

The Evolution of Courtship Behavior in Plethodontid Salamanders, Contrasting Patterns of Stasis and Diversification

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ABSTRACT: An examination of courtship in salamanders helps resolve the puzzling problem of long-term evolutionary stasis in behavior. To address the companion issues of stasis and diversification, we summarize and synthesize courtship observations in *Rhyacotriton* and 13 genera of plethodontids. We use a modular analysis of courtship to identify conservative, as well as variable behavioral elements and sequences. We use time-calibrated, molecular phylogenies to reconstruct the evolutionary history of key elements in courtship at different time levels. In deep time (40–175 mya), we reconstruct the evolution of courtship among plethodontid genera, using *Rhyacotriton* and *Ambystoma* as outgroups. On a shorter time scale (10–40 mya), we reconstruct courtship history among species within three genera (*Aneides*, *Plethodon*, and *Desmognathus*). These reconstructions reveal extraordinary stasis for greater than 130 million years (Myr) in the courtship modules that align sexual partners and accomplish sperm transfer. Although some aspects of courtship pheromone delivery predate the origin of plethodontids in the early Cretaceous (66 mya), other aspects of delivery and preliminary courtship have diversified in the last 20–30 Myr. We argue that intricate aspects of sexual communication generate multivariate stabilizing selection that is responsible for evolutionary stasis lasting 100 Myr or longer.

Key words: Ancient behavior; Evolutionary stasis; Functional complex; Mating behavior; Modular analysis; Phylogenetic history; Plethodontidae; Stabilizing selection; Urodela

BEHAVIORAL stasis on a 100-million-year (Myr) time scale is poorly documented in vertebrates and its causes are not well understood. Atz (1970) argued that vertebrates provided no example of even one million years of behavioral stasis. Greene (1994) refuted this argument, but the number of examples that can be offered in support of that refutation is indeed very small (Doten et al., in press). In this article, we show that courtship in salamanders, and plethodontids in particular, offers many examples of behavioral stasis lasting from 1–170 Myr. Although these examples are illuminating, the larger issue before us is the cause, rather than the fact, of such long-lasting stasis. We first review the facts, which provide some clues as to cause, and then turn to a general discussion of mechanisms that could generate long-lasting behavioral stasis.

Courtship behavior in plethodontids has been periodically reviewed over the last 50 yr (Salthe 1967; Arnold 1972; Houck and Verrell 1993; Houck and Arnold 2003). The earliest accounts from the 1920s and 1930s were based on observations of partial courtship sequences and often did not include illustrations (e.g., Mertens 1923; Noble 1927, 1929; Noble and Brady 1930; Branin 1935). In contrast, later accounts were often based on observations of complete courtship, including sperm transfer, and were accompanied by detailed illustrations (Stebbins 1949; Organ 1958, 1960a). More recently, accounts have included still photographs, motion pictures, and video records, all of which have greatly increased the ability to make detailed comparisons among taxa (e.g., Arnold 1972).

Courtship behavior has been described in varying degrees of detail in 13 genera of plethodontids representing all 10 tribes currently recognized in the family (Wake 2012; Appendix I). These descriptions are usually based on laboratory observations but sometimes on field observations

as well. In two cases (*Hemidactylum*, *Hydromantes*), the descriptions are so incomplete that they offer no or little evolutionary insight. Observations on the other 11 genera are based on observations of all stages of courtship, sometimes in multiple species. Most of this review is based on published accounts, but in some cases we supplemented those accounts with original, unpublished observations and links to online videos (for *Batrachoseps*, *Bolitoglossa*, and *Dendrotriton* in particular, see Appendix S1). These observations, together with those on outgroups, give us an overall picture of major events in the evolution of plethodontid courtship.

This review has three primary objectives:

- (1) To lay a conceptual foundation for understanding the evolution of plethodontid courtship, we surveyed all the behavioral elements recognized in the literature and organized them in modules.
- (2) To determine the time scale of major and minor innovations in the evolution of plethodontid courtship, we used time-calibrated phylogenies to reconstruct behavioral history, both among and within genera.
- (3) To understand the causes of the long-term stasis and relatively rapid evolution revealed in those reconstructions, we sought a single model of the evolutionary process that would explain both of these extremes and the many examples of behavioral differentiation that lie between them.

FIVE COURTSHIP MODULES

Salamander courtship has a modular structure. A module is a stereotyped sequence of behavioral elements, with the entire sequence being easily recognizable as a repeated instance of choreography (Scholes 2008). We organized courtship in *Rhyacotriton* and plethodontids into five distinct modules that occur in a characteristic temporal order: approach (AP), head contact (HC), tail-straddling walk

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(TSW), spermatophore deposition (SD), and positioning (POS; Fig. 1). Within each of these modules, courtship unfolds as a sequence of behavioral acts by the male and female. Although courtship consists of both female and male acts (Arnold 1976), we will mainly catalog the male acts, which can vary conspicuously among species (Fig. 2). These individual acts are repeatable and recognizable, but the precise order of acts within some modules can vary from courtship to courtship within populations. Furthermore, the order of modules can vary from courtship to courtship. For example, a male engaged in HC may revert to AP if the female runs off. The summary diagrams (Fig. 1) provide a general portrayal of the major themes of courtship choreography that have been observed in plethodontids.

The modular structure of salamander courtship is hierarchical. For example, during approach (AP), the male alternates among three behaviors (orient, follow, tap) as he pursues the female, but might then execute any one of five visual displays (jerk, forelimb, tail wag, dance, wag) before reverting to the first three behaviors (Fig. 2). We refer to these two sets of AP behaviors as submodules (pursuit and preliminary display). Likewise, the second and third courtship modules (HC and TSW) each consist of two submodules (Fig. 2). We use this hierarchical, modular scheme to present a general account of courtship in plethodontids (similarities and differences with *Rhyacotriton* are apparent in Fig. 1).

During approach (AP), the male appears to use both visual and olfactory cues as he follows the female. He visually orients to her movements and taps the substrate with his nasolabial cirri (projections that house ciliated grooves), transporting odorants into his nasal cavity, the location of olfactory sensory epithelia. He may also perform visually apparent displays with his tail and forelimbs (Fig. 2). A female typically runs away from the male during initial attempts at approach, causing him to repeat the AP act sequence many times.

The transition to head contact (HC) occurs if and when the female remains stationary when the male approaches and attempts to contact her with his head. Initial head contact with the female typically involves nudging or rubbing, or sliding the mental gland on the female's body. This first set of actions (the contact submodule) might accomplish transdermal delivery of mental gland secretions by simple diffusion through her epidermis (Picard 2005). Pheromone delivery is a more obvious possibility during the second submodule, transdermal pheromone delivery Type 1 (tpd1). During tpd1, secretions from the male's mental gland are likely delivered into the bloodstream as the male abrades the female's epidermis with his enlarged premaxillary teeth (snap, pull, rub; Fig. 3) or bites her epidermis (bite-seize, tug; Fig. 4). Transition to the next module—tail-straddling walk (TSW)—occurs when the male succeeds in crawling under the female's chin (under) so that her chin rests on his arched and laterally undulating tail base, or when the female actively places her chin on his tail base. In either case, the female steps astride the undulating tail and the pair moves forward in TSW (forward), which provides additional opportunity for male pheromone delivery.

During TSW, the male leads the female forward, as she straddles his undulating tail with her chin resting on his tail base. A simple communication system between the male and

female maintains the characteristic positions during TSW (Arnold 1976) and constitutes the tail-straddling walk submodule. A second submodule comes into play in some species as the male turns backwards the female (tbf) and delivers mental gland pheromones as part of the TSW. We refer to this submodule as transdermal pheromone delivery Type 2 (tpd2). In this submodule, the male delivers mental gland pheromones by abrading the female with his premaxillary teeth (pull, snap) or, in a submodule derived from tpd2, by slapping his mental gland on her nares (olfactory pheromone delivery [opd]; Fig. 5). After one or many such deliveries, the male eventually reverts to the walk submodule and then slides his vent against the substrate (vent), an event that indicates the onset of spermatophore deposition (SD).

The events that precede SD align the pair so that the male is positioned in a straight line in front of the female, with the femurs of his hind limbs aligned and perpendicular to his body (align). In this position, with the tempo of his tail undulations gradually increasing, he forms his spermatophore and attaches it to the substrate in front of her head (deposit). SD ends with a cessation of male tail undulations, as the male lifts his vent off his spermatophore (lift-off) and the pair begins the transition to positioning (POS; a video recording of this behavior is cataloged in Appendix S2).

In an apparent effort to position the female over the spermatophore during POS, the male first pulls his tail out from under the female by flexing it to one side (flex), and then moves forward with the female's chin resting on his tail base. As the male moves forward, he holds his body in a lateral sigmoid curve (sigmoid). The female typically follows the male while lifting her body over the spermatophore until her vent contacts the spermatophore. She then stops, lowers her body and inserts the sperm cap into her vent. Meanwhile, the male stops in front of her and straightens his body as he backs up under her chin and pectoral region (backup). Next, he rhythmically extends and flexes his hind limbs, pushing his tail base upward (extend) and arresting any further forward progress by the female. The male then typically ceases all movement (Fig. 4c), as the female remains in position with the spermatophore cap inserted in her vent. Eventually, she lifts her vent off the spermatophore and moves off with the sperm cap lodged in her vent. The sperm is later moved into the female's spermatheca, where it is stored until fertilization, which may occur many weeks or months after mating (Adams et al. 2005).

