Copeia, 2002(1), pp. 15–23

Geographic Variation of Multiple Paternity in the Common Garter Snake (*Thamnophis sirtalis*)

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The common garter snake, *Thamnophis sirtalis*, is the most widely distributed reptile species in North America. Although multiple paternity has been documented in this species, variation in reproduction and ecology suggests that the frequency of occurrence of multiple paternity may vary. We investigated the occurrence of multiple paternity in snakes on Vancouver Island with the following aims: (1) to detect the occurrence of multiple paternity at this location; (2) to determine whether life-history variation and single versus multiple paternity were associated; and (3) to determine whether local rates of multiple paternity differ in comparison to a previous study of this species. Sixteen females and their offspring were analyzed using three highly polymorphic microsatellite loci. Only six of 16 litters showed direct evidence of multiple paternity. Results also showed evidence of a trade-off between offspring size and number of offspring per litter and that females that were multiply mated generally made a higher reproductive investment than females that were singly mated. Rates of multiple paternity in this study and in the previous study differed and were significantly different when litters of fewer than five offspring were eliminated from the analysis. Although we cannot determine the causes of variation in multiple paternity given our data, we suggest two possible mechanisms, one genetic and one ecological, that may lead to different frequencies of multiple fertilizations in this species.

MULTIPLE paternity and sperm competition have been documented in numerous vertebrate taxa (see Birkhead and Møller, 1998). Because a single copulation usually transfers sufficient sperm to fertilize an entire clutch or litter, multiple mating by females in species is usually explained as either a way to acquire genetic benefits (e.g., avoidance of inbreeding, promotion of sperm competition, genetic bet-hedging) or as a means of eliminating costs accrued while attempting to prevent a subsequent copulation (Hosken and Blankenhorn, 1999). The benefits to males from multiple copulations, especially in species such as snakes in which males provide no postcopulatory parental investment, are a straightforward increase in fecundity: male reproductive success is a function of the number of females inseminated, regardless of whether females have been previously inseminated (Shine, 1988; Parker, 1992).

Many aspects of reproduction in snakes have been well studied (Seigel and Ford, 1987; Moore and Lindzey, 1992; Whittier and Tokarz, 1992), but investigations into paternity and multiple mating frequency have only just begun (see Höggren and Tegelström, 1995). The difficulty with assessing reproductive success of male snakes in a natural population is, in part, a result of their behavior. Most snakes are secretive and cryptic, and observations of natural behavior in the wild are difficult (Fitch, 1987). Data can be collected only by disturbing the animals, which may bias subsequent behaviors. Observation of mating is an unreliable method of assessing fertilization for various other reasons. Not all copulations result in fertilization, multiple matings can result in uneven fertilizations of a litter/clutch through sperm competition, and females can mediate sperm usage via cryptic choice (Höggren, 1995; Reyer et al., 1999).

Early suggestive evidence for the occurrence of multiple paternity in snakes was provided by analyzing offspring phenotypes (Gibson and Falls, 1975; Schuett and Gillingham, 1986). This was subsequently confirmed using allozyme electrophoresis (Stille et al., 1986; Schwartz et al., 1989; Barry et al., 1992). However, both techniques result in serious underestimations of the frequency of multiple paternity in litters, because of the relatively invariant nature of the markers themselves (Scribner et al., 1994). DNA fingerprinting (Jeffreys et al., 1985a,b) has also been used to provide evidence of multiple paternity in snake species (Höggren and Tegelström, 1995). In the past few years, PCR primers specific for single microsatellite DNA loci have been developed (McCracken et al., 1999; Prosser et al., 1999). This allows investigators to take advantage of the codominant and highly polymorphic nature of this type of genetic marker (Schlötterer and Pemberton, 1998) and deter-

mine paternity in litters or clutches when the actual father(s) is/are not sampled. Application of these PCR tools to gravid females and their offspring captured from a population of the common garter snake, *Thamnophis sirtalis*, resulted in the identification of heretofore undetected frequencies and levels of multiple paternity in this species (McCracken et al., 1999).

