# AN ABSTRACT OF THE THESIS OF

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Title: Courtship Behavior in the Salamander Genus *Aneides*.

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Salamander courtship coordinates the exchange of gametes. Plethodontids have evolved an elaborate means by which this is done. Throughout the family Plethodontidae a tail-straddling walk is performed to orchestrate the transfer of sperm. Typically this behavior is linear, however in the genus *Aneides* there are exceptions.

In the genus *Aneides* there are two species that have a circular tail-straddling walk; others perform the highly conserved linear tail-straddling walk. I observed courtship in three species of *Aneides* and used accounts of others to discern which members possessed circular tail-straddling walk and used a phylogeny to map the courtship characters found in the courtships of the members of the tribe plethodontini.

# Courtship Behaviors in the Salamander Genus *Aneides*.

by

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Jerod Rothwell Sapp, Author

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## Chapter I: General Introduction

Plethodontid salamanders have been used widely as models to study the evolution of morphological and ecological specializations (e.g., Wake 1966; Wake and Lynch 1976;), the speciation process (e.g., Arnold, et al 1993; Highton 1998; Wake 1997, 1998), developmental processes (e.g., Albrech and Albrech 1981; Hanken 1985; Wake and Marks 1993), as well as model systems to study ecological interactions (e.g., Hairston 1987). This group of salamanders is the most diverse ecologically and morphologically and the most speciose of extant caudate amphibians, with about 27 genera and over 250 species. (Petranka 1998; Wake 1966). Since Arnold's (1972; 1977) work on courtship behavior in salamanders, there have been several phylogenetic analyses of plethodontid courtship(e.g., Houck and Sever 1994; Verrell 1999).

Three families of salamanders have external fertilization; in the remaining seven families fertilization is internal (Pough et al 2001, Zug 2001). Salamanders with internal fertilization utilize different behaviors to coordinate sperm transfer during courtship (Salthe 1967, Arnold 1977, Halliday 1990). Plethodontids all have internal fertilization.

The courtship behavior of plethodontid salamanders is an elaborate process that involves sophisticated tactile and chemical communication between the sexes

(Houck & Sever 1994; Verrell and Mabry 2000). The tail-straddling walk (TSW) is a unique behavioral character in courtship behavior of plethodontids that distinguishes them from other salamanders (Noble 1931; Stebbins 1949; Arnold 1972; 1976). The male leads the female as she follows with her head resting atop his tail base. Insemination is accomplished after the male deposits a spermatophore on substrate. The female retrieves the sperm cap in the lips of her cloaca. Once inside the cloaca, enzymes break down the sperm cap membrane, and the sperm are stored in invaginations of the cloaca, the spermatheca. In many species of plethodontids, multiple matings are probably the rule, not the exception, and sperm can be stored for extended periods of time (Houck & Schwenk 1984). Internal fertilization is effected as ova pass into the cloaca via the oviducts just before oviposition (Dunn 1926; Noble 1931).

To orchestrate successful sperm transfer, a stereotyped series of behaviors occurs, with slight variation from species to species. I outline these behaviors in ethograms in Chapters II and III for four species of *Aneides*; but, in brief, courtship behavior can be broken down into general categories: an approach phase, a pursuit phase, and tail-straddling walk (TSW) followed by sperm transfer (Arnold 1972, 1977).

Early behavioral observations by Dunn (1926) and Noble (1931) hinted that courtship behavior might be used as a means to determine phylogenetic relationships among salamander families. With the advent of modern molecular techniques to generate phylogenies, behaviors and morphological aspects of the phenotype can be coup led with relationships elucidated by molecular analyses to gain a better understanding of presumed ancestral and derived character states. In this way it is possible to begin to erect and test hypotheses regarding the proximate and ultimate selective pressures involved in the evolution of complex courtship behaviors in plethodontids as well as other salamanders.

**Evolution of Courtship Behavior and Pheromone Delivery in Plethodontids**

Male plethodontid salamanders deliver pheromones to females during courtship by a variety of means. There are three ways by which males transfer pheromones to females: scratching, slapping, and biting (Arnold 1972; Houck 1980). Male plethodontids that scratch the female possess enlarged premaxillary teeth that they use to abrade the female's integument, presumably to aid in pheromone delivery (Houck & Arnold 1982). Pulling and snapping are two varieties of scratching. Pulling is a male behavior performed when the male bends his neck downward and presses his chin onto the female's body. While his chin is in contact with the female, the male pulls his chin backwards across her body. During this motion the elongated premaxillary teeth of the male can scrape the skin of the female allowing more rapid pheromone delivery. Snapping behavior occurs when the male's chin angles against the female's dorsum and then is drawn backward very rapidly with a sudden snapping motion of the male's body (Houck and Sever 1994). Slapping occurs in eastern *Plethodon* and involves the male turning back during tail-straddling walk to make contact with the female's nares with his chin (Arnold 1972).

There are three generalized types of mental glands in the family Plethodontidae: pad-shaped, anterior protrusion, and fan-shaped (Houck and Sever 1994). Different mental gland types characterize the tribes of plethodontids. This characterization is strengthened when coupled with the pheromone delivery mode. The predominant delivery mode is scratching (Houck and Sever 1994).

It is thought that ancestral plethodontids performed TSW; because all described plethodontid courtships include that behavioral character (Arnold 1972; 1977). TSW, mental glands, and sexually dimorphic premaxillary teeth are not found in any other family of salamanders. Derived behavioral and morphological characters of the Plethodontidae include biting or slapping delivery of courtship pheromones, physical restraint during courtship, and secondary loss of mental glands and the behaviors associated with courtship glands (Houck and Sever 1994).

Mental glands are found on sexually mature males and consist of a group of simple tubular or alveolar exocrine glands located on the male's chin. Mental glands secrete mucoproteins that are the pheromones used in courtship (Houck and Sever 1994). Some plethodontids lack mental glands. Plethodontids without mental glands also lack courtship behaviors such as pulling, snapping or restraint (Organ 1958; Arnold 1972; Beachy 1992).

**Phylogeny of Plethodontidae**

The family has been partitioned into two subfamilies, the Desmognathinae and Plethdontinae (Wake 1966). The Desmognathinae contains the genus *Desmognathus*, with 16+ species, and the monotypic genus *Phaeognathus*. From a life history standpoint, nearly all life history variation exhibited by the entire family can be found in the genus *Desmognathus*. On the other hand, desmognathines possess morphological features that may be less highly derived than plethodontines (e.g., four, rather than three epibranchials, a distinctive way of opening their mouths, and other morphological features).

The more diverse Plethodontinae is partitioned into three tribes (Wake 1966). Hemidactyliini is a group of 5 genera and that have gilled, aquatic larvae as well as several perenibranch forms. This group is thought to be a basal tribe of plethodontines because of the retention of aquatic larvae and by possessing several morphological characters that differ slightly from those found in the other two tribes (Wake 1966).

Genera in the two remaining tribes (Plethodontini and Bolitoglossini) all lack aquatic larval stages; courtship and oviposition occur in terrestrial situations (Wake 1966, but see Wake and Campbell (2001) for a possible exception). With the exception of two genera (*Batrachoseps* and *Hydromantes*), bolitoglossines are restricted to Mesoamerica. Two of the three genera of Plethodontines (*Aneides and Plethodon*) have centers of distribution in eastern North America and western

North America with three Rocky Mountain isolates (one species of *Aneides* and two of *Plethodon*).

The phylogeny of the Plethodontidae is not yet fully resolved. Highton (1962) first hypothesized that western *Plethodon* were more closely related to *Aneides* than to eastern *Plethodon*. Mahoney (2001) and others have used molecular techniques to add considerable strength to this hypothesis, indicating the probable paraphyly of the genus *Plethodon*. Herein, I will address the issue of the paraphyly in the tribe Plethodontini (*Ensatina*, *Plethodon*, and *Aneides)* using behavioral data.

There are at least three plausible arrangements of genera in the tribe plethodontini (Mahoney 2001). Early molecular studies suggested a paraphyletic *Plethodon*, with western species most closely related to the genus *Aneides* (Larson et al, 1981: Jackman, 1993). To fully resolve this paraphyly, a new genus would have to be erected for western *Plethodon*. Another possible arrangement of the tribe would place *Aneides* within *Plethodon*. In this treatment, *Aneides* and *Plethodon* would be considered names of clades, not genera (de Queiroz and Gauthier, 1994). The phylogeny tentatively endorsed by Mahoney (2001) is different than the previous two scenarios in the sense that *Plethodon* would be a treated as a metataxon (Plethodon*) and *Aneides* would become a sister clade to *Plethodon**.

