

Sexual size dimorphism of the tongue in a North American pitviper

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Abstract

Sexual dimorphisms – phenotypic dissimilarities between the sexes – are common and widespread among plants and animals, and classical examples include differences in body size, colour, shape, ornamentation and behaviour. In general, sexual dimorphisms are hypothesized to evolve by way of sexual selection acting on one sex through priority-of-access for sexual partners via mate choice and/or intra-sexual competition. In snakes, males are the mate-searching sex and one form of sexual selection involves male–male competition in locating females by following pheromone trails using their forked tongues, the structure used to sample environmental chemicals for transduction in the vomeronasal chemosensory system (VNS). Based on several lines of empirical evidence, increased tongue forking (bifurcation) in snakes (and some lizard taxa) appears to enhance chemical trail-following abilities through tropotaxis (the simultaneous comparison of stimulus intensities on two sides of the body) and thus aids in prey location and mate searching in males. We predicted that male copperheads, *Agkistrodon contortrix*, a North American pitviper, should have more deeply forked tongues than females owing to male–male competition for priority-of-access to widely dispersed females during the mating seasons. We examined formalin-fixed, ethanol-preserved museum specimens of adult *A. contortrix* for sexual size dimorphism (SSD) of the tongue. Tongue dimensions showed differences indicative of SSD, and the degree of bifurcation (i.e. mean tine length) was significantly greater in males. Various structures of the VNS and associated regions (e.g. muscles) in some vertebrate taxa show sexual dimorphism, but our study is the first to document dimorphism in the tongue of a tetrapod vertebrate.

Introduction

Sexual dimorphisms – phenotypic dissimilarities between the sexes – are widespread among plant and animal taxa (Darwin, 1871; Thornhill & Alcock, 1986; Andersson, 1994). The exact mechanism responsible for driving the evolution of sexually dimorphic traits is sometimes controversial (Shine, 1989; Andersson, 1994), but most commonly the explanation is that of sexual selection through the mechanisms of mate choice and/or intra-sexual competition (Birkhead & Møller, 1998; Simmons, 2001; Shuster & Wade, 2003). Accordingly, sexual dimorphisms are predicted to evolve when individuals with an enhanced trait show a fitness advantage over same-sex competitors (Lande, 1980; Andersson, 1994). Ultimately, the extent to which a sexually selected trait can be modified is limited when the selective costs (e.g. from natural selection) exceed the benefits (Andersson, 1994).

Although a wide range of sexually dimorphic traits is known in animals, dimorphisms in body size (Shine, 1993,

1994), coloration (Petrie & Halliday, 1994), shape (Schuett *et al.*, 2005), ornamentation (Thornhill & Alcock, 1986; Andersson, 1994) and behaviour (Kelley, 1988; Andersson, 1994; Schuett, Gergus & Kraus, 2001; Shuster & Wade, 2003) are the most conspicuous. In several lineages of vertebrates, such as mammals and reptiles, males attain relatively larger sizes than females owing to male–male competition for priority-of-access to females for mating (Andersson, 1994; Shuster & Wade, 2003). Similarly, ornamentation and bright coloration are typically most elaborate in males where reproductive success is linked to the outcome of mate choice by females (Andersson, 1994; Petrie & Halliday, 1994).

Sexual dimorphisms that are less conspicuous (and less well known) have been identified recently, such as those pertaining to the chemical senses (reviewed in Halpern & Martínez-Marcos, 2003). Chemical sense organs in most tetrapods comprise two distinct systems: the main olfactory system (MOS) and the accessory olfactory system [or the vomeronasal system (VNS)] (Halpern & Martínez-Marcos,

