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Novel Courtship Behaviors in Three Small Eastern *Plethodon* Species

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ABSTRACT.—Plethodontid salamanders provide an opportunity to investigate mating behaviors and pheromonal communication within the context of well-established phylogenetic relationships. Courtship in many members of the genus *Plethodon* has been well studied, but critical gaps exist in observations of pheromone delivery methods (vaccination or olfactory) in species phylogenetically intermediate between those having the two pheromone delivery methods. I observed courtship interactions in three small eastern plethodontids, *Plethodon cinereus*, *Plethodon angusticlavius*, and *Plethodon richmondi*. I documented several novel female behaviors, as well as interspecific variation in courtship patterns that otherwise have been highly conserved for over 70 million years. I confirmed that olfactory pheromone delivery occurs in *P. angusticlavius*, a species that is phylogenetically in the transition from vaccination to olfactory pheromone delivery within the genus *Plethodon*. The deviation from typical courtship patterns observed for the three study species revealed that females play a more active role in courtship than has previously been documented for *Plethodon* species.

Courtship in plethodontid salamanders, and particularly in the genus *Plethodon*, is typically initiated when the male orients to and approaches the female (Organ, 1961; Arnold, 1977). Initially, the male makes head contact with the female, and the pair later engages in TSW. During TSW, the female follows the male with her chin on the base of his tail and her forelimbs on either side of the male's tail (Noble and Brady, 1930; Stebbins, 1949; see fig. 2 in Arnold, 1977). During the courtship season, males develop a mental gland that secretes pheromone proteins that can alter female behavior (Houck and Reagan, 1990; Rollmann et al., 1999, 2000). Courtship pheromones are delivered to females before or during the TSW.

Most plethodontids maintain the ancestral mode of pheromone delivery by "vaccination." Vaccination occurs when the male scratches and abrades the skin on the female's dorsum with enlarged premaxillary teeth and subsequently rubs his mental gland over the same area (Organ, 1961; Arnold, 1972; Houck and Arnold, 2003). Also, males of several species use a rapidly executed snapping or pulling behavior (described below) to deliver pheromone to the female (Arnold, 1972; Marvin and Hutchison, 1996). These vaccination behaviors presumably allow the pheromone produced by the mental gland to enter the female's superficial circulatory system (Organ, 1961; Arnold, 1972; Houck and Arnold, 2003). In contrast, large eastern plethodontids have a derived mode of pheromone delivery in which the male "slaps" his mental gland directly on the female's nares (Arnold, 1976, 1977; and fig. 1 in Rollmann et al., 2000). Pheromonal stimulation by direct olfactory delivery is processed by the female's accessory olfactory system (Wirsig-Wiechmann et al., 2002). Regardless of the delivery mode, the majority of pheromone delivery occurs during TSW (Houck and Arnold, 2003).

During courtship, males are traditionally considered the more active participant, whereas females are more subtle. For example, the female initially demonstrates receptivity by no longer fleeing when the male approaches her and contacts her with his head. When the male initiates TSW, the female steps over his tail and places her chin on the base of his tail. The timing of spermatophore deposition presumably is coordinated by the ability of the male to assess the female's forward movement during linear TSW (Arnold, 1976). If a female moves in a coordinated fashion with the male (i.e., does not lag behind), the male is more likely to deposit a spermatophore without further courtship interactions. After spermatophore deposition, the female obtains the sperm mass by moving forward and lowering her cloaca onto the spermatophore, thus lodging the sperm mass in her cloaca.

Courtship in the Plethodontidae is highly stereotyped in that the same behaviors are observed in many species (Arnold, 1977; Marvin and Hutchison, 1996; Houck and Arnold, 2003). However, more recent observations of previously unstudied species have revealed novel variations in courtship behavior between species (Sapp, 2002). In particular, these recent studies have revealed unsuspected variation in the TSW. Species of *Aneides*, for example, engage in a TSW that proceeds in a circle, instead of in the traditional straight line (Sapp, 2002). *Aneides* females also exhibit behaviors previously considered stereotypical of males. For example, a female rubs the male's back with her chin, a behavior that in other species is typically performed only by males. Adding to the examples of variation in TSW and more active female behavior, I describe below the courtship behavior of *Plethodon cinereus*, *Plethodon angusticlavius*, and *Plethodon richmondi*.

Materials and Methods

I collected animals from two locations in Virginia and one location in Arkansas in August 2000 and February 2001. These included 28 *P. richmondi* and eight *P. cinereus* from Grayson County, Virginia (36°37.73′N, 81°35.23′W). Another 12 *P. cinereus* were collected from a second site in Grayson County, Virginia, on Mount Rogers (36°47′N, 81°32.96′W). Five *P. angusticlavius* were collected from Madison County Wildlife Management Area in Arkansas (36°14′42″N, 93°40′45W). Animals were sexed using the "candling" method (Gillette and Peterson, 2001). There were 14 *P. richmondi* of each sex and 14 females and six males of *P. cinereus*. For *P. angusticlavius*, there were four males and one female. Although the sample size for *P. angusticlavius* observations are small, the behavior of these salamanders highly stereotyped. In courtships of *P. angusticlavius* and *P. richmondi*, males and females used in trials were from the same population. I collected from two populations of *P. cinereus*. Consequently, males and females used in courtship observations may or may not have been from the same population because of the overall shortage of males.

