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COURTSHIP IN THE TORRENT SALAMANDER, *RHYACOTRITON*, HAS AN ANCIENT AND STABLE HISTORY

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***Abstract*.—Although many examples of behavioral homology have been documented in the vertebrate literature, these examples are skewed towards short timescales. In this article we report the case of complex behavior used by salamanders in sperm transfer that is at least 123–153 million years old. *Rhyacotriton* is an ancient salamander lineage with distant relationships to *Amphiuma* and plethodontids and an even more distant relationship to ambystomatids and salamandrids. Only fragments of *Rhyacotriton* courtship were known from past work, but they promised insights into the ancient sexual radiation of salamander families. Here we report laboratory observations of complete *Rhyacotriton* courtship that confirm that promise. We recorded ongoing courtship with still photography, audiotape recordings, and videography. Some elements of *Rhyacotriton* courtship were remarkably similar to plethodontid homologs (e.g., trail-straddling walks and sperm transfer behavior). The spermatophore of *Rhyacotriton* was likewise very similar to that of plethodontids and unlike that of other salamanders. As in plethodontids, courting *Rhyacotriton* males could mimic female behavior and dupe rival males into unprofitable spermatophore deposition. These observations suggest that key aspects of sexual behavior shared by these two salamander families have persisted for 123–153 million years.**

Key Words.—courtship behavior; deep homology; mating display; spermatophore; spermatozoon; sexual interference

INTRODUCTION

Examples of behavioral homologies in vertebrates on a timescale of 100 million years are extremely rare. Lorenz (1939, 1950) was confident of deep homology in only one vertebrate behavior, reaching over the forelimb to scratch with the hind foot. This style of head scratching is widespread in living groups of mammals, squamates, crocodilians, and birds, which shared a most recent common ancestor about 320 million years ago (Mya; Shen et al. 2016). On a shorter timescale, complex constricting behavior in modern snakes is approximately 100 Mya (Greene 1994; Zheng and Wiens 2016). Pigeons and sandgrouse (Columbimorphae) share a peculiar method of drinking (Mayr 1997), which dates at least to the time of their common ancestor, about 58 Mya (Prum et al. 2015). Undoubtedly, more examples of ancient homology will emerge as comparative behavioral data are mapped on time-calibrated molecular phylogenies.

The diagnosis of behavioral homology (Atz 1970; Wenzel 1992; Greene 1994) is strengthened if the behavioral traits are complex and unique to a clade of sister taxa. The importance of these criteria can be appreciated by analogy with plagiarism. The alternative to plagiarism is the independent literary origin of the passage in question, just as the alternative to homology is independent evolutionary origin by convergent or parallel evolution. A case for plagiarism is strengthened if the identical literary section is long and unique in the literature of a particular language. For this reason, identity of a single word seldom presents a case for plagiarism. Likewise, complex behaviors in an unusual sequence are unlikely to have independent origins, especially if they are unique to a pair of related taxa. Uniqueness contributes to the argument because it means that no examples of independent origin are known.

On these grounds, we present an especially strong and remarkable case for homology and evolutionary persistence of a complex behavior for more than 100 million years. The veracity of homology on this timescale is important, not only because of its rarity but because it argues for a particular model of behavioral evolution (Estes and Arnold 2007; Arnold et al. 2017). In particular, it argues for the persistence of stabilizing selection over hundreds of millions of years (Estes and Arnold 2007; Arnold and Houck 2016; Arnold et al. 2017).

The long problematic phylogenetic relationships of Torrent Salamanders (*Rhyacotriton*) have recently been resolved by DNA sequencing. A distinctive, virtually lungless, stream and seep-dwelling denizen of the Pacific Northwest, *Rhyacotriton* was initially thought to be a hynobiid (Gaige 1917; Czopek 1962; Whitford and Hutchison 1966). In later systematic analyses, *Rhyacotriton* was considered to be either an ambystomatid or the sister taxon of another Pacific

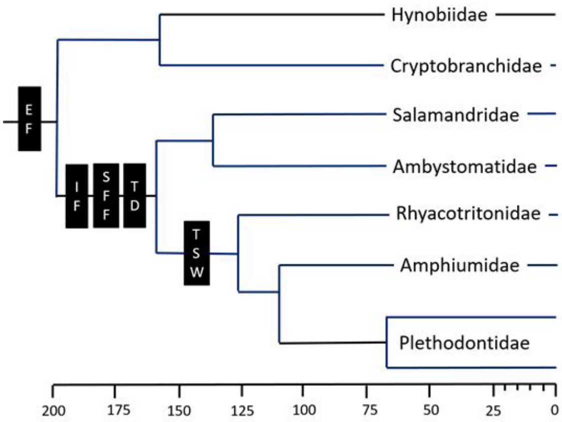

Figure 1. The evolutionary origin of key courtship traits in salamanders. Character origins are shown with solid boxes: EF = external fertilization; IF = internal fertilization; SFF = spermatophore deposition in front of the female; TD = tail fanning, tapping, undulation or wagging display in front of the female; TSW = tail-straddling walk. The time of origin for each trait is bounded by the times at the ends of the branch on which it resides (time scale in millions of years). The placement of EF is meant to indicate that external fertilization was present in the common ancestor of salamanders and anurans and in the common ancestor of extant salamanders. Time-calibrated phylogeny is based on Shen et al. (2016), using an independent-rate model (clock = 2). Proteids are not shown in this diagram, but other studies indicate that they are the sister group to rhyacotritonid-amphiumid-plethodontid clade (Zhang and Wake 2009; Pyron and Wiens 2011).