**Distribution and Natural History of *Aneides***

The genus *Aneides* has a discontinuous distribution in central North America. *Aneides aeneus* mainly occurs in the Appalachian Plateau and the Blue Ridge Province with disjunct populations in Tennessee and North and South Carolina. *Aneides aeneus*, a rock crevice-dwelling species, is found in sandstone, granite, and schist formations with deep, shaded crevices that are moist but not dripping wet (Bruce 1968; Gordon and Smith 1949; Mount 1975; Netting and Richmond 1932; Schwartz 1954).

*Aneides hardii* occurs in spruce and fir forests as well as alpine tundra in the Capitan, Sacramento, and White Mountains of New Mexico (Scott and Ramontnik 1992). This salamander principally inhabits moist north- or east-facing slopes with large downed timber (Schad et al. 1959).

The remaining members of *Aneides* range from northern Baja California to the Columbia River, west of the Cascade-Sierra Nevada axis (Wake 1974) to Vancouver Island.

*Aneides ferreus* is associated with large logs and talus in the coastal forests of Oregon and northern California (Corn and Bury 1991; Whitaker et al. 1986) and is one of the most arboreal salamanders in the United States. *Aneides ferreus* have been found 7-40 m above ground in trees (Leonard et al. 1993; Van Denburugh 1916) and also in rock faces or talus with deep cracks. *Aneides vagrans*, a sister species, occurs in the same habitats as *A. ferreus* but on Vancouver Island and in northern California.

*Aneides flavipunctatus* occurs in lowland mesic forests from extreme southern Oregon to central California (Lynch 1981; Nussbaum et al. 1983). Typical habitats for *A. flavipunctatus* are seeps in talus slopes and wet soil beneath logs or rocks along streams (Stebbins 1951).

*Aneides lugubris* inhabits costal oak forests from Baja California to northern California and is found on South Farrallon, Santa Catalina, Los Coronados, and Año Nuevo Islands. *Aneides lugubris* is found under rocks and woody surface cover, in stumps, logs, rodent burrows, and stonewalls (Rosenthal 1957; Stebbins 1951; Storer 1925). They can also be found 9-18 m above ground in trees (Ritter 1903; Stebbins 1951).

The diet of *A. aeneus* is comprised of arthropods (orthopterans, spiders, dipterans, hemipterans, and lepidopterans, as well as coleopterans, ants pseudoscorpions, mites, spiders, and snails (Lee and Norden 1973; Canterbury and Pauley 1990). The diet of *A. ferreus* and probably *A. vagrans* is very similar and includes isopods, insects, spiders, pseudoscorpions, and mites (Storm and Aller 1947; Whittaker et al 1986). *Aneides flavipunctatus* preys upon millipedes, beetles, termites, hymenopterans, flies, and collembolans (Lynch 1985). *Aneides hardii* seem to be a visually-oriented predator that preys on snails, spiders, mites, orthopterans, hemipterans, carabid and buprestid beetles, ants, and wasps (Johnston and Schad 1959). The largest adult *A. lugubris*, will sometime eat plethodontids, specifically *Batrachoceps sp.* (Miller 1944), but the majority of the diet consists of beetles, caterpillars, sow bugs, centipedes and ants, as well as termites millipedes, worms, and snails (Zweifel 1949; Bury and Martin 1973; Lynch 1985).

**Phylogenetic Relationship within *Aneides***

To date there exists no resolved phylogeny for *Aneides*. Based on molecular data, plausible, but different trees are generated when parsimony, maximum-likelihood, and strict consensus analyses are performed. Using the maximum likelihood analysis phylogeny (Mahoney 2001, fig.6) with *Ensatina* as an outgroup, I will compare the most basal lineage, *Aneides aeneus* courtship, with the three West Coast *Aneides*: *A. ferreus/A. vagrans*, and *A. flavipunctatus*, and *A. lugubris*.

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# Chapter II.

## The Circular Tail-straddling Walk of the Clouded Salamander, *Aneides ferreus* (Cope): A Deviation from the Highly Conserved Linear Tail-straddling Walk of the Plethodontidae.

Jerod R. Sapp

**Abstract**

Courtship in the family Plethodontidae is characterized by a linear tail-straddling walk, in which the female follows the male as he moves forward in a straight line. *Aneides ferreus* courtship differs from typical plethodontid patterns in three noteworthy ways: the duration of their courtship is long in comparison with other plethodontids, a circular tail-straddling walk precedes the typical plethodontid linear tail-straddling walk, and the female exhibits assertive behavior atypical of other female plethodontids. These behavioral differences of *Aneides ferreus* may be an outcome of limited space for courtship activities.

## Introduction

Salamanders use different behaviors to coordinate sperm transfer during courtship (Salthe 1967, Arnold 1977, Halliday 1990). In the family Plethodontidae, sperm transfer is orchestrated by a tail-straddling walk (TSW) in which the female follows the male with her chin resting on his sacral region (Noble & Brady 1930, Stebbins 1949). The tail-straddling walk is unique to plethodontids and has been observed in numerous genera in all of the major clades of the Plethodontidae, suggesting that this behavior arose early in the history of the family (Arnold 1977). Tail-straddling walk proceeds in a linear fashion, with the exception of bouts of pheromone delivery by the male or intervals during which the pair avoids obstacles. Tail-straddling behavior is consistently linear throughout all the plethodontid courtships so far described (Houck & Verrell 1993). *Aneides ferreus* deviates from this linear pattern by moving in a circular tail-straddling walk (Figure 1.) as a part of their courtship behavior. I refer to this behavior as circular tail-straddling walk (c-TSW), because the male positions his head atop the tail base of the female and the pair moves in a circular fashion.

This paper provides the first description of c-TSW and discusses its possible ecological and phylogenetic significance. I also analyze the durations of major components of courtship and compare these results to other courtship data for other plethodontids.

**Materials and Methods**

*Aneides ferreus* were collected from three localities: the Smith River drainage in Douglas County, OR (N43° 48.489' W123° 37.304'), Quosatana Butte in Curry County, OR (N42° 24.93' W124° 13.49'), and the Corvallis Watershed in Benton County, OR (N44° 30.63' W123° 28.03') from 1998 to 2000. *Aneides ferreus* participants were collected from October through May provided that the temperature was between 8-13° C. All salamanders were found at a minimum of 1 meter above the forest floor in either decaying timber (cull log piles from timber harvesting) or rock outcroppings. To ensure sexual maturity, I used salamanders with a SVL (snout-vent length) of 50 mm or greater. Any animals found in male-female pairs were also taken from the field. Salamanders were transported to Oregon State University and housed in a controlled climate room at 12-13.5° C with a natural photoperiod. Each animal was housed individually in a 12 x 17 x 6 cm plastic box. Boxes were lined with moist paper towels and had one crumpled towel to provide cover. The animals were fed small crickets (*Acheta domestica*) and wax worms (*Galleria nella*).

Courtship trials began in late October of each year when male and females were first found as pairs in the field. Trials were conducted until late November and resumed in February of the following year. Courtship trials were staged at night in the same room in which the animals were housed. Courtship arenas consisted of two glass plates separated by a Plexiglas frame 17x17x5 cm. The lower glass surface was covered with moist paper towels. Courtship trials were conducted for a period of 24 hours beginning at 1700 h. Successful courtship was scored by the presence of a spermatophore base on the substrate and a sperm cap in the vent of the female. Only successful courtships were used in analyses because I wanted to have a sequence from the onset of male's interest in courtship to the completion of sperm transfer in order to compare the sequences with other species. Courtships were taped using a low light camera (Panasonic VW BD400) and closed circuit time-lapse video tape recorder (Panasonic AG 6540). On a given night, four cameras were used to record four different pairs of salamanders on four video tape recorders at 4 images/second. Over a period of three years forty courting pairs were used in courtship trials. All courtships were conducted with both participants drawn from the same population to avoid any interpopulation interactions. Twenty successful courtships were videotaped. Scrutiny of the videotapes of the twenty successful courtships revealed no deviation in the order of behaviors of courtships among the three populations.

**Results**

A total of 74 courtship trials were conducted, and 25 complete sequences including spermatophore deposition were observed (33.7%). Females were successfully inseminated in all of the 25 complete courtships. Only twenty courtships were used in analysis due to poor quality of five of the tapes.

## Ethogram

**Male Behaviors**

**Approach-** The male consistently orients toward the female and follows her around the arena. This signals a shift in the male's interest in reproduction.

**Head contact (HC)-** The male touches his head to any aspect of the female's dorsum. Head contact is divided into three distinct categories.

