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Courtship Behavior of the Yonahlossee Salamander (*Plethodon yonahlossee*): Observations in the Field and Laboratory

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***Abstract.*—Comparative studies of salamander courtship have shed light on questions of broad biological and practical importance, such as the evolution of complex behaviors, molecular and behavioral foundations of prezygotic barriers, and the implementation of captive breeding programs. Unfortunately, most observations of courtship in plethodontid salamanders are made in the laboratory, raising the question of whether existing observations also pertain to courtship in the wild. We observed the courtship of *Plethodon yonahlossee* in the laboratory and field and found only minor differences in behaviors between these two settings. The courtship behaviors of *P. yonahlossee* were similar to those of other large eastern *Plethodon*. Male salamanders pursued females and initiated a period of head contact with the head and body of the female. After this preliminary contact, pairs engaged in a tail-straddling walk, which led to spermatophore deposition and sperm transfer. In the laboratory, courting males chased and bit other males. We observed courtship in the field in late August. Our observations reinforce current knowledge of *Plethodon* courtship behavior and inform captive breeding efforts that may be part of future conservation programs.**

Key Words.—captive breeding; Plethodontidae; reproduction; spermatophore; tail-straddling walk

Introduction

Much scientific attention has been given to courtship behaviors of plethodontid salamanders, particularly in the genera *Desmognathus* and *Plethodon*. Comparative studies have shed light on questions of broad biological significance, such as the evolution of complex behaviors and the molecular and behavioral foundations of prezygotic barriers (e.g., Dawley 1986; Houck et al. 1988; Houck and Arnold 2003; Verrell 2003; Hohenlohe and Arnold 2010). The most comprehensive set of field and laboratory observations has been on *Plethodon montanus* (Organ 1958; MacMahon 1964; Arnold 1976). However, courtship behaviors for most other large eastern *Plethodon* (e.g., *P. glutinosus*, Northern Slimy Salamander, and *P. kentucki*, Cumberland Plateau Salamander) have been described almost entirely from laboratory observations (e.g., Organ 1960; Arnold 1972; Marvin and Hutchinson 1996), raising the question of whether these observations also pertain to behaviors that occur in the wild (Houck and Verrell 1993).

Plethodontid salamanders accomplish internal fertilization using spermatophores to transfer sperm to females (Noble 1929; Noble and Brady 1930, Stebbins 1949). To facilitate this transfer, elaborate courtship behaviors have been shaped by sexual selection (Watts et al. 2004). As a prelude to sperm transfer, most male plethodontids deliver courtship pheromones to the female from a mental gland (Arnold 1977). These pheromones increase the sexual receptivity of the female (Houck and Reagan 1990; Rollmann et al. 1999; Wilburn and Swanson 2016), encouraging her to participate in a tail-straddling walk (TSW) in which she follows the male. The TSW ultimately aligns the pair to facilitate sperm transfer (Arnold 1977). Although much of the archetypal TSW has been conserved throughout the evolutionary history of plethodontids, spanning tens of millions of years, methods of pheromone delivery and other aspects of preliminary courtship vary among genera and species. This species-level behavioral variation has been especially well studied in *Desmognathus* (e.g., Verrell and Mabry 2000; Mead and Verrell 2002) and *Plethodon* (e.g., Marvin and Hutchinson 1996).

We report and compare field and laboratory observations of courtship in the Yonahlossee Salamander (*Plethodon yonahlossee*). The courtship behaviors of this species in a laboratory setting were described alongside those of other plethodontids in an

unpublished thesis (Arnold 1972). Brief excerpts from that thesis are published elsewhere (Arnold 1976, 1977). In this account, we provide a more detailed account of observations of the courtship behavior of *P. yonahlossee* in both laboratory and field settings and compare observations made in each setting. Furthermore, we compare courtship behaviors in *P. yonahlossee* to those of other large eastern *Plethodon*. Additionally, our courtship account is exceptionally complete because we document behavioral acts with still photography and videography, providing a baseline for future studies on courtship of species in the genus *Plethodon*.

Plethodon yonahlossee is the largest salamander in the genus and is endemic to the Blue Ridge Mountains of Tennessee, North Carolina, and Virginia, USA (Petranka 1998). This striking salamander is characterized by a large, chestnut-colored dorsal stripe (Dunn 1917). *Plethodon yonahlossee* is classified as a species of greatest conservation need in both Tennessee and Virginia (Tennessee Wildlife Resource Agency [TWRA] 2005; Virginia Department of Game and Inland Fisheries [VDGIF] 2005). In addition to having a limited distribution, *P. yonahlossee* may be locally threatened by timber harvest practices (Petranka et al. 1993, 1994; but see Ash and Bruce 1994). Knowledge of life history and phenology can inform management strategies in the field, and because captive breeding can be an effective conservation tool in addressing amphibian declines through repatriation efforts (e.g., Griffiths and Pavajeau 2008; Rija et al. 2010), knowledge of courtship behavior may provide considerable practical value.

