

Evolutionary insights into the regulation of courtship behavior in male amphibians and reptiles

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Abstract

Comparative studies of species differences and similarities in the regulation of courtship behavior afford an understanding of evolutionary pressures and constraints shaping reproductive processes and the relative contributions of hormonal, genetic, and ecological factors. Here, we review species differences and similarities in the control of courtship and copulatory behaviors in male amphibians and reptiles, focusing on the role of sex steroid hormones, the neurohormone arginine vasotocin (AVT), and catecholamines. We discuss species differences in the sensory modalities used during courtship and in the neural correlates of these differences, as well as the value of particular model systems for neural evolution studies with regard to reproductive processes. For example, in some genera of amphibians (e.g., *Ambystoma*) and reptiles (e.g., *Cnemidophorus*), interspecific hybridizations occur, making it possible to compare the ancestral with the descendant species, and these systems provide a window into the process of behavioral and neural evolution as well as the effect of genome size. Though our understanding of the hormonal and neural correlates of mating behavior in a variety of amphibian and reptilian species has advanced substantially, more studies that manipulate hormone or neurotransmitter systems are required to assess the functions of these systems.

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1. Introduction

Vertebrates inhabit a tremendous array of environmental niches, each requiring different morphological, physiological, and behavioral adaptations in order for individuals to survive and reproduce. Thus, it is not surprising that there is a corresponding diversity in the display and regulation of reproductive behaviors across vertebrate taxa, shaped by environmental and phylogenetic constraints. Species differ in the types of sensory signals they use to recognize and acquire mates as well as the hormonal and neural regulation

of courtship behaviors. Variation in the neural and behavioral phenotypes of different species under similar environmental conditions may represent unique or alternative solutions to particular ecological challenges, while similarities among many species may provide insight into characteristics that are more ancient or fundamental [1]. Consequently, the study of mating behavior and its neural substrate across a range of species can provide a context in which to consider species, population, or individual differences and help create a more widely applicable framework for understanding brain and behavior relationships [2].

Here we review some of the mechanisms underlying mating behavior in amphibians (anurans and urodeles) and reptiles (turtles, snakes, lizards, tuataras, and crocodiles), employing an evolutionary perspective to discuss species similarities and differences. Though there are many species in these taxa in which male reproductive physiology, neuroendocrinology, and behavior are similar to that in mammals and birds, there are also a number of species that differ dramatically from more traditional model systems and

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provide insight into alternative mechanisms for the control of sexual behavior. In addition, there are a number of amphibian and reptilian species that have resulted from hybridization events, and many of these hybrids are polyploid. Comparisons of these hybrid species with extant ancestral species allow us to investigate how speciation events alter neural and behavioral phenotypes. In this review we focus on the role of gonadal steroids, arginine vasotocin, and dopamine (DA), as well as their pertinent neural circuits, in the expression of courtship behavior in male amphibians and reptiles and discuss some evolutionary issues pertaining to species differences or similarities in these processes (see Ref. [3]).

2. Steroid hormone control of courtship behavior

The relationship between gonadal activity and mating behavior falls into three general categories [2,4,5]. In the first category, mating behavior is tied to gonadal hormone secretion as well as spermatogenesis, and this is called an associated pattern of reproduction. The majority of vertebrates fall into this category. In the second category are species in which mating behavior is not associated with gonadal hormone secretion or spermatogenesis, and this is called a dissociated pattern of reproduction. Finally, there is a third category characteristic of animals living in extreme environments in which mating behavior is displayed within minutes of a particular change in the environment (e.g., rainfall in deserts). In such animals the gonads are fully developed with gametes at or close to full maturation, and this state is maintained often times for years until the right conditions prevail. This is called a constant or opportunistic pattern of reproduction. Because there are very few studies of the behavioral neuroendocrinology of opportunistic breeding, particularly in amphibians and reptiles, we will restrict our comments to the associated–dissociated dichotomy.

Though the associated–dissociated framework has been extremely useful in describing patterns of mating behavior, there are many species that fall between these categories and these patterns are not mutually exclusive [6]. For example, some snakes have two periods of mating behavior, of which only one is associated with increases in gonadal steroid secretion (e.g., Ref. [7]), and in a number of amphibian and reptilian species reproductive behavior is associated with gonadal steroid secretion but not spermatogenesis (see below) (for a review on the selection pressures that could have led to the evolution of these different strategies: Refs. [2,3,6]).

2.1. Associated breeders—amphibians

The two main sex steroid hormones studied in relation to courtship and copulatory behavior are testosterone (T) and its reduced metabolite, dihydrotestosterone (DHT). Concentrations of both androgens are highest during the breeding

season in a variety of anurans (e.g., bullfrogs, *Rana catesbeiana*: [8,9]; Japanese toads, *Bufo japonicus*: [10, 11]; Mexican leaf frogs, *Pachymedusa dacnicolor*: [12]; edible frogs, *Rana esculenta*: [13]; desert spadefoot toads, *Scaphiopus couchii*: [14]) and urodeles (reviewed in Refs. [15–17]) (e.g., roughskin newts, *Taricha granulosa*: [18,19]; crested newts, *Triturus carnifex*: [20,21]; Japanese newts, *Cynops pyrrhogaster*: [22]; Jordan's salamanders, *Plethodon jordani*, and Allegheny mountain dusky salamanders, *Desmognathus ochrophaeus*: [23]; see Table 1). Moreover, administration of T and DHT can reinstate courtship behavior in castrated anurans such as male cricket frogs, *Acris gryllus* [24], South African clawed frogs, *Xenopus laevis* [25,26], and green treefrogs, *Hyla cinerea* [27], and urodeles such as roughskin newts [28], Japanese newts [29], and crested newts [30].