Salamanders were transported to Oregon State University, Corvallis, Oregon, and housed in a climate-controlled room (13–16°C) with a natural photoperiod. Animals were housed individually in plastic shoeboxes (12 × 17 × 6 cm) that were lined with moist paper towels, and several crumpled paper towels were added to provide cover. The animals were fed white worms (*Galleria nella*) and/or small crickets (*Acheta domestica*) weekly.

Separate from observed courtship stagings, males and females were paired randomly within each species and allowed to interact overnight to maintain levels of reproductive and social behavior in the laboratory. These unobserved courtships were conducted on nights when staged courtships were not recorded on video. Infrequently, and in all species, spermatophores were found the morning following unrecorded social interactions, indicating the maintenance of reproductive condition. Staged video taped courtship trials began in October 2000 and continued until behavioral interactions diminished (mid-December 2000 for *P. cinereus*, *P. richmondi*, through March 2001 for *P. angusticlavius*). The trials were staged at night, beginning at 1500–1900 h in the same room in which the animals were housed. Each courtship arena consisted of two glass plates separated by a plexiglass frame (17 × 17 × 5 cm). The lower glass surface was covered with a moist paper towel. Trials lasted until the following morning (0700–0900 h). Animals were given at least three days between staged interactions or after successful unrecorded courtships occurring between video taped encounters.

Courtships were recorded using a low light camera (Panasonic VW BD400) and a closed circuit time-lapse video tape recorder (Panasonic AG 6540). Up to four different pairs of animals were recorded each night, each at four images per second. Thirty-seven courtship interactions were recorded, including 13 spermatophore depositions. Behavioral observations are described using summary statistics (mean, SD, and range).

Results

Courtship behaviors are cataloged below in ethogram format for *P. cinereus*, *P. richmondi*, and *P. angusticlavius*. The catalog describes in detail all behaviors observed in the three species and generally follows nomenclature in previous ethograms from Arnold (1972, 1976). Following the catalog, I clarified which behaviors were performed by a particular species and by sex (Table 1). I also described the temporal relations of behaviors observed during courtship interactions for each species, and compare the duration of each stage of courtship among species (Tables 2–4).

Behaviors Performed by Males or Females.— Approach: One individual moves toward the other (within 2 cm); Retreat: One individual moves away after an approach by the other; Nudging: One individual moves its head along the sides of the other's body; Rubbing and Sliding; One individual moves its head in a side-to-side (rubbing) or forward-to-backward (sliding) motion while in contact with body/dorsum. When rubbing is performed by males, the premaxillary teeth may abrade the female's skin, accomplishing a type of vaccination pheromone delivery; Foot Shuffling: While stationary, the animal lifts one foot as the other descends. This behavior is confined to rear legs. It is similar to foot dancing (Arnold 1976), which includes all four legs. Cloacal Nudging: The snout of one animal contacts the cloacal region of the other salamander; Head Contact: Contact to the head is initiated by one individual (cheek to cheek, snout to cheek, snout to snout); Head Rubbing: Head to head contact is performed by one individual

TABLE 1. A comparison of female (F) and male (M) courtship behaviors for each species. See text for description of the behaviors. + = behavior present, − = behavior not present, 0 = behavior not observed, and ? = uncertainty caused by limited observations.

Behaviors	*P. cinereus*		*P. angusticlavius*		*P. richmondi*	
Female or Male	F	M	F	M	F	M
Approach	+	+	+	+	+	+
Retreat	+	+	+	+	+	+
Nudging	+	+	+	+	+	+
Rubbing/Sliding	+	+	+	+	+	+
Cloacal nudging	+	+	+	+	+	+
Head contact	+	+	+	+	+	+
Head rubbing	+	+	+	+	+	+
Foot shuffling	+	−	−	+	−	−
Tail wagging	+	+	+	+	+	+
Turning back	+	+	+	+	+	0
Female Only						
Cloacal rubbing	+		?		?	
High amplitude tail undulations	+		?		+	
Tail wagging	−		+		+	
High amplitude head swinging	+		−		+	
Pulling	−		−		+	
Stop and lower cloaca	+		+		0	
Pass over spermatophore	+		+		0	
Retreive sperm	+		+		0	
Resume tail straddling walk	+		+		0	
Male Only						
Snapping	+		0		+	
Pulling	+		0		?	
Slapping	−		+		−	
Head swinging	−		−		+	
Spermatophore deposition	+		+		0	
Eat spermatophore	0		0		0	
Tail undulation	+		+		+	
Tail wagging	+		+		+	
Crossing under	+		+		+	
Male and Female						
Linear tail-straddling walk	+	+	+	+	0	0
Female first tail-straddling walk	+	+	+	+	+	+
Circling	+	+	+	+	+	+
Joint head swinging	−	−	−	−	+	+

by making a side-to-side or forward-to-backward motion while its head is on top of the other's head; Turning Back: During female-first tail-straddling walk (ffTSW; see description below) or during linear TSW, the female or male turns back towards the other's dorsal and/or sacral region. When performed by a female, her body remains in contact with (or close to) the substrate. In contrast, a male turning back raises his body off the ground to better reach the female's nares for olfactory pheromone delivery; Tail Wagging: An individual undulates the distal portion of its tail (one-half to one-third of the tail). Tail wagging usually occurs throughout the courtship, often (but not necessarily) in contact with the other salamander [similar to tail undulation (see below) but faster and with only the tail tip moving].