**Rubbing-** The male rubs the female's dorsum with his chin by moving his head from side-to-side, not exceeding a ± 5° arc from the center of the male's head (Fig. II.2 A).

**Pulling**- The male repeatedly pulls his premaxillary teeth and his mental gland, in an anterior to posterior fashion (Fig. II.2 B), across some aspect of the female's dorsum, usually about 2mm.

**Pressing**- The male stops all head movement and presses his chin on the presacral region of the female's dorsum. Arching his head downward, more pressure appears to be applied during pressing than in pulling or rubbing.

**Foot shuffle**- The male picks up one hind foot and then places it back on the substrate and then picks up the opposite foot and puts it back on the substrate. The male strictly alternates movement of left then right, at a rate of 4 lifts/min. This behavior occurs in bouts that last up to 1-2 min and signals that the male will soon deposit a spermatophore.

**Tail undulation (TU)**- The male's tail undulates laterally when it is under the female's chin. The male's tail usually remains in contact with the female's chin during tail undulation. Two different types of tail undulation occur in courtship. During sperm transfer the tail base is elevated off the substrate while the male undulates his tail.

When tail undulation occurs prior to sperm transfer, the tail base remains in contact with the substrate.

**Spermatophore deposition (SD)-** The male ceases any forward motion and places his hind legs perpendicular to his body axis and this cue signals he is depositing a spermatophore on the substrate. During spermatophore deposition the male undulates his tail in a manner resembling a sinusoid curve with the tail base in contact with the substrate.

**Sperm Transfer (ST)-** After a spermatophore has been deposited on the substrate the male elevates his sacral region and pulls his tail out and to the side, then moves forward, leading the female over the spermatophore. When she is positioned over the spermatophore he will stop forward motion. At this time the male will rock his elevated tail base from side to side by pushing his hind limbs up and down and alternating from left to right, while he continues to undulate his tail.

## Female Behaviors

**Rubbing**- The female rubs the male's dorsum with her chin by moving her head from side-to-side, not exceeding a 10° arc. The female's head is positioned on the male's dorsum around the hind limbs but can also occur along the dorsum and on the male's head.

**Sperm mass pick-up-** With her chin resting on the male's tail base, the female moves over the spermatophore and positions her cloaca on the spermatophore. Typically, after 2-5 min she then lifts off the spermatophore with the sperm cap inside her cloaca. The spermatophore base remains on the substrate.

## Male and Female Behaviors

**Circular Tail-straddling walk (c-TSW)**- The male and female are in contact, each with its chin placed on the sacral area of the other. While in this position both the male and the female move in a circular pattern (Figure II.1).

**Linear Tail-straddling walk (l-TSW)**-The male leads the female in a linear fashion, while her chin is in contact with the sacral region of the male (Figure II.2 D) (Arnold 1976).

**Temporal Patterning**

All behaviors outlined in the description of temporal patterning occurred in all 20 courtships, in the same order presented. The average courtship lasted 454 min (SD=251.3min, range 169-1023 min). It takes animals a minimum of 20- 30 min and, more typically, an hour passes for the participants to adapt to the arena and for the male to repeatedly approach the female. Courtship of *Aneides ferreus* begins with an approach phase that leads into a phase of head contact by the male. During the approach phase, the male focuses attention towards the female by following her around the arena. The male continues to pursue the female, even if she moves away from him. The approach phase of courtship lasts 2-4 min (mean= 2.95, SD= 1.1min, range 1-5 min). After the approach phase, the male enters a sequence of courtship where his head is in prolonged contact with the female's body. Initially, the male rubs his chin on the female's dorsum. As head contact progresses, the male incorporates pulling by gradually replacing rubbing with pulling. A female can flee from all advances made by the male, thereby thwarting courtship and causing the male to revert to the approach phase. The head contact sequence lasts from 74-266 min (mean=202.9 min, SD= 171.8 min, range 45-448 min).

Female receptivity is signaled by a dramatic shift in behavior (entering c-TSW). On average the female's initial aversion to the male's pursuit persists for about 3 hrs then the onset of c-TSW signals female receptivity to courtship. In 20 out of 20 courtships, after persistent rubbing and pulling by the male, I observed females to no longer flee from the males. However this apparent response to head contact may be due to the fact that only successful courtships were used in the analysis of temporal patterning. The female's aversion to the male changes to an active female role in courtship. The female vigorously rubs her head from side-to-side on the male's dorsum, generally on his sacral region. During this time, the male is in constant contact with the sacral region of the female's dorsum. These behaviors initiate c-TSW. The c-TSW typically lasts for 1.5-6 hrs, provided the female does not terminate courtship. In c-TSW the male changes from pulling to pressing. When a male presses a female, she may respond by lifting her head upward. c-TSW can be interrupted if the pair become separated, causing the male to re-enter an earlier stage of courtship, or causing the female to immediately return to c-TSW, bypassing all of the earlier behaviors.

After c-TSW the pair shifts to l-TSW. During l-TSW the male will foot shuffle when he is ready to deposit a spermatophore. The duration of l-TSW was a small fraction of the courtship in comparison with the c-TSW; the pair will stay in l-TSW for 5-9 min. l-TSW leads to spermatophore deposition, which only lasts for approximately 5 min, and then sperm transfer and sperm mass pickup last about 6 min. Following insemination, the female retreats from the male and does not re-enter TSW with him. The male may continue to pursue the female after insemination, but multiple spermatophore depositions were not observed in the 24-hour time frame of the courtship trials.

**Statistical Analysis**

To facilitate comparison with other species, I separated courtship into five main components; Approach (AP), head contact (HC), circular tail-straddling walk (c-TSW), linear tail-straddling walk (l-TSW), spermatophore deposition and sperm transfer (SD/ST). The average durations for the five components were 2.95 min, 202.9min, 224.9 min, 11.2 min, and 11.05min respectively (Table II.1). Linear TSW showed the highest relative variability in duration with a coefficient of variation of 156%. The HC and c-TSW components of courtship comprise 95% of the courtship duration on average. However, components were not significantly correlated with one another ($p<0.05$). This analysis reveals that if a given pair spends more time in one component of courtship, that it does not reflect time spent in another. There is no relationship between the total time of courtship and the population from which the participants were drawn.

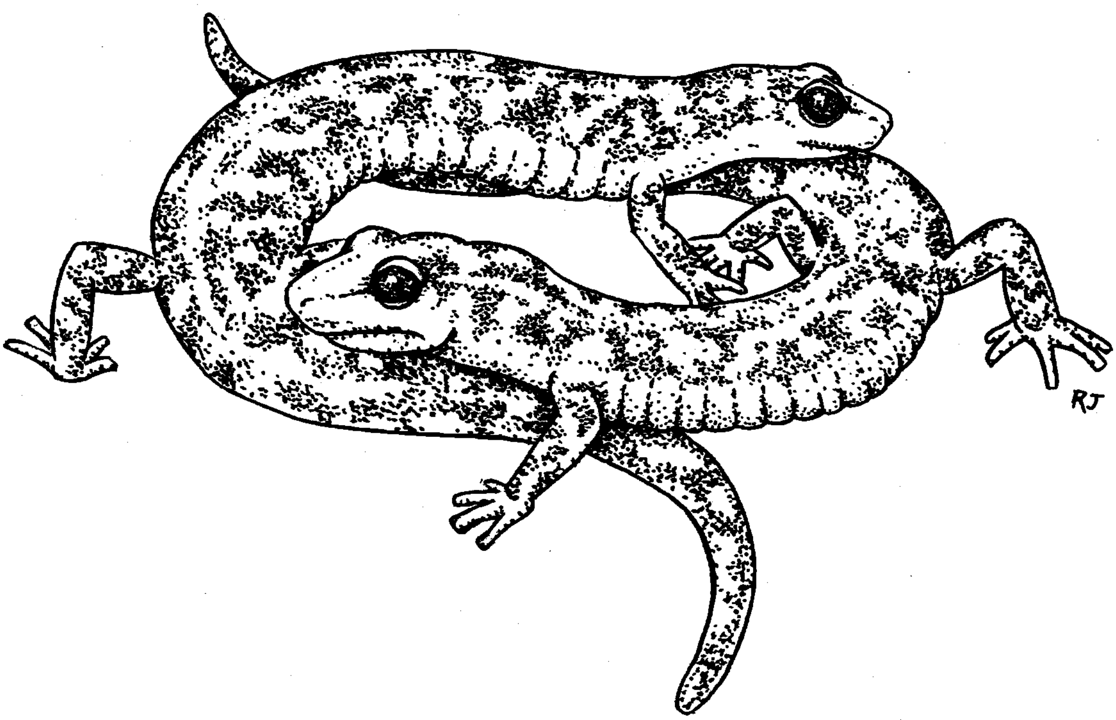


**Figure II.1.**
This illustration depicts a pair of Aneides ferreus in c-TSW, a manifestation of assertive female behavior. In the foreground the male maintains contact with the female's tail base; the female rubs the male as the pair performs c-TSW. (Illustration by Rick Jones)