The preceding account sketches the general flow of events during a typical plethodontid courtship. However, Fig. 1 also reveals some striking instances of stasis and diversification that are apparent even before we conduct a phylogenetic analysis. For example, the POS sequence flex → sigmoid → backup → extend is conserved across nearly the entire plethodontid radiation. In addition, this sequence is nearly identical with the corresponding sequence in *Rhyacotriton*: flex → undulate → backup → extend. Likewise, the SD module always includes a particular sequence (vent → align → deposit → lift-off) in plethodontids that differs only slightly from the sequence in *Rhyacotriton* (vent → tread → deposit → lift-off). In contrast, the head contact module (HC), while always ending with “under,” shows a variety of elements.

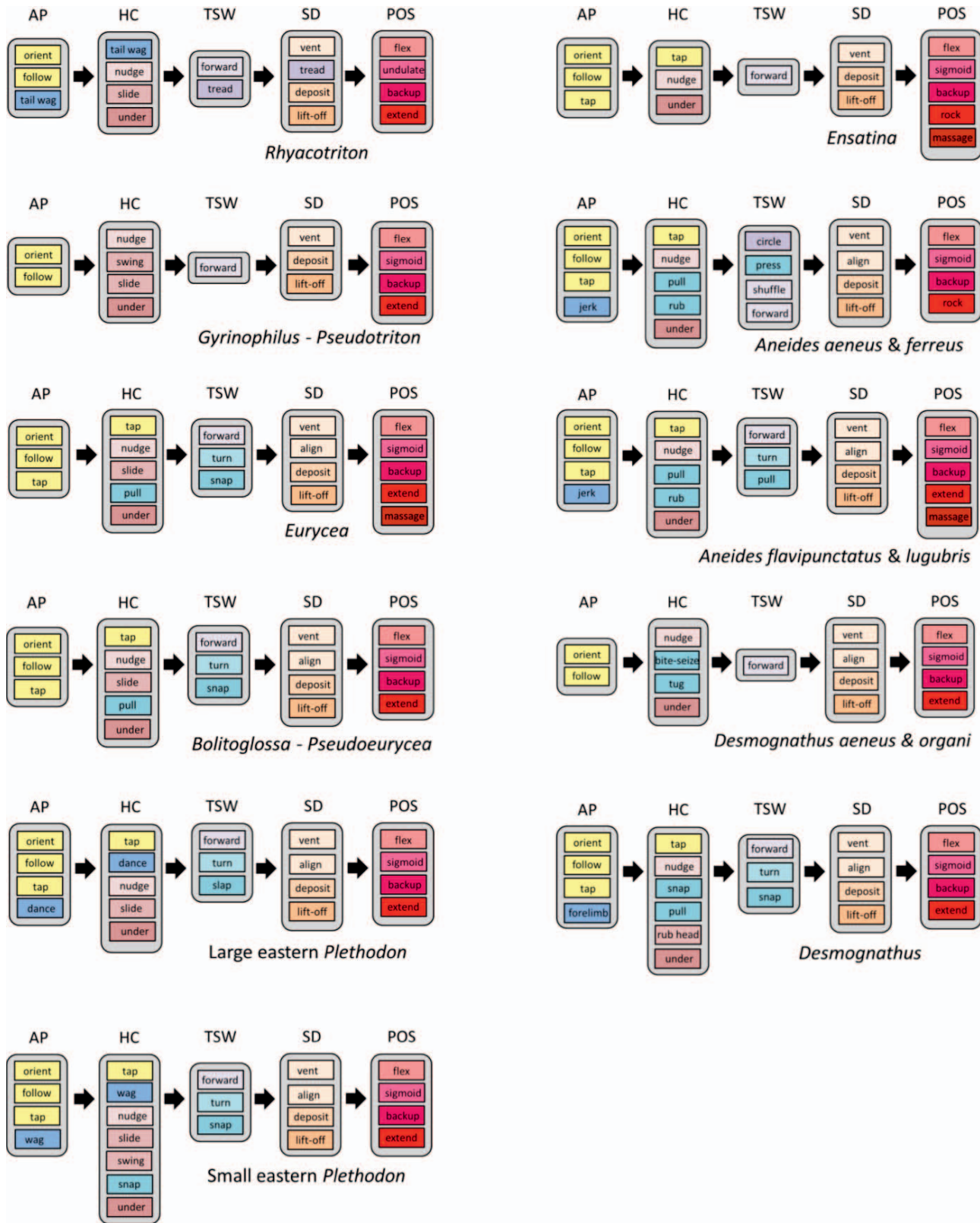


FIG. 1.—Modular analysis of courtship in *Rhyacotriton* and plethodontids. Modules are labeled: AP = approach, HC = head contact, TSW = tail-straddling walk, SD = spermatophore deposition, and POS = positioning. Courtship sequences flow from left to right (as indicated by large arrows) and from top to bottom within modules. Behaviors shown in the same color occur in the same temporal context. Behaviors shown with different shades of the same color occur in a predictable sequence; those in lighter shades occurring earlier than those shown in darker shades. See Fig. 2 for inventory of behavior by module and submodule.

Module	Submodule	Behavior long name	Behavior short name	Behavior abbreviation	Reference
AP	pur	Orienting	Orient		1
AP	pur	Following	Follow		1
AP	pur	Nasolabial cirrus tapping	Tap		2
AP	pd	Jerky movements	Jerk	jm	3,4
AP	pd	Fore limb movement	Forelimb	fm	3
AP	pd	Fore limb strokes	Strokes	fs	3
AP	pd	Tail wagging display	Tail wag		2,5
AP	pd	Foot dance	Dance	fd	2
AP	pd	Tail-wagging	Wag	tw	6,7
HC	con	Nudging	Nudge		2
HC	con	Head sliding	Slide		2
HC	con	Head swinging	Swing		2
HC	con	Rubs female head	Rub head	rfh	3
HC	con	Mouth grasping	Grasp	mgr	2,8
HC	con	Crawl under female chin	Under		2
HC	tpd1	Snapping	Snap	s	2,9
HC	tpd1	Pulling	Pull	p	2
HC	tpd1	Bite and seize	Bite-seize	bas	10
HC	tpd1	Rubbing	Rub		11
HC	tpd1	Tugging	Tug		10
TSW	walk	Moving forward in linear tsw	Forward	tsw	2
TSW	walk	Circular tail straddling walk	Circle	c-tsw	11
TSW	walk	Hind foot shuffling	Shuffle		11
TSW	walk	Hind limb treading	Tread		5
TSW	tpd2	Turning back to female	Turn	tbf	2
TSW	tpd2	Snapping	Snap	s	2,9
TSW	tpd2	Pressing	Press		11
TSW	opd	Slapping	Slap		2
SD		Vent sliding	Vent		2
SD		Align hind limbs	Align		2
SD		Deposit spermatophore	Deposit		2
SD		Lift vent off spermatophore	Lift-off		2
POS		Flex tail out to side	Flex		2
POS		Undulate trunk	Undulate		5
POS		Hold body in sigmoid curve	Sigmoid		2
POS		Backup under female	Backup		2
POS		Extend and flex hind limbs	Extend		2
POS		Rock laterally on hind limbs	Rock		11
POS		Massage female with tail	Massage	tm	2

FIG. 2.—Inventory of behavior patterns used in descriptions of plethodontid and rhyacotritonid courtship, showing their assignments to modules and submodules. Module abbreviations: AP = approach, HC = head contact, TSW = tail-straddling walk, SD = spermatophore deposition, POS = positioning. Submodule abbreviations: pur = male pursuit of female, pd = preliminary display, con = contact, tpd1 = transdermal pheromone delivery Type 1, walk = progress aspect of TSW, tpd2 = transdermal pheromone delivery Type 2, oph = olfactory pheromone delivery. Behaviors shown in the same colors occur in the same temporal context. Behaviors shown in shades of the same color occur in the sequence: lighter shade earlier, darker shade later. References for behavioral descriptions: 1 = this study; 2 = Arnold (1972); 3 = Verrell and Mabry (2003); 4 = Kiemnec-Tyburczy and Sapp (2017); 5 = Doten et al. (in press); 6 = Picard (2005); 7 = Organ (1960b); 8 = Organ (1960a); 9 = Organ (1961); 10 = Verrell (1999); 11 = Sapp and Kiemnec-Tyburczy (2011).