2003). While structurally similar, the VNS and MOS are functionally separate, and each has a different role in the reception and processing of chemical signals (Dennis *et al.*, 2003). In certain tetrapod lineages, especially squamate reptiles (lizards and snakes), the VNS has a particularly important role in mediating behavioural responses to prey and sex-specific chemicals (Wysocki & Meredith, 1987; Halpern, 1992; Schwenk, 1995; Zufall, Kelliher & Leinders-Zufall, 2002; Halpern & Martínez-Marcos, 2003; Huang *et al.*, 2006; see Baxi, Dorries & Eisthen, 2006). Several divergent lineages of vertebrates show sexual dimorphisms in the VNS (Dawley, 1992, 1998; Halpern & Martínez-Marcos, 2003). In a terrestrial plethodontid salamander *Plethodon cinereus*, for example, males have significantly larger vomeronasal organs (VNO) than females, and this has been suggested as being important to males in territorial recognition and courtship (Dawley, 1992, 1998; Dawley & Crowder, 1995). In mammals, sex differences occur in overall VNO volume, epithelial volume and number of bipolar cells, as well as differences in the accessory olfactory bulb to which the vomeronasal nerves project (Simerly, 1990; Segovia & Guillamón, 1993; Guillamón & Segovia, 1997). Furthermore, recent studies have revealed sexual dimorphism at an even finer scale – within the protein subunit of putative VNO receptors in the highly aquatic stinkpot turtle *Sternotherus odoratus* (Murphy, Tucker & Fadool, 2001).

In contrast to most other tetrapods, the VNS in snakes shows greater complexity than the MOS (Parsons, 1970; Wang & Halpern, 1980; Bertmar, 1981; Halpern, 1992; Halpern & Martínez-Marcos, 2003), and an intact VNS is critical for expression of a range of complex behaviours such as prey recognition and prey trailing (Burghardt & Pruitt, 1975; Ford & Burghardt, 1993), as well as for mate searching and male courtship (Kubie, Vagvolgyi & Halpern, 1978; Halpern & Martínez-Marcos, 2003). The principal structures of the VNS are the tongue, VNO and associated brain regions and their nuclei (Halpern, 1992; Schwenk, 1993, 1995; Halpern & Martínez-Marcos, 2003). Although the VNS is well developed in nearly all squamate reptiles, only some taxa possess a tongue that is deeply bifurcated, a signal feature of snakes and several lineages of lizards including helodermatids, teiids and varanids (Schwenk, 1988, 1994). The snake tongue serves to sample chemicals in the environment and deliver them into the oral cavity, where they enter the paired VNO ducts located in the anterior palate of the mouth (Halpern & Kubie, 1980). Subsequent signal processing occurs through the vomeronasal nerve and the accessory olfactory bulb, which projects to higher brain regions responsible for mediating behavioural responses to the chemical stimuli (e.g. Lanuza & Halpern, 1997, 1998; Martínez-Marcos, Lanuza & Halpern, 1999).

Based on several lines of evidence derived from empirical studies, Schwenk (1994) showed that increased tongue forking (bifurcation) in snakes (and some lizard taxa) appears to enhance trail-following abilities for locating prey and mates through tropotaxis – simultaneous comparison of stimulus intensities on two sides of the body (see Ford, 1986). Tropotaxis is only possible when the chemical gradient is

sufficiently steep for both sides of the body to be stimulated with different intensities – in the case of snakes, each tip of the forked tongue simultaneously samples a different point in the environment for assessment by ipsilateral VNO, thus increasing the likelihood of detecting a chemical gradient typical of a conspecific trail or odour plume. Consequently, enhancement of tongue bifurcation [i.e. increased tine length (TL)] in male snakes should increase mate-searching abilities. We thus predicted that TL should be a target of sexual selection and would exhibit sexual size dimorphism (SSD), with males having more deeply forked tongues. Sexual dimorphism has never been demonstrated for any tetrapod tongue, nor have any other components of the VNS been shown to be sexually dimorphic in squamate reptiles (but see Huang *et al.*, 2006).

