To the Graduate Council:

I am submitting herewith a thesis written by Julia Albright entitled "Microsatellite DNA markers, multiple paternity, and the inheritance of morphology and behavior in Butler's garter snake (*Thamnophis butleri*)." I have examined the final copy of this thesis for form and content and recommended that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Psychology.

Gordon M. Burghardt, Major Professor

We have read this thesis
and recommend its acceptance:

Gary F. McCracken

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Accepted for the Council:

Vice Provost and Dean of Graduate Studies

# Microsatellite DNA Markers, Multiple Paternity, and the Inheritance of Morphology and Behavior in Butler's Garter Snake (*Thamnophis Butleri*)

A Thesis
Presented for the
Master of Arts
Degree
The University of Tennessee, Knoxville

Julia Davene Albright
December, 2001

## Acknowledgments

I would like to thank the many people who helped make this project possible. First and foremost, I thank Dr. Gordon Burghardt, my major advisor, for giving me the opportunity to further explore and deepen my fondness for animal behavior. I appreciate his trust and patience over the past two years. Although I will be starting a new academic career, the knowledge I gained in the Reptile Ethology Lab will be invaluable in my future endeavors.

I would also like to thank Dr. Gary McCracken for his guidance and allowing me to perform my genetic studies in his lab. Many thanks to Dr. John Malone for serving on my committee and sharing his amusing stories about Voltaire.

Gary Casper of the Milwaukee Public Museum was extremely helpful in allowing me to use his snakes as subjects in my thesis, his hospitality during my stay in Wisconsin, and providing assistance whenever needed.

I thank Matt Lanier and Matthew Bealor for helping with data collection. I appreciate the coaching, patience and kindness of Leslie Saidak during my long hours in the McCracken lab. Janet Beets, Lara Eddington, and Andrea Strigley were instrumental my data collection and maintenance of my animals. I am also thankful for the comradery and sound advice from colleagues and friends Paul Andreadis, Mark Krause, John Placyk, Lynn Almli, Ben Keck, Melinda Harper and the "McCrackenettes": Sunitha Vege, Amy Russell, Jenny Holmes, and Nadia Ayoub.

Last, but not least, I would like to thank my family for their support throughout my academic career.

## Abstract

Variation in behavior can be the result of many environmental and genetic factors. Quantitative genetics has provided a contextual basis in which to study traits, such as behavior, that vary in a continuous manner. Estimating quantitative parameters with precision depends on complete knowledge of pedigrees. Molecular genetic techniques, such as microsatellite DNA markers, allow researchers to more accurately determine relatedness.

Garter snakes (*Thamnophis*) and other natricine snakes are suitable models for genetic studies due to the relatively large number of precocial offspring born to each dam. In addition, multiple paternity in *Thamnophis* litters is widespread. *Thamnophis butleri*, the subject of this thesis, is a threatened species in Wisconsin as a result of both human and genetic intrusion. The presence of morphological *T. radix- T. butleri* hybrids provides evidence that the parapatric species, *T. radix*, may be able to interbreed and genetically "swamp" *T. butleri*.

Preliminary studies found primers developed for *T. sirtalis* and *Nerodia sipedon* amplified *T. butleri* microsatellite DNA regions as well. Using this microsatellite information, I determined if multiple paternity was present in *T. butleri*. As expected, I found multiple paternity in 55% to 78% of the sample litters. In addition, I employed microsatellite allele distribution to deduce full and half-sibship with a litter.

Data was also collected on scale counts, body and head measurements, antipredator responses, and chemical extract and live prey preference. Disparity among

litters, or dam effects, was determined using an analysis of variance. Several characteristics, such as body size, head size, number of ventral scales, propensity to strike and flee when confronted with threatening stimuli, and preference for live fish was found to vary among litters.

Dam variance could be a result of maternal genetic, maternal environmental or additive genetic effects. I performed a nested analysis of variance to establish sire-within-dam effects, which are a consequence of genetics only; therefore, the difference between dam and sire-within-dam variances is attributable to maternal environment. I found no sire-within-dam differences for any traits measured. Low sample size for the sire-within-dam analysis probably masked any true differences.

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# Chapter 1

# Introduction

## General Introduction

Humans have realized for centuries that inheritance plays a role in behavior. Even in prehistoric times, humans domesticated animals, such as dogs, by selectively breeding those with favorable morphological and behavioral traits. In more recent times, laboratory researchers use animal models to document inherited behaviors. An example of this evidence is an elegant study by DeFries (1978) which describes how mice that reacted similarly in an open field study were bred selectively over 30 generations, resulting in two behaviorally distinct mice strains, high-active and low-active. With each subsequent generation, high lines became increasingly more active whereas low lines became less reactive. Inheritance played a key role in this dramatic divergence of characteristics.

Utilization of inbred strains and quantitative genetics methods have expanded the influence of behavioral genetics to many fields, including psychology and behavioral ecology. Behavioral characteristics are frequently under polygenic, or multiple gene control. Quantitative genetic principles rely only on phenotypes and the degree to which relatives resemble each other. This field of study is advantageous because it is not necessary to localize the genes responsible for trait expression (Boake, 1994).

Early ethologists such as Whitman (1899), Lorenz (1958), and Tinbergen (1941) realized that behavior can be as valuable as morphology when assessing relationships

between species. Skeptics insisted that one cannot determine ancestral lineages using traits, such as behavior, that are not left in the fossil record. By proving behavior has a genetic component, and is inherited through the same mechanism as morphological traits, quantitative geneticists validated behavior as a component in evolutionary processes (Brooks and McLennan, 1991).

According to Lande (1976), quantitative genetics serves to predict evolution of phenotypes (including behavioral patterns) and to deduce the rate and direction of prior evolution. More specifically, calculation of heritabilities, or amount of phenotypic variance attributable to genotypic variance, can lead to predictions about the evolution of traits. However, an important assumption of quantitative genetics is knowledge of parentage (Schwartz, 1989). Estimates of heritability can be skewed if proper pedigrees are not deciphered (Burghardt and Schwartz, 1999; King et al., 2001).

Research using microsatellite DNA markers has proved useful in quantifying relatedness among individuals (Avise, 1994). Microsatellites contain a core motif of one to six base pairs repeated up to one hundred times (Tautz, 1993). Mutations occur at high rates ($10^{-2}$ to $10^{-5}$ per generation) (Edwards et al., 1992) due to strand-slippage during DNA replication (Levinson and Gutman, 1987). During this slippage, either the replicating or the template strand loop out in the repetitive region, allowing repeats to be added or subtracted to the replicating strand (Schlötterer and Tautz, 1992). Previous studies have utilized microsatellites to detect multiple paternity in a wide range of animal taxa, such as *Drosophila* (Imhof et al., 1998), rodents (Baker et al., 1999), pipefish (*Syngnathus*) (Jones and Avise, 1997), sea turtle (*Lipidochelys*) (Kichler et al., 1999),

water snakes (*Nerodia sipedon*) (Barry et al., 1992), and common garter snakes (*Thamnophis sirtalis*) (McCracken et al., 1999).

Microsatellite variation exists in the number of tandem repeats at each allele, which varies from individual to individual (Palumbi, 1996). Since each parent contributes two alleles to an offspring, researchers can identify not only multiple paternity, but also determine full and half-sibs within a litter if multiple paternity is detected.

*Thamnophis* and other nactricine snakes are ideal model species for such studies due to their large, live-born litters of precocial offspring. Inheritance of morphological and behavioral traits can thus be examined independently from postnatal parental care (Burghardt and Schwartz, 1999), although prenatal maternal influences, such as a common uterine environment, may have been previously underestimated (King et al., 2001). Many aspects of *Thamnophis* ecology have been well characterized, specifically, chemosensory responses to skin substances of prey items (found to predict relative prey preference) and anti-predator behaviors (Burghardt and Schwartz, 1999 and Herzog et al., 1992).

This thesis documents multiple paternity in Butler's garter snakes, *Thamnophis butleri*, using microsatellite DNA markers (Chapter 2). Using this microsatellite data, I determine the degree to which full siblings and maternal half siblings differ with respect to morphological (Chapter 3) and behavioral traits (Chapter 4).

*T. butleri* is a unique species because it is a dietary worm specialist that will eat fish in a captive setting (Carpenter, 1952; Burghardt, 1967). The allopatric plains garter snake species, *T. radix*, is a dietary generalist and is thought to be the species from which

*T. butleri* recently derived (Schmidt, 1938; Rossman et al., 1996). Burghardt (1967) suggests *T. butleri*'s willingness to ingest fish may be retention of primitive perceptual mechanisms from a generalist ancestral species.

The snakes in my study are from north Milwaukee county, and several miles from a suspected hybrid zone in south Milwaukee where the range of a *T. radix* population meets that of an isolated *T. butleri* population in southeastern Wisconsin. In this hybrid zone, "genetic swamping", through which naturally discrete populations or species incorporate genetic material from other populations or species, is suspected (Casper, unpublished). Although Herzog et al. (1992) found that as neonates, both *T. butleri* from Michigan and *T. radix* from South Dakota behave in a similarly unreactive manner towards potential predators, Arnold and Bennet (1984) documented significant individual differences in avoidance responses to touching within a population of *T. radix*. Thus, there is considerable evidence that both chemosensory prey responses and anti-predator behaviors are heritable and likely to be under selective pressure (Schwartz, 1989).

## General Methods

All offspring used in this study were born to female *Thamnophis butleri* captured in Milwaukee County, Wisconsin by Gary Casper of the Milwaukee Public Museum (Fig. 1.1). Five litters used in this study were born August or September 1999 in the Reptile Ethology Laboratory (University of Tennessee, Knoxville) to pregnant females captured in 1998. The other six litters were born in June or July 2000 in the Reptile Ethology lab to females caught the previous fall, and presumably not pregnant at the time of capture.

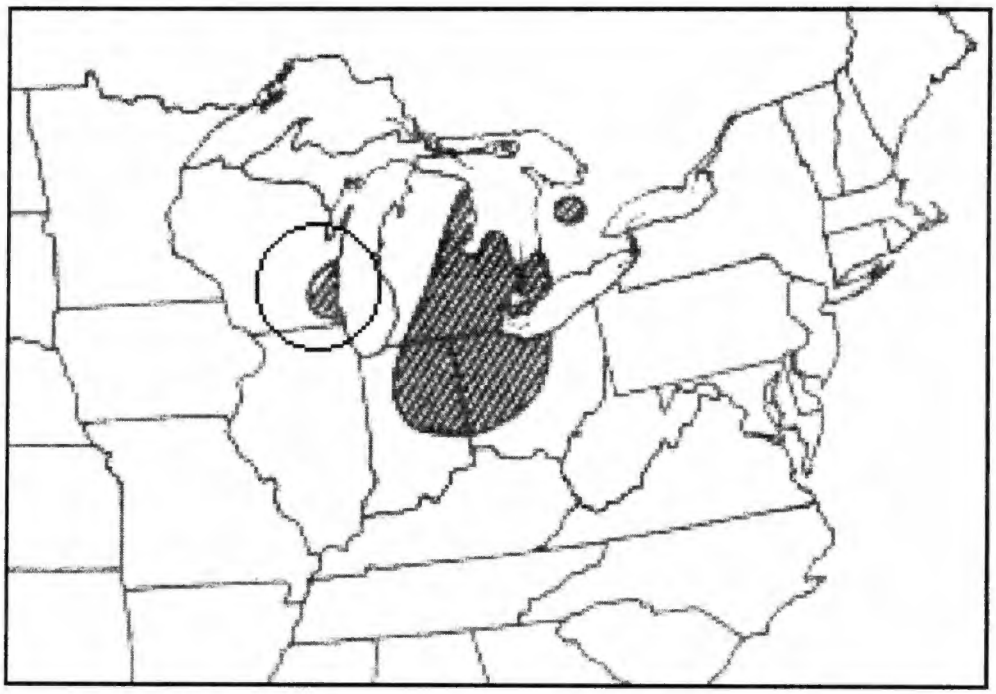

**Figure 1.1** Range of Butler's garter snake (*Thamnophis butleri*). Circled area is specific range of thesis population. Figure from Ernst and Barbour (1989).

The dams of the latter litters were presumably mated during mating studies performed by myself at the Milwaukee Public Museum in April 2000. These snakes were hibernated in a refrigerator (4°C) from October until April.

During hibernation, the males and females were housed together in seven boxes. One day after the snakes were removed from the refrigerator, I performed controlled mating studies. All snakes were given a unique water-based paint pen marking. Four males and four females were placed in each of 8 15-gallon aquarium for approximately eight hours. The following day, the combination of males and females in each aquarium was rotated so that after 10 days, all 32 females and 32 males in the study were placed together during two mating sessions. I recorded any instances of mating behavior.

Morphological (Snout-vent length, mass, tail length, head measurements, scale counts) and behavioral (prey choice test and anti-predator test) data were collected from both males and females at the Museum in 2000 (henceforth referred to as Cohort 2000). All 32 females from this cohort were brought back to the Reptile Ethology Lab, but only six gave birth. Dams of 1999 litters (Cohort 1999) were subject to morphological measurements, but they did not undergo behavioral assay. Morphological (head measurements, scale counts) and behavioral (chemosensory tests, prey choice tests, and anti-predator tests) data were recorded for offspring from both cohorts. Tissue samples (several millimeters of tail tips) were taken for all animals in the study for the molecular analysis. All adult females were housed in individual plastic boxes (31.5 x 16.5 x 9.5), offered 3-5 nightcrawlers (*Lumbricus terrestris*) twice a week, and given water *ad libitum*. Room temperature varied between 20-24°C. A 120 W heat strip was placed under half of the cage, bringing it to approximately 30°C so snakes could further

thermoregulate before parturition. Neonates were housed in plastic boxes (15 x 30 x 9 cm), fed 1-3 leafworms (*Lumbricus rubellus*) twice a week, and water was continuously available.

After birth, the masses and lengths were recorded for dams and neonates. Offspring were sexed and separated into the individual plastic boxes. Measurements of head length, jaw length, head width, and inter-ocular distances; and counts of superlabial, infralabial, and ventral scales were recorded for offspring within two weeks of birth and before the first feeding. Anti-predator and chemical prey choice tests were performed at one and ten days after birth, respectively.

For each trait of interest, analysis of variance was used to test for sex and dam effects among litters (full-sib analysis). A nested analysis of variance was used to test for sex, dam, and sire-within-dam effects on each characteristic as well (maternal half-sib analysis).

# Chapter 2

# Paternity Analysis

## Introduction

Until recently, researchers assumed single paternity in all snakes, especially in litters of *Thamnophis*, based on physical and behavioral evidence. Most significant to this assumption is the presence of a copulatory plug inserted by the male into the female oviductal orifices (Devine, 1975). In *T. sirtalis*, the plug is formed approximately 20 minutes after intromission (Blanchard and Blanchard, 1942) and can remain in the female for days to weeks, contingent on temperature (Devine, 1975; 1984). This plug is thought to be both a physical and chemical deterrent for subsequent matings (Devine 1975,1977), lending credence to the single paternity hypothesis (Ross and Crews, 1977;1978). Hemipenes of male snakes lack the ability to remove the plug (Devine 1984), and the plug's associated pheromones make the female unattractive to other suitors (Ross and Crews, 1977; 1978).

Matings of garter snakes are generally thought to occur in the spring. In areas with high population density, males emerge from hibernacula first and engage in intense competition for the later-emerging females. In *T. sirtalis*, many males may attempt to mate with a single female, resulting in a mating ball (Seigel, 1996). Females have a tendency to leave the mating area soon after copulation, although in one study,

researchers reported many female *T. sirtalis* mated again after the plug became dislodged (Whitter and Crews, 1986), which could make multiple paternity possible.

In addition, oviductal sperm storage has been documented in *Thamnophis* (Fox, 1956), thus allowing the opportunity for multiple mating and sperm competition. Halpert et al. (1982) detail the physiological and chemical mechanisms by which sperm is potentially stored throughout hibernation via infundibular and oviducal storage regions. Moreover, Blanchard and Blanchard (1941) found one female that did not mate in the spring, but gave birth that summer; thus indicating fall matings. Fall and multiple spring matings allow the possibility of multiple paternity.

Multiple paternity has now been documented for several species of snakes. Schuett and Gillingham (1986) found evidence for multiple paternity from skin color and patterning in captive bred copperhead snakes (*Agkistrodon Contortrix*). Protein electrophoretic analysis (alloyzmes) proved multiple sires in 16 of 32 litters of *Thamnophis sirtalis* (Schwartz et al., 1989) and 12 of 14 litters of northern water snakes (*Nerodia sipedon)* (Barry et al., 1992). DNA markers have been employed to reveal multiple paternity in the adder (*Vipera berus*) (Höggern and Tegelström, 1995), Kemp's ridley sea turtle (*Lepidochelys kempi*) (Kichler et al., 1999), and the common garter snake *Thamnophis sirtalis* (McCracken, et al. 1999).

Due to their high degree of polymorphism, microsatellites are well suited to investigate paternity and relatedness. Primers developed for both *Thamnophis sirtalis* (McCracken et al., 1999) and *Nerodia sipedon* (Prosser et al., 1999) have proven effective for *Thamnophis butleri*. In this study, I used these markers not only to detect

multiple paternity litters, but also to test the hypothesis of fall mating. As all females of Cohort 2000 were housed during hibernation with males, I analyzed the DNA of these males to determine if they are possible sires. The computer program KINSHIP (Goodnight and Queller, 1999) was utilized to establish full sibs within litters.

## Methods

### *Extraction*

Tissue samples (snake tails) of approximately 1-3 mg were chopped and suspended in 250 µL of extraction buffer (100 mM NaCl, 10mM Tris-HCl pH 8.0, 10mM EDTA pH 8.0, 0.5% SDS) with 50mg/ml of Proteinase K (Promega). Samples were digested overnight in a 55°C waterbath. DNA was extracted using a standard phenol: chloroform: isoamyl (25:24:1), ethanol precipitation procedure (Sambrook *et al.*, 1989). Extraction buffer volume was doubled before combining phenol: chloroform: isoamyl in a 1:1 ratio with the buffer volume (500µL). The samples were mixed for 10 minutes, centrifuged at 11, 000 rpm, and then supernant was pipetted off and placed in a clean tube. This procedure was repeated. DNA was precipitated with 0.2M final volume NaCl and 2X cold 100% ethanol overnight at -20°C. The samples were centrifuged at 14,000 rpm for 30 minutes. The ethanol was then discarded, and the DNA pellet was dried in a vacuum. DNA was resuspended in 1/10 TE (amount varied with pellet size) and

quantified using a Hoeffer DynaQuant 200 fluorometer. DNA was stored at 4°C until analysis.

***Polymerase Chain Reaction Application for Microsatellites***

Primers used in this study were from McCracken et al. (1999) for *Thamnophis sirtalis* and Prosser et al. (1999) for *Nerodia sipedon*. (See Table 2.1). PCR reactions for the primers contained 10 ng of DNA, 1 X Promega PCR buffer, 1.04 mM $MgCl_2$, 0.1 mM dNTP's, 10 picamoles of the downstream primer ($^{32}P$ labeled) and 1 picamole of the upstream primer, 1 unit Taq DNA polymerase, and brought to 12μL with sterile, distilled $H_2O$. PCR programs comprised an initial 2 minute denaturation at 95°C, then 30 cycles of 95°C for 1 minute, the optimum annealing temperature for 1 minute (Table 2.1), and elongated at 72°C for 1 minute. PCR products were then separated on a 6% polyacrylamide sequencing gel, run for 2-5 hours at approximately 85 V with 10 X TBE buffer. Autoradiograph film was then exposed to the gel for 4-24 hours.

*Data Analysis*

Alleles were scored using a known G-A-T-C sequence (Promega) and a 1Kb ladder (BioVentures), run simultaneously with the samples of interest. Overall allele frequencies were calculated using GENEPOP 3.3 (Raymond and Rousset, 2000). GENEPOP employs the Markov chain method to calculate unbiased exact *P*-values and determines Hardy-Weinberg equilibrium at each locus (Guo and Thompson, 1992). Since

**Table 2.1** Primer pair sequence, annealing temperature, product size range, repeat motif flanked by primer sequences, and number of alleles detected at each microsatellite loci.

| Locus | Primer sequence 5'-3' | Annealing Temp (°C) | Product size range | Repeat motif | Number of Alleles |
|---|---|---|---|---|---|
| $Ts2_a$ | F: GGCTAGCCCCTGTGTCCTT<br>R: CACAACTCCAAATATTGAAGATTA | 56 | 118-156 | $(AAT)_{10}+(AAT)_3$ | 8 |
| $Ns\mu2_b$ | F: TCCTCTTTGGCAGAGTAATAGT<br>R: AGCCGAGAACACACTAGTAAGT | 55 | 144-176 | $(AC)_{18}$ | 11 |
| $Ns\mu3_c$ | F: CTGACTCACTTCTGACCCTAAT<br>R: AATATTTGCTTGGCTCAAAC | 55 | 157-189 | $(ATCT)_{14}+AT(CA)_{20}$ | 8 |
| $Ns\mu9_d$ | F: AATGTGATTACATTTGTTTGG<br>R: GATGAAGAATGTTCAAAACC | 55 | 197-227 | $(AC)_3CC(AC)_{15}(AG)_4(AGAA)_2$ $(AG)_6AA(AG)_7A$ | 6 |

a – McCracken et al. (1999). GENEBANK ID: AF135964
b, c, d – Prosser et al. (1999).

the 22 adults are assumed to be unrelated, only their alleles were included in frequency calculations for the entire population. Incorporating many related individuals (offspring) in the calculation would severely bias the results. Apparent deficiency of heterozygotes causing deviation from Hardy-Weinberg could indicate the presence of a null allele, which result from either large increase in size or mutations in the primer sites. Heterozygotes for this null allele will appear to be homozygotes, skewing both genotype and allele frequencies (Dowling et al., 1996).

Multiple paternity for each litter was detected in two ways: 1) deduction using maternal and offspring genotypes and 2) the computer program KINSHIP. Since each parent contributes two alleles, the number of parents contributing to a litter will equal the number of alleles present divided by two (Garner, 1998). After I determined individual allele scores and allele frequencies, I used KINSHIP 1.3.1 (Goodnight and Queller, 1989) to further assess multiple paternity. The program compared a primary hypothesis, i.e., any two offspring of a litter are full-sibs, versus a null hypothesis that each pair are half-sibs. The program gave a significance value that the two individuals are full siblings against the background possibility of half siblings (Queller, personal communication). I also tested the reverse hypothesis that two offspring are half siblings against the background possibility of full siblings.

## Results

### *Hardy-Weinberg Equilibrium and Linkage*

Markov chain exact test employed by GENEPOP found only one locus, Nsμ3, not in Hardy-Weinberg equilibrium (rejection level $P \leq .05$) (Table 2.2). There did not appear to be a deficiency in heterozyogotes, nor was there direct evidence for a null allele at this loci. However, a null allele was suspected at locus Nsμ9, although it did not fall out of Hardy-Weinberg equilibrium. At this locus, Dam 1293 was homozygous for one allele and several of her offspring are homozygous for others. The presence of a null allele could explain these results. None of the loci showed evidence for significant linkage. Microsatellite data for the adults used in allele frequency calculation and for offspring are shown in Appendix I and II.