Unlike rodents and other mammals, there are relatively few examples in which estradiol (E2), the aromatized product of T, has been implicated in the display of courtship behavior. For example, seasonal changes in reproductive behavior are not correlated with seasonal changes in E2 in some newts [18,31], and E2 cannot reinstate courtship behavior in male South African clawed frogs [25], Japanese newts [29], crested newts [30], or roughskin newts [28]. However, despite the lack of E2 effects on the reinstatement of courtship behavior, brain aromatase activity and plasma E2 concentrations are highest during courtship behavior in male crested newts, suggesting a modulatory role of estrogens in this urodele [32,33].

Androgen-accumulating cells are distributed in limbic brain areas such as the preoptic area (POA), ventral hypothalamus, and amygdaloid nuclei in both anurans and urodeles [34–36], and, moreover, androgen implants into the POA can elicit courtship behavior [37]. Stimulation of the POA also elicits courtship vocalizations in anurans [38], and lesions of the POA abolish sexual behavior in anurans [39,40] and urodeles [41]. These nuclei have also been demonstrated to be androgen-sensitive and to be significant for the display of male sexual behavior in a range of other taxa [42,43].

2.2. Associated breeders—reptiles

Just as in amphibians, the two main sex steroid hormones associated with mating in reptiles are T and DHT. With regard to seasonal changes in steroid milieu and sexual behavior, both androgens increase prior to the onset of breeding in turtles (e.g., snapping turtles, *Chelydra serpentina*: [44,45]; painted turtles, *Chrysemys picta*: [46]; gopher tortoises, *Gopherus polyphemus*: [47]; desert tortoises, *Gopherus agassizii*: [48]; Kemp Ridley's sea turtles, *Lepidochelys kempi*: [49]; loggerhead sea turtles, *Caretta caretta*: [50]; musk turtles, *Sternotherus odoratus*: [51]; Galápagos turtles, *Geochelone nigra*: [52,53]), lizards (e.g., white-throated savanna monitors, *Varanus albigularis*: [54]; agamid lizards, *Amphibolurus nuchalis* and *A. caudicinctus*:

Table 1
Hormonal dependence of courtship behavior in amphibians and reptiles

Species name	Common name	Seasonal change in T correlated with breeding	T or DHT reinstates behavior in castrates
Amphibians			
<i>A. gryllus</i>	Cricket Frog		Y
<i>B. japonicus</i>	Japanese Toad	Y	
<i>H. cinerea</i>	Green Treefrog		Y
<i>P. dacnicolor</i>	Mexican Leaf Frog	Y	
<i>R. catesbeiana</i>	Bullfrog	Y	
<i>R. esculenta</i>	Edible Frog	Y	
<i>S. couchii</i>	Desert Spadefoot Toad	Y	
<i>X. laevis</i>	South African Clawed Frog		Y
<i>D. ochrophaeus</i>	Allegheny Mountain Dusky Salamander	Y	
<i>P. jordani</i>	Jordan's Salamander	Y	
<i>C. pyrrhogaster</i>	Japanese Newt	Y	Y
<i>T. granulosa</i>	Roughskin Newt	Y	Y
<i>T. carnifex</i>	Crested Newt	Y	Y
<i>A. tigrinum</i>	Tiger Salamander	N	
Reptiles			
<i>C. serpentina</i>	Snapping Turtle	Y	
<i>C. caretta</i>	Loggerhead Sea Turtle	Y	
<i>L. kempii</i>	Kemp Ridley's Sea Turtle	Y	
<i>S. odoratus</i>	Musk Turtle	Y	
<i>C. picta</i>	Painted Turtle	Y	
<i>G. nigra</i>	Galapagos Turtle	Y	
<i>G. agassizii</i>	Desert Tortoise	Y	
<i>G. polyphemus</i>	Gopher Tortoise	Y	
<i>A. mississippiensis</i>	American Alligator	Y	
<i>S. punctatus</i>	Cook's Strait Tuatara	Y	
<i>A. carolinensis</i>	Green Anole		Y
<i>A. sagrei</i>	Brown Anole	Y	
<i>S. jarrovi</i>	Mountain Spiny Lizard		Y
<i>U. stansburiana</i>	Side-Blotched Lizard		Y
<i>A. caudicinctus</i>	Ring-Tailed Dragon	Y	
<i>A. nuchalis</i>	Central-Netted Dragon	Y	
<i>P. barbata</i>	Eastern Bearded Dragon	Y	
<i>E. macularius</i>	Leopard Gecko		Y
<i>C. inornatus</i>	Little Striped Whiptail Lizard	Y	
<i>E. laticeps</i>	Broad-Headed Skink		Y
<i>N. ocellatus</i>	Ocellated Skink	Y	
<i>T. rugosa</i>	Shingleback Skink	Y	
<i>V. albicularis</i>	White-throated Savanna Monitor	Y	
<i>A. contortrix</i>	Cottonmouth	Y	
<i>V. aspis</i>	Aspic Viper	Y	
<i>T. sirtalis sirtalis</i>	Eastern Garter Snake	Y	
<i>T. sirtalis parietalis</i>	Red-Sided Garter Snake	N/Y	N

See text for references.