Behaviors Performed by Females Only.—Cloacal Rubbing: The female crawls over the male and rubs her cloaca with quick forward-backward movements while in contact with the male dorsum (usually toward the rear of the male); High Amplitude Tail Undulations: When the male is in contact with female's tail, usually with his chin touching her tail, she undulates her tail in a slow, high amplitude wavelike motion while her tail is either in full contact with the substrate or with the tail base slightly arched: High Amplitude Head Swinging: The female swings her head side to side at approximately 45°arcs. She may be a short distance from the male (<5 cm) and in visual contact, or the male may be rubbing or nudging the female's back or tail; Pulling: The female turns back toward the male, places her head on the posterior portion of the

TABLE 2. A summary of the duration (min) of the phases of courtship for *Plethodon cinereus*. The phases are separated into seven categories: approach, HC/TW = head contact and tail wagging, ffTSW = female-first tail-straddling walk, TSW = linear tail-straddling walk, SD = spermatophore deposition, ST = sperm transfer. See text for a more detailed description of these phases. The total time of each courtship sequence is presented (far right column), along with means, standard deviations, and percent of total for each courtship component (zeros were not included in calculations). The number in parentheses next to the sequence number denotes the number of multiple courtship sequences by the same pair that occurred sequentially on the same night. A blank in the ST column reflects a courtship without SD.

	Plethodon cinereus						
	Phases of courtship						
Sequence	Approach	HC/TW	ffTSW	TSW	SD	ST	Total (min)
1 (1)	14	103	0	2	5	No	124
2 (1)	0	5	0	5	5	No	15
3 (1)	0	0	0	22	6	No	28
4 (1)	0	0	0	35	6	No	41
5 (1)	240	0	0	240	0		480
6 (2)	2	9	0	3	6	Yes	20
7 (2)	2	450	25	0	0		477
8 (3)	1	150	54	0	0		205
9 (4)	1	287	39	0	0		327
10 (5)	1	520	1	0	0		522
11 (6)	1	62	1	0	0		64
12 (7)	1	243	1	0	0		245
13 (8)	60	30	0	2	4	No	96
14 (8)	0	0	0	11	5	Yes	16
15 (8)	0	420	0	0	0		420
16 (9)	10	8	0	3	6	No	27
17 (9)	0	6	0	14	5	No	25
18 (9)	0	0	0	26	5	Yes	31
19 (9)	0	0	0	58	6	No	64
20 (9)	0	690	0	64	0		754
21 (10)	1	291	6	0	0		301
22 (11)	1	245	83	0	0		329
23 (12)	1	195	249	0	0		445
24 (13)	1	238	1	0	0		240
Total	337	3952	460	485	59		5296
Mean	22.5	219.6	46	34.6	5.4		220.6
SD	62.1	199.5	76.6	62.6	0.7		224.5
% of Total	6.4	74.6	8.7	9.2	1.1		

male's body, and pulls her head quickly over the male's back. This behavior is similar to snapping (see male behaviors), except the female is not displaced from the male. Pulling usually occurs during ffTSW; Stop and Lower Vent: The female halts her forward movement and lowers her vent close to (or over) the spermatophore. She may or may not retrieve the sperm mass; Pass over Spermatophore: after the male has deposited a spermatophore, the female walks forward while elevating her body over the spermatophore. At this point, the male has moved his tail to one side, but the female continues forward with her chin on his tail base; Transfer Sperm Mass: After the female lowers her vent on to the spermatophore, she undulates her tail and sways forward and backward; Lift Off: The female lifts her cloaca upward after retrieving sperm and moves away; Resume Tail Straddling Walk: The female does not move away after spermatophore deposition but reengages in TSW. This reengagement occurs when the female is not inseminated and usually leads to additional spermatophore depositions (up to four).

Behaviors Performed by Males Only.—Snapping: The male's head moves backward over the female's dorsum in a very rapid pulling motion, either from the base of the female's tail (from ffTSW position) or in a position perpendicular to the female's body. This usually displaces the male 1–4 cm from the female (Arnold and Houck 1982). Snapping is one type of "vaccination" pheromone delivery in which the male's premaxillary teeth abrade the skin of the female; Slapping: While in TSW, the male curls back toward the female and "slaps" his mental gland on the female's nares (olfactory pheromone delivery); Crossing Under: The male walks under the female's chin until her chin is at the base of his tail. When the male positions himself so

TABLE 3. A summary of the duration (min) of each phase of courtship for *Plethodon angusticlavius*. See Table 2 legend for abbreviations The total time of each courtship sequence is presented (far right column) along with means, standard deviations, and percent of total of each courtship component. The number in parentheses next to the sequence number denotes the number of multiple courtship sequences by the same pair that occurred sequentially on the same night. A blank in the ST column reflects a courtship without SD.