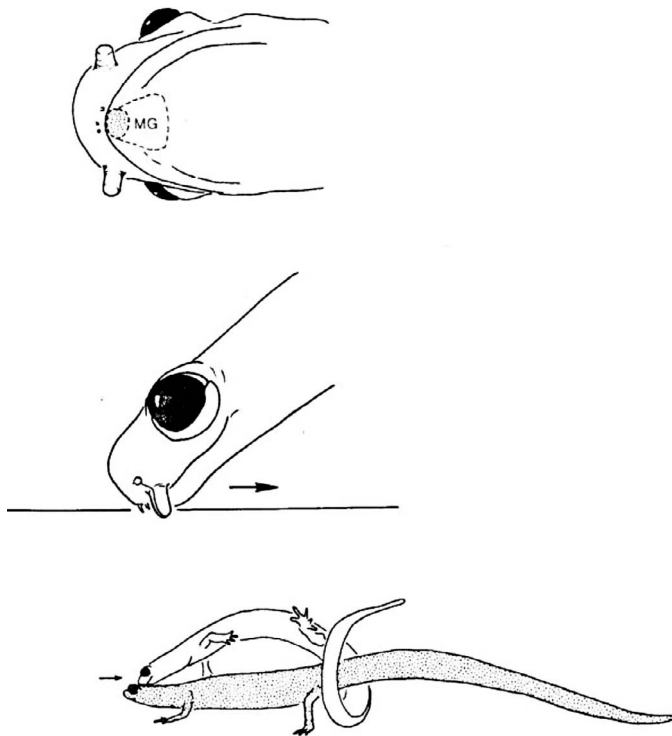


FIG. 3.—Transdermal pheromone delivery in *Eurycea wilderae* during an underwater courtship (from Arnold 1977). (A) Ventral view of the male's mental gland (MG), also showing three premaxillary teeth protruding through his upper lip. (B) The male's head during pulling, showing the backward motion that he uses to abrade the female's epidermis. (C) The male's position during pulling. The female is shaded. This image is based on a video recording cataloged in Appendix S2.

One of our primary aims in the analysis and discussion that follows is to understand why some modules are prone to extreme stasis while others are characterized by diversification. With that contrast in mind, we now turn to a phylogenetic reconstruction of courtship history.

COMPARATIVE EVOLUTION OF COURTSHIP BEHAVIOR: PATTERNS OF STASIS AND DIVERSIFICATION

We mapped courtship events for salamanders onto a time-calibrated molecular phylogeny (Shen et al. 2016). This mapping revealed major transitions in six behavioral traits over the last 175 Myr (Fig. 6). The most ancient transition, from external fertilization (in cryptobranchids and hynobiids) to internal fertilization (in salamandrids, ambystomatids, plethodontids, and their relatives) occurred ~160–200 million years ago (mya) (Doten et al., in press). In this time period, the ancestral male of extant modern salamander families (salamandrids, ambystomatids, rhyacotritonids, amphiumids, and plethodontids) performed a tail-fanning, tail-tapping, tail-wagging, or tail-undulation display in front of the female and then led her forward to a spermatophore deposition in front of her head (sff). The occurrence of the TSW in *Rhyacotriton*, as well as in 12 genera of plethodontids, indicates that this complex behavior originated about 130–160 Mya and has been retained in recognizable form ever since (Fig. 6). The ancestry of the immediate sister group to plethodontids (*Amphiuma*) dates to about 110 mya. However, the courtship of *Amphiuma* is virtually unknown

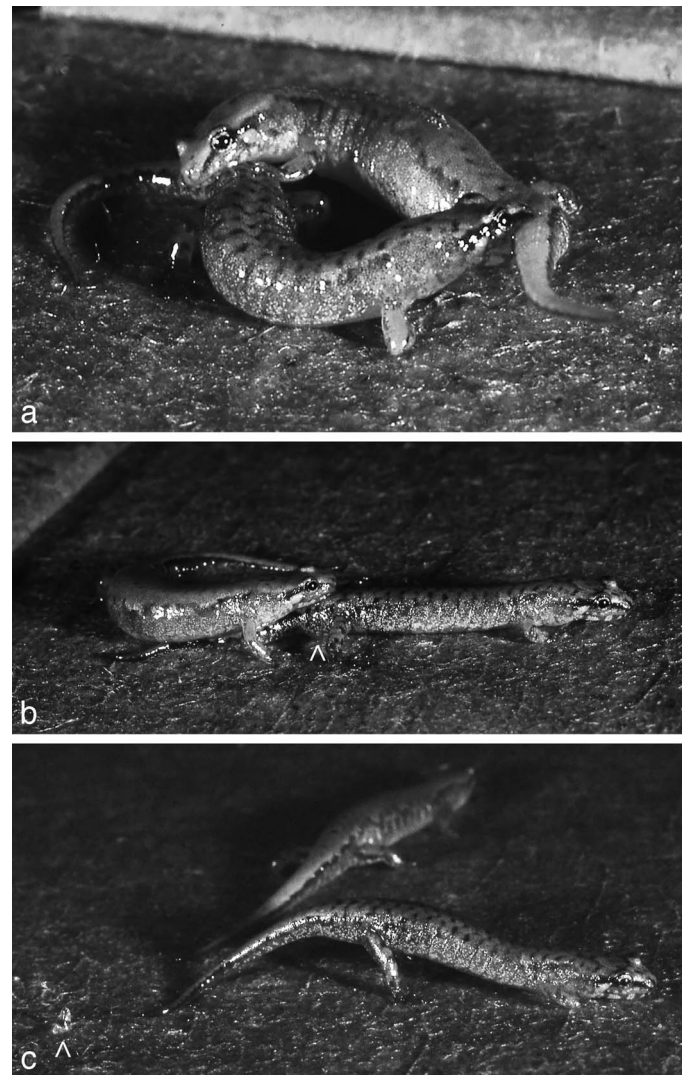


FIG. 4.—Courtship in *Desmognathus organi*. (a) During bite and seize (bas) the male (foreground) holds the female's tail base in his jaws. The female holds her chin on the male's tail base in an apparent attempt to elicit tail-straddling walk. (b) During spermatophore deposition (SD), the male (right) aligns his hind limbs perpendicular to his body (align). Position of spermatophore indicated with \wedge . The female holds her chin over his undulating tail base in TSW position. (c) The male has moved forward after SD and has helped positioned the female over the spermatophore. The female has now departed from the spermatophore (base visible above the \wedge), while the male continues to arch his tail and extend on his hind limbs (extend).

(Baker et al. 1947). Consequently, three courtship traits that are common to all major clades of plethodontids (tbf, tpd1, tpd2) could have arisen at any time in a long interval, between 70 and 130 mya. These traits are part of a functional complex used to deliver courtship pheromones to the female. Our reconstruction shows that, at some point in this 60-Myr interval, males began to turn back towards the female (tbf) during the TSW and deliver pheromones from the mental gland by scratching the female's epidermis with their enlarged premaxillary teeth (tpd2).

In contrast to the stability of this pheromone functional complex (TSW, tpd1, tbf, tpd2), throughout the 65 Myr history of plethodontids, other stable courtship traits have substantial but shorter evolutionary histories (Fig. 6). For

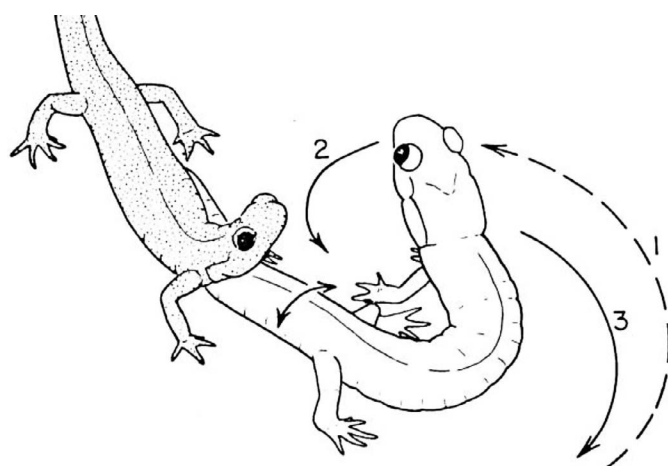


FIG. 5.—Olfactory pheromone delivery during turning back to the female (tbf) in *Plethodon shermani* (from Arnold 1976). (1) The dotted arrow shows the path of the male's head as he turns back towards the female during tail-straddling walk (TSW). (2) The path of the male's head as he slaps his mental gland across the female's nares. (3) The solid arrow shows the path of the male's head as he returns to TSW position. A video of a similar sequence is cataloged in Appendix S2.

example, one lineage of hemidactylines (*Pseudotriton* and *Gyrinophilus*) and one lineage of plethodontines (*Ensatina*) independently have simplified the TSW module by deleting tbf and tpd2. Both of these lineages also have lost both the mental glands and sexually dimorphic premaxillary teeth (Houck and Sever 1994). This combination of losses is 5–40 Myr old in the case of the *Pseudotriton* and *Gyrinophilus*, and as much as 40 Myr old in the case of *Ensatina*. The details of male and female behavior and posture during spermatophore pickup are similar throughout the 66-Myr plethodontid radiation. However, one small behavioral innovation (tm) appears to have evolved four times independently (in *Eurycea*, western *Plethodon*, *Ensatina*, and *Aneides*). In each of these four lineages, the male massages the female with his tail tip while she attempts to retrieve the sperm cap from the spermatophore (Figs. 2, 6). These convergent innovations occurred as long as 30–40 mya. Finally, another exception to the rule that transdermal pheromone delivery has been retained occurs in one lineage in the genus *Plethodon*. In this lineage (large eastern *Plethodon*), olfactory pheromone delivery has evolved from transdermal delivery (discussed below).