### *Multiple Paternity*

Deducing the number of sires from non-maternal alleles revealed multiple paternity in 5 of 9 litters (56%) examined. Three of the four litters from Cohort 1999 and two of five from Cohort 2000 must have been sired by more than one male (Table 2.3). However, KINSHIP significantly identified half-sibling groups in eight of the ten (80%) litters for which sample sizes were sufficient for analysis (Table 2.4). KINSHIP could significantly discriminate a maximum of only two sires in any litter. Litter size and multiple paternity did not seem correlated. Data from Table 2.3 indicated a slight correlation between sample size (not total litter size since some individuals did not

**Table 2.2** Observed and Expected Frequencies, *P*-values of probability test for Hardy-Weinberg equilibrium, and frequency of null alleles. Locus Nsμ3 was out of Hardy-Weingberg equilibrium, possibly due to a null allele.

| Locus | Heterozygote Obs/Exp | Homozygote Obs/Exp | *P*-value | $f_0$ |
|---|---|---|---|---|
| Ts2 | 15 / 17.1951 | 6 / 3.8049 | .0932 | .0598 |
| Nsμ2 | 14 / 16.9302 | 8 / 5.0698 | .0793 | .1082 |
| Nsμ3 | 17 / 17.1707 | 4 / 3.8293 | .0094* | .0051 |
| Nsμ9 | 6 / 7.1081 | 13 / 11.892 | .1823 | .2694 |

**Table 2.3** Litter alleles, maternal alleles and detection of multiple paternity of each litter at four microsatellite loci.

| Litter | Locus | Maternal Alleles | Litter Alleles | Multiple Paternity Detected? |
|---|---|---|---|---|
| 1289 (n=11) | Nsμ2 | 152,154 | 150,152,154,160 | No |
| (n=10) | Nsμ3 | 161,177 | 161,165,169,177 | No |
| (n= 8) | Nsμ9 | 217,227 | 217,227 | No |
| (n=11) | Ts2 | 124,154 | 124,142,152 | No |
| **1293** (n=16) | Nsμ2 | 154 | 154,162 | No |
| (n=15) | Nsμ3 | 169,173 | 169,173,181 | No |
| (n=13) | Nsμ9 | 227 | 201,225,227 | No |
| **(n=17)** | **Ts2** | **142,156** | **118,124,142,152,156** | **Yes** |
| **1294** (n=13) | Nsμ2 | 152,154 | 152,154 | No |
| **(n=13)** | **Nsμ3** | **169,173** | **161,165,169,173,177,181** | **Yes** |
| (n=13) | Nsμ9 | 227 | 227,201 | No |
| **(n=13)** | **Ts2** | **124,154** | **118,124,142,146,152** | **Yes** |
| **1298 (n = 9)** | **Nsμ2** | **152,154** | **148,152,154,160,176** | **Yes** |
| (n = 6) | Nsμ3 | 169,173 | 165,169,173 | No |
| (n = 4) | Nsμ9 | 227 | 227 | No |
| **(n = 7)** | **Ts2** | **146,152** | **118,124,146,148,152** | **Yes** |
| 1217 (n = 7) | Nsμ2 | 148,154 | 148,154 | No |
| (n = 7) | Nsμ3 | 157,177 | 157,165,177,181 | No |
| (n = 7) | Nsμ9 | 227 | 227 | No |
| (n = 7) | Ts2 | 124 | 124,142,152 | No |
| 1218 (n =12) | Nsμ2 | 148 | 148,154,156, | No |
| (n =11) | Nsμ3 | 165 | 165,169,189 | No |
| (n =11) | Nsμ9 | 201,227 | 197,201,217,227 | No |
| (n = 12) | Ts2 | 142,152 | 124,142,152 | No |
| **1220 (n = 9)** | **Nsμ2** | **148,150** | **144,148,150,156,176** | **Yes** |
| (n = 8) | Nsμ3 | 169,189 | 157,165,169,189 | No |
| (n = 8) | Nsμ9 | 227 | 227 | No |
| (n = 9) | Ts2 | 142,156 | 124,142,146,156 | No |
| 1226 (n =10) | Nsμ2 | 148 | 148,154,172 | No |
| (n =10) | Nsμ3 | 173,177 | 165,169,173,177 | No |
| (n = 9) | Nsμ9 | 197,227 | 197,227 | No |
| (n = 9) | Ts2 | 142 | 142,146,156 | No |
| **1228** (n =10) | Nsμ2 | 144,148 | 144,148,170 | No |
| (n =10) | Nsμ3 | 173,177 | 173,177,189 | No |
| (n =10) | Nsμ9 | 227 | 201,227 | No |
| **(n =11)** | **Ts2** | **142** | **124,142,152,156** | **Yes** |
| 1229 (n = 5) | Nsμ2 | Not known | 148,144 | No |
| (n = 5) | Nsμ3 | " | 165,177,181,189 | No |
| (n = 5) | Nsμ9 | " | 197,227 | No |
| (n = 5) | Ts2 | " | 124,142,146 | No |

**Table 2.4** Sireship based on KINSHIP full-sib and half-sib analysis.

| Litter | Sire | Individuals |
|---|---|---|
| 1289 | 1 | A, C, F, I, L, O |
| | 2 | H, P, N |
| 1290 | Not analyzed | |
| 1293 | 1 | B, D, J, K, O, Q, R, S, T |
| | 2 | H, I, |
| 1294 | 1 | A, B, C, D, E, F, I, L, M |
| | 2 | G, J |
| 1298 | 1 | G, C, I, L |
| | 2 | A |
| 1217 | 1 | A, C |
| | 2 | D, E, G |
| 1218 | 1 | A, B, C, E, F, G, H, J, M, D, L |
| 1220 | 1 | D, F |
| | 2 | B, C, H |
| 1226 | 1 | B, C, F, H |
| | 2 | A |
| 1228 | 1 | C, H, G, D, F, E |
| | 2 | B, I, J |
| 1229 | 1 | A, B, C, D, E |

amplify at some loci) and instance of multiple paternity. For litters with multiple paternity, the mean number of offspring was 11.3 and only 9.3 in litters without multiple paternity. However, this relationship is not significant according to a simple regression analysis ($r^2 = 2.43$, $p = 0.13$).

One litter, 1290 (Cohort 1999), was discarded from the data set because the genotype of the dam did not correspond to that of her assumed offspring. This could only be explained by an inadvertent swap with another female that must have occurred during feeding or cleaning in the laboratory. Also, some offspring carcasses from Litter 1289 were mislabeled, resulting in the exclusion of 5 of 19 neonates for genetic analysis. Dam 1228 gave birth to all stillborns, thus offspring were used for genetic analysis only. The DNA from 9 neonates never amplified, further reducing the number of offspring available for full and half-sib analysis.

***Full and Half-sibling Analysis***

Once a hypothesis was established concerning the relationship (full or half-siblings), KINSHIP calculated the likelihood that an individual shares a maternal or paternal allele. Two sets of hypotheses were tested: 1) primary hypothesis that a pair of offspring are full-siblings versus the null hypothesis of half-siblings, and 2) primary hypothesis that a pair of offspring are half-siblings with a null hypothesis of full-sibs. The program reported the ratio (primary/null) in a symmetrical matrix. A high ratio indicated a rejection of the null hypothesis in favor of the primary hypothesis. Tables 2.5

**Table 2.5** Litter 1289 KINSHIP matrices of likelihood ratios for each pair of offspring. **A**. Primary hypothesis = full-siblings; null hypothesis = half-siblings. **B**. Primary = half-siblings; null = full-siblings. KINSHIP arranged output so in A, full-siblings are grouped together and in B, half-sibs are grouped. Asterisk (*) indicates likelihood of full-sibs (A) or half-sibs (B) is significant.

**A**. If ratio $\geq 0.594$, $p \leq 0.05$

| | 89F | 89C | 89I | 89A | 89O | 89G | 89L | 89H | 89P | 89N | 89B |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **89F** | - | | | | | | | | | | |
| **89C** | **0.850*** | - | | | | | | | | | |
| **89I** | **0.974*** | **1.009*** | - | | | | | | | | |
| **89A** | **1.171*** | 0.068 | 0.405 | - | | | | | | | |
| **89O** | 0.570 | **0.933*** | -0.075 | -0.233 | - | | | | | | |
| **89G** | -0.019 | -0.143 | 0.564 | -0.019 | -0.143 | - | | | | | |
| **89L** | 0.114 | 0.149 | **0.910*** | 0.123 | -0.161 | 0.564 | - | | | | |
| **89H** | -0.159 | -0.116 | -0.398 | -0.159 | -0.116 | -0.301 | -0.689 | - | | | |
| **89P** | 0.157 | 0.360 | 0.132 | 0.371 | 0.059 | -0.301 | -0.159 | **2.203*** | - | | |
| **89N** | -0.275 | -0.24 | 0.521 | -0.061 | -0.541 | 0.564 | 0.230 | **1.454*** | **0.995*** | - | |
| **89B** | -0.113 | -0.194 | -0.194 | -0.113 | -0.194 | -0.143 | -0.194 | -0.113 | -0.113 | -0.194 | - |

**B**. If ratio $\geq 0.507$, $p \leq 0.05$

| | 89N | 89O | 89A | 89B | 89I | 89H | 89L | 89G | 89P | 89C | 89F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **89N** | - | | | | | | | | | | |
| **89O** | **0.541** | - | | | | | | | | | |
| **89A** | 0.061 | 0.233 | - | | | | | | | | |
| **89B** | 0.194 | 0.194 | 0.113 | - | | | | | | | |
| **89I** | -0.520 | 0.075 | -0.410 | 0.194 | - | | | | | | |
| **89H** | -1.450 | 0.116 | 0.159 | 0.113 | 0.398 | - | | | | | |
| **89L** | -0.230 | 0.161 | -0.120 | 0.194 | -0.910 | **0.689*** | - | | | | |
| **89G** | -0.560 | 0.143 | 0.019 | 0.143 | -0.560 | 0.301 | -0.560 | - | | | |
| **89P** | -1.000 | -0.060 | -0.370 | 0.113 | -0.130 | -2.200 | 0.159 | 0.301 | - | | |
| **89C** | 0.240 | -0.930 | -0.070 | 0.194 | -1.010 | 0.116 | -0.150 | 0.143 | -0.360 | - | |
| **89F** | 0.275 | -0.060 | -1.170 | 0.113 | -0.970 | 0.159 | -0.110 | 0.019 | -0.160 | -0.850 | - |

and 2.6 (Litter 1289 and 1294, respectively) are examples of the matrix provided by KINSHIP for each litter. KINSHIP also provided a 95% confidence value for the hypotheses. For example, in Litter 1289 if the likelihood ratio was below 0.594, one can be 95% confident the pairs of offspring are full siblings.

Given these data, I was able to construct a basic pedigree and infer sireship. Not every offspring of a litter fell within the 95% confidence level and therefore could not be assigned to a sire. Table 2.4 summarizes pedigree information for each litter using KINSHIP. For Litter 1289, KINSHIP identified the offspring pairs of F-C, F-I, F-A, C-I, C-O, and I-L as full-siblings. H-P, H-N and P-N were also identified as full-sibs. Using half-sibs as the primary hypothesis, N-O and H-L were marked as significant half-sibs. Therefore, by inference, two groups (F,C, I, O, L, A and H, P, N) must have been sired by different males. See Figure 2.1 for schematic depiction of KINSHIP results for Litter 1989. Figure 2.2 also depicts a schematic for relatedness and assumed sireship for Litter 1994. In this litter, many of the offspring were identified by KINSHIP as full-siblings. As with Litter 1989, half-sib analysis was able to establish two sires within Litter 1994.

**Table 2.6** Litter 1294 KINSHIP matrices of likelihood ratios for each pair of offspring. **A**. Primary hypothesis = full-siblings; null hypothesis = half-siblings. **B**. Primary = half-siblings; null = full-siblings. KINSHIP arranges output so in A, full-siblings are grouped together and in B, half-sibs are grouped. Asterisk (*) indicates likelihood of full-sibs (A) or half-sibs (B) is significant.

**A.** If ratio $\geq 0.515$, $p \leq 0.05$

| | 94A | 94E | 94C | 94D | 94B | 94L | 94M | 94F | 94I | 94G | 94J |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 94A | - | | | | | | | | | | |
| **94E** | **0.980*** | - | | | | | | | | | |
| **94C** | 0.273 | **0.754*** | - | | | | | | | | |
| **94D** | 0.327 | **0.809*** | **1.547*** | - | | | | | | | |
| **94B** | **0.592*** | -0.220 | **0.572*** | **0.572*** | - | | | | | | |
| **94L** | **0.646*** | -0.170 | **0.572*** | **0.626*** | **1.857*** | - | | | | | |
| **94M** | 0.342 | -0.470 | -0.470 | -0.470 | **0.815*** | **0.815*** | - | | | | |
| **94F** | -0.490 | -0.280 | **0.513*** | **0.513*** | **0.779*** | **0.779*** | -0.260 | - | | | |
| **94I** | -0.490 | -0.280 | **0.513*** | **0.513*** | **0.779*** | **0.779*** | -0.260 | **1.396*** | - | | |
| **94G** | -0.640 | -0.160 | 0.410 | 0.109 | -0.070 | -0.370 | -0.180 | -0.130 | -0.130 | - | |
| **94J** | -0.770 | -0.290 | 0.011 | -0.290 | 0.002 | -0.300 | 0.163 | -0.060 | -0.060 | **1.140*** | - |

**B.** If ratio $\geq 0.508$, $p \leq 0.05$

| | 94A | 94J | 94F | 94G | 94L | 94E | 94M | 94I | 94D | 94B | 94C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **94A** | - | | | | | | | | | | |
| **94J** | **0.772*** | - | | | | | | | | | |
| **94F** | 0487 | 0.057 | - | | | | | | | | |
| **94G** | **0.637*** | -1.140 | 0.1.0 | - | | | | | | | |
| **94L** | -0.650 | 0.299 | -0.780 | 0.372 | - | | | | | | |
| **94E** | -0.980 | 0.290 | 0.280 | 0.156 | 0.166 | - | | | | | |
| **94M** | -.0340 | -0.160 | 0.264 | 0.176 | -0.820 | 0.471 | - | | | | |
| **94I** | 0.487 | 0.057 | -1.400 | 0.130 | -0.780 | 0.280 | 0.264 | - | | | |
| **94D** | -0.330 | 0.290 | -0.510 | -0.110 | -0.630 | -0.810 | 0.471 | -0.510 | - | | |
| **94B** | -0.590 | 0.000 | -0.780 | 0.071 | -1.860 | 0.221 | -0.820 | -0.780 | -0.570 | - | |
| **94C** | -0.270 | -0.010 | -0.510 | -0.410 | -0.570 | -0.750 | 0.471 | -0.510 | -1.550 | -0.570 | - |

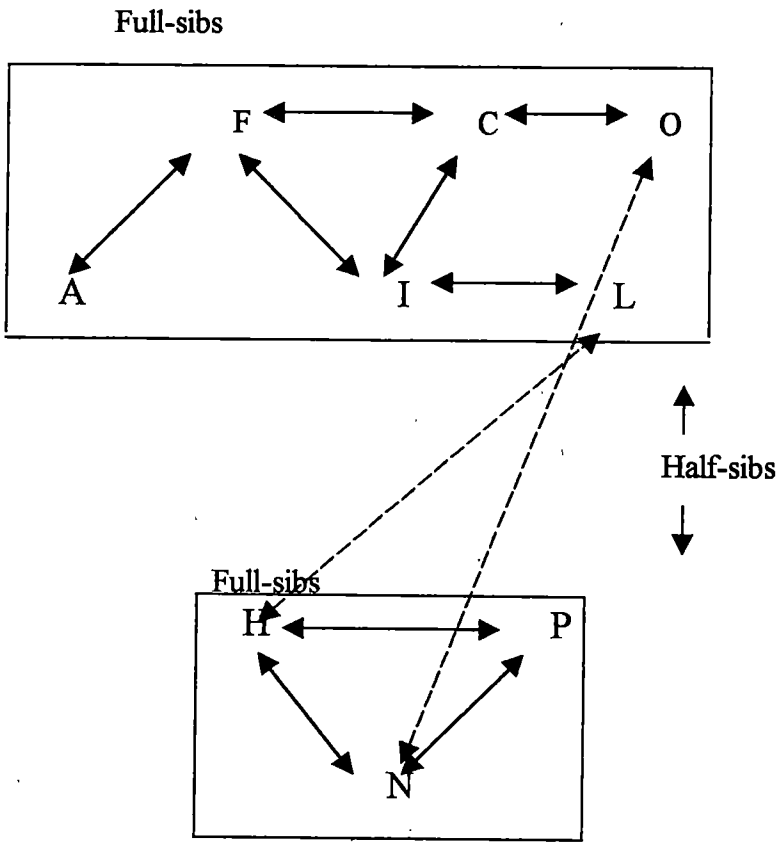


**Figure 2.1** Schematic of pedigrees from KINSHIP for Litter 1289. Solid lines = full sibs and dashed lines = half-sibs using significant likelihoods of relationship (see Table 2.4). Offspring F, C, I, O, L, and A were linked as full-sibs as are offspring H, P, and N. Individuals within each group are linked as half-sibs.

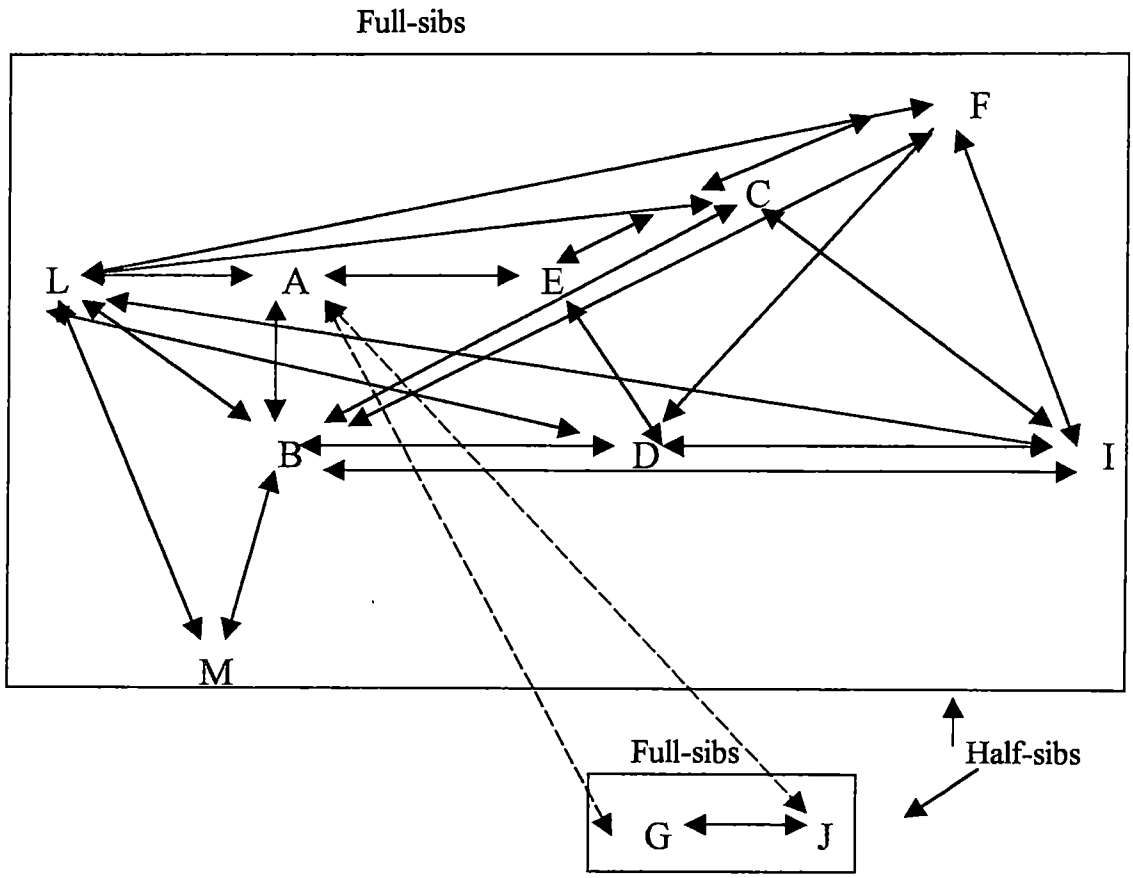


**Figure 2.2** Schematic of pedigrees from KINSHIP for Litter 1994. Solid lines = full-sibs and dashed lines = half-sibs according to significant likelihoods of relationship (See Table 2.5). Offspring A, B, C, D, E, F, I, L, M were linked as full-sibs as are offspring G, and J. Individuals within each group are linked as half-sibs.

***Controlled Mating Study***

Dams in Cohort 1999 were captured pregnant in the spring of 1999. However, females and males in Cohort 2000 were captured in the fall of 1999 prior to the Spring 2000 breeding season, providing the opportunity for controlled breeding studies. My original study proposal required me to observe the matings in aquaria. However, the subjects hid under the tank substrate or remained fairly motionless, apparently distracted by my presence. I witnessed 11 instances of courting behavior (males pushing females with snouts or performing undulating movements while on top of the female) but only two copulations (hemipenis penetration of female cloaca). I did not observe any copulatory plugs and had no way of confirming intromission during these matings.

Since offspring were born later that summer to six of the females in the study, three possibilities for fertilization exist: 1) Females mated the previous fall, before capture and hibernation 2) Mating occurred during hibernation since the sexes were housed together 3) Matings took place during the controlled mating study, and I did not observe the copulations resulting in fertilization. In four of the six litters, microsatellites ruled out litters being sired by males housed with dams during hibernation. Due to time constraints, I was not able to analyze all 32 males that had opportunity to mate with the females in the mating studies. Fall mating remains a possibility with these subjects.

Offspring in Cohort 2000 were born after an extremely short gestation period, two or three months earlier than those in Cohort 1999. Since females do not ovulate until after copulation, even if sperm stored from fall fertilized these eggs, it is unlikely the

resulting embryo would get a developmental "head start." It is more plausible that females were kept very warm (approximately 30°C) and well nourished, allowing offspring to grow more rapidly after fertilization (G. Casper, personal communication).

## Discussion

Although multiple paternity has been reported in *T. sirtalis* ( McCracken et al., 1999 and Schwartz et al., 1989), this is the first study to document multiple paternity in *T. butleri*. Of the 10 litters analyzed with KINSHIP, 8 (80%)showed clear evidence of multiple paternity. My data did not suggest a strong relationship between litter size and instances of multiple paternity as suggested by Schwartz (1989) and Garner et al. (in press) (Table 2.3). However, almost all litters were multiply sired according to KINSHIP. The suspected presence of null alleles at one locus could also be masking more instances of multiply sired litters and more than two sires per litter. I could not establish more than two sires in any litter, although McCracken et al. (1999) document a litter with at least three sires. Assigning paternity requires an accurate estimate of allele frequencies. Since adults used in frequency calculations were collected from one area, non-relatedness may not be a valid assumption. There is some evidence from other snakes and other species to suggest spatial proximity and relatedness are positively correlated (Holmes and Sherman, 1983; Lyman-Henley, 1991).

