

At large spatial scales, reptiles such as snakes show a much higher degree of genetic structure than more vagile organisms such as birds (Ward et al. 1993). However, snakes have a number of ecological, behavioral, and life-history characteristics that make it possible that gene flow could be quite restricted even at local scales. For example, in many species, critical habitat features such as overwintering hibernacula or microhabitats required by gestating females to maintain their body temperatures are heterogeneously distributed through space (Reinert 1993). There is also some evidence for local adaptations whose maintenance would be facilitated by restricted gene flow (Daltry et al. 1996). Genetic analyses at local geographic scales provide an indirect method for assessing whether such ecological factors have actually had an impact on realized gene flow. For difficult-to-observe species, such indirect approaches may be the best or only way of assessing past movements and mating behaviors of individuals.

In the following section we first briefly discuss the availability and usefulness of microsatellite DNA markers for genetic analysis of snake populations. Then, on a case-by-case basis, we review the results of four studies of fine-scale genetic structure in four ecologically diverse species. In general, all show evidence of genetic structure at small (2–20 km) geographic scales. However, there are also differences in the spatial patterning of differentiation that may be related to ecological differences between species. We discuss the implications of these results for understanding movement patterns and reproductive behaviors for each species, and we outline questions that should be addressed with future work.

Genetic Markers for Population Analyses

Until recently, allozyme loci provided the markers used most widely for genetic analysis of snake populations [see Dessauer et al. (1987) for a review]. However, highly variable microsatellite DNA loci are superior for both fine-scale analysis of genetic structure and parentage analysis (Quellar et al. 1993). A number of species-specific loci have been cloned in snakes, and some of these also amplify loci in other related species (Burns and Holden 1999; Gibbs et al. 1997 and unpublished; McCracken et al. 1999; Lougheed et al. 1999; Prosser et al. 1999; Villarreal et al. 1996).

Microsatellite loci appear to be superior to some other classes of DNA loci for fine-scale analyses of population structure (e.g., populations separated by $\geq$10 km). For example, Lougheed et al. (2000) used an assignment test developed by Paetkau et al. (1995) to compare the power of random amplified polymorphic DNA (RAPD) versus microsatellite DNA loci to correctly assign individual massasauga rattlesnakes to the five populations from which they had been collected. The probability of assigning individuals to their original population ranged from 92 to 100% for microsatellites, whereas for RAPD the values were much lower (29–74%). This higher resolution is likely related to the enhanced levels of variation at microsatellite loci and to the fact that these markers are codominant rather than dominant (Smouse and Chevillon 1998).

Kinship Analysis of Black Rat Snakes Within and Between Local Hibernacula

Many snakes in north temperate regions use communal hibernacula for overwintering (Gregory 1984), and individuals within species may exhibit high between-year fidelity to specific hibernacula (Larsen 1987). The black rat snake (*Elaphe obsoleta*) studied in the northern part of its range provides such an example. These snakes hibernate communally, and once an individual joins a communal hibernaculum, it almost invariably returns to the same overwintering site year after year (Blouin-Demers et al. 2000, in preparation; Prior and Weatherhead 1996; Weatherhead and Hoysak 1989). Adult rat snakes also use the same home ranges for a number of years (Stickel et al. 1980; Weatherhead and Hoysak 1989). This strong fidelity to summer and winter habitats raises the question of whether adult snakes in each hibernaculum are comprised of close kin (Gannon 1978). However, because rat snakes mate after dispersing from hibernacula, and because the home ranges of snakes from nearby hibernacula can overlap (Weatherhead and Hoysak 1989), it is also possible that there are high levels of intersite gene flow mediated by intermating between snakes from different overwintering sites.

We explored this issue by collecting blood samples from 6 to 43 (mean 23.8 $\pm$

12.0 SD) adult snakes identified as overwintering at nine hibernacula located in three subpopulations in eastern Ontario, Canada (Lougheed et al. 1999). Snakes were predominately captured following emergence, although some were also captured opportunistically during the breeding season. Within each subpopulation, hibernacula were all located within 6 km of one another (range 0.5–6 km). We assayed genetic variation in these samples using eight rat snake-specific microsatellite loci. We also sampled rat snakes from two hibernacula in suburban Maryland, but because the sites were in heavily disturbed habitats (Prior et al. 1997), we do not discuss those results here.