We tested whether the tongue of one snake species showed SSD in adults, especially with respect to TL. The study species was the copperhead *Agkistrodon contortrix*, a medium-sized pitviper of North America (Gloyd & Conant, 1990). The copperhead was selected for two main reasons: (1) there is an abundance of data on its natural history and reproductive biology (Gloyd & Conant, 1990; Schuett *et al.*, 1997) and (2) field studies showed that females are dispersed widely in the environment during the mating season, and that males sometimes search large distances to locate them (Fitch, 1960; Smith, 2007). Based on prior work, we assumed that females are a limited resource for males and thus there should be strong selection on males to locate females quickly when they are receptive (Duvall, Schuett & Arnold, 1993). Our prediction was that males should show a relatively greater TL than in females.

Methods

Specimens and morphological measurements

We used formalin-fixed, ethanol-preserved museum specimens (41 males, 41 females) of adult copperheads, *A. contortrix*, collected in Virginia, USA. All specimens were derived from the herpetological collection of the Carnegie Museum of Natural History and were selected for inclusion in this study based on their season of collection (late summer mating season), collection locality and body size. All the specimens that we used were killed on the date of collection. The mean (\pm SE) snout–vent lengths (SVL) for males and females were 68.8 ± 11.36 cm and 56 ± 0.68 cm, respectively. The tongues were carefully exposed through a longitudinal incision in the lower jaw and measured using vernier calipers accurate to 0.1 mm. Two measurements of the tongue were made from each specimen: TL (distance from the point of bifurcation to the tongue tip: depending on the condition that either the right or left line was measured) and tongue width (TW, distance across the tongue at the point of bifurcation). Five additional body measurements were made for potential use in the statistical analyses: SVL, head length (HL), maximum head width (HW), mid-head width (MHW) and tail length

(TAL). SVL (anteriormost point of the rostral scale to the caudal end of the cloacal scale) and TAL (caudal end of the cloacal scale to the tip of the tail) were measured using a cloth tape (± 0.1 cm). HL (distance from the articulation of the lower jaw with the quadrate bone to the anteriormost point of the rostral scale), head width (distance across the widest region of the head) and MHW (distance across the head at the supraocular scales) were measured using vernier calipers (± 0.1 mm). The total length was calculated as SVL + TAL. The condition of the specimens prevented measurement of HL and MHW in some cases, and these differences in sample size have been noted accordingly in the tables.

Statistical analysis

All data were inspected for outliers, normality (skewness and kurtosis) and equality of variance prior to performing statistical tests (Zar, 1996). Statistical analyses were performed using SAS Version 8.2 (SAS Institute, 1999) and JMP 5.01 (SAS Institute, 2002). There were significant (and marginally significant) differences between the sexes for all morphological measurements conducted (Table 1). This outcome thus precluded the use of these variables as covariates to control for body size differences between males and females in the TL analysis. Accordingly, statistical assumptions of lack of collinearity between the continuous and categorical predictor variables were violated (Quinn & Keough, 2002, p. 349). Consequently, to evaluate sex differences in TL while accounting for potential influential allometric effects, we used the residuals of the regression of MHW versus TL (both sexes included) as the dependent variable, and sex as the independent variable in a one-way ANOVA. This regression was highly significant ($F_{1,75} = 85.37$, $P < 0.0001$, $R^2 = 0.53$, slope = 1.19), as were regressions of TL versus SVL ($F_{1,81} = 103.05$, $P < 0.0001$, $R^2 = 0.56$, slope = 0.157), HW ($F_{1,55} = 3.85$, $P = 0.055$, $R^2 = 0.06$, slope = 0.21) and

HL ($F_{1,71} = 76.79$, $P < 0.0001$, $R^2 = 0.52$, slope = 0.53). We selected the residuals of MHW because of the proximity of this region to the tongue. The results of the analysis of variance on residual TL were highly significant ($P < 0.0001$), regardless of which residual set was used. In the above analyses, residuals were normally distributed and did not require transformation (Shapiro–Wilk goodness of fit: $W < 0.96$, $P > 0.19$).