Multiple matings, and therefore multiple paternity, is a result of female choice since forced copulations are improbable in snakes. Male hemipenes do not have a baculum and are unlikely to force apart scales covering the female cloaca (Devine, 1984).

Furthermore, snakes do not provide appreciable parental care. Females would not thus be seeking out males for the purpose of food provisions or protection. Genetic advantages to multiple mating must provide a selective advantage. Females may be guarding against the possibility of a sterile mate (Gibson and Jewell, 1982), possible genetic defects (Halliday and Arnold, 1987), or to insure more genetic diversity among offspring (Stockley et al., 1993). Multiple matings could thus be considered a form of genetic bet-hedging, in which females maximize the survival rate and reproductive viability of their own offspring (Parker, 1992).

Females may also engage in more than one copulation to bolster sperm competition. Madsen et al. (1992) found evidence of sperm competition in a mating study, in which multiple paternity reduced the rate of stillborn offspring in adders (*Vipera berus*). The mechanism of sperm competition is not completely clear, but Parker (1992) theorizes a "fit" male will ejaculate more sperm, rather than sperm of higher quality. The evolutionary strategy of sperm expenditure allows "fit" males to make sperm at lower cost than "unfit" males, enabling the former to produce proportionately more sperm and fertilize more ova.

Multiple paternity could also be the result from storage and fall matings, not just multiple spring copulations. Sperm storage (Devine 1984) and fall matings in *T. sirtalis* (Whittier and Crews, 1986) have been documented. Although sperm stored from fall matings is evacuated from storage receptacles and degrades within six hours after the subsequent spring mating (Halpert et al., 1982), Blanchard and Blanchard (1942) report a female *T. radix* gave birth in the summer without having mated during preceding spring

breeding season. My data do not rule out instances of sperm storage from fall matings. More controlled mating studies would be needed to corroborate Blanchard and Blanchard's (1942) successful fall mating finding.

# Chapter 3

# Morphology

## Introduction

The morphological characteristics of an individual snake arise from an interaction of genetics and ontogenetic pathways (Dohm and Garland, 1993). Meristic traits, such as scale counts, are usually fixed at birth and useful for phylogenetic purposes. Nevertheless, individual variation has been documented and this dissimilarity may be heritable (Dunn, 1942), most likely under polygenic influence. In contrast, body and head sizes, mensural traits, are quite plastic and may change with diet, prey availability, prey size and sex (Scudder-Davis and Burghardt, 1987; Shine, 1991; Lyman-Henley and Burghardt, 1995; Burghardt and Krause, 1999), as well as maternal condition (King et al., 2001).

Intraspecific variation in head sizes has been documented in European adders (*Vipera berus*) (Forsman, 1991) and European grass snakes (*Natrix natrix*) (Madsen and Shine, 1993). In both cases, snakes inhabiting islands with larger prey items had larger head and/or body sizes. However, in an experimental study, Krause (2000) reported that body size, but not head size, were influenced by diet. Scudder-Davis and Burghardt (1987) found *T. sirtalis* and *T. radix*, dietary generalists, grew faster when fed fish than when fed worms, probably due to higher calcium and phosphorous content of fish. In contrast, Lyman-Henley and Burghardt (1995) reported that *T. butleri*, earthworm specialist, grew more rapidly when reared only on worms as compared to those reared on

fish. Burghardt and Krause (1999) suggest *T. buterli* physiology has become adapted to worms and therefore will thrive on this diet. The relatively small head size of *T. butleri* has also probably developed to aid in earthworm foraging (Burghardt, 1993). Research has thus established the significance of dietary experience in relation to body and head size. Consequently, neonates with no dietary experience must be examined to establish the relative genetic influence on variation of corporal size.

Scalation is not subject to postnatal environmental factors and is thus quite appropriate for genetic analysis. Ventral and subcaudal scales correspond approximately one to one with body and tail vertebrae, respectively (Voris, 1975), and this ratio may relate to locomotor ability in *Thamnophis* (Arnold and Bennett, 1988). Moreover, a snake's cranial kinesis during prey ingestion may be influenced by the number of head scales (Dohm and Garland, 1993).

Although scalation is fixed after birth, intraspecific and intraindividual variation arise, and embryonic condition may be a factor in this variation. Scales develop via a complex interaction of developmental pathways during integument formation. The superficial epidermal layer is derived from the embryonic ectoderm, and the dermis originates from the mesoderm. According to Maderson (1985), scales form as an "outpushing" of the epidermis with a papilla of mesoderm tissue. The upper surface of this mesodermal papilla cornifices, forming a scale. Body scales appear earlier than head scales in embryonic development, and the two are thought to be a consequence of independent pathways.

Variation in timing of this ontogenetic pathway can cause variation. In addition, Fox (1948) found that changes in ambient incubation temperature directly influence the

number of scales in *T. elegans*. Fluctuations in embryonic environment may also be the cause of widely reported count disparity within an individual. In the same study, Fox found labial and ocular scale counts vary from one side of the body to the other. Rossman (1963) reported variation in a wide range of scale counts within *T. proximus* and *T. sauritus* individuals as well.

## Methods

Morphological data were collected on all females (n = 11) and their offspring (n = 115) in this study. Snout-vent length (SVL) and mass (in grams) was recorded immediately after birth. Head measurements, scored with .01 digital calipers, consisted of head length (HL), jaw length (JL), head width (HW) and inter-ocular distance (IOD). (Figure 3.1 and Table 3.1). Points of measurement were adapted from Krause (2000). For Cohort 1999, these measurements were taken several months after birth by another researcher. I recorded Cohort 2000 head measurements within two weeks of birth and before the first feeding. Scoring three adult females and three neonates three times (Table 3.2) assessed reliability for head measurements by the same observer. Ventral scales (as suggested by Dowling, 1951) and supralabial and infralabial scales on both sides of each snake's head were also counted. Since the number of labial scales has very little intraspecific variation, instances of lateral asymmetry were scored.

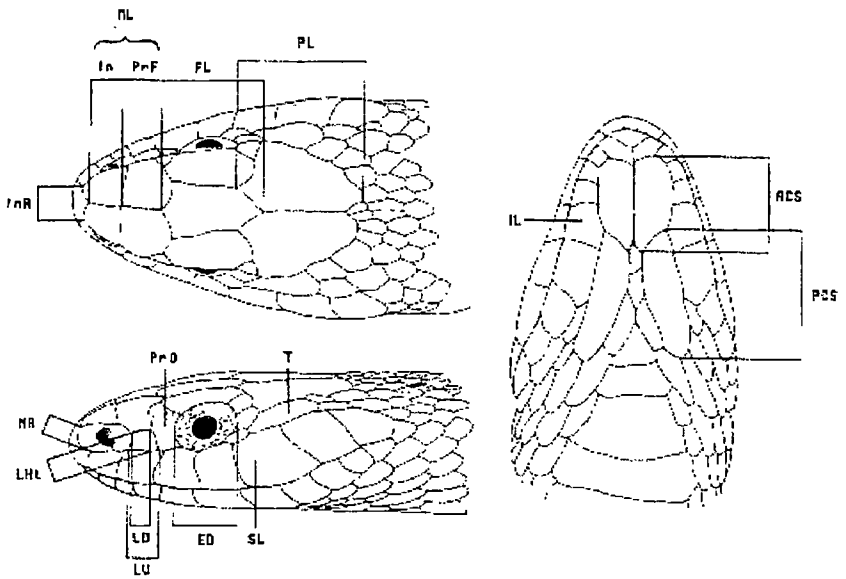


Fig. 1-11. Mensural features in garter snakes used to calculate various ratios found to have taxonomic significance in certain cases:

| | | | |
|---|---|---|---|
| InR | internasorostral contact | NR | nasorostral contact |
| Prf | prefrontal length | In | internasal length |
| FL | frontal length | PL | parietal length |
| ACS | anterior chin-shield length | ML | muzzle length |
| PCS | posterior chin-shield length. | LD | loreal length dorsally |
| LH | loreal height | LV | loreal length ventrally |
| ED | eye diameter | | |

**Figure 3.1** Schematic of head measurements and head scale counts. SL = supralabial, PL = parietal scale. Figure from Rossman et al. (1996).

**Table 3.1** Description of measurements of head length, jaw length, head width, and inter-ocular distance.

| | | |
|---|---|---|
| Head length | **HL** | distance between snout and posterior margin of the parietal scales |
| Jaw length | **JL** | distance between the snout and the posterior margin of the posterior-most upper labial scale on right side of head |
| Head width | **HW** | widest anterior point to posterior margin of parietal Scales |
| Inter-ocular distance | **IOD** | distance between junction point of supraocular Scale, parietal scale, and eye |

**Table 3.2** Repeatability for snout-vent length (SVL), head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) for 3 adult female and 3 neonate *T. butleri*.

| | HL | JL | HW | IOD |
|---|---|---|---|---|
| R | .9999 | .9998 | .9992 | .9988 |
| F-value | 835.2 | 4411.2 | 1266.3 | 840.4 |
| p-value | **<0.001** | **<0.001** | **<0.001** | **<0.001** |

***Statistics***

Cohort mass, tail length, and SVL differences were tested with a factorial ANOVA, with SVL as a covariate. Since head measurements for Cohort 2000 were recorded immediately after birth and Cohort 1999 approximately three months after birth, differences in cohort head measurements were not assessed. I used univariate F-statistics to test for the effects of SVL, litter, and sex on mass, tail length, SVL, HL, JL, HW, and IOD. King et al. (2000) found that full-sib analysis can overestimate genetic parameters. Thus, a half-sib analysis (nested ANOVA) is used to test sex, litter, and sire-within-litter effects. A simple linear regression was performed to find correlations between dam SVL and mass and offspring SVL, mass, and number of offspring. A simple regression was also performed to compare the relationship between offspring tail length and sex ratio for each litter. All data were natural log transformed (score +1) to reduce homogeneity of variance.

## Results

The data from 115 neonate garter snakes (73 in Cohort 1999 and 42 in Cohort 2000) were analyzed, although all 13 animals from litter 1290 were not incorporated into the sire-within-dam analysis. Descriptive statistics (Mean ± 1SE) on mass, snout-vent-length (SVL), tail length (TL), head length (HL), jaw length (JL), head width (HW), inter-ocular distance (IOD), ventral and labial counts, and labial asymmetry are in Appendix III and IV.

MANCOVA results for cohorts are reported in Table 3.3. SVL did covary with mass and tail length. SVL varied significantly between the cohorts with Cohort 2000 being longer. Cohort 2000 also had a longer tail length and larger mass, but these values were not significant. Since head measurements were recorded at different times relative to birth and by different researchers, the measurements were analyzed (full-sib) separately for each cohort. For Cohort 1999, females had greater jaw lengths (Figure 3.2). These measurements did not differ significantly for Cohort 2000. For Cohorts 1999, only mass, tail length, and jaw length varied among litters. Mass, SVL, HL, JL, HW and IOD varied among litters in Cohort 2000. In Cohort 2000, 26.2% of the neonates had instances of labial scale asymmetry, whereas only 8.57% of those in Cohort 1999 had any labial asymmetry. A chi-square test did find significant difference between cohorts ($\chi^2 = 25.26$, df = 1, $p < 0.001$).

SVL also covaried with mass, tail length, HL, HW, and IOD, but not with JL or number of ventral scales when testing for sex, litter, and sire-within-litter differences in Cohort 2000 (Table 3.4). Females did not differ from males in Cohort 2000 for any measurement (Figure 3.2). In Cohort 1999, mass, tail length, and jaw length differed among litters (Figure 3.3). All measurements but tail length varied significantly among litters in Cohort 2000 (Figures 3.4 and 3.5), while none differed significantly when sire-within-litter was examined. Nested analyses did not demonstrate any differences in sire-within-litters for either cohort.

Dams in Cohort 1999 had a mean and standard error of 15.00 (± 1.68) offspring and Cohort 2000 averaged only 8.40 (±1.33) offspring per litter. This difference is

**Table 3.3.** Results from MANCOVA testing effects of cohort on mass, SVL, and tail length. SVL is treated as a covariate with mass and tail length.

| Measure | Source | df | MS | F | p |
|---|---|---|---|---|---|
| SVL | Cohort | 1 | 0.179 | 76.45 | **<0.001** |
| Mass | Covariate (SVL) | 1 | 0.373 | 77.13 | **<0.001** |
| | Cohort | 1 | 0.009 | 1.95 | 0.166 |
| Tail | Covariate (SVL) | 1 | 0.110 | 8.30 | **0.005** |
| | Cohort | 1 | 0.044 | 3.31 | 0.071 |
| | | 110 | | | |

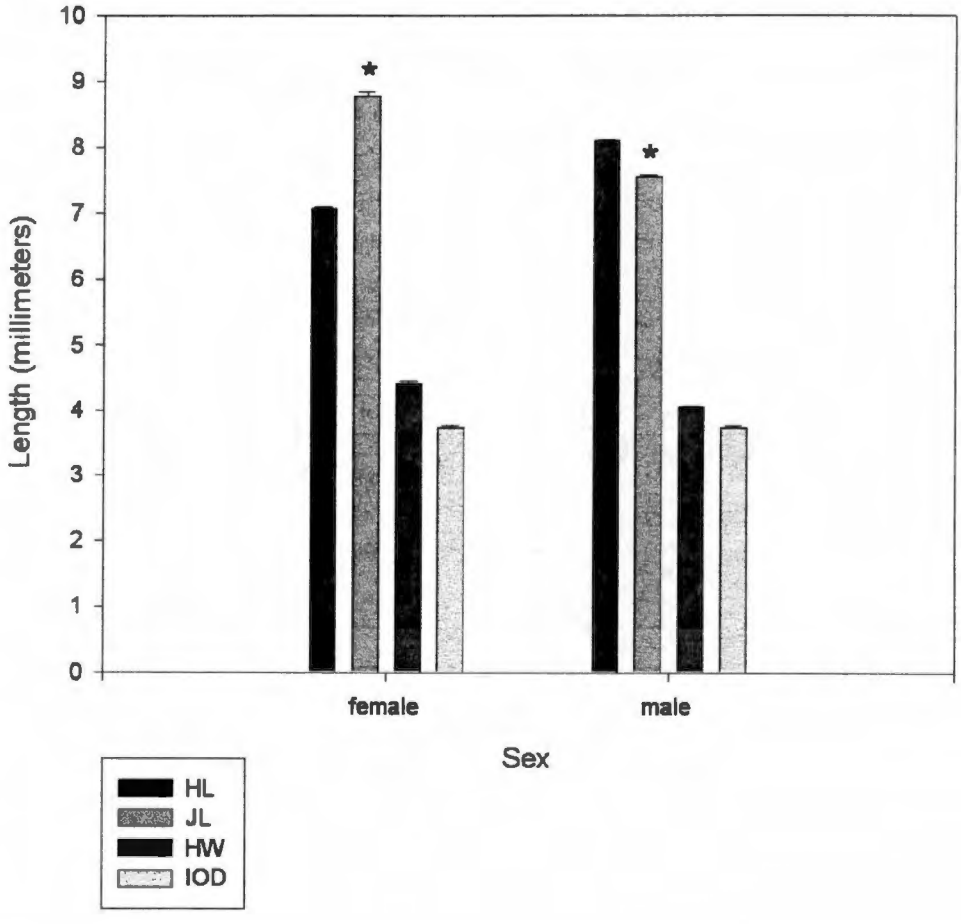


**Figure 3.2**. Sex differences in head measurements for Cohort 1999. JL is significantly higher in female these neonates ($F = 5.36$, $p = 0.030$).

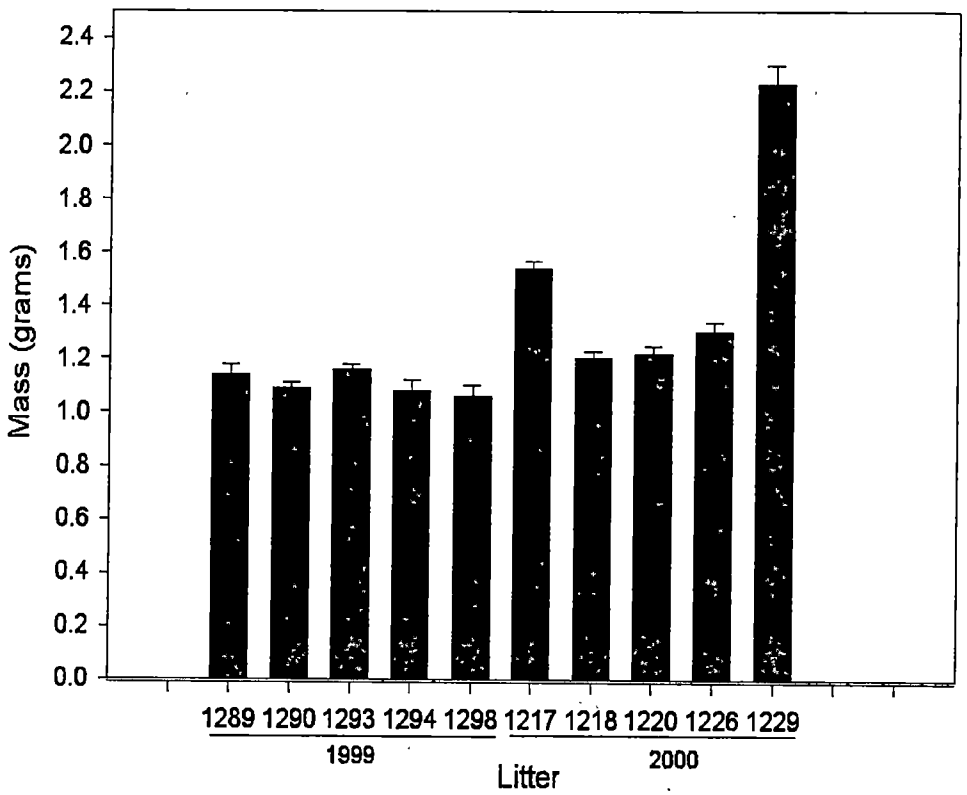


**Figure 3.3.** Average mass (grams) for litters. Cohort 1999 = 1289, 1290, 1293, 1294, and 1298 ($F = 5.31$, $p = 0.007$); Cohort 2000 = 1217, 1218, 1220, 1226,1229 ($F = 43.76$, $p < 0.001$).

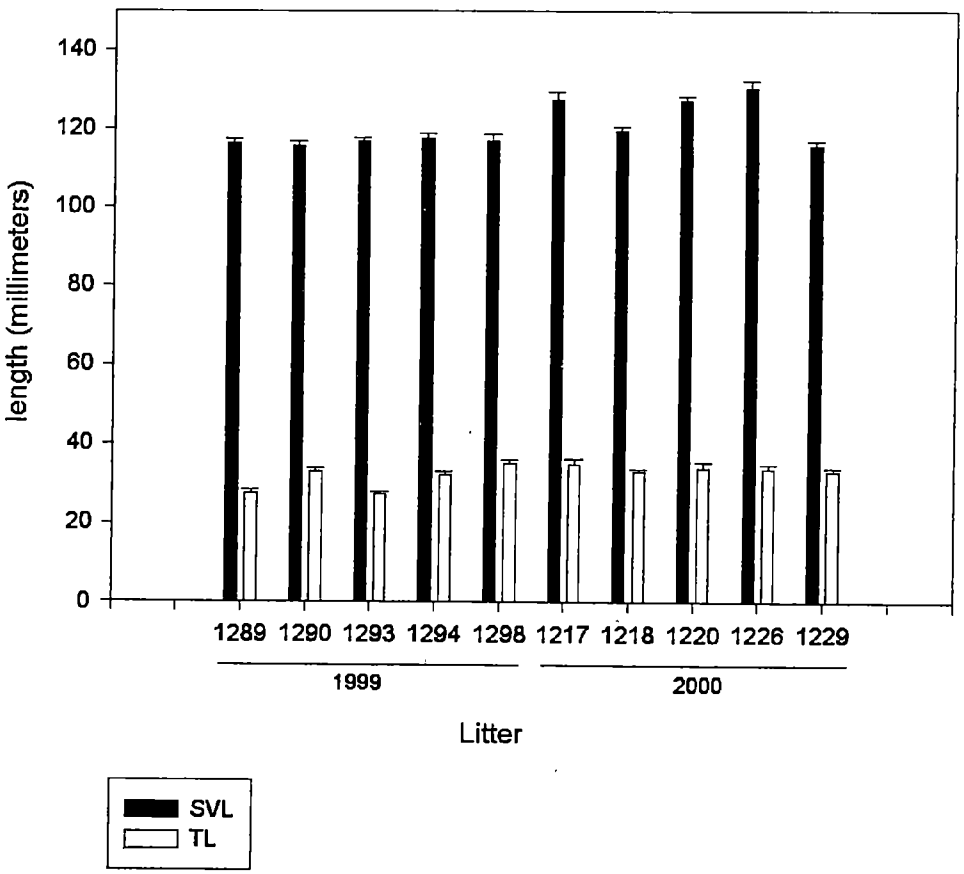


**Figure 3.4.** Average SVL and tail length for litters. Tail length varies significantly for litters in Cohort 1999 ($F = 6.05$, $p = 0.004$).

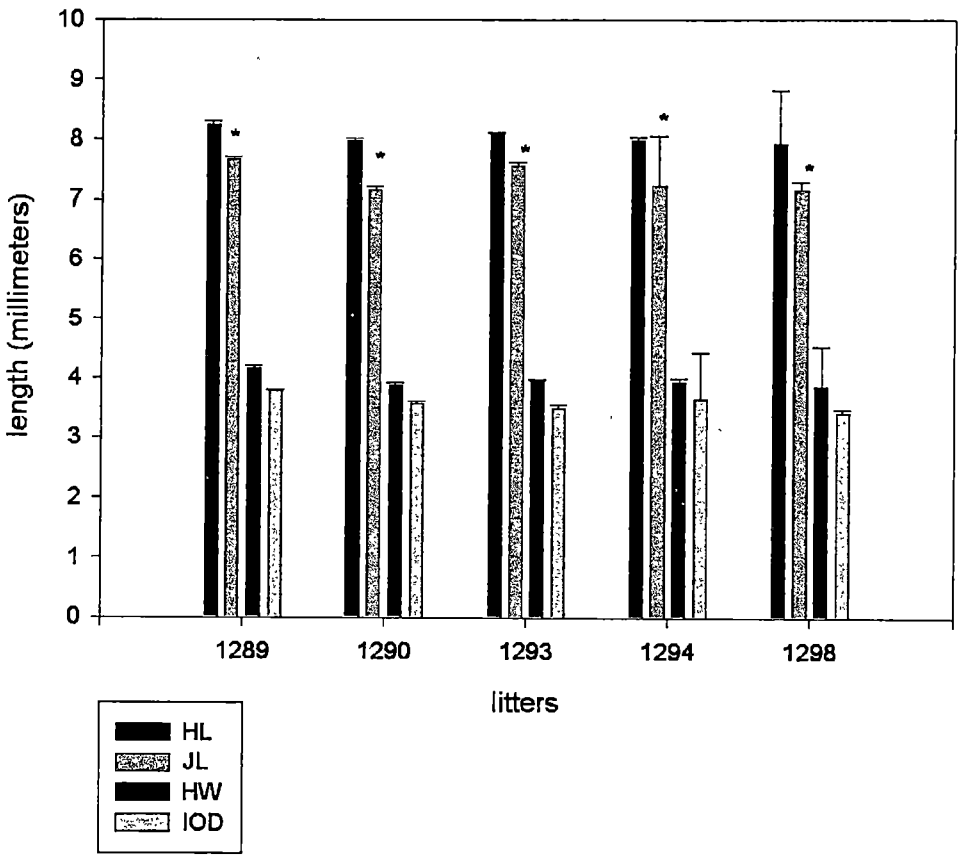


**Figure 3.5.** Average HL, JL, HW and IOD of litters in Cohort 1999. JL varies significantly ($F = 9.35$, $p < 0.001$).

significant according to an ANOVA test ($F = 10.62$, $df = 1,9$, $p = 0.012$). Dam mass had a significantly positive correlation with offspring number (Figure 3.6). Dam mass also has a positive relationship with offspring mass and SVL, although not significant (see Table 3.4 and Figures 3.7 and 3.8). Offspring mass has a negative correlation with number of offspring in a litter ($F = 8.44$, $df = 1,7$, $P = 0.027$) (Figure 3.9), so smaller litters were larger in mass. The tail length of neonates (a sexually dimorphic trait) does not differ with male to female offspring ratio for this sample population (Table 3.5).