Materials and Methods

We collected and observed *Plethodon yonahlossee* from the Mount Rogers National Recreation Area (MRNRA) in southwestern Virginia, USA (Grayson, Smyth, and Washington counties). We (SJA) staged laboratory trials with salamanders collected from the south side of Bluff Mountain (Grayson County, Virginia, USA) along a transect beginning at 36.6153, -81.5778 (1243 m) and ending at 36.6304, -81.5867 (1394 m) on 28 July 1968 and 5–29 August 1969. We collected salamanders by turning cover objects by day and searching the forest floor at night. Field observations took place over three independent field seasons. We observed in situ courtship in the field on the south slope of Bluff Mountain (Grayson County, Virginia, USA; 36.6153, -81.5778, 1243 m) on 23 August 1986, the south slope of Beech Mountain (Washington County, Virginia, USA; 36.6358, -81.6263, 1493 m) on 26 August 2012, and multiple sites along the Appalachian Trail on the northern slope of Hurricane Mountain (Smyth County, Virginia, USA; 36.7132, -81.5119, 1215 m; 36.7145, -81.5101, 1170 m; 36.7166, -81.5081, 1108 m) on 23 August 2015 and 26 August 2015.

For courtship trials in the laboratory, we housed and maintained *P. yonahlossee* as described by Arnold (1976). We housed pairs together in glass aquaria (51 × 25 × 30 cm) with substrates consisting of moistened paper towels and crumpled towels for retreats, and we maintained the aquaria at 13–16° C and on a natural photoperiod. In 1968, we housed multiple males and multiple females in each of four aquaria. In 1969, we housed a single male and a single female together in each of four aquaria. We fed the salamanders Houseflies (*Musca domestica*), blowflies (Callphoridae), or House Crickets (*Acheta domestica*) once a week. We observed courtship at night, and we used dim white light to illuminate aquaria, as such lighting did not interfere with courtship of *Plethodon montanus* (Northern Gray-Cheeked Salamander) and *P. shermani* (Red-Legged Salamander; Organ 1958; Arnold 1976) or *P. glutinosus* (Organ 1960) in previous studies. We described courtship into a tape recorder and later transcribed the descriptions. We observed and recorded 10 laboratory courtship sequences from seven different pairs, lasting a total of 158 min on nine nights in 1968 (30 July - 19 August) and 1969 (16 August - 4 September). We scored the presence of new spermatophores each morning so that dates of deposition could be recorded even when courtship was not directly observed.

During field observations in 1986, we used hand-written notes to record ongoing courtship. In 2012 and 2015, we used digital cameras to both photograph and record video segments of courtship. We watched video segments upon returning from the field to categorize and analyze courtship behaviors. In 2012, we used a HOBO ProV2 data logger (Onset, Bourne, Massachusetts) to record the temperature (°C) and relative humidity 1.5 cm above the forest floor, within 1 m of the courting pair. We determined the approximate time since sunset for the beginning of field courtship observations from the U.S. Naval Observatory sunset tables (available from http://aa.usno.navy.mil/data/docs/RS_OneDay.php [Accessed 8 September 2016]).

We carefully removed one spermatophore from an observation chamber immediately after deposition in 1968 by cutting a rectangular piece of paper towel that surrounded the spermatophore. We then immediately drew the spermatophore and measured it with dial calipers. We also obtained a photograph of a *Plethodon yonahlossee* spermatophore that was deposited on the night of 15 July 2010 in a captive breeding program (Tim Herman, pers. comm.). The spermatophore was photographed alongside a metric ruler for reference, and Image J (version 1.49 available from http://imagej.nih.gov/ [Accessed 15 October 2015]) was used to measure spermataphore height, length, cap height, and cap length.

We first present a summary of both field and laboratory observations of courtship. We then present a catalog of all courtship behaviors, with some contribution from behaviors observed in the field, as separate ethograms for males and females (Appendix 1) and a detailed summary of courtship sequences (Appendix 2). These ethograms and the description of temporal relationships between behaviors follow the structure of previous work on *P. montanus*, *P. shermani*, and *P. yonahlossee* (Arnold 1972, 1976) and define the behavioral vocabulary that we use in our account. Where appropriate, we reference specific figures from Arnold (1976), indicated by all lowercase letters (e.g., fig. 16), that correspond to our catalogued behaviors. Because our observations of courtship in the field typically included only abbreviated portions of the full courtship sequence, but took place in a natural setting, we describe these observations in greater individual detail in Appendix 3. Because of the absence of successful spermatophore transfer during laboratory courtship trials, we discuss these behaviors separately. Finally, we separately describe both the size and shape of spermatophores and observations of male-male interactions observed during laboratory courtship trials.

Results

Courtship.—In the laboratory, we observed 10 courtship sequences from seven different courting pairs, totaling 158 min and including three incidences of TSW and three incidences of spermatophore deposition, none of which resulted in successful spermatophore transfer. Divided into three categories of courtship behavior, these sequences were composed of approximately 86 min (54%) of preliminary actions, 55 min (35%) of TSW, and 17 min (11%) of spermatophore deposition and post-deposition positioning. Eight additional spermatophores were deposited when courtship was not observed (three from 10–17 August 1968; five from 15 August - 11 September 1969).

In the field, we observed eight courtship sequences from eight different courting pairs, totaling approximately 199 min and including five instances of TSW and three incidences of spermatophore deposition and transfer. Divided into three categories of courtship behavior, these courtship sequences were composed of approximately 109 min (55%) of preliminary actions, 65 min (33%) of TSW, and 25 min (13%) of spermatophore deposition and post-deposition positioning. A video of two field observations of *P. yonahlossee* courtship, the contents of which are summarized in Table 1, is available at https://goo.gl/y70vJU.

