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I am submitting herewith a dissertation written by James Michael Schwartz entitled "Multiple Paternity and Offspring Variability in Wild Populations of the Garter Snake, Thamnophis sirtalis (Colubridae)." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.

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MULTIPLE PATERNITY AND OFFSPRING VARIABILITY
IN WILD POPULATIONS OF THE GARTER SNAKE,
Thamnophis sirtalis (Colubridae)

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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James Michael Schwartz

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DEDICATION PAGE

To my family.

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During the course of my dissertation research, I discovered how much of a group achievement this work really is. I am deeply indebted to all the people who helped make this possible. I am sure that with my memory for names I will forget to mention a few, but to all, I am grateful.

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ABSTRACT

Information about the relationships among phenotypic and genetic traits is of increasing importance in biology. Analytical approaches to the study of evolutionary adaptations commonly use optimization techniques in order to make the mathematics tractable. The validity of these approaches depends on the assumption that the predicted response is genetically possible. Knowledge of underlying genetic correlations clarifies relevant tradeoffs for use in these theories. Quantitative genetic models for the evolution of phenotypic characters rely on the assumption that genetic and phenotypic covariance matrices remain relatively constant. Therefore, empirical data on covariance matrices can be used to test these assumptions.

The garter snake, Thamnophis sirtalis (Colubridae), was used to study the quantitative genetics of several behavioral, morphological and physiological traits. The assumption that members of a single litter are full-siblings was tested. Contrary to the earlier predictions, high levels (~50%) of multiple paternity were found in these natural populations of garter snakes. Data were also collected on scale counts, aggregative tendencies, antipredator behavior, responses to chemical prey extracts, and critical thermal minima to examine potential genetic correlations among the characters. Significant litter and population differences were found in nearly every trait. Most of the traits had significant

heritabilities. Inclusion of multiple paternity litters in full-sibling analyses leads to underestimates of heritabilities. The hypothesis that multiple paternity might increase variability within litters was examined. Variability was greatly increased for antipredator behavior in multiple paternity litters, but overall variability was not greatly within these litters was not enhanced.

Comparisons of the genetic and phenotypic correlation matrices suggest that the phenotypic correlations give a good estimate of the underlying genetic correlation but that whenever possible the genetic correlations should be determined.

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PART I

GENERAL INTRODUCTION

INTRODUCTION

The study of adaptation has been a traditional area of interest for evolutionary biologists (Dobzhansky, 1956; Lynch et al., 1988). Descriptive approaches to the study of evolution have been criticized (e.g. Gould and Lewontin, 1979), leading to the advance of analytical approaches such as "game theory" (Maynard Smith, 1982), theories of life history evolution (Stearns, 1977; Rose, 1984; Reznick, 1985; Giesel, 1986; Reznick et al., 1986), and optimal foraging theories (Pyke et al., 1977, Krebs et al., 1983; Schoener, 1987). These optimization techniques are widely used because they can make complex mathematics tractable, but the validity of these approaches depends on the assumption that the predicted response is genetically possible. This assumption has been the focus of considerable debate (e.g. Lewontin, 1979; Maynard Smith, 1978; Gould and Lewontin, 1979; Mayr, 1983; Tuomi et al., 1983) but still remains an important empirical issue. Knowledge of underlying genetic correlations would be useful for distinguishing biologically relevant tradeoffs for use in optimality theories. Thus, the documentation of significant genetic variation and covariation among traits is an important first step in investigating the action of natural selection (Arnold, 1986; Garland, 1988; Venable and Burquez, 1989) and provides useful information for selection experiments (Bakker, 1986).

Additionally, the demonstration of significant genetic correlations between various measures has important implications for theoretical studies (Turelli, 1988; Lofsvold, 1986). Quantitative genetic models for the evolution of phenotypic characters rely on the assumption that genetic and phenotypic covariance matrices remain relatively constant (Lande, 1976, 1979, 1980a, 1985; Lande and Arnold, 1983; Arnold and Wade, 1984). Therefore, any data on covariance matrices will be of great use in testing these assumptions as they accumulate for given taxa (Turelli, 1988).

Genetic correlations can arise from two sources: linkage disequilibrium and pleiotropy (Falconer, 1981). Linkage disequilibrium is the nonrandom gametic association of alleles at different loci (Hartl, 1980). Pleiotropy refers to the multiple effect of a single gene on several traits. Wright (1968) has argued that since virtually all genes appear to have numerous pleiotropic effects, most characters are likely to be correlated with some other characters. Pleiotropy may, therefore, help to explain the persistence of the high levels of genetic variation that is observed in metric traits that strongly affect fitness (Futuyma, 1986). Pleiotropy results in genetic covariances between traits and will produce correlated responses to selection. As selection changes the mean of one trait, it will also change the mean of unselected but correlated traits (Lande and Arnold, 1983). This can have a dramatic effect on both the rate (Dickerson, 1955; Antonovics, 1976;

Lande, 1982) and direction of evolution (Lande, 1980b; Via and Lande, 1985). Negative correlations between traits can result in resistance to selection and be responsible for considerable additive genetic variance persisting in characters that are under continuous selective pressure (see Arnold, 1988). Conversely, positive correlations among traits will result in an enhanced rate of change due to selection and low heritabilities. Although these effects have been known for a long time, they have often been ignored in studies of selection that focused on single traits (Haldane, 1954; Van Valen, 1965; O'Donald, 1968, 1970).

The main objective of this dissertation then, is to examine the genetic variances and covariances of selected morphological, physiological, and behavioral traits in the garter snake Thamnophis sirtalis (Colubridae).

For a study of this nature, care must be taken in choosing both the subject for study and the characteristics of interest. Because large numbers of relatives are required for suitable power in quantitative genetic studies, the study organism must be easy to obtain and maintain in the laboratory for the duration of the experiments. Thamnophis sirtalis fulfills these requirements. Large numbers of gravid females can be captured and will give birth to large litters (up to 85) in the laboratory. Neonates of this species are extremely precocial such that they require no parental care and can be isolated from birth. Ideally, both parents of a litter should be known in

order to determine maternal effects, but this would require a captive breeding program greatly complicating laboratory procedures and generalizations to natural populations. In the absence of such information, full-sibling analyses permit assigning upper bounds to the heritability of specific characters.

The traits of interest should be under selective pressure, and the measurement of variation in these traits must be easily quantified. The genetic analyses require continuous or metric variation, and as these techniques rely upon ANOVA statistics, the frequency distribution of a trait must not severely violate assumptions of normality or heterogeneity of variance. Given these restrictions, I measured traits which are likely to be under selective pressure and which generally meet the other requirements. Morphological, physiological, behavioral characters were examined including various scale counts (Part III), aggregative behavior (Part IV), chemical prey preferences (Part V), antipredator behaviors (Part VI), and cold tolerance (Part VII). The genetic and phenotypic relations among these characters were also examined (Part VIII).

An important assumption of many of the quantitative genetic analyses used in this study is that members of a single litter are full-siblings. This assumption is tested in Part II. One hypothesis for the occurrence of multiple mating is that it serves to increase variability among within a clutch or litter. Therefore, in each subsequent part, variability within and among

litters were compared.

GENERAL METHODS

Gravid Thamnophis sirtalis were commercially collected from two populations; Wayne County, Michigan and the Wolf River drainage, Wisconsin (Fig. 1a; note that all tables and figures mentioned in the text will be found in a separate appendix at the end of that part). Data were collected from these wild-caught animals and their laboratory-born offspring for a number of behavioral, morphological, and physiological traits before tissue samples were obtained for electrophoretic studies.

Female garter snakes were housed in individual plastic cages (31.5 x 16.5 x 9.5 cm), and offered six (1-1.5 gm) minnows (Pimephales promelas) once a week. Water was continuously available. The humidity varied between 60-80%; room temperature between 20-24°C. Additional heat was provided by 120 w heat strips (Wrap-On Co., Inc., 5550 West 70th Pl., Chicago, IL 60638) placed under one side of the cage. This allowed females to raise their body temperatures to 36°C. The young were housed in individual plastic boxes (18.5 x 13.5 x 5 cm) lined with a paper substrate and provided with water ad libitum.

After birth, the masses and lengths of postgravid females and their young were measured. The young snakes were sexed by applying pressure to the bases of their tails in order to protrude the hemipenes of the males (Fitch, 1987). Scale counts

were made (including preoculars, postoculars, loreals, pretemporals, supralabials, infralabials, ventrals, and subcaudals) on all animals. Subsequently, the young were individually marked and placed at 3-5 days of age into an arena for three days in order to determine their aggregative tendencies. Following these studies, chemical prey preferences were measured in 10 day old animals, using aqueous chemical extracts prepared from minnows (Pimephales promelas) and earthworms (Lumbricus terrestris), along with water controls. At 12 days of age, antipredator behavioral tests were conducted. Critical thermal minima (CTMin) were then determined using 14-day-old individuals. Finally, electrophoretic studies of protein polymorphisms were conducted in order to ascertain paternity within the litters. Since these studies were conducted at different times during the neonates' development, correlations among traits may be reduced as a result of ontogenetic changes. However, only 14 days elapsed between the first and last tests, so changes should be minor.

For each trait or group of traits examined, population, sex, age, and litter differences were tested. Nonparametric tests of significance (Wilcoxon 2-sample test, Kruskal-Wallis test) were employed whenever the data could not be transformed to meet the normality or homogeneity of variance assumptions of parametric tests (ANOVA, T-test). All analyses were performed with SAS Version 5 (SAS Institute Inc., 1985) using a IBM mainframe VM3081 computer.

Genetic analyses

Phenotypic variation in a trait (V_P) can be partitioned into the variation resulting from genetic factors (V_G) and the variation resulting from environmental factors (V_E). In the simplest case ignoring an interaction between V_G and V_E , $V_P = V_G + V_E$. Genetic components of variation include additive genetic variance (V_A), dominance variance (V_D), and the variance caused by the interaction of the two (V_I). In practice only the additive genetic variance can be readily estimated from observations of populations (Falconer, 1981). The non-additive genetic variance is usually included with environmental variance. The environmental variance includes all variation from non-genetic source including nutritional and climatic factors, maternal effects, and measurement errors. The heritability of a trait (h^2 ; the "narrow sense" heritability) is defined as the ratio of the additive genetic variance to the phenotypic variance ($h^2 = V_A / V_P$).

Mayr (1974) introduced the concepts of "open" and "closed" programs as a heuristic description of observable characteristics of learning. Open programs have a genetic program which allows for change. Closed programs, on the other hand, are unaffected by experience. Mayr realized that this distinction is not absolute, no program is completely closed nor open to modification. In this study I use variation in traits to assess the relative contribution of the additive genetic variance to these traits. Thus, the distinctions between closed

and open genetic programs do not correspond to differences between behaviors with high and low heritabilities. Programs that are closed, such as the number of fingers on a hand, may have no additive genetic variance (and therefore $h^2=0$). In this study I am restricting comparisons to the first few days of life to reduce the opportunity for experiential effects.

Almost all environmental sources of variation reduce the precision of genetic studies so every attempt should be made to reduce them. Maternal effects are particularly difficult to determine and may increase the similarity among littermates. Therefore, when the effects of a common maternal environment are unknown, heritabilities should be interpreted with caution. Because no information was available on the sires of litters in this study, it is not possible to assess maternal effects.

Analyses from groups of full-siblings can be used to estimate heritabilities of characters. Using analysis of variance (ANOVA) techniques, the phenotypic variance is partitioned into a within-family (var_w) and an among-family (var_a) component of variance. These components can be estimated from the appropriate mean square error terms (MSw and MSa , respectively where $MSw=var_w$ and $MSa = k var_a + var_w$; k = number of offspring per family) generated by the analysis. In full-sibling families, $h^2 = 2(var_a/(var_a+var_w))$. Further information and examples of the use of this technique can be found in Falconer (1981), Bulmer (1980), and Kempthorne, (1957). Calculations of the standard error of heritability are complex,

but well described in Osborne and Patterson (1952).

In this study heritabilities were calculated using full-sibling analyses both for the full data set, and separately for each population. Since evidence of multiple paternity was found in ~50% of the litters examined (Part II), full-sib heritabilities were also calculated separately for single and multiple paternity litters. Comparing the results from these calculations helps to assess the effects of untested assumptions of paternity used in previous studies (Arnold, 1981a, b; Garland, 1988). When the variance and mean of a trait are not independent, genetic calculations will be dependent upon the scale that is used (Bulmer, 1981; Falconer, 1981). Transformations were performed as needed to remove these effects.

Quantitative genetic analyses (full-sib heritabilities and genetic correlations) were performed using the SAS hierarchical analysis of variance and covariance procedure (PROC NESTED) with tests of significance determined using SAS general linear models methods for analysis of variance (PROC GLM). Standard errors of heritabilities were calculated from formulas in Osborne and Patterson (1952). Standard errors of genetic correlations were determined using formulas in Falconer (1981).

GENERAL RESULTS

During the summer of 1986, 28 wild-caught females gave birth in the laboratory to 408 neonates. Twenty-three litters

were obtained from females collected in Michigan, 5 from females collected in Wisconsin. The numbers of male and female offspring in each litter are given in Table Ia. There were no differences in the SVL or mass between male and female neonates (Table Ia; SVL: Wilcoxon 2 sample test $Z=0.010$, $P=0.992$; Mass: $Z=0.637$, $P=0.524$).

The results of the remaining experiments are presented in the chapters that follow. Each is presented as a separate study, but all will be discussed collectively in the final chapter. The first experiment demonstrates the presence of high levels of multiple paternity in these litters of garter snakes. The remaining sections measure genetic and phenotypic variability in the offspring of these females. In each experiment, significant population and litter differences were found. Some of the neonates died during these experiments (28 of 408), therefore the sample sizes in each separate experiment are not necessarily the same. These deaths were undoubtedly not random. Animals that died tended to be small (mean mass=1.25 gm). It is possible that these deaths could bias the results of these experiments, however, it is most probable that these snakes would have met the same fate in nature, surviving for only a few days after birth. Thus the results of these studies may well hold for snakes of the same ages in the wild.

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PART I

APPENDIX

Table Ia. Mean size and mass of litters of Thamnophis sirtalis from two populations¹.

Litter number	Litter						Postpartum	
	male	female	SVL(cm)	($\pm$ s.d.)	mass(g)	($\pm$ s.d.)	SVL of mother (cm)	mass of mother (g)
Michigan								
1	6	10	15.5	5.8	1.35	0.17	56.8	68.8
2	13	8	14.3	11.0	1.14	0.13	59.5	85.1
3	7	5	16.2	4.5	1.62	0.20	69.5	139.2
4	7	4	15.3	8.6	1.67	0.17	56.5	80.3
5	7	5	14.8	9.5	1.32	0.24	53.8	65.1
6	12	13	14.0	4.5	1.07	0.09	54.2	49.4
7	3	3	16.0	6.7	1.51	0.26	52.2	62.7
8	12	1	15.1	9.1	1.62	0.16	50.1	58.4
9	19	18	14.3	5.2	1.31	0.44	64.2	93.0
10	8	7	16.6	9.1	2.00	0.37	57.5	103.0
11	5	1	14.1	6.5	1.35	0.15	50.7	67.0
12	8	3	14.7	6.2	1.18	0.09	51.8	51.8
13	6	7	15.1	7.8	1.44	0.29	54.1	57.2
14	11	9	15.2	3.8	1.50	0.07	55.0	69.9
15	12	11	15.1	7.9	2.08	0.28	58.5	106.5
16	1	3	14.4	4.8	1.61	0.13	46.4	45.7
17	3	3	14.4	1.9	1.04	0.06	50.1	53.6
18	3	3	16.6	6.8	2.43	0.20	58.0	86.7
19	6	3	17.1	8.8	1.83	0.17	70.2	126.9
20	3	4	14.4	14.0	1.34	0.51	60.8	86.0
21	10	8	15.2	7.4	1.56	0.18	55.5	71.9
22	22	18	15.2	5.1	1.43	0.16	69.5	148.2
23	7	10	15.9	3.7	1.69	0.16	66.5	106.7
$\Sigma =$	191	157	$\bar{X} = 15.1$	10.3	1.50	0.36	57.4	81.9
Wisconsin								
1	8	2	15.1	5.3	1.27	0.07	48.6	38.4
2	6	3	12.4	2.4	0.68	0.15	45.6	28.1
3	9	8	15.2	6.5	1.38	0.15	60.3	71.6
4	11	2	13.2	8.1	0.90	0.18	46.8	28.2
5	3	8	14.6	11.5	1.23	0.18	53.7	51.9
$\Sigma =$	37	23	$\bar{X} = 14.7$	10.8	1.23	0.24	51.0	43.6
TOTAL	228	180						

1 - Litter information includes: the number of male and female offspring, the snout to vent length (SVL) and mass for postpartum females and neonate litter means.

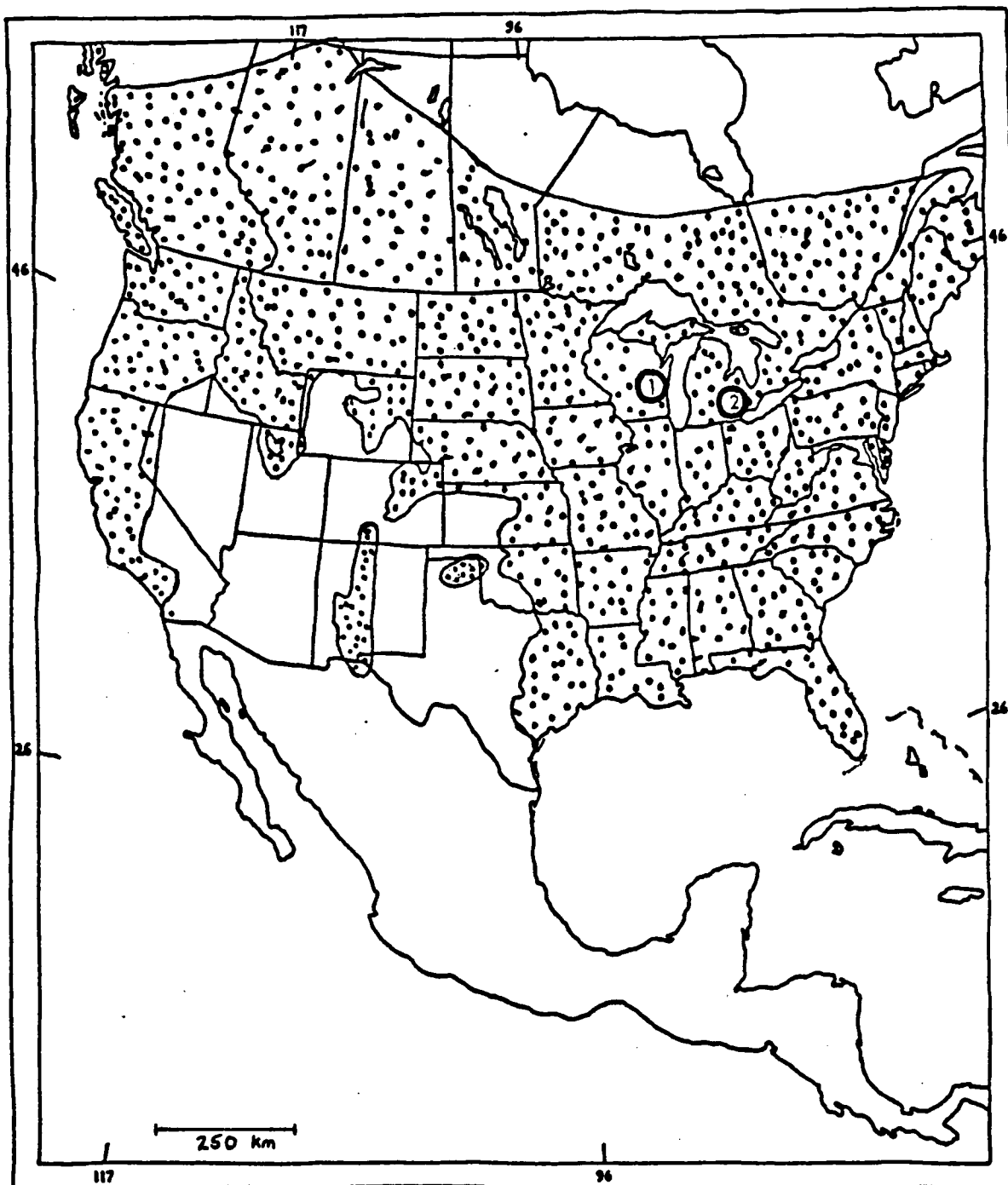


Figure Ia. Distribution of the garter snake, Thamnophis sirtalis in North America.

Modified from Fitch, 1965. The numbers represent the localities from which garter snakes were collected for this study: (1) Wisconsin, (2) Michigan.

PART II

MULTIPLE PATERNITY

INTRODUCTION

There have been several quantitative genetic studies of metric traits in litters of neonatal snakes of the genus Thamnophis. The heritability of feeding responses (Ayers and Arnold, 1983) and chemical prey preferences (Arnold, 1981a,c) have been measured in Thamnophis elegans. Genetic variation in antipredator behavioral displays has been studied in newborn T. radix (Arnold and Bennett, 1984). Garland (1988) has examined the genetic basis of variation in locomotor performance, activity metabolism, and antipredator behaviors in T. sirtalis. These studies all have assumed single paternity within litters. However, these paternity assumptions are untested and there are, in fact, no studies of paternity in any natural population of reptiles. Here, I use electrophoretic data to test these assumptions of paternity.

Although sperm storage and multiple matings are common in reptiles, direct evidence of sperm competition has not been shown in natural populations of any reptilian species (Devine 1984). Yet many reptiles are known to have adaptations for preventing sperm competition that presumably arose from such competition. The eastern garter snake, T. sirtalis, is perhaps the best studied example in reptiles. In these snakes, a copulatory plug is formed within 20 minutes of intromission (Blanchard and Blanchard 1942) and lasts from four days to two weeks depending on temperature (Devine 1975; 1984). In well-

studied populations in Manitoba, garter snakes mate on emergence from communal hibernacula, and females move to summer feeding grounds within a few days (Gregory 1974). Thus the plug, deposited by a successful suitor, is in place while the female is in the vicinity of the den where matings occur. The plug apparently acts as a mechanical block to additional matings. In addition, the plug contains an anti-aphrodisiac pheromone which further inhibits the reproductive activity of other males so that recently mated females are avoided (Ross and Crews 1977, 1978; Devine 1984).

Several studies suggest that copulatory plugs usually are effective in preventing females from remating (Parker 1970; Martan and Shepherd 1976; Ross and Crews 1977; Devine 1984; but see Parker 1984). In snakes, it is thought that plugs should be particularly effective because the hemipenes lack solid structure and cannot be used to dislodge the mass (Devine 1984). Therefore, formation of the copulatory plug, its associated anti-aphrodisiac pheromones, and the rapid movement of females away from the mating grounds provide especially strong indirect evidence for single paternity in T. sirtalis. This evidence evidently has served as the basis for assuming single paternity in this species, and many authors state that female garter snakes mate only once (Gregory 1974; Halliday 1980; Garstka et al. 1982; Halpert et al. 1982; Garstka and Crews 1985; Garstka et al. 1985; Joy and Crews 1985; Whittier et al. 1985; Halliday and Arnold 1987).

However, there is evidence that challenges the assumption of single paternity. First, Whittier and Crews (1986) report that approximately one third of the female garter snakes in a wild population remated after their copulatory plugs were shed. Second, multiple paternity has been documented in captive populations of T. sirtalis and 3 other snake species using either genetically based morphological characters (Agkistrodon contortrix, Schuett and Gillingham 1986; T. sirtalis, Blanchard and Blanchard 1941), or electrophoretic evidence (Lampropeltis getulus, Zweifel and Dessauer 1983; Vipera berus, Still et al. 1986). Third, Gibson and Falls (1975) found evidence that up to 55% of litters were multiply sired in a natural population of melanistic T. sirtalis. However, the validity of this field study of paternity relies upon the questionable assumption that melanism is inherited as a single Mendelian gene (Halpert et al. 1982). Here I utilize paternity exclusion techniques to examine the frequency of multiple paternity in wild populations and demonstrate that apparent adaptations to prevent sperm competition in T. sirtalis are not effective in preventing multiple paternity.