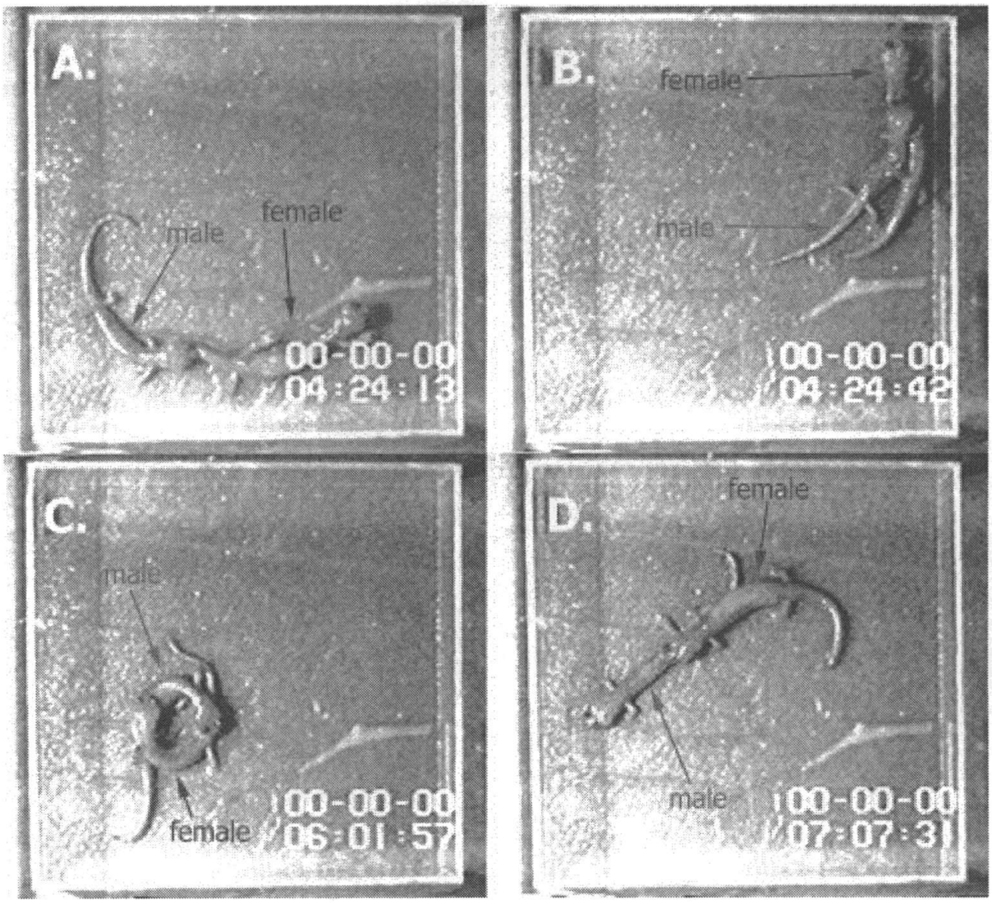


**Figure II.2. Video Images of *Aneides ferreus* courtship**

A) A non-receptive female avoids male advances, while he pursues her while rubbing in frame "A". B) Pulling by the male, during which he abrades her skin and applies pheromones. C) Circular TSW. D) Linear TSW, the male deposits a spermatophore.

**Duration of *Aneides ferreus* courtship phases**

| | AP | HC | c-TSW | l-TSW | SD/ ST | Total |
|---|---|---|---|---|---|---|
| | 2 | 45 | 109 | 3 | 10 | 169 |
| | 3 | 56 | 88 | 5 | 9 | 161 |
| | 3 | 74 | 68 | 8 | 24 | 177 |
| | 2 | 74 | 117 | 2 | 7 | 202 |
| | 4 | 176 | 18 | 10 | 10 | 218 |
| | 2 | 74 | 194 | 6 | 8 | 284 |
| | 4 | 115 | 126 | 8 | 12 | 265 |
| | 4 | 222 | 57 | 1 | 20 | 304 |
| | 5 | 243 | 75 | 2 | 17 | 342 |
| | 1 | 133 | 246 | 1 | 15 | 416 |
| | 3 | 192 | 153 | 47 | 6 | 401 |
| | 2 | 73 | 362 | 6 | 10 | 453 |
| | 2 | 73 | 431 | 3 | 9 | 518 |
| | 4 | 207 | 362 | 28 | 5 | 606 |
| | 3 | 150 | 447 | 1 | 7 | 608 |
| | 1 | 288 | 236 | 69 | 9 | 603 |
| | 3 | 312 | 332 | 5 | 8 | 660 |
| | 4 | 339 | 434 | 3 | 13 | 793 |
| | 4 | 764 | 91 | 4 | 14 | 877 |
| | 3 | 448 | 552 | 12 | 8 | 1023 |
| **mean (min)** | 2.95 | 202.9 | 224.9 | 11.2 | 11.05 | 454 |
| **SD (min)** | 1.1 | 171.8 | 160.2 | 17.5 | 4.9 | 251.3 |
| **% of courtship** | 0.6 | 44.7 | 49.5 | 2.5 | 2.4 | 99.8 |
| **Coefficent of Variation** | 37.3 | 84.7 | 71.3 | 155.9 | 44.1 | 55.4 |

**Table II.1.**
This table depicts the duration of five distinct phases in *Aneides ferreus* courtship. This table separates courtship into five categories [AP (approach), HC (head contact), c-TSW (circular TSW), l-TSW (linear TSW), and SD/ST (spermatophore deposition and sperm transfer)] and reports the mean, standard deviation, and coefficient of variation (CV) for 20 complete courtships.

**Courtship Character States of the tribe plethodontini**

| *Ensatina eschscholtzii* | *Plethodon idahoensis* | *Plethodon shermanii* | *Plethodon kentucki* | *Aneides ferreus* | *Aneides aeneus* | **Behavior Pattern** |
|---|---|---|---|---|---|---|
| + | + | + | + | + | + | linear TSW |
| - | - | - | - | + | + | circular TSW |
| - | - | - | - | + | ? | assertive female behavior |
| - | + | + | + | + | + | turning back |
| - | ? | - | - | + | ? | pulling |
| - | + | + | + | + | + | mental gland |
| 1 | 2 | 3 | 4 | 5 | 6 | *( reference)* |

**Table II.2.**

Comparison of courtship behaviors and morphology in the tribe Plethodontini are coded with a + for observed and a – for not observed. (1. Stebbins, 1949: 2. Lynch & Wallace, 1987; Brodie, E. D. Jr., 1968: 3. Arnold, 1976: 4. Marvin & Hutchison: 1996: 5. Cupp, 1971)

**Discussion**

Circular TSW may have arisen as a means of pheromone delivery by the male. In many plethodontid genera, pheromones are used to increase female receptivity (Houck and Sever, 1994). There are three primary modes of delivery: *slapping*, *scratching*, and *biting* (Arnold and Houck 1982, Houck 1986). In *Aneides ferreus*, pheromone delivery is accomplished by pulling, a form of scratching delivery. The premaxillary teeth abrade the female's body during pulling, and pheromones from the mental gland are applied to the abraded surface of the female's back. In most plethodontids, the male delivers pheromones during TSW by "turning back towards the female", a behavior pattern in which the male contacts the female's snout with his chin while maintaining the female's position astride his tail (Arnold, 1976; figure 18). In species with l-TSW, the male periodically interrupts linear progress to turn back and deliver pheromones and then reverts to l-TSW. c-TSW could be produced by the male remaining in a constant state of "turning back". Such a prolongation of "turning back" is the possible evolutionary origin of c-TSW. After the female becomes receptive towards the male's attempts to initiate courtship, the male remains turned towards the female, resulting in a circular TSW.

A congeneric species, *Aneides aeneus*, is the only other species of the family Plethodontidae known to perform c-TSW (Table II.2). Cupp (1971) observed *Aneides aeneus* courtship in the field in a rock crevice about 1 m above the ground. He observed c-TSW and also the shift to l-TSW prior to spermatophore deposition. *Aneides aeneus* and *A. ferreus* share the circular component of TSW in their courtships, and perform it in the same temporal context. Thus, c-TSW seems to have arisen only once and only in the genus *Aneides*.