Plethodon angusticlavius							
	Phases of courtship						
Sequence	Approach	HC/TW	ffTSW	TSW	SD	ST	Total (min)
1 (1)	68	64	9	8	6	No	155
2 (1)	4	3	3	0	0		10
3 (2)	9	59	21	3	6	No	90
4 (2)	0	8	8	8	0		24
Total	81	134	41	19	12		279
Mean	27	33.5	10.3	6.3	6		69
SD	35.6	32.5	7.6	2.9	0		66.7
% of total	29.0	48.0	14.7	6.8	4.3		

that his tail base is under her chin, he may straighten his hind limbs to lift up the female's chin. If the female is receptive, she will enter TSW from this position; Spermatophore Deposition and Sperm Transfer: The male stops, lowers his vent to the substrate, and undulates his tail. When deposition is complete several minutes later, the male flexes his tail to one side, raises his body off of the spermatophore and resumes forward motion with the female's chin on his tail base. When the female stops and lowers her cloaca onto the sperm mass, the male pushes backward under the female's chin by extending his hind limbs; Tail Undulation: The male arches and undulates the full length of his tail. This is usually performed when the base of the male's tail is in contact with any part of the female's body; Head Swinging: The male moves his head laterally in a side-to-side motion slightly above the substrate, alternating sides at a 15°arc.

Behaviors Performed Jointly by Males and Females.—Linear Tail Straddling Walk (TSW): The female follows the male with her head on his tail base, straddling his tail; Female-First Tail Straddling Walk (ffTSW): The male follows the female, initially with his head on the distal portion of her tail but moving toward the base of her tail, the male straddles her tail as he moves forward. An unreceptive female pulls her tail away from the male or walks quickly, preventing ffTSW; Joint Head Swinging: The male and female sway heads laterally, usually alternating sides relative to each other. The female's head moves in ± 45° arcs while the male's head swings at ± 15° arcs. The pair either faces one another or the male is on top of the female and facing the same direction; Circling: From ffTSW, the female turns back towards the male and the pair forms a circle. The female may or may not straddle the male's tail. If the female does straddle his tail, a circular form of TSW ensues later leading to the traditional linear TSW.

Temporal Relations

Plethodon cinereus.—I observed 11 complete courtship sequences (i.e., each sequence included spermatophore deposition and sperm transfer). Also, 13 observations were made of initial courtship interactions not leading to spermatophore deposition (Table 2). In total, these observations involved six males and 13 females and included over 90 h of courtship. Below, I categorize the courtship interactions using the three stages of courtship previously described for members of Plethodontidae (Organ, 1960a; Arnold, 1972): (1) orientation (approach); (2) persuasion (head

TABLE 4. A summary of the duration (min) of the phases of courtship for *Plethodon richmondi*. See Table 2 legend for abbreviations. The total time of each courtship sequence are presented (far right column) along with means, standard deviations, and percent of total for each courtship component. Tail-straddling walk, spermatophore deposition, and sperm transfer were not observed in this species.

Plethodon richmondi				
	Phases of courtship			
Sequence	Approach	HC/TW	ffTSW	Total (min)
1	1	447	84	519
2	1	299	1	330
3	1	377	17	577
4	18	33	2	61
5	7	132	1	139
Total	28	1288	105	1626
Mean	5.6	257.6	21	325.2
SD	7.4	171.8	35.9	226.6
% of total	1.7	79.2	6.5	

contact and pheromone delivery); and (3) sperm transfer (TSW, pheromone delivery and spermatophore deposition).

When first introduced into the courtship enclosure, both the male and the female explored the area. During the orientation stage, contact was initiated by males in 43% and by females in 56% of the staged encounters ($N = 24$). During orientation, the male and female usually made contact by nudging, head contact, and, in one case, cloacal rubbing. This stage of the courtship interactions lasted, on average, 22.5 min (SD = 62.1; $N = 24$).

The persuasion stage of courtship began after initial contact and both the male and female maintained participation in the courtship by slowing their movements around the arena, allowing for more direct contact. The main behavioral components of this stage were rubbing, tail wagging, and ffTSW. The male generally contacted the female's body, moving his head from posterior to anterior in a variety of ways, including rubbing, nudging, cloacal nudging, and snapping. Tail wagging by males usually occurred simultaneously with these behaviors, either while in contact with, or while following, the female. These male behaviors were often performed while in ffTSW. Snapping occurred sporadically, either while in the ffTSW position or from a position perpendicular to the female. While in ffTSW, a female arched and undulated her tail under the male's chin. A female performed high amplitude tail undulations, usually when in contact with the male. A female occasionally turned back toward the male while in ffTSW. In one courtship interaction, the female repeatedly turned back toward the male and the pair walked briefly in a circle. During the persuasion stage, females initiated rubbing, nudging, head contact and head rubbing. In courtship interactions that did not result in spermatophore deposition, the persuasion stage accounted for most of the time spent in courtship. In the 11 complete courtship sequences, the persuasion stage (as well as the entire courtship) was relatively short and many of the behaviors described above were not performed.