METHODS

Thirty-two gravid females were collected from two populations [22 from Wayne Co., Michigan and 10 from the Wolf River drainage, Wisconsin (extra females from Wisconsin were added to insure a sufficient number of litters for testing paternity in that population)]. These females gave birth to 442 young (litter sizes ranged from 5-40). Both blood and muscle tissue were collected and freshly processed for subsequent analysis. Blood was collected in heparinized capillary tubes and separated into cellular and plasma fractions by centrifugation. Muscle tissue was ground in a 1:1 weight to volume ratio of tissue to grinding buffer (Selander et al. 1971) and held for 90 min at 4°C. The homogenate was then centrifuged at 13,000 rpm for 40 min at 4°C, and the resulting supernatants saved for electrophoresis. All samples were stored at -80°C and electrophoresed within 6 months of collection. The genotypes of offspring and females were examined using standard horizontal starch-gel electrophoretic and staining techniques (Selander et al. 1971; Harris and Hopkinson 1978). A survey of 45 protein loci identified four easily scored polymorphic loci; one in blood plasma (Transferrin, Tff) and three in muscle tissue (6-phosphogluconate dehydrogenase, 6-PGD, Enzyme Commission number 1.1.1.44; Lactate dehydrogenase, LDH, E.C. 1.1.1.27; Mannose phosphate isomerase, MPI, E.C. 5.3.1.8). MPI was resolved on PGI phosphate (pH 6.7) buffer system; 6-PGD on tris citrate (pH

8.0); and Tff and LDH on Poulik discontinuous buffer system (Selander et al. 1971). Two triallelic loci, LDH and MPI, had a mean common allele frequency in the adult females of 71% and 61.3% respectively. Tff and 6-PGD had 5 and 7 alleles with a mean common allele frequency in the adult females of 70% and 37.5% respectively. Each allelomorph was scored independently by two researchers.

An examination of both the number of alleles and the number of distinct genotypes in each litter can be used to conservatively assess the incidence of multiple paternity (Akin et al. 1984; Avise and Shapiro 1986; Levine et al. 1987). When the genotype of the female parent is known, as it is in this study, the ability to detect multiple paternity greatly increases. For example, if the female is homozygous at a locus, the presence of three distinct alleles in the offspring indicates multiple paternity. It is even possible to detect the contributions of 3 or more males to a litter if a sufficient number of alleles (5 or more) are segregating at a locus.

Because males may share alleles with one another or with the female, the paternity exclusion technique is apt to underestimate the actual frequency of multiple paternity that occurs in a population. Akin et al. (1984) developed a method to estimate the real frequency of multiple paternity in cases where individuals share alleles. Their approach uses progeny sample size to calculate the probability of detection of multiple paternity for all possible combinations of parental

genotypes. The detection probability for each genotype combination is weighted by its probability of occurrence using the gene frequencies of alleles in the population. Females are assumed to have mated at most twice with equal paternal probabilities (i.e. no sperm displacement). I modified this method, which assumes no knowledge of parental genotypes, to include information on maternal genotypes in order to estimate the real frequency of multiple paternity in these litters.

This approach assumes that the loci assort independently. Tests for linkage disequilibria between all combinations of locus pairs were performed as follows. Assuming that the alleles of mothers assort independently, there should be an equal probability of finding each gametic combination in the offspring. The genotypes of offspring from mothers heterozygous at both loci were then used to calculate a G test goodness of fit statistic (Sokal and Rohlf, 1981) adjusted for small sample size. Samples from both populations were combined to increase sample sizes for these tests.

As an independent test for the presence of multiple paternity in litters I estimated the mean relatedness of littermates using the genotypic regression technique (Pamilo 1984). The correlation coefficients obtained in this analysis can be interpreted as the average degree of relatedness of individuals within litters (Pamilo 1984).

RESULTS

The allele frequencies for each polymorphic locus examined in this study are given in Table IIa. Examinations of litter and maternal genotypes uncovered evidence of multiple paternity in 16 of the 32 litters (Table IIb). Each locus scored revealed multiple paternity in 3 or more different litters, and in nine litters multiple paternity was detected at 2 or more loci. As seen in Table IIb, the ability to detect multiply sired litters increased with the number of alleles resolved at a locus. Litters from both populations were multiply sired (13/22 from Michigan, 3/10 from Wisconsin). There was no evidence of triple paternity in any litter, though only the 6-PGD and Tff loci had a sufficient number of alleles to permit its possible detection. Only one of the fourteen pairwise tests for linkage (between MPI and 6PGD; $G = 8.85$, $df=3$, $P < 0.05$) reached significance. Since one test in twenty is expected to be significant by chance effects alone, there is little evidence for linkage. Applying the approach of Akin et al. (1984), and combining the results from the four independent loci (assuming a mean litter size of 14), I estimate that the real frequency of multiple paternity in these litters was 72% (var = 6.9%).

For the genotypic regression analysis (Pamilo 1984), I separated the litters into two groups; those in which multiple paternity was detected, and those for which single paternity

could not be excluded. When a litter is composed of full-siblings the mean relatedness ($\bar{r}$) among individuals is 0.5, when composed entirely of half-siblings (i.e. each individual has a different unrelated father) $\bar{r}$ is 0.25. The estimated $\bar{r}$ ($\pm$ S.E.) in those litters lacking evidence of multiple paternity is 0.498 ± 0.022 (Table IIc). In the multiply sired litters, the estimated $\bar{r}$ is intermediate between its theoretical limits ($\bar{r} = 0.390 \pm 0.053$).

Litter size was correlated with the number of loci at which multiple paternity was detected (Fig. IIa; $r=0.807$, $p<0.0001$). All but one litter larger than 13 was found to have had two or more fathers. In all but one litter smaller than 13 no evidence of multiple paternity was found (Fig. IIa). To test whether this result is due to a reduced probability of detecting multiple paternity in smaller litters, I used the Akin et al. (1984) procedure. The probability of detection of multiple paternity with an average litter size of 7 (the harmonic mean of litter sizes less than 12) is 52% when it exists. Therefore, if all 16 litters with less than 13 neonates had two or more fathers, I should have detected multiple paternity in no less than 5 litters (binomial expansion, $\alpha=0.05$). Since I detected multiple paternity in only one litter, all 16 small litters could not have had two or more fathers. I would expect to detect multiple paternity in just one litter if at most 7 of the 16 litters had more than one father. The occurrence of multiple paternity in large litters (15/16) is significantly

different from this conservative estimate for small litters (7/16; $G=9.78$, $df=1$, $P < 0.01$).

DISCUSSION

The electrophoretic data revealed a high incidence of multiple paternity despite the presence of copulatory plugs and anti-aphrodisiac pheromones. Because garter snakes store sperm, occasionally for extended periods of time (Stewart 1972), it is possible that the multiple paternity that was observed is due not to a direct ineffectiveness of the copulatory plugs, but rather to an overlap of sperm in the female between subsequent mating seasons. For the following reasons, I suggest that this is not the case. First, although many females have abundant sperm in their oviduct in the spring after mating, no females have been found with live stored sperm during mid-summer, indicating that all available sperm supplies are used to fertilize the litter (Table II; Rahn 1940; Blanchard and Blanchard 1942; Fox 1956). Several researchers have observed autumn mating in this species (Blanchard and Blanchard 1941; Blanchard 1943; Aleksuk and Gregory 1974) and, in the fall, an increasing number of females are observed with sperm in their oviducts. However, there appears to be little chance that sperm from fall matings is responsible for the multiple paternity I observed. Halpert et al. (1982) have demonstrated that sperm from these autumn matings can move into specialized infundibular storage receptacles in the oviduct and remain viable throughout

the winter. Yet, these sperm are quickly evacuated from the receptacles and degenerate within 6 hours of a subsequent spring mating. Since ovulation and subsequent fertilization of the ova occurs 6-8 weeks after mating (Gregory 1977), it is unlikely that the evacuated sperm will fertilize ova.

At least four hypotheses can explain the apparent lack of multiple paternity in smaller litters. First, the method of Akin et al. (1984) assumes an equal mixing of the males' sperm. If this is not the case, the probability of detection of multiple paternity decreases by a factor of:

$$\frac{1-x_1^\pi - x_2^\pi}{1-2(1/2)^\pi}$$

where x_1 and x_2 are the proportion of ova fertilized by the first and second male respectively and π is equal to the litter size (Akin et al., 1984). For levels of sperm displacement as high as 80% and a litter size of 7, the probability of detection decreases by a factor of 0.7965. In this case, I would have found no less than 4 litters with multiple paternity. Therefore, unless high levels of sperm displacement occur, larger litters are more likely to be multiply sired than are small litters.

A second possibility is that little or no sperm mixing occurs. Instead sperm are used in order of fertilization. Such a "first-in, first-out" hypothesis is supported by anatomical evidence of the sperm storage system in garter snakes. Sperm

are stored in the lumina of the infundibulum of the oviduct until ovulation (Fox, 1956; Halpert et al., 1982). Mature follicle cells squeeze through the passage, stretching the walls of the uterus and releasing sperm. With the sperm of successive males packed in order from anterior to posterior, the eggs would first contact sperm of the initial male. The latter eggs would come in contact with sperm stored more and more caudad until they exhaust the first male's supply and come into contact with the second male's sperm. Small females may not have enough eggs to deplete the first male's sperm and would therefore have offspring from a single father.

Alternatively, the observed pattern of multiple paternity may simply be the result of mating tendencies. Small females do not always mate. Axtell (cited in Wright and Wright, 1957) found between 5 and 20% of female garter snakes in New York to be nonbreeders. He notes that many of these were just above the minimum size for maturity. Fitch (1965) found a strong relationship between size and breeding in snakes in Kansas; 58% of the females smaller than 580 mm snout to vent length (SVL) failed to mate, while only 24% of those larger than 668 mm SVL failed to mate. Whittier and Crews (1986) found that only one of five females smaller than 45 cm SVL had mated, while 12 of 13 larger than 45 cm SVL had mated. Gregory (1977) reports that 36.8% of females less than 500 mm snout to vent length (SVL) mated. In contrast, 93% of the females larger than 500 mm SVL mated. If small females are not likely to mate once, they are

even less likely to mate twice. Although females of litters with single paternity were smaller, there was considerable overlap in the sizes of both groups. The mean SVL of the single paternity females was 536.8 mm (range 456-695mm); multiple paternity females were larger (mean SVL = 589.5; range 501-695mm).

The converse is that larger females are preferred so males are more likely to mate with them once, and possibly twice. Female size in garter snakes is strongly correlated with potential fecundity (Gregory, 1977). Hawley and Aleksiuk (1976) found that the probability of courtship increases with increasing female body size. There should be a decided advantage for males that are able mate with large females. Although it is thought that males can discriminate between virgin and previously mated females (Blanchard and Blanchard, 1942), males may "choose" to mate with a recently mated female if the female is large enough even though they will "share" the young with another male. The high incidence of multiple paternity in large litters suggests that the advantage of mating with a large female is more than female size alone would predict.

Fourth, females may mate repeatedly in order to insure an adequate supply of sperm to fertilize their eggs. Males might not be able to fertilize all of the eggs of a large female either because their own stores have been depleted by frequent matings or because they are sterile. Although rare in animals,

this hypothesis is more likely in garter snakes due to their unique reproductive cycle. The testes of a male are only active during the summer when temperatures are high enough to support the necessary metabolic processes. Males then store sperm from the summer through the fall and winter until they mate in the spring. Should they deplete their supplies, they have to wait until the next summer to replenish their stores. Matings often do not result in fertilized eggs, and although this may be due to a number of factors, one is that males lack adequate sperm. There are no adequate data to test this idea, but Fitch (1965) found 1 of 3 males without viable sperm in the spring, and none in the summer.

Several adaptive explanations proposed to explain multiple mating by females do not apply to garter snakes. These include: nutrient transfer (Thornhill, 1976), paternal care of young (Davies, 1985), a reduction in sexual harassment (Gibson and Falls, 1975), and increasing competition among spermatozoa (Parker, 1970). There is no evidence that male garter snakes transfer nutrients to females or contribute to the care of the young. The third hypothesis can be eliminated by the observation that females return to a den area to remate (Whittier and Crews, 1986). Although multiple matings undoubtedly enhance sperm competition, the last hypothesis contradicts the evidence of the "first in, first out" fertilization hypothesis.

An alternative hypothesis is that females may mated

repeatedly to increase in variability in their progeny (Williams, 1975; Maynard Smith, 1978; Gladstone, 1979; and Walker, 1980). This hypothesis will be tested in the ensuing sections.

These results lead to at least two conclusions. First, the existence of adaptations to thwart multiple paternity clearly should not be accepted as proof that sperm competition does not occur. Rather, these adaptations provide evidence that sperm competition is a potential problem that males must face. There should be strong selective pressure on males of most species to prevent multiple paternity if possible, but their defenses may not be absolute. In some situations, such as in garter snakes where females appear to control the number of matings, male defenses may be surprisingly ineffective.

Second, the results point to the importance of testing assumptions of paternity before calculating heritabilities. Given these results, it seems likely that the inclusion of multiply sired litters may have led to underestimates of heritabilities in previous studies of quantitative genetics in garter snakes (e.g. Arnold 1981a, b, c; Ayres and Arnold 1983; Arnold and Bennett 1984; Garland 1988).

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PART II

APPENDIX

Table IIa. Allelic variation at 4 electrophoretic loci in adult female Thamnophis sirtalis from two populations¹.

Enzyme	Allele	Population	
		Michigan	Wisconsin
LDH		(21)	(8)
	A	0.310	0.188
	B	0.667	0.813
	C	0.024	0.000
MPI		(22)	(9)
	A	0.364	0.278
	B	0.568	0.722
	C	0.068	0.000
Tff		(20)	(10)
	A	0.025	0.100
	B	0.075	0.000
	C	0.125	0.300
	D	0.750	0.600
	E	0.025	0.000
6-PGD		(16)	(8)
	A	0.031	0.000
	B	0.563	0.000
	C	0.094	0.188
	D	0.094	0.625
	E	0.188	0.125
	F	0.031	0.063
	G	0.000	0.000

1 - Sample size is given in parentheses. See text for enzyme abbreviations.

Table IIb. Litters of Thamnophis sirtalis from two populations and loci with evidence of multiple paternity.

LITTERS WITH MULTIPLE PATERNITY					
POPULATION	LITTER	LOCUS (# OF ALLELES)			
		6-PGD(7)	Tff(5)	MPI(3)	LDH(3)
Michigan	1538	X	X	X	
	1520	X	X		X
	1502	X		X	
	1510	X	X		
	1513	X		X	
	1519	X			X
	1523			X	X
	1539	X	X		
	1501	X			
	1505	X			
	1512			X	
	1514	X			
	1537	X			
Wisconsin	1552	X	X		
	1543		X		
	1558	X			
Total #	16	13	6	5	3

Table IIc. Mean relatedness ($r \pm \text{s.e.}$) in litters of Thamnophis sirtalis with single and multiple paternity.

LOCUS ALLELES LITTERS			$\bar{r}$	$\pm$ SE
Single Paternity				
6PGD	7	16	0.528	0.061
Tff	5	15	0.544	0.081
MPI	3	16	0.471	0.111
LDH	3	16	0.450	0.141
MEAN			0.498	0.022
Multiple Paternity				
6PGD	7	16	0.465	0.046
Tff	5	16	0.359	0.077
MPI	3	16	0.482	0.085
LDH	3	16	0.254	0.110
MEAN			0.390	0.053

Table IIId. Frequency of female Thamnophis sirtalis found with sperm in oviducts throughout year.

SEASON	FREQUENCY (%)	
	W/O SPERM	W/ SPERM
Mar. 1 - June 6	16 (34.8)	30 (65.2)
June 7 - July 30	57+(96.6)	2 (3.4) ¹
July 31 - Oct. 8	122 (81.3)	28 (18.7)
Oct. 9 - Feb. 29	49 (51.0)	47 (49.0)

1 - Both females found between June and July contained dead sperm (Fitch 1965). Data compiled from Fox (1956), Fitch (1965), Aleksasuk and Gregory (1974), and Halpert et al. (1982). An unquantified number of additional snakes were sampled in June, but none were found with spermatozoa in the oviduct (Fitch 1965). Parturition normally occurs from late July to early August. Significantly fewer females were found with sperm in the oviducts between June and July (G test, $G = 76.47$, $P < 0.001$).

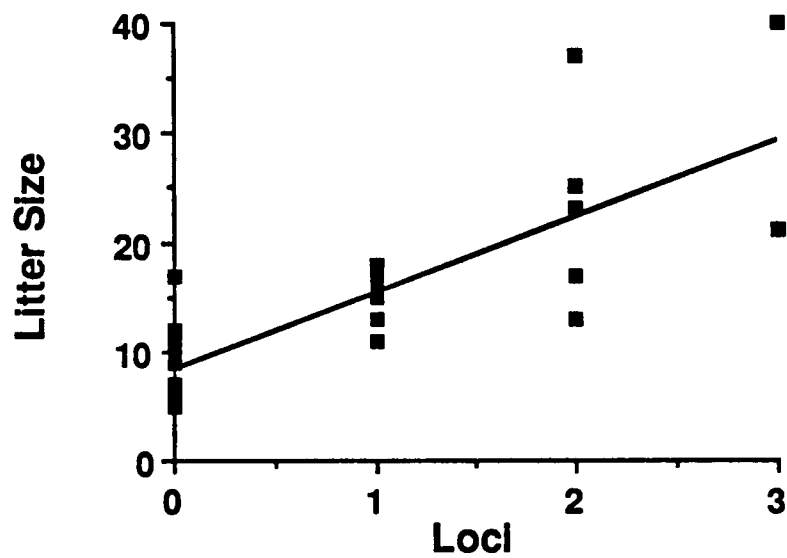


Figure IIa. Correlation of litter size in *Thamnophis sirtalis* with number of electrophoretic loci at which multiple paternity was detected.

PART III

SCALE COUNTS

INTRODUCTION

Because they are fixed in number at birth (Dunn, 1942), the number and patterns of scales often are used in the systematic classification of reptiles (see Camp, 1923; Smith, 1938; Fox, 1948a; Rossman, 1963; Voris, 1975; Rossman and Eberle, 1977; Toriba, 1985; Murphy et al., 1987). Although scalation has been an important taxonomic tool, considerable variation exists within species (Kerfoot, 1970) and numerous records of individuals with aberrant scale shapes and locations, or variation in numbers have been published (Ruthven and Thompson, 1913; Linsdale, 1936; Smith, 1938; Dunn, 1942; Inger, 1942, 1943; Fox, 1948a, b; Martof, 1954; Clark and Callison, 1967; Secoy, 1970; Vinegar, 1974; Funk and Tucker, 1978; Osgood, 1978; Plummer, 1980; Thorpe, 1984a, b; Murphy et al., 1987).

Based on evidence collected from litters, Dunn (1942) argued that individual variation in scales of snakes is genetic. He also argued from comparisons of scale counts in young and adult snakes that natural selection operates on this variation. Comparing distributions in young and old snakes, Inger (1943) found evidence that selection eliminates individuals with low numbers of ventral and subcaudal scales in Thamnophis radix. Murphy et al. (1987) suggest that the control of scalation is most likely to be polygenic based on the study of scale abnormalities in captive breeding experiments.

Yet not all scale abnormalities have a genetic basis; the

embryonic environment is also known to produce variation in scalation. Fox (1948b) found that changes in the average ambient temperature during development can alter the number of dorsal, ventral, subcaudal, ocular, and labial scales in T. elegans. Vinegar (1974) reported that the temperature of incubation influences development in Python molurus. Individuals that developed in colder than normal temperatures had abnormal labial and subocular scales as well as pattern and skeletal aberrations. Osgood (1978) demonstrated that the thermal environment in which embryos develop influences the number and variability of scales in Nerodia fasciata. Different scales were affected, including preocular, postocular, labial, ventral scales, while subcaudals remained unaffected by temperature.

In addition to individual differences in scale number, variation has been found within individuals; that is, the number of scales is not always the same from side to side. King (1959) found vertebrae with 3 articulating ribs in Natrix natrix, two on one side, one on the other, in contrast to the more usual pattern of 2 ribs articulating with the vertebrae, one on each side. Superficially, the ribs are attached to the ventral scutes by cutaneous muscles. In these snakes, the anomalous vertebrae are marked by "half-ventral" scales. King (1959) found similar "half-ventrals" in Coluber, Diadophis, Elaphe, Lampropeltis, Rhadinaea, and Thamnophis. Other scales can likewise differ from one side of the body to the other. Funk

and Tucker (1978) reported intraindividual variation in the subcaudal, temporal, nasal, and frontal scales in a litter of Tropidoclonion lineatum. Fox found considerable variation within individuals in the labial and ocular scales in T. elegans (1948b) and T. ordinoides (1948a), while Rossman (1963) recorded variation in the ocular, temporal, labial, and intergenial scales in T. proximus and T. sauritus. Martof (1954) found similar variation within individuals from a single litter of T. sirtalis in the labial, nasal, ocular, and temporal scales.

Geographic variation in scalation has been reported from field collections of adult animals in Natrix natrix (Thorpe, 1984a, b), T. proximus, T. sauritus (Rossman, 1963; Gartside et al., 1977), T. sirtalis (Fitch, 1941, 1965). To my knowledge, geographic variation in neonatal scale counts has only been reported in T. elegans (Fox, 1948b).

METHODS

General

I collected data on the number of supralabial, infralabial, loreal (as defined by Savage, 1972), preocular, postocular, ventral (as suggested by Dowling, 1951) and subcaudal scales (Fig. IIIa) of neonates and their mothers. I also found variation in scales located just caudal to the postoculars, just dorsal to the infralabials, and just anterior of the temporals, which I call "pretemporals" (Fig. IIIa). The scales of neonates were counted at one day of age, after the proto-integument had

been shed. Adult females were scored between 1-49 days postpartum as time permitted. Reliability of scoring scales was determined by recounting the scales of individuals from 2 litters. The reliability of head scale counts was high; 78 of 80 counts were identical. The reliability of the ventral and subcaudal scale counts was similar (ventral scales: 97.7%; subcaudal scales: 97.2%; over 40 counts).

Apparatus and Procedure

All scales on the head were counted on both sides as there is considerable asymmetric variation in scale number. A dissecting scope facilitated counts of small scales. The ventral and subcaudal scales were counted by placing the snake on a piece of clear Plexiglas, covering it with soft foam, and then carefully flipping the apparatus over. Pressure was maintained so as to keep the ventral surface of the snake upright. Additionally a small cube of foam was inserted near the head of the snake so as not to injure it. Scales were then counted using a felt-tip pen to mark off scales.

RESULTS

Scale count data were collected from 393 neonates (353 Michigan, 40 Wisconsin) and 28 adults (24 Michigan, 4 Wisconsin). The number of scales covering the body is positively correlated with body size. The number of ventral scales is correlated to both the number of subcaudals in both

populations (Michigan $r=0.445$, $P<0.0001$; Wisconsin $r=0.708$, $P<0.0001$) and to body length (snout to vent, SVL) in the Michigan population ($r=0.279$, $P<0.0001$). The length of the tail is also significantly correlated with the number of subcaudal scales in both populations (Michigan $r=0.557$, $P<0.0001$; Wisconsin $r=0.616$, $P<0.0001$).