However, before the proximate and ultimate causes of multiple paternity in snakes can be determined, local rates of multiple paternity need to be compared. In *T. sirtalis*, it is reasonable to assume that local frequencies will differ, because this is the most widely distributed North American reptile, inhabiting a wide array of environments (Gregory and Larsen, 1993). Furthermore, the reproductive characteristics of the common garter snake vary geographically (Fitch, 1985; Gregory and Larsen, 1993), and approximately 11 subspecies are currently recognized (Rossman et al., 1996).

In this study, we use single-locus microsatellite polymorphisms to detect the incidence and frequency of multiple paternity in wild-caught female *T. sirtalis* from the west coast of Canada. Various reproductive characteristics of both mothers and offspring also were analyzed to see whether multiple versus single paternity were significantly associated with life-history variation. Vancouver Island, on the west coast of Canada, affords garter snakes a mildly seasonal climate, where snakes are active even during the winter months (sensu Stewart, 1965). Continental locales, such as the study site of McCracken et al. (1999), instead expose garter snakes to much colder weather during the winter months. Cold winter weather has been linked to communal hibernation and consequent mass mating behavior at the hibernation site upon emergence in spring (Gregory, 1977, 1984). Such variation in ecology and demography could conceivably influence this species' reproductive behavior.

Materials and Methods

We captured gravid snakes during the summers of 1996 and 1997 at six sites on Vancouver Island, British Columbia. We measured the snout–vent length (SVL) of each mother to the nearest 0.5 cm. Gravid females were held in the laboratory until they gave birth. Offspring were measured (SVL) and weighed immediately after birth, and their sex was determined. The number of live versus dead offspring also was recorded. We sacrificed neonate snakes for tissue samples, but the mothers were sampled nondestructively by clipping tail tips.

We (TWJG, PTG, BJK) used various snake tissues (liver, muscle, whole body sections of dead neonates, and tail tips) for genomic DNA extraction. Genomic extractions were performed following Protocol I for isolating DNA from mammalian cells as outlined in Sambrook et al. (1989). We used genomic DNA from one Vancouver Island snake for construction of a subgenomic library; genomic DNA was prepared for ligation by overnight digestion with Hinc II (New England Biolabs). The 500–1000 base-pair size range of the digest was recovered from agarose using activated DEAE-cellulose membrane (Sambrook et al., 1989) and ligated into M13mp19 RF1 (Pharmacia-Biotech) using T4 DNA ligase (New England Biolabs). Library plates were screened using several simple sequence repeat oligonucleotides (GACA, CA, CAC and GATA repeats) end-labeled with ^{32}P. Positive clones were sequenced following the protocol for dye primer cycle sequencing (Perkin-Elmer Corp., 1995). A total of 14 primer sets were designed from positive clones containing suitable repetitive regions. We designed primers using OLIGO Primer Analysis Software, Version 1 (National Biosciences Inc., 1989). Of the 14 sets of primers, only two proved to generate consistent PCR products that appeared to be variable within populations (Primer sets 2 Ts, 3 Ts, GenBank accession numbers AF098738, AF098739, Table 1). Therefore, we augmented our primer sets with a set previously designed by GM and GMB (Primer set 5B Ts, referred to as Ts 1 in McCracken et al., 1999, GenBank accession number AF135963, Table 1).

We performed PCR of samples under the conditions specific for each primer set as noted in Table 1. All PCR products were separated using 14.5-cm-by-17-cm nondenaturing 8% polyacrylamide (19:1 acrylamide:bis-acrylamide) gels. Samples were electrophoresed using 2X TBE buffer and at 70V for 12–24 h, depending on the size range of PCR products. We loaded approximately 10 μl of PCR product for electrophoresis and stained gels with ethidium bromide for approximately 15 min after electrophoresis. We visually determined allelic diversity for each of 16 litters and mothers. Alleles present in littermates were identified as either maternal or paternal, depending on litter allelic diversity and the maternal allele profile. In some cases, shared alleles between mothers and fathers were evident. For each locus, litter alleles were counted and we estimated the number of fathers per litter as the total number of observed paternal alleles divided by two. This is based on the assumption that most members of the population are heterozygotes and that each

TABLE 1. PRIMER SEQUENCES, SUGGESTED ANNEALING TEMPERATURES, AND DETECTED POLYMORPHISM FOR MICROSATELLITE LOCI USED IN THIS STUDY. GenBank accession numbers for 2Ts and 3Ts are, respectively, AF098738 and AF098739. See McCracken et al. (1999) for third accession number. T_A = annealing temperature.