Northwest endemic, *Dicamptodon* (Tihen 1958). In contrast, recent systematic work based on DNA sequencing reveals a consistent phylogenetic pattern (Fig. 1). *Rhyacotriton* is most closely related to *Amphiuma* and plethodontids (Min et al. 2005; Zhang and Wake 2009; Pyron and Wiens 2011; Vietes et al. 2011; Shen et al. 2016). This consistency is all the more remarkable given the depth of the relationship. The split between *Rhyacotriton* and the *Amphiuma*-plethodontid lineage occurred about 128 Mya, the range of five estimates is 123-153 Mya (Shen et al. 2016). The more ancient origin of modern salamander families (Salamandroidea), all of which perform a tail fanning, tapping, undulation or wagging display in front of the female and later deposit a spermatophore in front of her (Houck and Arnold 2003), occurred about 160.5 Mya (Shen et al. 2016). In other words, any homologies observed between the sexual behavior of plethodontids and *Rhyacotriton* date to the late Jurassic or early Cretaceous. Unfortunately, the sexual behavior of *Amphiuma* is poorly known and offers no comparative insights (Houck and Arnold 2003). In this article we describe *Rhyacotriton* courtship and make the case for deep homology of sexual behavior and sperm delivery between *Rhyacotriton* and plethodontids.

Materials and Methods

We observed and recorded *Rhyacotriton* courtship in two settings: at the University of Michigan, Ann Arbor, in 1969–70 and at Oregon State University, Corvallis, in 2003 and 2014. In the sections below we separate observations made in these two settings and time periods.

Capture methods.—We obtained *Rhyacotriton* from three localities for the Ann Arbor observations: (1) *R. cascadae* at Wahkeena Falls State Park and Latourell Falls, Multnomah County, Oregon (several pairs in March-April, 1969 and four pairs in mid-April, 1970), (2) *R. variegatus* at 6.4–7.2 km up Fall Creek from Highway 34, Benton County, Oregon (several pairs, March-April 1969), and (3) *R. olympicus* at 21 km up the Quinault River from Quinault, Jefferson County, Washington (13 pairs, 28 March 1970). At the conclusion of the study, we euthanized animals and preserved them as vouchers in the Museum of Zoology at the University of Michigan, USA.

We collected *Rhyacotriton variegatus* at Parker Creek on Marys Peak, Benton County, Oregon, in 2003 and 2014 for the Corvallis observations. We found animals by overturning rocks along the edges of the creek and captured them by hand or with small aquarium nets. We measured snout-vent lengths (SVL) and visually assessed reproductive condition immediately after capture. We established that individuals were sexually mature using published SVL ranges found for gravid females and females that had recently oviposited (41–51.8 mm) and for sexually mature males (37.9–48.3 mm), as well as the presence of large ova in females and enlarged vent lobes on males (Sever 1988; Tait and Diller 2006). For videotaping of courtship in the laboratory on 18 April 2014, we transported two sets of animals (five males and three females and three males and three females) to Corvallis in a refrigerated cooler in separate 1L plastic bags containing damp moss. We setup animals for maintenance and videotaped them on the same day that we collected them. At the conclusion of the study, we euthanized the animals and preserved them as vouchers in the Herpetological Research Collection at Oregon State University, Corvallis, USA. In 2003, we collected eight animals on 9 April and videotaped a complete courtship of one pair a few days later.

Observational methods.—We maintained animals in Ann Arbor in a temperature-controlled room (18–19° C) with a 12–12 h photoperiod. We housed small sets of males and females together in 37.85 L (10 gallon) all-glass aquaria with glass lids and paper towel substrates. We observed and recorded courtship and other interactions at night in these same aquaria. We elevated one end of the aquarium, so that its long axis was tilted at about 5°, and filled with water so that the lowest half of the aquarium was under water. We provided a paper towel retreat at the unflooded end of the tank. We fed animals juvenile House Crickets (*Acheta domesticus*) and House Fly (*Musca domestica*) larvae. Observations of courtship were made in these same aquaria. We used dim white light to illuminate the aquaria at night. We described ongoing interactions into an audio tape recorder and photographed courting animals with a 35 mm Pentax SLR camera (Pentax Corporation, Tokyo, Japan) using an electronic flash.

We maintained animals in Corvallis in a temperature-controlled room (11–13° C) with a natural photoperiod. In 2014, we used the same containers for maintenance and observation and will refer them as observation chambers. We held animals in the observation chambers for 10 d after capture and fed four House Fly larvae per salamander once during that 10 d interval.

We placed animals in a clear plastic box (245 × 245 × 35 mm) for observation and videotaping of courtship. We created a gap between the sides and top of the box with a rolled paper towel (approximately 2 mm deep × 40 × 20 mm) to allow air circulation and prevent condensation on the inside of the top. We used moist paper towels as substrate. We placed two observation chambers side by side but separated by a vertical piece of opaque cardboard so that animals in one chamber could not see animals in the other chamber. We used a male-biased sex ratio (two males, one female) in each of the two observation chambers because previous observations showed that pairing a female with a single male usually did not result in courtship, but pairing multiple males with multiple females increased the chance of courtship. In one particularly successful session that yielded most recordings of courtship, two males (51 mm and 49 mm SVL) and one female (53 mm SVL) were placed in one chamber (video 1 on 18 April 2014). We conducted videotaping of male-male interactions in two sessions on 18 April 2014 (video 2): session 1 with two males and one female, and session 2 with three males and three females. We allowed a few minutes to elapse to allow animals to acclimate to one another and to the chamber and then we set up lights and video cameras to begin recordings.

We recorded courtship with digital video cameras mounted above observation chambers, beginning before sunset and continuing after sunset. We positioned each camera (Logitech WiLife Pro, Logitech International, Romanel-sur-Morges, Switzerland) so that the entire substrate of each chamber was in view (videos 1 and 3). We operated camera-controlling software (WiLife Command Center 2.5, Logitech International, Romanel-sur-Morges, Switzerland) by laptop computer. We positioned two red lights (7 W) above the observation boxes to illuminate the observation chambers during nighttime recording. We also recorded ongoing behavior (video 1) with cellular phone cameras (Droid 4, Motorola Mobility, Chicago, Illinois, USA, and Galaxy S3, Samsung Telecommunications, Suwon, South Korea).