Three genera (*Aneides*, *Desmognathus*, and *Plethodon*) provide case studies of the kinds of behavioral events that

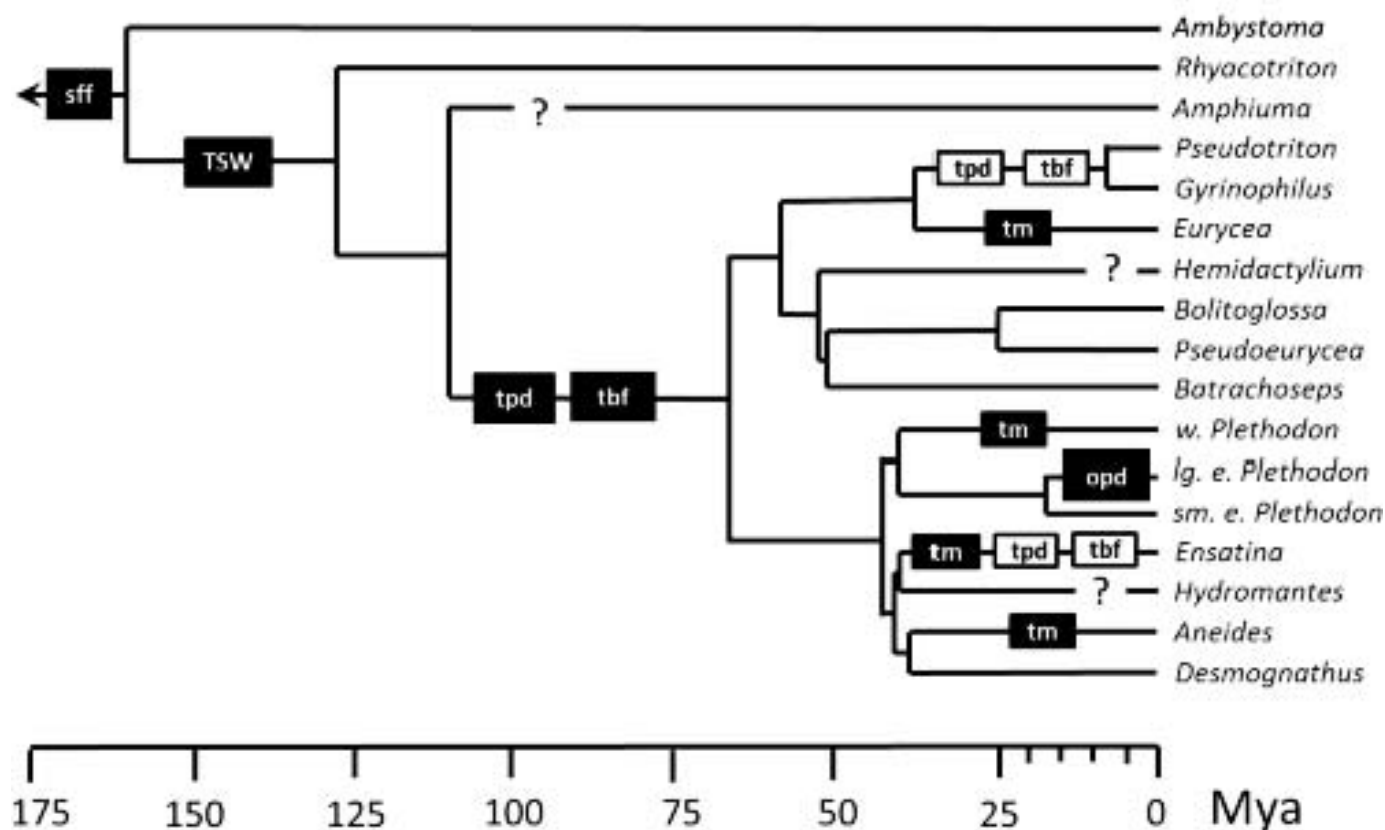


FIG. 6.—The evolutionary origin and loss of key courtship traits in *Rhyacotriton* and plethodontid genera. Character origins are shown with solid rectangles; character losses are shown with open rectangles: sff = spermatophore deposition in front of the female, TSW = tail-straddling walk, tpd = transdermal pheromone delivery (tpd1 and tpd2), tbf = turning back towards the female during TSW, tm = tail massage during spermatophore pickup, opd = olfactory pheromone delivery. The question marks on the *Amphiuma* and *Hydromantes* branches indicate that courtship behavior has not been adequately described. *Dendrotriton* is not shown on this tree but is the sister group to the *Bolitoglossa-Pseudoeurycea* lineage. The time of origin for each trait is bounded by the times at the ends of the branch on which it resides. Arrowhead pointing to the right indicates that the origin of traits on that branch precedes the date for the right end of the branch. Time-calibrated phylogeny based on Shen et al. (2016), using an independent-rate model (clock = 2). Time scale shown in millions of years.

TABLE 1.—Courtship trait distribution among plethodontid genera and outgroups. Table entries: 0 = absent, 1 = present, ? = unknown. Taxa: Cry = *Cryptobranchius* and *Andrias*, Amb = *Ambystoma*, Rhy = *Rhyacotriton*, Amp = *Amphiuma*, Pst = *Pseudotriton*, Gry = *Gyrinophilus*, Hem = *Hemidactylium*, Bol = *Bolitoglossa*, Pse = *Pseudotriton*, Den = *Dendrotriton*, Bat = *Batrachoseps*, wP = western *Plethodon*, seP = small eastern *Plethodon*, leP = large eastern *Plethodon*, Ens = *Ensatina*, Hyd = *Hydromantes*, Ane = *Aneides*, Des = *Desmognathus*. Courtship traits: tpd = transdermal pheromone delivery, opd = olfactory pheromone delivery, tsw = tail-straddling walk (forward), tbf = turning back towards female (turn), sff = spermatophore deposition in front of female, tm = tail massage during spermatophore pickup (massage). References: 1 = Kerbert (1904); Smith (1907); 2 = Houck and Arnold (2003); 3 = Doten et al. (in press); 4 = Organ and Organ (1968); 5 = Arnold (1972); 6 = Beachy (1997); 7 = Kozak (2003); 8 = Branin (1935); 9 = SJA, personal observations; 10 = Salthe and Salthe (1964); 11 = Lynch and Wallace (1987); Jones et al. (2001); 12 = Table 2, this study; 13 = Stebbins (1949); 14 = Mertens (1923); Noble and Brady (1930); Rehberg (1960); 15 = Cupp (1971); Sapp and Kiemnec-Tyburczy (2011); 16 = Table 2, this study.

Module	Trait	Cry	Amb	Rhy	Amp	Pst	Gry	Eur	Hem	Bol	Pse	Den	Bat	wP	seP	leP	Ens	Hyd	Ane	Des
HC, TSW	tpd	0	0	0	?	0	0	1	?	1	1	1	1	1	1	1	0	1	1	1
HC, TSW	opd	0	0	0	?	0	0	0	?	0	0	0	0	0	0/1	1	0	?	0	0
TSW	tsw	0	0	1	?	1	1	1	1	1	1	1	1	1	1	1	1	?	1	1
TSW	tbf	0	0	0	?	0	0	1	?	1	1	1	1	1	1	1	0	?	1	1
SD	sff	0	1	1	?	1	1	1	?	1	1	1	1	1	1	1	1	?	1	1
POS	tm	0	0	0	?	0	0	1	?	0	0	0	0	1	0	0	1	?	1	0
References		1	2	3		4,5	6	5,7	8	6,9	10	9	9	11	12	12	5,13	14	5,15	16

accompany diversification over 20–40 Myr time periods. Courtship has been described for 13 species of *Plethodon* (Table 2), 16 species of *Desmognathus* (Table 3), and 4 species of *Aneides*. In the following sections we will discuss these three case studies in detail.

We mapped the courtship transitions in the genus *Plethodon* (detailed in Table 2) onto a time-calibrated phylogeny (Fig. 7; Kozak et al. 2009), using three plethodontid genera (*Ensatina*, *Desmognathus*, and *Aneides*) as outgroups. That mapping suggests that no major behavioral transitions are associated with the split between western and eastern *Plethodon*, which occurred ~43 mya. Tail-wagging (tw) during the approach phase of courtship may have evolved during the interval 43–27 mya. But in any case, this behavioral pattern is a minor innovation.

On the other hand, several major transitions occurred within eastern *Plethodon* during the interval from about 28 through 11 mya (Fig. 7). During this period, small eastern *Plethodon* (the lineage that includes *P. cinereus* and *P. richmondi*) simplified the TSW by deleting the act of turning back towards the female (tbf). Since its origin over 48 Mya and up to the present, tbf has been used by all major clades of plethodontids (Fig. 7) as the principal avenue for transdermal delivery of male pheromones to the female (tpd). Small eastern *Plethodon* males continue to accomplish tpd by rasping the female's skin with their specialized premaxillary teeth. However, rasping occurs before the onset of TSW rather than during TSW.

In the sister group to the small eastern *Plethodon*, a major innovation in pheromone delivery occurred ~23–28 mya. During this interval, males apparently continued to deliver pheromones by rasping the female with their enlarged premaxillary teeth (i.e., by pulling and snapping). Additionally, males also slapped or slid their mental glands over the female's nares to accomplish olfactory pheromone delivery (opd; Picard 2005). Large eastern *Plethodon* evolved away from this biphasic delivery mode in the interval 23–14 mya by deleting snapping and pulling, and by reverting to sexually monomorphic premaxillary teeth. After this period of simplification, an ancestral large eastern *Plethodon* male used only opd by slapping his large mental gland over the female's nares.

