Y. Kanno, N. Davidson, *Science* **149**, 295 (1965).
19. J. D. Sheridan, M. G. Hammer, R. G. Johnson, *J. Cell Biol.* **67**, 395a (1975); R. S. Decker, *ibid.* **70**, 412a (1976).
20. B. Rose and W. R. Loewenstein, *Nature (London)* **254**, 250 (1975); J. Popowich and S. Caveney, *J. Insect Physiol.* **22**, 1617 (1976).
21. B. Rose and W. R. Loewenstein, *J. Membr. Biol.* **28**, 87 (1976); N. B. Gilula and M. L. Epstein, *Symp. Soc. Exp. Biol.* **30**, 257 (1976).
22. M. J. Berridge, *J. Cyclic Nucleotide Res.* **1**, 305 (1975).
23. V. C. Maino, M. N. Green, N. J. Crumpton, *Nature (London)* **251**, 324 (1974); S. Toyoshima, M. Iwata, O. Toshiaki, *ibid.* **264**, 447 (1976).
24. S. D. Balk, J. F. Whitfield, T. Youdale, A. C. Braun, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 675 (1973).
25. R. W. Tsien and R. Weingart, *J. Physiol. (London)* **242**, 95P (1974); R. W. Tsien and R. Weingart, *ibid.* **260**, 117 (1976).
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Hemipenile Preference: Stimulus Control of Male Mounting Behavior in the Lizard *Anolis carolinensis*

Abstract. *The urogenital system of squamate reptiles is represented by separate, bilaterally symmetrical tracts. Males alternate in their use of the right and left hemipenes. Sensory feedback from the hemipenis and, to a lesser extent, from the ipsilateral testis is important in determining which hemipenis the male will use for mating.*

The squamate reptiles (lizards and snakes) are unique among amniote vertebrates in having bilaterally symmetrical intromittent organs. These structures, known as the hemipenes, can be highly ornamented, possessing numerous papillae, calyces, and spines. Indeed, hemipenile structure has long been used by herpetological systematists as an aid in classifying snakes (*1*). The functional significance of the paired hemipenes is less well known, however. I now report several experiments which indicate that, in the lizard *Anolis carolinensis*, sensory feedback from the hemipenis is important both in the male's initial orientation during mounting and in the termination of copulation. Further, propioceptive feedback from the testes has a similar but less pronounced influence on male copulatory posture. These findings suggest that the hemipenile alternation observed in successive copulations in *A. carolinensis* is due to feedback from both the testes and hemipenis. A possible function of this process might be to monitor the ability of each testis to deliver mature sperm.

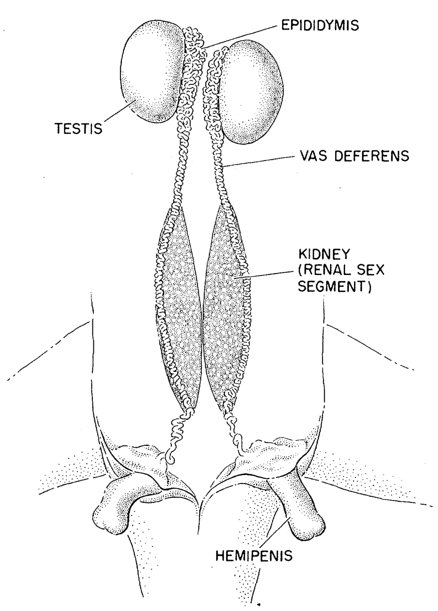

Fig. 1. Urogenital system of the male lizard *A. carolinensis*.

Courtship and copulation in the lizard *A. carolinensis* has been described in detail elsewhere (*2*). Briefly, the male advances toward the female, pausing to perform the courtship display. This display consists of a species-typical up-and-down bobbing movement of the body coordinated with extension of a brightly colored red throat fan or dewlap. If sexually receptive, the female will stand for the male, arching her neck as the male takes a neck grip (*3*). The male then straddles the female, swinging his tail beneath the female's to appose the cloacae. The male then inserts a single hemipenis into the female's cloaca; copulation lasts approximately 10 minutes, although individual males have characteristic mating times (*4*). If the male mounts on the female's right side, he will evert the left hemipenis and vice versa. Since the male curves his tail beneath the cloacal openings, it is possible to determine quickly which hemipenis he has inserted by the angle of deflection of his tail; for example, the male's tail will be curved to his right if he has intromitted with the right hemipenis.