We viewed and analyzed video recordings using Windows Media Player (Microsoft Corporation, Redmond, Washington, USA). We used the Play Speed Settings Tool under Enhancements to play the footage up to 16 times faster than real time to quickly bypass intervals without courtship or other kinds of interaction. In 2003, we conducted maintenance, observation, and video recording as described by Verrell (1997).

Sperm preparation method.—We macerated the sperm mass from a spermatophore recently deposited in Corvallis in 2014 in Modified Ham's F-10 medium as described by Friesen et al. (2014). We viewed and videotaped the resulting preparation at 100X and 400X with a Zeiss Axiostar Plus Transmitted-light Microscope (Zeiss, Oberkochen, Baden-Wűrttemberg, Germany).

Spermatophores.—In the course of studies of salamander courtship, one of us (SJA) examined recently deposited spermatophores of 30 species representing 15 genera (number of species shown in parentheses): *Ambystoma* (8), *Aneides* (1), *Bolitoglossa* (5), *Chioglossa* (1), *Cynops* (1), *Desmognathus* (2), *Eurycea* (1), *Hemidactylium* (1), *Hydromantes* (1), *Notophthalmus* (1), *Plethodon* (4), *Pleurodeles* (1), *Pseudoeuyrcea* (1), *Salamandra* (1), and *Salamandrina* (1). Many of these examinations were brief, made during a failed attempt to save long-lasting voucher specimens. In the case of seven species, however, SJA examined the spermatophores under a Wild Stereo-Microscope (Wild, Heerbrugg, Switzerland) at 10X and sketched gross morphology using *camera lucida*: *Ambystoma maculatum*, *A. tigrinum*, *Bolitoglossa subpalmata*, *Ensatina eschscholzii*, *Plethodon shermani*, *P. yonahlossee*, and *Rhyacotriton cascadae*. The *Rhyacotriton* spermatophore was one of three complete spermatophores that SJA retrieved on 20 April 1970 a few hours after they were deposited by a courting male *R. cascadae* from Wakeena Falls, Oregon.

RESULTS

We detected no differences in the behavior of *R. cascadae*, *R. olympicus*, and *R. variegatus*, and so make no references to species in the descriptions that follow.

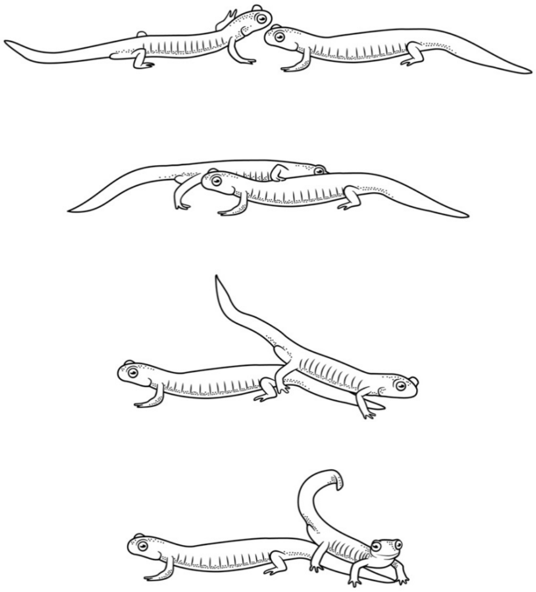

Figure 2. A male *Rhyacotriton* sp. approaches a female, nudges her flank, crawls over her tail and performs a tail-wagging display (after Arnold 1972, based on a sequence photographs taken at night of courting animals).

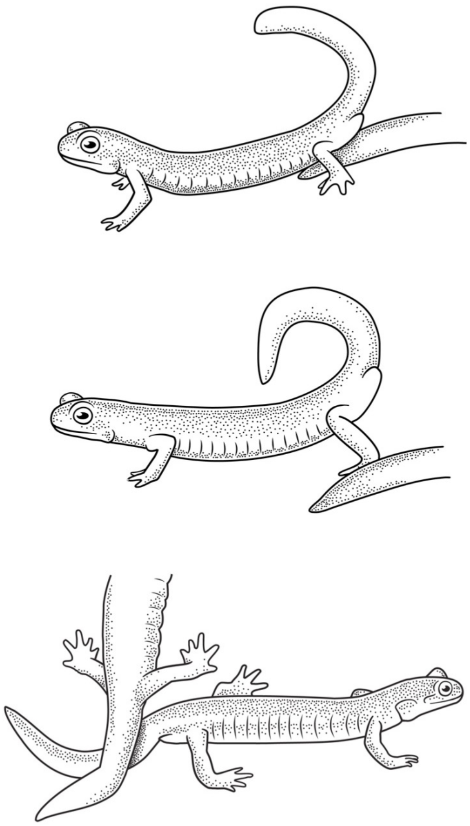

Figure 3. Approach phase display and postures of male *Rhyacotriton* sp. (Top) A male curls his tail over his trunk and then assumes the tail-wagging display posture in which his tail tip wags from side to side. (Bottom) A male has crawled under the tail of a female (vertical on the container wall) and then pauses while arching and undulating his tail base (after Arnold 1972, from photographs of courting animals).