Circular TSW may be an adaptation to courtship in constrained spaces. *Aneides ferreus* and *Aneides aeneus* occupy analogous ecological nich?s. Both typically usually occur in rock or bark crevices, and both have flattened morphologies, suggesting a structural adaptation to their crevice dwelling habitat. Analysis of the courtship behavior of other plethodontids that occupy similar constrained habitats would provide further tests of this hypothesis.

Another aspect of *A. ferreus* courtship behavior that is remarkable is the active role that the female plays in courtship. As in other plethodontids, *A. ferreus* females must become receptive towards males for courtship to occur and progress. Most plethodontid females become receptive to courtship and follow the male during TSW, with the only feedback cue for the male being where the female's head is positioned upon the male's tail base (Arnold 1976). Typically this is the only means by which a male could possibly assess female receptivity, with an exception being *Plethodon cinereus* females that will initiate TSW by leaving odor trials that males can use to find potential mates (Gergits & Jager 1990). By asserting that female *Plethodon cinereus* initiate TSW simply by leaving odor trials that males may follow to find females with which to mate, Gergits & Jager cannot limit this assertion to a single species. In *A. ferreus*, however, receptive females play an assertive role. Besides a shift from showing no reaction to the male's advances to following him in TSW, the female *A. ferreus* vigorously rubs her chin over the dorsum of the male. In the described courtships of species in the tribe Plethodontini this would be considered more typical of male-like behavior, but the female performs this behavior throughout TSW. "Head sliding" or rubbing is found in *Plethodon kentucki*, *P. montanus*, *P. glutinosis*, *P. yonaholosse*, *P. ouachitae*, *P. caddoenisis*, *P. welleri*, *P.cinereus*, *P. richmondi*, and *P. idahoensis* (Marvin and Hutchison, 1996; Table II.1) and is only performed by males. Deviating from the typical female behavior in the tribe plethodontini, *A. ferreus* females perform "male-like" behaviors. Furthermore, while the female is rubbing the male's dorsum, his role in courtship dampens so that the female becomes the more active participant in courtship. In all other accounts of courtships, the female plays a more passive role even when receptive, following male cues up to insemination. In contrast, the *A. ferreus* female continues to be the more active participant during c-TSW by rubbing the male until l-TSW begins, at which time the male resumes the more active role for the duration of courtship. The shift in behavior is an obvious visual cue to the observer that the female has become receptive.

*Aneides ferreus* has the longest courtships recorded for the Plethodontidae. Plethodontid courtships typically last less than 120 min and often less than 30 min (Arnold 1976 Fig 30, Sapp unpublished data). In *Plethodon kentucki*, a large eastern *Plethodon*, courtship lasts on average 99.0 min (Marvin & Hutchinson 1996). In other large eastern *Plethodon*, *P. montanus* and *P. shermani*, courtships last on average 55.9 min (Arnold 1976). *Ensatina*, a genus closely related to *Aneides*, is an exception, with courtship lasting as long as 300 min (Stebbins 1949). Plethodontid courtships can have a spermatophore deposited without successful sperm transfer. *Aneides ferreus* courtships last an average of 465 minutes (n=20); coupled with the fact that sperm transfer was 100% successful, perhaps lengthy courtships allow for higher rates of successful insemination (Arnold 1976).

It is unclear why the females of *A. ferreus* are so dramatically different from the currently described *Plethodon* females during courtship and why the duration of courtship is so long in comparison with other members of the tribe Plethodontini. One hypothesis for the long courtship duration and the circularity of the TSW found in *A. ferreus* centers on the habitat in which they occur, these behaviors could have arisen as a result of the confined spaces (crevices) which *A. ferreus* and *A. aeneus* inhabit. With added protection from living in unexposed environments, pressures from predation may not be as strong as those on animals exposed during courtship, thus allowing for lengthy courtships when compared to animals that court on the forest floor. This hypothesis also could explain the presence of circularity in the TSW phase of courtship. If animals are confined in a small area (a rock/bark crevice or elevated substrate) linear TSW may not be physically possible or may not effectively coordinate the pair into successful courtships. To test this hypothesis one could examine the courtships of plethodontids that occupy similar habitats; *Plethodon petraeus*, for example, is a rock crevice obligate (Wynn et al. 1988) that may exhibit similar behaviors.

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# Chapter III

## Courtship behavior of the Black Salamander, *Aneides flavipunctatus* (Strauch), and the Arboreal Salamander, *Aneides lugubris* (Hallowell).

Jerod Sapp

**Abstract**

In the family Plethodontidae, a linear tail-straddling walk (TSW) characterizes courtship. During tail-straddling walk the female follows the male as he moves forward in a straight line. *A. flavipunctatus*, a member of the family Plethodontidae, exhibits courtship behavior typical of the family. Courtship of *A. flavipunctatus* is similar to accounts of *Plethodon* courtship (Noble and Brady 1930; Organ 1958, 1966; Arnold 1976; Houck and Verell 1993; Lynch and Wallace 1987; Marvin and Hutchison 1996) in which the male leads the female in TSW in a linear fashion. Two species of *Aneides*, that occupy habitats above the forest floor, have a circular component to their TSW (*A. ferreus* and *A. aeneus*), but *A. flavipunctatus* does not. The lack of a circular component to TSW in *A. flavipunctatus*, a species that is associated with the forest floor and riparian areas, illustrates vivid differences in courtship behaviors within the genus. To test if the circular TSW is an adaptation to an arboreal mode of existence I examined the courtship behaviors of the most arboreal of the *Aneides*, *A. lugubris*. *Aneides lugubris* did not have a modified TSW, but rather exhibited typical plethodontid courtship. I conclude that circular TSW may be an adaptation to salamanders that are crevice-dwellers rather than arboreal.

**Introduction**

The genus *Aneides* is a member of the family Plethodontidae, the largest and most evolutionarily and ecologically diverse group of extant caudate amphibians (Pough 2001). Currently six species of *Aneides* are recognized: one eastern North American species, *A. aeneus*, a cohort of four western North American species; *A. ferreus*, *A. vagrans*, *A. flavipunctatus*, and *A. lugubris*, and a Rocky Mountain isolate, *A. hardii* (Wake 1974).

Salamanders utilize different behaviors to coordinate sperm transfer during courtship (Salthe 1967, Arnold 1977, Halliday 1990). In plethodontids, sperm transfer is orchestrated by a tail-straddling walk (TSW) in which the female follows the male with her chin resting on his tail base or sacral region (Noble & Brady 1930, Stebbins 1949). The tail-straddling walk is unique to plethodontids and has been observed in numerous genera in all clades of the family, suggesting that this behavior is ancestral (Arnold 1977). Tail-straddling walk proceeds linearly, with both partners moving in a straight line, except for bouts of pheromone delivery by the male or intervals during which the pair avoids obstacles. Some members of the genus *Aneides* have a different type of tail-straddling walk, one that is circular. This paper describes courtship in two western species, *A. flavipunctatus* and *A. lugubris*.

*Aneides flavipunctatus* and *A. lugubris* are associated with different adaptive zones (Wake 1966). *Aneides flavipunctatus* is typically associated with riparian areas and talus slopes within the terrestrial adaptive zone and can be found in wet soil beneath logs or rocks along streams and beneath rocks and logs in pastures (Staub 1993, Stebbins 1951, Wood 1936). *Aneides lugubris* is found in stumps, in trees, under rocks, under woody surface cover, and in mine shafts, rodent burrows, and stone walls (Rosenthal 1957, Stebbins 1951, Storer 1925). *Aneides flavipunctatus* appears not to be a proficient climber, as are most other members of *Aneides*. Conversely, *A. lugubris* exhibits morphological specializations associated with the arboreal adaptive zone. A prehensile tail and flattened toe tips allow this salamander to maneuver and maintain position in small crevices and in other habitats located above the forest floor. Despite their arboreal adaptations, *Aneides lugubris* are sometimes on the ground, under cover objects and migrating across roads during warm rains (pers comm. Westphal).

The other species in the genus also show contrasting ecologies. *Aneides aeneus*, the eastern member of the genus is an obligate rock crevice-dwelling species typically found in sandstone, granite, and schist formations with deep, moist shaded crevices (Bruce1968; Gordon & Smith 1949; Mount 1975; Netting & Richmond 1932; Schwarz 1954). *Aneides ferreus* and *A. vagrans* are abundant where rock faces or talus provide deep cracks for foraging, resting and nesting. Adults are also found beneath loose bark and in cracks of decaying logs and stumps, and as high as 40 m in tree canopies (Bury and Corn 1988, Fitch 1936, Stebbins 1951, Stemlock and Harstead 1979).