There are significant population differences in both the number of ventral scales ($F_{1,27}=12.76$; $P=0.001$) and the number of subcaudals ($F_{1,27}=6.36$; $P=0.018$). Wisconsin neonates had a higher mean number of ventral and subcaudal scales (Table IIIa). There were no significant differences between the populations in the distribution of the various types of threshold scales (Table IIIb).

There are significant differences in ventral and subcaudal scales between litters from Michigan (ventrals: $F_{23,328}=7.72$, $P<0.0001$; subcaudals: $F_{23,328}=6.68$, $P<0.0001$). Litter differences were present in ventral scales in the Wisconsin population ($F_{4,35}=6.83$, $P<0.0004$), but not in the subcaudal scales ($F_{4,35}=2.42$, $P=0.0666$). Significant litter differences exist for preocular ($X^2=137.4$, $df=52$, $P<0.0001$), postocular ($X^2=104.7$, $df=52$, $P<0.0001$), supralabial ($X^2=81.7$, $df=52$, $P<0.01$), loreal ($X^2=162.2$, $df=52$, $P<0.0001$), and pretemporal ($X^2=54.9$, $df=26$, $P<0.001$) scales.

Significant sex differences (Table IIIc) are present in newborn garter snakes in the number of subcaudal scales and ventral scales using litter means for each sex. Male garter

snakes have a greater number (ventral: $F_{1,44}=10.51$, $P=0.002$; subcaudal: $F_{1,44}=24.58$; $P < 0.0001$) of these scales than do females. Individuals that could not be sexed with confidence by protruding hemipenes also fell between the means of the other two groups for these characters (Table IIIc).

There was a significant difference between the sexes in the distribution of preocular scales (Table IIIb; $G=13.20$, $df=2$, $P < 0.01$). Males tended to have a greater number of preoculars than expected, while females tended to have fewer than expected. There were also sex differences in the distribution of infralabial scales (Table IIIb; $G=7.81$, $df=2$, $P < 0.05$). Females had more infralabial scales than expected, males had fewer. There were no other significant sex differences in scalation on the head (Table IIIb).

Some age differences in the number of ventral and subcaudal scales exist (Table IIId). Although there were no significant differences between the number of ventral scales of adult female and neonatal snakes (Michigan: $F_{1,46}=1.64$; $P=0.207$; Wisconsin: $F_{1,7}=0.62$, $P=0.457$), significant differences were present between the two age groups in the number of subcaudal scales (Michigan: $F_{1,46}=8.16$; $P=0.006$; Wisconsin: $F_{1,7}=8.36$, $P=0.023$). This is not surprising given that the adult animals are all female and there are significant differences between the sexes in the subcaudal number. When I examined age effects in just female snakes separately by each population, an interesting result appeared. There were no significant differences between

the number of ventral ($F_{1,45}=0.08$; $P= 0.776$) or subcaudal scales ($F_{1,45}=2.12$; $P= 0.152$) of neonate and adult Michigan females. Wisconsin females displayed the same pattern with no difference present between the number of ventral scales ($F_{1,6}=0.05$; $P= 0.830$), nor between the numbers of subcaudals ($F_{1,6}=2.90$; $P= 0.140$) in young and old females. The fact that adult females tended to have fewer subcaudal scales may be the result of tail loss in the older animals. Nearly every adult female examined had lost some small portion near the tip of the tail together with the subcaudal scales in that region.

Too few adults from the Wisconsin population were sampled to permit statistical testing of age differences in the head scales. However, adequate numbers of Michigan adults were scored. The distributions of supralabial (Table IIIb; $X^2 = 10.8$ $df=2$, $P= 0.005$), and preocular (Table IIIb; $X^2 = 17.6$, $df=2$, $P< 0.0001$) scales are significantly different between adult and neonatal animals. Neonates tended to have fewer supralabial and more aberrant preocular scales than expected. Since there were also sex differences in the number of preocular scales, I tested the distributions for these scales with just the females. The age differences in distribution of preocular scale number remained significant ($X^2 =9.703$, $df=2$, $P< 0.05$).

Heritabilities

Much of the variation in scale number is heritable (Table IIIe). The heritabilities of the ventral and subcaudal scales are significantly different from zero in both populations. In

general, the heritability of the ventral scales are higher than those for the subcaudals. Excluding litters with multiple paternity increases the estimate of the heritability of subcaudal scales in the Wisconsin population. Since there were significant sex differences in the number of subcaudal and ventral scales, I also calculated the heritabilities of the ventral and subcaudal scales using sex as a covariate. The heritabilities increased for the Michigan population (ventrals: $h^2=0.663 \pm 0.261$; subcaudals: $h^2=0.817 \pm 0.267$), but decreased for the Wisconsin population (ventrals: $h^2=0.5535 \pm 0.476$; subcaudals: $h^2=0.778 \pm 0.489$). Sex differences in heritabilities were examined using all litters in order to insure an adequate sample sizes for comparison. No sex differences in the heritability of ventral scales were found in snakes from Michigan (males: $h^2=0.788 \pm 0.179$; females: $h^2=0.624 \pm 0.183$) or Wisconsin (males: $h^2=0.658 \pm 0.451$; females: $h^2=1.477 \pm 0.384$) populations. Likewise, no sex differences were found in the heritability of subcaudal scales of snakes from the Michigan (males: $h^2=0.669 \pm 0.174$; females: $h^2=0.939 \pm 0.186$) or Wisconsin (males: $h^2=0.415 \pm 0.424$; females: $h^2=1.110 \pm 0.559$) populations.

There are no differences in the coefficient of variation between single and multiple paternity litters for either the number of ventral (single paternity CV=2.39; multiple paternity CV=2.32) or subcaudal scales (single paternity CV=10.92, multiple paternity CV=9.32).

Threshold Characters

Calculation of heritabilities for the remaining scale counts is more complicated. Rather than being continuously distributed metric characters, these traits fall into discrete categories. It is possible to estimate the heritability of this class of traits by treating them as threshold characters (Falconer, 1981). An underlying assumption of this approach is that many genes, each with a relatively small effect, influence the expression of these traits. When the contribution of the genes is below the threshold, the trait is of one phenotype. When the contribution of the genes exceeds the threshold, the trait is of the other phenotype. This approach compares the frequency of expression of the threshold characters in one generation with its expression in the next. Other assumptions include lack of a common environmental influence and, for traits with two thresholds, a logical ordering of the traits with respect to the underlying distribution of liability. The heritabilities of the threshold scales is presented in Table IIIIf.

DISCUSSION

Although neonates used in this study were reared in a common environment, there is considerable variation among litters in the two populations in both the number and shape of many different scales. The differences among the litters in the

number of half-ventrals, aberrant preocular, supralabial, and loreal scales suggest that some litters of snakes may have greater developmental lability under the conditions of this experiment or might have lower thresholds for the expression of these aberrant scales.

Significant sex differences were present in the number of subcaudal scales at birth. Males have, on average, six more pairs of subcaudals than do females. This difference can be explained by the 3mm longer mean tail length of the male at birth. While Martof (1954) found similar results, this is somewhat less than the difference of 10 subcaudal scales he reported in garter snakes collected in Washtenaw County, Michigan and may reflect additional geographic variation in scale counts.

The sex differences found in the number of ventral scales agrees with previous studies in Thamnophis brachystoma (Barton, 1956), T. elegans (Klauber, 1943; Fox, 1948b), T. radix (Klauber, 1943), T. ordinoides (Linsdale, 1936; Klauber, 1943), T. proximus, T. sauritus (Rossman, 1963), and T. sirtalis (Martof, 1954) where sex differences were observed. Unlike the sex differences in subcaudal scales there were no differences in SVL between the sexes at birth (see Part II).

These data do not support the finding of Dunn (1942) and Inger (1943) of age differences in the number of ventral scales. However, the presence of significant age differences in the numbers of preocular and supralabial scales may be the result of

selection on these traits.

The heritability of the scale counts for the Michigan population are quite similar to those reported by Arnold (1986) for T. elegans (ventral: $h^2=0.59 \pm 0.19$; subcaudal: $h^2=0.57 \pm 0.18$).

No support was found for the hypothesis of selection on ventral scales in either population. The coefficient of variation was, in fact, lower in Michigan neonates (CV=3.038) than in the adults (CV=3.297). However, in the Wisconsin population, the difference between young (CV=2.255) and old (CV=1.626) females in variation in ventral scale numbers was in the expected direction and is only suggestive of selection given the small sample size.

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PART III

APPENDIX

Table IIIa. Population differences in ventral and subcaudal scales of neonate Thamnophis sirtalis from two populations.

Population	N	Ventrols ¹		Subcaudals ¹	
		Mean	+SE	Mean	+SE
Michigan	352	150.1	0.24	65.8	0.38
Wisconsin	40	154.6	0.63	70.6	0.80

1- $P < 0.0001$

Table IIIb. Population, sex and age differences of threshold scale counts in Thamnophis sirtalis from two populations scored by type.¹

Scale		Population Differences		Sex Differences		Michigan Age Differences	
		Mich.	Wisc.	Male	Female	Neonate	Adult
Half-Ventrals	0	267	39	151	129	247	20
	1-4	96	4	59	35	93	3
	≥5	14	0	6	7	13	1
Unpaired Subcaudals	0	364	44	209	166	340	24
	≥1	13	0	7	5	13	0
Preoculars	Loss	84	2	34	50	84	0
	1-1	272	42	167	118	250	22
	Gain	16	0	13	3 **	14	2 **
Postoculars	Loss	22	5	14	12	22	0
	3-3	311	38	174	147	291	20
	Gain	42	1	27	12	38	4
Supralabials	Loss	61	0	34	24	60	1
	7-7	281	44	171	130	264	17
	Gain	34	0	11	11	28	6 **
Infralabials	Loss	54	20	39	27	52	2
	10-10	297	24	171	127	277	20
	Gain	24	0	6	16 *	22	2
Loreals	Loss	48	2	22	28	48	0
	1-1	280	42	162	128	257	23
	Gain	47	0	31	15	46	1
Pretemporals	0-0	296	43	183	154	273	23
	Gain	75	1	33	45	78	1

* - $P < 0.05$
 ** - $P < 0.01$
 *** - $P < 0.001$

1- Half-ventrals and unpaired subcaudals scored by number present. Remaining scales scored as typical pattern or as a gain or loss of a scale on either side of the body.

Table IIIc. Sex differences in ventral and subcaudal scales of neonate Thamnophis sirtalis from two populations.

Sex	N	<u>Ventrals</u>		<u>Subcaudals</u>	
		Mean	<u>±SE</u>	Mean	<u>±SE</u>
Michigan					
Male	190	151.4	0.33	68.5	0.50
Indeterminate	5	149.8	2.46	65.8	2.03
Female	157	148.6	0.33	62.5	0.49
Wisconsin					
Male	26	156.6	0.53	73.3	0.66
Female	14	151.1	0.91	65.6	1.00

Table IIIId. Age differences in ventral and subcaudal scales of neonate Thamnophis sirtalis from two populations.

Age	N	<u>Ventrals</u>		<u>Subcaudals</u>	
		Mean	<u>+SE</u>	Mean	<u>+SE</u>
Michigan					
All Neonates	352	150.1	0.24	65.8	0.38
Adult Female	23	148.3	1.01	60.1	1.00
Neonate Female	157	148.6	0.33	62.5	0.49
Wisconsin					
All Neonates	40	154.6	0.63	70.6	0.80
Adult Female	4	153.8	1.25	59.8	4.05
Neonate Female	14	151.1	0.91	65.6	1.00

Table IIIe. Full-sib heritabilities in ventral and subcaudal scales of neonate Thamnophis sirtalis from two populations.¹

Population	N	<u>Ventrals</u>		<u>Subcaudals</u>	
		h ²	<u>±SE</u>	h ²	<u>±SE</u>
All Litters					
Michigan	352	0.6364	0.1492**	0.5657	0.1428**
Wisconsin	40	0.9015	0.3958*	0.3337	0.3232
Single Paternity Litters					
Michigan	80	0.5345	0.2492**	0.4091	0.2312**
Wisconsin	23	0.7809	0.4892*	0.9359	0.4794*
Multiple Paternity Litters					
Michigan	272	0.6706	0.1965**	0.6288	0.1917**

1 - Tests of significance of $h^2 > 0$ were conducted by analysis of variance among and within litters, * = $P < 0.05$, ** = $P < 0.01$.

Table IIIf. Heritability of threshold scale counts in neonate Thamnophis sirtalis from two populations.

Scale	Heritability
Half-Ventrals	0.002
Unpaired Subcaudals	-
Preoculars	0.7449
Postoculars	0.3363
Supralabials	0.2456
Infralabials	0.1809
Loreals	-
Pretemporals	1.5694

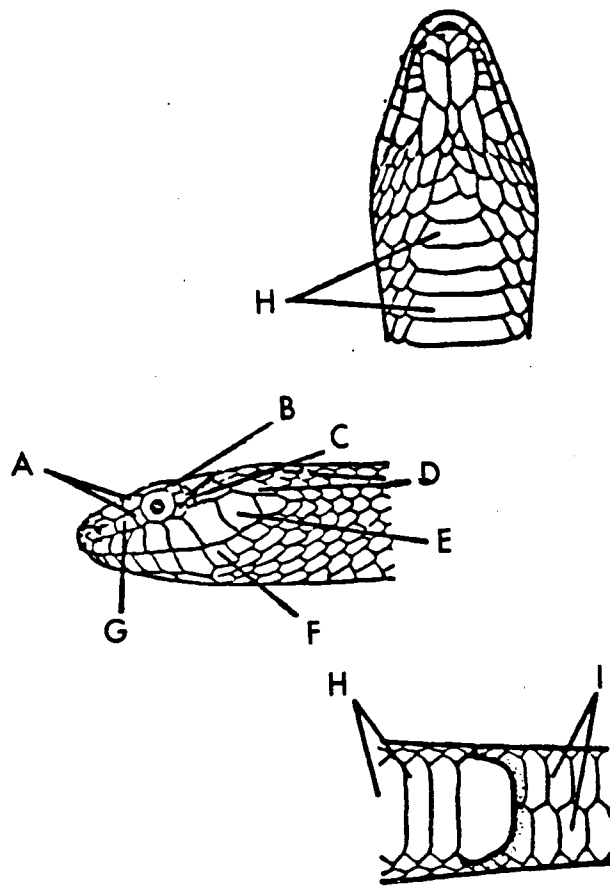


Figure IIIa. Scales used in the study of variation in Thamnophis sirtalis. A-Preocular, B-Postocular, C-Pretemporal, D-Temporals, E-Supralabials, F-Infralabials, G-Loreals, H-Ventrals, I-Subcaudals.

Modified from Stebbins, 1966.

PART IV

AGGREGATION BEHAVIOR

INTRODUCTION

Due to their secretive nature, snakes are considered by some researchers as the least social of the reptiles (Brattstrom, 1974; Wilson, 1975). However, Gillingham (1987) recently reviewed the literature on snake social behavior and concluded that snakes can be recognized as social on the basis of interaction between individuals with regard to courtship, combat, and aggregative behaviors.

The formation of mating aggregations and the use of shared hibernacula during the winter season are common in some snake species and may involve thousands of individuals (Aleksuik, 1977; Aleksuik and Gregory, 1974; Swain and Smith, 1978; Parker and Brown, 1973; Finneran, 1949; Gregory, 1975, 1984; Graves et al., 1986). Nevertheless, aggregations occur in several species at other times of the year (Duvall et al., 1985; Fitch, 1965; Huheey and Stupka, 1967; Klauber, 1956; Noble and Clausen, 1936). Prater (1933) believed that these aggregations formed as a consequence of animals finding and occupying the same "preferred" habitat, but recently several researchers have established that snakes congregate for other reasons as well.

Burghardt (1983) demonstrated that neonatal Thamnophis sirtalis and Storeria dekayi have a tendency to associate with conspecifics. This response is mediated in part by a tendency to prefer areas soiled by conspecifics (Porter and Czaplicki, 1974; Heller and Halpern, 1981, 1982a, b). Snakes often return

to occupy locations previously "conditioned" by conspecifics relying on vomeronasal cues to locate sites (Dundee and Miller, 1968; Burghardt, 1980, 1983; Heller and Halpern, 1982b).

Snakes also may directly benefit by aggregating. Noble and Clausen (1936) demonstrated a 20-25% decrease in water loss in Storeria dekayi and a 40% reduction in water loss in T. butleri when in aggregation. The respiratory rate of snakes in aggregations drops as well. Clausen (1934) and Clausen and Mofshin (1936) demonstrated that snakes in aggregations actually had reduced oxygen consumption when compared to isolated individuals. This reduction in consumption of oxygen was not simply the result of lower activity and reduced surface exposure of aggregated snakes (Clausen and Mofshin, 1936). Some snakes aggregate in response to cold temperatures (Aleksuik, 1977). Myres and Eells (1968) tested the thermal stability of snakes in aggregation and found that they lost heat more slowly than did individual snakes. Similarly, snakes free to coil or change body posture cooled less rapidly than did restrained individuals. Graves and Duvall (1987) report similar findings in a study of thermoregulation and aggregation in Crotalis viridis viridis. Snakes cooled more slowly when in aggregation than when alone.

Burghardt and Rand (1985) found that sleeping group size was positively correlated with growth in hatchling Iguana iguana. Schwartz and Allen (submitted) tested the hypothesis that aggregation might similarly affect growth in 16 neonate

Nerodia sipedon from a single litter. They found that aggregation can lead to increased growth, but may also increase competitive interactions among the group members.

Species differences have been found in the tendency to aggregate. Burghardt (1983) found that neonate Thamnophis sirtalis had a greater tendency to group than did neonate Storeria dekayi. Noble and Clausen (1936) observed considerable variation between species of snakes in their tendency to aggregate. These differences may represent species-specific differences in response to environmental conditions of heat and humidity.

Given these benefits for group formation, the tendency to aggregate should be under selective pressure. Here, I report on the aggregative behavior of the garter snake, Thamnophis sirtalis. Both litter and population differences were evaluated and the heritability of this behavior was measured.

METHODS

Littermates were placed into a common enclosure to ascertain their tendency to aggregate. To identify individuals, small pieces of colored waterproof tape, marked with a unique letter, were placed on the heads of the animals covering the parietal scales.

Apparatus

For the group enclosure, I used a one m diameter pool with

a gravel substrate and six cover objects (Fig. IVa). The cover objects consisted of a 16 cm diameter plastic base and a removable wooden top (Fig. IVb). Each base was supported by three 1 cm circular feet, to provide sufficient room for the snakes to crawl beneath the cover object. By removing the wooden top, I could examine the snakes while minimizing the disturbance to the animals. A transparent grid overlay was used to locate the positions of the snakes to the nearest millimeter.

Water was continuously available in the center of the arena in three petri dishes. The snakes were maintained on a 12:12 L:D cycle. The relative humidity was between 54 and 74%; the air temperature ranged from 25.5 to 26.5°C. Animals were 4-8 days old when placed into the enclosure.

Procedure

Snakes were released into the center of the testing apparatus and their positions recorded twice a day for three days. Observations occurred between 0900-1200 and 1400-1730 with at least four hours between successive observations. At each observation the relative humidity, air temperature and time were recorded. Snakes were disturbed as little as possible so as to record their "normal" aggregation tendencies. One difficulty in determining a snake's position relative to others lies in deciding from which part of the body to measure. Nearest neighbor distances were calculated to the nearest mm using the location of the snake's head to represent the snake's position in space. When snakes were found under cover objects,

this was determined using a plastic overlay. The overlay was divided into 10 mm² blocks and each was labeled with letters so that the snake's location could be identified by letter code. Within each block, the number of mm from each axis was recorded to pinpoint the location. Finally, the letter designation of the cover object and the total number of snakes beneath it were noted. When a snake was not under a cover object, the direction (to the nearest 10°) and distance (to the nearest 5 mm) to the nearest cover object were estimated using a protractor to aid in determining the angle, and objects in the arena of known length to help estimate distance. Nearest neighbor distance data were transformed ($\ln(\text{distance} + 1)$) to meet ANOVA assumptions of normality and homogeneity of variance.

RESULTS

Cover Object Use

There were significant litter differences in Wisconsin animals in the percent of observations that a snake was found under cover, the mean number of changes in cover objects, and the number of different cover objects visited (Table IVa). Significant litter differences were also found in these measures in the Michigan snakes. Additionally, litter differences in the maximum number of times individuals were found under a given cover object in Michigan snakes (Table IVa) were significant.

Nearest Neighbor Distance

The litter means of the nearest neighbor distances were negatively correlated with litter size (Pearson's $r=-0.6333$, $P=0.002$). Analyses of covariance were used in subsequent analyses to remove these effects. Significant differences were found among litters in mean nearest neighbor distance ($F_{20,340}=5.26$; $P=0.0001$). However, after removing litter size effects, no significant differences were found between populations in the mean litter nearest neighbor distances ($F_{1,18}=0.01$; $P=0.9291$; Table IVb). The nearest neighbor distances of animals found underneath cover objects was significantly lower than that of snakes found in the open ($F_{1,35}=45.28$; $P<0.0001$). No sex differences in nearest neighbor distances were found ($F_{1,38}=0.02$; $P=0.8911$), nor were there differences between single and multiple paternity litters in the mean nearest neighbor distance ($F_{1,20}=0.02$, $P=0.882$).

Population Differences

There were, however, population differences (Table IVa). The Michigan litters were significantly more likely to be found in the open (Wilcoxon 2-sample test $Z=1.9566$, $P=0.05$). In addition, after adjusting for litter differences in the number of snakes in the enclosure, the Michigan animals were found in larger groups underneath the cover objects. There did not appear to be population differences in preferences for a given cover object as there were no significant differences in the maximum number of times a particular snake was found under a

cover object ($P=0.360$), nor were there population differences in the total number of different cover objects used ($P=0.618$).

Coefficients of Variation

There were no differences between the mean litter coefficients of variation ($\pm$ SD) in the nearest neighbor distances of either single ($CV=24.8 \pm 4.0$) or multiple ($CV=25.5 \pm 5.6$) paternity litters.

Heritabilities

The means of the six measures of nearest neighbor distance for each individual were used to calculate heritabilities (Table IVc). Significant heritabilities in nearest neighbor distance were found in Michigan snakes ($h^2=0.26 \pm 0.11$), but not in Wisconsin snakes ($h^2=0.06 \pm 0.17$). There were only 3 litters large enough to be tested in the Wisconsin population. Sex differences in heritability were tested in the Michigan population (males: $h^2=0.42 \pm 0.16$; females: $h^2=0.18 \pm 0.14$), but no significant differences were found. The small sample size precluded testing Wisconsin snakes for these differences. Excluding litters with multiple paternity increased the estimate of the heritability of nearest neighbor distance in both populations (Table IVc).

DISCUSSION

These results demonstrate that the tendency to form groups in snakes has a heritable basis for at least one population.

This is the first time that a genetic basis for aggregative behaviors in snakes has been demonstrated.

Population differences in the percent of time found under cover suggest that Michigan animals moved about more in the arena. Differences among populations in the movement patterns of Thamnophis sirtalis in the field have been reported (Macartney et al., 1988). One could argue that the smaller size of Wisconsin animals might influence the metabolic requirements of these animals and, in order to conserve energy, Wisconsin snakes might spend more time under cover, however, there was no effect of body mass on the number of observations that a snake was found under cover ($F_{1,317}=2.07$, $P=0.1511$). No population differences in nearest neighbor distance were found, but given the large variation in this measure and the small number of Wisconsin litters tested, this result is not conclusive.

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PART IV

APPENDIX

Table IVa. Litter differences in cover object use of neonate Thamnophis sirtalis in aggregation.