Catalog of courtship actions.—We denote observations in Ann Arbor and Corvallis as A and C, respectively. We illustrate the following descriptions of courtship with a 16 min sequence, videotaped simultaneously with three different cameras in Corvallis (video 1, https://www.youtube.com/watch?v=lMbK1e1Z3p4&t=8s). We reference video segments by number (1–18) in Table 1. We denote intervals of elapsed time as min:s. The types of behaviors were: Nudging (C, A) - A salamander uses its snout to nudge the side of the body of another salamander (Fig. 2, segments 2 and 16); Head-Swinging (C, A) - A salamander arhythmically swings its head from side to side with its chin in contact with the dorsum another salamander (female in segment 5); Head-Sliding (C, A) - A salamander slides its chin along the dorsum of another salamander as it moves forward; Lifting (A) - The male turns his head under the chin of the female and lifts his head contacting the dorsum of his head to the chin of the female; Tail-wagging display (C, A) (TWD) - The male slowly raises the tip of his tail, gradually curling the entire tail upward until the tail tip is directly above the his midbody (segments 1 and 16). With the tail curled in this position, the male slowly and rhythmically swings the last centimeter of his tail back and forth (Figs. 2 and 3); Tail-straddling Walk (C) (TSW) - The male and female walk forward with the female straddling the tail of the male and with her chin resting on the base of his tail, sacrum, or mid trunk. The male may move his hind limbs in a treading motion (see Spermatophore Deposition). The female may perform head-swinging. The male vertically arches his midbody and undulates his tail so that only the tip of his tail touches the substrate (segment 4); Spermatophore Deposition (C) (SD) - Both the male and female are stationary with the female straddling the tail of the male. Initially, the hind limbs of the male are stationary but then for the rest of SD, he continuously raises and lowers one or the other hind leg, alternating between left and right legs, sweeping them backward and forward in a characteristic treading motion. His tail is positioned slightly off-center to the venter of the female, the distal end undulating laterally. The female remains stationary but may perform head-swinging with her chin rested on the tail base or mid torso of the male (segment 5); and Positioning (C) - The male walks forward after SD with the chin of the female resting on his sacrum and his trunk undulating from side to side (segment 6). When the female stops with her vent over the spermatophore, he stops and arches his sacrum upward (segment 7). He undulates his trunk as he backs up under the venter of

Table 1. Summary of a temporal alignment of video records of *Rhyacotriton variegatus* courtship from three cameras of a courtship sequence recorded on 18 April 2014 (video 1). Onset times represent time points on the video available at https://youtu.be/lMbK1e1Z3p4. Parentheses indicate an action in progress, so the time point is not aligned with other records. Duration is computed as the average of 1–3 records. Abbreviations are C1O = camera 1 onset, C2O = camera 2 onset, and C3O = camera 3 onset.

Segment	Event	Action illustrated	C1O min:s	C2O min:s	C3O min:s	Duration min:s
1	male approaches female with TWD	TWD	0:14			
2	female turns towards male	female nudging	2:36			2:22
3	male tail arched and undulated under female chin	initiation of TSW	3:04			0:28
4	tail-straddling walk	TSW	3:12	(20:20)		0:08
5	spermatophore deposition	SD	5:00	20:29	(30:28)	3:06
6	male lifts off spermatophore and moves forward	Positioning	8:05	23:36	32:41	0:10
7	female vent over spermatophore	Positioning	8:15	23:47	32:53	0:10
8	male backs up under female	Positioning	8:25	23:57	33:03	0:11
9	male and female stationary	Positioning	8:38	24:07	33:15	1:41
10	female starts to move off		10:19	25:51	34:55	0:02
11	male starts to move off	Positioning	10:21	25:53	34:57	0:52
12	female lifts vent off spermatophore		11:11	26:47		0:48
13	female moves away from spermatophore		12:01	27:33		0:22
14	female crawls over male		12:23	27:55		1:00
15	male moves forward, end of positioning pose		13:24	28:55		0:41
16	male stationary, begins TWD while nudging female	TWD	14:06	29:36		0:21
17	female crawls over male		14:25	29:59		0:19
18	male bites conspecific		16:13			
	live spermatozoa				35:00	

the female, flexing and extending his hind limbs until his trunk is positioned under the pectoral girdle of the female (segment 8). He assumes a stationary pose with his hind limbs extended and blocks forward progress by the female, who is likewise stationary with her vent located over the spermatophore (segment 9).

Courtship in *Rhyacotriton* follows four phases, previously described for plethodontid salamanders (Arnold 1977; Verrell and Arnold 1989; Arnold et al. 2017): a preliminary pursuit and approach phase in which the male follows and slowly approaches the female, a period of physical contact in which the male contacts the body of the female with his head, an invitation to TSW in which the male performs TWD, and finally a TSW in which the male leads the female forward, deposits a spermatophore, and then positions her over it. Actual sequences depart from this idealized scheme in a variety of ways, as illustrated in the following accounts.

Temporal relations.—In Ann Arbor, courtship encounters were nocturnal and invariably took place on land (the non-submerged portion of the substrate) even though pairs were maintained in sloping aquaria with half of the substrate submerged and half exposed. Spermatophores were discovered on several occasions and were always attached to the substrate on land. Usually one (but on one occasion three) spermatophore was deposited in a single night by a male housed with a female. Preliminary courtship took the following, rather variable, course. A male approached a female as she moved about. In close proximity to the female, the male sometimes abruptly stopped, if the female remained stationary, and performed TWD without contacting her. Alternatively, he sometimes approached the female and began contacting her with his head (via nudging, head-swinging or head-sliding) as he moved along her length. If she remained stationary as the male contacted her with his head, the male sometimes moved away from her, stopped, and performed TWD while facing away from her (Fig. 2). On several occasions, males performed lifting when they reached the head of the female, after first moving along the length of her body. The male then crawled forward under the chin of the female, paused when the dorsal, proximal part of his tail contacted her chin (or some other region of her body), and then arched and undulated his tail (Fig. 3 shows this male tail arching under the trunk of the female). In all cases the female remained stationary. Then, the male sometimes ceased tail arch and undulation, and resumed head contact with the female, or he sometimes moved forward and initiated TWD. On many occasions males initiated and performed TWD without approaching females or other animals. Frequently a male moved about the container, stopped and performed TWD, and then terminated the action after a few minutes, moved forward again and resumed TWD. Although TSW and SD were never observed, spermatophores were deposited on five occasions, always on the terrestrial portion of observations chambers.