Results

TL was significantly greater in males than in females (Table 1, Figs 1 and 2a). Importantly, males had significantly longer TLs relative to females even after accounting for allometric effects (i.e. the residuals of TL vs. MHW; $F_{1,75} = 61.9$, $P < 0.0001$). This result indicates a robust sex difference in TL independent of other morphological sex differences. Similar to TL, all other morphological variables included in the analysis followed the same trend with males greater than females (Table 1). With the exception of HW and tongue parameters, all morphological variables were significantly correlated with each other and in each sex (Table 2).

There was a significant positive relationship between SVL and TL in both males ($F_{1,39} = 12.61$, $P = 0.001$, $R^2 = 0.24$) and in females ($F_{1,39} = 5.20$, $P = 0.028$, $R^2 = 0.12$) (Fig. 2b). The slopes of the male and female regressions (SVL vs. TL) did not differ ($t_{78} = 0.51$, $P = 0.62$) (Fig. 2b). Elevation of the regression line for males was significantly greater than in females ($t_{79} = 25.30$, $P < 0.0001$) (Fig. 2b).

Discussion

The copperhead, *A. contortrix*, like many species of pitvipers, shows SSD (male biased) as adults in overall size, particularly with respect to total length (Fitch, 1960; Shine, 1978, 1993, 1994). By controlling for potentially

Table 1 Sex differences in morphological measurements of adult *Agkistrodon contortrix*

Parameter	Transform	F-value	d.f.	P-value	True means (N)	Relationship
Tine length	LN	738.95	1, 80	<0.0001	F: 10.24 \pm 0.09 (41) M: 13.95 \pm 0.10 (41)	M > F
Tongue width	LN	36.87	1, 80	<0.0001	F: 1.91 \pm 0.02 (41) M: 2.08 \pm 0.02 (41)	M > F
Head width	None	3.81	1, 55	0.056	F: 21.45 \pm 0.42 (26) M: 22.68 \pm 0.46 (31)	M > F
Head length	LN	61.63	1, 71	<0.0001	F: 28.89 \pm 0.26 (37) M: 32.46 \pm 0.39 (36)	M > F
Mid-head width	LN	63.49	1, 75	<0.0001	F: 12.31 \pm 0.11 (40) M: 13.89 \pm 0.17 (37)	M > F
Tail length	None	92.18	1, 80	<0.0001	F: 7.84 \pm 0.12 (41) M: 9.95 \pm 0.19 (41)	M > F
SVL	LN	79.94	1, 80	<0.0001	F: 56.01 \pm 0.68 (41) M: 68.79 \pm 1.36 (41)	M > F
Total length	LN	90.79	1, 80	<0.0001	F: 63.85 \pm 0.76 (41) M: 78.74 \pm 1.48 (41)	M > F

Many of the variables were natural log-transformed (LN) to achieve normality but true means ± 1 SE are presented. F, female; M, male; N, number of individuals measured; SVL, snout–vent length.

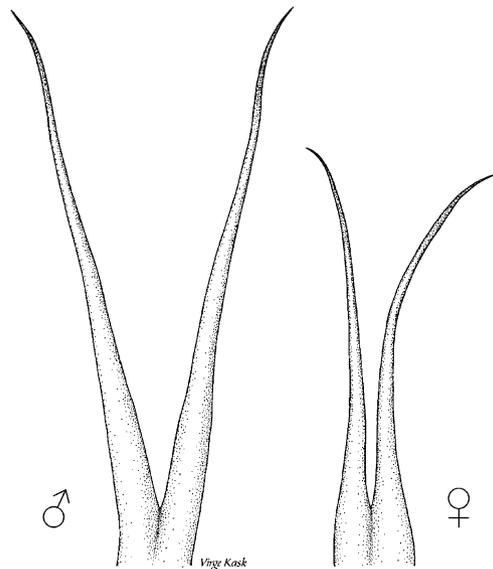


Figure 1 Illustration drawn from preserved specimens demonstrating the relationship between tine lengths and tongue widths in male (left) and female (right) copperheads *Agkistrodon contortrix*. Specimens depicted were equal in body size (snout–vent length).