Locus	Primer sequence (5′ to 3′)	T_A	No. alleles
2Ts	tacacgtgccggaaatatgctag	54C	22[a]
	tgaataacacctctgggtcagtctatc		
3Ts	ggtcacttaaatacaacgaaattggttagct	58C	12[a]
	cggacagctctggctcccttg		+1 null
5B Ts[b]	cggcataaatcttatctagc	54C	13[c]
	acttttcaggctgatgttc		

[a] As determined in another study (see Garner, 1998).
[b] Primer set referred to in McCracken et al. (1999) as Ts 1.
[c] As detected in this study.

parent that contributes to a litter will contribute both its alleles. Exceptions will include cases in which a male fathers only one offspring in a litter and cases in which mother and father are closely related and therefore share alleles. Thus, measurement at a single locus will result in some underestimation of parental contributions. If sufficient loci are used, and loci analyzed are highly polymorphic, as in this case, such underestimation will be minimized. This method is the same used by McCracken et al. (1999) to detect frequency of occurrence of multiple paternity in their study litters.

To determine whether litters without observed multiple paternity met expected Mendelian frequencies, we tested paternal allele distributions of these litters at each locus not exhibiting direct evidence of multiple paternity using a Fisher's exact test. Probabilities were generated assuming one heterozygous father. Loci where only one paternal allele was detected were not subjected to this test, for obvious reasons. Only litters that contained at least six offspring were tested; we considered litters smaller than this to have too few offspring to use in this analysis. Litters in which multiple paternity was observed at one or more loci were also tested as a control. For these cases, tests were restricted to the loci at which multiple paternity was not detected.

We used parametric statistical analyses to test relationships among life-history variables (correlation analysis) and to test for differences in life-history traits between litters with single versus multiple fathers (analysis of variance, analysis of covariance). We used multiple regression to test for trade-offs between traits while holding other relevant variables constant. To better meet the assumptions of parametric tests, we log-transformed all variables, except proportion of live young, which we arcsine-square root transformed. All analyses were performed using SAS 6.12

To determine whether the frequency of multiple paternity detected in snakes captured on Vancouver Island differed significantly from that detected by McCracken et al. (1999), a Chi-square test for differences was performed on the total number of litters analyzed and on the subset of litters that had greater than four offspring. We did this because at least three offspring must be analyzed to even detect multiple paternity, and at least four offspring must be sampled to distinguish the full range of potential paternal alleles from two fathers. As a further comparison, the frequencies detected with the one locus used in both studies were also analyzed using a chi-square test for differences. In all statistical tests, we used a nominal significance level of $\alpha = 0.05$

RESULTS

Gravid snakes ranged in size from 480–730 mm SVL. Litter sizes ranged from 3–19 (mean = 8.5, s = 5.07) and mean offspring mass per litter varied from 1.60–2.81 g. These counts included stillborn young (cf. Farr and Gregory, 1991; Gregory et al., 1992), which ranged in number from 0–6; one litter of three was 100% stillborn (Table 2).

The maximum number of alleles detected at a locus in a single litter was five (e.g., locus 2 Ts and locus 3 Ts in litter BQ 1), whereas the smallest was one (locus 3 Ts in litter Q 2). Six of the 16 litters exhibited at least three paternal alleles at one of the loci scored, clearly showing that more than one male contributed to each of these litters.

All of the litters in which multiple paternity was not directly detected met Mendelian expectations of allele frequencies at every locus (Table 3). Fisher's exact tests of the multiply sired litters showed that three of the litters deviated from Mendelian at a locus at which no multiple paternity could be detected (BQ 1, EL 2, Q 3;

TABLE 2. STATISTICAL SUMMARY OF VALUES OF LIFE-HISTORY TRAITS OF *Thamnophis sirtalis* BY PATERNITY GROUP. Given are mean (above) and range and standard deviation (below). * = unweighted statistics of litter averages, not individual neonate values.