In Corvallis, the approach phase of courtship was observed on numerous occasions, with and without video recording. During this phase, males performed nudging, head-sliding, and tail wagging display while following an individual female as she walked around the chamber. The TWD of the male was a characteristic behavior of the approach phase, performed with the male facing towards or away from the female, and often alternated with physical contact with the female (nudging and head-sliding). Females typically responded to these actions by standing still or slowly walking forward.

One instance of TSW resulting in spermatophore deposition is illustrated in 16 min video 1 (Table 1). Videotaping began with the male behind the female, performing TWD (segment 1). The female moved forward, and the male followed. The male crawled diagonally over the body of the female and stopped with his trunk on top of her torso and with his tail undulating. The female then nudged the body of the male as he continued forward and he stopped in front of her (segment 2). The female placed her chin on the base of the tail of the male, which was undulating and arched (segment 3). The male and female then walked forward in a tail-straddling walk (segment 4). During TSW, the male walked forward as the female followed with her chin resting on base of the tail of the male. The base of the tail of the male shifted from side to side during TSW, and his trunk undulated slightly from side to side. During TSW, which lasted 1 min 48 sec, the pair moved forward one body length.

The transition from TSW to spermatophore deposition occurred as the male continued walking forward, and, with the chin of the female resting on his sacrum, he repeatedly lowered his hip so that the vent of the cloaca contacted the substrate. SD began while the male remained stationary and with his vent in contact with the substrate and with the female in the same TSW position as before. The transition to spermatophore deposition was noticeable as the male aligned his femurs perpendicular to his body axis and 20 sec later began the characteristic treading motion of SD (segment 5).

During the 3 min 05 sec duration required for spermatophore deposition, both partners were stationary in a TSW posture. The male continuously raised and lowered one or the other hind leg, alternating between left and right legs, sweeping them backward and forward, in a characteristic treading motion. His tail was positioned slightly to the left of and parallel to the body of the female, the distal end undulating laterally. Meanwhile, the female periodically and arhythmically head-swung on the sacrum of the male and shifted slightly forward.

SD ended when the male walked forward and lifted his vent off of a fully formed spermatophore, his tail to the left of the body of the female (segment 6). The male and female moved forward in TSW position with the trunk of the male undulating from side to side. The pair stopped when the vent of the female was directly above the spermatophore. At this time, the male was in front of the female, and her chin rested on his sacrum (segment 7). Next, the male arched and undulated his trunk as he backed up under the chin and chest of the female, lifting her forelimbs off the substrate and blocking her forward progress (segment 8). Both partners remained motionless in this final position with the vent of the female over the spermatophore for 1 min 41 sec (segment 9). Periodically, the male renewed the arching of this trunk as he rested in front of the stationary female with his tail undulating slightly. The female started to move off of the spermatophore but paused (segment 10) and almost immediately the male moved forward but maintained the positioning pose (segment 11). The female lifted off of the spermatophore (segment 12) and moved away from it (segment 13), crawling over the male (segment 14). The male moved forward, ending the positioning pose (segment 15). While stationary, the male nudged the female, now in front of him, and began TWD (segment 16). The female departed from the male, crawling over him (segment 17). About 2 min later, the male nudged and attacked a salamander positioned in front of him (segment 18).

We illustrate a second instance of tail-straddling walk leading to spermatophore deposition with video 2 (Table 2). This video includes two transitions from head contact to tail-straddling walk. In both examples, the male crawls under the chin of the female, while arching and undulating his tail. The female turns parallel to the axis of his tail and steps astride, initiating the tail-straddling walk (segments 2–3 and 5–6). Both TSWs are of longer duration than those in video 1. The first lasts a little over 9 min and breaks off when the female departs (segment 3). The second lasts nearly 9 min and terminates as the male transitions into SD (segment 6). The view during SD and Positioning is partially obscured by condensation on the glass cover of the chamber, but hind limb treading is clearly visible during SD. The view of positioning is also obscured because the male climbs the vertical corner of the chamber as he leads the female over the spermatophore.

Male-male interactions.—In Ann Abor, when several males were housed together with females, males frequently pursued and bit other males. We

Table 2. Summary of a video record of *Rhyacotriton variegatus* courtship recorded in April 2003 (video 2). The recording was made in time lapse mode. Consequently, the time units for adjacent columns labeled Onset and Duration correspond to the elapsed time on the recording, which is not real time. Real time is given by the time stamp on the video (columns labeled Time Stamp and Duration) which is in units of hours, minutes and seconds. Other conventions as in Table 1. The video is available at https://www.youtube.com/watch?v=HNu_Hh_mUvY&t=45s

Segment	Event	Action Illustrated	Onset	Duration	Time Stamp h:min:s	Duration h:min:s
1	male pursuit of female with nudging and tail-wagging display	Pursuit and Head Contact	(0:00)	0:45:00	1:19:46	0:09:51
	male breaks off pursuit		0:45	0:13:00	1:29:37	0:02:58
2	male pursuit of female with nudging and tail-wagging display	Pursuit and Head Contact	0:58	0:24:00	1:32:35	0:05:01
3	tail-straddling walk	TSW	1:22	0:41:00	1:37:36	0:09:03
4	TSW breaks off, male resumes pursuit		2:03	0:21:00	1:46:39	0:05:17
	recording breaks off		2:24	0:04:00	1:51:56	1:29:06
5	recording resumes, male continues pursuit	Pursuit and Head Contact	(2:28)		3:21:02	
6	tail-straddling walk	TSW	3:22	0:41:00	3:32:43	0:08:56
7	view obscured by condensation on glass	SD, Positioning	4:03	0:30:00	3:41:39	0:06:35
8	female departs		4:33		3:48:14	0:00:20
	recording ends		4:35		3:48:34	3:35:14
	position of spermatophore marked by tip of videographer's finger				7:23:48	

never observed this type of interaction between males and females or between females. We did not observe TSWs involving two males or other kinds of sexual interference in Ann Arbor.