In contrast to major innovations in pheromone delivery, a suite of mostly minor behavioral transitions characterize the lineage of eastern *Plethodon* since their origin about 28 mya

(Fig. 7). For example, a spasmodic movement of male feet during the approach phase (fd) arose during the interval 28–23 mya and was later lost in the *P. welleri* lineage. Loss of tail-wagging (tw) characterizes the entire lineage of large eastern *Plethodon*. Finally, males of at least one species in the *P. glutinosus* complex (i.e., *P. cylindraceus*) grasp the female's trunk with their jaws during preliminary courtship (mgr).

Two significant behavioral transitions occur in the history of the plethodontid genus *Aneides* (Fig. 7). In the behaviors of this genus, we see a major change in the tail-straddling walk, an otherwise highly stable feature of plethodontid sexual choreography (Sapp 2002; Sapp and Kiemnec-Tyburczy 2011; Kiemnec-Tyburczy and Sapp 2017). Two species of *Aneides* (*A. aeneus*, *A. ferreus*) execute a circular tail-straddling walk (c-tsw) in which the couple moves in a circular path, while maintaining a symmetric posture in which both the male and female rest their chins on each other's tail base. Furthermore, the females in each of these species assume a mobile and active role in courtship, such that features of HC (rub, pull) as well as c-tsw, are sexually symmetric (Sapp 2002; Sapp and Kiemnec-Tyburczy 2011). In *A. flavipunctatus* and *A. lugubris*, TSW is linear (as it is in all other plethodontids and *Rhyacotriton*), and female sexual participation is not as active as it is in *A. aeneus* and *A. ferreus* (Kiemnec-Tyburczy and Sapp 2017). Below, we discuss the issue of why female behavior might evolve in parallel with c-tsw, as well as alternative reconstructions for the history of c-tsw.

Major convergent transitions in courtship also occur in the genus *Desmognathus*, possibly 20–28 mya (Fig. 8). Two diminutive species (*D. aeneus*, *D. organi*) have independently evolved an otherwise unique mode of pheromone delivery. In these two species, the male bites the female and holds on to her with his specialized premaxillary teeth (Fig. 4a), while swinging his head from side to side (tug). During this bite and seize (bas), the male secretes pheromone from his mental gland through a duct that empties at his mandibular symphysis, inside his mouth (Sever 1976). This anatomical peculiarity is not found in any other plethodontids. The pheromone apparently makes its way into the wound created by bas and hence into the circulatory system of the female. In parallel with these transitions, both species have lost pulling and snapping, as well as the otherwise universal plethodontid feature of rubbing or nudging the

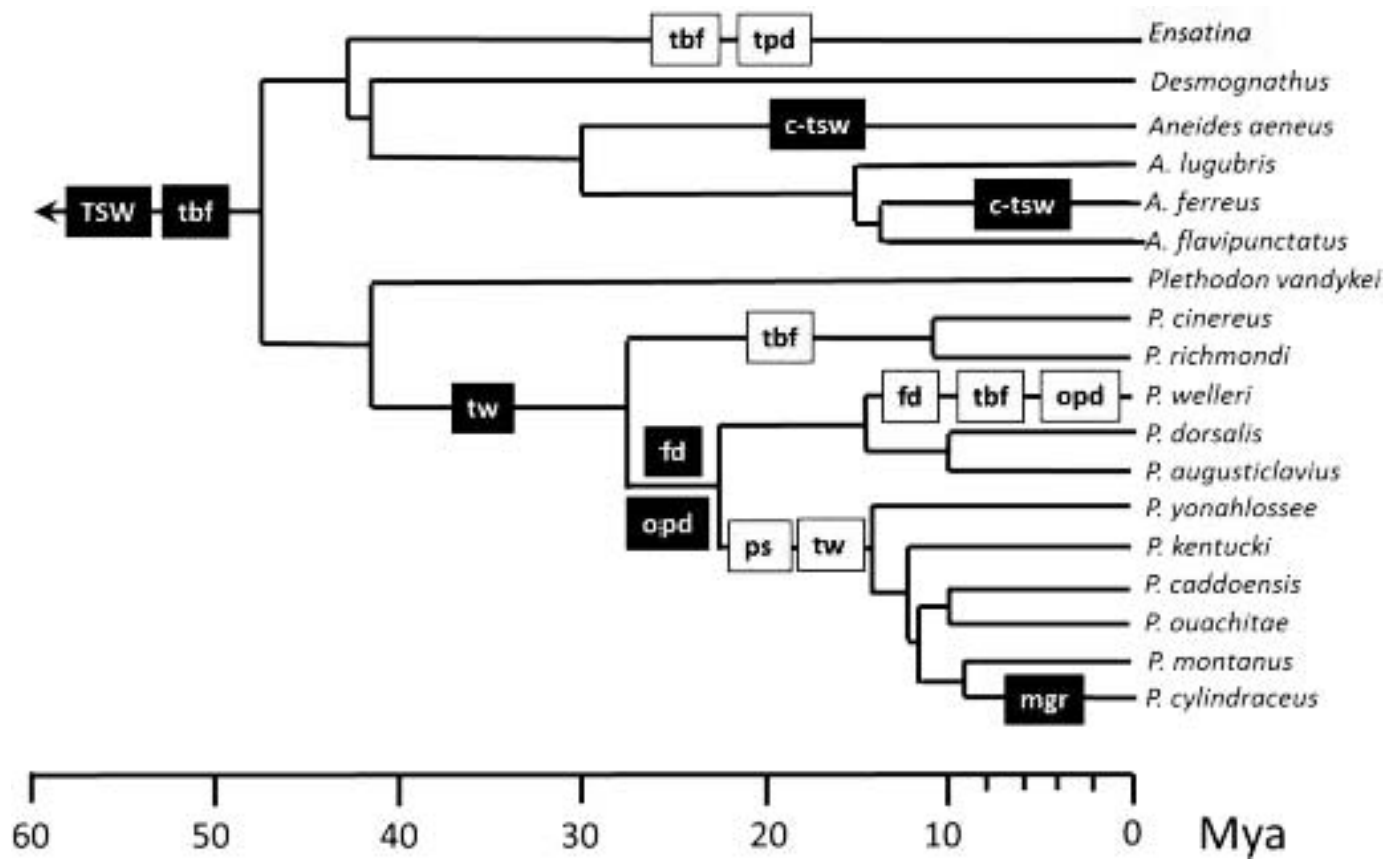


FIG. 7.—The evolutionary origin and loss of key courtship traits in *Ensatina*, *Aneides*, and *Plethodon*. Character origins are shown with solid rectangles; loss is shown with open rectangles: TSW = tail-straddling walk module, c-TSW = circular tail-straddling walk, tbf = turning back towards the female during TSW, tpd = transdermal pheromone delivery (tpd1 and tpd2), opd = olfactory pheromone delivery, fd = foot dance, ps = pulling and snapping, tw = tail wagging, and mgr = mouth grasping. The time of origin or loss for each trait is bounded by the times at the ends of the branch on which it resides. Time-calibrated phylogeny based on Kozak et al. (2009); time scale shown in millions of years.

female’s head during preliminary courtship. After bas, the male releases the female and the pair engages in a completely typical TSW (Fig. 4b) that leads to normal sperm transfer and POS by the male (Fig. 4c). An alternative

reconstruction of bas history will be considered in a later section.

Aside from the bite-seize innovation in *D. aeneus* and the *D. wrighti* group that includes *D. organi*, courtship

TABLE 2.—Courtship trait distribution in the genus *Plethodon* (based on Marvin and Hutchison 1996). Table entries: 0 = absent, 1 = present, ? = unknown. Species denoted: van = *P. vandykei* and *P. vehiculum*, cin = *P. cinereus*, ric = *P. richmondi*, wel = *P. welleri*, dor = *P. dorsalis*, aug = *P. augusticlavius*, yon = *P. yonahlossee*, ken = *P. kentucki*, cad = *P. caddoensis*, ouc = *P. ouchitae*, mon = *P. montanus*, she = *P. shernani*, cyl = *P. cylindraceus*. Positioning refers to the sequence, flex–sigmoid–backup–extend. References: 1 = Lynch and Wallace (1987); Jones et al. (2001); 2 = Arnold (1972); 3 = Gergits and Jaeger (1990); 4 = Dyal (2006); 5 = Arnold (1976); 6 = Picard (2005); 7 = Pierson et al. (2017); 8 = Marvin and Hutchison (1996); 9 = Organ (1958); 10 = Organ (1960b); 11 = Organ (1960a).