## Discussion

Several instances of cohort, sex, and litter (dam) variation for morphological traits were found. Cohort 2000 had a larger mass and SVL, but smaller litter sizes than Cohort 1999, possibly due to differences in prenatal maternal environment. Females were captured near the same site and brought into captivity for most of the gestation period, but heat strips were placed under Cohort 2000 dams, allowing maximum thermoregulation. Cohort 2000 also had a higher incident of labial asymmetries. Gutzke and Packard (1987) report a higher instance of morphological abnormalities in bull snakes hatchlings incubated at higher temperatures (but see also Burger et al., 1987). The higher prenatal temperature of Cohort 2000 could explain the increased number of labial scale abnormalities present in this group.

In addition, previous studies involving oviparous lizards (Servino and Adolph, 1989) and pine snakes (Burger et al., 1987) indicate certain intermediate temperature ranges (below approximately 32°C and above approximately 22°) manifest larger

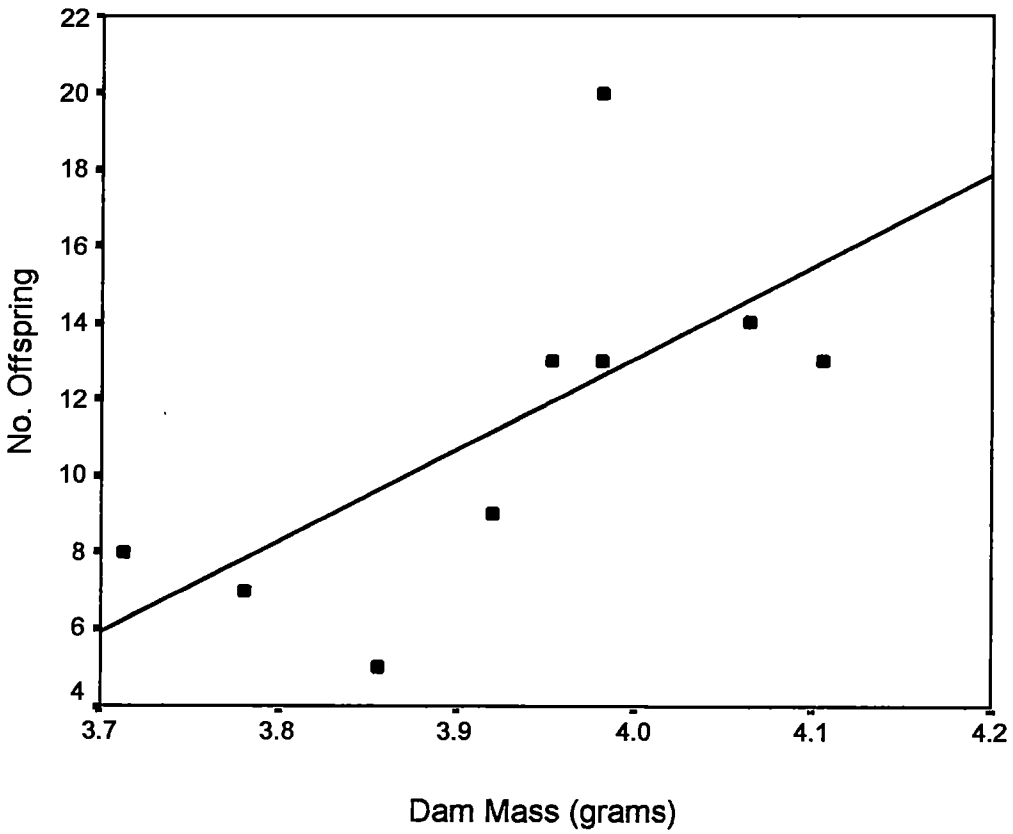


**Figure 3.6**. Average offspring mass versus number of offspring in the litter ($r^2 = 0.442$, $p = 0.027$). Regression equation is Dam Mass = 3.7 + 0.67 Number of Offspring.

**Table 3.4.** Regression analyses of dam SVL and mass and offspring SVL, mass, and number of offspring in each litter.

| | r | $r^2$ | F | df | p |
|---|---|---|---|---|---|
| Dam SVL/Offspring SVL | -0.122 | 0.014 | 0.087 | 1,7 | 0.778 |
| Dam SVL/Offspring mass | -0.042 | 0.002 | 0.011 | 1,7 | 0.920 |
| Dam SVL/No. Offspring | 0.182 | 0.033 | 0.241 | 1,7 | 0.638 |
| Dam mass/Offspring SVL | -0.667 | 0.455 | 4.806 | 1,7 | 0.071 |
| Dam mass/Offspring mass | -0.636 | 0.404 | 4.066 | 1,7 | 0.090 |
| Dam mass/No. offspring | 0.665 | 0.442 | 5.555 | 1,7 | **0.051** |
| Offspring mass/ No.offpring | -0.765 | 0.585 | 8.441 | 1,7 | **0.027** |

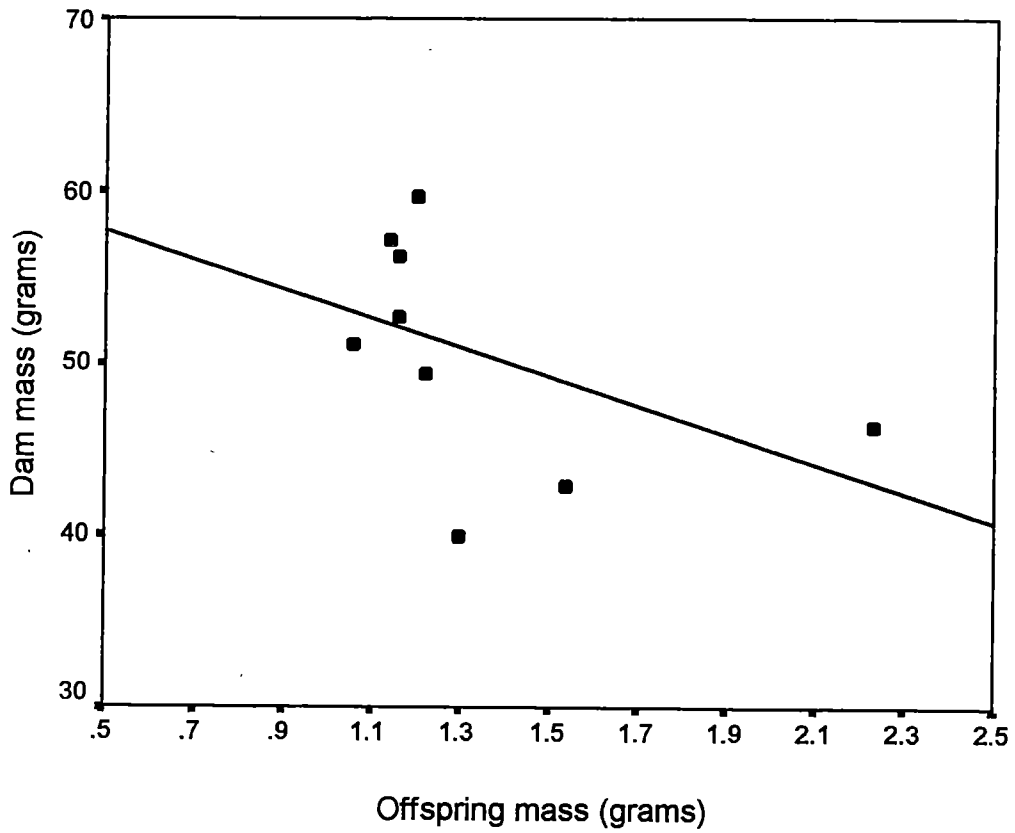


**Figure 3.7**. Dam mass versus average mass of her offspring. This is not a significant correlation ($r^2$ = 0.404, P = 0.090). Regression equation is Dam Mass = 4.3 – 0.64 Offspring Mass.

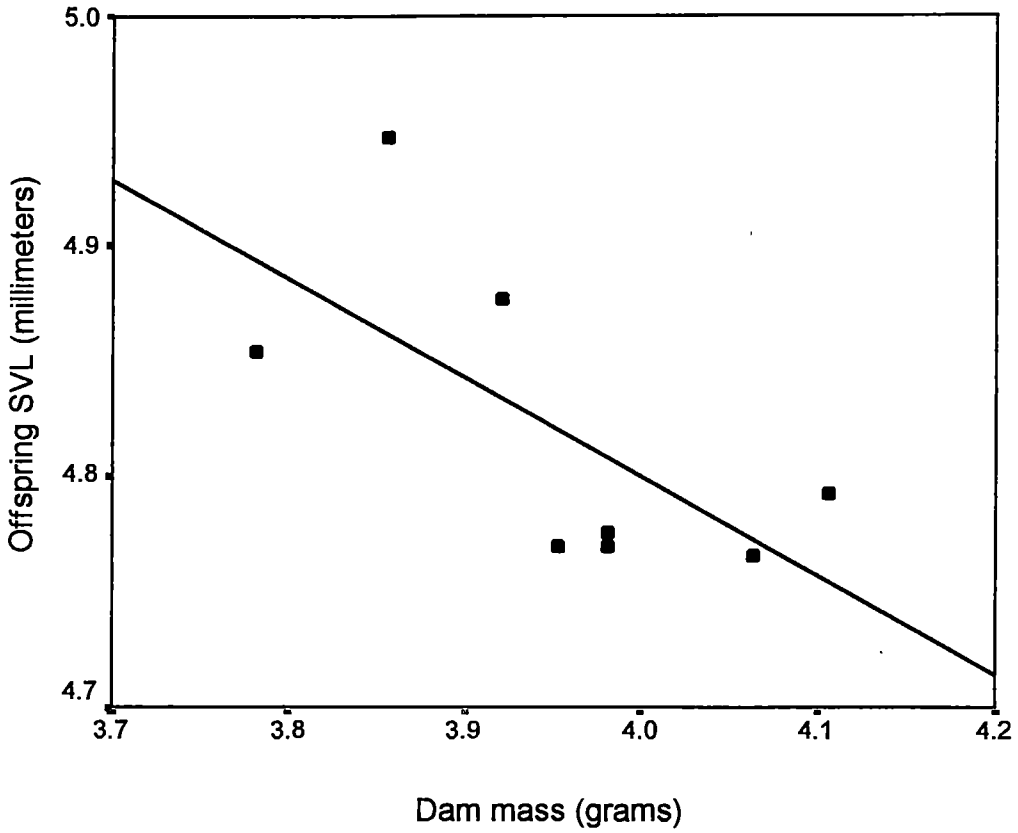


**Figure 3.8** Dam mass versus average SVL of her litter. Correlation not significant ($r^2$ = 0.455, P = 0.071). Regression equation is Dam Mass = 3.7 - 0.66 Offspring SVL.

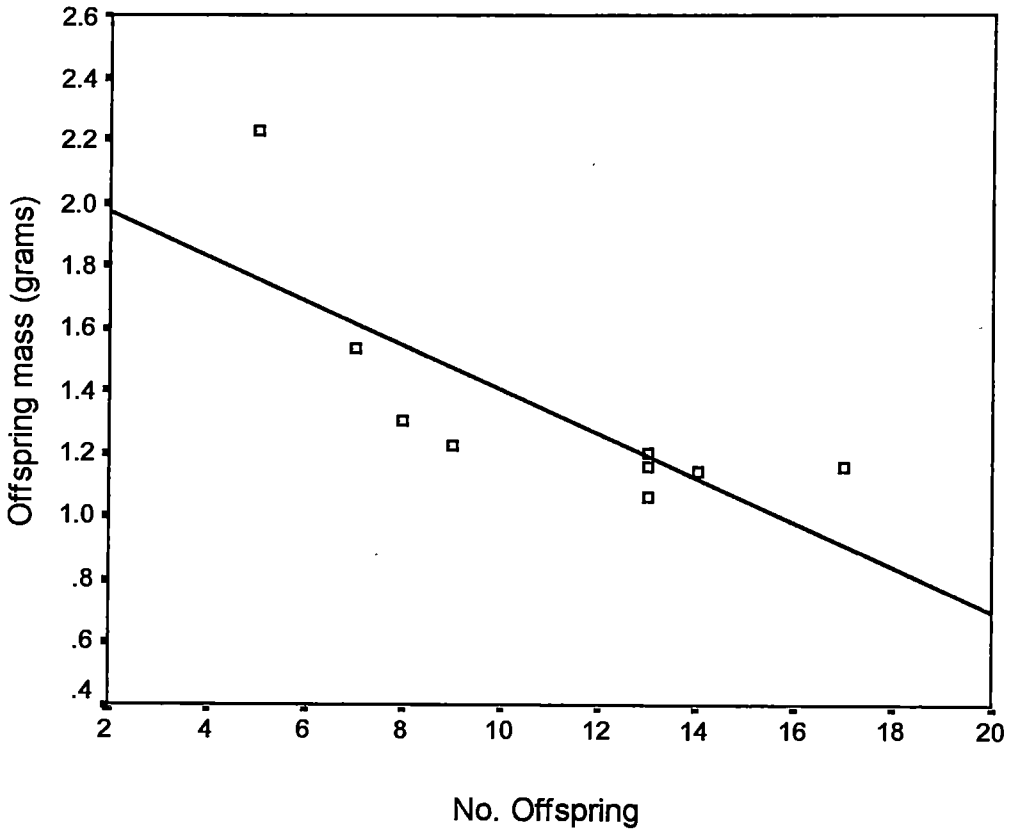


**Figure 3.9**. Offspring mass versus number of offspring in the litter. Correlation is significant ($r^2 = 0.442$, $p = 0.051$). Regression equation is Offspring Mass = 1.1 – 0.76 Number of Offspring.

**Table 3.5**. Regression analyses of average offspring tail length and sex ratio of offspring from each litter

| r | $r^2$ | F | df | p |
|---|---|---|---|---|
| 0.270 | 0.073 | 0.626 | 1,110 | 0.452 |

hatchlings. For this study, Cohort 2000 offspring had larger masses and SVL; however, dams in Cohort 2000 had less offspring per litter than dams in Cohort 1999. Krause (2000) and Ford and Killabrew (1983) also found larger female garter snakes gave birth to more offspring.

Ford and Killabrew (1983) suggest that dam size and litter size interact to determine offspring size. In their study, large female *T. butleri* could have both small and large litters with little effect on offspring size. However, small females were more likely to compromise neonate size when litter size increased. Since garter snakes are viviparous and clutch size is determined at ovulation, the number and size of the neonates are decided in part by the amount of nutrients the dam has available throughout gestation. Thus large females can allot more energy to each young regardless of the number of offspring in that litter (Ford and Killewbew, 1983). This ability would explain the non-significant trend in my data of females with larger masses having offspring with larger masses and SVL.

In my study, litters born early (Cohort 2000) had larger young with less young per litter. Litters born late had a larger number of offspring per litter. This is in contrast to the findings of King (1993) in which litters of viviparous brown snakes consisted of smaller and more numerous neonates if they were born earlier in the summer. Since females do not grow during gestation, King posits females are accumulating resources during gestation to increase offspring size. Perhaps my results do not correspond because Cohort 2000 dams, although not larger than Cohort 1999, may have been in better condition during ovulation. Cohort 2000 animals were in captivity and kept warmer and

better nourished than they normally would have been outside in Wisconsin during early spring.

Characteristics other than size and number of offspring can be influenced by maternal environment in viviparous snakes. Sex hormones may have an effect on the timing and degree to which sexual dimorphic characteristics are expressed (Osypka and Arnold, 2000). Sexual dimorphism in natricine snakes is known to be widespread in adults (Shine, 1993; Shine and Crews, 1988), as well as in neonates (King et al., 1999). Females usually develop larger bodies because fecundity increases with body size (Seigel and Ford, 1987). Conversely, male garter snakes have smaller body sizes because their mating success is not dependent on size (Shine, 1993). King et al. (1999) found male tail length to be longer for other natricine snakes *Nerodia sipedon, Storeria dekayi, T. radix,* and *T. sirtalis*. This was consistent with my full-sib analysis data. Several other researchers and I have found ambiguous results in neonates. Krause (2000) reported *T. sirtalis* males to have longer SVL and tail length, but smaller mass than females. King et al. (1999) found *T. radix* neonatal females to have slightly larger body masses and longer SVLs, whereas *T. sirtalis* males had a longer SVL and equal body masses than females.

I did find head measurements to be consistently larger for females than males in Cohort 2000. Shine and Crews (1988) suggest testosterone has an inhibitory effect on growth of head dimensions. Osypka and Arnold (2000) found levels of sex hormones circulating in the uterine environment could affect sexually dimorphic traits, such as tail length. Although they expected female offspring in litters with a high male to female ratio to have masculinized features (such as increased number of subcaudal scales), the

opposite was found. They suggest female neonates may be able to aromatize testosterone into estrogen, thus resulting in a further feminization of traits.

Although sex differences appear in a full-sib analysis, half-sib (nested) analysis does not indicate such differences. Sex differences may be erroneously found or masked in a one-way ANOVA because of family differences (King et al., 1999). Although I did not find differences using a nested ANOVA, a small sample size may have obstructed any true differences. As with all my other morphological and behavioral data, a more complete pedigree would have provided more insight into any true differences. As King (2001) emphases, dam effects can be attributable to maternal genetic, maternal environmental, and additive genetic effects. Variation in sire-within-dams analysis is due solely to genetic effects. Therefore, the product dam variance minus sire-within-dam variance indicates the degree of non-genetic maternal environment, such as common uterine environment.

# Chapter 4

# Behavior

Both antipredator responses and responses to chemical extracts of prey items have been well studied in the genus *Thamnophis*. These behaviors vary both intra- and interspecifically. Previous studies have reported individual stability in responses to prey (Burghardt 1975; Arnold, 1981) and in defense reactions to threatening stimuli (Herzog and Burghardt, 1988) in several species of *Thamnophis*. Behaviors that are stable within individuals yet vary within a population or litter lend themselves well to studies of habitability (Burghardt, 1993).

## Anitpredator Behavior

### *Introduction*

Predator avoidance is an essential component of the behavior of most animals. Many types of antipredator responses, along with morphological features specialized for defense, have been reported (Greene, 1988). Most notable are the tail rattling of *Crotalus* (Klauber, 1956), the hood spreading of *Naja* (Bogert, 1943), and death feigning of *Heterodon* (Edgren, 1955). The Japanese natricine snake *Rhabdophis tigrinus* has special nuchal glands on its neck with skin covering that breaks fairly easily, releasing noxious cardiac steroid secretions (Mori and Burghardt, 2000). Behaviors such as freezing, fleeing, and tail waving are more widespread among natricine snakes, including *Thamnophis*. Even so, the predisposition to perform antipredator behaviors can vary among and within populations and even within individuals (Greene, 1988). Temperature

(Arnold and Bennett, 1984, Mori et al., 1996, and Hertz et al., 1982) and type of stimulus (Arnold and Bennett, 1984; Greene, 1988; Herzog et al., 1989; Herzog et al., 1992) alter responses to threatening stimuli, but individuals tend to react consistently to a given stimuli (Arnold and Bennett, 1984; Herzog and Burghardt, 1988).

Species within the genus *Thamnophis* exhibit varying types of defensive responses toward predatory stimuli, attributable to disparate ecologies (Herzog et al. 1992). The wide-ranging *T. sirtalis* is a dietary generalist and at birth often acts passively in antipredator tests, but behavior changes over a matter of days. *T. melanogaster*, indigenous to Mexico, is one of the most aquatic garter snakes and behaves quite aggressively, readily biting and striking as a neonate. In contrast, *T. butleri*, a northern terrestrial garter snake, is a relatively passive animal. Some species show ontogenetic shifts, e.g. *T. sirtalis* displays increasing amounts of striking and biting weeks after it is born (Herzog et al., 1992). Herzog et al. (1992) suggest feeding ecology may play a role in this difference. *T. melanogaster* prey upon rapidly moving fish, thus may be more reactive to visual stimuli in general. *T. butleri* feed upon the more easily captured earthworms. *T. radix* and *T. butleri* reacted similarly to threatening stimuli in previous studies (Herzog and Burghardt, 1986; Herzog et al., 1989a). However, only still and non-moving, not touching stimuli was used to elicit responses. Subsequent research has shown *T. radix* will respond with more strikes than *T. butleri* when presented with a touching stimulus.

Variation in antipredator responses is common among different populations of the same species (Schwartz, 1989; Herzog and Schwartz, 1990; and Burghardt and Schwartz, 1999). Herzog and Schwartz (1990) report identical differences in behavior of adult and

neonate snakes of the same species. Interspecific and consistent individual differences give strong evidence that antipredator responses are heritable. Many researchers have demonstrated heritablility (Arnold and Bennett, 1984; Garland, 1988; Schwartz, 1989) and anti-predator responses are correlated with other physiological characteristics such as speed and stamina in *T. sirtalis* (Garland, 1988).

***Methods***

My test procedures were adapted from Herzog and Burghardt (1986, 1988) and Mori et al. (1996). Neonates were tested for defensive responses the day after birth. Each snake was gently removed from its home cage and placed in a test arena (58.5 x 50.5 x 51.5 cm). After the subject was placed in the testing arena, it was left undisturbed for 30 seconds before the threatening stimulus, a human finger, was presented. Herzog et al. (1989b) report that a human finger is as effective in eliciting antipredator behavior as is a model of natural predators. The right index finger was brought within 1 to 2 cm from the snake's snout. For Trial 1 (nonmoving stimulus), the finger was held stationary, but maintained in front of the snout. In Trial 2 (moving stimulus), the investigator presented an oscillating (3 to 4 times a second) index finger. The snake was gently tapped behind the head approximately once every second for Trial 3 (tapping stimulus). Each trial lasted for one minute, and 30 seconds elapsed between each trial (Mori et al., 1996). Instances of "strike", "bite", "flee", "flatten", and "tail wave" were recorded during each trial. These are several salient defensive behaviors common in *Thamnophi* described by other researchers (Arnold and Bennett, 1988; Herzog and Burghardt, 1986; Herzog et al.,

1989; Mori et al. 1996). Striking and fleeing have obvious defensive roles. Body flattening, in which the animal may be increasing apparent size, is a type of bluff. Tail waving is an attempt by the animal to lure predators away from the head (Greene, 1988). This caudal luring is often accompanied by head hiding (Bowers et al., 1993).