*Aneides hardii*, occurs in well-rotted logs and beneath bark, logs, rocks, and rubble in the Capitan, Sacramento, and White Mountains of New Mexico (Johnston and Schad 1959, Lowe 1950, Schad et al. 1959, Stebbins 1951). Thus, two of six species (*A. flavipunctatus* and *A. hardii*) are terrestrial and are not found in habitats above the forest floor.

I predict that *Aneides* that inhabit different adaptive zones (terrestrial, semi-arboreal, and arboreal) may have evolved different courtship behaviors, in part, because of the different selective pressures associated with different adaptive zones. The first step in testing this hypothesis is to compare courtship behavior among members of the genus. Specifically, will *Aneides* that are found in the semi-arboreal/arboreal adaptive zone have a modified TSW, presumably an adaptation to arboreal modes of existence? And, conversely, do terrestrial species (e.g. *A. flavipunctatus*) exhibit the typical plethodontid TSW, like most *Plethodon*?

**Materials and Methods**

*Aneides flavipunctatus* were collected from three localities: Hanley Gulch, Josephine Co. OR (N42° 04.716', W123° 01.881'), Seiad Creek, Siskiyou CO. CA (N41° 51.087', W123° 10.405'), and Usal Road, Mendocino Co., CA (N39° 49.192', W123° 50.637') between the fall of 2000 and the spring of 2001. *Aneides lugubris* used in this paper were collected from Contra Costa Co. California in September of 1970 (Arnold, 1972), and from Santa Cruz, California. Salamanders were transported to Oregon State University and housed in a controlled climate room at 12-13.5° C with a natural photoperiod. Each animal was housed individually in a 12 x 17 x 6 cm plastic box. Boxes were lined with moist paper towels and each had one crumpled towel to provide cover. The animals were fed small crickets (*Acheta domestica*) and wax worms (*Galleria nella*).

Courtship trials were staged at night in the same room in which the animals were housed. Courtship arenas consisted of two glass plates separated by a Plexiglas frame 17x17x5 cm. The lower glass surface was covered with a moist paper towel. Courtship trials lasted for a period of 24 hours beginning at 1700 h. Successful courtship was scored by the presence of a spermatophore base on the substrate and a sperm cap in the vent of the female. Only successful courtships were used in analyses. Courtships were taped using a low light camera (Panasonic VW BD400) and a closed circuit time-lapse video tape recorder (Panasonic AG 6540). On a given night, two cameras were used to record two different pairs of salamanders on video tape recorders at 4 images/second. Five courting pairs were used in the courtship trials for *Aneides flavipunctatus* and one pair for *A. lugubris*. All courtships were conducted with both participants drawn from the same population. Courtship trials began in late March of 2001and ended in May 2001 for *Aneides flavipunctatus* and began and ended in January 2002 for *Aneides lugubris*. Sequences observed by Stevan J. Arnold were also used to compare my observation of courtship in *Aneides lugubris*.

## Results

**Ethogram for *Aneides flavipunctatus*.**

**Behavioral Definitions:**

Male behaviors

**Approach-** The male consistently orients toward the female and follows her around the arena.

**Head contact (HC)** - The male will touch his head to any aspect of the female's dorsum. Head contact is divided into four distinct categories.

**Rubbing-** The male rubs the female's dorsum with his chin by moving his head from side-to-side, not exceeding a 10° arc

**Pulling-** The male repeatedly pulls his premaxillary teeth and his mental gland, in an anterior to posterior fashion, across some aspect of the female's dorsum. The male places his chin on the female and applies force while pulling his chin backward = 3mm across the dorsum of the female. The male usually begins pulling behavior on the female's tail because the female is usually retreating from the male's advance.

**Pulling at mid-body-** As the male continues to pull his chin (mental gland) across the female she is less apt to retreat and this allows him to pull on other areas besides the tail. It is the same behavior as \ initial pulling, but more force is applied to the female

**Turning back to pull-** As the pair is in TSW the male breaks from the forward linear movement in order to pull his chin across the female's mid-body. The male's body is curved with his head facing the tail of the female while he abrades the female's dorsum with his chin. After turning back to pull the male resumes the forward movement of TSW.

**Tail undulation (TU)-** The male's tail undulates laterally when it is under the female's chin. The male's tail usually remains in contact with the female's chin during tail undulation. Two different types of tail undulation occur in courtship. During sperm transfer the tail base is elevated off the substrate while the male undulates his tail. When tail undulation occurs during spermatophore deposition, the male's tail base will remain in contact with the substrate.

**Spermatophore deposition (SD)-** The male ceases any forward motion and places his hind legs perpendicular to his body axis. The male undulates his tail in a manner resembling a sinusoid curve with the tail base in contact with the substrate.

**Sperm Transfer (ST)-** After a spermatophore has been deposited on the substrate the male elevates his sacral region and pulls his tail out and to the side, then moves forward, leading the female over the spermatophore. When she is positioned over the spermatophore he will stop moving forward. At this time the male will rock his elevated tail base from side to side by pushing his hind limbs up and down and alternating from left to right, while he continues to undulate his tail beneath the female's chin.

Female behaviors

**Retreat-** Prior to TSW, the female moves away from the male while he persistently pulls his mental gland over her body.

**Chin up-** After pulling at the mid-body by the male, the female may elevate her chin perpendicular to the substrate, apparently indicating female receptivity.

**Sperm mass pick-up-** With her chin resting on the male's tail base, the female moves over the spermatophore and, while stationary, positions her cloaca on the spermatophore, inserting the spermatophore in her vent. Typically, after 2-5 min she then lifts off the spermatophore with the sperm cap inside her cloaca. The spermatophore base remains on the substrate.

Male and female behavior

**TSW-** The male and female participate in tail-straddling walk (TSW). The male leads the female in a linear fashion, with her chin is in contact with the sacral region of the male (Arnold 1976).

**Temporal Patterning**

Courtship of *Aneides flavipunctatus* begins with an approach phase that leads into a phase of pulling by the male. When animals are first placed in the courtship arena, no courtship behaviors occur for a minimum of 30 minutes before the male repeatedly approaches a female. During the approach phase, the male will focus all attention towards the female, following her throughout the arena. The male will continue to pursue the female while she persistently moves away from him. The male will pull his mentum on some area of the female's tail as she evades him. He will continue to pull on her tail and will attempt to pull on other parts of the femake's dorsum. As she becomes more receptive to the male he begins to pull with more force and on other places than the female's tail, including the head and mid-body.

The female signals her receptivity by pointing her chin upward, perpendicular to the substrate. This chin up behavior occurs while the male is pulling across the mid-body. When the female puts her chin up, she is receptive to TSW. To initiate TSW, the male crawls forward and situates his tail base under the elevated chin of the female. At this time she will either lower her chin and enter TSW, or she will not enter TSW in which case the male will resume pulling at mid-body. At this time a circle is formed when the male is turning back and the female has her head on his tail base. This posture is reminiscent of circular tail-straddling walk, found in *A. ferreus* and *A. aeneus*; but, it differs in the sense that the pair does not maintain this orientation when the male moves forward. The circle is rapidly broken and the pair moves forward in a straight line. When the pair is in TSW the male leads the female, turning back periodically to pull his mentum across the mid-body of the female. As courtship progresses the male will turn back less frequently and the pair will stay in a forward linear TSW. If TSW is broken, the male will TU without raising his tail base; this may be a cue for the female to re-enter TSW. If she does, the courtship will progress to spermatophore deposition; if not, the male will return to the approach phase.

Spermatophore deposition (SD) is the next behavior to occur if TSW is not broken. After the spermatophore is deposited on the substrate he leads the female over the sperm mass. When she is situated on the spermatophore he will TU and FS while she picks up the sperm mass with her cloaca. After the female has picked up the sperm mass she will move away from the male, leaving the spermatophore base on the substrate. Following insemination, the female will retreat from the male and does not re-enter TSW with the male. The male may continue to pursue the female after insemination, but multiple spermatophore depositions were not observed in the 24-hour time frame of the courtship trials.

## Ethogram for *Aneides lugubris*:

The behaviors defined are based on accounts described in Stevan J. Arnold's doctoral thesis (1972) as well as my observation of a single courtship.

Male Behaviors

**Approach-** The male consistently orients toward the female and follows her around the arena. This signals a shift in the male's interest in reproduction.

**Head contact (HC)-** The male touches his head to any aspect of the female's dorsum. Head contact is divided into three distinct categories.

**Nudging-** The male contacts his snout to the female's body.

**Pulling**- The male repeatedly pulls his premaxillary teeth and his mental gland, in an anterior to posterior fashion, across some aspect of the female's dorsum, usually about 2mm.