[55]; shingleback skinks, *Tiliqua rugosa*: [56]; brown anoles, *Anolis sagrei*: [57]; little striped whiptail lizards, *Cnemidophorus inornatus*: [58,59]; ocellated skinks, *Niveoscincus ocellatus*: [60]; eastern bearded dragons, *Pogona barbata*: [61]), the Cook's strait tuatara, *Sphenodon punctatus*: [62]), crocodiles (American alligators, *Alligator mississippiensis*: [63]), and snakes (e.g., cottonmouths, *Agkistrodon contortrix*: [64]; aspic vipers, *Vipera aspis*: [7,65]; eastern garter snakes, *Thamnophis sirtalis sirtalis*: [66]; Table 1). A direct causal role of T, where castration leads to the loss of sexual behavior and T replacement reinstates behavior, has been documented in the brown anole [67], green anole, *Anolis carolinensis* [68–70], leopard gecko, *Eublepharis macularius* [71], mountain spiny lizard, *Sceloporus jarrovi* [72], broad-headed skink, *Eumeces laticeps* [73], side-blotched lizard, *Uta stansburiana* [74], and little striped whiptail lizard [58]. Further, blocking AR or androgen synthesis can inhibit the expression of courtship behavior in intact male brown anoles [75].

As in amphibians, E2 is relatively ineffective at inducing courtship behaviors in reptiles [58,67,69–71,73,74,76]. Similarly, in green anoles, blocking the conversion of T to DHT, but not the conversion of T to E2, attenuates courtship behaviors [77,78]. However, intracranial implants of E2 have been found to induce courtship behavior in green anoles [79], and the discrepancy between these conflicting results has not been adequately resolved.

Relatively few studies have investigated AR protein or mRNA expression and regulation in the reptilian brain, but there appears to be considerable similarity among the species examined in AR distribution. For example, AR or AR mRNA is found in the POA, external nucleus of the amygdala (AME; also known as the ventromedial nucleus of the amygdala), and ventromedial hypothalamus (VMH) in whiptail lizards [80], leopard geckos [81], Tokay geckos, *Gekko gecko* [82], green anoles [83,84], and eastern fence lizards, *Sceloporus undulatus* [85]. Implants of androgens into the POA restore courtship behavior in gonadectomized whiptail lizards [86,87] and green anoles [88]. Further, lesions of areas such as the POA and AME lead to decrements in the display of courtship behavior [89–91], and neurons in the POA show increased metabolic activity during the display of courtship behavior [92,93]. Thus, in species where T appears significant in activating or priming the display of copulatory behaviors, the POA and AME may represent primary sites of action for T.

2.3. Dissociated breeders—amphibians

In neotenic tiger salamanders, *Ambystoma tigrinum*, males breed during March and April, while spermatogenesis occurs during May and June, and plasma androgens peak between September and November [94]. Consequently, both spermatogenesis and mating behavior are dissociated from elevations in androgen concentrations in male tiger sala-

manders. The environmental cue that triggers reproduction in these salamanders is unknown, although it is but plausible that temperature, a cue that has been implicated as a major cue initiating reproduction in amphibians [95–97], plays a significant role in the activation of courtship behavior in this species.

As mentioned earlier, the distinction between associated and dissociated patterns of reproduction is not always clear. For example, androgen elevations are associated with reproduction but spermatogenesis occurs outside the breeding season in edible frogs [13], desert spadefoot toads [14], Japanese toads [10], plethodontid salamanders [23], crested newts [21], and roughskin newts [18,19]. Therefore, spermatogenesis but not mating is dissociated from sex steroid hormone production. Despite the seasonal association between androgens and courtship behavior, the causal role of androgens remains to be tested, and in some species such as desert spadefoot toads, courtship behavior persists for at least 7 weeks after castration, suggesting that androgens might not be necessary for the maintenance of clasping [98].