	Putative single paternity $n = 10$	Multiple paternity $n = 6$
SVL of females	565.5	615.8
	(480–715, 82.75)	(515–730, 74.66)
Litter size	7.3	10.5
	(3–19, 5.17)	(6–17, 4.64)
Average SVL of	165.04	173.44
littermates (mm)*	(145.00–181.75, 11.24)	(156.65–190.14, 11.34)
Average mass of	2.38	2.40
littermates (g)*	(1.60–2.85, 0.39)	(2.00–2.81, 0.33)
Proportion of live	0.63	0.67
young in litter	(0.0–1.0, 0.41)	(0.3–1.0, 0.35)

Table 3). In the other three litters, the loci that did not exhibit direct evidence for multiple paternity all met expected allele frequencies.

Only a few of the life-history traits were significantly correlated in the dataset overall (n = 16 in all cases). Longer females had bigger litters (r = 0.72, P = 0.002), and bigger litters had a higher proportion of live young (r = 0.53, P = 0.03). Average SVL and average mass of littermates were strongly correlated (r = 0.65, = 0.007), but neither was correlated with any other traits (although average mass of littermates approaches a significant relationship with litter size; r = 0.45, P = 0.08).

TABLE 3. TESTS FOR MENDELIAN DISTRIBUTIONS OF PATERNAL ALLELES AT LOCI WHERE ONLY TWO PATERNAL ALLELES WERE DETECTED. Litters with six or greater offspring were tested.

Litter	Litter size	Detected paternity	Locus	*P*-value
BQ 1	15	multiple	5B Ts	0.036
IH 4	6	multiple	3 Ts	0.688
			5B Ts	0.219
IH 3	7	multiple	3 Ts	0.453
EL 2	11	multiple	5B Ts	0.006
Q1	7	multiple	2 Ts	0.453
			3 Ts	0.125
Q 3	17	multiple	3 Ts	0.013
			5B Ts	0.332
Q 2	19	single	2 Ts	1.000
			3 Ts	[a]
			5B Ts	1.000
SL 1	6	single	2 Ts	1.000
			3 Ts	1.000
			5B Ts	0.688
Q 8	6	single	2 Ts	1.000
			3 Ts	0.688
			5B Ts	1.000
Q 6	14	single	2 Ts	0.182
			3 Ts	1.000
			5B Ts	1.000
Q 9	6	single	2 Ts	0.688
			3 Ts	1.000
			5B Ts	1.000
DP 1	7	single	2 Ts	1.000
			3 Ts	[a]
			5B Ts	0.125

[a] Only a single paternal allele detected at the locus.

Although none of the differences between singly and multiply sired litters was significant (all df 1,14), the latter had longer mothers (F = 1.64, P = 0.22), larger litters (F = 2.66, P = 0.13) and longer offspring (F = 2.05, P = 0.17). Average mass of offspring and proportion of live young were only slightly higher in litters with multiple fathers. Because litter size was significantly correlated with SVL of mother overall, we compared litter size between the two groups by analysis of covariance, using female SVL as a covariate (slopes equal); the two groups still did not differ ($F_{1,13}$ = 0.90, P = 0.36). Our samples were small, so we considered the possibility that failure to show a significant difference might have been the result of a lack of power. Using formulas in Cohen (1977), we computed the power of the analyses of variance comparing female SVL, litter size, and average SVL of littermates between the two groups. We also calculated the minimum sample size that would have been required for the result that we obtained to have been declared significant at α = 0.05 at a power of 0.80. Power of the comparisons of SVLs of mothers was 0.26 and the minimum sample size required would have been 28 snakes per group. Respective values for the comparisons of litter sizes (adjusted for SVL of mother) were 0.17 and 60, and for average SVL of littermates, 0.70 and 9.

We also compared average mass of littermates

between groups using average SVL of littermates and litter size as covariates (both the latter being positively correlated with the former). Both covariates had strongly significant effects when considered together (slopes equal) and the difference between the two groups approached significance ($F_{1,12} = 3.51$, $P = 0.09$). The reverse comparison of average SVL of littermates, with average mass of littermates and litter size as covariates, was even stronger ($F_{1,12} = 4.61$, $P = 0.053$), although the effect of litter size was not significant. Adjusted means from these comparisons showed that offspring in singly sired litters were shorter for a given mass and litter size than those in multiply sired litters, but were heavier (i.e., had higher body condition).