X $\pm$ SE*				
N	% of time under cover	mean # of changes in cover object	max # of times under given cov. obj.	# of different cov. obj.
Michigan				
17	58.0 $\pm$ 0.05 (0.001)	3.4 $\pm$ 0.10 (0.032)	3.1 $\pm$ 0.08 (0.046)	3.3 $\pm$ 0.08 (0.012)
Wisconsin				
4	76.6 $\pm$ 0.06 (0.042)	3.1 $\pm$ 0.30 (0.017)	3.0 $\pm$ 0.12 (0.404)	3.3 $\pm$ 0.17 (0.078)

* - P values given in parentheses.

Table IVb. Population, sex, exposure, and paternity differences in mean litter nearest neighbor distance ($\pm$ SE) of Thamnophis sirtalis during aggregation tests.

Contrast	N	Nearest Neighbor Distance (mm)	
Population (NS)			
Michigan	17	83.8	+ 6.0
Wisconsin	4	97.1	<u>±</u> 12.9
Sex (NS)			
Male	21	86.2	+ 5.6
Female	20	86.5	<u>±</u> 6.1
Exposure *			
Covered	19	67.5	+ 4.6
Uncovered	19	122.8	<u>±</u> 8.0
Paternity (NS w/ litter size as covariate)			
Single	7	103.5	+ 10.1
Multiple	14	77.8	<u>±</u> 5.2

* - $P < 0.0001$

Table IVc. Full-sibling heritabilities of two populations of neonate Thamnophis sirtalis in mean nearest neighbor distance.

Population	N	Heritability ¹
All Litters		
Michigan	315	0.260 ± 0.109*
Wisconsin	47	0.064 ± 0.167
Single Paternity		
Michigan	44	0.760 ± 0.402*
Wisconsin	30	0.087 ± 0.229
Multiple Paternity		
Michigan	271	0.122 ± 0.081*

- 1 - Tests of significance of $h^2 > 0$ performed by analysis of variance among and within litters:
 * = $P < 0.01$. Heritabilities were calculated using the residuals from a regression of nearest neighbor distance and litter size.

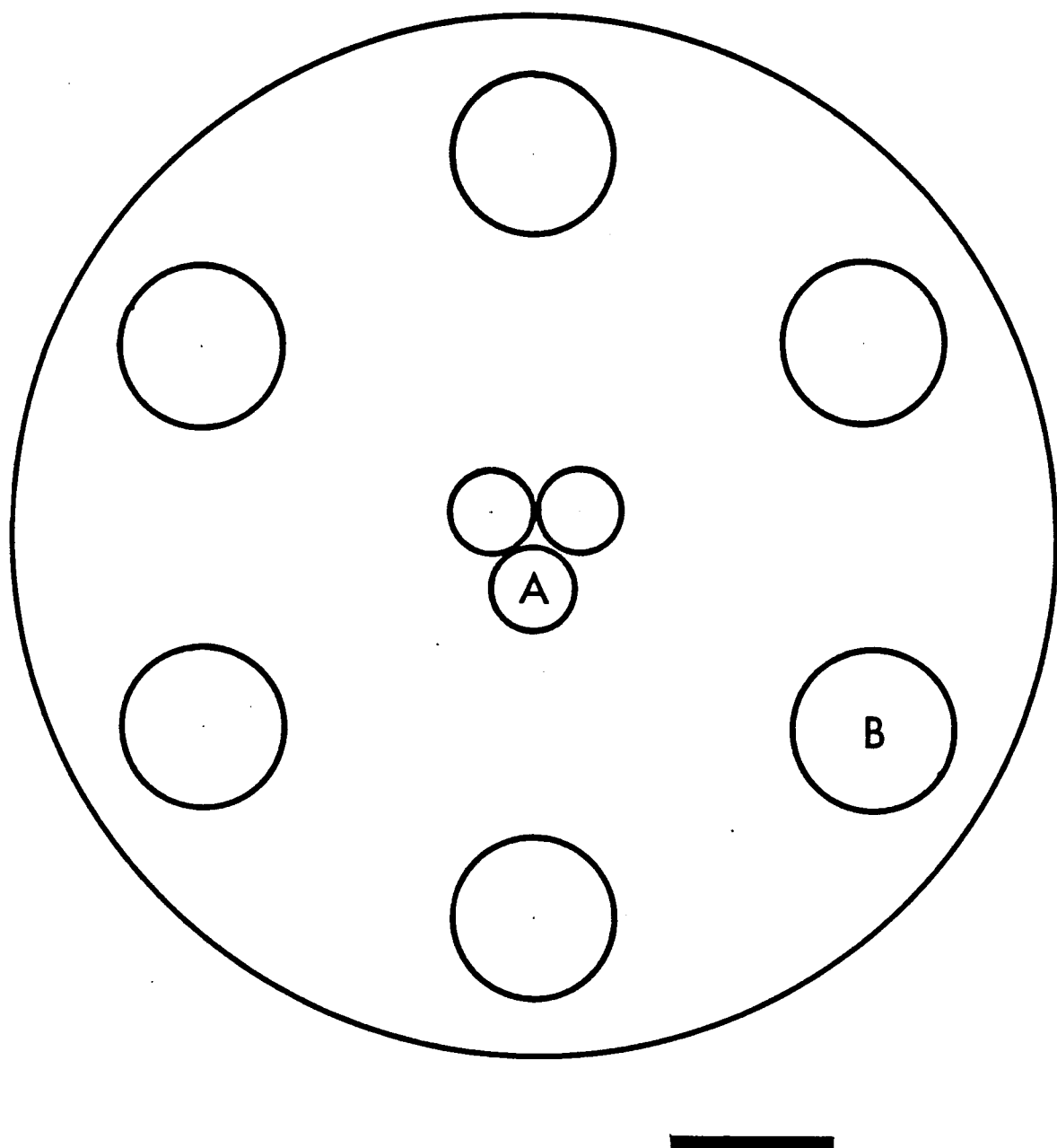


Figure IVa. Diagram of the arena used for testing aggregative behavior in Thamnophis sirtalis. A-Water dishes, B-Cover objects. Scale bar= 15cm.

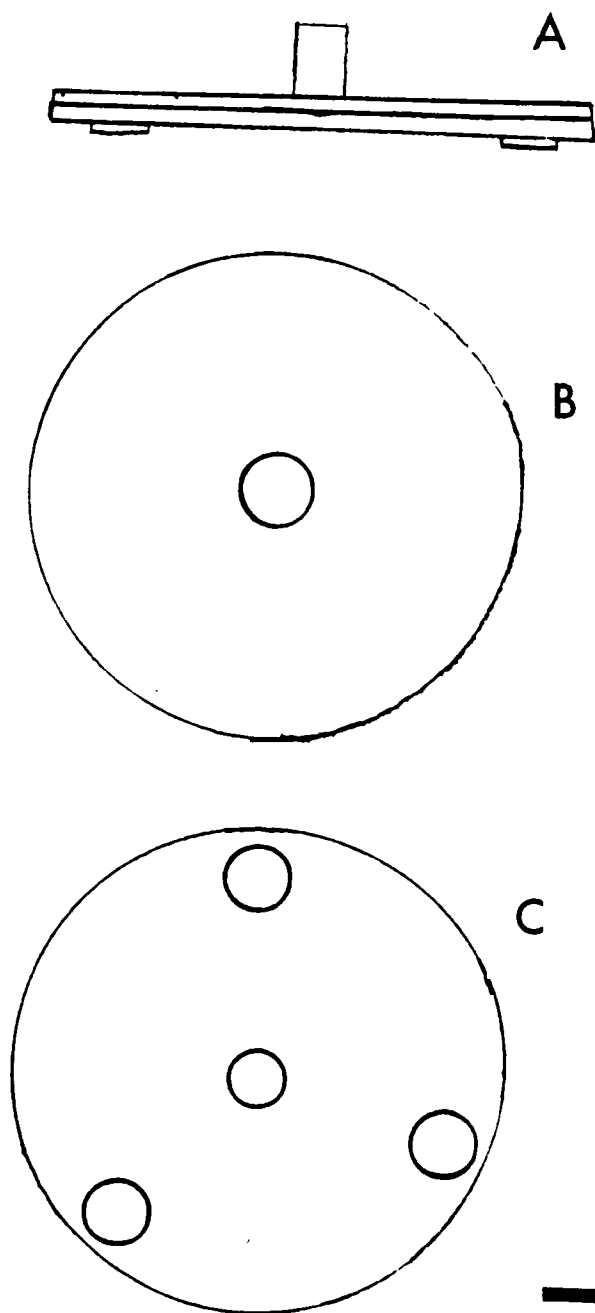


Figure IVb. Diagram of a cover object used during aggregation tests of Thamnophis sirtalis. A-Side view, B-wooden disk, C-Plastic bottom with feet. Scale bar= 5mm.

PART V

CHEMICAL PREY PREFERENCES

INTRODUCTION

Geographic differences in the feeding preferences of snakes has been well documented in some species. Dix (1968) examined the food preferences of T. sirtalis and Nerodia sipedon in adults and neonates. Thamnophis sirtalis were collected from Everglades, Florida, Potomac, Maryland, and Wayland, Massachusetts. Nerodia sipedon were collected from Everglades, Florida, Potomac, Maryland., and Great Swamp, New Jersey. Dix found that the choices of newborn snakes were the same as adults. Between sites, snakes had significantly different prey preferences.

Drummond and Burghardt (1983) compared the foraging behavior between a coastal and an inland population of the garter snake, Thamnophis elegans, and found significant differences in their tendency to eat fish and as well as in their aquatic foraging tactics. Snakes from both populations use the same behavioral repertoire, but they differ in their tendencies to perform many of the behaviors. A significantly higher proportion of the inland population ate fish and spent a greater portion of time performing aquatic foraging behaviors (e.g. craning, cruising, diving). In addition, snakes from this population made significantly more aerial and underwater attacks than did snakes from the coastal population. Kephardt (1982) examined the diets of T. sirtalis and T. elegans at 22 sites in northern California. The two species differed in diet, although

variation within species was so great that differences among sites was often greater than differences between the species. The slug feeding tendency of T. elegans also varies across its range; Arnold (1981c) found that the proportion of snakes that attacked and ate Ariolimax (a common prey item in coastal populations) varied among thirteen populations in California. The variation in response corresponded with the distribution of the slug. Populations outside of the range of the slug were much less likely to attack the prey than were snakes sympatric with the slugs. These geographic differences hold for neonates as well (Arnold, 1977), and are ontogenetically stable across the first year of life regardless of past feeding experience (Arnold, 1981c).

Burghardt and Hess (1968) also found evidence that suggests that responses of neonate snakes to chemical extracts is stable regardless of past feeding experience. They raised 2 groups of neonate Thamnophis sirtalis from a single litter, force-fed a diet consisting exclusively of strained beef liver for 64 and 191 days. Garter snakes do not normally encounter beef liver and will not eat it unless force-fed. The responses of these littermates to various chemical extracts were then tested. Snakes fed a single food diet did not alter their responses to other prey extracts. Subjects tested at ages 64 and 191 days did not differ from naive neonates tested at 4 days in their preferences to particular extracts. The response of snakes to beef liver extracts was indistinguishable from that to

water controls. In addition, there was no change in the response of snakes forced-fed liver when compared to newborns.

Many snakes utilize chemical cues to discriminate among various prey (Burghardt, 1970b, 1980; Arnold, 1981c; Chiszar et al., 1981), and the response of these snakes to chemical prey extracts has been found to be a simple and reliable method for ascertaining feeding preferences. Burghardt (1967) found species differences in response to chemical extracts from prey in unfed newborn Thamnophis radix, T. butleri, T. couchii, Opheodrys vernalis, and Storeria dekayi that correspond to the normal feeding preferences of each species. Burghardt (1969) found similar results in a comparative study of T. sirtalis, T. butleri, T. radix, T. ordinoides, and T. couchii. Burghardt (1968) also reports comparable findings from a study of the chemical prey preferences of neonates of Nerodia sipedon, Regina grahamii, and Regina septemvittata. Intraspecific differences in chemical prey preferences have also been found to correspond to intraspecific differences in feeding preferences. Gove and Burghardt (1975) examined the responses of several populations of the water snake, Nerodia s. sipedon, to chemical prey extracts. They found evidence that newborn snakes have innate predispositions for sympatric prey species normally consumed. Arnold (1981c) found that the chemical prey preferences of T. elegans in coastal and inland populations correspond to their feeding preferences in those populations.

Burghardt (1975) found that individual differences in

preferred prey extract are consistent among members of the same litter. These preferences can, however, be modified by feeding (Fuchs and Burghardt, 1971; Burghardt et al., 1973), especially in neonates (Gove and Burghardt, 1975).

There is some evidence of population differences in response to chemical prey extracts in T. sirtalis. Burghardt (1970a) tested 4 litters from three populations, 2 from northern Illinois, and one each from western Iowa and northern Wisconsin. Both Illinois litters gave strong responses to both fish and worm extracts. The Wisconsin litter preferred fish extracts, while the Iowa litter gave greater responses to worm extracts.

The variation in feeding and chemical prey preferences has been found to have a heritable basis. Arnold found heritable variation in the response of neonates to both chemical prey extracts (1981a, c) and prey animals (1981b, c) in a coastal and an inland population of T. elegans. There does not appear to be an influence of the maternal environment on these behaviors. Burghardt (1971) found no effect of the maternal diet during gestation on the subsequent chemical prey preferences of the offspring in T. sirtalis. Likewise, Arnold (1981b) did not find maternal effects on slug feeding preferences in crosses between two populations of T. elegans.

METHODS

General

Using the techniques of Burghardt (1975), chemical prey preferences were tested with extracts prepared from surface substances of potential prey (fish and earthworm). Neonatal snakes were tested at 9-12 days of age; adult females at 1-2 months postpartum. Snakes were tested at $25 \pm 2^{\circ}\text{C}$.

Extracts

The extracts were prepared by placing the prey animals in distilled water at 60°C for 2 min; 3 g of prey/10 ml of water. The solution was then decanted and centrifuged for 10 min at 2500 rpm (Burghardt, 1969). Extracts were frozen (-4°C) until use. Two prey species were used, fathead minnows (Pimephales promelas) and earthworms (Lumbricus terrestris). The amount of material obtained by this technique ranges from 2 to 3 g/liter (Burghardt et al., 1988).

Procedure

Subjects were all tested using a fish and worm extract, as well as a distilled water control. Each individual was tested once for response to seven samples in the following order: water, worm, fish, water, fish, worm, water. Burghardt (1969) demonstrated that the response of a snake to a particular stimulus can be affected by the response to previous stimuli. Snakes which attack one stimulus have significantly higher

scores to the next even when it is a water control (Burghardt, 1969). The use of the seven sample sequence insures that each stimulus precedes and follows every other, so that such "carry-over" effects can be identified. At least twenty, but no more than thirty minutes, elapsed between successive presentations of an extract to any one individual. The stimulus was presented on a cotton swab dipped into the extract and placed approximately 1 cm from the tip of the snake's snout. The number of tongue flicks directed both toward and away from the swab during a 30 s presentation of the stimulus were recorded. The trial ended if the snake attacked or attempted to ingest the swab. When this occurred, the latency of the attack, and the number of tongue flicks until the attack were recorded. If the subject did not tongue flick within 20 s of presenting the stimulus, it was tapped lightly on the top of the head with the end of the swab. Every effort was made to keep the swab 1 cm in front of the snake if it moved about the arena but occasionally the snake would escape from the test arena. The snake was then picked up and replaced in the arena. The timing of the trial was not interrupted.

Statistics

Many authors have used an index to analyze both tongue flick and attack data as a single composite measure of responsiveness to a particular stimulus. The tongue flick attack score (TFAS) first used by Burghardt (1967) has been

found to be a highly effective measure of response strength for discriminating among extracts (Cooper and Burghardt, in press). For this measure, an attack is weighted more than are tongue flicks, as it seems reasonable to treat an actual attack to the extract as an indication of a greater response to the stimuli than any given number of tongue flicks. If no attack occurs, the score is equal to the number of tongue flicks directed toward the swab in 30 secs. To give greater weight to an attack, the attack latency is subtracted from 30 secs and the result is added to the maximum number of tongue flicks elicited from any snake to any stimulus, so that the score for an attack will be at least as high as those in which no attack occurred. In an attempt to normalize the distribution and increase the homogeneity of variance of the TFAS, data were subjected to a natural log (score + 1) transformation. Analysis of variance techniques were used to test whether the snakes were discriminating between stimuli or displayed a preference for a particular extract.

RESULTS

Litter differences

There were litter differences in the mean TFAS to the various stimuli (Fig. Va). There were significant differences among the litters in TFAS to all stimuli in both the Michigan (Fish: $F_{22,327}=3.08$, $P<0.0001$; Worm: $F_{22,327}=3.11$, $P<0.0001$; Water: $F_{22,327}=2.92$, $P<0.0001$) and Wisconsin populations (Fish:

$F_{4,51}=4.56$, $P=0.003$; Worm: $F_{4,51}=3.12$, $P<0.023$; Water: $F_{4,51}=3.36$, $P=0.017$). Litter differences were also found in the response to most of the different presentations of the stimuli (Michigan: #1,2,3,4,5,6, $P\leq 0.005$; Wisconsin: #2,4,5,6, $P\leq 0.04$; #3, $P=0.073$). Therefore, all subsequent analyses used litter means to control for these effects.

Tongue flick attack scores

Snakes from the two populations were able to discriminate among the prey extracts and the water controls (Fig. Vb; Michigan: $F_{1,21}=21.39$, $P<0.0001$; Wisconsin: $F_{1,4}=5.82$, $P=0.017$). Snakes responded significantly less to the water controls than to the prey extracts (Adults: $F_{2,25}=26.65$, $P<0.0001$; Neonates: $F_{2,25}=31.53$, $P<0.0001$).

Some effects of the order of presentation of the same stimuli were found (Fig. Vc). There were significant differences between the response of Michigan neonates to the three different presentations of the water controls (Kruskal-Wallis test $X^2=6.54$, $df=2$, $P=0.038$). There were no significant differences among the different presentations of the same stimuli in the Wisconsin animals.

Significant population differences were found in the response to fish but not worm extracts. Wisconsin neonates responded more to fish extracts than Michigan neonates (Wilcoxon 2-sample test $Z=2.4594$, $P=0.014$).

Age differences were also present (Fig. Vd). The adult females from Michigan gave significantly higher responses to the

three stimuli than did Michigan neonates (Fish: $Z = 5.1883$; $P < 0.0001$; Worm: $Z = 3.3719$; $P < 0.001$; Water: $Z = 2.3733$; $P = 0.018$). Only the response of Wisconsin neonates to the water controls was significantly higher than the response of adult females ($Z = 2.3270$; $P = 0.020$). There were no other significant differences between the responses of the young and old snakes from Wisconsin.

Attacks

Population differences in the number of attacks to the different stimuli are shown in Figure Ve. The Wisconsin neonates gave significantly more total attacks to fish than did the Michigan snakes (Wilcoxon 2-sample test $Z = 2.4918$; $P = 0.013$). Population differences in the number of attacks to the various presentations of the stimuli are presented in Figure Vf. Both fish stimuli (#3 and 5) elicited significantly more attacks from the Wisconsin population than the Michigan population (#3: $Z = 2.8212$; $P = 0.005$; #5: $Z = 2.2807$; $P = 0.023$). There were no significant differences between the populations in attacks to the two worm extracts (#2 and 6) or the water controls (#1, 4, and 7).

The number of attacks made by the neonate and adult snakes to the various stimuli were also compared (Fig. Vf). Although there were no significant differences between the two age classes in response to either of the prey extracts, Michigan adults were less likely to attack the water controls (Wilcoxon

2-sample test $Z=2.7537$, $P < 0.006$). Only the presentations of water following the presentation of a prey extract showed significant differences between the age groups (Seq. #4: $Z=2.0066$, $P=0.045$; Seq. #7: $Z=2.006$, $P=0.045$). There were no differences between the Wisconsin age groups in response to different stimuli.

Coefficients of Variation

The coefficients of variation of single and multiple paternity litters did not differ for the total of responses to each extract (single paternity: 33.8 ± 13.6 ; multiple paternity: 34.1 ± 9.7). Similarly, the coefficients of variation did not differ in response to fish (single paternity: 40.0 ± 17.5 ; multiple paternity: 44.5 ± 14.8), and worm (single paternity: 47.3 ± 19.0 ; multiple paternity: 42.8 ± 10.3) prey extracts, nor to the water controls (single paternity: 33.8 ± 13.6 ; multiple paternity: 34.1 ± 9.7).

Heritabilities

The heritabilities of the TFAS are presented in Table Va. Mean response scores to all presentations of a given stimulus were used to calculate a measure of heritability for each stimulus. The TFAS to both prey extracts in the two populations have significant heritable variation. After excluding litters with multiple paternity, only the heritability estimate of the response to the water control in the Michigan population is not significant. Interestingly, the responses to the preferred

chemical prey extracts in both population have the highest heritabilities. In general, the heritabilities of the responses to prey extracts are higher than to water controls.

In order to increase the sample size, all litters were used to calculate heritabilities of TFAS of both sexes to the extracts. No differences were found for the overall score in either the Michigan (males: $h^2=0.168 \pm 0.114$; females: $h^2=0.303 \pm 0.151$) or the Wisconsin (males: $h^2=0.746 \pm 0.452$; females: $h^2=0.863 \pm 0.635$) populations. Similarly, there are no sex differences in the heritability of the responses to fish (Michigan males: $h^2=0.164 \pm 0.113$, females $h^2=0.385 \pm 0.161$; Wisconsin males: $h^2=0.332 \pm 0.408$, females $h^2=0.330 \pm 0.680$) or worm (Michigan males: $h^2=0.270 \pm 0.132$, females $h^2=0.277 \pm 0.147$; Wisconsin males $h^2=0.473 \pm 0.434$, females $h^2=0.642 \pm 0.673$) prey extract nor to the water (Michigan males: $h^2=0.256 \pm 0.129$, females: $h^2=0.162 \pm 0.128$; Wisconsin males: $h^2=0.351 \pm 0.412$; females: $h^2=0.985 \pm 0.602$) controls.

Attacks to chemical stimuli could be treated as a threshold character in order to calculate heritabilities. However, the age differences in the probability to attack are such that adult females are much more likely to attack chemical extracts from prey stimuli than are neonates. Since the logical choice for the "affected" trait is an attack, the resulting heritabilities will have a negative sign.

DISCUSSION

These results agree with similar studies of the prey preferences of T. sirtalis from Wisconsin and Michigan populations. Burghardt (1970a) found Wisconsin neonates prefer fish extracts. In my study 52% of the individuals attacked the fish stimuli as compared to 34% in Burghardt's study. This difference may reflect age and presumably hunger level differences in the unfed snakes when tested. I used 9-12 day old snakes, Burghardt (1970a) tested 4-5 day old snakes. Burghardt found a mean TFAS of 44.2 to fish stimuli and 36.1 to worms (using 60 s trials), compared to 34.3 to fish and 27.0 to worms in my study with 30 s trials. The extract preferences observed in neonates from Michigan match the feeding preferences of neonates from the same population. Batts and Allen (in Burghardt, in press) tested 23 neonates from a litter of T. sirtalis collected in Wayne County, Michigan. The snakes were given a choice of earthworm, Lumbricus terrestris, or mosquito fish, Gambusia affinis. Of the 15 snakes that ate 20 or more food items, 7 significantly preferred worms, 5 snakes ate significantly more fish, and 3 snakes showed no preference for either prey item. In my study, the Michigan population gave a greater mean response to worm extracts than to fish, and gave a greater mean response to fish than to the water controls. Some snakes showed preferences for the worm extracts, some for the fish extracts, and some showed no preference.

Significant litter differences were found in both populations. Previous studies of population and species differences in responsiveness to prey chemicals in T. sirtalis (Burghardt, 1970a, 1975; Gove and Burghardt, 1975) have relied on subjects from one or two litters as representing the population. If the results of my study are generalizable to other populations and other species, care must be taken when sampling populations. An effort must be made to test animals from several litters within a population and several populations within a species before making comparisons among groups.