Spermatophore transfer and description.—We observed three instances of spermatophore deposition in the laboratory, each between a different pair of animals. In each sequence, the female successfully located the spermatophore with her vent but then failed to successfully remove the sperm mass. In two pairs, the female departed from the male after pausing with her vent in contact with the spermatophore. In both cases, the male initially remained motionless after the female departed and then moved forward tapping the substrate, but no additional courtship interactions occurred. In the third pair, the female moved forward slowly and intermittently over the spermatophore, rather than moving forward rapidly and continuously as in the other two courtships. The female eventually paused with her cloaca in contact with the sperm cap, and the male executed the series of actions described in Appendix 1 with his tail flexed to one side. Both animals then moved forward in TSW. The male then turned back towards the female and slapped his mental gland on the head of the female. The female then departed from the male, and

Table 1. Sequences of *P. yonahlossee* (Yonahlossee Salamander) courtship videotaped in the field. Times in the onset (min:s) and duration (min:s) columns refer to time points on the video, available at https://goo.gl/y70vJU. Time points at which actions were in progress are shown in parentheses. (A) A sequence videotaped on 23 August 2015 at Hurricane Mountain, Virginia. (B) A sequence videotaped on 26 August 2015.

Sequence/ Segment	Description	Action	Onset	Duration	Figure
A / 1	Attempt to continue TSW	Tail-straddling walk	0:00	0:30	-
A / 2	Male performs foot dance as he approaches females and lifts her chin	Foot dance; Lifting	0:31	1:41	2
	Female departs	—	2:12	-	-
A / 3	Spermatophore deposition in progress	SD	2:46	1:51	-
A / 4	Male moves away from deposited spermatophore	Moving forward with tail flexed	4:38	0:05	-
A / 5	Male leads female towards and over spermatophore		4:43	0:26	-
B / 6	Male flexes hindlimbs under chin of female	Stationary with tail flexed	5:10	0:17	-
B / 7	Male is stationary with female over spermatophore		5:27	0:47	-
B / 8	Close-up showing vent of female over spermatophore	Spermatophore pickup	6:16	0:29	3

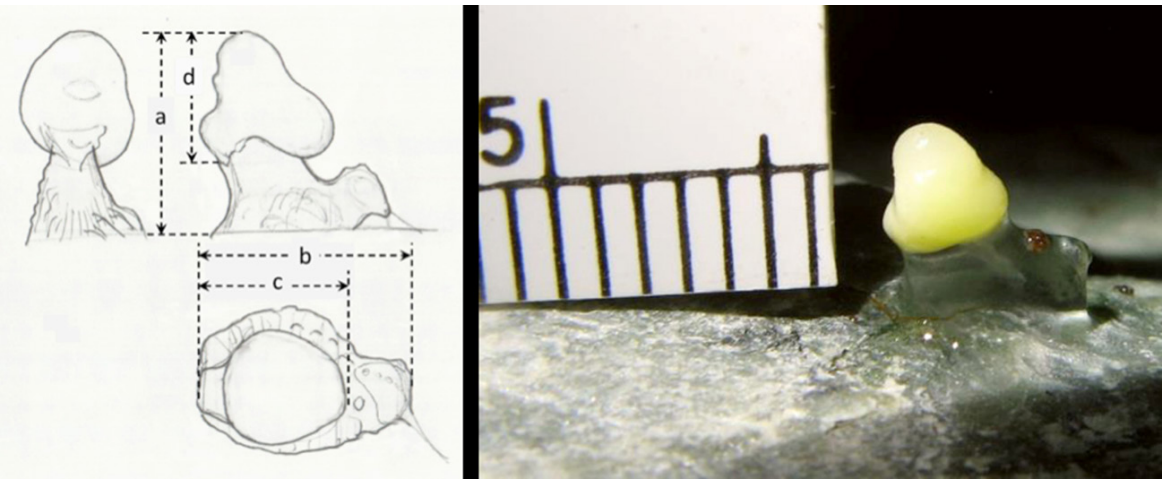

Figure 1. (Drawing) *Plethodon yonahlossee* (Yonahlossee Salamander) spermatophore deposited in the laboratory on 12 August 1968. Spermatophore dimensions are shown in Table 2. (Drawing Left) End view. (Drawing Top right) Side view, anterior on the left side. (Drawing Bottom right) Top view, anterior on left side. (Photograph Right) *Plethodon yonahlossee* (Yonahlossee Salamander) spermatophore deposited in the laboratory on 15 July 2010 (Photographed by Tim Herman).

the male did not deposit any additional spermatophores that evening.

The spermatophore base inserts deeply into the cap (Fig. 1). As in other large eastern *Plethodon*, the cap is mushroom-shaped, and the base is colorless (Organ and Lowenthal 1963). The two spermatophores we measured had heights of 5.0 and 4.4 mm, respectively (Table 2).

Male-male interactions.—In laboratory courtship trials, we observed agonistic interactions on four occasions. These occurred over a 15-d period (2–17 August 1968) in a tank in which multiple males were housed with multiple females. On one occasion, a male that had been courting a female approached another male, tapped his tail, and then lunged and grabbed the tail of the other male in his mouth and held on for 10 s while the other male struggled. After being released, the attacked male rapidly ran off. On a second occasion, a male lunged and bit the tail of another male, who then ran off. On a third occasion, a male that had been courting a female approached another male, placed his chin on the tail of the other male, and then lunged and bit his tail, causing the attacked male to run off. On a fourth occasion, one male grabbed the tail of another male in his mouth, causing both males to writhe about, until they finally flipped free of one another.

Discussion

Comparisons of field and laboratory observations.—Courtship behaviors observed in the laboratory and the field were similar. In particular, the postures and actions during the TSW and spermatophore deposition and transfer observed during laboratory courtship (1968–1969) were identical to those we observed in the field in 1986, 2012, and 2015. Laboratory and field observations of courtship behaviors were also similar for Red-backed Salamanders (*P. cinereus*; Jaeger et al. 2016).