The sperm transfer stage began when the male crossed under the female's chin, moved forward, and the female entered TSW. While in TSW, the male undulated his tail under the female's chin and body as the pair walked forward in tandem. The amount of time in TSW ranged from 2–240 min. The mean amount of time in TSW was 35 min (SD = 62.6; $N = 14$) when courtships with more than two spermatophore depositions were included. Because the length of TSW increased with each successive spermatophore deposition, a few observations of multiple spermatophore deposition increased the mean substantially. If courtships with more than two spermatophore depositions are excluded, the mean amount of time in TSW was 5.7 min (SD = 4.82; $N = 7$). After the TSW, the pair ceased moving forward as the male lowered his vent. While the male's vent was lowered, tail undulations increased in speed, particularly as spermatophore deposition was nearing completion, which was within 6 min. The male then raised his vent and shifted his tail to one side at a 90° angle to his body (cf. Arnold, 1976). The pair moved forward until the female was positioned over the spermatophore. The female lowered her cloaca and swayed/rocked on the spermatophore to lodge the sperm mass in her cloaca. While the female retrieved sperm, she foot shuffled. In one instance, the female then crawled over the male and rubbed her cloaca on the base of the male's tail, dislodging the sperm mass from her cloaca. A male typically deposited one spermatophore, but occasionally 2–4 spermatophores were deposited in the course of a single courtship interaction.

Plethodon angusticlavius.—I observed four courtship sequences with spermatophore deposition occurring in two of these courtships. Two of the four courtship sequences involved the same male and I had only one female in the study. Observations included over 4.5 h of courtship (Table 3).

When introduced into the courtship arena, a pair initially remained stationary and in close proximity, usually with one animal on top of the other. Once the pair began exploring the area, both the male and female approached one another repeatedly. The male attempted to make head contact with the female while tail wagging, in a manner similar to that described above for *P. cinereus*. The approach phase of courtship accounted for 29% of courtship duration (mean = 27 min; SD = 36 min, $N = 4$).

When the female became more receptive, the male began head contact by rubbing and sliding his head on the female, beginning at the tip of her tail and working his way to her head. While the male rubbed the female's head, he seemed to focus on and around the nares of the female, perhaps accomplishing olfactory pheromone delivery. The female would slow her walk, allowing for ffTSW. The mean time spent in ffTSW was 10.25 min (SD = 7.6 min; $N = 4$). While the male straddled the female's tail, she undulated her tail in response to the male's contact. From ffTSW, either the male broke contact and moved axially along the female and attempted to cross under her chin, or else the female curled back toward the male's tail base (forming a circle), and then the pair entered linear TSW. If the male broke contact, he initiated TSW by crossing under the female's chin while walking forward, thereby giving the female an opportunity to step over his tail and enter TSW.

Tail-straddling walk lasted an average of 6.3 min (SD = 2.9 min) for the two complete sequences with spermatophore deposition. During TSW, the male undulated his tail under the female's body. In one sequence, the male turned back toward the female's head and seemed to tap her nares in a manner similar to that observed for pheromone delivery in *Plethodon shermani* (see fig. 1 in Rollmann et al., 2000). However, because of the angle of the view, I was unable to confirm if the male made contact with the female's nares. I also did not observe snapping or pulling in *P. angusticlavius*. After a short period of linear TSW, the male stopped forward movement and lowered his cloaca onto the substrate and deposited a spermatophore. Spermatophore deposition consistently took 6 min. Once the spermatophore was deposited, the pair moved forward. The female in these two courtships did not stop and lower her cloaca onto the spermatophore, and consequently, she was not inseminated.

Plethodon richmondi.—I observed five initial courtship interactions. Spermatophore deposition only occurred during courtship encounters that were not recorded on videotape. My observations involved two females and two males and included over 27 h of courtship (Table 4). I describe below the male-female interactions during the orientation and persuasion courtship stages because sperm transfer behaviors were not observed.

A male and female were equally likely to initiate contact by approaching the other. During this stage, the male and female made contact by nudging or head contact. This phase of courtship was relatively brief lasting on average 5.6 min (SD = 7.4 min; $N = 5$).

Most of the courtship interactions occurred during the persuasion stage and primarily included head contact and tail wagging. On average, this stage lasted 257 min (SD = 172 min; $N = 5$) and included behaviors such as male rubbing and sliding, head rubbing, male and female tail undulations, high amplitude head swinging, joint head swinging, cloacal nudging, ffTSW, and snapping. A male initiated the head contact stage by following the female axially along her dorsum, rubbing and sliding on all parts of the female's body, approaching the female's head last. Once the male reached the female's head, the female often performed high amplitude head swinging and, in one courtship, the pair performed joint head swinging. The male focused his rubbing around the cloacal region of the female. During this stage, the male and female undulated their tails when contact occurred.