As in previous studies (Arnold, 1981a, c), variation in the response of neonate garter snakes to chemical extracts derived from prey stimuli has significant heritability. Although different species were tested to different prey extracts, the results are similar. Arnold (1981c) reports an average heritability of $.35 \pm .20$ in response to prey odors and $.05 \pm .11$ in response to controls in coastal T. elegans. Inland snakes averaged $.21 \pm .12$ to prey odors and $.12 \pm .09$ to controls.

Removing half-sib families from my analyses increased the heritabilities of all but the response to the water control in the Michigan population. If, therefore, previous studies (Arnold, 1981a, c) of the heritability of the response of garter snakes to chemical prey extracts included half-sib families in the analyses, the heritabilities reported underestimate the additive genetic variation in the population.

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PART V

APPENDIX

Table Va. Heritability of TFAS in neonate Thamnophis sirtalis from two populations to water controls and fish and worm prey extracts.¹

Population	N	Fish			Worm			Water		
		h ²	±	s.e.	h ²	±	s.e.	h ²	±	s.e.
All Litters										
Michigan	350	0.2437	0.0968**		0.2472	0.0974**		0.2277	0.0936**	
Wisconsin	52	0.5212	0.3288**		0.3469	0.2810*		0.3782	0.2810*	
Single Paternity										
Michigan	79	0.2999	0.2110*		0.7022	0.2700**		0.0400	0.1368	
Wisconsin	35	0.7622	0.4206**		0.4582	0.3680*		0.5319	0.3866*	
Multiple Paternity										
Michigan	271	0.2436	0.1161**		0.0848	0.0684*		0.2620	0.1210**	

1 - Tests of significance of $h^2 > 0$ were performed by analysis of variance among and between litters: * = $P < 0.05$, ** = $P < 0.01$.

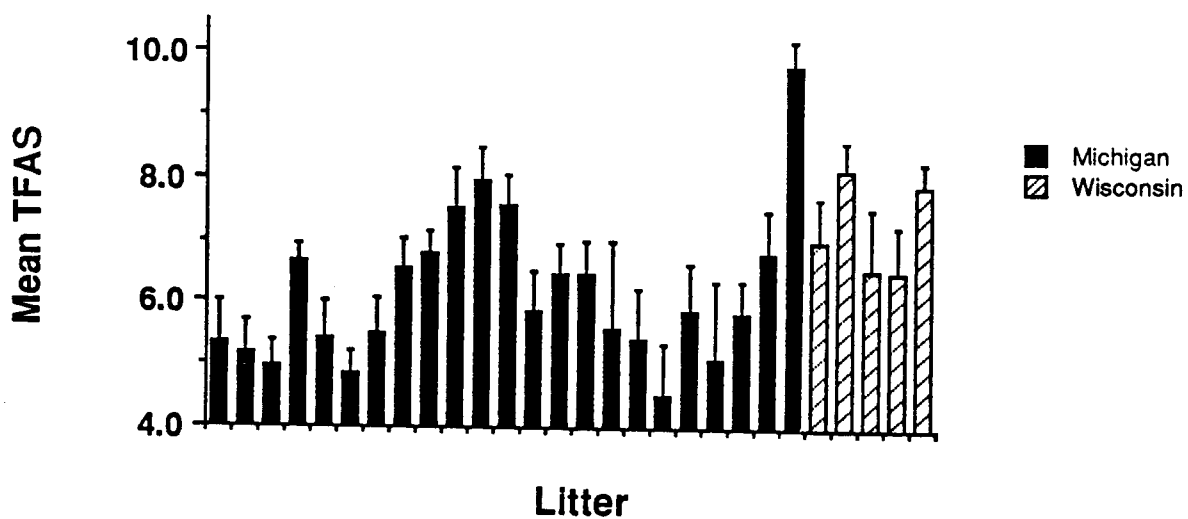


Figure Va. Litter comparisons of neonate *Thamnophis sirtalis* from two populations in mean natural log transformed tongue-flick attack scores (TFAS $\pm$ SE) to all extracts.

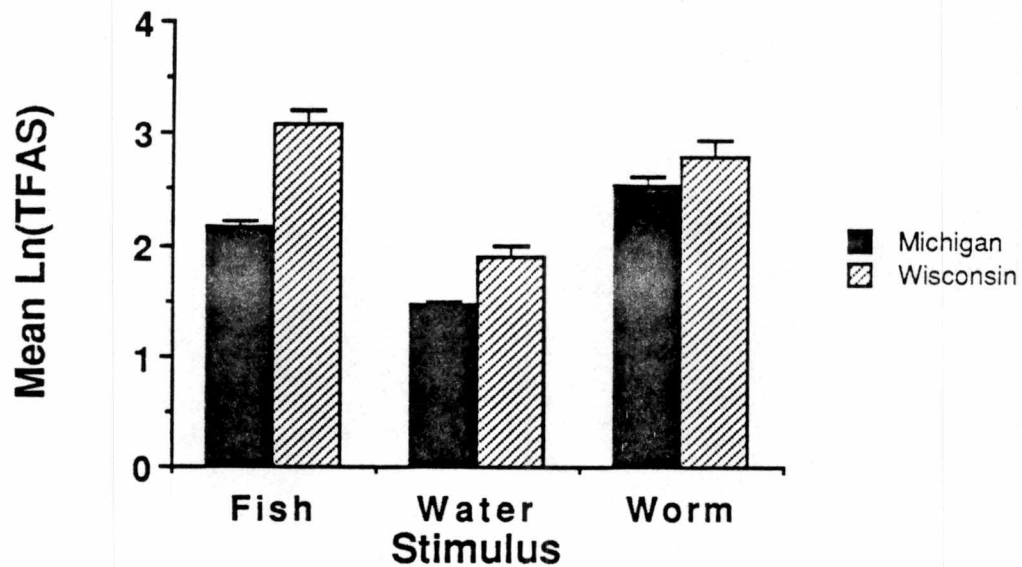


Figure Vb. Comparison of the mean natural log transformed tongue-flick attack scores (TFAS $\pm$ SE) of neonate *Thamnophis sirtalis* from two populations to water controls and fish and worm prey extracts.

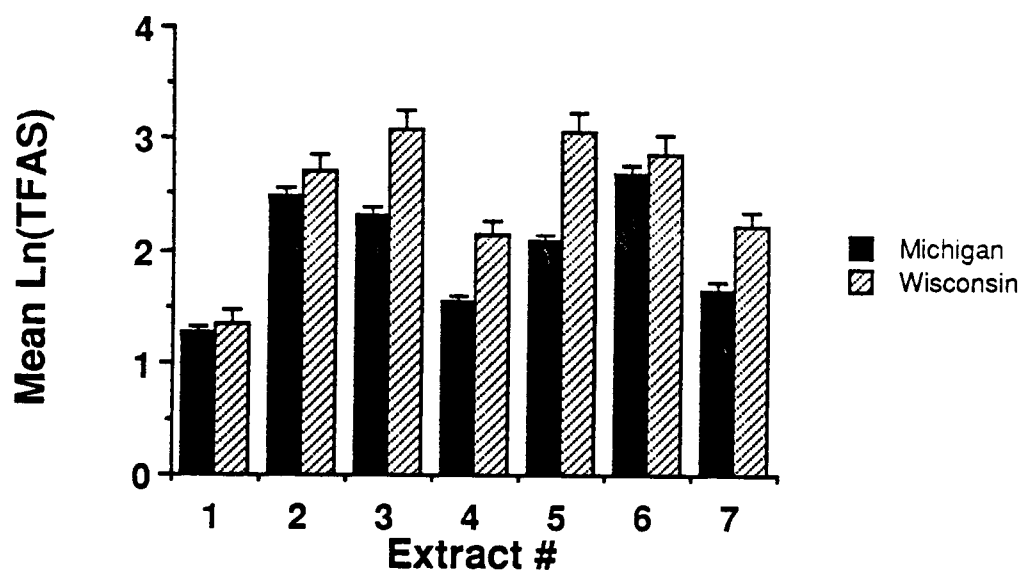


Figure Vc. Comparison of the mean natural log transformed tongue-flick attack scores (TFAS $\pm$ SE) of neonate Thamnophis sirtalis from two populations to different extracts in order of presentation.

Extracts: (1,4,7) water controls, (2,6) worm, (3,5) fish.

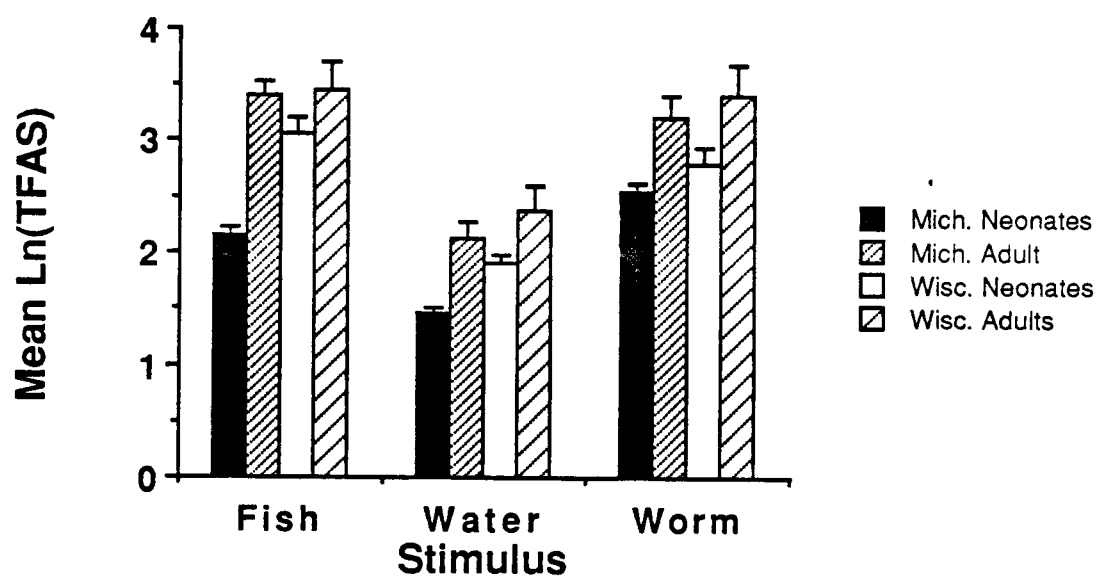


Figure Vd. Comparison of mean natural log transformed tongue-flick attack scores (TFAS $\pm$ SE) of neonate and adult *Thamnophis sirtalis* from two populations to water controls and fish and worm prey extracts.

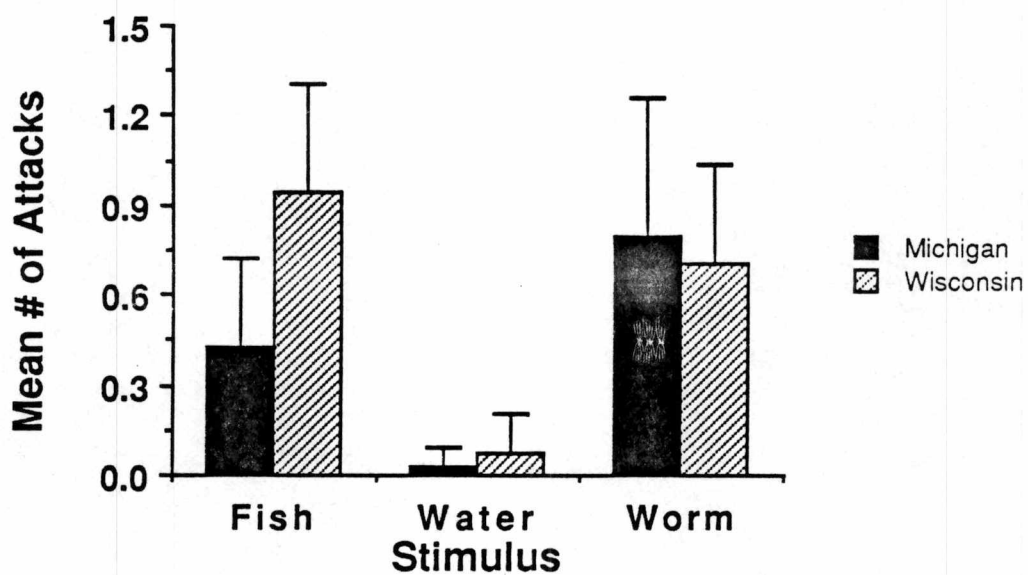


Figure Ve. Comparison of the mean number of attacks ($\pm$ SE) of neonate *Thamnophis sirtalis* from two populations to water controls and fish and worm prey extracts.

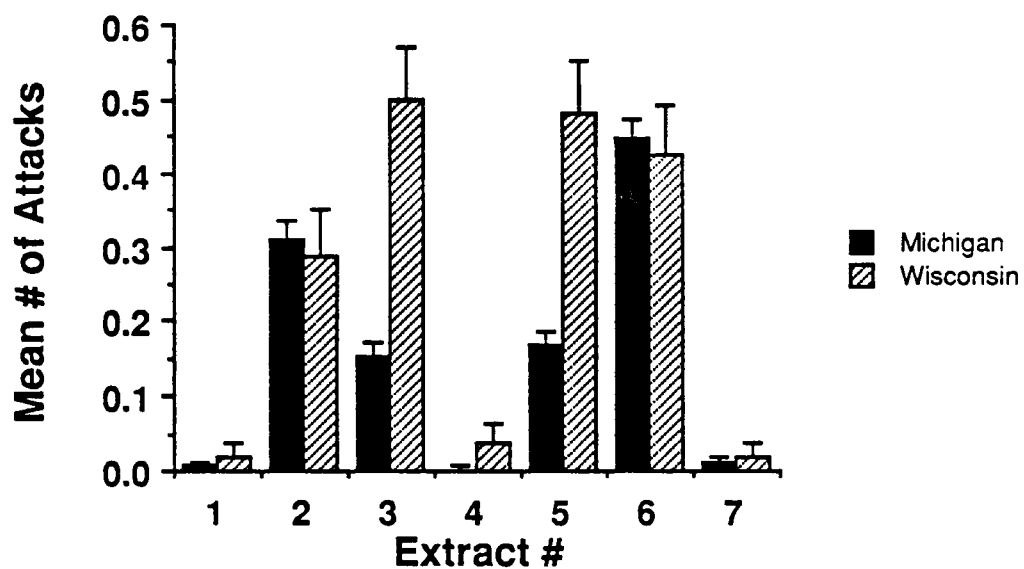


Figure Vf. Comparison of the mean number of attacks ($\pm$ SE) of neonate Thamnophis sirtalis from two populations to different extracts in order of presentation.

Extracts: (1,4,7) water controls, (2,6) worm, (3,5) fish.

PART VI

ANTIPREDATOR BEHAVIOR

INTRODUCTION

The defensive behaviors of snakes are quite varied and include both morphological and behavioral adaptations, yet surprisingly, few physiological defensive adaptations are known (Greene, 1988). Examples of morphological modification are numerous; the hood spreading behavior of cobras (e.g. Naja, Bogert, 1943) and the tail-rattling displays of crotalids (e.g. Crotalus, Sistrurus, Klauber, 1956) are among the most famous. Such displays are, however, not limited to venomous snakes. Greene (1979) lists 129 species in 77 genera and 5 families that utilize some form of tail or head display. The taxonomic distribution of behavioral displays such as death feigning (e.g. Heterodon platirhinos, Edgren, 1955), rigidity (e.g. Trachyboa boulengeri, Lehmann, 1970), constriction of the predator (e.g. Charina bottae, Peabody et al., 1975), and forming a ball (e.g. Calabaria reinhardtii, Bustard, 1969) has been compiled by Greene (1988).

The antipredator behaviors of several natricine snakes have been studied. Scudder and Burghardt (1983) observed differences in the defensive behavior of three species of water snakes (Nerodia cyclopion, N. fasciata, and N. rhombifera). Herzog and Burghardt (1986) have found species differences in the antipredator behaviors of both adult and neonatal T. sirtalis, T. butleri, and T. melanogaster. Arnold and Bennett (1984) report considerable individual neonatal variation in

antipredator behaviors within a population of T. radix.

Examination of the development of these behaviors has shown that both the species and individual differences remain constant over at least the first year of life in T. melanogaster (Herzog and Burghardt, 1988). Herzog and Schwartz (submitted) report geographic differences in antipredator behavior in neonate T. sirtalis that suggests genetic differences between the populations.

Several investigators examining antipredator behaviors have found considerable heritable variation ($h^2 = 0.40$ to 0.60) in the defensive responses of laboratory born neonates from wild-caught females in Thamnophis radix (Arnold and Bennett, 1984); Thamnophis sirtalis (Garland, 1988; Schwartz and Herzog, in prep); Thamnophis ordinoides (Brodie, pers. comm.). Heritable variation of this magnitude suggests that antipredator responses in garter snakes should be capable of rapid evolutionary change (Arnold and Bennett, 1984).

Environmental factors are also known to affect the antipredator behavior of snakes. Since snakes are ectotherms, it is perhaps not too surprising that temperature has been found to have effects in several species. Greenwald (1974) noted that gopher snakes (Pituophis catenifer) with body temperatures of 18°C sometimes exhibit defensive behavior toward prey. This temperature corresponds with the lower threshold for normal locomotion in juvenile gopher snakes (Brattstrom, 1965). Chadwick and Rahn (1954), and Martin and Bagby (1972) found that

rattling frequency increases with increasing body temperatures in Crotalus atrox and C. viridis. Natrix maura is more likely to exhibit balling, head and body flattening, and striking behaviors as ambient temperature drops (Hailey and Davies, 1986). In Regina septemvittata, the flight distance has been found to increase with increasing body temperatures (Layne and Ford, 1984).

The internal state of an animal can also influence its defensive behavior. Hailey and Davies (1986) report that fasted Natrix maura are less likely to resort to escape behavior than regularly fed animals. This behavioral change correlates with a significant reduction in the maximal activity metabolic rate, endurance, and lactate production in these snakes. Herzog and Bailey (1987) found that juvenile Thamnophis sirtalis had significantly higher defensive scores after ingesting ~50% of their body mass in food. This corresponds to a reduction in the ability of recently fed snakes to locomote (Garland and Arnold, 1983). These animals appear to minimize this problem by adjusting their behavior to compensate for the loss in escape ability. A similar change in defensive behavior is dependent upon the reproductive state of female garter snakes (Brodie, 1989). Female snakes lose mobility with both increasing relative clutch size (Shine, 1988) and reproductive state (Brodie, 1989), thereby increasing the chance of predation. Behavioral compensation reduces this risk by shifting toward antipredator behaviors that are less dependent upon locomotion

(Brodie, 1989). Other behavioral changes occur in oviparous snakes. In the Malayan pit viper, Calloselasma rhodostoma, "body-jerking" behavior is exhibited by brooding females upon tactile stimulation (York and Burghardt, 1988). This behavior is observed in the presence of eggs and disappears within two days after the hatching of the neonates.

METHODS

General

The methods for testing defensive behavior were adapted from Herzog and Burghardt, 1986, 1988; Herzog and Bailey, 1987) and consist of presenting the subject with a threatening stimulus (the right index finger of the right hand) while recording the number of attacks directed to that object. Herzog et al. (in press) have determined that the human finger is as effective as more natural predator models in eliciting defensive behavior. All neonates were tested between 12-15 days of age. Adult females were tested 1-2 months postpartum. In order to reduce diurnal effects, all tests were conducted between 1200 and 1430 hours. Although only one observer conducted the tests, inter-observer reliability of strike scoring is high ($r=0.99$, over 76 trials).

Apparatus

The test arena consisted of a glass aquarium (51 x 59 x 25 cm) with brown indoor/outdoor artificial turf covering the

bottom. This covering was washed between tests of different litters. The sides of the apparatus were lined with a single piece of white cardboard eliminating corners in the arena and providing a more uniform test environment. The number of strikes was recorded with a hand counter and a Graylab 451 timer was used to time trials.

The test arena for the adult females was similar to that used for testing the neonates with the exception that it was larger (57 x 87 x 57 cm).

Procedure

Subjects were taken from their home cage and placed by hand into the center of the arena. After a 30 s acclimation interval, the right forefinger of the experimenter was placed approximately 2 cm in front of the snake's snout and held stationary for 60 s. Subjects that moved were followed so that the stimulus would remain about 2 cm in front of the snout. The number of strikes directed to this stationary stimulus were recorded as non-moving stimulus scores. Both open- and closed-mouth strikes were counted regardless of whether they made contact with the finger. At the conclusion of this trial, the finger was removed and the snake left undisturbed for 30 s. A second trial (moving stimulus) was conducted and was identical to the first with the exception that the stimulus finger was repeatedly moved back and forth in front of the snake at a rate of 3-4 oscillations/sec throughout the trial.

The same procedure was used for testing the adults except

that the experimenter wore a rubber glove to protect the finger and a greater distance was used between the finger and the snout (3-5 cm). The initial acclimation period was also increased to 1 min.

Statistics

Following the procedures of Herzog and Burghardt (1986; 1988), Herzog and Bailey (1987), and Herzog et al. (in press), all analyses were performed on natural log transformed data ($\ln(\text{defensive score} + 1)$) in order to reduce the heterogeneity of variance.

RESULTS

As in previous studies, no sex differences were found in in the antipredator behavior of males and females (Table VIa; Wilcoxon 2-sample test $Z = 0.956$, $P = 0.339$). The mean female defensive score was 7.8 (sd = 15.4). The mean male defensive score was 9.2 (sd = 17.0).

There were significant population differences ($Z = 2.88$, $P = 0.004$) in defensive behavior (Table VIa). The mean number of strikes for the Wisconsin animals was four greater than that of Michigan animals. Significant litter effects were found in both populations (Fig. VIa; Wisconsin: $F_{4,47} = 2.809$, $P = 0.036$; Michigan: $F_{22,325} = 8.504$, $P < 0.0001$). A two-way repeated measures ANOVA (population x movement condition) confirmed that there was a significant population effect ($F_{1,26} = 20.58$, $P <$

0.0001). There was no significant overall effect of movement condition ($F_{1,26} = 0.10$, $P = 0.753$), nor was there a significant movement x population interaction ($F_{1,26} = 1.82$, $P = 0.189$).

No age differences in defensive behavior were found (Table VIa): the mean defensive score of neonates was not different from that of adult females (Michigan: Wilcoxon 2-sample test, $Z = 1.055$, $P = 0.292$; Wisconsin: $Z = 0.1225$, $P = 0.902$). This result held even after removing the males from the analysis (Michigan: $Z = 1.546$, $P = 0.122$; Wisconsin: $Z = 0.3674$, $P = 0.713$).

Estimates of the heritabilities of antipredator behaviors are presented in Table VIb. Full-sibling heritabilities are relatively high for the Michigan population. After removing the litters with multiple paternity, the heritability estimate increases for the Michigan population, however, the estimate for the Wisconsin population is no longer significant. The mean estimate of the heritability of antipredator behavior ($h^2 = 0.47 \pm 0.34$) is quite similar to that found in T. radix by Arnold and Bennett (1984) of $h^2 = 0.45 \pm 0.26$, and that of Garland (1988) in T. sirtalis of $h^2 = 0.42 \pm 0.11$. Sex differences in heritability were measured on all litters to increase sample size. There were no sex differences in the heritability of antipredator behavior in Michigan snakes (males: $h^2 = 0.675 \pm 0.175$; females: $h^2 = 0.795 \pm 0.188$) or in the Wisconsin population (males: $h^2 = -0.144 \pm 0.237$; females: $h^2 = 0.666 \pm 0.670$).