We used two approaches to assess levels of relatedness among snakes within individual hibernacula. First, we calculated F_{IS} values, which identify nonrandom associations between alleles, and tested whether they were significantly different from zero using FSTAT (Goudet 1995). Second, we used KINSHIP (Goodnight and Quellar 1999) to estimate relatedness (r; Quellar and Goodnight 1989) between all pairs of individuals within single sites, and then compared the distribution of observed values with simulated distributions expected given (1) no relatedness among individuals (expected $r = 0$) and (2) full-sib relationships (expected $r = 0.5$). We used allele frequencies pooled across hibernacula within each subpopulation for this analysis because we found no evidence for genetic differentiation among hibernacula (see below). We also determined the levels of differentiation between hibernacula by calculating all pairwise values of F_{ST} using ARLEQUIN (Schneider et al. 1997) and R_{ST} values (Slatkin 1995) using RSTCALC (Goodman 1997). Permutation procedures were used to test whether a given value was significantly different from zero.

All F_{IS} values (pooled across all six loci) were small (0.00–0.13) and not significantly different from zero, suggesting that levels of inbreeding within single hibernacula are low to nonexistent. This result was paralleled by our analysis of r values between snakes within hibernacula, where the distributions of observed relatedness values invariably were almost identical to the distribution of r values expected if individuals were unrelated (as opposed to, for example, being full sibs) (Lougheed et al. 1999).

Consistent with the results above, we also found no evidence of genetic differentiation between hibernacula within subpopulations. All F_{ST} (mean 0.01, range 0.00–0.027) and R_{ST} (mean 0.006, range 0.00–0.026) values were small and not significantly different from zero. Thus the lack of inbreeding and absence of differentiation between local hibernacula suggest that levels of gene flow are high and that effective population sizes are large (N_e 600–1000; Lougheed et al. 1999) relative to the number of snakes typically found in a single hibernaculum. However, significant differentiation was observed between subpopulations 15–50 km apart (mean F_{ST} 0.059, mean R_{ST} 0.062), indicating that these snakes show structure over relatively fine geographic scales.

These results suggest that adult site fidelity at hibernacula is unimportant in determining the genetic structure of local populations, and that mating between males and females from different hibernacula and/or juvenile dispersal are significant mechanisms for gene flow in these snakes. We are currently exploring this further using DNA-based studies of parentage and hibernaculum choice by young snakes relative to the hibernacula of their parents (Blouin-Demers G, et al., in preparation). Assessing the geographic scale over which these behaviors occur in relation to habitat heterogeneity may clarify why rat snake populations start to show evidence of genetic differentiation between populations separated by more than 15 km (Lougheed et al. 1999). They also may indicate how human-induced habitat fragmentation can impact the demographic and genetic connectedness of rat snake populations at different spatial scales.

Population Structure in Eastern Massasauga Rattlesnakes

The eastern massasauga rattlesnake (*Sistrurus c. catenatus*) is a small (<75 cm), venomous, live-bearing species found in both wetlands and dry upland habitats throughout its range in eastern to central North America (Conant and Collins 1991; Weatherhead and Prior 1992). This species is especially abundant on the eastern shore of Georgian Bay and on the Bruce Peninsula, where the populations appear to be more-or-less continuously distributed (Wellar and Oldham 1993). In the rest of its range these snakes exist in small, habitat-isolated populations (Johnson and Menzies 1993). They do not hibernate in large aggregations, but rather as individuals in separate locations. Telemetry data indicate that individual home ranges cover maximum distances of about 1 km on average, and that although these snakes are somewhat sedentary, individuals have been recorded traveling more than 1 km in 1 day (Weatherhead and Prior 1992).

Gibbs et al. (1997) used six microsatellite DNA markers to conduct genetic analysis of samples (n = 25–80 individuals per population) collected from five regional populations (all ≥50 km apart) in Ontario, New York, and Ohio. All samples were collected opportunistically from snakes during the reproductive season in each population. They found significant differentiation: overall F_{ST} was 0.19 between all populations, and each population contained a substantial portion (mean 23%) of unique or private alleles seen nowhere else. Significant differentiation was also detected within regional populations between samples collected at sites approximately 2–35 km apart. Most impressive was the fine-scale genetic structure observed between samples collected from two sites whose geographic centers are roughly 1.5 km apart [Twin Points (n = 27 snakes) and Blind Bay (n = 41)] within Killbear Provincial Park. For these sites, significant differences in allele frequencies (all $P < .0033$; overall $P < .0001$) were documented at five of six loci and the overall F_{ST} value (0.04) was significantly different from zero ($P < .001$). In contrast to the results for rat snakes, these analyses indicate that the microgeographic differentiation in snakes can exist on a scale of less than 5 km within the continuous range of a species. Low levels of gene flow and genetic isolation at very small spatial scales may be the evolutionary norm for this species, and this may be a reason why these snakes persist even as isolated populations in small, recently fragmented landscapes (Gibbs et al. 1997).