Female-first tail-straddling walk also occurred during the persuasion phase of courtship. A receptive female allowed the male to rub and slide while straddling her tail. An unreceptive female kept her tail curled away from the male in response to his rubbing her tail, preventing ffTSW. The male moved forward over her tail until he reached the tail base. Snapping typically occurred while in this position, although males infrequently snapped while perpendicular to the female. During ffTSW, the female sometimes turned back toward the male's tail base and nudged the male. The male arched and undulated his tail, presumably in an attempt to persuade the female to enter linear TSW. When not in ffTSW, the male occasionally lifted his tail base under the female's chin and arched and undulated his tail. The female usually broke contact with the male and he then ceased tail undulations. On average, ffTSW lasted 21 min (SD = 36 min; $N = 5$).

Discussion

Courtship behaviors observed in three *Plethodon* species represent a significant deviation from the typical courtship pattern previously described for this salamander genus. The typical pattern involves a linear TSW led by the male, a pattern found in all major clades of the family (Houck and Arnold, 2003). This type of TSW is consistently linear except when the male turns back for pheromone delivery (e.g., fig. 1 in Rollmann et al., 2000) or turns for obstacle avoidance. In contrast, the courtship behaviors of *P. cinereus*, *P. angusticlavius*, and *P. richmondi* all included a novel, female-led tail-straddling walk. Moreover, females of these species more actively participated in courtship than did females of other *Plethodon* species. The females that I observed initiated courtship as often as did males, performed tail undulations or tail wagging, and also approached, nudged, and rubbed males. Additionally, these females occasionally turned back towards the male while in ffTSW. Other notable female behaviors seen in at least one of these species included head swinging (*P. cinereus* and *P. richmondi*), cloacal rubbing (*P. cinereus*) and pulling (*P. richmondi*). Although small sample sizes in two species, *P. angusticlavius* and *P. richmondi*, provided limited observations, plethodontid salamanders exhibit highly stereotyped behavior within a species. Consequently, my results provide strong predictions of female behavior under normal field conditions.

The ffTSW seen in these three species may represent a less frequent segue into the male-led linear TSW (Fig. 1), which typically leads to spermatophore deposition. Such a sequence of behavior happened when, during ffTSW, the female turned back towards the male (forming a circle) and placed her chin on the base of his tail.

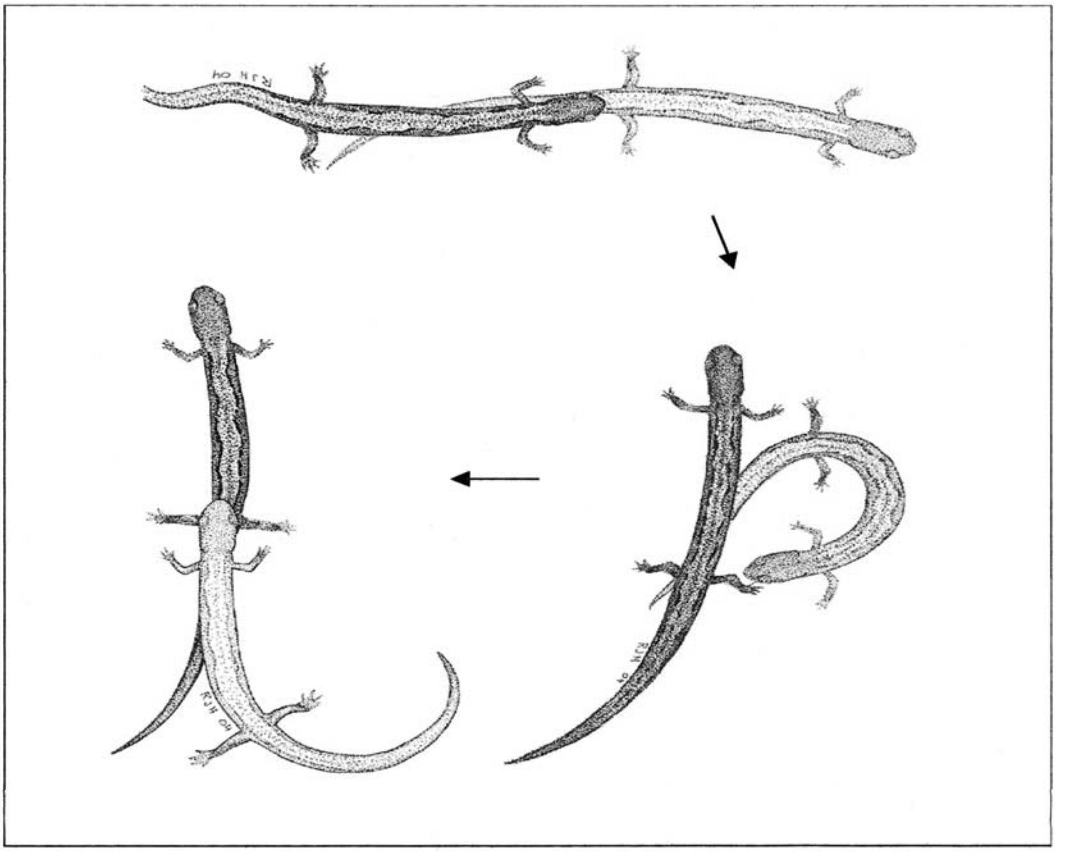

FIG. 1. Courtship sequence for *Plethodon angusticlavius*. This sequence began with female-first tail-straddling walk (top). The female (lighter animal) turned around toward the male, formed a circle (bottom right). From this position, the female entered tail-straddling walk (bottom left). This drawing has been created from videotaped footage of *P. angusticlavius*. The depicted courtship led to spermatophore deposition.