We illustrate descriptions of male-male interactions observed in Corvallis with videotaped sequences totaling 68 min (video 3, https://youtu.be/3HF99DH3_z0). Video segments are referenced by number (19–64) in Table 3. We observed multiple incidences of male-male aggression consisting of rapid approach with and without biting. Usually, an aggressive male approached and contacted another male that was courting a female. The aggressor male oriented toward the courting male and approached him, sometimes nudging various parts of the body with their snout. After contact, the aggressor male bit the courting male on the limbs or side of the body, sometimes shaking his head back and forth while maintaining his hold. Sometimes the aggressor male rotated about his long axis while holding a body part in his mouth. Aggression ended when the aggressor male released the previously courting male and ran away or simply stopped attacking the other. In some cases, males attacked females, but those incidences were uncommon. Most aggressor males were larger than the male they attacked. Aggressor males tended to conduct long sequences of attacks. Some males acted aggressively toward other males without courting females. We illustrate six bouts in which a male bit another animal one or more times in videos 1 (segment 18) and 2 (segments 28, 34, 49, 56, and 64).

We videotaped three bouts of sexual interference in the form of female mimicry on 18 April 2014 (video 2). During each observation, a following male (FM) behaved like a female during TSW and SD with a leading male (LM). The video recording for the first event began with TSW already in progress (segment 19). The record of the second event began with two males performing TWD and with one male biting the other (segment 48). The record for the third event began with two males approaching each other and other animals (segment 54). Each of these three sequences transitioned to TSW in which one male straddled the tail of the other male. In one case, the FM elicited three spermatophore deposition by the leading male (segments 20, 38, and 41) and in the other case one deposition (segment 51). Male-male TSW closely resembled male-female TSW, but FMs tended to move more anterior to the LM’s sacrum, each time causing the LM to move rapidly forward. Sometimes male-male TSW was interrupted by the departure of the FM (segments 36) or because the FM bit the LM (segment 56). When the FM departed, the LM continued arching his sacrum and undulating his tail, resulting in resumption of TSW (segment 37).

We observed SD four times during two mimicry episodes (segments 20, 38, 41, 51). During SD the LM performed the characteristic treading motion of his hind legs while the MM straddled his tail, sometimes performing head-sliding on the dorsum of the LM. On two occasions the FM departed during SD (segments 22 and 52), but the LM continued treading. On one of these occasions, the FM returned to straddle the tail of the LM

Table 3. Summary of video records of three sessions (noted as cameras on video) of male-male interactions in *Rhyacotriton variegatus* videotaped sequentially on 18 April 2014 (video 3). Onset time points represent points on the video available at https://youtu.be/3HF99DH3_z0. Parentheses indicate an action in progress. Two males and one female are present in session 1. Three males and three females are present in session 2.

Session	Segment	Event	Onset h:min:s	Duration min:s
1	19	male-male TSW	(0:09)	
1	20	male-male SD	1:20	0:21
1	21	FM begins departure, but then resumes TSW position	1:41	1:11
1	22	FM begins second departure	2:52	1:00
1	23	LM lifts off spermatophore and immediately approaches FM	3:52	0:12
1	24	LM begins TWD while approaching FM	4:04	0:06
1	25	break in videotaping	4:10	
1	26	LM approaches FM with TWD	(4:35)	
1	27	LM approaches female with TWD	5:44	0:53
1	28	LM repeatedly bites female, then follows her with TWD	6:37	1:36
1	29	LM head slides on female trunk, then TWD	8:13	1:16
1	30	LM nudges FM tail	9:29	0:22
1	31	LM crawls over female	9:51	0:29
1	32	LM crawls over FM	10:20	1:02
1	33	LM begins TWD	11:22	0:26
1	34	FM bites LM	11:48	0:28
1	35	second episode of male-male TSW begins, LM in front	12:16	1:15
1	36	FM nudges trunk of LM, end of TSW	13:31	1:13
1	37	male-male TSW resumes	14:44	0:29
1	38	second male-male SD	15:13	2:56
1	39	LM lifts off spermatophore, pair moves forward in TSW	18:09	2:00
1	40	LM arches and undulates trunk, leading to third TSW	20:09	9:08
1	41	LM begins third male-male SD	29:17	3:00
1	42	LM lifts off spermatophore, pair moves forward, past spermatophore	32:17	0:31
1	43	switch to red light	32:48	0:03
1	44	LM back up under FM	32:51	0:13
1	45	LM stationary in positioning pose	33:04	0:41
1	46	LM departs from FM	33:45	0:24
1	47	FM follows and nudges LM, leading to fourth TSW	34:09	7:13
1		videotaping ends	41:22	
2	48	two males approach each other and other animals while performing TWD	(41:27)	
2	49	larger active male bites smaller active male, twice	49:46	
2	50	male-male TSW, with larger FM performing TWD	49:50	0:18
2	51	male-male SD	50:08	0:21
2	52	FM departs, LM continues with SD	50:29	2:19
2	53	LM lifts off spermatophore, moves forward and begins positioning with no FM behind him	52:48	0:37
2		videotaping ends and then resumes in next segment	53:25	
2	54	two active males follow each other and other animals	(53:31)	
2	55	larger FM follows smaller LM, places chin on tail base and elicits TSW	54:37	0:41
2	56	FM bites LM, twice, TSW ends	55:18	0:21

Table 3 (continued). Summary of video records of three sessions (noted as cameras on video) of male-male interactions in *Rhyacotriton variegatus* videotaped sequentially on 18 April 2014 (video 3). Onset time points represent points on the video available at https://youtu.be/3HF99DH3_z0. Parentheses indicate an action in progress. Two males and one female are present in session 1. Three males and three females are present in session 2.