Module	Traits	van	cin	ric	wel	dor	aug	yon	ken	cad	ouc	mon	she	cyl
AP, HC	Tail-wagging	0	1	1	1	1	1	0	0	0	0	0	0	0
AP, HC	Tapping	1	1	1	1	?	?	1	1	1	1	1	1	1
AP, HC	Foot dance	0	0	0	0	1	1	1	1	1	1	1	1	1
HC	Nudging	?	1	1	1	?	?	1	1	1	1	1	1	1
HC	Head sliding	1	1	1	1	1	1	1	1	1	1	1	1	1
HC	Mental gland tapping	0	0	0	0	?	?	0	1	0	0	1	1	1
HC	Head swinging	0	1	1	0	?	0	0	0	1	1	0	0	0
HC	Mouth grasping	0	0	0	0	0	0	0	0	0	0	0	0	1
HC, TSW	Pulling and snapping	0	1	1	0	1	0	0	0	0	0	0	0	0
HC	Lifting	1	1	1	1	1	1	1	1	1	1	1	1	1
TSW	Turning back	1	1	0	0	0	1	1	1	?	1	1	1	1
TSW	Slapping	0	0	0	0	1	1	1	1	?	1	1	1	1
SD	Vent sliding	?	?	?	0	?	?	1	1	?	1	1	1	1
SD	Spermatophore deposition	1	1	1	1	1	1	1	1	?	1	1	1	1
POS	Positioning	1	1	?	1	1	1	1	1	?	1	1	1	1
References		1	2,3,4	2,4	5,2	6	4	2,7	8	2	2	9,10	10	2,11

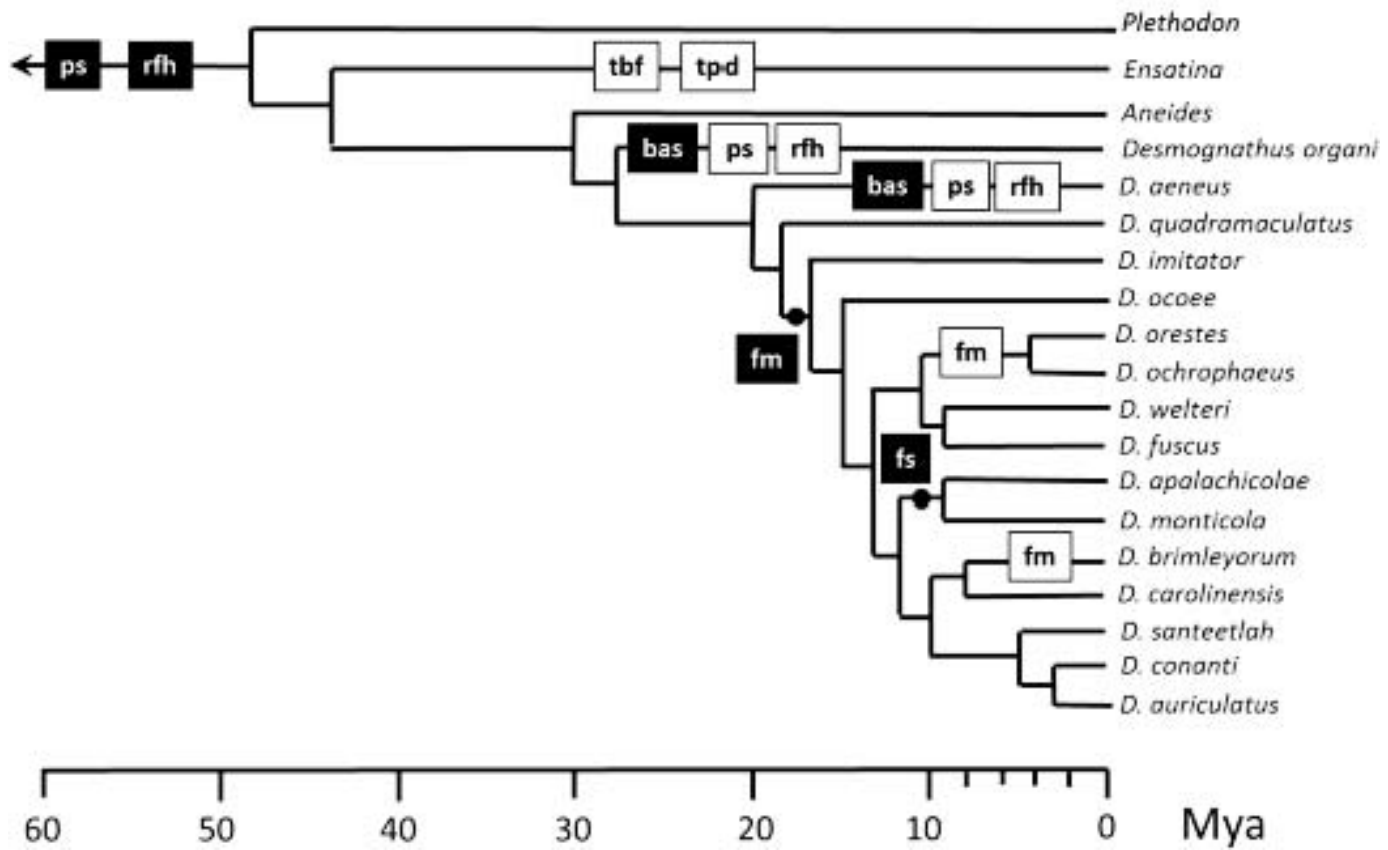


FIG. 8.—The evolutionary origin and loss of key courtship traits in the genus *Desmognathus*. Character origins are shown with solid rectangles; losses are shown with open rectangles: tbf = turning back towards the female, tpd = transdermal pheromone delivery (tpd1 and tpd2), ps = pulling and snapping, rfh = rub female head, bas = bite and seize, fm = forelimb movement, and fs = forelimb strokes. Small solid circle denotes branch of origin for adjacent trait box. The time of origin or loss for each trait is bounded by the times at the ends of the branch on which it resides. Time-calibrated phylogeny based on Kozak et al. (2009); time scale shown in millions of years.

behavior in *Desmognathus* is remarkable for its stability over the 28 Myr history of the genus (Table 3; Fig. 8). All aspects of TSW, sperm transfer, and positioning remain unchanged during this entire period. The few transitions that do occur are restricted to the preliminary stages of courtship. In particular, forelimb movements (e.g., one resembling the butterfly stroke in a swimming human) originated in a period about 17–18 mya in the sister group

to *D. quadramaculatus*. These forelimb movements (fm) were retained in most lineages but were lost in the *orestes/ochrophaeus* and *brimleyorum* lineages several million years later. One lineage (*apalachicola/monticola*) continued to perform forelimb movements (fm) in the original context but also executed fm in such close proximity to the female that the male’s forelimbs stroked her head, limbs or body. Finally, the case of jerky movements during the

TABLE 3.—Courtship trait distribution in the genus *Desmognathus* (after Verrell and Mabry 2003). Table entries: 0 = absent, 1 = present, 0/1 = presence varies from report to report. Species designations: org = *D. organi*, aen = *D. aeneus*, qua = *D. quadramaculatus*, imi = *D. imitator*, oco = *D. ocoee*, ore = *D. orestes*, och = *D. ochrophaeus*, wel = *D. welteri*, fus = *D. fuscus* (southern), apa = *D. apalachicola*, mon = *D. monticola*, bri = *D. brimleyorum*, car = *D. carolinensis*, san = *D. santeetlah*, con = *D. conanti*, aur = *D. auriculatus*. References: 1 = Houck (1980); 2 = Verrell (1999); 3 = Promislow (1987); 4 = Verrell (1994b); 5 = Herring and Verrell (1996); 6 = Mead and Verrell (2002); 7 = Verrell and Mabry (2003); 8 = Uzendoski and Verrell (1993); 9 = Verrell (1994a); 10 = Brock and Verrell (1994); 11 = Verrell (1997b); 12 = Maksymovitch and Verrell (1992); 13 = Verrell (1995); 14 = Verrell (1997a).

Module	Traits	org	aen	qua	imi	oco	ore	och	wel	fus	apa	mon	bri	car	san	con	aur
AP	Jerky movements	0	0	0	0	0	0	0	0	1	0	0/1	1	0	1	1	0
AP	Forelimb movement	0	0	0	1	1	0	0	1	1	1	1	0	1	1	1	1
AP	Forelimb strokes	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
HC	Rubs female head	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HC	Bite and seize	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HC	Pulling	0	0	0	0	1	1	1	1	1	1	0/1	1	1	0	0	1
HC, TSW	Snapping	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
References		1,2	3	2	4	5	5	6	7	8	9	10	11	5	12	13	14

approach phase (jerk or jm), illustrates an instance of chaotic evolutionary history. Jerky movements have been observed in five species of *Desmognathus* (*fuscus*, *conanti*, *santeetlah*, *brimleyorum* and some populations of *monticola*). One would have to invoke multiple origins and losses to account for the history of jerky movements—consequently that history is not shown on Fig. 8. Furthermore, although such jerky movements are unusual in plethodontids; they also occur in the courtship of some *Bolitoglossa* and *Aneides* species.

COURTSHIP AS A FUNCTIONAL COMPLEX WITH MODULAR ORGANIZATION

Viewing courtship as a functional complex (Olson and Miller 1958; Cheverud 1982; Watts et al. 2004) is the key to understanding the evolutionary stability of plethodontid courtship behavior as well as the evolutionary modes that characterize its diversification. A functional complex is a set of phenotypic traits that: (1) interact functionally, and (2) achieve a particular endpoint. In the present case, the endpoint of courtship is insemination. To achieve that endpoint, plethodontid courtship involves various interacting behavioral components, as well as interacting morphological structures such as the male's mental gland and his specialized premaxillary teeth, as well as the female's neuro-anatomical substrates for sensory perception and processing. The mental and other courtship glands produce pheromone cocktails composed of multiple proteins (Wilburn et al. 2017), and these cocktails are also part of the functional complex. Watts et al. (2004) stressed the point that evolution at the level of courtship pheromones is rapid and episodic, but also decoupled from the more stable evolutionary pattern of the morphological elements in the functional complex. Our point here, however, is that the functional coupling of behavioral and morphological elements, both within and between the sexes, is probably responsible for key features of courtship evolution, in particular for its remarkable stability. Before we take up the causes of long-term stability, it will be useful to review and summarize the results of our phylogenetic surveys (Figs. 6–8).