There were, however, some minor differences between observations of courtship in the field and laboratory. For example, we never observed foot dance in the laboratory, but we observed it once in the field (Video Segment 2). In this instance, the female may have been unreceptive as indicated by TSW ending after 20 min. The male performed foot dance as he approached the female and re-initiated courtship. In *P. shermani*, the

Table 2. Spermatophore dimensions (mm) for large Eastern *Plethodon* spp. Two sets of measurements are provided for *P. yonahlossee* (Yonahlossee Salamander), representing those shown in Fig. 1 (left and right, respectively). The larger value for spermatophore height in the first set (*) at least partially reflects the higher position of the sperm cap on the spermatophore base.

	P. yonahlossee				
Trait	1968	2010	*P. glutinosus*[1]	*P. kentucki*[2]	*P. montanus*[3]
Height (a)	5.0*	4.4	4.0	3.5	3.6
Base length (b)	5.3	5.3	4.0	3.3	-
Cap length (c)	3.6	2.9	2.7	1.6	2.5
Cap height (d)	3.4	2.9	2.5	1.6	1.6

[1](Organ 1960), [2](Marvin and Hutchinson 1996), [3](Organ 1958).

Figure 2. (Top) A courting male *Plethodon yonahlossee* (Yonahlossee Salamander) (left) foot dancing as he attempts to renew tail-straddling walk with a female (right) at Hurricane Mountain, Virginia, USA, 23 August 2015. (Photographed by Will Lattea). (Bottom) A female *Plethodon yonahlossee* (left) following a male (right) during the tail-straddling walk at Hurricane Mountain, Virginia, USA, 26 August 2015. (Photographed by Evin T. Carter).

foot dance appears to increase the probability that a pair will initiate TSW (Eddy et al. 2012).

The rarity of foot dance in our field observations is not surprising because we mostly failed to observe preliminary courtship actions, which is the stage in which foot dance typically occurs in other *Plethodon* (Organ 1958, 1960; Arnold 1976; Marvin and Hutchinson 1996). Our failure to observe foot dance in the laboratory is more revealing, because this behavior is routinely observed in laboratory courtship by species with foot dance in their repertoires (Organ 1958, 1960; Arnold 1976; Marvin and Hutchinson 1996). We suspect that foot dance might be a relatively rare element in the repertoire of *P. yonahlossee*. Finally, successful spermatophore transfer occurred during all field observations (n = 3) that included spermatophore deposition but never in similar circumstances in the laboratory (n = 3). Because of our small sample size, we hesitate to make any strong conclusions, but this may suggest that some artifact of the laboratory setting prevented successful spermatophore transfer.

***Comparisons with other* Plethodon**.—We found many similarities between the courtship behaviors of *P. yonahlossee* and those of other large eastern *Plethodon*. For example, the overall temporal scheme of *P. yonahlossee* courtship was identical to those of other large eastern *Plethodon*. As with other large *Plethodon*, male *P. yonahlossee* initiated courtship by approaching the female and making contact with his head and/or mental region along the dorsum or flanks of the female (Organ 1958, 1960; Arnold 1976; Marvin and Hutchinson 1996). However, *P. montanus*, *P. glutinosus*, and *P. kentucki* often perform a foot-dance following nudging or lifting (Organ 1958, 1960; Arnold 1976; Marvin and Hutchinson 1996) and prior to the TSW. As with other *Plethodon*, male *P. yonahlossee* stopped moving forward if the chin of the female slipped

Figure 3. A female *Plethodon yonahlossee* (Yonahlossee Salamander) with a spermatophore inserted into her cloaca (spermatophore base visible) at Hurricane Mountain, Virginia, USA, 26 August 2015. (Photographed by Will Lattea).

posteriad on the tail of the male during the TSW (Organ 1958, 1960; Arnold 1976). Both male *P. glutinosus* and *P. kentucki* often bite the female salamander during initial phases of courtship (Arnold 1976; Marvin and Hutchinson 1996). However, we did not record this biting behavior in either laboratory or field observations of *P. yonahlossee* courtship. One marked difference between the courtship behaviors of *P. yonahlossee* and other large *Plethodon* is in post-deposition positioning. When male *P. yonahlossee* positioned the female over the spermatophore, they engaged in a few vigorous, coordinated flexions and extensions of all four limbs. In contrast, other large eastern *Plethodon* males merely extend their limbs once as they push their tail base up under the chin of the female (Marvin and Hutchinson 1996).