*Statistics*

All data were natural log transformed (score+1) in order to reduce heterogeneity of variance. Paired t-tests were performed to evaluate flee and strike behavior differences among the types of stimuli. Then responses across trials were pooled for each stimulus to obtain a single antipredator measure per subject. Analysis of variance was performed to detect any difference between cohorts, sexes, and dams. Sex, dam, and sire-within-dam variations were tested with a nested analysis of variance. Although all snakes were tested in the same fashion, different experimenters performed the trials for Cohort 1999 and Cohort 2000. Therefore, statistical analyses were conducted without comparing cohorts. In addition, bites, which were rare, and were combined with strikes for statistical analyses.

***Results***

Using a full-sib analysis, no sex differences were found for any of the behaviors for either cohort. See Table 4.1 for results of Univariate F-test. For Cohort 1999, tail waves differed among litters ($F = 11.45$, $df = 3$, $p = 0.020$). In Cohort 2000, strikes ($F = 130.95$, $df = 4$, $p < 0.001$) and flees ($F = 2.34$, $df = 4$, $p = 0.033$) showed a significant

**Table 4.1.** Results of Univariate F-test for sex, litter, and sire-within-litter effects. Cohorts are analyzed separately.

| Source of variation | | df | Hypothesis MS | Error MS | F | p |
|---|---|---|---|---|---|---|
| Cohort 1999 | | | | | | |
| Sex | Strike | 1 | 7.17 | 4.51 | 1.59 | 0.220 |
| | Flee | 1 | 4.98 | 17.70 | 0.28 | 0.601 |
| | Flatten | 1 | 0.00 | 0.00 | - | - |
| | Tail Wave | 1 | 0.05 | 19.15 | 0.01 | 0.959 |
| | | 23 | | | | |
| Litter | Strike | 3 | 5.02 | 2.13 | 2.36 | 0.213 |
| | Flee | 3 | 35.02 | 2.59 | 13.51 | **0.015** |
| | Flatten | 3 | 0.00 | 0.00 | - | - |
| | | 70 | | | | |
| Sire within litter | Strike | 4 | 2.13 | 4.51 | 0.47 | 0.756 |
| | Flee | 4 | 2.59 | 17.70 | 0.15 | 0.963 |
| | Flatten | 4 | 0.00 | 0.00 | - | - |
| | Tail Wave | 4 | 5.023 | 19.15 | 0.26 | 0.899 |
| Cohort 2000 | | | | | | |
| Sex | Strike | 1 | 1.57 | 3.28 | 0.57 | 0.459 |
| | Flee | 1 | 6.72 | 18.69 | 0.36 | 0.555 |
| | Flatten | 1 | 0.98 | 2.85 | 0.34 | 0.564 |
| | Tail Wave | 1 | 0.61 | 7.20 | 0.08 | 0.775 |
| | | 26 | | | | |
| Litter | Strike | 4 | 3.73 | 0.03 | 130.95 | **<0.001** |
| | Flee | 4 | 342.97 | 27.76 | 12.34 | **0.033** |
| | Flatten | 4 | 3.04 | 4.48 | 0.68 | 0.651 |
| | Tail Wave | 4 | 20.54 | 4.11 | 5.00 | 0.109 |
| | | 41 | | | | |
| Sire within litter | Strike | 4 | 0.03 | 3.28 | 0.01 | 0.999 |
| | Flee | 4 | 27.75 | 18.69 | 1.57 | 0.249 |
| | Flatten | 4 | 4.48 | 2.85 | 1.57 | 0.228 |
| | Tail Wave | 4 | 4.11 | 7.20 | 0.57 | 0.641 |
| | | 41 | | | | |

litter effect. Figure 4.1 depicts the mean number strikes, flees, flattens, and tail waves for litters in Cohort 1999 (Litter 1290 is not included in half-sib analysis) and Figure 4.2 depicts the mean number of responses for litters in Cohort 2000. Figure 4.3 illustrates strike behavior variation among the different types of stimuli. Snakes in Cohort 1999 tended to strike during the initial still phase (still-moving: $t = 2.270$, $df = 40$ $p = 0.026$; still-touching: $t = 2.34$, $df = 40$, $p = 0.022$). This suggests the animals became habituated to the stimulus. Neonates from Cohort 2000 tended to strike at the touching stimulus (touching-still: $t = 2.008$, $df = 40$, $p = 0.051$; touching – moving: $t = 2.197$, $p = 0.034$).

Both cohorts were more likely to flee during the touching stimulus phase (Figure 4.4). Both cohorts also produced the least number of fleeing behaviors when the moving stimulus was presented. All paired t-tests produced significant results. No neonates in Cohort 1999 performed flattening behaviors, and in Cohort 2000 there were no significant differences in the number of flattening responses among the three types threatening stimuli. Cohort 2000 exhibited far more tail waves when presented with the touching stimulus than either the still ($t = 7.032$, $df = 40$, $p < 0.001$) or the moving stimulus ($t = 6.577$, $df = 40$, $p < 0.001$) (Figure 4.5). The same pattern is exhibited by individuals in Cohort 2000 (touching-still: $t = 8.041$, $df = 69$, $p < 0.001$; touching-moving: $t = 8.061$, $df = 69$, $p < 0.001$) although less tail waves were displayed by this cohort. Neither sex differences nor sire-within-litter differences were found for any antipredator behavior examined (totals across stimuli) (Table 4.1).

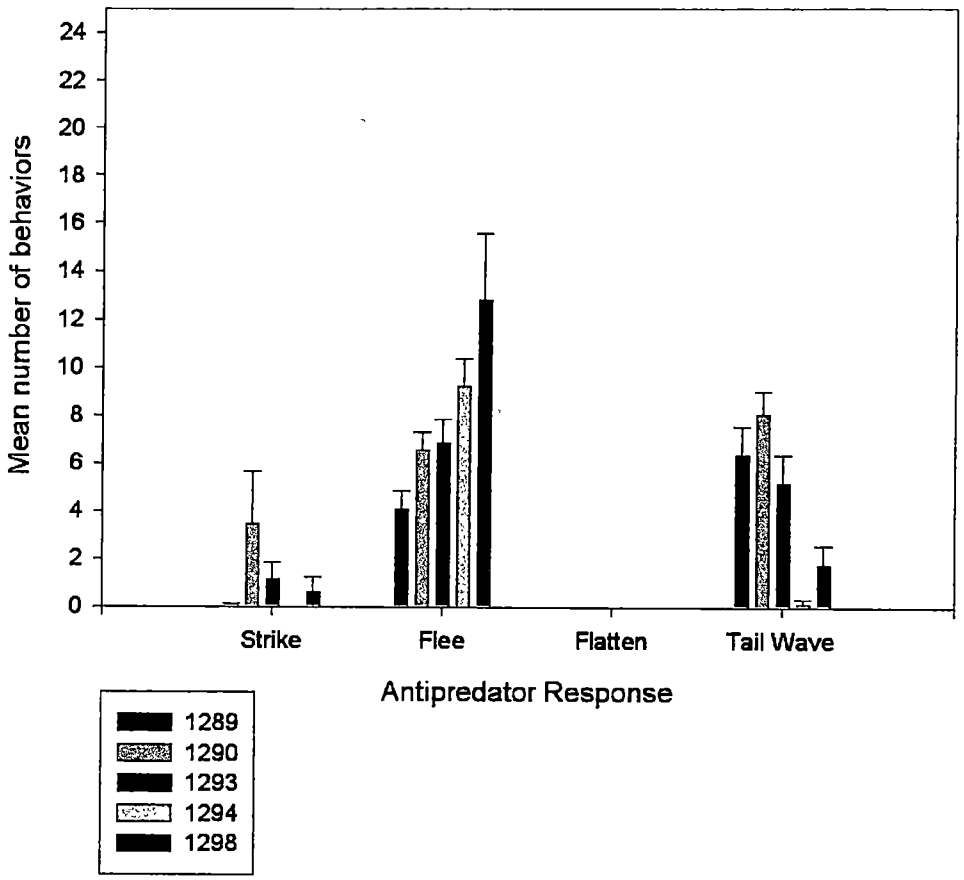


**Figure 4.1.** Mean number of Strikes, Flees, and Tail Waves for Cohort 1999. Flee responses differed significantly among litters ($F = 12.34$, $p = 0.033$).

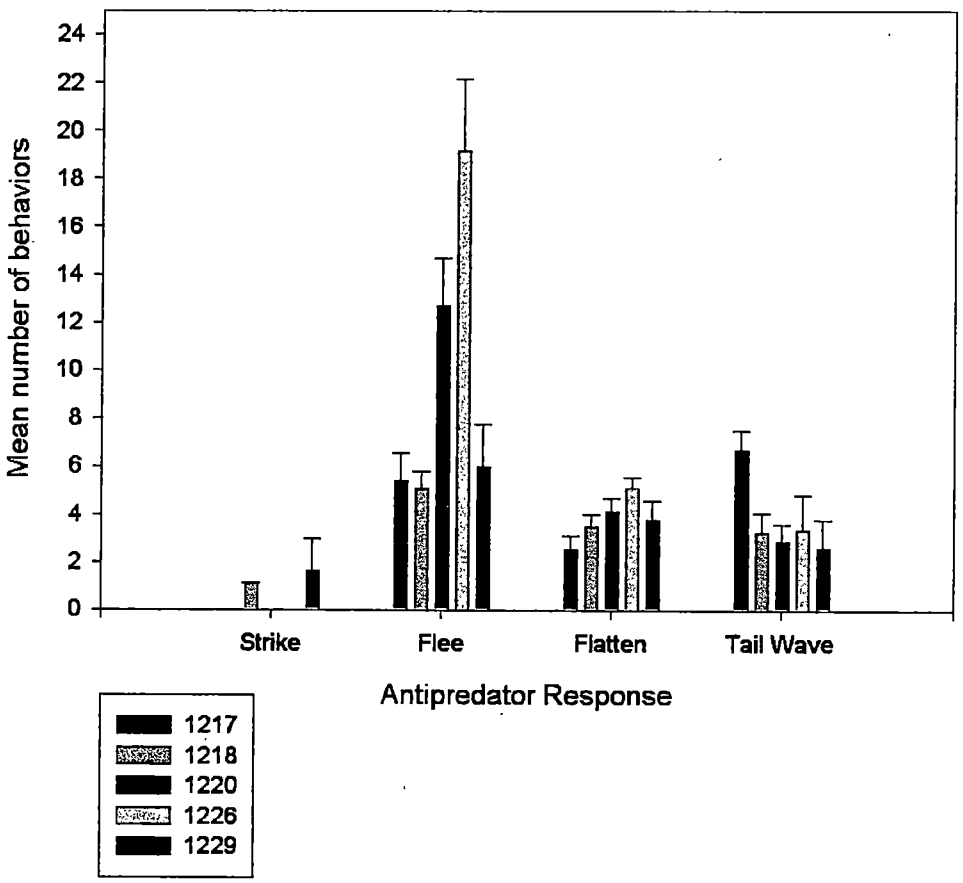


**Figure 4.2.** Mean number of Strikes, Flees, and Tail Waves for Cohort 2000. Strikes (130.95, $p < 0.001$) and flee responses ($F = 12.34$, $p = 0.033$) differed significantly among litters.

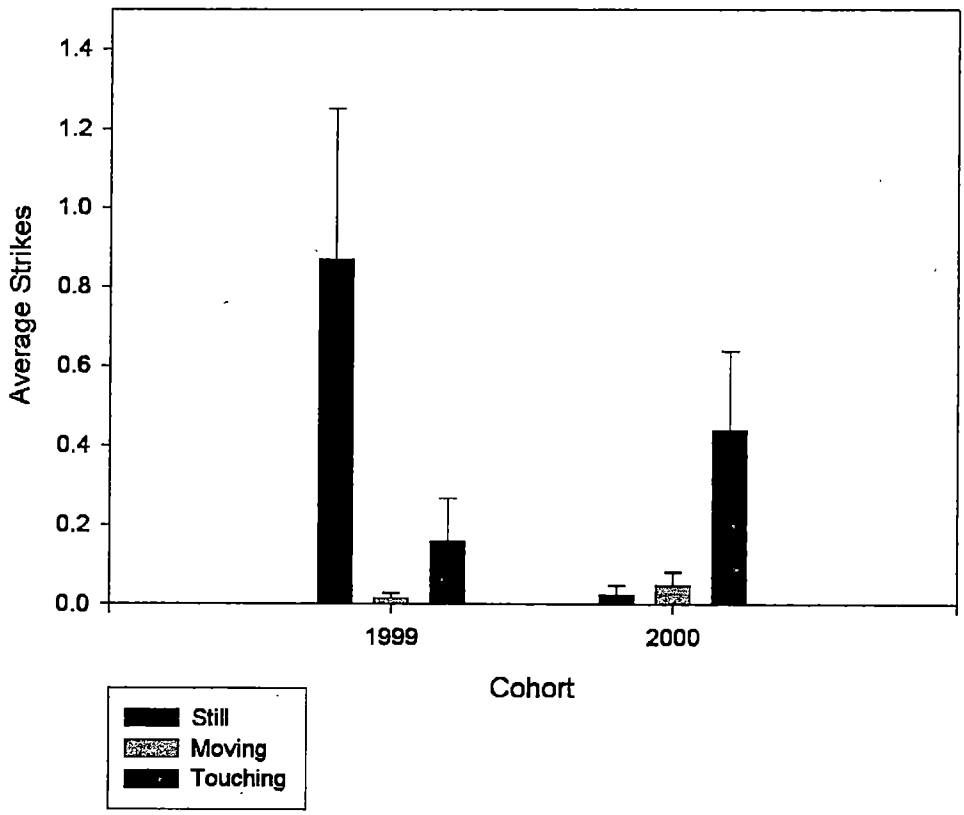


**Figure 4.3.** Average number of strikes elicited during each stimulus presentation. Cohort 1999 struck more during the initial still stimulus, and Cohort 2000 struck most during the final touching stimulus. All differences are significant.

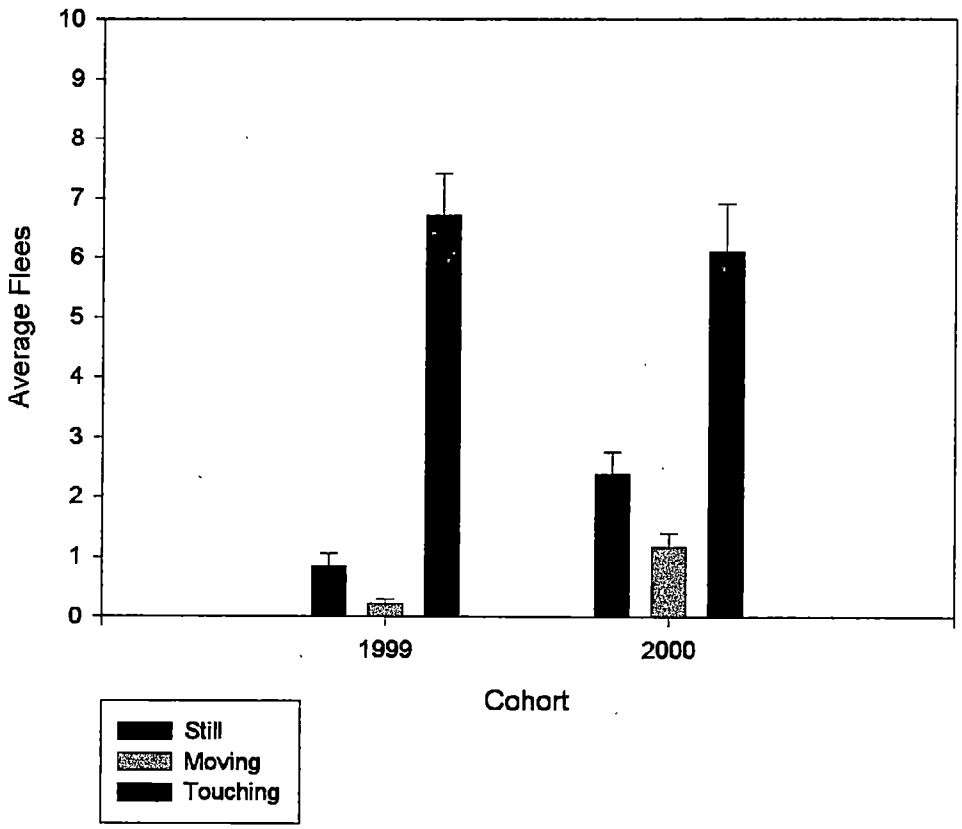


**Figure 4.4.** Average number of flees exhibited by each cohort during each stimulus phase. Both cohorts were more likely to flee during the touching phase. All differences are significant.

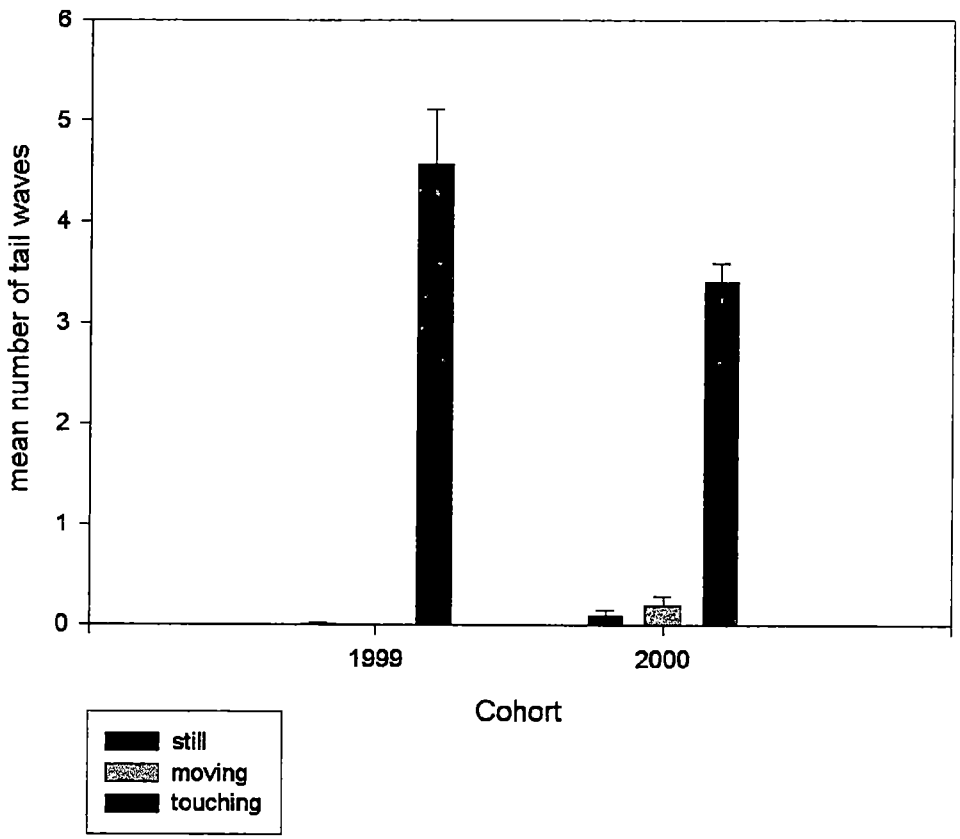


**Figure 4.5.** Average number of tail waves elicited during each stimulus phase by both cohorts. Both cohorts performed the most tail waves during the final touching stimulus presentation. Tail waves during the touching phase are significantly different from both the still and moving stimuli phases.

***Discussion***

My data corroborate many previous studies concerning the defensive behavior in *T. butleri*. Herzog et al., (1989a, 1992) and Herzog and Burghardt (1988) found *T. butleri* direct a low number of strikes and flees towards a threatening stimulus. This pattern seems to continue into adulthood (Herzog and Burghardt, 1986) and may be attributable to *T. butleri*'s consistent feeding ecology: a worm-specialist, in comparison to a piscivore, will not need to pursue prey actively. This energetic adaptation may carry over from predatory to defensive behaviors (Herzog et al., 1992).

I found no sex differences, although male neonate *T. butleri* in Herzog and Burghardt's (1986) studies performed more strikes than females. Evidence did show litter differences in striking and fleeing responses, suggesting a genetic influence on these behaviors. However, I found no sire-within-litter differences using the nested ANOVA. Once again, a larger sample size of offspring with known paternity would possibly show true variation.

Although different researchers tested the cohorts, strikes and flees are distinct behaviors, and I feel comfortable making generalizations about these behavioral patterns due to their distinct nature. Discrete trends in striking behavior appeared for the two cohorts. Cohort 2000 exhibited more strikes to the touching stimulus, which could be considered more threatening. Also, both cohorts exhibited more flees from the touching stimulus. This is consistent with the findings of Mori et al. (1996) in which *Rhabdophis tigrinus* performed more defensive behaviors to the touching than non-touching stimuli. However, Cohort 1999 struck at the initial nonmoving stimulus, but made relatively few

strikes towards the subsequent moving and touching stimuli. Neonatal *T. melanogaster* and some *T. butleri* in the study by Herzog et al. (1989a) demonstrated habituation in defensive behavior, although most *T. butleri* did not. The litter and individual variation within *T. butleri* is further evidence for the role of genetics in antipredator responses.

## Response to Chemical Cues and Live Prey

### *Introduction*

Many of the garter snake's behaviors involve foraging for prey items and the use of chemoreception. Molecules are captured by the tongue and brought to the vomeronasal organ. Pit-like structures in the roof of the mouth have richly enervated epithelia and are responsible for the snake's highly sensitive chemoreception (Burghardt, 1970b). Even neonate snakes that have no feeding experience demonstrate tongue flicking and sometimes attack toward a cotton swab dipped in extracts from surface substances of species-specific prey (Burghardt, 1966, 1967, 1969, 1971). The degree to which they respond to a certain extract usually corresponds with their natural diet (Burghardt, 1969).

Experience can also modify feeding behavior. For instance, feeding experience in *T. sirtalis* can both enhance and inhibit future response to prey (Fuchs and Burghardt, 1971). Furthermore, in a study by Lyman-Henley and Burghardt (1995), *T. sirtalis*, a dietary generalist garter snake, responded strongly to both fish and worm chemical extracts, regardless of previous diet (exclusively fish or worm). The same study showed that ingestively naïve neonate *T. butleri*, earthworm specialist, had higher response to

worm initially, but if fed exclusively fish, would favor fish extract in subsequent chemosensory tests. Nevertheless, response to worm extract remained high in these *T. butleri*. Since there has not been a single report of *T. butleri* eating fish in the field, Burghardt (1993) suggests that this species has retained the ancestral perceptual mechanisms of a dietary generalist.