Given the general lack of significant differences between singly and multiply sired litters in univariate tests, we tested overall differences in litter characteristics between the two groups by multivariate analysis of variance. Litter size, average SVL of littermates, average mass of littermates, and proportion of live young in litters were the dependent variables. Although this test fell short of significance ($F_{4,11} = 2.79$, $P = 0.08$), the single discriminant canonical variable associated with it was most heavily weighted by litter size and average SVL of littermates (both negatively). We then compared the canonical variable between the two paternity groups by analysis of covariance, with the female SVL as covariate (slopes homogeneous), and found a significant negative pooled regression ($F_{1,13} = 7.92$, $P = 0.01$), as well as a significant difference between groups ($F_{1,13} = 11.89$, $P = 0.004$); we used type III sums of squares in these tests. The mean adjusted value of the canonical variable was lower in the multiply sired group than in the putatively singly sired group, which meant that, for females of a given SVL, litters showing multiple paternity were larger or consisted of longer, larger offspring than those showing only single paternity.

This last result suggested that multiple paternity was associated with litters of snakes that allocated more resources to reproduction. If so, this should be reflected in a higher "trade-off" curve between litter size and average offspring size for snakes that showed multiple paternity (see van Noordwijk and de Jong, 1986; Olsson and Shine, 1997). Because bigger snakes tend to have both bigger litters and bigger babies, demonstrating this trade-off requires correction for other influential factors (Gregory and Skebo, 1998). We used a variation of Gregory and Skebo's (1998) causal model to test this relationship as follows. We did a multiple regression

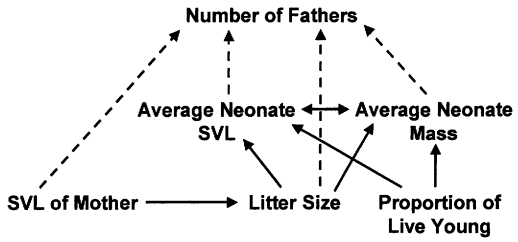

Fig. 1. Hypothesized causal relationships between life-history variables in the form of a "path" diagram, with direction of arrows indicating presumed direction of influence ("cause"). Relationships between SVL of mother, litter size, and average size of neonates as posited in Gregory and Skebo (1998), with presumed influence of proportion of live young added. Dashed lines show factors that might influence attractivity of females to males and hence the number of potential fathers of a litter.

of average neonate SVL on litter size, average neonate mass, and proportion of live young in litter, all factors hypothesized to influence average neonate SVL (Fig. 1). This regression was significant ($F_{3,12} = 3.79$, $P = 0.04$), and indicated a negative (albeit nonsignificant) semipartial relationship between average SVL of littermates and litter size, as should be the case if there is a trade-off between these two traits. To illustrate this semipartial relationship, we then expressed litter size as a residual from a regression on average mass of neonates and proportion of live young (i.e., adjusting litter size for all other "causative" variables; Thorndike, 1978; Nie et al., 1975) and then plotted it against average SVL of littermates. It is clear that all those litters showing multiple paternity occur along the upper edge of the relationship (Fig. 2). Although the linear regression between average neonate SVL and adjusted litter size is not significant for putatively singly sired litters, it was significant for the multiply sired litters ($F_{1,4} = 12.11$, $P = 0.025$).

A chi-square test for differences in frequency of multiple paternity between the two studies yielded a nonsignificant result ($\chi^2 = 0.083$, df = 1), but when litters with fewer than five offspring were eliminated from the analysis, the frequencies did differ significantly ($\chi^2 = 0.019$, df = 1). Analysis of the one locus used in both studies shows a marginally significant result ($\chi^2 = 0.055$, df = 1)

Discussion

In this study, we found clear evidence of the occurrence of multiple paternity in *T. sirtalis* from Vancouver Island. At a minimum, multiple