The male then began the linear male-led TSW. I did not observe this complete sequence in each of the three species, but *P. angusticlavius* performed this sequence of behaviors and the courtship led to insemination. Although *P. richmondi* performed ffTSW, I did not observe the male-led TSW or insemination. In *P. cinereus*, pairs that performed ffTSW either did not engage in male-led TSW or terminated the ffTSW and only later entered the male-led TSW. However, females in all three species turned back towards the male during ffTSW. Recent observations of plethodontids in the genus *Aneides* also revealed a circular TSW that led to the traditional male-led linear form (Sapp, 2002). Given the phylogeny of these salamanders (Larson et al., 2003), it seems possible that the circular entry into TSW has evolved more than once within this group (Fig. 2).

The delivery of pheromones by male *Plethodon* salamanders typically occurs during the linear TSW (Arnold and Houck, 1982; Houck, 1986). In these three species, however, most pheromone delivery occurred by scratching during ffTSW. The male scratched the female's tail from the tip until he arrived at the base of her tail. Scratching is one type of vaccination pheromone delivery. When the male reached the base of the female's tail, the male snapped (a quick pulling back of his teeth across her dorsum), which is another type of vaccination pheromone delivery. As courtship progressed, the female walked more slowly, allowing for the maintenance of contact during the ffTSW. In *P. angusticlavius*, one courtship interaction included a "slap" from the male as he led TSW. Also, *P. richmondi* and *P. angusticlavius* exhibited persistent head-head rubbing, which may result in olfactory pheromone delivery to the female's nares.

In an earlier description of courtship in *P. cinereus*, Gergits and Jaeger (1990) observed 10 courtships and four inseminations in the field. They concluded that the courtship sequence of this species generally followed the pattern of

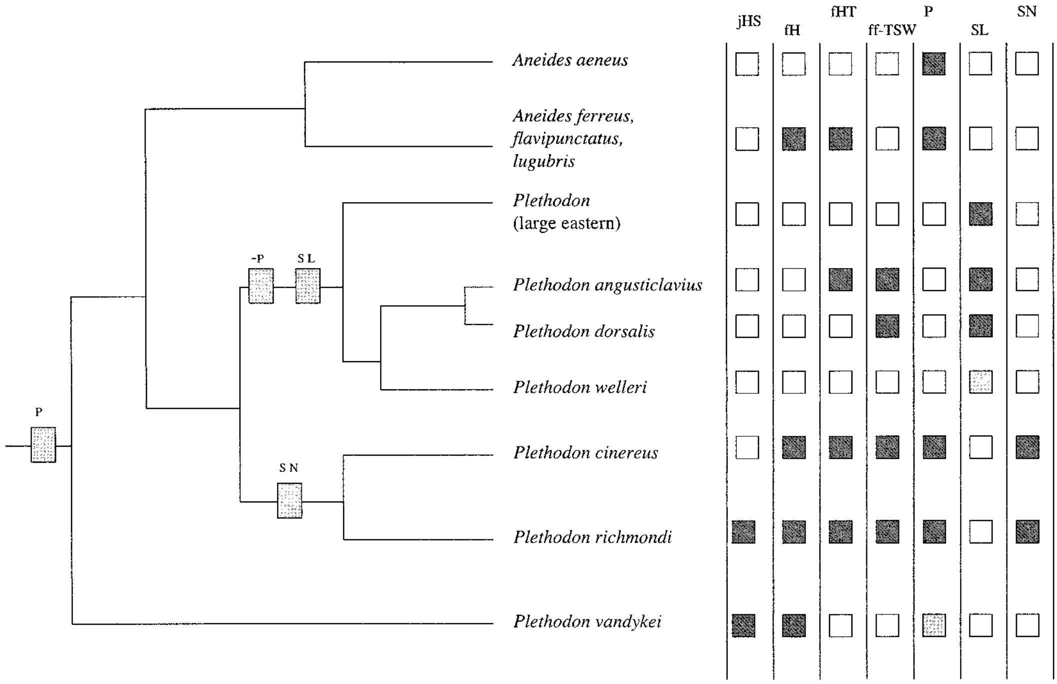

FIG. 2. Character states of behavioral features mapped onto a partial phylogeny of *Plethodon* and the related genus *Aneides*: P (pulling), TSW (linear tail-straddling walk), jHS (joint head swinging), SN (snapping), SL (slapping), ff-TSW (female-first tail-straddling walk), fH (female head rubbing), and fHT (female head rubbing and/or tail wagging). Behaviors are described in the text. Minus sign denotes evolutionary loss. This tree is based on information in Mahoney (2001) and Larson et al. (2003). Dark squares denote observed behaviors, gray squares denote hypothesized behavior presence, and open squares indicate a lack of observations or absence of behavior. Gray rectangles represent proposed phylogenetic transitions.