Intraspecific response differences to chemical extracts have been widely reported and have been found to correspond to the disparate prey consumed by populations (Burghardt, 1993; Gove and Burghardt, 1975; Arnold, 1981; Schwartz, 1989; Arnold, 1992; Arnold and Phillips, 1999). Stable litter and individual differences also occur (Burghardt, 1975; Lyman-Henley and Burghardt, 1995), suggesting chemosensory responses to prey are heritable. Definitive evidence for heritablitity of chemical prey extract and live prey responses in *T. elegans* comes from Arnold (1981) and in *T. sirtatlis* from Schwartz (1989). However, Burghardt et al. (2000) report that initial responses of *T. sirtalis* to are not heritable, but changes in relative preference after feeding experience are heritable. Neither Arnold (1981) nor Burghardt (1971) found that maternal diet affects offspring chemical or prey preferences.

***Methods***

Techniques used in the chemical prey preference component of the study were taken from Burghardt (1975, 1992). Subjects consisted of all neonate garter snakes, tested 10 days after birth and prior to the first feeding, which occurred approximately 10 to 14 days after birth. Adults were not subjected to chemical preference tests.

*Extract Preparation*

Extracts were made by placing three grams of prey items (misquito fish or leafworm) in 10 milliliters of distilled water. The mixture was allowed to heat for 2 minutes at 60°C. The supernant was poured off and centrifuged at 2500 rmp for 10 minutes. Extracts were kept frozen (-4°C) until use (Burghardt, 1969).

*Chemical Test Procedure*

Each snake was exposed to a control (distilled water), fish extract and worm extract in the following order: control, worm, fish, control, fish, worm. At least 20, but no more than 30, minutes elapsed between stimulus presentations. The extracts were presented to the subjects on cotton swabs placed approximately one centimeter from the snakes' snouts. The number of tongue flicks toward the swab and total number of tongue flicks made in 30 seconds was recorded for each snake and each stimulus. If a snake attacked the swab, the trial ended and attack latency was noted. Occasionally an individual would not tongue flick during stimuli presentation. In this instance, the snake was touched on the snout with the swab after 10 seconds.

*Scoring*

The tongue-flick attack score (TFAS) used in this study was first developed by Burghardt (1969). TFAS determines strength of response to a stimulus by combining both number of tongue flicks and attack latency into one index (Cooper and Burghardt,

1990). The TFAS is based on the premise that an attack is a stronger predatory response than any amount of tongue flicking (Burghardt, 1970a). If no attack occurs, the TFAS was equal to the number of flicks in the 30-second trial. If the snake did bite, the TFAS was the attack latency subtracted from 30 seconds, and this result was added to the maximum number of tongue-flicks the snake emitted to any of the stimuli (Cooper and Burghardt, 1990).

*Prey Choice Test Procedure*

Each snake was given a choice of earthworm or fish for the first feeding. Individuals were tested in their cages. The prey items were placed in small petri dishes and presented to the snakes simultaneously. I watched the trial for the first hour and recorded any prey item(s) consumed and attack latency within that hour. I also noted prey items consumed by each snake after 2 hours and 24 hours of initial prey presentation.

*Statistics*

As with the morphology and antipredator behaviors, all TFAS scores were natural log transformed (score + 1). For the chemical prey, preference was measured by TFAS to the first presentation of worm and fish, averaging TFAS for the two presentations, and by subtracting fish TFAS from worm TFAS for each individual (W-F). This latter index reflects the direction and strength of chemical prey preference. A repeated measures ANOVA was performed to detect any order effect for stimuli presentation. A repeated measures ANOVA was used to identify significant differences between test stimuli (fish

and worm) and the control (water) and litter effects. Full-sib analysis was performed for detecting cohort, sex, and dam differences on the W-F score. Also, sex, dam, and sex-within-dam differences were tested with half-sib (nested) analysis. Analyses were performed using both TFAS from the 1st presentation of each extract and the average TFAS of the two presentations. In addition, difference in number of attacks each individual made toward a swab was evaluated with a chi-square test. Live prey choice preference was compared with chemical extract tests with a binomial and chi-square test.

***Results***

Overall, the snakes exhibited higher average TFAS scores toward worm than fish stimulus, although not significantly ($t = 1.39$, $df = 1,111$, $p = 0.16$). Table 4.2 reports MANOVA results for cohorts. Cohort 1999's worm TFAS (both average and 1st stimulus presentation) was higher than Cohort 2000. Cohort 1999 also had a higher fish TFAS (1st presentation) than Cohort 2000 ($F = 4.94$, $df = 1,110$, $p = 0.036$). There was no difference in Worm TFAS – Fish TFAS score. Cohort 1999 attacked the swab more frequently than Cohort 2000 ($\chi^2 = 9.03$, $df = 3$, $p = 0.026$). Only Litter 1229 in Cohort 2000 exhibited any attacks.

Because the snakes were exposed to each stimulus in two different trials, a nested repeated measures ANOVA was performed to detect any differences in response to each stimuli between trials. Figure 4.6 demonstrates an overall decreased responsivity from the first round of stimuli presentation to the second round, although only the worm TFAS differed significantly ($F = 2.23$, $df = 9,102$, $p = 0.026$). For each stimuli, litters differed

**Table 4.2.** Cohort effects on TFAS and Worm TFAS – Fish TFAS for both the average of two stimuli presentations and the 1st presentation. Difference between average of presentation and 1st presentation did not significantly differ.

| | df | MS | Error | F | p |
|---|---|---|---|---|---|
| Av. Worm TFAS | 1 | 7.98 | 0.99 | 8.03 | **0.005** |
| Av. Fish TFAS | 1 | 11.50 | 0.850 | 13.52 | **<0.001** |
| Av. Worm TFAS- Av. Fish TFAS | 1 | 9.63 | 99.29 | 0.097 | 0.756 |
| Worm TFAS (1st presentation) | 1 | 5.61 | 1.56 | 4.85 | **0.030** |
| Fish TFAS (1st presentation) | 1 | 4.96 | 1.10 | 4.94 | **0.036** |
| 1st Worm TFAS – 1st Fish TFAS | 1 | 0.02 | 1.16 | 1.16 | 0.893 |
| | 110 | | | | |

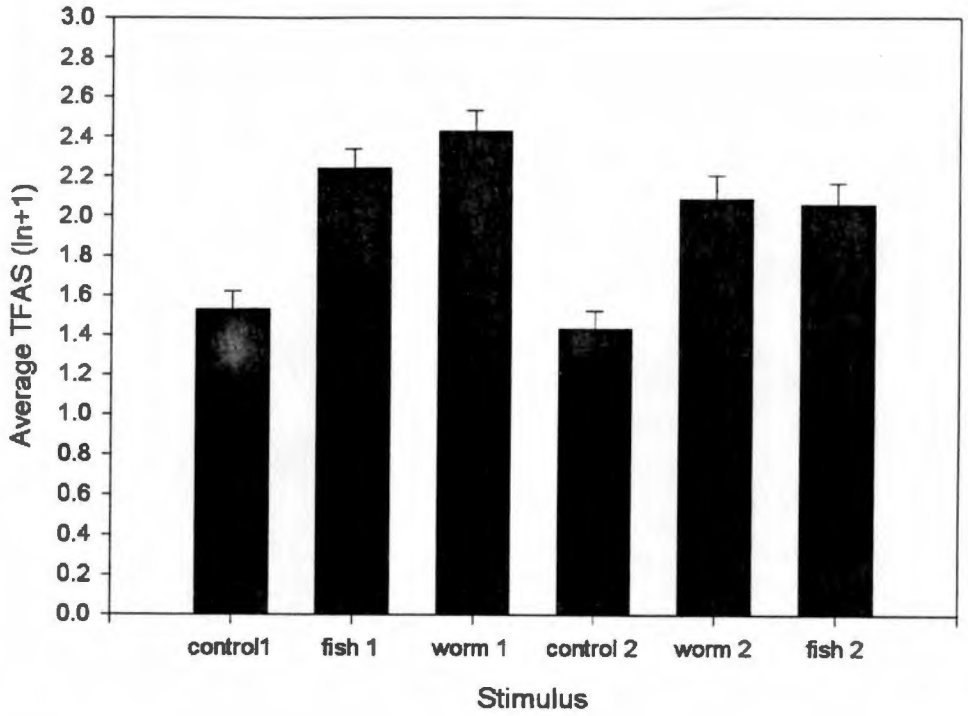


**Figure 4.6.** TFAS for each stimulus presentation. Repeated measures ANOVA found only worm scores differed between the 1st and 2nd presentation ($F = 2.23$, $df = 9,102$, $p = 0.026$). There was a litter effect for each extracts stimulus presentation: (worm: $F = 3.28$, $df = 9, 102$, $p = 0.002$; fish: $F = 3.33$, $df = 9, 102$, $p = 0.001$; control: $F = 2.69$, $df = 9, 102$, $p = 0.008$).

in the degree to which order effected their response. Fish and worm TFAS significantly varied from the control TFAS (fish-control: $t = 6.89$, $df = 111$, $P = <0.001$; worm-control: $t = 7.66$, $df = 111$, $P = <0.001$). As in the defensive test, the snakes seem to have become habituated to the extracts. Table 4.3 reports Univariate F-test results for sex, litters, and sire-within-litters. Using a full-sib analysis, litter differences were found only for worm and fish TFAS for both the first stimulus presentation and average of the presentations (See Figure 4.7). Although 9 of 10 litters had a positive W-F score ($\chi^2 = 13.0$, $df = 1$, $p < 0.001$), I found no discernable litter differences in W-F score, indicating no strong preference for either stimulus in these neonates (Figure 4.8). There were no sex differences for worm TFAS, fish TFAS, or W-F score. Nested analysis did not find TFAS or W-F score differences between sexes or among dams (litters). For the live prey choice test, 60% of the snakes ate the worm first and only 8% consumed the fish first. In Cohort 1999, most (65%) neonatal *T. butleri* consumed only the worm ($\chi^2 = 0.003$, $df = 1$, $P <0.001$). Only one of the five snakes in this cohort that did eat the fish ate the worm first. Snakes in Cohort 2000 were most likely not to eat any of the prey items ($\chi^2 = 9.44$, $df = 1$, $0.05 < p <0.001$).

Although many Cohort 2000 neonate snakes chose neither prey, those that did eat chose fish more often than litters from Cohort 1999. In fact, 7% of Cohort 1999 snakes and 29% of Cohort 2000 snakes ate fish. Five of the seven fish-eating snakes in Cohort 2000 consumed the fish before the worm. The most common eating practice of both males and females was to consume worms only, as compared to eating of fish only, fish and worms, or neither prey item. Only litters 1226 and 1229 (Cohort 2000) did not show a significant preference for worms.

**Table 4.3**. Results of Univariate F-test analyzing sex, litter, and sire-within-litter differences in TFAS. Both the average and 1st presentation scores are given. Only litter differences in Worm TFAS were significant.

| Source of variation | | df | Hypothesis MS | Error MS | F | p |
|---|---|---|---|---|---|---|
| Sex | Av.Worm TFAS | 1 | 1.30 | 0.88 | 1.48 | 0.231 |
| | Av. Fish TFAS | 1 | 1.41 | 1.04 | 1.35 | 0.251 |
| | Av.Worm TFAS-Av.Fish TFAS | 1 | 100.56 | 106.29 | 0.94 | 0.336 |
| | 1st Worm TFAS | 1 | 1.30 | 0.88 | 1.48 | 0.231 |
| | 1st Fish TFAS | 1 | 1.41 | 0.05 | 1.35 | 0.251 |
| | 1st Worm TFAS-1st Fish TFAS | 1 | 0.00 | 1.59 | 0.00 | 0.970 |
| | | 45 | | | | |
| Litter | Av.Worm TFAS | 8 | 366.45 | 33.84 | 10.83 | **0.003** |
| | Av. Fish TFAS | 8 | 317.35 | 145.12 | 2.19 | 0.159 |
| | Av. Worm TFAS-Av.Fish TFAS | 8 | 32.37 | 83.21 | 0.39 | 0.895 |
| | 1st Worm TFAS | 8 | 2.16 | 0.29 | 7.41 | **0.008** |
| | 1st Fish TFAS | 8 | 1.09 | 0.97 | 1.12 | 0.446 |
| | 1st Worm TFAS-Fish TFAS | 8 | 1.36 | 0.79 | 1.72 | 0.245 |
| | | 111 | | | | |
| Sire-Within Litter | Av.Worm TFAS | 7 | 33.84 | 111.30 | 0.30 | 0.948 |
| | Av. Fish TFAS | 7 | 145.12 | 90.62 | 1.60 | 0.160 |
| | Av.Worm TFAS-Av. Fish TFAS | 7 | 83.21 | 106.29 | 0.78 | 0.605 |
| | 1st Worm TFAS | 7 | 0.29 | 0.88 | 0.33 | 0.936 |
| | 1st Fish TFAS | 7 | 0.968 | 1.05 | 0.92 | 0.497 |
| | 1st Worm TFAS-1st Fish TFAS | 7 | 0.79 | 1.59 | 0.50 | 0.832 |
| | | 45 | | | | |

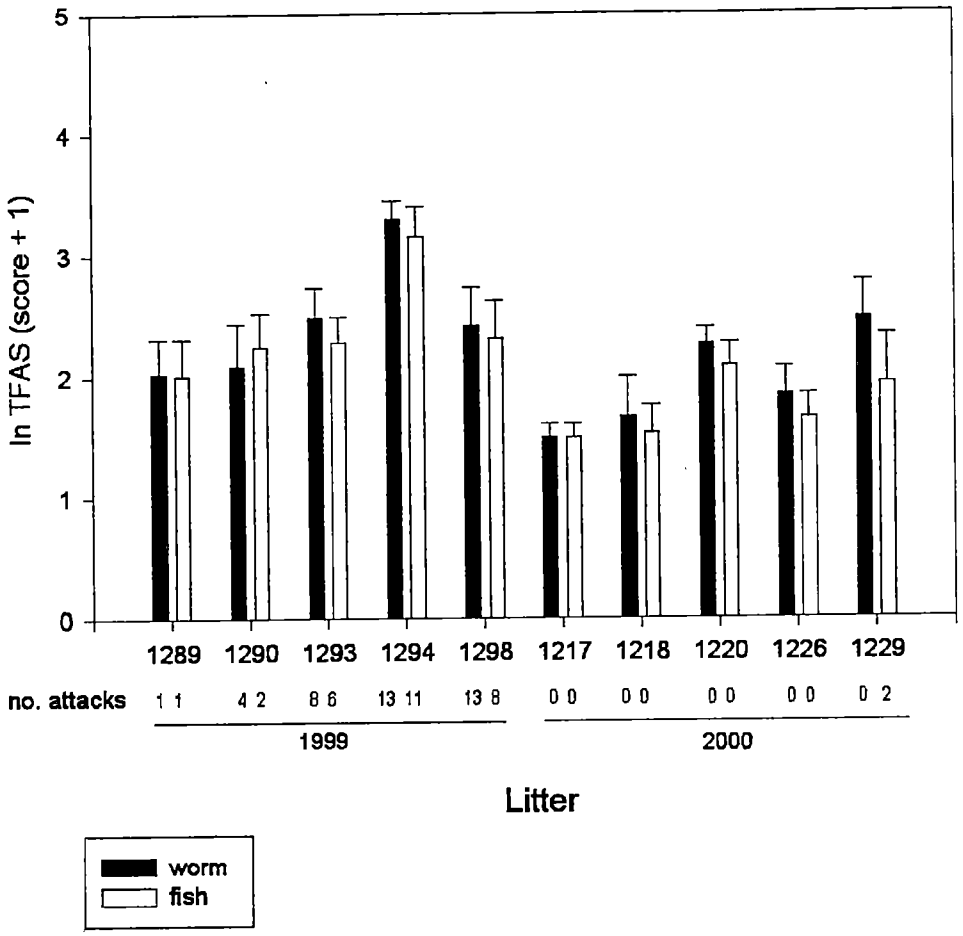


**Figure 4.7.** Average TFAS (ln score +1) and number of attacks toward each stimulus by litter. Cohort 1999 = 1289,1290,1293,1298. Cohort 2000 = 1217, 1218, 1220, 1226, 1229.

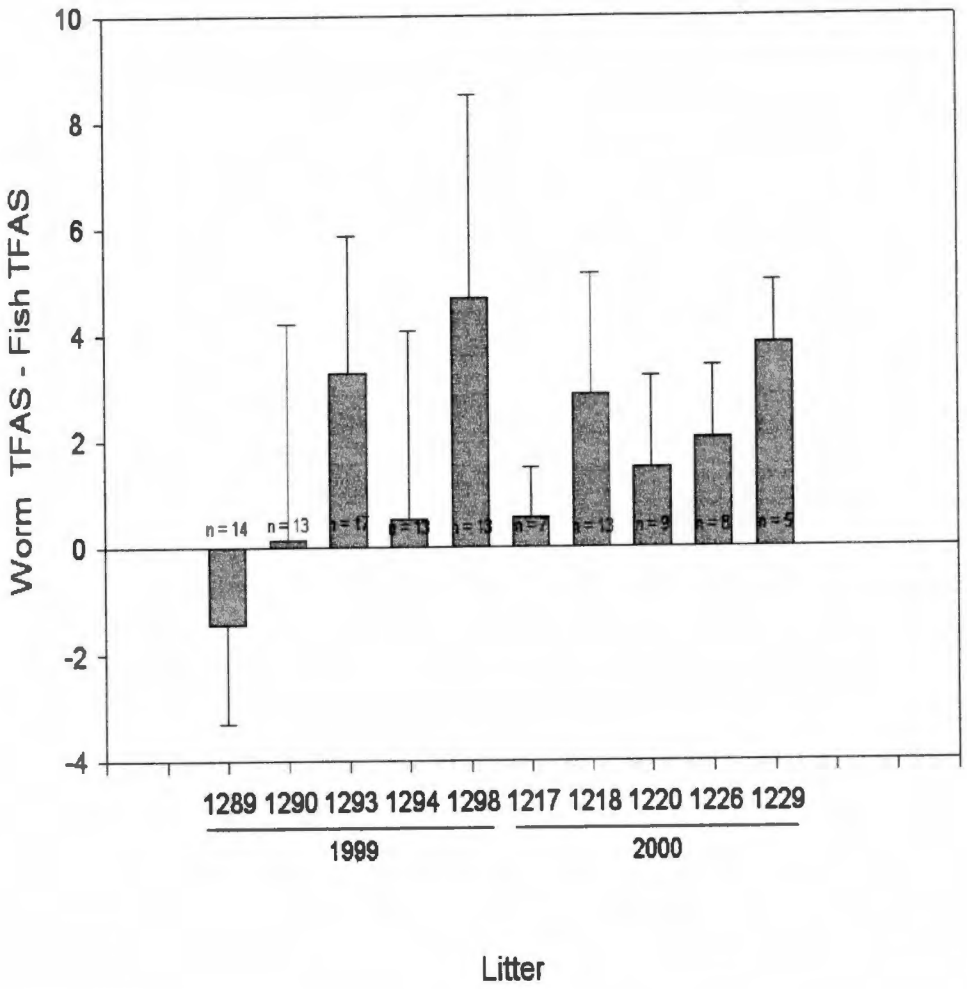


**Figure 4.8.** Average Worm TFAS – Average Fish TFAS. Sample size is given in the bars. No differences are significant.

*Comparison of Chemical Prey Responses and Live Prey Preferences*

A binomial test indicated that responses to chemical extract of prey and prey items consumed first are not necessarily related in neonate *T. butleri* (Figure 4.9). In the snakes that had a larger TFAS score for worms, there was a non-significant preference for worm ($n = 64$, $p = 0.061$). Snakes that responded more strongly to fish extracts ($n = 9$) displayed no preference for fish or worm either ($p = 0.508$). A small sample size for this group could be masking any significant preferences. Table 4.4 reports the first prey choice of individuals in each litter.

A chi-square test revealed a significant preference for live worms as the first meal regardless of TFAS preference for both sexes. Both cohorts were more likely to consume worms regardless of TFAS (Cohort 1999: $\chi^2 = 62.76$, $df = 1$, $p < 0.001$). Moreover, both sexes and cohorts tended to have both a chemical extract and live prey preference for worm. The trend is weaker in Cohort 2000, probably due to a lack of consuming either prey during the live prey choice test. Nevertheless, 17% (4 of 23) of Cohort 2000 neonates chose fish as their first meal whereas only 5% (2 of 46) of those in Cohort 1999 preferred the worm.

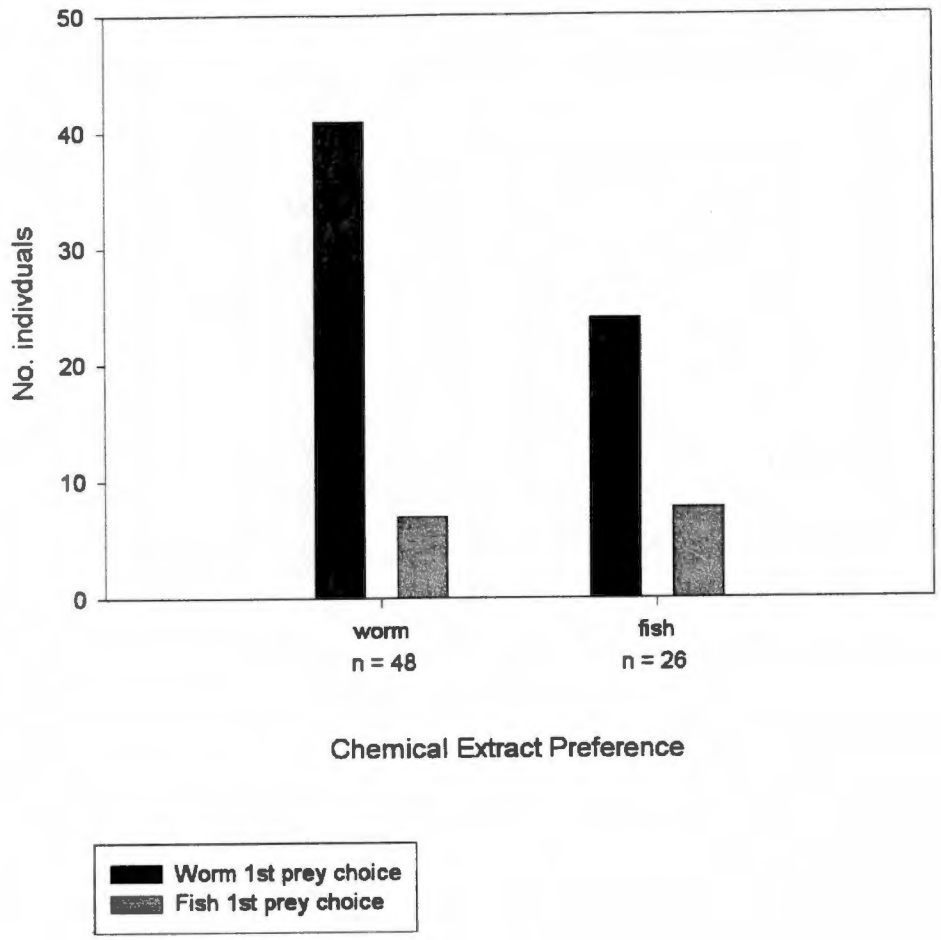


**Figure 4.9**. First prey item consumed by neonates preferring either worm of fish chemical extracts.

**Table 4.4**. Number of individuals choosing to consume worm, fish, or neither as first prey choice.

| Litter | Worm | Fish | None |
|---|---|---|---|
| 1289 (n=14) | 12 | 0 | 2 |
| 1290 (n=13) | 9 | 0 | 4 |
| 1293 (n=19) | 13 | 1 | 5 |
| 1294 (n=13) | 9 | 3 | 1 |
| 1298 (n=13) | 10 | 0 | 3 |
| 1217 (n= 7) | 4 | 0 | 3 |
| 1218 (n=13) | 9 | 0 | 4 |
| 1220 (n= 9) | 2 | 2 | 5 |
| 1226 (n= 7) | 2 | 1 | 4 |
| 1229 (n= 5) | 3 | 2 | 0 |

*Attack comparisons*

Individuals in Cohort 1999 (mean 0.94 ± 0.14) were more likely to attack the swab than those in Cohort 2000 (mean 0.05 ± 0.05). Cohort 1999 also struck more during defensive testing, however, this is a weak relationship. Only 4 of 111 individuals attacked during the defensive tests and the chemical extract tests.