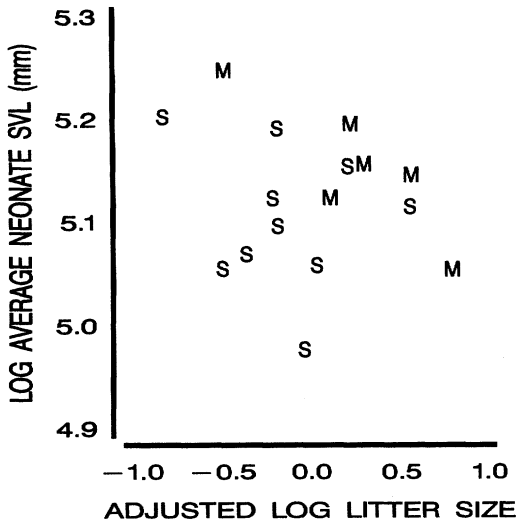

Fig. 2. Plot of "adjusted" litter size versus average SVL of littermates. This graph shows the semipartial relationship between neonate SVL and litter size from the multiple linear regression equation, neonate SVL = constant + litter size + neonate mass + proportion live young, as dictated by the causal relationships in Figure 1. Litter size was adjusted by linearly regressing it on the other independent variables (neonate mass and proportion live young) in the original regression equation, thereby holding them constant statistically, and then expressing it as a residual from that relationship (for method of calculating semipartial relationships, see Nie et al., 1975). M = multiply sired litter, S = singly sired litter.

insemination occurred in 37.5% of the litters examined. Fisher's exact tests do not suggest that any of the purportedly singly sired litters actually were multiply sired (Table 3). McCracken et al. (1999) analyzed litter profiles from eight females captured on Beaver Island, Charlevoix Country, Michigan. Litter sizes in their study ranged from 4–13 (mean = 7.5), and six of the eight litters showed direct evidence of multiple paternity. Therefore, McCracken et al. (1999) found direct evidence of multiple paternity in 75% of the analyzed litters and at least one case of triple paternity. Analysis of the subset of litters where multiple paternity is likely to be detected shows a significant difference between the two sites. The one locus used in both studies also suggests differences between the two locations. Given these results and the detection of one case of triple paternity in the McCracken et al. (1999) study, multiple paternity seems to occur more frequently in snakes captured at the Michigan site than those sampled on Vancouver Island.

Assessment of the relationship between degree of paternity and life-history variables was hampered by small samples. Estimates of minimum sample size required indicated that we would have needed many more snakes to conclude that some of the differences we found were statistically significant. Our conclusions in this area therefore should be viewed mainly as hypotheses for future tests. Nonetheless, when we combined all litter characteristics via canonical discriminant analysis and compared them between singly and multiply sired litters with female SVL as a covariate, we found a highly significant difference.

We also found evidence that offspring in multiply sired litters were longer than those from litters with one father. The trade-off between allocating resources to length rather than mass has received little attention in snakes. However, Forsman and Lindell (1996) found that juvenile adders (*Vipera berus*) increased in length at the expense of body condition, even when food was limited. They surmised that body length had a stronger positive effect on survivorship than did body reserves. Thus, one consequence of multiple paternity might be offspring that are more fit, on average, but this remains to be tested.

Female attractiveness presumably is affected by pheromones that are potential indicators of a female's fecundity (Gartska and Crews, 1981; Mason et al., 1989). This is correlated with female body size, because larger females produce more yolk, with an associated increase in pheromone production (Gartska et al., 1982). Our data suggest that multiple paternity in *T. sirtalis* is associated with high reproductive investment by females, resulting in large litters or large offspring. Such an interpretation is consistent with Figure 2, which indicates that there is a trade-off between offspring size and litter size. The diagonal of such a relationship usually represents a limiting factor (Thomson et al., 1996), similar to the situation described by Olsson and Shine (1997) for the trade-off between litter and offspring size in the lizard, *Lacerta agilis*. In that case, the upper limit was set by the size of the female's abdominal cavity, with trade-off relationships below that limit set by variable acquisition of resources. In Figure 2, all the litters known to have had multiple fathers are at or near the upper limit of the trade-off relationship and exhibit a strong negative relationship. All the litters that are well below the diagonal are putatively singly sired; they simply may not have acquired the necessary resources to attract multiple mates. But what about those putatively singly sired litters that also are on or near the diagonal? Two possibilities exist that are consistent with the attractivity hypothesis. One is that those litters actually had multiple fathers but

that our methods failed to detect it. The second is that, although these females may have had multiple mates and therefore been multiply inseminated, the sperm of only one male actually fertilized the ova. Obviously, careful experimentation and larger samples will be required to sort this out. In the meantime, it is noteworthy that in the poeciliid fish, *Gambusia affinis*, the incidence of multiple insemination appears to be related to size of female, larger females being more fecund (Greene and Brown, 1991).