other plethodontid salamanders. In contrast, my observations show that female behavior in this species is much more variable than previously thought. The difference between field observations and my laboratory observations of courtship behaviors may be a result of laboratory artifact. However, studies of agonistic behavior in *P. cinereus* conducted in the field and laboratory indicate that laboratory-based inferences are a reasonable representation of field behavior, at least in *P. cinereus* (Gergits and Jaeger, 1990). Another explanation for the differences between my observations and those of Gergits and Jaeger (1990) may be the unusual weather conditions that led to their observation of 10 courtships in one night. Prior to their field observations, multiple weeks of dry conditions led to limited courtship opportunities. After one rainy day, the density and activity of salamanders on the surface dramatically increased and the females apparently were highly receptive. Courtships in which the female is highly receptive often do not include pheromone delivery (Arnold, 1977; Halliday, 1990; Houck and Reagan, 1990). Thus, it is not surprising that the courtship observations of Gergits and Jaeger (1990) did not include pheromone delivery and the associated behaviors of snapping and ffTSW.

Previous attempts to observe the complete courtship of these three species have had limited success (Arnold, 1972). In general, my observations of male behavior in *P. cinereus* are in agreement with earlier observations of Arnold (1972). However, the female behaviors I observed in this species have not been reported previously. Similarly in *P. richmondi*, many of my observations are novel, but in general my observations of male behavior agree with those of Arnold (1972). Interestingly, the courtship observations of *Plethodon vandykei*, a western species, reveal some similarities to those of *P. richmondi* (Lynch and Wallace, 1987). In particular, these are the only two *Plethodon* species that exhibit joint head swinging. Similar to my observations and those of *Aneides*, female *P. vandykei*, also have a more active role in courtship than most plethodontids (Sapp, 2002; Lynch and Wallace, 1987).

Prior observations of *P. angusticlavius* were not available, but following my observations, a colleague observed courtship in a northern population of a closely related species *Plethodon dorsalis*; both species are within the *P. dorsalis* complex

(Picard, 2005). These observations are in general agreement with those presented here. In particular, slapping was observed as the mode of pheromone delivery but occurred prior to, instead of during, the male-led TSW. In addition, ffTSW was observed, but females did not turn back toward the males. These populations are now considered to be different species within the *P. dorsalis* complex (Highton, 1997), perhaps accounting for differences between courtship behaviors. The courtship of *Plethodon welleri*, closely related to *P. angusticlavius*, has been observed, but the mode of pheromone delivery is still unclear (Organ, 1960b; Arnold, 1972). Based on observations of pheromone delivery in *P. angusticlavius*, I would expect to see olfactory delivery in *P. welleri* as well.

Documentation of courtship pheromone delivery suggests that most small eastern *Plethodon* species have the ancestral delivery mode of "vaccination," whereas large eastern *Plethodon* have a derived mode of olfactory pheromone delivery. My additional observations of pheromone delivery correspond to the phylogeny established from molecular data for the eastern *Plethodon* species (Fig. 2; Highton and Larson, 1979; Larson et al., 1981; Highton, 1991; Mahoney, 2001). In particular, *P. cinereus* and *P. richmondi* are among the vaccinating *Plethodon* species, and my observations of scratching, pulling and snapping in these species confirm their more basal placement in the group. *Plethodon angusticlavius* lies in an intermediate position on the phylogeny in that this species is more closely related to large eastern *Plethodon* species than to most other small eastern *Plethodon* species. My observations suggest that the *P. angusticlavius* method of pheromone delivery is intermediate between those having vaccination and olfactory delivery because males perform behaviors associated with both delivery modes (i.e., rubbing and slapping, respectively). In addition, mental gland morphology, premaxillary teeth, and behavioral observations of slapping prior to linear TSW supports the placement of this species as intermediate between vaccination and olfactory pheromone delivery (Highton, 1962; Coss, 1974; Picard, 2005).

As compared to other well-studied *Plethodon*, females of these three species participated more actively in courtship during the course of this study. The more assertive courtship behaviors of these females could have social and phylogenetic implications. In particular, courtship behaviors performed by females of these species may represent a derived condition, one reflected in salamanders that have a more complex social structure relative to other conspecifics. Many laboratory and field studies have revealed such a complex social structure for *P. cinereus* from Virginia. Females and males each maintain territories that often overlap, some adults appear to be socially monogamous, both males and females codefend territories during the breeding season and females are less aggressive toward familiar females than toward unfamiliar females (Gabor and Jaeger, 1995; Gillette et al., 2000; Lang and Jaeger, 2000; Jaegar et al., 2002; Jaeger and Peterson, 2002). Male *P. cinereus* are more aggressive toward polyandrous females than toward monogamous females during the courtship and noncourtship season (Jaegar et al., 2002). In addition, individuals in pairs codefending territories are less aggressive toward opposite-sex intruders than toward same-sex intruders, suggesting a potential conflict between male and female remating strategies (Jaeger et al., 2002). If active female participation in courtship is a derived condition corresponding to a more complex social structure, I predict that western species of the genus *Aneides* would have independently evolved more active female behaviors (e.g., Sapp, 2002) and a complex social structure similar to that found in *P. cinereus*. Future studies of social and courtship behavior in *Aneides* could test this prediction.

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