***Discussion***

Although some studies have found *T. butleri* have a significantly higher TFAS for worm than fish (Lyman-Henley and Burghardt, 1995), this was not the case with my data in general. Burghardt (1967, 1969) also found little difference in neonatal *T. butleri* reaction toward fish and worm extracts. This species' chemical prey preference is also

more liable to feeding experience than *T. sirtalis*, a dietary generalist (Lyman-Henley and Burghardt, 1995). Although these snakes from north Milwaukee showed no strong preferences for chemical extracts of prey, there was a strong preference for live worms meals. However, data show snakes from the suspected hybrid zone in southern Milwaukee will eat live fish more readily (Burghardt, unpublished).

This apparent contradiction may be a result of the retention of ancestral perceptual mechanisms and constraints of morphology posed by Burghardt (1993). In other words, *T. butleri* has retained its generalist ancestral ability to recognize fish as a food source, but is limited to consuming worms because its small head and slow nature do not facilitate capturing quick prey such as fish (Burghardt, 1969). Prey preference for worm and fish are independent of one another. Studies have supported the idea of separate receptor mechanisms for recognition of different prey species (Burghardt, 1992) and indicate discrete neural tissue may be devoted to detection of each (Burghardt, 1993). Arnold (1981) and Schwartz (1989) found that chemical extract preferences are heritable. For the *T. bulteri* in my study, only worm TFAS showed significant litter differences, suggesting response to worm is a heritable trait. However, Burghardt et al. (2000) suggest response to fish is the more heritable characteristic in *T. sirtalis*.

# Chapter 5

## Conclusions

Multiple paternity was detected in 55% to 80% of the litters in my study population of *Thamnophis butleri*. It is unlikely copulatory plugs prevent multiple matings in breeding seasons, although I could not rule out fall matings in this sample of litters. Future studies might include more controlled mating studies in which females are allowed to mate in the fall and kept isolated in captivity to prevent mating the following spring. Any offspring would thus been sired by sperm from the fall. Also, observance of the order in which males mate with specific females and then analyzing paternity would be helpful to determine the effects of this order. For instance, such procedures may help resolve the issue of which male has the largest proportion of offspring, i.e. the male who mates first, last, or somewhere in between. Perhaps it is not order that determines proportion of offspring, but males with certain characteristics that win the sperm competition.

I did not find evidence to support the hypothesis that maternal half-sibs have less variation among characteristics than full-siblings. Several factors contributed to the incomplete pedigrees in my study. This incomplete knowledge of paternity has negative implications, such as concealing real trait variation in maternal half-siblings. First, poorly isolated DNA probably caused the lack of amplification experienced in several individuals. Also, the microsatellite loci I was able to amplify did not show much allelic diversity. Therefore many sires might carry the same genotype at several loci, masking

true sireship. I could only identify a maximum of two sires per litter, but triple paternity has been documented in *T. sirtalis* (McCracken et al., 1999)

Although I never found any sire-within-dam effects, litter differences were found for several morphological and behavioral traits. Litter effects, in part, may be the result of the heritable nature of traits. According to my findings, body mass, body length, and jaw length vary among some litters. In the antipredator tests, propensity to strike, flee, and tail wave when presented with a threatening stimulus also differed among litters. Response to worm chemical extracts and to live fish prey items varied among litters and therefore, may also have a genetic influence.

Evidence suggests that some morphological and behavioral traits may have a genetic correlation. Garland (1988) found aggressive antipredator behaviors to be correlated with high endurance in *T. sirtalis*. Brodie (1989) reports *T. ordinoides* with stripes tend to flee from predators whereas those with spots will exhibit more cryptic, passive behaviors. *T. butleri* have similar coloration, patterning, and life history to *T. ordinoides* and also elicited fleeing as the primary defense behavior. According to Brown (1931), it is difficult for predators to focus on snakes with longitudinal stripes and uniform coloration.

Morphology may be affecting foraging behavior as well. Snakes with larger and longer heads are better suited to consuming larger prey items (Pough and Groves, 1983; Forsman, 1991). In my study, neonates with larger heads, although not larger relative head size, consumed more fish. The larger size of these snakes combined with the fact the mosquito fish were confined to small petri dishes suggests these neonates were able to subdue the quick prey more easily than the smaller snakes. No instance of *T. butleri*

consuming fish in the field has ever been reported. This dietary specialization could be due to both inability to capture fish, or the lack of fish and abundance of earthworm in *T. butleri*'s damp microhabitat (Carpenter, 1952). My thesis data and other studies (Burghardt, 1967, 1969, Lyman-Henley, 1995) have proven *T. butleri* does have the perceptual mechanisms in place to recognize fish as a food source, but has somehow become detached from the species' actual consumption of fish.

Also, snakes that ate fish in the live prey preference test also had higher rates of fleeing in defensive tests. This supports the hypothesis that piscivorous snakes exhibit more active behaviors because they must expend more energy to capture fish (Herzog et al., 1986, 1989). Perhaps those snakes that ate only worms did not have energetic capacity to capture the more active fish. Therefore, it can be inferred that not only the preference for fish, but also the metabolic capacity required to subdue fish, are heritable.

One litter, 1229, is particularly noteworthy. It consisted of only five offspring with an average mass of 2.2 grams. The next heaviest litter averaged only 1.5 grams. Neonate Litter 1229 possessed relatively larger heads, heavy labial barring, distinct post-cranial cresents, and a slightly orange hue to the dorsal stripes, all characteristic traits of *T. radix* (Rossman et al., 1996). Individuals in litter 1229 were quite aggressive, averaging not only the highest number of strikes in the defensive test, but also striking at research assistants performing routine cleaning or simply walking past the snakes' cages. During the prey choice test, four of the five snakes ate the fish prey item, the highest percentage in any litter analyzed. Chemoreceptive and defensive testing later in the snakes' development could be quite telling. For instance, *T. radix* has a defensive ontogeny than *T. butleri*..

Although this litter is from a "pure" *T.butleri* area in north Milwaukee, Wisconsin, the characteristics described above are characteristic of the parapatric *T.radix*. This similarity could be because the occurrence of "gene swamping," resulting in *T. butleri – T. radix* hybrids, as suggested by morphological evidence in southern Milwuakee. Further genetic evidence could confirm the presence of the hybrids. Both mitochondrial DNA and microsatellite DNA studies are underway at the present time. Thus, utilization of *T. butleri* as a model in quantitative genetic studies allows us to simultaneously gather valuable morphological and behavioral data that may eventually save the species from complete extirpation.

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# Appendices

## Appendix I.

Adult *T. butleri* used to calculate allele frequencies

| subject # | Ts2 | Ner 2 | Ner 3 | Ner 9 |
|---|---|---|---|---|
| 89 | 152/124 | 154/152 | 177/161 | 227/217 |
| 90 | 146/142 | 154 | 177/169 | 227 |
| 93 | 156/142 | 154 | 173/169 | 227 |
| 94 | 152/124 | 154/152 | 173/169 | 227 |
| 98 | 152/146 | 154/152 | 173/169 | 227 |
| 17 | 124 | 154/148 | 177/157 | 227 |
| 18 | 152/142 | 148 | 165 | 227/201 |
| 20 | 156/142 | 150/148 | 189/169 | 227 |
| 26 | 142 | 148 | 177/173 | 227/197 |
| 28 | 142 | 148/144 | 177/173 | 227 |
| 29 | | 148/146 | | |
| 29 | 152/118 | 148/145 | 173/165 | 227 |
| 30 | 142 | 172/154 | 165 | 227 |
| 31 | 124 | 154 | 173/169 | 227/197 |
| 32 | 152/124 | 148 | 169/157 | 227/201 |
| 33 | 142 | 144 | 176 | 227 |
| 34 | 146/124 | 154 | 165/157 | |
| 35 | 144/140 | 154/148 | 173/169 | 213 |
| 36 | | 148/145 | | 227 |
| 37 | 148/124 | 148 | 165 | 227 |
| 38 | 148/124 | 148/145 | 173/157 | 227/201 |
| 39 | 148/118 | 176 | 177/173 | 227 |
| 40 | 124 | 152/148 | 173/169 | 227 |
| 41 | 146/142 | 154/148 | 173/165 | |

Appendix II

Microsatellite DNA data: alleles for dams and offspring

| subject # | Ner 2 | Ner 3 | Ner 9 | Ts 16B |
|---|---|---|---|---|
| 1289 | 154/152 | 177/161 | 227/217 | 152/124 |
| 89A | 150/152 | 165/161 | 227 | 152/124 |
| 89B | 154/152 | | | 152 |
| 89C | 160/154 | 177/161 | | 152/124 |
| 89F | 152/150 | 177/161 | 227/217 | 152/124 |
| 89G | 154/150 | | | |
| 89H | 160/152 | 161 | | 152/142 |
| 89I | 154/150 | 177/161 | 227 | 152/124 |
| 89L | 154/150 | 177/169 | 227 | 152/124 |
| 89N | 154/150 | 161 | 227 | 152/142 |
| 89O | 160/154 | 169/161 | 217 | 152/124 |
| 89P | 160/152 | 161 | 227 | 152/124 |
| | | | | |
| 1290 | 154 | 177/169 | 227 | 146/142 |
| 90A | 154 | 169 | 227/217 | 156/118 |
| 90B | 154/146 | 169 | 227/217 | 156/146 |
| 90C | 146 | 169 | 227 | 156/142 |
| 90D | 152/146 | | 227 | |
| 90E | 154 | 169 | 227 | 156/118 |
| 90F | 154 | 169 | 227 | 156/118 |
| 90G | 154/146 | | 227 | 156/142 |
| 90H | 154 | | 227 | 156/118 |
| 90I | 154 | 169/165 | 227/217 | 156/146 |
| 90J | 154/152 | 173/165 | 227 | 156/118 |
| 90K | 154 | | | |
| 90L | 154 | 173/169 | 227/225 | 156/152 |
| 90M | 154 | 169/165 | 227/217 | 152/146 |
| | | | | |
| 1293 | 154 | 173/169 | 227 | 156/142 |
| 93B | 154 | 169 | 201 | 156 |
| 93C | 154 | 169 | 227 | 156/152 |
| 93D | 154 | 169 | | 156/142 |
| 93F | 154 | | | 142/124 |
| 93G | 154/146 | 173/165 | 227/217 | 152/118 |
| 93H | 162/154 | 173/169 | 227/225 | 152/142 |
| 93I | 162/154 | 173/169 | 225 | 152/142 |
| 93J | 162/154 | 173/169 | 225 | 156/152 |
| 93K | 154 | 173/169 | 225 | 142/118 |
| 93L | 154 | 181/173 | 227/201 | 142/118 |
| 93M | 154 | 181/173 | | 156 |
| 93O | 154 | 173/169 | 227 | 156/118 |
| 93Q | 162/154 | 173/169 | 227/201 | 152/142 |

Appendix II (continued)

| subject # | Ner 2 | Ner 3 | Ner 9 | Ts 16B |
|---|---|---|---|---|
| 1294 | 154/152 | 173/169 | 227 | 152/124 |
| 94A | 152 | 173/169 | 227 | 152 |
| 94B | 152 | 173/165 | | 152/118 |
| 94C | 154/152 | 173/169 | | 152/118 |
| 94D | 154/152 | 173/169 | 227 | 152/118 |
| 94E | 154/152 | 173/169 | 227 | 152 |
| 94F | 154 | 173/165 | | 152/118 |
| 94G | 154/152 | 181/173 | 201 | 124/118 |
| 94H | 154/152 | 177/161 | | 152/124 |
| 94I | 154 | 173/165 | | 152/118 |
| 94J | 154/152 | 173/165 | 201 | 142/124 |
| 94K | 154/152 | 165/161 | | 142/124 |
| 94L | 152 | 173/165 | 227 | 152/118 |
| 94M | 152 | 173/165 | | 146/124 |
| | | | | |
| 1298 | 154/152 | 173/169 | 227 | 152/146 |
| 98A | 154 | 173/169 | 227 | 152/118 |
| 98C | 160/152 | | | 146/124 |
| 98E | 152 | 173/169 | 227 | |
| 98F | 154/148 | | | 146/124 |
| 98G | 176/152 | 173/165 | | 146/124 |
| 98H | 176/152 | 169/165 | | 124/118 |
| 98I | 176/152 | 169/165 | 227 | 152/148 |
| 98K | 176/152 | 169/165 | | 148/146 |
| 98L | 176/152 | | 227 | |
| | | | | |
| 1217 | 154/148 | 177/157 | 227 | 124 |
| 17A | 154/148 | 181/177 | 227 | 124 |
| 17B | 148 | 181/177 | 227 | 142/124 |
| 17C | 154/148 | 181/157 | 227 | 124 |
| 17D | 154/148 | 181/157 | 227 | 142/124 |
| 17E | 154/148 | 165/157 | 227 | 152/124 |
| 17F | 148/144 | 165/157 | 227 | 124 |
| 17G | 154/148 | 165/157 | 227 | 142/124 |
| | | | | |
| 1218 | 148 | 165 | 227/201 | 152/142 |
| 18A | 148 | 169/165 | 227/197 | 152/124 |
| 18B | 154/148 | 169/165 | 227/201 | 142/124 |
| 18C | 156/148 | 169/165 | 227 | 142/124 |
| 18D | 148 | 189/165 | 227/201 | 142/124 |
| 18E | 156/148 | 169/165 | 201 | 152/124 |
| 18F | 148 | | 227/197 | 142/124 |
| 18G | 148 | 169/165 | 227/201 | 152/124 |
| 18H | 148 | 169/165 | 227/197 | 142/124 |

Appendix II (continued)

| subject # | Ner 2 | Ner 3 | Ner 9 | Ts 16B |
|---|---|---|---|---|
| 18I | 148 | 165 | 227 | 142/124 |
| 18J | 154/148 | 169/165 | 227 | 142/124 |
| 18L | 154/148 | 169/165 | 227/197 | 152/142 |
| 18M | 148 | 169/165 | | 142/124 |
| 1220 | 150/148 | 189/169 | 227 | 156/142 |
| 20A | 176/150 | 189/165 | 227 | 142/124 |
| 20B | 148/144 | 189/169 | 227 | 142/124 |
| 20C | 156/148 | 189/157 | 227 | 142/124 |
| 20D | 150 | 169/165 | 227 | 142 |
| 20E | 148/144 | 169/157 | 227 | 156/146 |
| 20F | 150 | 189/165 | 227 | 156/146 |
| 20G | 148/144 | | | 142 |
| 20H | 148/144 | 189/157 | 227 | 142/124 |
| 20I | 148 | 169/165 | 227 | 142/124 |
| 1226 | 148 | 177/173 | 227/197 | 142 |
| 26A | 154/148 | 173/169 | 227/197 | 156/142 |
| 26B | 172/148 | 177/165 | 227 | 146/142 |
| 26C | 172/148 | 177/165 | 227/197 | 142 |
| 26D | 172 | 173/165 | 227/197 | 156/142 |
| 26E | 172/148 | 173/169 | | |
| 26F | 172/148 | 177/165 | 227 | 142 |
| 26G | 172 | 173/169 | 227 | 156/142 |
| 26H | 172/148 | 173/169 | 227 | 142 |
| 26I | 154 | 177/165 | 227 | 156/142 |
| 26J | 172 | 173 | 227 | 156/142 |
| 1228 | 148/144 | 177/173 | 227 | 142 |
| 28A | | 189/177 | | 142 |
| 28B | 148/144 | 189/177 | 227/201 | 142/124 |
| 28C | 148/144 | 189/173 | 227/201 | 152/142 |
| 28D | 148/144 | 177/173 | 227/201 | 152/142 |
| 28E | 148 | 173 | 227/201 | 152/142 |
| 28F | 148 | 177/173 | 227/201 | 152/142 |
| 28G | 148/144 | 177/173 | 227/201 | 152/142 |
| 28H | 170/148 | | 227/201 | 152/142 |
| 28I | 148/144 | 189/177 | 227/201 | 156/142 |
| 28J | 148 | 189/177 | 227 | 156/142 |
| 28K | 148 | 177/173 | 227 | 152/142 |
| | | | | |
| 1229 | 148/146 | | | |
| 29A | 148/144 | 177/165 | 227 | 142/124 |
| 29B | 148/144 | 189/181 | 227 | 146/142 |
| 29C | 148/144 | 189/181 | 227 | 124 |
| 29D | 148 | 189/181 | 227/197 | 124 |
| 29E | 148 | 189/165 | 227 | 146/124 |

Appendix III

Descriptive statistics Mean (± 1 SE) by cohort and sex for mass, snout-vent length (SVL), tail length (TL), head length(HL), jaw length (JL), head width (HW), inter-ocular distance (IOD) for neonate *T. butleri*.

| Cohort | Sex | mass (0.1g) | SVL (1.0mm) | TL (1.0mm) | HL (0.01mm) | JL (0.01mm) | HW (0.01mm) | IOD (0.01mm) |
|---|---|---|---|---|---|---|---|---|
| **1999** | **M** n = 26 | 1.10(0.02) | 116.5(1.09) | 33.5(0.08) | 7.93(0.05) | 7.23(0.07) | 3.89(0.04) | 3.51(0.05) |
| | **F** n = 44 | 1.11(0.01) | 116.8(0.60) | 29.4(0.50) | 8.13(0.03) | 7.48(0.05) | 4.02(0.02) | 3.63(0.03) |
| | **Total** n = 70 | 1.11(0.01) | 116.7(0.55) | 30.9(0.50) | 8.05(0.03) | 7.38(0.04) | 3.97(0.22) | 3.58(0.03) |
| **2000** | **M** n = 23 | 1.50(0.09) | 129.5(1.72) | 35.5(0.69) | 8.10(0.07) | 7.80(0.08) | 4.12(0.03) | 3.97(0.04) |
| | **F** n = 19 | 1.29(0.04) | 123.8(1.37) | 33.1(0.71) | 8.00(0.04) | 7.72(0.04) | 4.11(0.03) | 3.96(0.05) |
| | **Total** n = 42 | 1.40(0.05) | 126.9(1.20) | 34.4(0.52) | 8.05(0.04) | 7.76(0.05) | 4.11(0.02) | 3.96(0.03) |

## Appendix IV

Descriptive statistics Mean (± 1SE) by litter and sire-within-litter for mass, snout-vent length (SVL), tail length (TL), head length(HL), jaw length (JL), head width (HW), inter-ocular distance (IOD) for neonate *T. butleri*.

| Litter | Sire | mass (0.1g) | SVL (1.0mm) | TL (1.0mm) | HL (0.01mm) | JL (0.01mm) | HW (0.01mm) | IOD (0.01mm) | dam mass | dam SVL | number offpring |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1289** | **1** n = 5 | 1.14(0.04) | 119.6(1.36) | 28.6(1.21) | 8.16(0.04) | 7.66(0.07) | 4.11(0.03) | 3.79(0.04) | | | |
| | **2** n = 3 | 1.11(0.05) | 113.7(0.88) | 26.3(0.33) | 8.40(0.02) | 7.67(0.16) | 4.12(0.02) | 3.77(0.03) | | | |
| | **Total** n =14 | 1.14(0.02) | 116.4(1.03) | 27.9(0.67) | 8.24(0.07) | 7.67(0.05) | 4.17(0.03) | 3.78(0.03) | 57.2 | 477.0 | 14 |
| **1290** | **Total** n = 13 | 1.09(0.02) | 115.7(1.11) | 33.1(0.97) | 7.97(0.05) | 7.16(0.06) | 3.89(0.03) | 3.58(0.03) | - | - | |
| **1293** | n = 6 | 1.15(0.01) | 116.2(1.35) | 26.8(0.98) | 8.07(0/03) | 7.50(0.07) | 3.95(0.03) | 3.51(0.06) | | | |
| | **2** n = 2 | 1.13(0.05) | 116.0(3.00) | 30.5(2.50) | 8.05(0.03) | 7.70(0.12) | 3.96(0.02) | 3.43(0.04) | | | |
| | **Total** n = 17 | 1.16(0.02) | 116.9(0.87) | 27.5(0.63) | 8.09(0.02) | 7.56(0.05) | 3.96(0.02) | 3.51(0.04) | 56.2 | 424.0 | 20 |

Appendix IV (continued)

| Litter | Sire | mass (0.1g) | SVL (1.0mm) | TL (1.0mm) | HL (0.01mm) | JL (0.01mm) | HW (0.01mm) | IOD (0.01mm) | dam mass | dam SVL | number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1294** | **1** n = 9 | 1.07(0.04) | 118.1(1.56) | 31.9(1.24) | 7.92(0.09) | 7.26(0.10) | 3.91(0.07) | 3.63(0.11) | | | |
| | **2** n = 2 | 1.01(0.12) | 115.5(6.50) | 32.0(1.00) | 8.12(0.06) | 7.16(0.38) | 4.01(0.22) | 3.71(0.21) | | | |
| | **Total** n = 13 | 1.08(0.04) | 117.5(1.34) | 32.2(0.89) | 7.98(0.06) | 7.23(0.83) | 3.94(0.06) | 3.66(0.77) | 52.6 | 421.0 | 13 |
| **1298** | **1** n = 4 | 1.12(0.03) | 119.3(0.75) | 36.8(1.44) | 8.08(0.06) | 7.20(0.12) | 4.01(0.09) | 3.50(0.12) | | | |
| | **2** n = 1 | 1.07(-) | 122.0(-) | 34.0(-) | 8.05(-) | 7.39(-) | 4.05(-) | 3.82(-) | | | |
| | **Total** n = 13 | 1.06(0.04) | 116.8(1.91) | 35.2(0.78) | 7.93(0.89) | 7.16(0.13) | 3.87(0.67) | 3.42(0.07) | 51.1 | 400.8 | 13 |
| **1217** | **1** n = 2 | 1.56(0.11) | 125.0(6.00) | 36.5(2.50) | 8.08(0.02) | 7.71(0.00) | 4.18(0.06) | 4.08(0.19) | | | |
| | **2** n = 3 | 1.50(0.23) | 129.3(3.84) | 35.5(2.73) | 7.90(0.61) | 7.71(0.29) | 4.18(0.02) | 4.11(0.03) | | | |
| | **Total** n = 7 | 1.54(0.03) | 127.3(2.23) | 35.0(1.44) | 8.00(0.06) | 7.72(0.05) | 4.19(0.03) | 4.09(0.06) | 42.8 | 430.0 | 7 |