Several modes of evolutionary change can be recognized within the functional complex that organizes plethodontid courtship. First in complexity are simple additions and deletions of courtship elements, such as forelimb movement (fm), tail massage (tm), and mouth grasping (mgr).

Second in complexity are elaboration or simplification of elements, such as the conversion of *Desmognathus* forelimb movement (fm) into stroking of the female (fs) by performing fm so close to the female that the male's forelimbs stroke her head or body.

Third in complexity are deletions and temporal rearrangement of entire modules. By modules, we mean stereotyped sequences of behavioral elements, with the entire sequence being easily recognizable as a repeated instance of choreography (Scholes 2008). For example, a male turning back toward the female during the TSW (tbf) is a submodule in which the male moves his head back towards the female's head or body (while in the TSW), pauses with his head in close proximity to her head or body, executes a particular kind of pheromone delivery (pulling, snapping, or slapping), and then returns to the original TSW position

(Fig. 5). This submodule involves participation by the female for she must maintain her position in the TSW throughout tbf (indeed, in the case of opd, she may even raise her head to a position that appears to facilitate slapping). The complex tbf module is at least 66 Myr old but is still recognizable in seven major clades of plethodontids (Fig. 6). Despite the apparent importance of the submodule for pheromone delivery, it has been entirely deleted in at least two major clades. Our point here is that such submodule deletion is undoubtedly more significant than the deletion of a minor courtship element such as fm.

Finally, remodeling of an entire module is more significant still and amounts in some cases to what Simpson (1944, 1953) called megaevolution. The conspicuous examples of module remodeling in plethodontids are (1) the conversion of transdermal pheromone delivery Type 1 into bite and seize (bas) pheromone delivery in *Desmognathus*, (2) the conversion of TSW into circular TSW (c-tsw) in *Aneides*, and (3) the conversion of transdermal pheromone delivery Type 2 into olfactory delivery (opd) in *Plethodon*. The two examples of pheromone delivery conversion involve extensive remodeling of the morphological (glandular and dentary) as well as the behavioral components of the module.

Comparative examinations of these four evolutionary modes (Tables 1–3; Fig. 1) reveals that simple modifications of elements (addition, deletion, elaboration, and simplification) are more common than changes in whole modules (deletion, rearrangement, or remodeling). Why is this so? Why are modules so apparently resistant to change, especially to remodeling, compared with the elementary acts of courtship? The answer probably has to do with the premium on retaining the functionality of modules and their roles in the overall functionality of courtship. Put another way, the parts of a module must interact in a particular way to secure its overall function. The TSW is a case in point. Arnold (1976) showed that the positions of the male and female in the walk are maintained because of simple rules governing forward progress by the male. If the female decelerates, the male slows down and stops, until she moves forward. If the female moves too far forward, the male accelerates, so that her chin drops back into position on his tail base. The functionality of the TSW module depends on the integrity of these rules and their set points. In other words, the behavioral algorithm that regulates the TSW is probably under stabilizing selection that is both intense and that promotes coordination between the sexes. From this perspective, it is not surprising that the TSW has been so stable and has been remodeled only in the genus *Aneides* with the change to c-TSW. Similar arguments apply to the stability of tpd and tbf, which have only rarely been remodeled (Figs. 1, 3, and 4). Like the TSW itself, these submodules depend upon coordinated interactions between the sexes, the kind of interaction that produces multivariate stabilizing selection and results in evolutionary stability.

Stabilizing selection has long been recognized as the leading candidate explanation for evolutionary stasis. Charlesworth et al. (1982) concluded that all other mechanisms of stasis (e.g., gene flow, developmental constraint, canalization, restricted genetic variation) are themselves subject to modification or lack empirical support as causes of stasis. More recently, evaluations of alternative models for phenotypic diversification have shown that stabilizing selection is

the most plausible explanation for stasis (Estes and Arnold 2007; Uyeda et al. 2011; Arnold and Houck 2016). In the present case, we wish to understand stasis in a functional complex that mediates sexual communication between the sexes. In models of this process, the key feature that can halt diversification and promote stasis is stabilizing selection, especially strong stabilizing selection on the female side of the complex (Arnold and Houck 2016).

In the case of the conversion of TSW into c-TSW, we have some ecological understanding of what might have driven the remodeling of an otherwise stable module. The two species of *Aneides* with c-TSW (*A. aeneus*, *A. ferreus*) have different habitat preferences from the two species with standard, linear TSW (*A. flavipunctatus*, *A. lugubris*). *Aneides aeneus* is a rock crevice-dwelling species, and *A. ferreus* is strongly arboreal, residing under the bark and in crevices of Douglas Fir (*Pseudotsuga douglassi*) and Coast Redwood (*Sequoia sempervirens*), sometimes dozens of meters off the ground. In contrast, *A. flavipunctatus* is terrestrial, and *A. lugubris*, although associated with the Coast Live Oak (*Quercus agrifolia*), is also commonly found in terrestrial habitats. This correspondence between habitats and style of TSW might suggest that c-TSW is an adaptation to courtship in confines of small crevices (Sapp and Kiemnec-Tyburczy 2011). Further examination of the genus is necessary, however, because the lack of c-TSW in the arboreal *A. lugubris* complicates the correspondence between habitat and behavior (Kiemnec-Tyburczy and Sapp 2017).

COEVOLUTION OF COURTSHIP BEHAVIOR AND MORPHOLOGY

The case of coevolution of the mental gland and courtship rests on three broad observations:

- (1) For those cases of mental gland loss for which we have observations of courtship behavior (*Ensatina*, *Pseudotriton-Gyrinophilus*, *Eurycea rathbuni*), loss of pheromone delivery behaviors accompanies gland loss and reversion to sexually monomorphic premaxillary teeth.
- (2) A unique ducted release of mental gland pheromones inside the male's mouth (Sever 1976) is associated with the conversion of transdermal pheromone delivery (tpd) to bite and seize delivery (bas) in two diminutive *Desmognathus* (*D. aeneus*, *D. organi*).
- (3) The evolution of the anterior protrusion-type mental gland (Sever et al. 2016) in *Desmognathus* is associated with a particularly vigorous form of snapping. Moving beyond these broad-brush observations, the coevolutionary process between the mental gland and courtship behaviors deserves further study. In an analysis of mental gland evolution based on the one nuclear gene tree (*Rag 1*), Sever et al. (2016) found that the mental gland was probably present in the ancestral plethodontid. Particular gland morphologies characterize sister tribes (e.g., the large pad type of plethodontines and bolitoglossines). Convergence has occurred on a few occasions; the independent loss of the entire gland is also a recurrent theme. However, we have little detailed understanding of how premaxillary teeth, the mental gland, and pheromone delivery behaviors coevolve. The strength of selection on male premaxillary teeth is evident in the genus *Thorius*. In some species in this genus of diminutive bolitoglossines, all teeth have been lost in

both sexes, except for one or two premaxillary teeth in males (Hanken and Wake 1994). These remaining teeth have apparently been retained to deliver courtship pheromones. The genus *Eurycea* also provides fertile territory for exploring coevolution. Two mental gland morphologies are common in this genus and a third is present in *E. lucifuga* (Sever et al. 2016). Currently, we do not know enough about *Eurycea* courtship behaviors to interpret these morphological differences.

MOLECULAR, BEHAVIORAL, AND MORPHOLOGICAL COEVOLUTION

In contrast to the stasis and modest rates of diversification that characterize behavioral and morphological evolution in plethodontids, the molecular evolution of courtship pheromones is strikingly different. Plethodontid pheromones have been recruited from at least two and perhaps three different protein families (Wilburn et al. 2017). In each of these families, the general structure of the protein (its folding configuration) is conserved throughout the plethodontid radiation. Against this background of stasis, certain parts of each protein undergo a completely different mode of evolution, one that finds no parallel in the morphological or behavioral components of the functional complex (Watts et al. 2004). In the protein domains involved in docking of pheromone with female receptors, evolution is rapid and incessant (Wilburn et al. 2017). Models of coevolution suggest that this evolutionary mode is a consequence of weak stabilizing selection on the configuration of the receptor proteins in the female (Arnold and Houck 2016). The same models suggest that the restrained evolution of behavior and morphology, ranging all the way to extreme stasis, reflects correspondingly strong stabilizing selection on female behavior and morphology.

SEXUALLY SYMMETRIC COURTSHIP BEHAVIOR

Sexually symmetric behavioral roles are aspects of plethodontid courtship that deserve more attention. In the genus *Aneides*, females play such an active role that courtship is sexually symmetric (Sapp 2002; Sapp and Kiemnec-Tyburczy 2011; Kiemnec-Tyburczy and Sapp 2017). During *Aneides* courtship, sessions in which the male actively courts a stationary, immobile female with head-swinging and rubbing, can alternate with sessions in which an active female courts a stationary, immobile male using those same elements. Bechler (1988) described a similar situation in *Eurycea rathbuni*, in which either the male or the female can head rub the partner during preliminary courtship. Mutual head rubbing is also a feature of *Batrachoseps* courtship (see Appendix S1). In small eastern *Plethodon*, the female sometimes leads the male in a tail-straddling walk (Picard 2005; Dyal 2006). This type of active female behavior might be facultative, because it is observed in some courtship sessions but not in others. Taken together, these observations indicate that symmetric female behavior in plethodontids ranges from an occasional tendency to actively initiate TSW (which apparently occurs in all plethodontids) to symmetrical courtship behavior, most extremely expressed in *Aneides*. Although this continuum is apparent, the factors that drive transitions along the continuum are not clearly understood. Sexually symmetric

displays in birds are well known (Huxley 1914; Kirkpatrick et al. 1990) and might serve as a model of understanding analogous behavior in plethodontids. Studying the origin of sexually symmetric courtship in plethodontids would be a fruitful area for future behavioral research.