Plethodon yonahlossee is also similar to other large eastern *Plethodon* in the duration of TSW, distance traveled during TSW, and rate of tail undulations during TSW. We measured distance traveled only once in a field setting, in which the pair traveled 3.2 m in 29 min. However, we did not observe the initiation of the TSW, and thus, the potential distance traveled was likely greater than 3.2 m. During a prolonged laboratory trial, *P. glutinosus* traveled 5.2 m (Organ 1960). *Plethodon jordani* (Red-Cheeked Salamander) traveled approximately 3 m in a circular path during 25 min of TSW (Green and Richmond 1944). In another study, the mean distance traveled by *P. kentucki* in TSW was 1.65 m (0.01–8.28; Marvin and Hutchinson 1996). In field conditions, we never observed the onset of the TSW, but the observed durations ranged from 15–29 min. The mean duration of TSW for *Plethodon kentucki* was similar to our observations, at 20.4 min (1.1–72.9; Marvin and Hutchinson 1996). The TSW of *P. jordani* has been shown to last as long as 55.9 min (Arnold 1976), which is considerably longer than our observation of 29 min. However, we did not observe the beginning of the TSW and thus cannot directly compare these data. The rate of tail undulations during courtship was initially slower than other large eastern members of *Plethodon*, but was comparable at the time of spermatophore deposition. The initial rate of undulations (0.24–0.83 waves/s) was slower than *P. glutinosus* and *P. kentucki*, which exhibit mean frequencies of 0.95 waves/s and 0.60 waves/s, respectively (Organ 1960; Marvin and Hutchinson 1996). However, tail undulation frequencies of *P. yonahlossee* at spermatophore deposition (1.25 waves/s) were similar to *P. kentucki* (1.20 waves/s; Marvin and Hutchinson 1996).

The spermatophore of *P. yonahlossee* also resembles those of other large eastern *Plethodon* (Organ 1958, 1960; Arnold 1976, Marvin and Hutchinson 1996). A clear gelatinous base inserts deeply into the apical sperm cap. A comparison of the two spermatophores from *P. yonahlossee* that we obtained shows that in one, the sperm cap sits high on the base, but in the other, the cap sits low on the stalk so that the posterior lip of the cap rests in a depression on the base. Houck and Arnold (2003, fig. 10.1D) photographed a *P. shermani* spermatophore moments after the male departed and before the female contacted the sperm cap, revealing that the low position of the cap on the stalk is characteristic of freshly deposited spermatophores. It seems likely that the higher position we found is a consequence of a female partially removing the cap with her vent. In any case, the position of the cap on the stalk affects the overall height of the spermatophore. Spermatophore

dimensions of *P. yonahlossee* were slightly larger than other large eastern *Plethodon*. Spermatophore size in plethodontids appears to be correlated with salamander snout-vent length (Organ and Lowenthal 1963). Thus, it is not surprising that spermatophores of *P. yonahlossee* appear to be larger.

Our observations suggest that *P. yonahlossee* courtship likely occurs from late July through September in southwestern Virginia and coincide with the observation by Pope (1950) that gravid females are active on the surface in August. According to Pope, almost 50% of female *P. yonahlossee* salamanders collected on Whitetop Mountain from 11–17 August 1949 had large, mature ova, and males collected on 11 August had well-developed mental glands. At the same locality, Organ (1990) collected large, gravid females (84–88 mm SVL) in August 1957 and 1958. We collected a large male (75 mm SVL) with a well-developed mental gland on the north slope of Whitetop Mountain (1,036 m) on 14 August 2009 (M. Kevin Hamed, pers. obs.). Furthermore, a recently captured male *P. yonahlossee* released a spermatophore cap while shedding a layer of skin on 7 August 1958, indicating spermatophore production is underway in early August (Organ and Lowenthal 1963). Other large eastern *Plethodon* have a similar courtship season. For example, *P. montanus* engaged in courtship from mid-July to early October on Whitetop Mountain (Organ 1958; Arnold 1976), and we observed a courting pair of *P. montanus* in close proximity to a courting pair of *P. yonahlossee* on 26 August 2015. Approximately 100 km north (Giles Co., Virginia, USA), *P. glutinosus* has been observed courting on 19 August 1949 (Pope 1950), and we also observed courtship on 20 September 2013 (Will Lattea, pers. obs.). Captive *P. glutinosus* collected from Whitetop Mountain courted from 12–30 September 1958 (Organ 1960). Thus, the timing of courtship for *P. yonahlossee* appears to be similar to that of both *P. montanus* and *P. glutinosus* from nearby localities.

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Appendix 1

Ethogram of male behavior during courtship.—The following catalog of male behaviors is a cumulative description of stereotyped behavior from laboratory and field observations. Here we define the behavioral actions referred to in the main text.

(1) Male preliminary actions:

Stationary. The male neither approaches nor moves away from the female.
Approach. The male moves towards the female, following her if she moves forward.
Tapping. The male lowers and raises his head, momentarily touching his nasolabial cirri to the dorsum of the female or to the substrate. This action transports chemical substances up the nasolabial grooves into the nares (Brown 1968; Arnold 1976).
Nudging. The male contacts his snout to the lateral part of the body of the female.
Head sliding. The male moves forward holding his chin in contact with the dorsal surface of the body of the female and his head level. He does not move his head from side to side.
Lifting. The male turns his head towards the female and places it under her chin. He then raises his head, contacting the chin of the female with his snout, eyelid, or the top of his head (Appendix Fig. 1).
Foot Dance. The male raises and lowers his forelimbs and hindlimbs in a rhythmic fashion (Organ 1958; Arnold 1976; Video Segment 2). Either one limb is raised at a time, or a forelimb and the alternate hindlimb are raised simultaneously.

(2) Male actions during the TSW (Arnold 1976, fig. 16c):

Stationary with tail arched and undulating. While stationary, the male arches the proximal part of his tail upward and undulates his tail. The vent is held above the substrate (Appendix Fig. 1).
Moving forward with tail arched. The male moves forward with the proximal part of this tail arched slightly upward and with his vent above the substrate. He may undulate his tail (Appendix Fig. 2).
Turning back towards the female. The male turns his head back towards the head of the female and approaches her by stepping laterally with his forefeet so that his body axis forms a "U" (Arnold 1976, fig. 18). As his head approaches the female, he accentuates the upward arch of his tail, increases the frequency of tail undulations, and holds his head high.
Slapping. While in the position just described, the male rapidly moves his head in a downward and backward motion, so that his mental gland makes momentary contact with the top of the head of the female (Arnold 1976, fig. 18).
Sliding vent in contact with the substrate. While moving forward, the male holds his vent in contact with the substrate. He holds his tail level and may undulate it slightly.
Spermatophore deposition (SD). The entire venter of the male (with the exception of his chin) is in contact with the substrate. The femurs of the male are oriented at right angles to the long axis of his body. The tail of the male undulates continuously and is in contact with the venter of the female (Arnold 1976, fig. 19a).