Although there was substantial variability in antipredator behaviors, the coefficient of variation ($\pm$ s.e.) within multiple

paternity litters ($CV=186.2 \pm 18.0$) was greater than in singly sired litters ($CV=136.5 \pm 13.5$).

These results demonstrate that variation in antipredator behaviors between these populations of garter snakes has a heritable basis and therefore has great potential for rapid evolution.

DISCUSSION

These experiments demonstrated that there are significant differences between two populations of garter snakes in the tendency to strike at a threatening stimulus. Since naive animals were tested, these geographic differences in antipredator behavior are not due to differential experience with predators. Quantitative genetic analyses reveal that the difference in the antipredator behavior between the two populations has a heritable basis. To my knowledge, this is the first report of heritable population differences in the antipredator behavior of a terrestrial vertebrate.

The results also support Arnold and Bennett's (1984) conjecture that the substantial heritabilities of antipredator responses in garter snakes would permit rapid behavioral evolution in the species. At present, however, it is not known what factors are responsible for maintaining the behavioral differences between populations. There are a number of explanations which could account for these differences. Possible non-adaptive explanations include genetic drift and

founder effects. Differential selection by predators in the two populations is a hypothesis which needs to be examined, and more information is needed on the defensive behaviors in snakes from intervening populations.

These results further indicate that researchers making comparisons between species should be careful about making generalizations based on animals drawn from a single locality. Caution should be used in concluding that species differ (or are similar) in behavior if all representatives come from a single population.

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PART VI

APPENDIX

Table VIa. Population, sex and age differences in antipredator behaviors of Thamnophis sirtalis from two populations.

Contrast	N	Total Score	Stimulus	
			Non-moving	Moving
Sex				
Male	223	9.2 + 17.0	4.3 + 8.5	4.9 + 9.7
Female	117	7.8 + 15.4	3.4 + 7.3	4.4 + 8.8
Population **				
Michigan	349	5.8 + 12.5	2.4 + 5.7	3.4 + 7.7
Wisconsin	52	25.9 + 24.6	13.0 + 12.6	12.9 + 13.4
Age *				
Neonates	401	8.4 + 16.1	3.8 + 7.9	4.6 + 9.2
Females	28	12.1 + 14.0	7.4 + 11.9	8.8 + 12.9

* - Wilcoxon 2 sample test, $P < 0.01$

** - Wilcoxon 2 sample test, $P < 0.0001$

Table VIb. Full-sibling heritabilities of antipredator behaviors in two populations of neonate Thamnophis sirtalis.¹

Population	N	Heritability $\pm$ SE		
		Total	Non-moving	Moving
All Litters				
Michigan	348	0.671 $\pm$ 0.154**	0.508 $\pm$ 0.138**	0.698 $\pm$ 0.156**
Wisconsin	52	0.303 $\pm$ 0.266*	0.481 $\pm$ 0.320*	0.249 $\pm$ 0.246
Single Paternity Litters				
Michigan	79	0.949 $\pm$ 0.272**	0.462 $\pm$ 0.242**	1.045 $\pm$ 0.266**
Wisconsin	35	0.272 $\pm$ 0.306	0.650 $\pm$ 0.408	0.036 $\pm$ 0.192
Multiple Paternity Litters				
Michigan	269	0.558 $\pm$ 0.182**	0.528 $\pm$ 0.178**	0.524 $\pm$ 0.177**

1 - Tests of significance of $h^2 > 0$ were performed by analysis of variance among and within litters: * = $P < 0.05$, ** = $P < 0.01$.

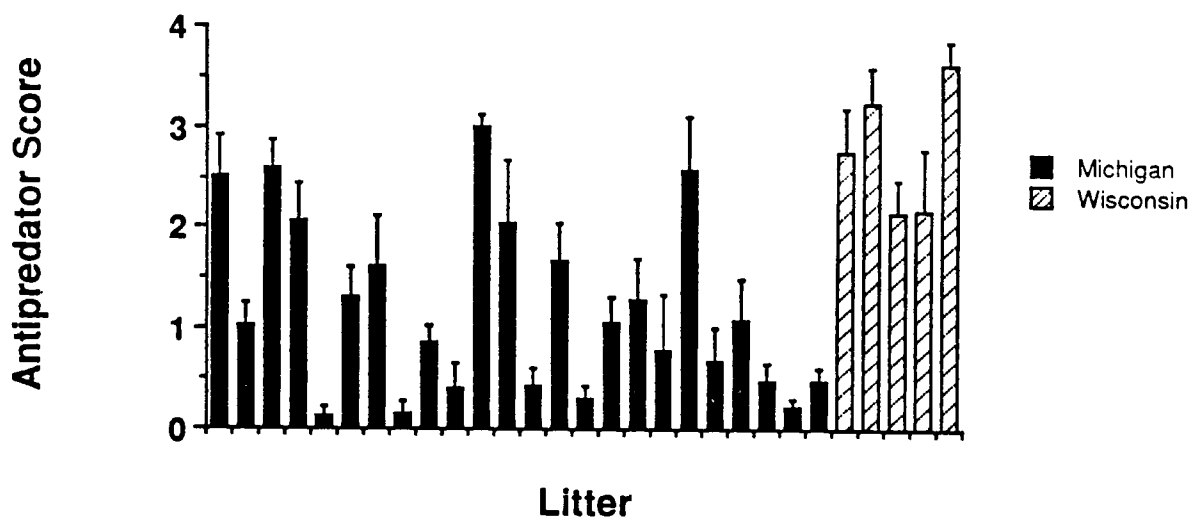


Figure VIa. Litter comparisons of neonate Thamnophis sirtalis from two populations in mean ($\pm$ SE) natural log (+1) transformed antipredator scores.

PART VII

CRITICAL THERMAL MINIMA

INTRODUCTION

The development of new terminology in the 1930's to describe reptiles as poikilothermic, endothermic, ectothermic, heliothermic, thigmothermic, and heterothermic illustrates both the enormous range of adaptations of these creatures to the thermal environment and the interest in these relations (Bogert, 1949). Evolutionary adaptation to this environment has lead to modifications in the distribution (Bailey, 1949; Saint-Girons and Saint-Girons, 1956; Dunson and Ehlert, 1971; Graham et al., 1971), physiology (Aleksiuk, 1971a, b; Barone and Jacques, 1975; see also Spellerberg, 1976), behavior (Bustard, 1967, 1968; Viittanen, 1967; Prieto and Whitford, 1971), reproductive modes (Saint Girons and Kramer, 1963; Hock, 1964; Neill, 1964; Viittanen, 1967; Smyth and Smith, 1968; Fitch, 1970; Prestt, 1971) and even the body size and shape (Heatwole et al., 1969; Spellerberg, 1971) of reptiles.

The responses of temperate reptiles to cold temperatures are perhaps the most critical. Variability in spring and fall temperatures can be dramatic and unpredictable, and reptiles, as ectotherms, can easily fall victim to freezing temperatures that prevent locomotion. Physiologists have developed a measure that can be used to examine the cold tolerance of reptiles in detail. The Critical Thermal Minimum (CTMin) is defined as the lowest body temperature at which an animal loses the ability to locomote or to right itself (Jacobson and Whitford, 1970;

Spellerberg, 1971, 1973). It is quite likely that this physiological measure is under selective pressure, since animals unable to locomote will more likely perish than those that can move to warmer surroundings. Vincent and Secoy (1978) found that Thamnophis sirtalis are more cold resistant in very cold years. Other traits are also thought to be shaped by cold environments. Viviparity is common among reptiles found in north temperate zones or high elevations and is believed to be an adaptation to the extremely cold temperatures found in these environments (Hock, 1964). Seasonal changes in reptilian physiology (Aleksiuk, 1971a,b) and thermoregulation (Spellerberg, 1973) are also thought to be adaptations to the thermal environment since reptiles have a low oxidative metabolism relative to endotherms. Lillywhite (1987) reviews several species differences that appear to be related to the geographic ranges of snakes and suggests that thermal tolerances are adaptively determined. The viper, Vipera berus, has a similar northern distribution to T. sirtalis and has been found to be limited by low ground and sub-surface temperatures (Viittanen, 1967). Jacobsen and Whitford (1970) found that the CTMin of Nerodia rhombifera was higher than T. proximus and that the distribution of T. proximus extended farther north.

Thamnophis sirtalis undoubtedly encounters cold temperatures throughout most of its northern range. In Manitoba, some 300 kilometers south of the northern limits of T. sirtalis, the ground is frozen to a depth of 90 cm in January,

surface temperatures are often -40°C or lower (Gartska et al., 1982; Gregory, 1984), and snow cover may last from September through May. The longest period of killing frost-free days ($\leq 1.4^{\circ}\text{C}$) may be as few as 95 (Gregory, 1977) suggesting that this species is frequently exposed to harsh cold temperatures. In the hibernacula, the temperature hovers around -1°C (Lynch, 1983).

Although it is difficult to compare the results of many of the earlier temperature studies due to disparate methodologies, Thamnophis spp. appear to be more tolerant of cold temperatures than many other snakes (Carpenter, 1956; Tables VIIa and b). Thamnophis sirtalis is probably the last snake species to enter hibernation and the first to emerge in populations in both Michigan (Carpenter, 1952) and Kansas (Fitch, 1956). Brown et al. (1974) found a low body temperature of 4.1°C in hibernating T. elegans. Leuth (1941) found that T. radix could survive for more than 24 hours at 1.5°C , while Bailey (1949) suggested that this species can withstand a minimum temperature of approximately -2°C . Fitch (1956) reports finding six garter snakes caught in traps that had survived exposure to -4°C . Stewart (1965) reports CTMins of 3.4°C for T. sirtalis acclimated to 8°C and 5.9°C for snakes acclimated to 32°C . For T. ordinoides, he found CTMins of 3.8°C for 8°C acclimation and 7.0°C for 32°C acclimation. Jacobson and Whitford (1970) found a mean CTMin of 7.8°C in T. proximus when acclimated to 30°C and 4.2°C when acclimated to 15°C , while N. rhombifera has a mean

CTMin of 8.6°C when acclimated to 30°C and 6.0°C when acclimated to 15°C. They also found that the acclimation affected both heart rate and oxygen consumption. Changes in oxygen consumption at cooler temperatures after acclimation have been found in Diadophis punctatus (Buikema and Armitage, 1969), Pituophis catenifer affinis (Greenwald, 1971), T. cyrtopsis (Fleharty, 1963), T. elegans (Fleharty, 1963; Seidel and Lindeborg, 1973), T. ordinoides (Stewart, 1965), T. radix (Seidel and Lindeborg, 1973) and T. sirtalis (Stewart, 1965; Aleksiuk, 1971a). While studying lactate dehydrogenase kinetics, Aleksiuk (1971b) found an enzymatic basis for cold compensation in T. sirtalis.

In lizards, Wilson and Echternacht (1987) found geographic variation in the CTMin of the green anole, Anolis carolinensis, along a latitudinal gradient. The variation in CTMin followed the pattern of minimum ambient temperatures with more cold tolerant lizards being found in colder climates. Spellerberg (1976) found a similar pattern in Lacerta agilis from Europe and England. Studies of population differences in CTMin of snakes are lacking. If, as the evidence I have presented suggests, the CTMin are under selective pressure, population or subspecies differences in such a widely distributed species in CTMin should be expected.

METHODS

During the summer of 1986, 34 female T. sirtalis, collected from two sites (Wayne County, Michigan and the Wolf River drainage, Wisconsin), gave birth to 432 neonates. These animals were maintained at $27 \pm 2^{\circ}\text{C}$. Since feeding can affect the thermal behavior of snakes (Lysenko and Gillis, 1980), the 20-21 day-old neonates had never been fed at the time of testing. Adult females were tested 1-2 months post-partum. As daily variations have been found in CTMins (Spellerberg and Hoffmann, 1972), all animals were tested between 1300 and 1700 EST.

Testing followed the general procedures of Spellerberg (1971, 1973). Snakes were cooled on a plexiglas plate inside a Sears Kenmore 5.1 cu ft freezer until they were unable to right themselves within 30 secs after being placed on their back. From the results of earlier testing, snakes locomote extensively when first placed into the chamber. They lose their locomotory ability before their righting reflex. The snakes were tested by turning them over whenever they ceased movement for 5-10 secs. Usually animals that could right themselves would begin within 5 secs. If no movement occurred within 15 secs, the ventral surface was lightly stimulated with the end of a pen.

Body temperatures were monitored with a Micromite Thermo Electric potentiometer with a resolution of 0.1°C . All tests were made using either a 36 gauge (neonates) or 30 gauge (adult females) copper-constant thermocouple inserted ca 1 cm into the

cloaca and taped to the base of the tail. Care was taken to avoid impeding body movements with the instrumentation. The rate of cooling is known to influence the absolute value of CTMin observed (Spellerberg, 1973). All animals were cooled at an average rate of 2-4°C/min. Although this rate of cooling may seem high for accurate behavioral tests, the cooling rate is much more rapid at the start of the test and falls off as the animal approaches the substrate temperature. As the animal approached its CTMin, its temperature was dropping slowly enough for precise measurements to be recorded. A total of 24 litters (19 Michigan, 5 Wisconsin) comprising 375 neonates were tested.

RESULTS

Repeated measures of additional individuals were used to determine the accuracy of these methods; 22 snakes were each tested a second time (Doughty, unpubl. data). The mean intra-individual difference was 0.096°C (± 0.382 s.e.) with a range from -4.6°C to 3.6°C ($r=0.57$, $P<0.01$).

CTMin was significantly correlated with mass in both populations (Michigan $r=-0.2814$, $P<0.0001$; Wisconsin $r=-0.5233$, $P<0.001$). Other investigators have not found this to be the case. However, most previous studies have tested no more than 30 snakes of a given species. Therefore, in all subsequent tests of CTMin, body mass was used as a covariate.

There was a non-significant difference between the two populations in mean CTMin (Table VIIC, $F_{1,29}=3.55$, $P=0.060$).

Michigan snakes had a mean ($\pm$ s.d.) CTMin of $7.2^{\circ}\text{C} \pm 1.6^{\circ}\text{C}$. Wisconsin snakes, from the colder region, had a mean CTMin of $6.6^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$. There were no sex differences in CTMin (Table VIIc).

Significant age differences were present (Wilcoxon $Z=5.418$, $P<0.0001$). Females had much lower CTMins than their offspring (Table VIIc). This difference is the result of their larger size, for after controlling for differences in mass, the CTMins of neonates and adults are not significantly different ($P=0.6971$).

Litter differences in CTMin are presented in Figure VIIa. These differences are significant, even after removing the litter effects of body mass (Student's $t=4.87$, $p<0.0001$).

Variation in CTMin is heritable in both populations (Table VIIId). After removing litters with multiple paternity, the estimate of heritability in CTMin is still significant in both populations, though lower in the Wisconsin population. Using all litters to increase the sample size, sex differences in heritability were also investigated. There were no sex differences in the CTMin of either the Michigan (males: $h^2=0.700 \pm 0.177$; females: $h^2=0.409 \pm 0.167$) or in the Wisconsin (males: $h^2=0.758 \pm 0.452$; females: $h^2=0.771 \pm 0.654$) neonates.

Likewise, paternity did not appear to affect the amount of variability in CTMin. In multiple paternity litters, the mean $CV=15.52$; in single paternity litters, the mean $CV=16.31$.

DISCUSSION

Body mass was found to be correlated with CTMin in Thamnophis sirtalis. Although other researchers have not found this result, Heatwole et al. (1969) found suggestive evidence in a study in which they found strong species differences in the ability of several tropical anoline lizards to survive cold temperatures. Within a given species of Anolis, low temperature tolerance was strongly correlated with body size. This relationship was not simply related to body mass and cooling time, and is quite similar to my finding. Webb and Heatwole (1971) also found that the circulatory system of snakes can greatly affect the distribution of heat within an animal. Head and body temperatures deviate by as much as 7°C (Strelnikov, 1944 cited in Webb and Heatwole, 1971), while the difference between surface and core temperatures of the head may reach 15°C (Webb and Heatwole, 1971).

The Wisconsin T. sirtalis population comes from an area that averages 3°C cooler throughout the year than the Michigan population (Table VIIe). Snakes from Michigan are exposed to a mean of 184 days of frost, while snakes from the Wisconsin area face 204 days of frost. The mean last day of frost in Michigan is April 25, while the mean first day of frost in the fall is October 23. In Wisconsin the mean last frost is May 6, while the mean first day occurs on October 13. Thus, on average, snakes in Michigan have 11 more frost free days in the spring,

and 10 extra frost free days in the fall than Wisconsin snakes. These results tend to support the hypothesis of Kitchell (1969) who suggested that the different temperature relationships and behavior of snakes may be correlated to their distribution and that these differences may be adaptive. Because of the considerable variation in CTMin found in these animals, a greater number of litters need to be collected to test whether populations differ in their mean CTMin.

Gibson and Falls (1979) found significant body temperature differences between male and female T. sirtalis captured in the field. I did not find sex differences in the CTMin of neonatal animals from Michigan or Wisconsin populations. Hirth and King (1969) found seasonal differences in preferred body temperatures in Crotalis viridis. They also noted a difference of 2°C in preferred body temperatures of gravid females during the spring and summer. Thus, the sex differences found in the field may be the result of acclimation differences caused by sex differences in thermal history.

To my knowledge this is the first report of heritable variation in CTMin in any animal. Additional data on the clinal variation in the CTMin of Thamnophis sirtalis need to be collected. Studies of the selection on this trait should also be investigated as it provides a unique opportunity for examining the mechanisms of evolution.

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PART VII

APPENDIX

Table VIIa. Voluntary minimum temperatures of various snake species.

Species	Temperature	Authority
Acrochordidae		
<u>Acrochordus arafurae</u>	24	11
Boidae		
<u>Boa constrictor</u>	21	12, 13
<u>Charina bottae</u>	12.2	7
<u>Python spilotes</u>	25	14, 15, 16
Colubridae		
<u>Arizona elegans</u>	15.0	7
<u>Carphophis amoenus</u>	14	17
<u>Chionactis occipitalis</u>	17.8	7
<u>Coluber constrictor</u>	15.0	7
<u>Contia tenuis</u>	16.0	7
<u>Coronella austriaca</u>	10	18
<u>Diadophis amabilis</u>	18.2	7
<u>D. punctatus</u>	11.7	7
<u>Elaphe obseleta</u>	18.2	7
<u>Haldea valeriae</u>	23.5	7
<u>Heterodon platyrhinos</u>	22.0	10
<u>H. nasicus</u>	21	10
<u>Hypsiglena torquata</u>	27.0	7
<u>Lampropeltis calligaster</u>	30.4	8
<u>L. doliata</u>	22.3	7
<u>L. getulus</u>	15.1	7
<u>Masticophis anthonyi</u>	25.2	9
<u>M. flagellum</u>	24.0	7
<u>M. lateralis</u>	28.0	7
<u>M. taeniatus</u>	18	19
<u>Nerodia erythrogaster</u>	24	20, 21
<u>Nerodia rhombifera</u>	25.8	7
<u>N. sipedon</u>	16.0	7
<u>Opheodrys vernalis</u>	18.0	7
<u>Phyllorhynchus decurtatus</u>	24	22
<u>Pituophis catenifer</u>	16.4	7, 23, 24
<u>Rhinocheilus lecontei</u>	18.0	7
<u>Salvadora hexalepis</u>	30	25
<u>Spalerosophis cliffordi</u>	20	26
<u>Storeria dekayi</u>	23.8	7
<u>S. occipitomaculata</u>	24.3	7
<u>Tantilla gracilis</u>	20	17
<u>Thamnophis ordinoides</u>	12.6	7
<u>T. sirtalis</u>	9.0	7
<u>T. cyrtopsis</u>	22	27
<u>T. sauritus</u>	12.6	7
<u>T. butleri</u>	12.4	7
<u>T. elegans</u>	14.2	7
<u>T. radix</u>	21	28
<u>T. rufipunctatus</u>	20	27
<u>Toluca lineata</u>	20.0	7
<u>Tropidoclonion lineatum</u>	19	17
<u>Uromacer catesbyi</u>	28	29
<u>U. oxyrhynchus</u>	28	29
Elapidae		
<u>Acanthophis antarcticus</u>	27	30
<u>Austrelaps superbus</u>	26	30, 31, 32
<u>Notechis scutatus</u>	23	30, 31, 32
<u>Oxyuranus scutellatus</u>	39	39
<u>Pseudechis porphyriacus</u>	26	40, 31, 32
<u>Pseudonaja nuchalis</u>	30	32
<u>P. textilis</u>	29	30, 32
<u>Unechis flagellum</u>	24	32

Table VIIa (cont.).

Species	Temperature	Authority
Leptotyphlopidae		
<u>Leptotyphlops dulcis</u>	16	17
Viperidae		
<u>Aqkistrodon contortrix</u>	17.5	7
<u>A. piscivorus</u>	24.6	7
<u>Crotalus atrox</u>	18.0	7
<u>C. cerates</u>	14	22, 33, 34
<u>C. horridus</u>	21.2	7
<u>C. mitchelli</u>	19	34
<u>C. pricei</u>	18.0	7
<u>C. scutulatus</u>	22.2	7
<u>C. viridis</u>	15	19
<u>Vipera aspis</u>	16?	35, 36, 37
<u>V. ammodytes</u>	16?	35, 36
<u>V. berus</u>	14?	35, 36, 38
<u>V. latastei</u>	16?	36
<u>V. seoanei</u>	16?	35, 36
<u>V. ursinii</u>	16?	35, 36, 38

- | | |
|-------------------------------|--------------------------------------|
| 1- Spellerberg, 1976 | 21- Mushinsky et al. 1980 |
| 2- Spellerberg, 1972 | 22- Cowles & Bogert 1944 |
| 3- Lueth, 1941 | 23- Cunningham 1966 |
| 4- Carpenter, 1953 | 24- Dill 1972 |
| 5- Bailey, 1949 | 25- Jacobsen & Whitford 1971 |
| 6- This study | 26- Dmi'el & Borut 1972 |
| 7- Brattstrom, 1965 | 27- Fleharty 1967 |
| 8- Fitch, 1956 | 28- Hart, 1979 |
| 9- Brattstrom, 1965 | 29- Henderson 1982 |
| 10- Platt, 1969 | 30- Lillywhite 1980 |
| 11- Shine and Lambeck (1985) | 31- Shine 1979 |
| 12- McGinnis & Moore 1969 | 32- Heatwole 1976 |
| 13- Montgomery & Rand 1978 | 33- Mosauer 1936 |
| 14- Cogger & Holmes 1960 | 34- Moore 1978 |
| 15- Webb & Heatwole 1971 | 35- Saint Girons 1975 |
| 16- Johnson 1972 | 36- Saint Girons 1978 |
| 17- Clark 1967 | 37- Naulleau 1979 |
| 18- Spellerberg & Phelps 1975 | 38- Saint Girons & Saint Girons 1956 |
| 19- Hirth & King 1969 | 39- Johnson 1975 |
| 20- Gehrmann 1971 | 40- Heatwole & Johnson 1979 |

Table VIIb. Cold temperature relations of various snake species.