Genetic Differentiation in Northern Water Snakes

Based on microsatellite DNA analyses, the northern water snake also shows evidence for local population differentiation. This species is ecologically different from the terrestrial species described above because small populations frequently occur in isolated bodies of water (such as beaver ponds) separated by extensive stretches of land. Thus restricted dispersal by this largely aquatic species may lead to genetic differentiation between its relatively isolated populations.

Prosser et al. (1999) used eight species-specific microsatellite loci to analyze samples of roughly 50 individual northern water snakes collected during the breeding season from each of three ponds in east-

ern Ontario, all within 2 km of one another. Based on mark-recapture analyses at two of the ponds, each population contained ≤100 adults total (Brown and Weatherhead 1999a). Using F_{ST}, small but significant levels of differentiation were detected over all populations ($F_{ST} = 0.006$; $P = .009$) and in each pairwise comparison (all $F_{ST} = 0.006$; all $P < .02$). However, R_{ST} values showed no evidence for differentiation. The fact that these different measures gave contrasting results argues that these populations are at the lower limit with respect to the geographic scale over which pond-based populations of this species may differ detectably. At this spatial scale, migration between ponds may be sufficiently limited that allele frequencies in populations of this size can begin to drift apart.

Genetic Differentiation in Timber Rattlesnakes

The timber rattlesnake (*Crotalus horridus*) is found in mountainous regions in the eastern United States. Small numbers of snakes overwinter in hibernacula in rocky areas with southern exposures, and individuals may reuse the same hibernacula in multiple years (Conant and Collins 1991). In southeastern Pennsylvania, the ecology and demography of these snakes has been studied for more than 15 years (Reinert 1984, 1993). Recently microsatellite DNA markers (Villarreal et al. 1996) were used to examine patterns of differentiation between snakes sampled from five hibernacula located between 500 and 3000 m apart within a 6000 ha study site (Bushar et al. 1998). Although sample sizes were small (≤8 specimens were collected from four of the hibernacula), evidence was found for significant between-hibernacula differentiation. Based on a measure of identity probability developed by Paetkau and Strobeck (1994), individuals using the same hibernacula were genetically more similar to one another than they were to randomly chosen individuals from the local population. Furthermore, F_{ST} values for 5 of 10 possible pairwise comparisons of hibernacula were both positive (range 0.05–0.12) and had 95% confidence intervals that did not overlap zero. Possible mechanisms that may lead to this differentiation are a high frequency of mating between individuals from the same hibernaculum, and the possibility that juveniles may find hibernacula by trailing their mothers to overwintering sites (Brown and McLean 1983; Reinert and Zappalorti 1988). If correct, these mechanisms would illustrate the importance of habitat heterogeneity (patchy distribution of suitable basking and overwintering sites) in restricting interhibernacula gene flow. These results need to be confirmed with larger samples, but they do provide preliminary evidence of genetic differentiation at a small geographic scale in yet another taxonomically and ecologically distinct species of snake.

Implications of Results

Several implications follow from the evidence that four ecologically different snakes all show genetic differentiation at microgeographic scales. First, the findings reveal the power of genetic analyses in studying ecologically relevant behaviors of difficult-to-observe species. The suggestion that individuals show limited dispersal over relatively short distances is not predicted from data on individual movement patterns based on telemetry data for any of these species (see below). Thus DNA-based analyses should be considered a powerful tool that complements more conventional techniques for studying certain aspects of snake movement and mating behavior.

Second, the disparity between the potential movement patterns of these snake species based on telemetry data and the realized dispersal based on gene flow is in striking contrast to the pattern found in other vertebrates (Koenig et al. 1996). In some birds and mammals for example, genetic analyses commonly suggest that the incidence of long-distance dispersal exceeds that predicted using behavioral methods such as mark-recapture studies (Dobson 1994; Edwards 1993; Waser and Elliott 1991). The snakes described here all show the opposite pattern. Radiotelemetry studies on each species have shown that individuals can move more than 1 km in a day (although average movements may be much less than this; Blouin-Demers G and Weatherhead PJ, unpublished; Brown GP and Weatherhead PJ, unpublished; Bushar et al. 1998; Weatherhead and Hoysak 1989; Weatherhead and Prior 1992). However, all species show evidence of genetic structure at a scale that is small relative to the distances that individuals can move. Perhaps movements during the active season are not necessarily indicative of gene flow mediated by movements during the mating season, particularly in species such as the massasauga rattlesnake that exhibits genetic structure at the finest scale. These results also suggest that juvenile dispersal is extremely limited in at least some of these snakes, suggesting that dispersal may be disproportionately costly for snakes relative to other vertebrates. If so, the differences in scale of population structure shown by the four species also suggest that dispersal costs might vary substantially among species.