But why do relative frequencies of multiple paternity differ between our sample and that analyzed by McCracken et al. (1999)? Population density and sex ratio can alter sexual behavior in many taxa (e.g., territorial versus group spawning in fishes, Kodric-Brown, 1988; searching versus calling in male anurans, Arak, 1983; frequency and duration of mating in water striders, Arnqvist, 1992; Rowe, 1992), and the rate of extrapair fertilizations (EPFs) has been shown to increase with population density in many bird species (Westneat and Sherman, 1997). Increases in local breeding density resulted in a higher reported rate of extrapair copulations in *Pica nuttalli* (Birkhead et al., 1992), *Uria aalge* (Hatchwell, 1988), *Hirundo pyrrhonota* (Brown and Brown, 1996), and *Hirundo rustica* (Møller, 1991). If paternity rates are strictly a function of population density or sex-ratio bias and if the potential for a harrassed female to be captured by a potential predator is high (Gregory, 1984), then perhaps females mate multiply because of convenience polyandry (Thornhill and Alcock, 1983) so as to reduce the costs of resisting a second mating attempt (Rowe, 1992).

We do not have comparative data on density and sex ratio of mating groups for our site and that of McCracken et al. (1999). However, in garter snakes, cold winter climates often are associated with communal hibernation and mass-mating behavior at the hibernation site upon emergence in spring (Gregory, 1984; Sexton, 1992; but see Shine et al., 2001). Sex ratios during mating in such cases are heavily biased toward males (Gregory, 1984), although most mating groups actually consist of relatively small numbers of males with a single female (Shine et al., 2001). Given the difference in climate, we would predict that communal denning is more common in Michigan than on Vancouver Island. In fact, garter snakes on Vancouver Island often spend the winter singly or in small groups (Lawson, 1991; Norman, 1999); the mild winter even allows snakes to change sites on occasion (Norman, 1999). Thus, females on Vancouver Island should encounter fewer males in spring and fewer at any time. Certainly, mating behavior is much less obvious on Vancouver Island, compared to the "mating balls" seen elsewhere (Aleksiuk and Gregory, 1974). Whether these differences are real and sufficient to account for the difference in degree of multiple paternity between the two sites awaits rigorous testing.

Another possible explanation for variable rates of multiple paternity is that the two study populations may differ in genetic diversity. Female birds seek out extrapair fertilizations more frequently in populations that are more genetically diverse (Petrie et al., 1998). Female snakes exert a strong influence over the occurrence of intromission, as the female must gape her cloaca before the male hemipenis can be inserted. Although this female control does not preclude the occurrence of convenience polyandry, it does suggest a degree of female choice that may be more indicative of a "good genes" strategy (Halliday and Arnold, 1987) rather than a male-driven reproductive strategy. This latter hypothesis would be relatively easy to test, using existing garter snake microsatellite markers to assess genetic diversity in study populations where rates of multiple paternity vary.

We have demonstrated that multiple paternity does occur on Vancouver Island, and, more important, that local rates of multiple paternity vary. The reasons for this variation are not known, but we suggest two speculative explanations, one ecological (convenience polyandry) and one genetic (population genetic diversity). Further experimental and correlational work will be needed to test these ideas.

Acknowledgments

We thank S. Wischniowski for assistance in the field. D. Duffus, A. Gibson, and D. Hosken made helpful comments on this and an early version of this manuscript. This research was supported by a Research Grant from the Natural Sciences and Engineering Research Council of Canada to PTG. All animals and tissue samples were collected according to the standards of the animal care unit of the University of Victoria (University of Victoria animal care permits 68–95 and 51–96) and under a British Columbia Ministry of Environment, Land and Parks permit C025201.

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