**Lifting**- The male raises his head and turns his head under the female's chin.

**Tail undulation (TU)**- The male's tail undulates laterally when it is under the female's chin. The male's tail usually remains in contact with the female's chin during tail undulation. Two different types of tail undulation occur in courtship. During sperm transfer the tail base is elevated off the substrate while the male undulates his tail. When tail undulation occurs prior to sperm transfer, the tail base remains in contact with the substrate.

**Spermatophore deposition (SD)**- The male ceases any forward motion and places his hind legs perpendicular to his body axis and this cue signals he is depositing a spermatophore on the substrate. During spermatophore deposition the male undulates his tail in a manner resembling a sinusoid curve with the tail base in contact with the substrate.

**Sperm Transfer (ST)**- After a spermatophore has been deposited on the substrate the male elevates his sacral region and pulls his tail out and to the side, then moves forward, leading the female over the spermatophore. When she is positioned over the spermatophore he will stop forward motion. At this time the male will rock his elevated tail base from side to side by pushing his hind limbs up and down and alternating from left to right, while he continues to undulate his tail.

Female Behaviors

**Female tail undulation**- The female will undulate the distal aspect of her tail when the male has become separated from her prior to tail-straddling walk

**Chin up**- The female will point her chin upward; this can happen while the male is face to face with her or while he is pulling.

**Sperm mass pick-up**- With her chin resting on the male's tail base, the female moves over the spermatophore and positions her cloaca on the spermatophore. Typically, after 2-5 min she then lifts off the spermatophore with the sperm cap inside her cloaca. The spermatophore base remains on the substrate.

Male and Female Behaviors

**Linear Tail-straddling walk (l-TSW)**-The male leads the female in a linear fashion, while her chin is in contact with the sacral region of the male (Arnold 1976).

**Temporal patterning**

The male initiates contact with the female by nudging her with his chin. The female initially retreats from the male's advances. He continues to pursue her and nudge or pull his chin across some aspect of the female's dorsum. Pulling can occur as a single behavior or can be performed in a series of successive movements.

As the male continues HC and the courtship progresses the female becomes less apt to flee from the male and head contact from the male increases. If the male is distracted or stops the head contact and begins to move away, female tail undulation may occur to re-orient the male to the female's position. Pulling and nudging by the male plus female tail undulation ultimately culminates with the male turning his head under the female's chin and performing lifting. If the female moves forward with the male, tail-straddling walk is established. If the female does not move with the male the behaviors prior to lifting will resume or courtship behavior may cease all together.

During TSW, the female's chin rests atop the male's tail base. Forward progress by the male is contingent with the placement of the female's head. If the female situates her head too far behind his tail base, then he will slow forward movement to allow her to align her head with the apex of his tail base. Conversely, if the female's head is too far in front of the tail base, he may speed the forward movement to align her head with the apex of his tail base. TSW can take a very short time (Table III.1.). Following TSW, spermatophore deposition occurs. During spermatophore deposition the male stops all forward motion and places his hind limbs at a 90° angle to his body axis. His entire venter is in complete contact with the substrate. The male undulates his tail while the female's head remains in contact with the male's tale base.

After depositing a spermatophore, the male resumes forward movement and leads the female over the spermatophore. The spermatophore contacts the female's venter as she move forward. She lifts her body and slides over the top of the sperm mass. When the sperm mass contacts her vent, she lowers her vent upon it, inserting the sperm mass inside her cloaca. As the female sits atop the spermatophore the male will vigorously undulate his tail while his tail base is elevated from the substrate. His hind feet will rock back as he undulates his tail under the female's chin. After she has move off the spermatophore base with the sperm cap, the male may attempt to re- initiate courtship. However, only single spermatophore depositions were observed in an evening.

**Duration of *Aneides flavipunctatus* and *Aneides lugubris* courtship phases**

| *A. flavipunctatus* SEQUENCE | AP | HC | TSW | SD/ST | min |
|---|---|---|---|---|---|
| **1** | 5 | 77 | 61 | 7 | 150 |
| **2** | 7 | 34 | 54 | 18 | 113 |
| **3** | 6 | 17 | 60 | 1 | 84 |
| **4** | 1 | 29 | 35 | 8 | 73 |
| **5** | 1 | 286 | 85 | 11 | 383 |
| mean | 4 | 88.6 | 59 | 9 | 160.6 |
| SD | 2.8 | 112.7 | 17.9 | 6.2 | 127.8 |
| % of courtship | 2.4 | 55.2 | 36.7 | 5.7 | |

| *A. lugubris* | AP | HC | TSW | SD/ST | |
|---|---|---|---|---|---|
| **1** | 3 | 140 | 2 | 10 | 155 |
| % of courtship | 1.9 | 90.3 | 1.3 | 5.9 | |

**Table III.1**. The duration of four phases of courtship in *Aneides flavipunctatus* and *Aneides lugubris*: [AP (approach), HC (head contact), TSW (tail-straddling walk), and SD/ST (spermatophore deposition and sperm transfer). The mean, standard deviation, and percent of the courtship are reported for each phase.

**Character States of Courtship in the plethodontini**

| | AFB | pulling | c-TSW | l-TSW | crevice | arboreal | lengthy | scratching | gland | TB | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | - | - | + | - | - | + | - | - | - | *Ensatina eschscholtzii* |
| 2 | | | + | + | + | + | | + | + | + | *Aneides aeneus* |
| 3 | + | + | + | + | + | + | + | + | + | + | *Aneides ferreus* |
| 4 | - | + | - | + | - | - | + | + | + | + | *Aneides flavipunctatus* |
| 5 | + | + | - | + | | + | + | + | + | + | *Aneides lugubris* |
| | | | | | | | | | | | *Aneides hardii* |
| 6 | - | - | - | + | - | - | - | - | + | + | Large eastern *Plethodon* (*Plethodon shermani*) |
| 7 | - | + | - | + | - | - | - | + | + | + | Small eastern *Plethodon* (*Plethodon cinereus*) |
| 8 | - | + | - | + | - | - | - | + | + | + | Small eastern *Plethodon* (*Plethodon welleri*) |
| 9 | - | | - | + | - | - | - | | + | + | western *Plethodon* (*Plethodon idahoensis*) |

**Table III.2.**
Distribution of character states for behavioral, morphological and ecological features among species and clades in the tribe Plethodontini. Abbreviations for character states are: AFB (assertive female behavior), pulling (pulling pheromone delivery), c-TSW (circular tail-straddling walk), l-TSW (linear tail-straddling walk), crevice (crevice-dwelling), arboreal (arboreal habits), lengthy (lengthy courtship), scratching (scratching pheromone delivery), gland (mental gland), TB (turning back). + symbols represent the presence of the character, - symbols represent the absence of the character, empty spaces denote no data for the character state. Courtship observations: 1(Stebbins 1949, Arnold 1972), 2 (Cupp 1971), 3 (Sapp),4 (Sapp), 5 (Arnold 1972, Sapp), 6 (Arnold 1972, 1976), 7 (Arnold 1972), 8 (Arnold 1972), 9 (Lynch and Wallace 1987).