A third implication, discussed by Gibbs et al. (1997), is relevant to snake conservation. The size of demographically independent management units (Moritz 1994) defined by the population genetic structure in the snakes described here is very small. If this is true for snakes generally, then any natural repopulation of areas where a species is in decline or is extinct may be very slow. Thus active translocations may be needed to reestablish extirpated populations, even in the midst of what might appear to be continuously distributed populations. Also, the source of donor individuals for such translocations would have to be carefully considered in light of the observed genetic distinctiveness of populations. Caution in this regard seems warranted until we learn whether local population differentiation represents simple drift effects on neutral variation or is indicative of some type of local adaptation (see below).

DNA-Based Analysis of the Mating System and Sexual Selection in Northern Water Snakes

Virtually all aspects of sexual selection research (e.g., identifying reproductive strategies, characterizing mating systems, understanding the basis for sexual dimorphism) ultimately require knowledge of individual reproductive success. Of specific interest are the means and variances of offspring production among individuals. For females, quantifying true reproductive success is relatively straightforward in many animals. For males, however, it has been necessary to use the number of social mates, copulations, offspring produced by social mates, or some other indirect measure of reproductive success. DNA-based methods of paternity analysis now permit verification of whether these indirect measures of reproductive success are reliable, and if not, to re-address the questions of interest using more accurate measures. In this section we review how we have used paternity analysis to investigate several aspects of sexual selection in the northern water snake. Details of this research are presented by Prosser et al. (manuscripts in review).

This research program, initiated in 1990, had two major goals: (1) to quantify the

extent to which females produce litters sired by multiple males and to understand the adaptive basis for multiple mating, and (2) to understand the factors that contribute to variance in male reproductive success and, specifically, to determine how the relatively smaller body size of males (Brown and Weatherhead 1999c) might be related to male reproductive success. The research has been conducted in eastern Ontario, where water snakes are abundant in most bodies of water. By using study populations in relatively isolated beaver ponds, it was feasible to mark most juvenile and adult water snakes individually. For long-term identification snakes were either scale branded or PIT tagged, and for short-term identification (e.g., within a mating season) snakes were marked with unique patterns of acrylic paint (Barry et al. 1992; Brown and Weatherhead 1999b; Weatherhead et al. 1995).

Barry et al. (1992) used allozymes to investigate the incidence of multiple paternity within litters. They captured adult females from a number of beaver ponds and held them in the laboratory until they gave birth. Multiple paternity was detected either directly (more than two paternal alleles present in a litter) or indirectly (only two paternal alleles present but genotype frequencies significantly different from those expected with a single father). Barry et al. (1992) estimated that 12 of 14 (85.7%) litters were multiply sired. Prosser et al. (in review) used microsatellite markers (Prosser et al. 1999) to investigate multiple paternity. This technique, combined with an intensive sampling regime focused on just two pond populations, allowed the exact number of sires in each litter to be determined. Multiple paternity was detected in 26 of 48 (54.2%) litters. Most litters had only one or two sires, but the maximum number recorded for a single litter was six. The incidence of multiple paternity was higher where females were spatially more clumped, in years with shorter mating seasons, and in larger females. There was no evidence that multiple mating reduced the risk to females that not all their ovules would be fertilized, or that multiple mating by females reduced the incidence of stillborn young [as had been reported by Madsen et al. (1992) for the European adders (*Viperus berus*)].