2.4. Dissociated breeders—reptiles

In most temperate turtles and several snakes, mating occurs at a time when the gonads are not producing gametes or circulating levels of androgens are low [2,4,6,16,53]. One of the most studied reptilian models of dissociated reproduction is the red-sided garter snake (*Thamnophis sirtalis parietalis*) found in Manitoba, Canada [99]. The mating season for the red-sided garter snake is an abbreviated 1-month period that occurs immediately after emergence from hibernation. In initial studies, androgen concentrations were found to be low or basal during winter dormancy and following emergence when most mating occurs [4,100]. However, subsequent studies found that androgen concentrations were elevated upon emergence and rapidly decreased over the course of 2 weeks [99,101–103], and the degree of elevation in androgen concentrations upon emergence varied across populations and years as well as with time of sampling. Despite the elevated androgen concentrations, experiments revealed that circulating androgens are not required for courtship behavior in the spring [104,105]. Castration prior to hibernation or after emergence from hibernation does not eliminate courtship behavior, and androgen administration to castrated males does not induce courtship behavior [99]. Testicular recrudescence, spermatogenesis, and elevations in androgen concentrations occur after the mating season, and spermatogenesis and elevations in androgens are also dissociated. It is hypothesized that sexual behavior is dissociated from androgens and spermatogenesis in this species because of the truncated breeding season, and similar arguments have been made for other species with short but predictable breeding periods [2]. Interestingly, the environmental cue that triggers courtship behavior seems to be increases in temperature in the spring

[99], and temperature is an important signal for reproductive biology in a number of reptiles (e.g., Refs. [5,16,63,106]).

Androgens, however, are not unimportant for courtship behavior in male red-sided garter snakes. Courtship behavior slowly declines over years in castrated males, suggesting that periods of androgenic stimulation are important in reorganizing the neural substrates of mating behavior for the spring [107]. Further, treating long-term castrates with androgen for 8 weeks during the summer (at the time when intact males usually experience a surge in androgens) leads to an increase in courtship behavior in the next spring following hibernation [107]. Similar long-term or “organizing” effects of androgens in adulthood on the display of sexual behavior have been implicated in other snakes (e.g., Ref. [7]). These model systems will be invaluable in understanding how the “memory” of sex steroid actions is maintained.

It has also become clear that steroids are synthesized de novo in the brain and that neural production of steroids can affect behavior (reviewed in Refs. [108–110]). It is possible that male red-sided garter snakes castrated prior to hibernation demonstrate courtship behavior in the spring because sex steroid hormones are synthesized in hypothalamic brain areas and function to maintain the integrity of the neural circuits underlying courtship behavior. In this respect, it will be exciting to assess neurosteroid production across seasons and to assess the effects of androgen and estrogen receptor antagonists on courtship behavior in red-sided garter snakes.

As in associated breeders, the POA is integral to the display of courtship behaviors in species with a dissociated pattern of reproduction. Lesions of the POA-anterior hypothalamus (AH) continuum in the red-sided garter snake reduce courtship and copulatory behavior [101,111], and the POA-AH shows increases in metabolic activity during courtship [92]. Given that the POA is an evolutionarily conserved nucleus in the control of thermoregulation and that temperature is important in activating courtship behavior in this species, it is possible that POA lesions disrupt the ability to transduce information about temperature into appropriate behavioral responses [101]. Halpern et al. [112] found androgen-accumulating cells in the POA as well as other areas such the amygdala and septum in garter snakes (subspecies not reported), and it is plausible that the long-term effects of androgens in red-sided garter snakes are mediated, in part, by androgenic stimulation in these areas.

Just as in amphibians, there are examples in which the associated–dissociated categorizations fail to accurately depict patterns of reproductive physiology and behavior. In some snakes such as copperheads, mating occurs in the spring when the testes are in their final stage of regression but T concentrations are elevated [64]. Spermatogenesis occurs following the mating season. Such a dissociation, termed post-nuptial spermatogenesis [113,114], has been found in other snakes and turtles such as the Japanese rat

snake, *Elaphe climacophora* [115] and eastern garter snakes, *T. sirtalis sirtalis* [66]. In the Cook's strait tuatara, androgen elevations are associated with breeding activity, and although peak spermatogenic activity occurs during the breeding period, spermatogenesis occurs throughout the year [62]. In the red-sided garter snake, although the bulk of mating activity occurs in the spring following emergence from hibernation, individuals have been observed to mate during the autumn when androgen levels are elevated, suggesting a mixed strategy [99], and it will be important to assess the androgen dependence of fall courtship.

3. Arginine vasotocin

3.1. Amphibians

Arginine vasotocin (AVT) has been implicated as a major neurohormone modulating the expression of courtship behavior in anurans as well as urodeles. Injections of AVT induce calling in a variety of anurans including Great Plains toads, *Bufo cognatus* [116], green treefrogs [117,118], bullfrogs [119], and cricket frogs [120], and differences in neural AVT expression have been found between calling and noncalling male cricket frogs [121]. In the roughskin newt and Japanese newt, AVT induces amplexus [122–124], and circulating AVT concentrations are correlated with differences in the willingness to clasp females within the reproductive season and across seasons [19,125,126]. In the roughskin newt, AVT increases the amount of T that crosses the blood–brain barrier [127]. The secretion of pheromones that are important for increasing female receptivity is also induced by AVT in Japanese newts [124,128].