Appendix IV (continued)

| Litter | Sire | mass (0.1g) | SVL (1.0mm) | TL (1.0mm) | HL (0.01mm) | JL (0.01mm) | HW (0.01mm) | IOD (0.01mm) | dam mass | dam SVL | number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1218** | **Total** n = 13 | 1.20(0.03) | 119.5(1.00) | 33.2(0.63) | 8.05(0.04) | 7.62(0.06) | 4.14(0.04) | 4.02(0.05) | 59.7 | 470.0 | 13 |
| **1220** | **1** n = 2 | 1.23(0.08) | 129.5(2.50) | 34.5(1.50) | 7.98(0.06) | 7.74(0.03) | 3.92(0.09) | 3.69(0.02) | | | |
| | **2** n = 3 | 1.29(0.02) | 128.0(2.51) | 36.0(1.73) | 7.97(0.07) | 7.66(0.13) | 4.00(0.03) | 3.83(0.03) | | | |
| | **Total** n = 9 | 1.22(0.03) | 127.2(1.15) | 34.2(1.20) | 7.92(0.05) | 7.63(0.05) | 4.02(0.04) | 3.77(0.03) | 49.4 | 415.0 | 9 |
| **1226** | **1** n = 1 | 1.32(-) | 132.0(-) | 34.0(-) | 7.76(-) | 7.84(-) | 4.05(-) | 3.93(-) | | | |
| | **2** n = 4 | 1.35(0.04) | 133.0(2.82) | 32.2(1.31) | 7.99(0.08) | 7.79(0.08) | 3.94(0.06) | 3.83(0.07) | | | |
| | **Total** n = 8 | 1.30(0.04) | 130.3(2.09) | 33.8(1.03) | 7.90(0.07) | 7.73(0.05) | 3.98(0.03) | 3.84(0.04) | 39.9 | 398.0 | 8 |
| **1229** | **Total** n = 5 | 2.23(0.07) | 139.8(2.62) | 38.2(1.85) | 8.62(0.10) | 8.42(0.12) | 4.33(0.04) | 4.20(0.03) | 46.3 | 435.0 | 5 |

Appendix V

Descriptive statistics Mean (± 1SE) by cohort and sex for ventral scales, infralabial and supralabial scales on both sides of the head for neonate *T. butleri*.

| Cohort | Sex | Ventral | Right Infralabial | Left Infralabial | Right Supralabial | Left Supralabial |
|---|---|---|---|---|---|---|
| **1999** | **M** | 141.4(1.70) | 7.7(0.10) | 7.7(0.10) | 6.1(0.01) | 6.2(0.09) |
| n = 69 | n = 27 | | | | | |
| | **F** | 140.3(1.06) | 7.7(0.09) | 7.7(0.09) | 6.2(0.08) | 6.2(0.08) |
| | n = 44 | | | | | |
| | Total | 140.7(0.92) | 7.7(0.07) | 7.7(0.07) | 6.1(0.06) | 6.2(0.06) |
| **2000** | **M** | 137.3(1.28) | 8.5(0.15) | 8.3(0.14) | 7.0(0.02) | 7.0(0.04) |
| n = 42 | n = 23 | | | | | |
| | **F** | 135.2(1.28) | 8.5(0.15) | 8.3(0.15) | 7.0(0.08) | 7.0(0.08) |
| | n = 19 | | | | | |
| | Total | 136.3(0.91) | 8.5(0.14) | 8.3(0.10) | 7.0(0.04) | 7.0(0.04) |

Appendix VI

Descriptive statistics Mean (± 1SE) by litter and sire within-litter for ventral scales, infralabial and supralabial scales on both sides of the head for neonate *T. butleri*.

| Litter | Sire | Ventral | Right Infralabial | Left Infralabial | Right Supralabial | Left Supralabial | Individuals w/ labial asymmetry |
|---|---|---|---|---|---|---|---|
| **1289** | **1** n = 5 | 137.0(2.51) | 7.8(0.20) | 7.8(0.20) | 6.4(0.25) | 6.4(0.25) | |
| | **2** n = 3 | 129.7(1.33) | 7.8(0.17) | 8.0(0.00) | 6.7(0.33) | 7.0(0.00) | |
| | **Total** n =14 | 136.2(2.18) | 7.8(0.11) | 7.7(0.13) | 6.4(0.13) | 6.4(0.14) | 2 |
| **1290** | **Total** n =13 | 147.5(1.07) | 7.5(0.14) | 7.5 (0.14) | 6.0(0.09) | 6.1(0.08) | 1 |
| **1293** | **1** n = 9 | 138.4(2.15) | 7.7(0.17) | 7.7(0.17) | 6.0(0.21) | 6.1(0.20) | |
| | **2** n = 2 | 149.5(5.50) | 7.5(0.50) | 7.5(0.50) | 5.5(0.00) | 6.0(0.50) | |
| | **Total** n = 17 | 140.9(1.59) | 7.7(0.11) | 7.7 (0.11) | 5.9(0.13) | 6.0(0.13) | 3 |
| **1294** | **1** n = 9 | 143.2(3.38) | 7.4(0.17) | 7.4(0.17) | 6.0(0.00) | 6.0 (0.00) | |
| | **2** n = 2 | 140.0(10.00) | 7.5(0.50) | 7.5(0.50) | 6.0(0.00) | 6.0(0.00) | |
| | **Total** n = 13 | 142.0(2.65) | 7.5(0.14) | 7.5(0.14) | 5.9(0.07) | 5.9(0.07) | 0 |

Appendix IV (continued)

| Litter | Sire | Ventral | Right Infralabial | Left Infralabial | Right Supralabial | Left Supralabial | Individuals w/ labial asymmetry |
|---|---|---|---|---|---|---|---|
| **1298** | **1** n = 4 | 139.5(1.55) | 8.5(0.29) | 8.5(0.29) | 6.4(0.24) | 6.4(0.24) | |
| | **2** n = 1 | 138.0(-) | 9.0(-) | 9.0(-) | 7.0(-) | 7.0(-) | |
| | **Total** n = 13 | 137.2(1.31) | 8.3(0.14) | 8.3(0.14) | 6.5(0.14) | 6.5(0.14) | 0 |
| **1217** | **1** n = 2 | 139.00(6.00) | 7.50(0.00) | 7.50(0.00) | 7.00(0.00) | 7.00(0.00) | |
| | **2** n = 3 | 132.67(2.85) | 9.00(0.58) | 8.67(0.33) | 7.33(0.33) | 7.33(0.33) | |
| | **Total** n = 7 | 136.14(2.66) | 8.57(0.41) | 8.29(0.42) | 7.14(0.14) | 7.14(0.14) | 3 |
| **1218** | **Total** n = 13 | 135.00(1.80) | 8.58(0.19) | 8.50(0.20) | 7.00(0.00) | 7.00(0.00) | 1 |
| **1220** | **1** n = 2 | 133.50(0.50) | 8.50(.050) | 8.00(0.00) | 7.00(0.00) | 7.00(0.00) | |
| | **2** n = 3 | 137.67(1.45) | 8.33(0.00) | 8.00(0.00) | 7.00(0.00) | 7.00(0.00) | |
| | **Total** n = 9 | 134.67(1.01) | 8.33(0.17) | 8.11(0.11) | 6.94(0.06) | 6.83(0.12) | 4 |

Appendix VI (continued)

| Litter | Sire | Ventral | Right Infralabial | Left Infralabial | Right Supralabial | Left Supralabial | Individuals w/ labial asymmetry |
|---|---|---|---|---|---|---|---|
| **1226** | **1** n = 1 | 133.00(-) | 9.00(-) | 9.00(-) | 7.00(-) | 7.00(-) | |
| | **2** n = 4 | 137.50(3.77) | 8.75(0.25) | 8.25(0.25) | 7.00(0.00) | 7.00(0.00) | |
| | **Total** n = 8 | 139.00(2.54) | 8.65(0.18) | 8.25(0.16) | 6.81(0.13) | 6.88(0.13) | 3 |
| **1229** | **Total** n = 5 | 138.8(2.31) | 8.40(0.25) | 8.40(.25) | 7.00(0.00) | 7.00(0.00) | 0 |

Appendix VII

Descriptive statistics Mean ( ± 1SE) by cohort and sex for total defensive behaviors (toward still, moving, and touching stimuli) for neonate *T. butleri*.

| Cohort | Sex | Strike | Bite | Flee | Flatten | Tail wave |
|---|---|---|---|---|---|---|
| **1999** | **M** n = 26 | 1.23(0.92) | 0.58(0.41) | 8.38(1.08) | 0.00(0.00) | 4.08(0.86) |
| | **F** n = 44 | 0.93(0.51) | 0.55 (0.31) | 7.41(0.94) | 0.00(0.00) | 4.57(0.71) |
| | **Total** n = 70 | 1.04(0.47) | 0.56(0.25) | 7.77(0.71) | 0.00(0.00) | 4.39(0.54) |
| **2000** | **M** n = 23 | 0.77(0.41) | 0.64(0.36) | 9.86(1.55) | 3.59(0.43) | 3.59(0.68) |
| | **F** n = 19 | 0.21(0.14) | 0.21(0.14) | 9.42(1.81) | 4.11(0.33) | 3.84(0.71) |
| | **Total** n = 42 | 0.51(0.23) | 0.44(0.20) | 9.66(1.17) | 3.83(0.28) | 3.71(0.49) |

Appendix VIII

Descriptive statistics (M ± 1SE) by litter and sire for total defensive behaviors (toward still, moving, and touching stimuli) for neonate *T. butleri*.

| Litter | Sire | Strike | Bite | Flee | Flatten | Tail Wave |
|---|---|---|---|---|---|---|
| **1289** | **1** n = 5 | 0.20(0.20) | 0.00(0.00) | 4.00(1.14) | 0.00(0.00) | 5.20(1.85) |
| | **2** n = 3 | 0.00(0.00) | 0.00(0.00) | 3.00(1.00) | 0.00(0.00) | 7.00(4.04) |
| | **Total** n =14 | 0.07(0.07) | 0.00(0.00) | 4.07(0.76) | 0.00(0.00) | 6.36(1.17) |
| **1290** | **Total** n =13 | 3.46(2.18) | 1.77(1.09) | 6.54(0.77) | 0.00(0.00) | 8.08(0.95) |
| **1293** | **1** n = 6 | 3.00(1.91) | 1.67(1.05) | 7.17(1.58) | 0.00(0.00) | 6.83(2.23) |
| | **2** n = 2 | 0.00(0.00) | 0.00(0.00) | 10.00(1.00) | 0.00(0.00) | 5.50(5.50) |
| | **Total** n = 17 | 1.12(0.73) | 0.59(0.40) | 6.82(1.04) | 0.00(0.00) | 5.18(1.17) |
| **1294** | **1** n = 9 | 0.00(0.00) | 0.00(0.00) | 8.00(1.05) | 0.00(0.00) | 0.22(.022) |
| | **2** n = 2 | 0.00(0.00) | 0.00(0.00) | 8.50(2.50) | 0.00(0.00) | 0.00(0.00) |
| | **Total** n = 13 | 0.00(0.00) | 0.00(0.00) | 9.23(1.10) | 0.00(0.00) | 0.15(0.15) |
| **1298** | **1** n = 4 | 0.00(0.00) | 0.00(0.00) | 8.75(4.19) | 0.00(0.00) | 3.75(2.25) |
| | **2** n = 1 | 0.00(-) | 0.00(-) | 9.00(-) | 0.00(-) | 0.00(-) |
| | **Total** n = 13 | 0.62(0.62) | 0.46(0.46) | 12.77(2.75) | 0.00(0.00) | 1.77(0.79) |
| **1217** | **1** n = 2 | 0.00(0.00) | 0.00(0.00) | 6.50(0.50) | 2.50(0.00) | 6.00(2.00) |
| | **2** n = 3 | 0.00(0.00) | 0.00(0.00) | 3.33(0.67) | 2.00(1.00) | 7.33(0.33) |
| | **Total** n = 7 | 0.00(0.00) | 0.00(0.00) | 5.43(1.11) | 2.57(0.57) | 6.71(0.79) |

Appendix VIII (continued)

| Litter | Sire | Strike | Bite | Flee | Flatten | Tail Wave |
|---|---|---|---|---|---|---|
| **1218** | **Total** n = 13 | 1.08(0.0051) | 1.00(0.46) | 5.08(0.73) | 3.50(0.54) | 3.25(0.85) |
| **1220** | **1** n = 2 | 0.00(0.00) | 0.00(0.00) | 13.50(2.50) | 6.00(1.50) | 2.00(0.50) |
| | **2** n = 3 | 0.67(0.67) | 0.00(0.00) | 17.00(3.51) | 3.00 (1.15) | 1.00(0.58) |
| | **Total** n = 9 | 0.00(0.00) | 0.00(0.00) | 12.67(2.03) | 4.11(0.61) | 2.89(0.72) |
| **1226** | **1** n = 1 | 0.00(-) | 0.00(-) | 30.00(-) | 5.00(-) | 0.00(-) |
| | **2** n = 4 | 0.00(0.00) | 0.00(0.00) | 15.33(5.21) | 5.00(1.15) | 2.00(0.58) |
| | **Total** n = 8 | 0.00(0.00) | 0.00(0.00) | 19.13(3.03) | 5.13(0.40) | 3.37(1.49) |
| **1229** | **Total** n = 5 | 1.60(1.36) | 1.20(1.20) | 6.00(1.76) | 3.80(0.80) | 2.60(1.21) |

Appendix IX

Descriptive statistics Mean (±1SE) by cohort and sex of average worm and fish TFAS (natural log transformed), average worm TFAS – fish TFAS, and average attacks toward swabs for neonate *T. butleri*.

| Cohort | Sex | lnTFAS worm | lnTFAS fish | TFAS worm -TFAS fish | av. attacks |
|---|---|---|---|---|---|
| **1999** n=71 | **M** n = 26 | 2.43(0.24) | 2.31(0.12) | +5.94(1.74) | 1.00(0.24) |
| | **F** n = 44 | 2.48(0.15) | 2.44(0.14) | +1.56(2.30) | 0.91(0.17) |
| | **Total** n = 70 | 2.46(0.13) | 2.39(0.12) | +1.53(1.41) | 0.94(0.14) |
| **2000** n = 42 | **M** n = 22 | 1.99(0.16) | 1.87(0.13) | +2.20(1.19) | 0.09(0.09) |
| | **F** n = 19 | 1.81(0.20) | 1.56(0.16) | +1.95(1.63) | 0.00(0.00) |
| | **Total** | 1.91(0.13) | 1.73(0.10) | +2.14(0.82) | 0.05(0.05) |

Appendix X

Descriptive statistics M( ±1SE) by cohort and sex for the 1st presentation of of worm and fish TFAS (natural log transformed), worm TFAS – fish TFAS, and average attacks toward swabs for neonate *T. butleri*.

| Cohort | Sex | lnTFAS worm | lnTFAS fish | TFAS worm -TFAS fish |
|---|---|---|---|---|
| **1999** n=71 | **M** n = 26 | 2.66(0.24) | +2.25(0.25) | +3.22(158) |
| | **F** n = 44 | 2.56(0.17) | +2.48(0.17) | +1.56(2.30) |
| | **Total** n = 71 | 2.46(0.13) | +2.39(0.12) | +3.22(1.58) |
| **2000** n = 42 | **M** n = 22 | 2.10(0.16) | +2.10(0.15) | +2.20(1.19) |
| | **F** n = 19 | 1.95(0.22) | +1.80(0.19) | +1.95(1.63) |
| | **Total** | 1.91(0.13) | +1.73(0.10) | +1.96(0.12) |

Appendix XI

Descriptive statistics Mean (± 1SE) by litter and sire of average worm and average fish TFAS (natural log transformed), worm TFAS – fish TFAS, and average attacks toward swabs for neonate *T. butleri*.

| Litter | Sire | lnTFAS worm | lnTFAS fish | worm TFAS - fish TFAS | av. attacks |
|---|---|---|---|---|---|
| **1289** | **1** n = 5 | 2.25(0.63) | 2.11(0.60) | +2.40(5.06) | 0.20(0.20) |
| | **2** n = 3 | 3.12(0.20) | 3.40(0.13) | -6.67(3.84) | 0.33(0.33) |
| | **Total** n=14 | 2.01(0.37) | 2.11(0.39) | -2.07(2.21) | 0.14(0.36) |
| **1290** | **Total** n=13 | 2.30(0.25) | 2.32(0.25) | +1.65(3.74) | 0.46(0.78) |
| **1293** | **1** n = 6 | 2.35(0.44) | 2.13(0.38) | +2.74(4.75) | 1.00(0.38) |
| | **2** n = 2 | 2.72(0.42) | 2.20(0.00) | +7.50(6.50) | 0.50(0.50) |
| | **Total** n = 17 | 2.31(0.25) | 2.32 (0.23) | -0.19(2.51) | 1.85(1.46) |
| **1294** | **1** n = 9 | 3.55(0.16) | 3.05(0.39) | +9.33(6.96) | 2.22(0.46) |
| | **2** n = 2 | 3.58(0.04) | 1.65(1.65) | +21.95(11.45) | 2.00(1.00) |
| | **Total** n = 13 | 3.54(0.11) | 2.90(0.36) | +9.84(5.21) | 1.85(1.46) |
| **1298** | **1** n = 4 | 2.96(0.45) | 3.05(0.33) | +0.60(7.83) | 2.00(0.71) |
| | **2** n = 1 | 3.38(-) | 2.40(-) | +18.4(-) | 1.00(0.00) |
| | **Total** n = 13 | 2.93(0.27) | 2.45(0.27) | + 8.60(3.24) | 1.62(1.33) |

Appendix XI (continued)

| Litter | Sire | lnTFAS worm | lnTFAS fish | worm TFAS - fish TFAS | av. attacks |
|---|---|---|---|---|---|
| **1217** | **1** n = 2 | 1.94(0.14) | 1.96(0.35) | -0.50(1.50) | 0.00(0.00) |
| | **2** n = 3 | 1.79(0.70) | 1.83(0.37) | +2.67(4.70) | 0.00(0.00) |
| | **Total** n = 7 | 1.86(0.29) | 1.92(0.16) | +1.00(2.01) | 0.00(0.00) |
| **1218** | **Total** n = 13 | 1.85(0.36) | 1.75(0.29) | +2.00(2.96) | 0.00(0.00) |
| **1220** | **1** n = 2 | 2.25(0.31) | 2.59(0.19) | -3.50 (0.50) | 0.00(0.00) |
| | **2** n = 3 | 2.38(0.28) | 2.26(0.34) | +1.00(5.03) | 0.00(0.00) |
| | **Total** n = 9 | 2.31(0.17) | 2.29(0.16) | +0.33(1.91) | 0.00(0.00) |
| **1226** | **1** n = 1 | 2.70(-) | 1.10(-) | +12.0(-) | 0.00(-) |
| | **2** n = 4 | 2.32(0.28) | 1.73(0.21) | +5.50(3.59) | 0.00(0.00) |
| | **Total** n = 8 | 2.18(0.22) | 1.75(0.20) | +3.88(2.33) | 0.00(0.00) |
| **1229** | **Total** n = 5 | 2.79(0.25) | 2.27(0.50) | +3.66(2.35) | 0.40(0.89) |

Appendix XII

Descriptive statistics M( ± 1SE) by litter and sire of 1st worm and 1st fish TFAS (natural log transformed), 1st worm TFAS – 1st fish TFAS.

| Litter | Sire | lnTFAS worm | lnTFAS fish | worm TFAS - fish TFAS |
|---|---|---|---|---|
| **1289** | **1** n = 5 | 2.01(0.63) | 2.11(0.60) | +2.40(5.06) |
| | **2** n = 3 | 3.12(0.20) | 3.40(0.13) | -6.67(3.84) |
| | **Total** n=14 | 2.01(0.37) | 2.11(0.39) | -2.07(2.21) |
| **1290** | **Total** n=13 | 2.30(0.25) | 2.32(0.25) | +1.65(3.74) |
| **1293** | **1** n = 6 | 2.35(0.44) | 2.13(0.38) | +2.74(4.75) |
| | **2** n = 2 | 2.72(0.42) | 2.20(0.00) | +7.50(6.50) |
| | **Total** n = 17 | 2.31(0.25) | 2.32 (0.23) | -0.19(2.51) |
| **1294** | **1** n = 9 | 3.55(0.16) | 3.05(0.39) | +9.33(6.96) |
| | **2** n = 2 | 3.58(0.04) | 1.65(1.65) | +21.95(11.45) |
| | **Total** n = 13 | 3.54(0.11) | 2.90(0.36) | +9.84(5.21) |
| **1298** | **1** n = 4 | 2.96(0.45) | 3.05(0.33) | +0.60(7.83) |
| | **2** n = 1 | 3.38(-) | 2.40(-) | +18.4(-) |
| | **Total** n = 13 | 2.93(0.27) | 2.45(0.27) | + 8.60(3.24) |

Appendix XII (continued)

| Litter | Sire | lnTFAS worm | lnTFAS fish | worm TFAS - fish TFAS |
|---|---|---|---|---|
| **1217** | **1** n = 2 | 1.94(0.14) | 1.96(0.35) | -0.50(1.50) |
| | **2** n = 3 | 1.79(0.70) | 1.83(0.37) | +2.67(4.70) |
| | **Total** n = 7 | 1.86(0.29) | 1.92(0.16) | +1.00(2.01) |
| **1218** | **Total** n = 13 | 1.85(0.36) | 1.75(0.29) | +2.00(2.96) |
| **1220** | **1** n = 2 | 2.25(0.31) | 2.59(0.19) | -3.50 (0.50) |
| | **2** n = 3 | 2.38(0.28) | 2.26(0.34) | +1.00(5.03) |
| | **Total** n = 9 | 2.31(0.17) | 2.29(0.16) | +0.33(1.91) |
| **1226** | **1** n = 1 | 2.70(-) | 1.10(-) | +12.0(-) |
| | **2** n = 4 | 2.32(0.28) | 1.73(0.21) | +5.50(3.59) |
| | **Total** n = 8 | 2.18(0.22) | 1.75(0.20) | +3.88(2.33) |
| **1229** | **Total** n = 5 | 2.79(0.25) | 2.27(0.50) | +3.66(2.35) |

## Vita

Julia Davene Albright was born in Singapore in 1976, but grew up in Nashville, Tennessee, the home state of both of her parents, David and Judy Albright. She received her Bachelor of Science degree in Psychology from Vanderbilt University in 1998. While at Vanderbilt, and the following year at Cornell University, Julie was involved in human and non-human primate vocalization studies. She returned to Tennessee to study animal behavior and herpetology with Dr. Gordon Burghardt in 1999, and in 2001, received her Masters of Arts degree in Psychology. Julie is currently attending the University of Tennessee College of Veterinary Medicine, where she intends to further examine animal behavior and/or herpetology in an applied setting.