(3) Male actions after spermatophore deposition:

Moving forward with tail flexed. The male holds the distal portion of his tail at an approximate 90° angle to the long axis of his body (Appendix Fig. 3; Video Segments 4 and 5). He flexes his trunk in a sigmoid curve and holds his vent high above the substrate as he moves forward (Arnold 1976, fig. 19d).
Stationary with tail flexed (positioning). The male holds the distal portion of his tail at an angle approximately 90° to the long axis of his body. The trunk is straight as the male flexes and extends both his forelimbs and hindlimbs so that his tail base is repeatedly raised and lowered under the chin of the female (Appendix Fig. 3; Video Segments 6 and 7).

Ethogram of female behavior during courtship.— The following catalog of female behaviors is a cumulative description of stereotyped behavior from laboratory and field observations.

(1) Female preliminary actions:

Moving away. The female moves away from the male.
Stationary. The female neither approaches nor moves away from the male.
Approach. The female moves towards the male.

Raise head. The female raises her head from level to nearly a 45° angle in response to male contact with her chin (Fig. 2).
Contact chin to male tail. The female lowers her head, placing her chin in contact with the arched and undulating tail of the male.
Stepping astride. With her chin in contact with the arched and undulating tail of the male, the female steps over the tail of the male with one forelimb so that her chest is astride the tail of the male (Arnold 1976, fig. 16b). Usually, she faces in the same direction as the male.

(2) Female actions during the TSW (Arnold 1976, fig. 16c):

Stationary during the TSW. The female remains motionless while astride the tail of the male (Fig. 2).
Moving forward during the TSW. The female moves forward while astride the tail of the male, holding her chin in contact with the dorsum of his tail.
Stationary during spermatophore deposition. The female remains motionless astride the tail of the male, with her chin in contact with the dorsum of his tail base (Arnold 1976, fig. 19a).

(3) Female actions after spermatophore deposition:

Moving over the spermatophore. The female moves forward over the spermatophore so that her venter slides in contact with the sperm mass and so that her chin is in contact with the tail base of the male (Arnold 1976, fig. 19c; Video Segment 5).
Spermatophore pickup. With her cloaca situated over the spermatophore, the female lowers her sacrum, inserting the sperm mass into her cloaca (Fig. 3, Video Segment 8). She may slowly undulate her tail base while the sperm mass is inserted (Arnold 1976, fig. 19e). To complete pickup, she lifts her sacrum clear of the spermatophore base, while holding the sperm mass in her cloaca.

Appendix Figure 1. (Top) The male (left) turning back towards the female (right) during the tail-straddling walk of *Plethoodon yonahlossee* (Yonahlossee Salamander) at Hurricane Mountain, Virginia, USA, 23 August 2015. (Photographed by Will Lattea). (Bottom) *Plethodon yonahlossee* in tail-straddling walk, with the chin of female (right) on the arched and undulating tail of male (left) at Hurricane Mountain, Virginia, USA, 23 August 2015. (Photographed by Will Lattea).

Appendix Figure 2. (Top) Tail-straddling walk of *Plethodon yonahlossee* (Yonahlossee Salamander) with the female (left) following the male (right) at Beech Mountain, Virginia, USA, 26 August 2012. (Photographed by M. Kevin Hamed). (Bottom) A pair of *Plethodon yonahlossee* maintaining contact during the tail-straddling walk while crossing woody debris from Beech Mountain, Virginia, USA, 26 August 2012. (Photographed by M. Kevin Hamed).

Appendix Figure 3. (Top) A male *Plethodon yonahlossee* (Yonahlossee Salamander) (left) with its tail at right angle to the body, with the female (right) positioned with her vent over the spermatophore at Hurricane Mountain, Virginia, USA, 26 August 2015. (Photographed by Todd W. Pierson). (Bottom) A stationary male *Plethodon yonahlossee* (right) positioning the female (left) over the spermatophore at Hurricane Mountain, Virginia, USA, 26 August 2015. (Photographed by Evin T. Carter).

Appendix 2

Temporal relations in laboratory observations of P. yonahlossee courtship (from Arnold 1972).—Upon making contact with a female, the male moves along her length while contacting her intermittently with his head. These contacts by the male take the form of tapping, head-sliding and nudging. The male usually moves along beside the female, but he may also crawl along her dorsum. The male moves either anterior or posterior along the length of the female. The female may remain motionless or actively approach the male, or she may move away from the male as he contacts her with his head.

Whenever the female departs from the male, he follows her by moving forward in rapid bursts each time the female moves forward. When in close proximity to the female, the male approaches her slowly. As, or just after, the male establishes contact with the female, he may undulate his tail laterally with or without arching his tail. When the male reaches the end of the tail of the female while moving posteriad along her length, he either turns around and moves anteriad, without breaking contact with her, or he moves beyond the tip of her tail. In the latter case, the male taps the substrate while turning his head from side to side and then moves anteriad along the tail of the female, if he contacts it. When the male reaches the head of a stationary female, he turns his head towards the female and places it under her chin, and he performs lifting.