The effects of AVT are modulated by hormonal milieu. For example, AVT enhances courtship behaviors in intact and androgen-treated castrated roughskin newts but not in cholesterol-treated castrates [123,129], and androgens are required for AVT-induced calling in green treefrogs [117]. Androgen-accumulating cells have been found in AVT-immunoreactive (AVT-ir) areas, and androgen affects AVT-ir and AVT binding [119,130,131]. Further, treating males with the stress hormone, corticosterone, inhibits AVT effects on clasping in roughskin newts [132] and calling in green treefrogs [118].

These effects of AVT could be mediated by sexually dimorphic neuronal populations in the bed nucleus of the stria terminalis, amygdala, and/or POA; male roughskin newts and bullfrogs have greater AVT-ir than females in these populations [131,133]. Arginine vasotocin seems to facilitate courtship behavior in the roughskin newt by influencing sensorimotor processes toward arousing stimuli [134]. The enhanced response to arousing visual stimuli following AVT injections could be due to AVT acting in the optic tectum (OT), and there is preliminary evidence supporting this notion [135]. A similar influence on sensorimotor processing has been found in green treefrogs

[117] and has been suggested in bullfrogs [119]. Steroidal environment also affects AVT cell number in the pretrigeminal nucleus, which acts as the vocal pattern generator, and could thereby increase courtship vocalizations in anurans in a manner similar to that seen with exogenous AVT injections [119].

3.2. Reptiles

Relatively little work has been done on the functional significance of AVT on the display of courtship behavior in reptiles. On the other hand, the distribution of AVT cells and fibers has been described in ball pythons, *Python regius*, red-eared slider turtles, *Trachemys scripta elegans*, and tokay geckos [136]. The pattern of distribution of AVT in these reptiles appears similar to that seen in mammals [136] and amphibians [137]. Moreover, the expression of AVT is sexually dimorphic in a number of nuclei that have been implicated in the display of male-typical sexual behavior. For example, in the ball python and red-eared slider turtle, AVT innervation of the lateral septum, ventral amygdala, midbrain periaqueductal gray, ventral tegmental area, and substantia nigra is greater in males than in females [136]. In the tokay gecko, there are more AVT fibers in the lateral septum and the nucleus sphericus (NS) in males than in females [138]. Finally, because temperature has been found to regulate the expression of AVT in nuclei such as the paraventricular hypothalamus of the wall lizard, *Lacerta muralis* [139], it is possible that AVT is involved in reproduction in species that use temperature cues to initiate courtship or gonadal recrudescence.

4. Dopamine

4.1. Amphibians

Catecholamines like dopamine (DA) modulate the display of copulatory behaviors in mammals and birds [140,141]. However, very few studies have manipulated the dopaminergic system and assessed effects on courtship behavior in amphibians. Malacarne et al. [142] injected bromocriptine, a DA agonist, into male crested newts and found that it suppressed courtship behavior. This effect, however, was attributed to the inhibitory effect of bromocriptine on prolactin production (for review of prolactin effects on courtship in amphibians: Refs. [124,143]).

On the other hand, sex differences as well as the effects of androgens on the expression of tyrosine hydroxylase (TH), a rate-limiting enzyme in catecholamine synthesis, in limbic brain areas have been studied in Northern leopard frogs [144,145]. In general, males have more TH-immunoreactive (TH-ir) cells in the POA and caudal hypothalamic areas relative to females, and TH-ir expression in these same areas decreases following castration and increases following systemic androgen treatment. Cells in the POA of South

African clawed frogs both accumulate androgens [34] and are TH-ir [146], suggesting that androgens might regulate TH-ir in this anuran as well.

4.2. Reptiles

Though the distribution of catecholamine-synthesizing neurons in the brain has been characterized in a number of reptiles (reviewed in Refs. [147,148]), the function of catecholamines such as DA in sexual behavior in male reptiles is relatively unknown. Only in whiptail lizards and leopard geckos have the effects of DA agonists or antagonists on courtship behavior been investigated. Systemic injections of a DA D1 receptor agonist into gonadectomized male little striped whiptail lizards and parthenogenetic desert grassland whiptail lizards, *C. uniparens*, significantly increase the display of mounting behavior [149]. Moreover, relative to little striped whiptail lizards, gonadectomized desert grassland whiptail lizards require a 10-fold lower dosage to elicit mounting behavior. The fact that the desert grassland whiptail is triploid, whereas the little striped whiptail is diploid, could contribute to this difference; for instance, variation in the amount of D1 expression due to ploidy differences could mediate such a difference in sensitivity.

In the leopard gecko, systemic injection of a DA D1 receptor antagonist inhibited the display of courtship behavior in castrated, T-implanted males (J.T. Sakata, S.C. Woolley, and D. Crews, unpublished data). Interestingly, males from different incubation temperatures varied in their sensitivity to DA antagonism: only the highest dosage (8 mg/kg) inhibited courtship behavior in adult males hatched from eggs incubated at 30 °C, whereas both medium and high dosages (4 and 8 mg/kg) inhibited courtship in males from 32.5 °C. Thus, males from 30 °C seem to be less disrupted by DA receptor antagonism than are males from 32.5 °C, and this pattern is consistent with other phenotypic differences between males from these incubation temperatures (reviewed in Refs. [150,151]); for example, males from 30°C show more sexual behavior following castration and following identical T treatment [71,152].