**Character States Mapped on a Phylogeny of the tribe plethodontini.**

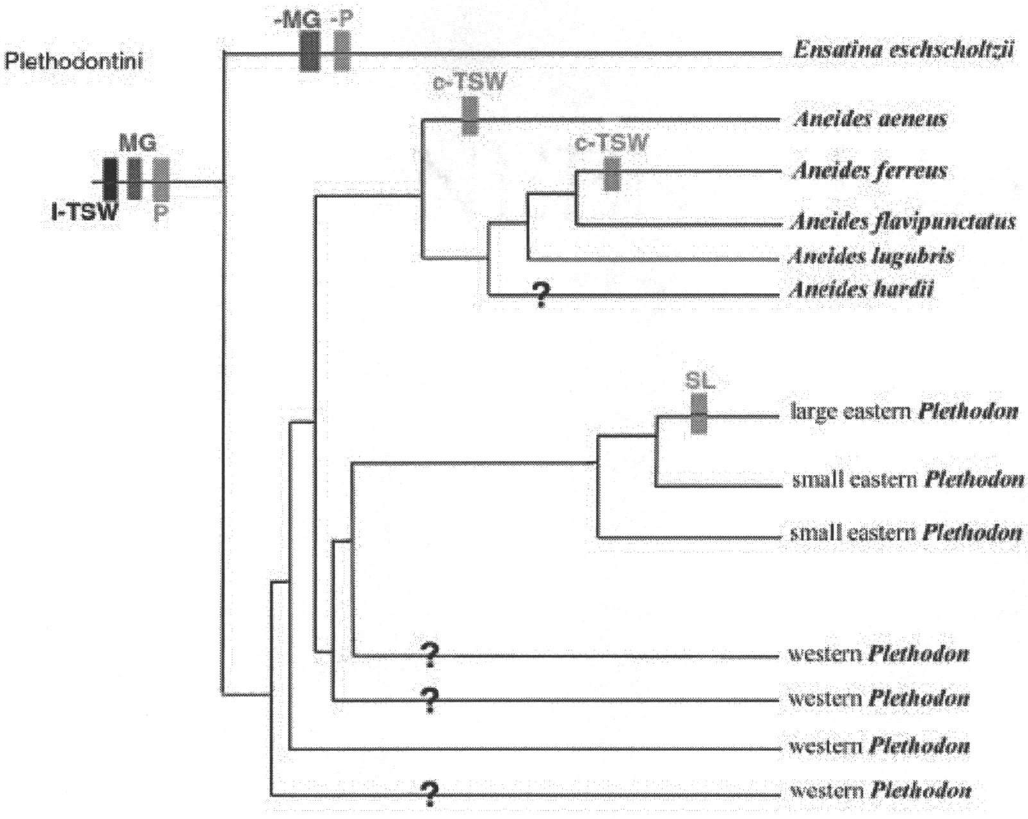


**Figure III.1.**
Character states of behavioral and morphological features mapped to a phylogeny of the tribe Plethodontini: L-TSW (linear tail-straddling walk), MG (mental gland), P (pulling), c-TSW (circular tail straddling walk), and SL (slapping) are denoted by hatch marks on the tree. Minus signs denote evolutionary loss. The tree is based on Mahoney (2001, fig. 6).

**Discussion**

I hypothesized that a circular tail-straddling walk could be an adaptation to an arboreal adaptive zone. However, neither, *Aneides lugubris* nor *Aneides flavipunctatus* perform a circular tail-straddling walk (c-TSW). *Aneides flavipunctatus* would be expected not to exhibit this behavior, but *Aneides lugubris* would. My hypothesis was based on the courtship behavior of two congeners that occupy habitats off the forest floor, and have a c-TSW, *Aneides ferreus* and *Aneides aeneus* (Fig. III.1). Although the number of observations of *A. lugubris* is low, the fact that two separate observers agree on temporal patterning and behaviors, strengthens the observation's validity. c-TSW may not be an adaptation for an arboreal lifestyle but may be an adaptation to crevice-dwelling salamanders. *Aneides lugubris* and *A. flavipunctatus* do not perform c-TSW (Fig. III.1). A plausible explanation for this is that their courtship does not take place in crevices or confined areas but rather in less constrained areas in the arboreal or terrestrial adaptive zones. Field observations are needed for all members of *Aneides* to determine when and where courtship occurs.

*Aneides flavipunctatus* and *Aneides lugubris* courtships have many similarities and only one potential difference. Both species have linear tail-straddling walks and perform pulling. *Aneides lugubris* female was observed undulating her tail for extended periods of time, presumably to focus the male's interest. This behavior was not seen in *Aneides flavipunctatus*.

There is a disparity in the time spent in the phases of courtship of the western *Aneides*. *Aneides ferreus* spends more time in TSW when compared to *A. flavipunctatus* or *A. lugubris*. In my observations the mean courtship duration of *A. ferreus* was 454 min, while the other two western species exhibited considerably shorter durations (*A. flavipunctatus* 160.6 min, and *A. lugubris* 155 min). The courtship of *Aneides ferreus* are the longest recorded plethodontid courtships. Apart from having longer courtships *A. ferreus* they possess a modified TSW (circular tail-straddling walk) that is not seen in the other western *Aneides*. This suggests the modification of the highly conserved tail-straddling walk, observed in *A. ferreus*, could be an adaptation to courtship performed in a different environment from western congeners. To determine if the confined spaces in which the two species of *Aneides* that perform circular TSW (c-TSW) increases courtship duration, observations of *Aneides aeneus* must be recorded and compared to *A. ferreus*. Courtship observations of *Aneides flavipunctatus* collected from various habitats throughout their extensive range may reveal behavioral differences within the species. The two chromosomal races of *Aneides lugubris* may possibly exhibit behavioral differences in courtship. However, if my observations of *A. lugubris* and *A. flavipunctatus* are typical, then there are two avenues by which c-TSW arose twice within the genus: the ancestral state may have been to perform c-TSW and all species have lost that character except for *A. ferreus* and *A. aeneus*, or that it has independently arisen twice in the crevice dwelling members of *Aneides*.

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# Chapter IV: Conclusions

## Summary

I have explored the courtship behaviors of two species of *Aneides* for which there was no courtship behavior previously described (*A. ferreus*, and *A. flavipunctatus*). I also re-examined courtship in a species for which only a single sequence had been recorded (*A. lugubris*). I found that *A. ferreus* perform a circular tail-straddling walk that had been seen in *A. aeneus* but not fully described or defined. When this behavior is mapped on to the current phylogeny for *Aneides,* there are two possible scenarios for the evolution of c-TSW. The circular tail-straddling walk has arisen twice in the genus, or it is a basal character that the other species of *Aneides* have lost. My initial interpretation was that c-TSW is an adaptation to an arboreal or semi-arboreal lifestyle. If this were the case the most arboreal member of *Aneides* would also possess the c-TSW or some modification of courtship to accommodate the arboreal adaptive zone. Typical plethodontid courtships have a linear tail-straddling walk, and these salamanders all court on the forest floor. Salamanders that occupy niches that constrain the space for courtship should exhibit modified characters that are adapted to the niche in which they exist. However, the most arboreally adapted of the *Aneides*, *A. lugubris*, has a linear tail-straddling walk, just as do the forest floor dwelling plethodontids. Closer evaluation of the natural history of the two species that possess c-TSW indicates that they live in the crevices of large rocks or of trees. Therefore, c-TSW may be an adaptation to confined spaces in which these salamanders live.

The evolution of the c-TSW can be linked to pheromone delivery. I postulate the c-TSW is a constant state of "turning back". After the female becomes receptive towards the male's attempts to initiate courtship via pheromone delivery, the male remains turned towards the female, resulting in a circular TSW.

Additional characters were found for *A. ferreus* such as the assertive nature of the females, and the lengthy duration of courtship. They have the longest courtship thus described in the Plethodontidae. The duration of courtship could reflect the adaptation to living under or in deep crevices that provide more shelter from predation than if courtships were performed on the forest floor. Their habitat lends support to the c-TSW being an adaptation to the physical restraints crevice-dwellers face. Linear TSW may not be physically possible or may not effectively coordinate the pair into successful courtships.

Habitats in which the members of *Aneides* reside may also explain the differences in their tail-straddling walks. *Aneides aeneus* has been recorded performing c-TSW in the field (Cupp 1970); *A. ferreus* also exhibits this behavior. These two species have very similar habitats. The sister species *A. ferreus* and *A. vagrans* provide yet another opportunity to look for a c-TSW within the genus. *Aneides flavipunctatus* occupies a very different niche from the aforementioned species. They are primarily found on the forest floor and can be associated with riparian areas, although, they are also found in mesic oak forests with rock outcroppings. These salamanders exhibit a linear TSW, like the majority of Plethodontidae. *Aneides flavipunctatus* are active on the forest floor during the breeding season and most likely court on the forest floor substrate. *Aneides lugubris* do not have c-TSW, but rather a typical plethodontid linear TSW. These are characteristically large salamanders that may require more space in which to court, thereby utilizing some other adaptive zone to perform courtship.

**Future Considerations**

The most important additional work to be considered is field observations of the courtships of not only *Aneides*, but of all the western plethodontids. The eastern plethodontids have been observed courting in the field (Petranka 1998). Such observations provide a benchmark for laboratory observation to be compared against. Will the courtship durations be similar in the field? Are any of the laboratory observations artifacts of being in confined areas with no means of escape? The next step should be to perform field detailed observations to find when and where these salamanders are reproducing.

Recent molecular work has produced trees that are not fully resolved. In order to map behavioral characters with confidence, more work needs to be done on the relationships in the plethodontini.

Agonistic behaviors have been recorded in *Aneides vagrans* (Davis 2002). Many agonistic behaviors described are similar to courtship behaviors that I observed. Are some behaviors used in courtship as well as agonistic confrontations?

There exist different races within *Aneides* in two of the species I have discussed. There may be cryptic species among the *A. flavipunctatus* races and the chromosomal races of *A. lugubris.* Behavioral observation of courtship as well as molecular analyses could reveal whether races of *Aneides lugubris* and *flavipunctatus* are reproductively isolated.

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