Session	Segment	Event	Onset h:min:s	Duration min:s
2	57	larger male performs TWD and approaches female	55:39	6:44
2	58	larger and smaller males crawl over each other without interacting	1:01:23	1:50
2	59	larger and smaller males again crawl over each other without interacting	1:03:13	0:47
2	60	smaller and larger males both perform TWD	1:04:00	0:30
2	61	larger male approaches female and performs TWD while nudging her	1:04:30	0:36
2	62	female moves away from displaying male, larger male continues TWD	1:05:06	2:05
2	63	larger and smaller male both perform TWD in close proximity	1:07:11	0:49
2	64	active male bites another animal, three times	1:08:00	0:30
2		videotaping ends	1:08:30	

(segment 21). When the departed FM did not return to the SD position, the LM immediately approached the FM after SD (segment 23) or proceeded with positioning even though the FM did not straddle his tail (segment 53). FMs consistently walked past the spermatophore after SD (segments 39 and 42) rather than stopping when their vent contacted it, as a female would.

***Spermatophore and Spermatozoa*.**—Spermatophores (five) were all deposited on the terrestrial portions of observation chambers that provided both terrestrial and aquatic sites. Each spermatophore consisted of a laterally-compressed, clear gelatinous stalk abruptly rising from a flattened, oval base. The apex of the stalk was inserted deeply into a large, dome-shaped sperm mass, which sat firmly on the stalk (i.e., the upper 40–50% of the stalk was inserted into the sperm mass).

Live sperm of *Rhyacotriton* have not been described, although Zalisko and Larsen (1988) and Scheltinga and Jamieson (2003) described the vas deferens and associated sperm and Sever et al. (2004) described sperm storage in females. Live swimming *Rhyacotriton* sperm are shown at the end of video 1 (35:00). An undulating membrane that spans almost the entire length of the spermatozoon is clearly visible.

Discussion

***Courtship evolution in Rhyacotriton and its relatives*.**—The many unique and close similarities between the courtships of *Rhyacotriton* and plethodontids argue strongly for homology. The most striking courtship similarity between these two related lineages is in the tail-straddling walk that precedes and accomplishes sperm transfer. The TSWs are similar in many ways: (1) the walk is initiated by the male crawling under the females chin or by the female turning towards the tail base of the male, (2) the female remains with her chin on the body of the male during a lengthy spermatophore deposition, (3) afterwards the pair moves forward in a coordinated march, (4) and when the female stops over the spermatophore, the male backs up under her chin, apparently stabilizing her position over the spermatophore. All of these characteristics are found in *Rhyacotriton* and in all the diverse plethodontids that have been observed (*Gyrinophilus*, *Pseudotriton*, *Eurycea*, *Desmognathus*, *Bolitoglossa*, *Chiropterotriton*, *Ensatina*, *Aneides*), but not in other salamanders (Arnold et al. 2017). The exclusivity of these four similarities argues strongly for homology.

At the same time, the TSWs of *Rhyacotriton* and plethodontids differ in subtle ways. During spermatophore deposition male *Rhyacotriton* perform a unique treading motion with their hind limbs; whereas, plethodontid males hold their hind limbs stationary with femurs perpendicular to the body axis. After spermatophore deposition, plethodontid males pull their tail out to the side and hold it at a 45° angle as they move forward (Arnold 1972; Houck and Arnold 2003). In contrast, the *Rhyacotriton* male does not pull his tail out to the side. It remains under the body of the female as she moves forward over the top of the spermatophore. When the vent of the female is positioned over the spermatophore, the plethodontid male stops and arches his tail base under the chin of the female. The *Rhyacotriton* male stops and backs up under the female, so that his sacrum ends up about midway under her trunk. Among plethodontids, only male *Aneides* and *Ensatina* back up under the female to a comparable extent, but unlike *Rhyacotriton*, they then massage the sacral region of the female with their tail tip (Stebbins 1949; Arnold 1972; Sapp and Kiemnec-Tyburczy 2011). However, all of these differences between *Rhyacotriton* and plethodontids are minor variations on the same basic TSW theme.

The reader may be concerned by our small sample size for observations of TSW in *Rhyacotriton*, but such concern is easily allayed. In plethodontid salamanders, literally dozens of observations of TSW have been made in each of more than 20 species (Arnold et al. 2017). In those intensively sampled species, TSW is so stereotyped that even a single observation yields an accurate picture of TSW, especially if the behavior is captured by video or a sequence of still photographs. Stereotypy is so strong that TSWs observed under field and laboratory conditions agree even in small details (Pierson et al. 2017).

It is important to consider the unique tail-wagging display of *Rhyacotriton* in relation to two courtship traits that are shared by modern families of salamanders with internal fertilization (the Salamandroidea) and that are likely to have been present in the common ancestor of those families 144–177 Mya. First, all of those modern families for which we have good observations (salamandrids, ambystomatids, rhyacotritonids, and plethodontids) execute a tail-motion display (TD) in front of the female as a part of preliminary courtship: a tail-fanning display in salamandrids, a tail-tapping display in ambystomatids, a tail-undulation display under the chin of the female in plethodontids and rhyacotritonids, and a tail-wagging display in rhyacotritonids (Houck and Arnold 2003). Within their respective families, each of these types of display has persisted for tens of millions of years, and yet relatively small steps in execution would be necessary to transform one form of display into any other. Major transitions between these displays appear to be very rare, but these transitions may eventually be resolved as observations include more and more species within families. In the case of *Rhyacotriton*, the male executes a tail-undulation display (previously known only in plethodontid species) as well as a unique tail-wagging display.

Second, representatives of all major families deposit a spermatophore in front of the female (SFF), an event that is usually preceded by a tail-nudging or tail-straddling walk (Houck and Arnold 2003). Many salamandrids employ a tail-nudging walk in which the tail is S-shaped or bent to one side, exposing pheromone-releasing papillae on the cloacal walls of the male (Houck and Arnold 2003). The walk in ambystomatids is similar, but the male lifts his tail vertically to expose his papillae (Houck and Arnold 2003). The tail-straddling walks of plethodontids and rhyacotritonids are strikingly different from these other families, with the female astride the tail of the male, perhaps responding to pheromones released from the dorsal tail base, but the basic choreography of the walk is retained across all four families. This contrast between the evolutionarily stable choreography of walks and the variable manifestation of tail-motion displays follows the general rule that extreme stasis is associated with a premium on coordination between the sexes (Arnold et al. 2017).