The role of DA in courtship behavior in reptiles is further highlighted in recent studies on the expression of TH in limbic brain areas [153,154]. In little striped whiptail lizards, sexually vigorous males have more TH-ir cells in the dorsal hypothalamus than sexually sluggish males, and when housed with females, sexually vigorous males have more TH-ir cells in the substantia nigra pars compacta (SNpc) than isolated males. In the parthenogenetic desert grassland whiptail lizard, mounting behavior is displayed only following ovulation, and post-ovulatory parthenogens had more TH-ir cells in the SNpc relative to individuals without follicles. Interestingly, just as mounting behavior is rarely displayed by female little striped whiptails across the reproductive cycle, there is no significant change in TH-ir

across reproductive cycle in any nucleus in female little striped whiptails [153].

5. Species differences in courtship signals and their neural correlates

Across amphibian and reptilians species, there is considerable variability in the use of different sensory modalities in courtship behavior and in detecting the presence of a female [155,156]. For example, many species use pheromonal cues to discriminate between sexes and species [157]. A preference for the odor of female conspecifics has been demonstrated in male amphibians such as *Ambystoma jeffersonianum* [158], *Desmognathus imitator* and *D. ochrophaeus* [159], species of *Taricha* [160], and a number of *Plethodon* species [161,162], and in reptiles such as the Iberian wall lizard, *Podarcis hispanica* [163], broad-headed skink [73,164,165], leopard gecko [166,167], and red-sided garter snake [168]. Visual signals are used extensively by *Anolis* lizards; for example, green anoles use dewlap extensions to display to females as well as to other males (reviewed in Ref. [169]). Anurans are famous for their calling abilities, and one of the primary functions of these calls is to attract females. There are also a few reptiles, such as the tokay gecko, that use acoustic signals during courtship [82,170]. *Triturus* newt species differ in the amount of somatosensory feedback from the female required to induce spermatophore deposition (reviewed in Ref. [156]). Further, many species depend on multiple modalities during courtship. Male Iberian wall lizards use visual cues at long distances and pheromonal cues at short distances to detect females [163], and red-sided garter snakes use pheromonal, visual, and thermal cues to locate females [171].

It is likely that hormones and neurotransmitters that modulate the expression of courtship behavior act, in part, by affecting sensory and motor systems [155,172]. Further, depending on the degree to which sensory characters are phylogenetically constrained, it is possible that the distribution of hormone receptors and/or neurotransmitters in the central nervous system vary between species that rely on different modalities for female detection and courtship displays. In both reptiles and amphibians, species differences in the distribution and abundance of AR as well as catecholamines (e.g., DA) have been found in sensory or motor areas [82,148,173], which is in contrast to the conserved expression of AR and catecholamines in preoptic, hypothalamic, septal, and amygdaloid nuclei. For example, garter snakes depend more on pheromonal cues for courtship than do green anoles, and their accessory olfactory system is much more developed and accumulates more androgens than the olfactory system of green anoles [83,112]. On the other hand, green anoles have more androgen-accumulating cells in the cortex and motor nucleus of the trigeminal nerve relative to garter snakes

[83]. The motor nucleus of the trigeminal nerve is important for the display of neck grips, which is exhibited by green anoles but not garter snakes.

There are also instances where AR expression may be more constrained by phylogenetic history than local adaptation. For example, the tokay gecko has high levels of AR in nuclei involved in vocal production as well as the torus semicircularis (TS) [82], and stimulation of the TS produces vocalizations similar to advertisement calls [82]. However, the TS of green anoles, a species that does not produce vocalizations, also expresses AR [82,84]. Consequently, more information is necessary to determine whether the presence AR in the TS and auditory nuclei of the tokay gecko is a derived trait specifically associated with vocal production or an evolutionarily conserved trait co-opted for use in vocal courtship signals. Further phylogenetic investigation of the role of androgenic stimulation in these nuclei will help understand evolutionary changes in AR expression.

There are also some differences in the distribution of androgen-accumulating cells that do not correlate with species differences in courtship behavior. For example, both South African clawed frogs and leopard frogs vocalize during courtship, but only South African clawed frogs have androgen-accumulating cells in the pretrigeminal nucleus and motor neurons of the cranial nerve IX-X [34,35,174].

Catecholaminergic fibers have been documented in the OT, TS, and NS in a number of reptiles and amphibians. Interestingly, the level of dopaminergic innervation of the OT and TS is greater in the tokay gecko and the ball python than in the red-eared slider turtle [147], which could indicate a greater modulation of responses to visual or auditory signals by catecholamines in the tokay gecko and ball python. The ball python also has greater catecholaminergic innervation of the NS [147], the primary target of neurons in the vomeronasal organ [175], than the tokay gecko and this suggests species differences in catecholaminergic regulation of pheromonal signals. Anurans and urodeles have catecholaminergic fibers in OT and TS, even though the groups differ dramatically on the use of visual vs. auditory signals during courtship [146]; therefore, catecholamine innervation into these sensory areas might be a product of phylogenetic constraint. Just as some have assessed the role of AVT in sensory processing (e.g., Refs. [134,135]), it will be important to compare the behavioral effects of catecholamine manipulations across these species.