If the female departs, the male approaches her. If she remains stationary, the male crawls forward under her chin. If the chin of the female chin fails to contact the dorsum of his tail as he crawls forward under her chin, the male continues forward and moves posteriad along the opposite side of the female and resumes head contact with her. If the chin of the female does contact his tail, the male arches his tail upward and undulates his tail laterally in contact with the chin of the female. When the chin of the female contacts his tail, the male continues forward while arching and undulating his tail until the point of contact slips posterior to a point approximately one vent-length posterior to the rear margin of the vent of the male. The male then stops and continues to arch and undulate his tail. If the female departs, the male lowers his tail, ceases tail undulations, and approaches her. If she remains motionless for several minutes, the male also lowers his tail, ceases tail undulations, and approaches her. However, if the female turns her head axial on the tail of the male and slides anteriad with her chin in contact with his dorsal tail while stepping astride the tail of the male with her forelimbs, the male moves forward, arching and undulating his tail in contact with the chin of the female. Both animals may then move forward intermittently in TSW.

The TSW may be initiated in another way. The female may actively approach the male as he crawls beside her and place her chin on the dorsum of the body or tail of the male. In such instances, the male does not lift under the chin of the female, but instead crawls forward immediately and then arches and undulates his tail if the chin of the female contacts the dorsum of his tail. The female may then step astride and slide anteriad along the tail of the male.

During the TSW, the snout of the female usually rests on the dorsum of the tail of the male, within a region one or two vent-lengths posterior to the insertion of the hindlimbs of the male. The male tends to move forward whenever the snout of the female is anterior to a point approximately one vent-length posterior to the rear margin of the vent of the male vent. The male stops when the snout of the female slips posterior to this point and accentuates the upward arch of his tail while undulating his tail laterally in contact with the chin of the female. The female maintains an axial orientation of her chin on the tail of the male by actively turning her head towards the tail when it slips laterally off the midline of her chin (e.g., when the male turns to avoid obstacles in his path).

In some courtships, the male moves forward on a straight course following the initiation of the TSW and then stops and deposits a spermatophore after moving forward a few centimeters. In other courtships, however, the male pauses intermittently during the TSW and turns back towards the female and then resumes forward progress on a straight course. In this way, the male alternates between moving forward and turning back towards the female (and slapping her anterior head with his mental gland) before stopping and depositing a spermatophore.

If the female departs from the male during the TSW, he lowers his tail, ceases tail undulations, and approaches the female. If, however, the female remains astride the tail of the male and continues to actively move forward, the male begins sliding his vent in contact with the substrate and pauses intermittently. Finally, after pausing with his vent in contact with substrate, the male aligns his hindlimbs and begins spermatophore deposition with the female straddling his tail and with her snout directly above or within a few mm of his vent. During spermatophore deposition, the female remains nearly motionless astride the tail of the male while his tail undulates in contact with her venter from her chin to her vent. She may slide her snout anterior or posterior a few millimeters, but she does not actively swing her head from side to side.

Following spermatophore deposition, the male withdraws his tail from underneath the female by flexing his tail to one side. The male then moves forward on a straight course with his tail still flexed to one side. The female moves forward behind him with her chin resting on the dorsum of his proximal tail. As she moves forward, the spermatophore lies directly in her path, and it slides posteriad in contact with her ventral midline. If the spermatophore contacts her vent, she stops and lowers her vent upon it and thereby inserts the spermatophore into her cloaca. She may undulate her proximal tail slowly as her vent rests over the spermatophore. Almost simultaneously, the male stops in front of her and, with rhythmic extensions and flexions of his forelimbs and hindlimbs, he forces the head of the female upward.

Appendix 3

Detailed observations of courtship in the field.—We observed eight pairs of *P. yonahlossee* courting in the field. Three sequences resulted in sperm transfer, and the remaining five were incomplete. Of these eight pairs, we encountered six already in TSW, and each sequence was slightly different. Here, we describe them chronologically:

At 2051 on 23 August 1986 (102 min post-sunset), we encountered a pair of *P. yonahlossee* in the process of depositing a spermatophore along a road (Hwy 600) on Bluff Mountain, Virginia. The observation site was rock talus partially covered with grass and vegetation. A light rain fell during the period of observation, which lasted until 2053. We illuminated the pair with the dim white light of a headlamp throughout the observation period. The spermatophore-deposition site was at the junction of two sloping rocks, so that the body of the male sloped downward and away and the body of the female sloped upward and towards the deposition site. The female straddled the tail of the male in SD position with her snout on top of the tail of the male at about the posterior margin of his vent. The hindlimbs of the male were perpendicular to the axis of his body. After about 2 min, the female moved forward about 1 cm, just before the male lifted his vent off of the spermatophore, revealing a white sperm cap. The male pulled his tail out from under the female so that it formed an approximate 45° angle to his body as he moved forward. The female followed with her chin resting on the arched tail base of the male and stopped with her vent over the spermatophore. The male stopped in front of her, backed up under her chin, performed extensions and flexions of his hindlimbs (and possibly of his forelimbs) and then remained stationary and motionless with his tail base arched under her chin and with all four limbs fully extended and angled back towards the female. After a short period, the female lifted her vent high over the spermatophore base while undulating her tail base from side-to-side. Next, she lifted her chin off of the tail of the male and quickly departed from him in the opposite direction. The sperm cap was now missing from the spermatophore base. A total of 2 min and 29 sec had elapsed since we first encountered the pair.