The spermatophore of *Rhyacotriton* closely resembles the spermatophores of plethodontids and proteids but differs from those found in other salamander families. The gelatinous bases of plethodontid spermatophores have a large oval stalk that inserts deeply into a large oval/spherical sperm mass (Organ 1958, 1960; Organ and Lowenthal 1963; Arnold 1976; Zalisko et al. 1984), features shared with *Rhyacotriton*. As in *Rhyacotriton*, the plethodontid female inserts the entire sperm mass into her cloaca and removes the entire sperm cap during sperm transfer. Likewise, the spermatophore base of *Proteus* insert deeply into the sperm cap (Briegleb 1961). In contrast, ambystomatid spermatophores have a stalk that is crowned with four knobs. A domed sperm mass sits on the platform formed by these four knobs (Uzzell 1969; Arnold 1976). Female *Ambystoma* typically remove only a portion of the sperm mass. The spermatophores of salamandrids are of two types, both of which differ from *Rhyacotriton* in numerous ways. The spermatophore bases of some salamandrids (e.g., *Pleurodeles*, *Notophthalmus*) consists of a slender stalk rising from a broad, flat base, crowned by a relatively small sperm mass (Jordan 1891; Zeller 1905; Smith 1910; Zalisko et al. 1984). The spermatophore base of other salamandrids (e.g., *Cynops*, *Taricha*, *Triturus*) is a delicate tent-like structure with numerous folds, ridges, and two prominent apical lobes. A small, spherical sperm mass is attached to the apex of a curved, terminal stalk situated between two apical lobes of the spermatophore base (Zeller 1905; Arnold 1972). *Pleurodeles* and *Salamandra* insert the entire spermatophore (base and sperm mass) into the cloaca during sperm transfer, but a female *Notophthalmus*, *Cynops*, *Taricha* and *Triturus* lightly brushes the sperm mass with her cloacal lips (Arnold 1972). The transferred sperm mass adheres to her cloacal lips as she departs from the spermatophore and later moves into her cloaca, perhaps by ciliary action. In summary, rhyacotritonids, plethodontids, and proteids share a unique spermatophore morphology that may have arisen in the late Jurassic (Zhang and Wake 2009; Pyron and Wiens 2011; Shen et al. 2016).

Two modes of sexual interference in *Rhyacotriton* and plethodontids are similar but both are shared with one or two other families. Female mimicry has been observed in four related families (rhyacotritonids, plethodontids, ambystomatids, and salamandrids). In this mode, interfering males follow rival males during the tail-straddling or tail-nudging walk that precedes spermatophore deposition and dupe them into profitless depositions (Arnold 1976). This behavior has been observed in *Ambystoma tigrinum* (an ambystomatid), *Ensatina*, *Plethodon*, *Pseudotriton* (all plethodontids), *Cynops*, *Notophthalmus*, and *Triturus* (all salamandrids), as well as in *Rhyacotriton* (Arnold 1977; Verrell 1989; Sparreboom 1996). Biting and other agonistic interactions between male rivals are less common but have been observed in many plethodontid genera (*Aneides*, *Eurycea*, *Desmognathus*, *Plethodon*, and *Pseudotriton*) and several salamandrid genera (*Cynops*, *Paramesotriton*, *Salamandra*, and *Triturus*; Verrell 1989; Sparreboom 1996). These observations suggest that the origins of female mimicry and male-male biting predate the divergence of plethodontids and *Rhyacotriton* and may have evolved independently in those two families.

Our laboratory tried for several years to videotape *Rhyacotriton* courtship before achieving the modest success reported here. We believe that three factors contributed to eventual success. First, staging interactions immediately after capture appears to be important. Although TWD was commonly observed days and even weeks after capture, the probability of TSW and SD appears to fall off rapidly with time. Second, including multiple males and females in an observation chambers appears to increase the probability that sexual interactions will occur. Third, use of body size criteria for sexual maturity of Tait and Diller (2006) insures that sexual interaction is at least a possibility.

The importance of various sensory modalities in the sexual communication of *Rhyacotriton* needs to be investigated (Sever 1988). Vision is probably an important channel of communication, especially during the approach phase of courtship. On many occasions, we watched a male suddenly turn towards and approach a moving animal. Furthermore, the TWD may be partly a visual display. Male posture during this display prominently exhibits the light-colored ventral surface of his tail and vent lobes. In addition, these male-specific lobes house glandular tissue that may play a role in the production of a courtship pheromone (Sever 1988).

The possibility of species differences in courtship among the four, extant species of *Rhyacotriton* (Good and Wake 1992) also deserves exploration. Although we observed the approach phase of courtship in three of these species (*R. cascadae*, *R. olympicus*, and *R. variegatus*) and did not detect differences among them, we observed complete courtship only in *R. variegatus*. We may have failed to detect interspecific differences in the preliminary stages of courtship, and differences may exist in the later stages of courtship as well. In this article we have described and argued for deep homology between the courtships of *Rhyacotriton* and plethodontid salamanders. In a companion article we address the causes for the remarkable persistence of courtship similarity between these two families (Arnold et al. 2017).

***Implications for conservation*.**—Global climate change threatens *Rhyacotriton*, especially on the southern limits of its distribution (Bury 2015). Our observations inform the possibility of breeding *Rhyacotriton* in captivity in a couple of ways. First, we had the best success obtaining successful courtship and insemination when we staged courtship with multiple males and females, rather than with single pairs. Second, although *Rhyacotriton* adults are often viewed as thoroughly aquatic, courtship and spermatophore deposition occurred on the terrestrial rather than in the submerged parts of aquaria. Third, Rhyacotrition males are aggressive during the courtship season and should be maintained in separate enclosures.

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