Species	Temperature	Authority
Colubridae		
<u>Arizona elegans</u>	-3.0	7
<u>Coronella a. austriaca</u>	3.5	1
<u>C. girondica</u>	5.0	1
<u>Heterodon platyrhinos</u>	7.0	9
<u>H. nasicus</u>	7.0	9
<u>Lampropeltis getulus</u>	-2.0	7
<u>Masticophis flagellum</u>	-3.0	7
<u>Natrix maura</u>	4.0	1
<u>N. natrix helvetica</u>	3.9	1
<u>N. natrix natrix</u>	3.4	1
<u>N. tessellata</u>	6.5	1
<u>Nerodia sipedon</u>	> 1.5	3
<u>N. rhombifera</u>	6.0	8
<u>N. rhombifera</u>	8.6	8
<u>Pituophis catenifer</u>	-3.0	7
<u>Rhinocheilus lecontei</u>	-2.0	7
<u>Salvadora hexalepis</u>	7	10
<u>Thamnophis radix</u>	> 1.5	3
<u>T. radix</u>	> -2.0	5
<u>T. radix</u>	-2.0	7
<u>T. proximus</u>	4.2	8
<u>T. proximus</u>	7.8	8
<u>T. ordinoides</u>	4	11
<u>T. sirtalis concinnus</u>	4	11
<u>T. sirtalis</u>	-2.0	7
<u>T. sirtalis</u>	> 1.5	4
<u>T. sirtalis</u>	4.4	6
<u>T. sirtalis neonates</u>	7.3	6
Elapidae		
<u>Austrelaps superbus</u>	1-3.0	1,2,12
<u>Notechis scutatus</u>	1-2.2	2,12,13
<u>Pseudonaja nuchalis</u>	7	12
<u>Pseudonaja textilis</u>	3.5	2,12,13
<u>Suta flagellum</u>	3.0	2,13
<u>Suta flagellum</u>	-4.2	2
Viperidae		
<u>Agkistrodon contortrix</u>	4-9	14,15
<u>Crotalis cerastes</u>	-2.0	7
<u>C. mitchelli</u>	-2.0	7
<u>Vipera aspis</u>	5-6	16,17
<u>Vipera berus</u>	3.0	1
<u>Vipera ursinii</u>	4-5	16,18

- | | |
|------------------------------|---------------------------------------|
| 1- Spellerberg, 1976 | 10- Jacobson & Whitford, 1971 |
| 2- Spellerberg, 1972a | 11- Stewart, 1965 |
| 3- Lueth, 1941 | 12- Lillywhite, 1980 |
| 4- Carpenter, 1953 | 13- Heatwole, 1976 |
| 5- Bailey, 1949 | 14- Fitch, 1960 |
| 6- This study | 15- Sanders & Jacob, 1981 |
| 7- Brattstrom, 1965 | 16- Saint Girons, 1975, 1978 |
| 8- Jacobson & Whitford, 1970 | 17- Naulleau, 1979 |
| 9- Platt, 1969 | 18- Saint Girons & Saint Girons, 1956 |

Table VIIc. Population, sex and age differences in two populations of neonate Thamnophis sirtalis in Critical Thermal Minima (CTMin °C).

Contrast	N	CTMin $\pm$ (s.d.)
Population (NS)		
Michigan	355	7.2 $\pm$ 1.6
Wisconsin	44	6.6 $\pm$ 1.5
Age (NS w/ mass as covariate)		
Offspring	375	7.3 $\pm$ 1.4
Females	24	4.4 $\pm$ 1.7
Sex (NS)		
Male		7.4 $\pm$ 1.5
Female		7.3 $\pm$ 1.3

Table VIId. Full-sibling heritabilities of two populations of neonate Thamnophis sirtalis in Critical Thermal Minima.

Population	N	Heritability ¹
All Litters		
Michigan	340	0.564 $\pm$ 0.072**
Wisconsin	52	0.776 $\pm$ 0.198**
Single Paternity		
Michigan	79	0.873 $\pm$ 0.136**
Wisconsin	35	0.458 $\pm$ 0.230*
Multiple Paternity		
Michigan	261	0.439 $\pm$ 0.162**

1 - Tests of significance of $h^2 > 0$ performed by analysis of variance among and within litters:
 * = $P < 0.05$, ** = $P < 0.01$.

Table VIIe. Annual temperatures at Michigan and Wisconsin Thamnophis sirtalis collection sites.¹

		Detroit Michigan	Green Bay Wisconsin
January	Mean Temp. (°C)	-3	-8
	Mean Max.	1	-4
	Mean Min.	-7	-13
	Extreme Max.	19	13
	Extreme Min.	-27	-38
	Days Max. $\leq 0^{\circ}\text{C}$	18	21
	Min. $\leq 0^{\circ}\text{C}$	29	30
April	Mean Temp. (°C)	9	6
	Mean Max.	14	11
	Mean Min.	3	1
	Extreme Max.	31	29
	Extreme Min.	-13	-14
	Days Max. $\leq 0^{\circ}\text{C}$	0.5	7
	Min. $\leq 0^{\circ}\text{C}$	10	24
July	Mean Temp. (°C)	23	22
	Mean Max.	30	28
	Mean Min.	17	15
	Extreme Max.	40	40
	Extreme Min.	9	6
	Days Max. $\leq 0^{\circ}\text{C}$	0	0
	Min. $\leq 0^{\circ}\text{C}$	0	1
October	Mean Temp. (°C)	12	9
	Mean Max.	18	16
	Mean Min.	6	4
	Extreme Max.	32	29
	Extreme Min.	-6	-13
	Days Max. $\leq 0^{\circ}\text{C}$	0	0
	Min. $\leq 0^{\circ}\text{C}$	5	4

1- From 1986 United States National Oceanographic and Atmospheric Administration EDIS, National Climatological Center, Ashville, North Carolina.

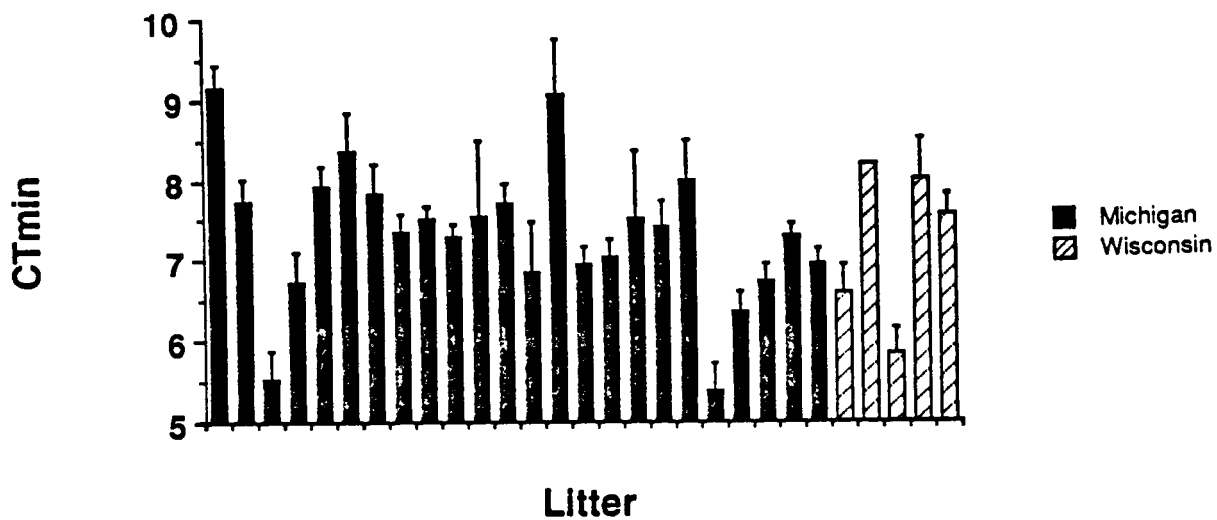


Figure VIIa. Litter comparisons of neonate *Thamnophis sirtalis* from two populations in mean Critical Thermal Minima (CTMin $\pm$ SE °C).

PART VIII

CORRELATIONS

INTRODUCTION

In addition to polygenic effects, genes affecting quantitative traits can also have pleiotropic effects; that is, a single gene can affect several different characters. Such pleiotropic effects will lead to genetic correlations between these traits (Bulmer, 1980).

Correlated characters are of great interest to evolutionary biologists since selection on one trait will bring about changes in the distribution of the correlated character (Lande, 1979; Falconer, 1981; Cheverud, 1982, 1984). Linkage disequilibrium can likewise result in genetic correlations, but unlike the former case, such correlations rapidly break down with recombination unless very tightly linked. In populations at equilibrium under stabilizing selection, therefore, the principle cause of genetic correlations is pleiotropy (Futuyma, 1986). Considerable genetic variation and high heritabilities can be maintained by these pleiotropic effects of genes.

METHODS

Phenotypic correlations between traits will be the result of both genetic and environmental factors (Manly, 1985). The phenotypic correlation (r_p) is equal to the phenotypic covariance (cov_p) divided by the square root of the product of the phenotypic variance in trait X (var_{pX}) and the phenotypic variance in trait Y (var_{pY}).

$$r_P = \text{cov}_P / \sqrt{(\text{var}_{PX} \text{var}_{PY})}$$

The phenotypic covariance can be partitioned into the additive genetic covariance (cov_A) and the environmental covariance (cov_E).

$$\text{cov}_P = \text{cov}_A + \text{cov}_E$$

Like "narrow sense" heritabilities, the environmental covariance includes non-additive genetic components. Substituting the correlations and standard deviations for the covariances yields:

$$r_P \sqrt{\text{var}_{PX} \text{var}_{PY}} = r_A \sqrt{\text{var}_{AX} \text{var}_{AY}} + r_E \sqrt{\text{var}_{EX} \text{var}_{EY}}$$

Since $\text{var}_A = h^2 \text{var}_P$ and $\text{var}_E = e^2 \text{var}_P$ (where $e^2 = h^2 + 1$) with a little algebra, this leads to

$$r_P = h_X h_Y r_A + e_X e_Y r_E$$

The genetic correlation is a measure of the extent that two characters are affected by the same genes. It is defined as

$$r_A = \text{cov}_{XY} / \sqrt{(\text{var}_X \text{var}_Y)}$$

where cov and var are the covariance and variance components of the traits X and Y respectively.

Genetic correlations can be estimated from sibling analyses. Full-sibling analyses include effects due to maternal and dominance effects. Both approaches assume: 1) the genotype and environment are randomly associated and additive in their

effects, and 2) the population under consideration is assumed to mate randomly with equal viability and survival of all genotypes (Kempthorne, 1957).

Phenotypic correlations and their standard errors were calculated using SAS regression procedure (PROC REG; SAS Institute, Inc., 1985). Genetic correlations were determined using the SAS hierarchical analysis of variance procedure (PROC NESTED). Standard errors for the genetic correlations were calculated using the formulas in Falconer (1981). Cheverud (1988) argues that phenotypic correlations should be computed when possible because they are good estimates of the underlying genetic correlations and have the advantage of being easier to calculate and are statistically more powerful. The magnitudes of the phenotypic and genetic correlation matrices are compared using an average correlation (the sum of the squared correlations), while the patterns of the matrices are compared by computing the correlation between the phenotypic and genotypic correlation matrix elements (Cheverud, 1988). The effective sample size (N_{es}) for these comparisons is given as the product of the geometric mean heritability and the number of families.

RESULTS

Correlations for the various characters measured in this study are presented in Tables VIIIA and b. For each population, 66 phenotypic and genotypic correlations were computed (The

genetic correlations of nearest neighbor distances in the Wisconsin population were not calculated as the heritability of this trait is negative, Part III). At $\alpha=0.05$, one out of 20 will be significant by chance alone; therefore, for further comparisons alpha will be reduced to 0.001. Many of the significant phenotypic correlations in the Michigan population are easily explained. For instance, it is no surprise that measures of size (SVL, TL) are correlated with mass. Measures of length (SVL, TL) are correlated with the number of scales (ventral and subcaudal scales, respectively) covering that part of the body. Mass was found to be strongly correlated with CTMin (Part VII), and so are SVL and TL. In the Wisconsin population, the phenotypic measures of size are significantly correlated (mass with SVL and TL). The only measures of length that are correlated are TL and the number of subcaudals. As in the Michigan population, mass and CTMin are phenotypically correlated.

Many of the genetic correlations are significantly different from zero. Some are positive like the genetic correlation between the number of ventral and subcaudal scales indicating that selection for an increase in the mean of either trait will result in selection for an increase in the mean of the other trait as well. Antipredator behavior is negatively correlated with chemical prey preferences in both populations. This result supports the hypothesis (Burghardt, 1970) that attacks to prey extracts are "ingestive" rather than defensive

in nature.

The genetic and phenotypic correlations are remarkably similar given the small effective sample sizes. The difference between the genetic and phenotypic correlation matrices is smaller in the Michigan (mean genetic correlation=0.1725, phenotypic=0.0757, difference=0.0968, N_{es} =8.8) than in the Wisconsin population (mean genetic correlation=0.5906, phenotypic=0.1487, difference=0.4419, N_{es} =2.5). The genetic correlations tend to be higher, and indication of their lower precision (Cheverud, 1988). The correlation between the genetic and phenotypic correlation matrices is 0.73 (Pearson's r) for both populations. These values suggest that, at least for the Michigan population, the genetic correlation matrix is fairly accurate. The high consistency between the phenotypic and genetic correlation matrices suggests that the phenotypic matrix gives a good indication of the underlying genetic correlations. However, when possible, rather than rely on inferences drawn from phenotypic correlations, genetic correlations should be computed as there is no causal association between the matrices. For instance, in this study there is highly significant genetic correlation between mass and the number of ventral scales in both populations, but no significant phenotypic correlation.

Likewise some of the genetic and phenotypic correlations are opposite in sign in the Wisconsin population. For instance,

antipredator behavior and both SVL and TL, and the number of ventral scales and TL have genetic and phenotypic correlations opposite in sign. Thus, although phenotypically tail length and the number of ventral scales are positively correlated, selection on either one of these traits for an increase in the mean of the trait will select for a decrease the the mean of the other trait.

There are also differences between the two populations. The genetic correlations between mass with SVL and TL are opposite in sign. Wisconsin snakes are typically thinner for a given SVL than are Michigan animals (see Part I) and this may explain this result.

DISCUSSION

There are many significant genetic correlations among these traits in both populations of garter snakes. The negative correlations, such as between antipredator behavior and the response to chemical prey extracts, will resist selection on those traits if both contribute to fitness. The substantial variability in these traits and their high heritabilities can, in part, be explained by the correlations between these traits. Future studies of selection will need to consider genetic correlations. Phenotypic correlations, in general, give a good approximation of the underlying genetic correlation.

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PART VIII

APPENDIX

Table VIIIa. Genetic and phenotypic correlations among traits in neonate *Thamnophis sirtalis* from Michigan.

Michigan*

	Ventral	Subcaudal	Mass	Antipred.	CTMin	Chemical	SVL	TL	Fish	Worm	Water	NND
Ventral		0.4464	0.5354	-0.124	-0.6224	0.364	0.4594	0.4202	0.3771	0.127	0.4742	-0.136
Subcaudal	0.4454		0.175	-0.169	-0.4092	0.326	-0.240	0.3731	0.248	0.110	0.5583	-0.210
Mass	0.2764	0.082		0.017	-0.4344	0.210	0.7464	0.7804	0.103	0.149	0.3512	0.225
Antipred.	-0.060	-0.1261	0.041		0.289	-0.6544	0.107	-0.080	-0.5954	-0.5613	-0.3951	0.257
CTMin	-0.1662	-0.1281	-0.2814	0.1181		-0.203	-0.4604	-0.8044	0.075	-0.143	-0.6144	0.037
Chemical	0.023	0.004	0.1381	-0.1181	-0.1321		0.137	0.212	0.7894	0.8734	0.7654	0.304
SVL	0.2794	-0.041	0.7444	0.043	-0.2484	0.1722		0.6444	0.160	-0.033	0.292	-0.275
TL	0.3624	0.5574	0.4474	-0.104	-0.2814	0.009	0.4604		-0.028	0.008	0.7614	-0.286
Fish	-0.011	-0.020	0.096	-0.1301	-0.073	0.8154	0.1632	-0.024		0.4681	0.4181	0.182
Worm	-0.021	-0.025	0.1071	-0.079	-0.067	0.8504	0.096	-0.020	0.4914		0.5913	0.237
Water	0.1301	0.083	0.1422	-0.069	-0.2003	0.7094	0.1723	0.097	0.4224	0.4394		0.239
NND	0.038	0.011	-0.038	0.008	-0.009	0.039	-0.018	-0.057	0.017	0.017	0.071	

* - Genetic correlations are given above the diagonal, phenotypic correlations below. 1 - $P < 0.05$, 2 - $P < 0.01$, 3 - $P < 0.001$, 4 - $P < 0.0001$. Traits include the number of ventral and subcaudal scales, mass, total anti-predator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

Table VIIb. Genetic and phenotypic correlations among traits in neonate *Thamnophis sirtalis* from Wisconsin.

Wisconsin*

	Ventral	Subcaudal	Mass	Antipred.	CTMin	Chemical	SVL	TL	Fish	Worm	Water	NND
Ventral		0.9894	-0.5523	0.356	0.5021	0.537	-0.4101	-0.289	0.162	0.6582	0.7994	-
Subcaudal	0.7084		-0.271	-0.175	-0.059	1.1804	-0.110	-0.076	0.7311	1.4474	1.3224	-
Mass	-0.124	0.023		-0.426	-0.9974	0.4951	0.9994	1.0184	1.0514	0.247	0.290	-
Antipred.	-0.077	0.030	0.043		0.8294	-0.470	-0.293	-1.1714	-0.459	-0.531	-0.223	-
CTMin	0.253	0.125	-0.5233	0.267		-0.554	-1.0054	-1.2084	-0.8424	-0.453	-0.422	-
Chemical	0.233	0.205	0.3601	-0.177	-0.4082		0.716	0.8674	0.8474	0.9954	0.9454	-
SVL	0.068	-0.3791	0.9004	0.091	-0.3791	0.301		1.0194	1.2554	0.4861	0.5141	-
TL	0.262	0.6164	0.5774	0.115	-0.194	0.238	0.6694		1.2414	0.6972	0.7652	-
Fish	0.066	0.047	0.3881	-0.090	-0.3391	0.8314	0.3461	0.216		0.512	0.6882	-
Worm	0.176	0.138	0.216	-0.221	-0.3691	0.8744	0.128	0.134	0.5544		1.2184	-
Water	0.3871	0.3891	0.265	-0.109	-0.248	0.6754	0.276	0.250	0.3411	0.4802		-
NND	0.056	-0.005	-0.156	-0.148	-0.113	-0.269	-0.196	-0.108	-0.204	-0.3591	-0.016	

* - Genetic correlations are given above the diagonal, phenotypic correlations below. 1 - $P < 0.05$, 2 - $P < 0.01$, 3 - $P < 0.001$, 4 - $P < 0.0001$. Traits include the number of ventral and subcaudal scales, mass, total anti-predator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

PART IX

CONCLUSIONS

MULTIPLE PATERNITY

These studies of multiple paternity and offspring variability in litters of Thamnophis sirtalis demonstrate that contrary to the earlier predictions based on physiology and behavioral observations, high levels of multiple paternity occur in natural populations of garter snakes. The mechanisms developed by males to prevent multiple matings by females are ineffective in this species and should not be used as evidence that multiple paternity does not exist in a population.

Litter size was correlated with the number of loci at which multiple paternity was detected. Larger litters were nearly all multiply sired, while all but one litter of less than 13 individuals was singly sired. Although the probability of detecting multiple paternity when it exists decreases with decreasing sample size, there was a significant difference in the occurrence of multiple paternity between large and small litters. These results suggest several future lines of investigation into the dynamics of sexual selection in this species. First, the electrophoretic data yield no information about sperm precedence. Such information will be important in determining optimal mating behavior "strategies" males would be predicted to follow. Second, the hypothesis of sperm depletion needs to be tested. If male sperm supplies are not always sufficient to fertilize an entire female's litter, female choice will be altered. The observed pattern of multiple paternity may

also reflect mating preferences of these animals.

COEFFICIENT OF VARIATION

It does not appear that multiple sires increase the amount of variation within the offspring of a litter. A comparison of the mean coefficients of variation in litters of single and multiple paternity litters reveal that multiple sired litters had greater variation for only 6 of 12 traits (Table IXa). The mean difference was 4.0, but this is due to the large magnitude of the difference in the coefficient of variation in antipredator behavior. The mean difference for the other 11 traits is -0.1.

Several caveats need to be mentioned. First, the various traits may have been measured with more or less precision. Second, the traits considered may not be the ones under the strongest selective pressure. Third, some of the single paternity litters probably had multiple sires reducing the power of the comparisons.

Nonetheless, while variation was considerably higher for some traits (e.g. antipredator scores) in multiple paternity litters, in general, variation appears to be as great in singly sired litters. Ultimate questions regarding the reasons for multiple paternity remain unanswered.

VARIATION

Significant litter and population differences were found

in various scale counts, measures of aggregation behavior, responses to chemical prey extracts, and antipredator behavior. Litter differences result primarily from the heritable nature of the traits. Additional studies need to be completed to determine the extent and source of these population differences throughout the range of this species. Future species comparisons must include information from several litters within populations and several populations within the species.

Age differences were found for some traits (e.g. preocular and supralabial scale counts, chemical prey preferences). For traits that are invariant with age (such as scale counts), selection may account for the observed differences. Other traits (like the chemical prey preferences and antipredator behavior) are known to have experiential effects. A few studies have examined the development of these behaviors in Thamnophis sirtalis, but more need to be done. It would be particularly interesting to see whether variation in developmental changes have a significant heritable component.

HERITABILITIES

A comparison of heritabilities calculated for a number of different characters is given in Tables IXb and c. Quantitative genetic studies that assume single paternity within litters, but which are based on the inclusion of litters of multiple paternity, clearly underestimate the true heritabilities. In 17 of the 22 comparisons, the heritability computed for single

paternity litters is higher than for multiply sired litters. The theoretic bias in the estimate of heritability obtained by including multiple paternity litters in the analysis is not great. Assuming that 72% of the litters were multiply sired with an average relationship of 0.39, heritabilities and their standard errors will increase by a factor of 1.35x. This is not a large change, but it could be the difference between a significant and a non-significant result.

Maternal and dominance effects for most characters have not been examined. Since full-sibling heritabilities were calculated, these values should be treated as upper limits to the real heritability. Heritability values, in theory, range from 0 to 1. In some cases, the reported heritabilities exceed 1.0. Since the real value can be no greater than 1.0, the excess can give an indication of the precision of these estimates.

There did not appear to be any consistent differences between the heritability of traits in males and females in either population (Tables IXd and e). This suggests that the metric variation in these characters is the result of the simultaneous segregation of multiple autosomal genes.

This is one of the few studies to examine the genetic basis of thermal relations in animals. Significant heritable variation in the thermal behavior in both populations was found. The significant heritability in nearest neighbor distances in the aggregation studies suggests that the social behavior of

this species has a significant genetic component. Selection, then given the proper conditions, could increase or decrease the gregariousness of this species. Considerable heritable variation in scale numbers, antipredator behavior, response to chemical prey extracts, antipredator behavior, and susceptibility to cold temperatures was documented providing the basis for future studies on the action of natural selection on these traits.

CORRELATIONS

Significant genetic correlations exist among many of the traits examined. The negative correlations, such as between antipredator behavior and the response to chemical prey extracts, will resist selection on those traits if both contribute to fitness. The substantial variability in these traits and their high heritabilities can, in part, be explained by the correlations between these traits.

A comparison of the phenotypic and genetic correlations indicates that for many traits, phenotypic values give a good indication of the underlying genetic relationships between the traits. However, for some traits (e.g. CTMin and antipredator behavior in both populations, and TL and subcaudal scales in Wisconsin snakes), genetic and phenotypic correlations are quite different. Thus, whenever possible, both phenotypic and genetic correlations should be calculated.

The demonstration of significant genetic variation and

covariation among traits provides necessary information for construction of optimality models. Biologically relevant assumptions of the tradeoffs between traits can be incorporated resulting in better models and more valid tests of evolutionary theory.