confounding factors, we showed that tongue dimensions in adult male and female *A. contortrix* exhibit significant differences indicative of SSD. Specifically, TW and TL (degree of bifurcation) were greater in males than females. Although certain muscles (e.g. masseter) and structures of the head show SSD in other vertebrates (e.g. Widmer, English & Morris-Wiman, 2007), none detected SSD (or other types of SD) of the tongue *per se* (Schwenk, 1988; Maeda *et al.*, 1993; Iwasaki, 2002). Thus, to the best of our knowledge, we are the first to document SSD of the tongue in a tetrapod vertebrate.

Unlike other types of sexually dimorphic structures (e.g. antlers) that show seasonal changes and steroid dependence, we cannot at this point determine whether this is true for TL in male *A. contortrix*. Morphological changes related to season have been documented in other components of the VNS (Dawley, 1998; Halpern & Martínez-Marcos, 2003; Woodley, 2007). For example, significant seasonal changes in VNS cell volumes occur in some species of terrestrial salamanders (Dawley & Crowder, 1995; Dawley, 1998; Dawley *et al.*, 2000; Woodley, 2007). Thus, it is possible that similar annual modifications occur in TL in male snakes. Because all the specimens tested in this study were captured, euthanized and preserved during the mating season, a time when plasma sex steroids are elevated (Schuett *et al.*, 1997; C. F. Smith, 2007), further testing outside of the mating season will be required to address this question. Additionally, as noted by Fitch (1960), SSD is present in newborn *A. contortrix*, with males having significantly longer tails than females (for similar examples, see Schuett *et al.*, 2005; Krause & Burghardt, 2007). Thus, testing TL differences in neonates and juveniles of both

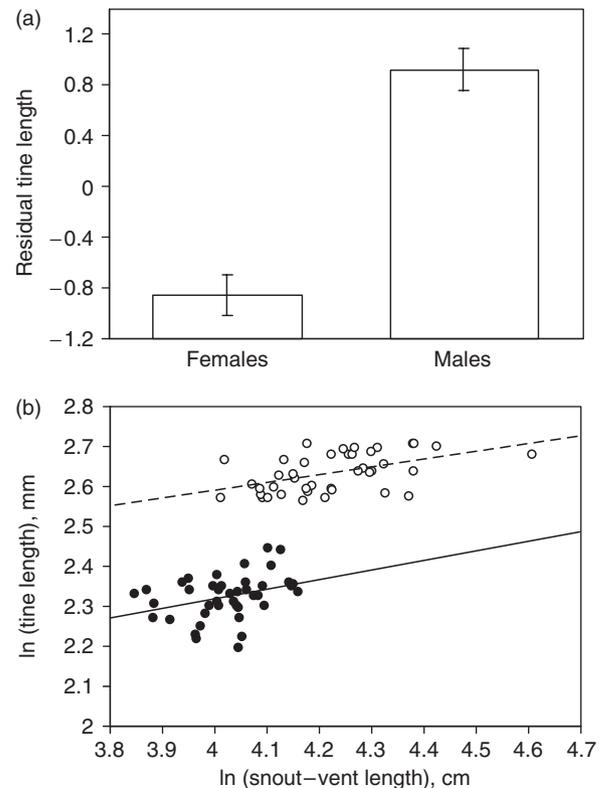


Figure 2 (a) Sex differences in residual tine length in adult male and female *Agkistrodon contortrix*. Values represent mean \pm 1 se. (b) Relationship between SVL and TL in male (open circles, dashed line) and female (solid circles, undashed line) *A. contortrix*. The axes represent natural log-transformed values for SVL and TL. The relationship for males was described by the equation: $TL = 1.81 + 0.196SVL$. The relationship for females was described by the equation: $TL = 1.36 + 0.240SVL$. Regression for untransformed values yielded similar results. Males: $TL = 11.38 + 0.037SVL$. Females: $TL = 7.64 + 0.046SVL$. SVL, snout–vent length; TL, tine length.

sexes will be necessary to address hypotheses regarding potential ontogenetic shifts in tongue form.