In male lizards and snakes, the retracted hemipenes are paired membranous pouches in the base of the tail attached to the posterior wall of the cloaca (*1*). During mating, intromission is achieved with the eversion of one hemipenis through the male's cloacal opening. Dissections of the urogenital system of squamate reptiles reveal that the vas deferens from the paired abdominal testes do not enter into a common urogenital sinus as in other vertebrates. Instead, sperm are transported by each vas deferens to a seminal groove on the surface of the ipsilateral hemipenis (Fig. 1); thus, male lizards and snakes have two separate and functional reproductive tracts.

In my examination of the stimulus control of male copulatory behavior in this lizard, I have monitored the sexual behavior of individual males to determine whether they exhibit a preference for either the left or right hemipenis (*5*). In general, males alternate in their use of the right and left hemipenis (Table 1). Although some males exhibited a preference if given an extended series of tests (for example, males B and C), most males rarely mated with one hemipenis three or more times in succession [intercopulation interval (ICI): $\overline{X} = 1.28$ days, standard error (S.E.) = 0.10]. Differences in copulation times for the right and left hemipenis were not significantly different within individuals, although males did show individually specific copulation times [see also (*4*)].

Removal of one hemipenis dramatically altered male mating patterns (Table 1) (*6*); unilaterally hemipenectomized males assumed only that posture which enabled them to use the remaining hemipenis. The transition from preoperative alternation behavior to a postoperative preference was immediate, without any obvious "trial and error" on the part of

Table 1. Pattern of alternation in use of right (R) and left (L) hemipenis exhibited by intact and unilaterally hemipenectomized *A. carolinensis* during successive matings.

Lizard	Hemipenis used	Total Right	Total Left
	Intact normal		
2	LLRLRLRLRLR	5	6
3	LLRLRL	2	4
5	LRLLRLL	2	5
6	LLRLRRLR	4	4
A	RLRLRR	4	2
B	RRRLRR	5	1
C	RRRLRR	5	1
F	LRRRLR	4	2
G	RLRRLL	3	3
H	RLLRRL	3	3
J	RLRRLR	4	2
	Unilateral hemipenectomy		
3	RRRRRRRR	8	0
5	RRRRRRRR	8	0
6	RRRLRRRR	7	1
B	LLLLLL	0	6
C	LLLLLLLL	0	8
J	LLLLLLLL	0	8

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Table 2. Pattern of alternation in the use of right (R) and left (L) hemipenis exhibited by male *A. carolinensis* following different surgical alterations of the urogenital system. Since incision through the body wall might influence hemipenile preference, some animals were castrated through an incision on the contralateral side. Two of the unilateral castrations were ipsilateral (I) to the unilateral hemipenectomy, and two were contralateral (C).

Lizard	Intact testis	Hemipenis used	Total	
			Right	Left
		Bilateral hemipenectomy		
1		LLRLRR	3	3
2		RRLRLLR	4	3
		Unilateral castration		
3	R	RRLRRRLRLRLR	8	4
4	L	RRLLLRLLL	3	6
5	L	LLLLLRLL	1	7
6	R	RLRLRRLRRRLRR	9	4
7	L*	RLLLRLLLRLRRLLLRL	6	10
8	R*	LRLRLRRLRR	6	4
9	L*	LLRLRLLRL	3	6
10	R*	LRRLLRRRLRRLLRLR	9	7
		Bilateral castration		
11		LRRLRL	3	3
12		LRRRLRRLR	6	3
13		RRLRLRRLLRLRRLR	9	6
		Unilateral castration plus hemipenectomy		
14-I	R	RRRRLRRRRRRRRRRRL	15	2
15-C	L	RLLRRLR	4	3
16-I	L	LLLL	0	4
17-C	L	RRRLRRLRLL	6	4

*Incision contralateral to side of castration.

the male. In the single instance in which the male used the hemipenectomized side, the copulation time was shorter (6.15 minutes) than that exhibited normally (mating time: $\overline{X}$ = 14.23 minutes; range = 13.00 to 15.25). Unilaterally hemipenectomized males also tended to mate less often than intact males (ICI $\overline{X}$ = 3.24 days; S.E. = 0.61). Finally, duration of copulation was unchanged and was within the range of preoperative mating times for each male.