PART IX

APPENDIX

Table IXa. Comparison of mean coefficients of variation in litters of multiple and single paternity in Thamnophis sirtalis from Michigan and Wisconsin.¹

	Ventral	Subcaudal	Mass	Antipred.	CTMin	Chemical	Fish	Worm	Water	SVL	TL	NND	Mean
Multiple Paternity	2.3	9.3	8.4	186.2	15.5	34.1	44.5	42.8	34.1	4.8	8.7	25.5	34.7
Single Paternity	2.4	10.9	8.9	136.5	16.3	33.8	40.0	47.3	33.8	4.9	8.5	24.8	30.7
Difference	-	-	-	+	-	+	+	-	+	-	+	+	4.0

1 - Comparisons include the number of ventral and subcaudal scales, mass, total antipredator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

Table IXb. Comparison of full-sibling heritabilities calculated using litters of single and multiple paternity in Thamnophis sirtalis from Michigan.

Michigan¹

	Ventral	Subcaudal	Mass	Antipred.	CTMin	Chemical	Fish	Worm	Water	SVL	TL	NND	Mean
Single Paternity	0.534	0.409	1.361	0.949	0.873	0.293	0.300	0.702	0.040	1.252	0.909	0.760	0.698
Multiple Paternity	0.671	0.629	1.348	0.558	0.439	0.241	0.243	0.084	0.262	0.998	0.290	0.122	0.491
Difference	-	-	+	+	+	+	+	+	-	+	+	+	0.207

1 - Comparisons include the number of ventral and subcaudal scales, mass, total antipredator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

Table IXc. Comparison of full-sibling heritabilities calculated using litters of single and mixed paternity in Thamnophis sirtalis from Wisconsin.

Wisconsin¹

	Ventral	Subcaudal	Mass	Antipred.	CTMin	Chemical	Fish	Worm	Water	SVL	TL	NND	Mean
Single Paternity	0.781	0.936	1.448	0.272	0.458	0.689	0.762	0.458	0.532	1.302	1.064	0.087	0.732
Mixed Paternity	0.902	0.334	1.442	0.103	0.776	0.503	0.521	0.347	0.378	1.242	0.609	0.064	0.602
Difference	-	+	+	+	-	+	+	+	+	+	+	+	0.130

1 - Comparisons include the number of ventral and subcaudal scales, mass, total antipredator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

Table IXd. Comparison of full-sibling heritabilities calculated separately for male and female neonates in litters of Thamnophis sirtalis from Michigan.

Michigan¹

	N	Ventral	Subcaudal	Mass	Antipred.	CTMin	Chemical	Fish	Worm	Water	SVL	TL	NND	Mean
Males		0.788	0.669	1.378	0.675	0.700	0.168	0.164	0.270	0.256	1.162	0.290	0.424	0.579
Females		0.624	0.939	1.290	0.795	0.409	0.303	0.385	0.277	0.162	1.055	0.558	0.184	0.582
Difference	+	-	-	+	-	+	-	-	-	+	+	-	+	-0.003

1 - Comparisons include the number of ventral and subcaudal scales, mass, total antipredator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

Table IXe. Comparison of full-sibling heritabilities calculated separately for male and female neonates in litters of Thamnophis sirtalis from Wisconsin.

Wisconsin¹

	N	Ventral	Subcaudal	Mass	Antipred.	CTMin.	Chemical	Fish	Worm	Water	SVL	TL	NND	Mean
Males	0.658	0.415	1.585	-0.144	0.758	0.746	0.332	0.473	0.351	1.463	0.609	-0.197	0.587	
Females	1.477	1.110	1.057	0.666	0.771	0.863	0.330	0.642	0.985	1.046	-0.592	-0.624	0.644	
Difference	-	-	+	-	-	-	+	-	-	-	+	+	+	-0.057

1 - Comparisons include the number of ventral and subcaudal scales, mass, total antipredator behavior score, CTMin temperature, total tongue-flick attack score to fish and worm prey extracts and water controls plus the total response to these three stimuli (Chemical), snout vent length (SVL) tail length (TL), and nearest neighbor distance (NND) during aggregation studies.

APPENDIX

Descriptive statistics for mass in litters of Thamnophis sirtalis.

Mass (gm)						
Litter	N	Mean	+SD	Min	Max	C.V.
1501	15	0.85	0.07	0.7	1.0	8.6
1502	19	0.76	0.06	0.6	0.8	8.2
1504	12	0.96	0.07	0.8	1.1	7.8
1505	10	0.98	0.06	0.6	0.8	8.2
1509	12	0.84	0.11	0.6	1.0	12.8
1510	25	0.73	0.05	0.6	0.8	6.4
1511	6	0.92	0.03	0.9	1.0	4.0
1512	13	0.96	0.10	0.7	1.1	10.7
1513	37	0.84	0.07	0.6	1.0	8.6
1514	15	1.09	0.15	0.8	1.3	13.8
1515	0	-	-	-	-	-
1516	6	0.85	0.06	0.8	1.0	7.3
1517	7	0.78	0.04	0.7	0.8	5.5
1519	13	0.88	0.12	0.7	1.0	14.0
1520	20	0.92	0.03	0.9	1.0	3.0
1523	23	1.12	0.1	0.8	1.2	8.9
1524	3					
1526	5	0.71	0.03	0.7	0.7	4.3
1529	6	1.23	0.06	1.1	1.3	5.1
1534	9	1.04	0.06	0.9	1.1	6.1
1536	7	0.83	0.21	0.6	1.1	25.2
1537	18	0.94	0.07	0.8	1.0	8.0
1538	40	0.88	0.06	0.7	1.0	7.4
1539	17	0.99	0.06	0.9	1.1	6.3
1548	10	0.82	0.03	0.8	0.9	3.6
1556	1	0.52	-	0.5	0.5	-
1558	17	0.86	0.06	0.7	0.9	7.5
1562	7	0.64	0.09	0.5	0.8	14.6
1565	5	0.80	0.08	0.7	0.9	10.1

Descriptive statistics for snout vent length in litters of Thamnophis sirtalis.

Snout Vent Length (mm)						
Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	15	155.1	5.8	143	163	3.7
1502	23	143.1	11.0	109	156	7.7
1504	12	162.3	4.5	154	168	2.8
1505	11	153.2	8.6	131	165	5.6
1509	12	147.8	9.5	133	161	6.4
1510	25	139.5	4.5	128	146	3.2
1511	6	159.8	6.7	148	168	4.2
1512	13	150.9	9.1	131	164	6.0
1513	37	142.6	5.2	129	153	3.7
1514	15	166.2	9.1	148	181	5.5
1515	2	144.5	13.4	135	154	9.3
1516	6	141.0	6.5	132	152	4.6
1517	11	147.0	6.2	136	156	4.2
1519	13	150.6	7.8	138	162	5.2
1520	21	151.8	3.8	145	161	2.5
1523	23	151.4	7.9	130	162	5.2
1524	4	144.2	4.8	137	147	3.4
1526	5	144.0	1.9	142	146	1.3
1529	6	165.5	6.8	160	177	4.1
1534	9	170.9	8.8	153	186	5.1
1536	7	144.0	14.0	129	167	9.8
1537	18	151.9	7.4	136	162	4.9
1538	40	152.2	5.1	141	164	3.3
1539	17	158.8	3.7	151	164	2.3
1548	10	151.0	5.3	139	157	3.5
1556	1	124.0	-	124	124	-
1558	17	151.5	6.5	134	161	4.3
1562	7	131.7	8.1	122	159	6.1
1565	5	146.4	11.5	131	163	7.8

Descriptive statistics for tail length in litters of Thamnophis sirtalis.

Tail Length (mm)						
Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	15	36.3	3.1	32	43	8.6
1502	23	38.8	3.8	31	45	9.8
1504	12	42.2	3.0	39	48	7.1
1505	11	41.6	3.4	35	47	8.1
1509	12	37.3	4.0	31	44	6.4
1510	25	37.1	2.8	32	42	7.6
1511	6	42.8	3.6	39	47	8.4
1512	13	40.6	2.8	36	46	6.8
1513	37	40.4	3.2	33	48	7.9
1514	15	39.9	4.9	31	49	12.3
1515	2	37.5	0.7	37	38	1.9
1516	6	36.8	6.0	29	44	16.2
1517	11	35.8	3.4	31	41	9.4
1519	13	38.6	5.1	31	47	13.3
1520	21	40.3	3.9	33	47	9.6
1523	23	41.7	3.1	32	46	7.5
1524	4	38.0	3.4	36	43	8.8
1526	5	36.6	3.6	33	41	10.0
1529	6	45.8	3.9	40	50	8.4
1534	9	45.1	4.2	39	52	9.4
1536	7	39.3	2.2	37	43	5.6
1537	18	41.8	4.4	33	49	10.5
1538	40	41.1	3.9	34	49	9.6
1539	17	41.0	2.6	35	46	6.4
1548	10	44.3	2.2	41	48	5.0
1556	1	35.0	-	35	35	-
1558	17	44.3	4.6	32	50	10.5
1562	7	39.4	2.7	36	44	6.8
1565	5	40.4	3.8	34	44	9.4

Descriptive statistics for ventral scales in litters of Thamnophis sirtalis.

Ventral Scales

Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	15	149.7	2.7	145	155	1.8
1502	23	149.2	3.8	143	157	2.5
1504	12	155.4	4.0	152	163	2.6
1505	11	146.4	2.4	142	151	1.6
1509	12	151.6	3.0	148	159	2.0
1510	25	146.0	4.3	138	154	3.0
1511	6	148.2	2.5	145	151	1.7
1512	13	152.0	1.8	149	156	1.2
1513	37	150.4	4.6	136	159	3.1
1514	15	151.1	3.2	143	155	2.1
1515	2	155.0	4.2	152	158	2.7
1516	6	150.5	2.8	146	154	1.9
1517	11	148.2	5.1	137	153	3.4
1519	13	146.0	3.7	138	151	2.5
1520	21	152.2	3.1	146	157	2.0
1523	23	154.3	3.0	150	159	1.9
1524	4	147.8	5.4	141	153	3.6
1526	5	147.2	3.0	142	149	2.1
1529	6	148.5	3.9	141	152	2.6
1534	9	155.1	7.5	147	167	4.8
1536	7	149.3	4.5	140	153	3.0
1537	18	145.8	4.4	138	157	3.0
1538	40	150.1	2.8	144	158	1.9
1539	17	151.2	3.8	144	158	2.5
1548	10	158.2	2.8	153	162	1.8
1556	1	156.0	-	156	156	-
1558	17	152.4	3.3	146	159	2.2
1562	7	156.6	2.4	152	159	1.6
1565	5	152.4	3.8	149	157	2.5

Descriptive statistics for subcaudal scales in litters of Thamnophis sirtalis.

Subcaudal Scales

Litter	N	Mean	<u>+SD</u>	Min	Max	C.V.
1501	15	56.4	8.9	40	72	15.7
1502	23	66.0	5.6	57	78	8.5
1504	12	70.3	5.8	63	78	8.2
1505	11	63.8	5.6	53	73	8.8
1509	12	65.9	6.2	58	76	9.4
1510	25	62.0	5.4	49	70	8.7
1511	6	64.5	7.7	56	76	11.9
1512	13	67.2	3.3	61	73	4.9
1513	37	73.1	5.0	62	81	6.9
1514	15	63.9	5.2	56	72	8.1
1515	2	66.0	7.1	61	71	10.7
1516	6	68.5	7.7	59	79	11.2
1517	11	59.8	6.7	51	70	11.2
1519	13	63.6	6.3	52	73	10.0
1520	21	66.5	6.6	54	76	9.9
1523	23	69.7	3.7	61	75	5.3
1524	4	69.8	7.2	63	76	10.4
1526	5	58.0	4.1	51	61	7.0
1529	6	66.2	7.1	56	75	10.8
1534	9	63.4	5.6	58	73	8.9
1536	7	68.7	5.8	63	80	8.4
1537	18	62.8	8.2	47	80	13.1
1538	40	66.6	7.1	54	82	10.7
1539	17	63.0	4.9	53	71	7.8
1548	10	74.0	3.2	69	80	4.3
1556	1	74.0	-	74	74	-
1558	17	69.5	6.0	61	77	8.6
1562	7	70.6	3.4	65	75	4.8
1565	5	66.8	3.6	63	70	5.3

Descriptive statistics for nearest neighbor distance in aggregation tests in litters of Thamnophis sirtalis.

Mean Nearest Neighbor Distance (Log Transformed)

Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	16	3.98	1.0	1.9	5.8	25.2
1502	23	4.11	0.9	1.9	6.0	22.0
1504	12	4.31	1.1	1.6	5.9	25.8
1505	11	4.34	1.1	1.8	6.1	25.2
1509	12	4.25	0.8	2.6	5.9	18.7
1510	25	3.63	0.9	1.4	5.7	26.1
1511	6	-	-	-	-	-
1512	13	4.23	0.8	2.6	5.9	19.1
1513	37	3.82	0.8	1.1	5.5	21.7
1514	15	4.16	1.0	1.9	6.2	22.8
1515	0	-	-	-	-	-
1516	6	-	-	-	-	-
1517	11	4.45	1.0	2.5	6.4	23.7
1519	13	4.12	1.0	2.4	6.1	23.1
1520	20	3.67	1.0	1.1	5.8	28.0
1523	23	3.72	1.0	2.1	5.9	26.2
1524	4	-	-	-	-	-
1526	6	-	-	-	-	-
1529	6	-	-	-	-	-
1534	9	3.56	1.0	2.2	6.1	27.4
1536	7	-	-	-	-	-
1537	18	3.63	1.1	1.1	6.0	30.4
1538	40	3.33	1.4	0.0	5.8	41.5
1539	17	3.79	1.0	1.4	5.9	25.9
1548	10	3.96	1.2	0.0	6.0	31.3
1556	9	4.47	1.0	2.7	6.2	22.0
1558	17	4.02	0.8	2.1	5.8	19.4
1562	7	-	-	-	-	-
1565	11	4.02	1.0	1.4	5.8	25.1

Descriptive statistics for total tongue-flick attack scores
(fish + worm prey extracts + water controls) in litters of
Thamnophis sirtalis.

Chemical Extract Score (Log transformed)

Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	16	5.4	2.7	2.5	9.8	49.9
1502	23	5.2	2.4	0.4	8.6	45.5
1504	12	5.0	1.4	2.0	6.8	27.5
1505	11	6.7	0.9	5.0	8.3	13.3
1509	12	5.4	2.2	1.8	8.6	39.9
1510	25	4.9	1.8	0.5	8.0	36.1
1511	6	5.5	1.4	3.8	7.9	25.0
1512	13	6.5	1.7	4.3	9.9	26.6
1513	37	6.8	2.0	3.2	11.5	25.6
1514	15	7.5	2.5	3.2	10.4	33.1
1515	0	-	-	-	-	-
1516	6	8.0	1.3	6.0	9.3	16.3
1517	11	7.6	1.6	3.2	9.5	21.5
1519	13	5.8	2.4	0.0	10.0	41.3
1520	20	6.5	2.2	2.5	10.6	33.4
1523	23	6.5	2.4	1.7	10.4	36.6
1524	4	5.6	2.8	3.0	9.4	49.5
1526	6	5.4	2.0	2.5	7.6	36.6
1529	6	4.5	1.9	2.1	6.8	42.9
1534	9	5.8	2.4	1.9	10.4	41.2
1536	7	5.1	3.2	0.3	9.2	63.1
1537	18	5.8	2.3	1.1	10.2	39.4
1538	40	7.9	2.5	0.0	11.2	31.3
1539	17	6.8	2.9	0.2	10.0	42.3
1548	10	9.8	1.3	6.9	11.2	13.4
1556	9	7.0	2.0	5.0	11.4	23.5
1558	17	8.1	1.9	4.6	10.8	23.4
1562	7	6.5	2.6	2.1	10.1	39.2
1565	9	6.5	2.2	3.0	10.2	34.2

Descriptive statistics for tongue-flick attack scores to fish prey extracts in litters of Thamnophis sirtalis.

Fish Extract Score (Log transformed)

Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	16	2.1	1.2	0.3	4.0	59.7
1502	23	1.8	1.1	0.0	4.1	59.8
1504	12	1.5	0.6	0.5	2.3	39.9
1505	11	2.0	0.1	1.9	2.3	5.7
1509	12	2.8	1.2	0.9	4.2	41.7
1510	25	1.5	0.8	0.0	2.7	55.1
1511	6	2.6	0.9	1.6	4.1	35.8
1512	13	2.1	1.0	0.8	4.2	46.1
1513	37	2.4	0.8	0.7	4.2	35.7
1514	15	3.0	1.3	0.3	4.2	43.0
1515	0	-	-	-	-	-
1516	6	2.9	1.0	1.7	4.0	33.7
1517	11	2.2	0.6	1.2	3.0	26.3
1519	13	2.2	1.1	0.0	4.1	47.0
1520	20	2.2	0.6	1.1	3.4	26.5
1523	23	2.2	1.1	0.7	4.2	49.9
1524	4	2.5	1.4	0.9	4.1	55.2
1526	6	2.0	1.0	0.8	3.4	49.8
1529	6	1.2	0.5	0.5	1.7	45.2
1534	9	1.9	1.1	0.0	4.0	55.1
1536	7	1.9	1.3	0.3	4.0	67.5
1537	18	1.8	0.8	0.3	3.2	47.3
1538	40	2.8	1.0	0.0	4.2	34.0
1539	17	2.4	1.4	0.0	4.1	59.2
1548	10	3.8	0.5	2.6	4.2	14.1
1556	9	2.2	0.9	1.1	4.2	42.8
1558	17	3.4	1.0	0.7	4.2	31.0
1562	7	2.5	1.1	0.9	4.1	44.8
1565	9	3.0	0.9	1.4	4.2	30.7

Descriptive statistics for tongue-flick attack scores to worm prey extracts in litters of Thamnophis sirtalis.

Worm Extract Score (Log transformed)

Litter	N	Mean	$\pm$ SD	Min	Max	C.V.
1501	16	2.1	1.4	0.0	4.3	67.4
1502	23	2.2	1.1	0.0	4.1	51.5
1504	12	1.8	0.6	0.7	2.6	32.0
1505	11	2.6	0.8	1.4	4.1	31.7
1509	12	1.5	1.2	0.0	4.1	79.6
1510	25	2.4	1.0	0.5	4.2	40.1
1511	6	1.5	0.7	0.5	2.4	45.8
1512	13	2.8	1.0	1.5	4.2	37.3
1513	37	2.7	0.9	1.0	4.2	34.6
1514	15	3.1	1.1	1.5	4.2	34.2
1515	0	-	-	-	-	-
1516	6	3.5	0.8	2.3	4.2	24.0
1517	11	3.8	0.6	2.0	4.2	16.6
1519	13	2.4	1.3	0.0	4.2	54.2
1520	20	2.6	1.2	0.7	4.2	45.8
1523	23	2.8	1.2	0.0	4.2	43.7
1524	4	2.1	1.4	0.5	3.8	67.5
1526	6	2.0	1.2	1.0	4.0	58.4
1529	6	2.0	1.1	0.5	3.2	54.1
1534	9	2.3	1.2	1.0	4.1	49.6
1536	7	2.2	1.4	0.0	4.0	64.0
1537	18	2.5	1.2	0.0	4.2	49.2
1538	40	3.2	1.2	0.0	4.2	36.6
1539	17	2.7	1.2	0.0	4.1	44.9
1548	10	3.6	0.8	1.8	4.1	21.5
1556	9	2.7	0.9	1.4	4.2	34.4
1558	17	2.9	0.9	1.6	4.1	29.7
1562	7	2.4	1.4	0.0	4.1	61.1
1565	9	2.0	1.1	0.5	3.8	53.2

Descriptive statistics for antipredator behaviors (moving + non-moving stimuli) in litters of Thamnophis sirtalis.

Antipredator Score

Litter	N	Mean	+SD	Min	Max	C.V.
1501	16	24.8	21.7	0.0	61.0	87.8
1502	21	4.2	8.6	0.0	36.0	202.0
1504	12	23.5	24.4	4.0	84.0	103.9
1505	11	10.6	9.5	0.0	28.0	89.0
1509	12	0.2	0.6	0.0	2.0	248.6
1510	25	9.4	15.3	0.0	3.1	163.8
1511	6	11.3	15.3	1.0	41.0	137.4
1512	13	0.3	0.8	0.0	3.0	277.8
1513	37	4.0	8.4	0.0	34.0	208.9
1514	15	1.9	5.2	0.0	19.0	276.8
1515	0	-	-	-	-	-
1516	6	20.7	25.8	0.0	63.0	124.6
1517	11	0.6	1.0	0.0	3.0	161.4
1519	13	10.0	10.9	0.0	31.0	108.9
1520	20	0.6	1.3	0.0	5.0	201.4
1523	23	5.1	9.9	0.0	45.0	193.2
1524	4	1.5	1.9	0.0	4.0	127.6
1526	6	5.5	12.5	0.0	31.0	227.3
1529	6	20.5	24.5	1.0	52.0	119.5
1534	9	2.4	3.7	0.0	10.0	153.2
1536	7	2.8	4.8	0.0	13.0	167.2
1537	18	1.0	2.4	0.0	10.0	228.6
1538	40	0.5	1.4	0.0	6.0	262.3
1539	17	0.5	1.1	0.0	4.0	201.6
1548	10	24.5	25.6	0.0	88.0	104.4
1556	9	34.4	27.4	1.0	94.0	79.6
1558	17	15.2	14.9	0.0	48.0	98.5
1562	7	16.6	15.5	0.0	38.0	93.6
1565	9	46.6	29.5	11.0	94.0	63.3

Descriptive statistics for CTMin in litters of Thamnophis sirtalis.

CTMin (°C)						
Litter	N	Mean	±SD	Min	Max	C.V.
1501	15	9.2	1.03	7.3	10.6	11.2
1502	19	7.7	1.22	5.7	10.2	15.8
1504	12	5.5	1.22	3.5	8.1	22.1
1505	10	6.7	1.15	5.0	8.8	17.1
1509	12	7.9	0.93	6.6	9.5	11.8
1510	25	8.4	2.38	9.0	13.0	28.5
1511	6	7.8	0.89	6.7	9.3	11.4
1512	13	7.3	0.79	5.8	8.6	10.8
1513	37	7.5	0.85	4.9	9.1	11.3
1514	15	7.3	0.56	6.5	8.5	7.7
1515	2	7.5	2.62	13.5	15.4	7.9
1516	6	7.7	0.61	6.8	8.6	7.9
1517	7	6.8	1.61	5.8	10.2	23.5
1519	13	9.1	2.47	6.4	15.4	27.3
1520	20	7.0	0.96	5.2	8.6	13.8
1523	23	7.0	1.05	5.7	9.4	14.9
1524	3	7.5	1.49	5.8	8.6	19.9
1526	5	7.4	0.67	6.6	8.1	9.1
1529	6	8.0	1.28	6.5	10.0	16.1
1534	9	5.4	1.06	4.0	7.7	19.8
1536	7	6.3	0.66	5.5	7.3	10.5
1537	18	6.7	0.85	5.2	8.5	12.7
1538	40	7.3	0.99	5.8	9.8	13.6
1539	17	6.9	0.78	5.7	8.7	11.3
1548	10	6.6	1.10	5.1	8.5	16.7
1556	1	8.2	-	8.2	8.2	-
1558	17	5.8	1.24	3.3	7.8	21.2
1562	7	8.0	1.39	6.2	10.0	17.4
1565	5	7.6	0.58	7.1	8.3	7.6

VITA

James Michael Schwartz was born in Ames, Iowa on May 10, 1960. He traveled extensively as a child, but entered the University of Washington in September 1978, where he received a Bachelor of Arts degree in Zoology in June 1982. After a year of research at Friday Harbor Marine Laboratory, he entered the Graduate School of the University of Tennessee, Knoxville in September, 1983 and received the Doctor of Philosophy degree with a major in Life Sciences, and a minor in Ethology in May 1989. Eager to explore new problems and interesting taxa, he will study Drosophila in Hawaii after completion of his degree.