Conventional (i.e., nongenetic) approaches to quantifying male reproductive success in water snakes indicated that approximately one-third of all sexually mature males did not attempt to mate within a given year (Weatherhead et al. 1995). Where females were dispersed, males had larger home ranges and the number of females encountered by males increased with home range size, whereas the opposite patterns were found where females were spatially clumped (Brown and Weatherhead 1999b). Furthermore, where females were dispersed, males that were larger and in better condition were observed in more mating aggregations, but where females were clumped, no advantage to size or condition was found. We then used microsatellite analyses of paternity to reexamine these results (Prosser et al., in review). One discovery was that the behavioral and genetic estimates of male reproductive success were poorly correlated. Many males observed in mating aggregations failed to sire any offspring, whereas some males not seen in aggregations did so. There was also little agreement between which males were observed courting a given female and those that actually sired her offspring. The variance in male reproductive success was substantially higher when estimated from genetic paternity data than from mating behavior. This high variance in male success indicated that the opportunity for selection on males was more than 70 times higher than on females. Despite this strong sexual selection pressure, none of the behavioral or morphological attributes of males that we examined (e.g., home range size, number of females courted, body size, relative tail length, or condition) reliably predicted male reproductive success (Prosser et al., in review). Thus our considerable progress in obtaining accurate estimates of male reproductive success has not yet been matched by progress toward understanding the causes or consequences of sexual selection in this species.

Directions for Future Research

The genetic results reviewed here raise questions about important aspects of the biology of the species studied to date and of other snake species. One obvious question is whether the patterns of population genetic structure detected thus far are typical of snakes in general. Perhaps the fine-scale structure observed is merely a unique feature of the populations examined. However, this seems unlikely. For the massasauga rattlesnake and the black rat snake, this seems unlikely because the same pattern of population structure was observed at more than one location. We are less sure of the generality of the results for the other two species because each was studied in a single site, and outcomes might differ elsewhere. For example, water snakes living along the shore of a large lake might be less likely to display the fine-scale genetic structure that was observed for this species in relatively isolated beaver ponds. Analyses of populations from ecologically and geographically distinct locations are required to address such issues. In addition, comparisons of the patterns of structure reported here, which are based on biparentally inherited markers, with those from sex-specific markers (e.g., female-specific mtDNA) may provide insights into whether there are differences in dispersal between males and females (e.g., Seielstad et al. 1998).

A second issue concerns the nature of selection pressures that may have resulted in restricted dispersal and gene flow in these species. For all of the species, dispersal costs may be related to high risks of predation, or the risk of losing contact with an essential but patchily distributed resource such as a hibernaculum. Understanding such risks will be an important step toward understanding the selection pressures that shape snake movement patterns. For venomous species, an intriguing possibility is that genetic structure may reflect local adaptation in venom composition matched to local prey. Daltry et al. (1996) found significant geographic variation in venom composition in the Malayan pit viper (*Calloselasma rhodostoma*), a result which they presumed was genetically based and locally adaptive, reflecting differences in prey specialization by snakes in different areas. If so, variation in venom composition should map onto the genetic structure of the population. The only other study to examine this possibility found no evidence that geographic allozyme variation in Mojave rattlesnakes (*Crotalus s. scutulatus*) matched the spatial pattern of venom variation (Wilkinson et. al 1991). Additional studies using more sensitive measures of variation in both venom and genetic structure among snake populations are needed.

The paternity analyses also raise important questions. In the northern water snake, what factors explain variance in male reproductive success? More generally, are behavioral estimates of male reproductive success in other snake species as unreliable as we found for water snakes? It seems reasonable to expect that where copulations are scored (rather than just mating attempts), the behavioral observations should be more reliable. How-

ever, multiple paternity appears to be quite common in snakes (Olsson and Madsen 1998). Thus to use copulations to predict reproductive success will probably require knowing how sperm competition is affected by such things as the relative timing of each copulation and the duration of the copulation events.

For most snakes, the reliability of behavioral estimates of reproductive success has not been an issue simply because systematic observations of behavior in the field have not been feasible. The good news from our paternity analyses is that DNA-based techniques for determining paternity are well suited to studying snake reproductive biology. Obtaining insights into sexual selection by this approach requires the development of highly variable microsatellite markers (quite feasible in our experience) coupled with logistic considerations. Typically, gravid females must be captured and held until they give birth or lay eggs, so that blood from neonates can be sampled for DNA. Also, a substantial proportion of the potential sires in the local population ideally must be included in the genetic analyses. Relatively closed populations (such as water snakes in beaver ponds) are ideally suited for this kind of research, but any population could prove suitable particularly if mark-recapture data are available that would allow assessment of whether most snakes in a population have been sampled. For various reasons, the use of snakes as model organisms in ecological research has increased substantially in recent years (Shine and Bonnet 2000). The ability to study sexual selection in snakes using DNA-based parentage analyses can only contribute further to this trend.

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