Beyond demonstrating the anatomical fact that male *A. contortrix* have greater TLs, the following question will require clarification: why do males of *A. contortrix* have larger tongues (TL) than females? Although multiple hypotheses will need to be tested, we currently favour the notion that increased TL in male *A. contortrix* (and possibly in other snake species) is a mechanism for increasing the ability to trail and locate sexually receptive females. Both male and female snakes use their tongues in searching for and locating prey, which, in some species, can be a substantial investment of time (Greene, 1997); however, we detected no sex difference in feeding frequency or diet composition in a Connecticut population of *Agkistrodon contortrix* snakes (Smith, 2007). In all snake mating systems studied thus far, males are the mate searching sex (Gillingham, 1987; Slip & Shine, 1988; Duvall *et al.*, 1993; see Kokko & Wong, 2007). In some cases, mate searching by males involves movements in the magnitude of several

Table 2 Correlations among morphological measurements for adult (A) female and (B) male *Agkistrodon contortrix*

	Tongue width	Head width	Head length	Mid-head width	Tail length	SVL	Total length
<i>(A)</i>							
Tine Length	0.4330 0.0047* 41	0.0807 0.6953 26	0.2299 0.1710 37	0.3048 0.0558 40	0.2549 0.1078 41	0.3604 0.0206* 41	0.3625 0.0198* 41
Tongue width		0.3119 0.1208 26	0.3170 0.0559 37	0.3715 0.0183* 40	0.5352 0.0003* 41	0.3176 0.0430* 41	0.3675 0.0181* 41
Head width			0.5647 0.0027 26	0.4587 0.0184* 26	0.6880 0.0001* 26	0.3574 0.0731 26	0.4241 0.0308* 26
Head length				0.8287 <0.0001* 37	0.7255 <0.0001* 37	0.7318 <0.0001* 37	0.7645 <0.0001* 37
Mid-head width					0.6220 <0.0001* 40	0.7459 <0.0001* 40	0.7651 <0.0001* 40
Tail length						0.6209 <0.0001* 41	0.7113 <0.0001* 41
SVL							0.9926 <0.0001* 41
<i>(B)</i>							
Tine Length	0.4802 0.0015* 41	0.1308 0.4830 31	0.4303 0.0088* 36	0.4628 0.0039* 37	0.3232 0.0393* 41	0.4854 0.0013* 41	0.4867 0.0013* 41
Tongue width		0.2878 0.1164 31	0.6425 <0.0001* 36	0.6983 <0.0001* 37	0.2378 0.1344 41	0.6305 <0.0001* 41	0.6093 <0.0001* 41
Head width			0.7065 <0.0001* 31	0.5587 0.0013* 30	0.5944 0.0004* 31	0.6203 0.0002* 31	0.6467 0.0001 31
Head length				0.8878 <0.0001* 35	0.5917 0.0001* 36	0.8634 <0.0001* 36	0.8651 <0.0001* 36
Mid-head width					0.5071 0.0014* 37	0.8650 <0.0001* 37	0.8548 <0.0001* 37
Tail length						0.6040 <0.0001* 41	0.6807 <0.0001* 41
SVL							0.9950 <0.0001* 41

The top number in each cell represents Pearson's correlation coefficient (r), the middle number the P -value and the bottom number the sample size for each correlation.

*Statistically significant correlation.

SVL, snout-vent length.

kilometres (Duvall *et al.*, 1993; Duvall & Schuett, 1997). Thus, the tongue and other structures of the VNS in male snakes (and some lizard taxa) are potential targets of sexual selection (Cooper, 1997).