CONCLUSIONS

The Need for Additional Observations

The lack of observations of courtship in key taxa hampers our vision of courtship evolution in plethodontids. In particular, we need observations of taxa that are the sole surviving representatives of ancient lineages (e.g., *Amphiuma*, *Hemidactylum*, *Karsenia*, *Phaeognathus*, *Urspeleperpes*, *Stereochilus*, and *Hydromantes*). We also have little understanding of courtship evolution in bolitoglossines, the most structurally diversified and speciose clade of plethodontids. No observations of courtship have been made in the following nine bolitoglossine genera (some of which have dozens of species): *Bradytriton*, *Chiroptero-triton*, *Cryptotriton*, *Ixalotriton*, *Nototriton*, *Nyctanolis*, *Oedipina*, *Parvimolge*, and *Thorius*. Just on the basis of habitat occupancy, one might expect that interesting modifications of courtship have accompanied the bolitoglossine radiation in fossorial and arboreal adaptive zones. Two examples illustrate the kinds of surprises we might encounter. *Bolitoglossa occidentalis*, a small arboreal species with fully webbed feet, inhabits banana trees on coffee plantations. In the laboratory, males of this species can court on smooth vertical surfaces and even while upside down (see Appendix S1). *Batrachoseps* is a semifossorial to fossorial genus in a sister clade to bolitoglossines. In *B. major*, an elongate species with small limbs, the TSW has been modified in a revealing way. The female straddles the male's tail base with just her forelimbs, using her hind limbs to propel her body along beside the male's tail rather than on top of it (see Appendix S1). We might expect convergent modification of the TSW in other elongate, small-limbed taxa (e.g., *Amphiuma*, *Phaeognathus*, and *Oedipina*), if indeed they retain the TSW.

The Future

A phylogenetic approach to behavioral evolution has become increasingly popular as molecular systematics has developed powerful new techniques for delineating relationships among populations, species, and higher taxa (Martins 1996). Multispecies comparisons of courtship behavior, including ancestral character state reconstruction, can be investigated by combining observational studies with robust phylogenies (e.g., Houck and Sever 1994; Houck and Arnold 2003; Verrell and Mabry 2003; Sever et al. 2016). In this article, we used time-calibrated phylogenies to provide a time scale for assessing stasis and diversification. In the process, we uncovered examples of behavioral stasis in salamanders that appear to be unprecedented in the vertebrate literature. This time-based discovery exercise also highlights the need to understand the factors that govern stasis on the one hand, and promote diversification on the other. One avenue to unravel the relevant factors is experimental study of plethodontid courtship, a topic we hope to review in a future article.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at <http://dx.doi.org/10.1655/Herpetologica-D-16-00068.S1> and <http://dx.doi.org/10.1655/Herpetologica-D-16-00068.S2>.

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APPENDIX

Species of Plethodontid and Rhyacotritonid Salamanders for which Partial or Complete Courtships have Been Observed. Acronyms as Defined in Fig. 2.

Species	Behavioral delivery of mental gland pheromones	Video links ^a	Courtship illustrations ^a	References
<i>Rhyacotriton variegatus</i>	None	AP-POS	Drawing	Doten et al. (in press)
<i>Pseudotriton ruber</i>	None	TSW	Drawing, photos	Organ and Organ (1968); Arnold (1972, 1977)
<i>Gyrinophilus porphyriticus</i>	None			Beachy (1997)
<i>Eurycea bislineata</i>	Swing, pull, snap		Drawing	Noble (1929); Arnold (1972)
<i>E. cirrigera</i>	Swing, pull, snap			Kozak (2003)
<i>E. lucifuga</i>	Swing		Drawing	Organ (1968)
<i>E. neotenes</i>	Unknown		Drawing	Bogart (1967)
<i>E. rathbuni</i>	Unknown			Bechler (1988)
<i>E. sosorum</i>	Unknown	TSW		
<i>E. wilderae</i>	Pull, snap	HC		Arnold (1972); Kozak (2003)
<i>Hemidactylium scutatum</i>	Unknown			Branin (1935)
<i>Bolitoglossa flavimembris</i>	Slide, snap			Arnold (1972)
<i>B. occidentalis</i>	Pull, snap		Drawing	Arnold (personal observations)
<i>B. resplendens</i>	Pull, snap		Drawing	Arnold (personal observations)
<i>B. subpalmata</i>	Slide, pull, snap			Arnold (1972)
<i>Pseudoeurycea belli</i>	Pull, snap			Salthe and Salthe (1964)
<i>Dendrotriton bromeliacius</i>	Swing, pull, snap		Drawing	Arnold (personal observations)
<i>Batrachoseps attenuatus</i>	Pull, snap	AP-HC		Arnold (personal observations)
<i>Batrachoseps major</i>	Pull, snap	AP-POS		Arnold (personal observations)
<i>Plethodon vandykei</i>	Slide		Drawing	Lynch and Wallace (1987)
<i>P. vehiculum</i>	Unknown			Jones et al. (2001)
<i>P. caddoensis</i>	Slide, swing			Arnold (1972)
<i>P. cylindrawingaceus</i>	Slide, slap			Organ (1960a); Arnold (1972)
<i>P. montanus</i>	Slide, slap	AP-POS	Drawing	Organ (1958); Arnold (1976)
<i>P. kentucki</i>	Slide, slap		Drawing	Marvin and Hutchison (1996)
<i>P. ouachitae</i>	Slide, swing, slap			Arnold (1972)
<i>P. melavenstris</i>	Slide, swing	TSW-AP		Arnold (1972)
<i>P. shermani</i>	Slide, slap	HC, SD, SD-POS, AP, AP-POS, TSW-POS, TSW-POS	Drawing, photos	Arnold (1976, 1977); Houck and Arnold (2003)
<i>P. yonahlossee</i>	Slide, slap	AP-POS	Drawing, photos	Arnold (1972); Pierson et al. (2017)
<i>P. angusticlavius</i>	Slide, slap			Dyal (2006)
<i>P. dorsalis</i>	Slide, pull, snap, slap			Picard (2005)
<i>P. cinereus</i>	Slide, swing, pull, snap	AP-POS		Arnold (1972); Gergits and Jaeger (1990); Dyal (2006)
<i>P. richmondi</i>	Slide, swing, pull, snap			Arnold (1972); Dyal (2006)
<i>P. welleri</i>	Slide, swing		Drawing	Organ (1960a); Arnold (1972)
<i>Ensatina eschscholtzii</i>	None	AP-POS	Drawing, drawing	Stebbins (1949); Arnold (1972)
<i>Hydromantes genei</i>	Swing		Drawing	Mertens (1923); Noble and Brady (1930); Noble (1931); Rehberg (1960)
<i>H. italicus</i>	Unknown			Durand (1970)
<i>Aneides aeneus</i>	Pull, rub, press			Cupp (1971)
<i>A. lugubris</i>	Pull, rub, press		Drawing, drawing	Arnold (1972); Kiemnec-Tyburczy and Sapp (2017)
<i>A. ferreus</i>	Pull, rub, press	TSW, AP-POS, AP-HC		Sapp and Kiemnec-Tyburczy (2011)
<i>A. flavipunctatus</i>	Pull, rub, press	POS-SD, TSW, HC		Kiemnec-Tyburczy and Sapp (2017)
<i>Desmognathus organi</i>	Bite-seize, tug		Photos	Organ (1961); Houck (1980); Verrell (1999); this article
<i>D. aeneus</i>	Bite-seize, tug		Photos	Promislow (1987)
<i>D. apalachicola</i>	Pull, snap			Verrell (1994a)
<i>D. auriculatus</i>	Pull, snap			Verrell (1997a)

APPENDIX
Continued.

Species	Behavioral delivery of mental gland pheromones	Video links ^a	Courtship illustrations ^a	References
<i>D. brimleyorum</i>	Pull, snap			Verrell (1997b)
<i>D. carolinensis</i>	Pull, snap			Herring and Verrell (1996)
<i>D. conanti</i>	Snap		Drawing	Verrell (1995)
<i>D. fuscus</i> (southern)	Pull, snap			Uzendoski and Verrell (1993)
<i>D. imitator</i>	Tpd (snap)			Verrell (1994b)
<i>D. monticola</i>	Pull, snap			Brock and Verrell (1994)
<i>D. ochrophaeus</i>	Pull, snap			Herring and Verrell (1996)
<i>D. ocoee</i>	Pull, snap	HC	Photos	Herring and Verrell (1996); Mead and Verrell (2002)
<i>D. orestes</i>	Pull, snap			Herring and Verrell (1996)
<i>D. quadramaculatus</i>	Pull, snap			Verrell (1999)
<i>D. santeetlah</i>	Snap			Maksymovitch and Verrell (1992)
<i>D. welteri</i>	Pull, snap			Verrell and Mabry (2003)

^a The online version of this table contains embedded hyperlinks to web-based content for most taxa having an entry in this column.