At 2155 (111 min post-sunset) on 26 August 2012, we encountered a pair of *P. yonahlossee* in TSW on Beech Mountain, Virginia. Initial ground temperature was 15.65° C and cooled to 14.46° C by the time of spermatophore deposition, while relative humidity increased from 86.1% to 92.7%. The TSW lasted at least 29 min, but we did not observe the preliminary actions. The female maintained snout contact on the male dorsum even while crossing woody debris and rocks (Figs. 2, 3). The pair covered 3.2 m, but the path was not a straight line and weaved around rocks. In the 29 min, we did not observe the male turning back towards the female, suggesting this behavior does not occur with every encounter or occurred prior to our initial field observations. During TSW, the initial frequency of male-tail undulations was 0.26 waves/s (n = 1 wave), but in the last 5 min of the TSW, the rate increased to 0.83 waves/s (n = 1 wave). Immediately prior to spermatophore deposition, tail undulations increased to 1.25 waves/s (n = 1 wave). The male stopped for < 1 min, then placed his vent in contact with the substrate, and finally deposited a spermatophore after a few minutes. Next, the male moved forward with his tail held approximately at a 70° angle and paused when the vent of the female was over the spermatophore. The female lowered her cloaca and picked-up the spermatophore cap. The female then broke contact with the male and quickly moved 1.3 m away into a rock crevice. Observations concluded at 2224.

(1) At 2150 (102 min post-sunset) on 23 August 2015, we encountered a pair of *P. yonahlossee* in TSW on Hurricane Mountain, Virginia. The female straddled the tail of the male, which was slightly arched and undulating. After several minutes, the male began moving forward slowly with the female following. The female began rhythmically moving her pelvis from side-to-side with the same rhythm as the lateral movement of the tail of the male. At 2156, after moving forward approximately 10 cm, the male quickly turned back towards the female (Appendix Fig. 1). The female raised her head, and the male slapped his snout against her chin multiple times while remaining in the tail-straddling position. The pair moved forward approximately 10 cm and repeated the lifting and slapping motion. At 2205, the male attempted to turn back and slap for a third time, but the female quickly fled into a root hole at the base of a Yellow Birch (*Betula alleghaniensis).* At 2217, the female came out of another root hole approximately one meter from the initial one she retreated into with her head lifted high in the air. We made no further observations of this pair.

(2) At 2215 (127 min post-sunset) on 23 August 2015, we observed a pair of *P. yonahlossee* in the preliminary actions of courtship on Hurricane Mountain, Virginia. When the male approached the female (< 7 cm away), the female quickly moved approximately 60 cm away from the male. The male then slowly followed the same path. When the male was within a few cm of the female, she again moved away; this happened several times. Observations concluded at approximately 2300.

(3) At 2340 (212 min post-sunset) on 23 August 2015, we observed a pair of *P. yonahlossee* in TSW on Hurricane Mountain, Virginia. The male had his rear legs extended and his tail arched as it undulated along the arched portion (0.24 waves/s; n = 6 waves), with his tail tip under the female. The female straddled the rear half of his tail with her head raised and chin touching the arched portion of the tail of the male. The pair remained in this position for the

next 20 min. At 0001 (24 August 2015), the male slowly turned clockwise towards the female and broke contact. The male continued to undulate his tail base as the base was contacting the substrate (0.29 waves/s; n = 18 waves). The male also performed a foot dance every few seconds in a rhythmic fashion, alternating between all four feet and sometimes lifting both a front foot and opposite back foot at the same time. The male lifted his head below the chin of the female while continuing to undulate his tail and foot dance. The male continued foot dance for approximately three min. until the female broke contact and quickly left the area. The male moved in the same direction as the female, but we made no further observations. Observations concluded at approximately 0005.

(4) At 2157 (113 min post-sunset) on 26 August 2015, we observed a pair of *P. yonahlossee* in the process of spermatophore deposition on Hurricane Mountain, Virginia. When we discovered the pair, the female was positioned over the entire length of the tail of the male, which was flat to the ground and undulating laterally along its entire length. The mean frequency of tail undulations was 0.40 waves/s (n = 44 waves). Approximately 10 min later, the male lifted his vent off the ground, moved his tail out from under the female, and moved his tail to the side. He did this while moving forward slightly, with the female following as her chin remained resting on the base of his tail. The spermatophore deposited by the male became visible when he moved forward. When the vent of the female was over the spermatophore, the male stopped and repeatedly lifted his pelvis against the chin of the female with his tail flexed to one side. A few minutes later (2200), the female lifted her vent off the spermatophore, holding the sperm cap in her cloacal lips (Fig. 2). Observations concluded at 2215.

(5) At 2230 (146 min post-sunset) on 26 August 2015, we observed a pair of *P. yonahlossee* in TSW on Hurricane Mountain, Virginia. However, as soon as a flashlight was focused on the pair, they separated. The male then repeatedly approached the female, but she moved quickly away each time. We observed this approach-flee behavior periodically over the course of approximately one hour (until 2330), when the pair disappeared from view.

(6) At 2300 (176 min post-sunset) on 26 August 2015, we observed a pair of *P. yonahlossee* in TSW on Hurricane Mountain, Virginia. However, they terminated courtship behaviors as soon as the beam of the flashlight reached them, and they never resumed. We observed a pair of *P. montanus* courting in close proximity to this pair, and they also dispersed shortly after being reached by the flashlight beam.