In snakes, bifurcation of the tongue allows two points on a chemical trail (e.g. female pheromones, prey urine) to be sampled simultaneously (Schwenk, 1994). Moreover, this

anatomical arrangement allows the concentration gradient of the chemical trail between these two points to be assessed, and adjustments can be made towards the strongest signal to maintain contact with the trail (Ford, 1986; Schwenk, 1994). This type of movement is termed tropotaxis (Fraenkel & Gunn, 1961). Increased depth of the tongue fork (i.e. increasing TL) allows increased distance between sampling

points, with the actual distance between the two sampling points (the tongue tips) being a function of the absolute tongue size, TW, depth of the fork and the degree to which the tongue is spread during sampling (Schwenk, 1994). In some species of snakes, the distance between the tongue tips is wider than the head (Ford, 1986).

Based on this information, one mechanism for increasing the efficacy of the tongue in chemosensory searching (e.g. mate searching) is to increase the distance between the sampled points; specifically, to increase the distance between tongue tips, thus allowing a greater difference in signal strength between the two points sampled (Ford, 1986; Schwenk, 1994). Increasing the difference between the points sampled could be achieved in tongue structure by increasing TL (fork depth). As in other snake species, male *A. contortrix* search for females by tracking their pheromone trails (Schuett & Gillingham, 1988), a behaviour not documented in females (Gillingham, 1987; Duvall *et al.*, 1993). During the mating season, the females of a Connecticut population were widely dispersed and sexually receptive for a relatively brief period (c. 6 weeks) in late summer (Smith, 2007). Based on prior work, there should be strong intrasexual selection on males to locate receptive females quickly and efficiently (Duvall *et al.*, 1993). Thus, in accord with sexual selection theory, we would predict this heightened modification only in males, which is supported in this study (see Ford, 1986; Duvall *et al.*, 1993).

A question remains about whether males follow ground-based or air-borne pheromone trails to locate females. The ability of snakes to follow ground-based scent trails (of prey and conspecifics) using the forked tongue-VNS mechanism is well established (see Halpern, 1992; Schwenk, 1994), but their ability to follow air-borne chemical cues, in contrast, is poorly understood. In particular, the role of the forked tongue in locating and following such trails remains largely uninvestigated (Waters, 1993; Aldridge, Bufalino & Reeves, 2005; Parker & Kardong, 2005, 2006). We know that crustaceans such as lobsters and crabs use paired chemosensory appendages to detect and follow turbulent odour plumes in water much as snakes use their forked tongues (e.g. Koehl, 2006), and one study has demonstrated that snakes can use the forked tongue-trailing mechanism to the detect directionality of air-borne odours (Waters, 1993). Male snakes searching for mates in the field frequently encounter ground-based pheromone trails left by passing females and these trails can be followed directly to the female using the forked tongue and VNS (e.g. O'Donnell *et al.*, 2004). However, we know from anecdotal evidence that males can also locate receptive females that have not left any ground-based trails because they were only recently placed in the field by the investigator and their movements tracked (C. F. Smith, and G. W. Schuett & R.A. Repp, unpubl. data). In both such cases for which we have the most complete information, the male located a female that was upwind, according to the prevailing winds in the area for that day. It is possible that the need for detection and trailing of air-borne pheromones would result in even stronger selection on tongue fork depth in males than

ground-based odour tracking owing to the breadth, turbulence and temporal transience of such odour plumes (e.g. Atema, 1996; Weissburg, 2000; Moore & Crimaldi, 2004).

In conclusion, based on multiple lines of evidence derived from empirical studies, we suggest that greater TL in male *A. contortrix* increases trail-following abilities and thus increases opportunities to locate sexually active females (e.g. Ford, 1986; Schwenk, 1994; O'Donnell *et al.*, 2004). Male mate searching behaviour has been documented in many snake species; hence, SSD of the tongue is likely to be more widespread, especially in taxa where long-distance mate searching and trailing is common (Slip & Shine, 1988; Duvall *et al.*, 1993). While other hypotheses not directly related to sexual activities are important to test regarding tongue SSD in *A. contortrix*, mate trailing is most likely to be a critical factor.

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