6. Hybridizations, ploidy, and other species comparisons

Amphibians and reptiles are excellent model systems to study the process of evolution because there are a number of hybrid species, where, unlike most other taxa, ancestral species are still extant. Consequently, comparisons between hybrids and their ancestral species can provide a unique

opportunity to investigate correlated changes in biological organization through speciation.

In amphibians, there exist many interspecific hybrids in the genera *Ambystoma* (mole salamanders), *Rana* and *Hyla*. A multitude of hybrids have been formed from matings among *Ambystoma jeffersonianum*, *A. laterale*, *A. texanum*, and *A. tigrinum* (reviewed in Ref. [176]). The only species in which seasonal changes in sex steroid hormones in males have been measured is the tiger salamander, *A. tigrinum*, a species with a dissociated pattern of reproduction (see above). *Ambystoma tigrinum*, as well as *A. laterale* and *A. texanum*, were involved in the hybridization events leading to the evolution of *A. nothagenes*, and it will be interesting to determine if *A. nothagenes* also shows a dissociated pattern of reproduction. Further, it would also be interesting to test the contribution of the *A. tigrinum* genome by comparing the reproductive biology of *A. nothagenes* to other hybrids that involved *A. laterale* and *A. texanum* but not *A. tigrinum*. Dawley and Dawley [158] report that male *A. jeffersonianum* prefer the odors of conspecific females to those of female *A. platineum*, a hybrid species which possess two copies of the *A. jeffersonianum* genome. An interesting question is whether *A. jeffersonianum* males prefer the odors of female *A. platineum* over female *A. tremblayi*, a hybrid that possesses only one copy of the *A. jeffersonianum* genome [176]. In other words, does genetic distance correlate with male odor preferences?

Among reptiles, approximately one third of extant whiptail lizard species (genus *Cnemidophorus*) are all-female (parthenogenetic) species that resulted from hybrid unions of sexual species [177]. Many lineages of these parthenogenetic species complexes such as the *sexlineatus*, *neomexicanus*, and *tesselatus* complexes, arose from a single or only a few hybridization events [178,179]. Moreover, studies of desert grassland lizards and other parthenogens have found that they display both male- and female-like pseudosexual behavior depending on reproductive state [180,181]. (Some all-female amphibian hybrids, such as that between *A. platineum* and *A. texanum*, have also been found to display courtship-like behaviors [182]).

We have studied the evolution and neuroendocrine control of sexual behaviors in the desert grassland whiptail lizard for several decades (reviewed in Ref. [183]). The parthenogenetic desert grassland whiptail lizard, *C. uniparens*, is a hybrid species that descended from interspecific matings involving little striped whiptail lizards, *C. inornatus*, and displays male-typical pseudosexual behavior following ovulation when progesterone (P) concentrations are elevated [181,184]. Further, P administration to ovariectomized parthenogens can induce pseudosexual courtship behavior [185]. Female little striped whiptail lizards, on the other hand, rarely display mounting behavior following ovulation. However, P modulates courtship behavior in male little striped whiptails, and although circulating concentrations of P do not change seasonally in males of this species [59], exogenous P can reinstate courtship behavior

in a subset of castrated males (P-sensitive males [186–188]). Because P affects courtship behavior in male but not female little striped whiptails, it has been proposed that a P-sensitive male was involved in the hybridization event, thereby conferring the capacity to display male-typical sexual behavior in response to P, and that the post-ovulatory surge of P was co-opted to trigger male-typical courtship behavior in the descendant desert grassland whiptail lizard.

In addition to the opportunity to make comparisons between ancestral and descendant species, hybrids often differ from closely related species in genome size, as many hybrids are polyploid; for example, the desert grassland lizard is triploid, while its ancestral species are diploid. Consequently, these comparisons allow the opportunity to study directly the effects of a larger genome size on neural organization and function. Such comparisons have been made in salamanders, in which brain morphology of polyploid species is less complex than would be predicted by phylogenetic relationships alone [189]. Neural organization and structure appear to be secondarily simplified in salamanders: the simple brains of salamanders are thought to be derived from a more complex ancestral state.

Iberian-ribbed newts, *Pleurodeles waltl*, exist as either diploid or triploid individuals, and while both show similar seasonal changes in androgen concentrations, the concentration of androgens in the triploid is consistently ~40% lower than that of the diploid [190]. Similarly, across the reproductive cycle, triploid desert grassland whiptails have lower circulating concentrations of E2 than female little striped whiptail lizards, their diploid maternal ancestor, and they require lower E2 dosages to induce receptive behavior (reviewed in Ref. [184]). Further, the triploid parthenogen expresses higher ER mRNA expression in some limbic nuclei [184]. It would be interesting to test whether the triploid Iberian-ribbed newt requires lower dosages of androgens to elicit courtship behavior or has more AR in limbic brain areas than the diploid form. As mentioned above, the triploid desert grassland whiptail requires lower levels of DA agonists to induce mounting behavior relative to the male diploid little striped whiptail, and differences in DA receptor expression have been postulated [149].