In a series of experiments to extend these findings, males were either hemipenectomized or castrated, and their mating behavior was observed (Table 2). Bilaterally hemipenectomized males continued to exhibit alternation in terms of copulation posture but mating frequency decline markedly (ICI $\overline{X}$ = 13.17 days; S.E. = 2.61); when mating did occur, copulation was prolonged as reported previously (*4*). Bilateral castrates failed to exhibit a preference for the right or left hemipenis (ICI $\overline{X}$ = 4.54 days; S.E. = 0.68), but unilateral castration resulted in a shift in hemipenile preference similar to but less dramatic than that exhibited by unilateral hemipenectomized males (ICI $\overline{X}$ = 2.67 days; S.E. = 0.22); unilaterally castrated males mounted normally but they tended to mate with the hemipenis contralateral to the castrated side (χ^2 = 9.32, $P < .01$).

Evidence that neural feedback from the testes is important in determining which hemipenis is used comes from experiments in which unilaterally castrated males were also unilaterally hemipenectomized on the contralateral or ipsilateral side. The two ipsilaterally castrated animals behaved like unilaterally hemipenectomized males and mounted almost exclusively on the side with the intact hemipenis (ICI $\overline{X}$ = 3.17 days; S.E. = 0.75). However, unilaterally hemipenectomized males failed to show this hemipenile preference if the contralateral testis was removed (ICI $\overline{X}$ = 3.25 days; S.E. = 0.70), which suggests that mounting behavior is influenced by neural feedback from both the hemipenile and testicular levels. The physiological basis of the alternation is unknown at this time, but the alternation observed in sexually active lizards may reflect the ability of each testis to provide adequate numbers of sperm for each copulation (*7*).

A number of studies indicate that sensory feedback from the penis is necessary for normal mating behavior in mammals. Surgical denervation or anesthetization of the glans penis of sexually experienced mammals results in the loss of erection and ejaculation and a decline in intromission frequency due to improper orientation by the male (*8*). The results of this study indicate that sensory feedback from the hemipenes is important in the initial orientation as well as in the termination of copulation in the male lizard. The existence of a unilateral neural connection between each testis and the contralateral magnocellular paraventricular and arcuate nuclei of the hypothalamus has been demonstrated recently in mammals which may play a role in neuroendocrine feedback (*9*). The finding that proprioceptive feedback from the testes may also play a role in male mating behavior is, to the best of my knowledge, unique. The discovery of a complete bilateral representation of the genital tract in squamate reptiles demonstrates the lizards and snakes to be a potential model system with which to study central versus peripheral control of behavior.

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References and Notes

1. E. D. Cope, *Rep. U.S. Natl. Mus.* **1898**, 153 (1900); H. G. Dowling and J. M. Savage, *Zoologica (N.Y.)* **45**, 17 (1960).
2. D. Crews, *Herpetologica* **31**, 37 (1975).
3. ——, *Horm. Behav.* **4**, 307 (1973).
4. ——, *Physiol. Behav.* **11**, 463 (1973).
5. Males were housed singly in wire mesh cubicles (22 by 30 by 8 cm) in a controlled environmental chamber (Sherer-Gillet-48-LLTP) programmed to provide a daily photothermal cycle (LD 14:10; 32°:23°C); relative humidity was maintained at 70 percent. Cubicles contained a water bottle, sticks for perching, and a food dish; mealworms and crickets were available at all times. A sexually receptive female was introduced into the male's cage each day and observed for 2 hours. If the male copulated with the female, the hemipenis used and the duration of copulation were noted. In those experiments with castrated males, sexual activity was maintained by a single subcutaneous 1.0 cm Silastic (Dow Corning) implant (inside diameter, 0.025 inch; outside diameter, 0.047 inch) containing approximately 5 mg of free testosterone. Testing was resumed within 1 week of castration. Both intact preovulatory females and ovariectomized, estrogen-induced females were used as stimuli females.
6. The hemipenis removed was chosen randomly for those males that had not exhibited an obvious preference; in those males with an apparent preference (males B and C), the preferred hemipenis was removed.
7. D. Crews, unpublished observations.
8. S. G. Carlsson and K. Larsson, *Z. Tierpsychol.* **21**, 854 (1964); N. T. Adler and G. Bermant, *J. Comp. Physiol. Psychol.* **61**, 240 (1966); L. R. Aronson and M. L. Cooper, in *Perspectives in Reproduction and Sexual Behavior*, M. Diamond, Ed. (Univ. of Indiana Press, Bloomington, 1966), pp. 51–82; F. A. Beach, *Hormones and Behavior* (Hoeber, New York, 1947).
9. A. N. van den Pol, paper presented at the 5th annual meeting, Society for Neurosciences, New York, 2 to 6 November 1975 (Abstract No. 708); I. Gerendal, K. Racz, B. Halasz, paper presented at the 5th International Congress on Endocrinology, Hamburg, 18 to 24 July 1976 (Abstract No. 592).
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