The effects of differences in ploidy on calling behavior have been investigated in *Hyla versicolor*, a hybrid species whose ancestral species is the diploid *H. chrysoscelis*. There exist both triploid and tetraploid *H. versicolor* males, and the mean pulse rate of the advertisement calls declines as ploidy increases [191,192]; both hybrid types have lower pulse rates than males of the ancestral species. Interestingly, hybrids involving other *Hyla* species produce calls with pulse rates intermediate to those of the ancestral species [193]. This system provides a fascinating opportunity to examine the neural correlates of these hybridization events and evolutionary changes in hormonal control of social behavior. It would be interesting to assess the role of AVT in altering call parameters (e.g., Ref. [120]) or to compare

whether ploidy correlates with sensitivity to the effects of AVT on calling.

Finally, just as in other taxa, comparisons between closely related species are also important for understanding ecological and phylogenetic influences on reproductive phenotype. Two closely related species can display very different reproductive profiles despite similar ecological conditions, or related species may display similar reproductive strategies despite different ecological constraints. For example, whereas the European viper, *Vipera berus*, shows only a single peak of mating and T concentrations in a year, the closely related aspic viper, *V. aspis*, displays mating and T elevations both in the spring and autumn (see Refs. [7,65]). Similarly, the terrestrial Southern copperhead, *A. contortrix*, and the semiaquatic Eastern cottonmouth, *A. piscivorous*, both mate in the late summer and spring, but only in the Southern copperhead are there two peaks of T corresponding with the mating periods [64]. Whereas the red-sided garter snake, *Thamnophis sirtalis parietalis*, has a truncated breeding season, its Mexican congener, *T. melanogaster*, has a long breeding season, and there is less selection pressure for mating behavior to be dissociated from testicular activity under these circumstances. However, both have dissociated patterns of reproduction, suggesting a prominent role of phylogeny in shaping these processes [194]. Two agamid species, the central netted dragon, *A. nuchalis*, and the ring-tailed dragon, *A. caudicinctus*, in Australia occupy the same ecological niche but differ in the degree to which precipitation induces testicular and mating activity: the central netted dragon behaves more opportunistically than the ring-tailed dragon [55].

7. Conclusion

In this review, we highlight the similarities and differences in the regulation of the display of courtship behaviors by testicular hormones, the neurohormone AVT, and catecholamines such as dopamine in male amphibians and reptiles. In our discussion of the role of androgens, we utilize the associated–dissociated categorization, and it is evident that many species lie more along a continuum than in disparate categories. There are species in which only particular aspects of testicular activity—androgen production or spermatogenesis—are tied to mating behavior, and other species in which different mating periods are associated with different aspects of testicular function. In light of the work on garter snakes, *Thamnophis sirtalis parietalis*, the need to characterize the dependence of behavior on androgens through hormonal manipulations is emphasized. For example, in eastern garter snakes, *T. sirtalis sirtalis*, a species with a breeding season shorter than that of red-sided garter snakes, androgens are elevated at the time of mating [66]. This suggests that the display of courtship behavior is dependent on circulating androgens, and a similar conclusion might be drawn from field studies

in red-sided garter snakes [101]. However, because exhaustive studies manipulating peripheral androgen concentrations at different times of the year were done in red-sided garter snakes, we know that circulating androgens are not required for spring mating in red-sided garter snakes (reviewed in Ref. [99]). The relative independence of courtship behavior on testicular androgens is also highlighted in studies on desert spadefoot toads, where males continue to court females at high levels as long as 7 weeks following castration [14,98]. Therefore, empirical tests of the dependence of courtship behavior on circulating androgens in species in which seasonal changes in mating behavior are correlated with seasonal changes in androgens are essential. It is also important to compare the effects of androgen manipulations on neural phenotypes across species that differ in their behavioral dependence on androgens. Finally, given the role of neurosteroids in the regulation of social behaviors in mammals and birds, it will be important to assess the influence of neural steroid production on courtship behavior in species with associated and dissociated patterns of reproduction.

In our review of the effects of AVT and catecholamines in amphibians and reptiles, it is evident that experiments manipulating AVT in reptiles and manipulating catecholamines in both reptiles and amphibians are greatly needed. The characterizations of AVT neural phenotypes in anurans and urodeles and AVT manipulations have significantly contributed to our understanding of the conserved importance of this neurohormone in reproductive behaviors in vertebrates, and similar endeavors in reptiles are critical. Similarly, there are a number of reptilian species in which catecholaminergic populations have been characterized, and these studies have highlighted the conservation in the distribution of catecholamine-synthesizing cells in preoptic and hypothalamic areas across vertebrates (reviewed in Ref. [148]). However, now it is increasingly important to assess the functional contributions of catecholamines in reptiles (e.g., Refs. [149,153,154]) as well as in amphibians. It is evident that AVT and catecholaminergic systems are influenced by sex steroid hormones, and given the diversity of hormone–behavior relationships across male amphibians and reptiles, we anticipate much diversity in the mechanism and function of variation in these systems as well.

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