Sexual Parasitism in the Green Tree Frog (*Hyla cinerea*)

Abstract. *Noncalling adult male tree frogs were found in close association with about 16 percent of the calling males in a pond in Georgia. In 13 of 30 field experiments a noncalling satellite male intercepted and achieved amplexus with a gravid female moving toward the calling male. This mating strategy, which conserves energy required for calling, resembles the strategy employed by other vertebrates.*

Many male anurans attract mates by means of species-specific calls (*1*). In some hylids, ranids, microhylids, and bufonids noncalling males have been observed in close association with calling males (*2*). Garton and Brandon (*3*) and Fellers (*4*) observed noncalling male *Hyla cinerea* in association with calling adults. We observed these "satellite" males (*5*) in *Hyla cinerea* and *Hyla squirella* in Georgia and *Hyla versicolor* in Missouri. Two hypotheses regarding the biological significance of these associations have been suggested (*6*): (i) satellite males are waiting for call sites to become available after the calling male achieves amplexus (or stops calling for some other reason), and (ii) satellite males intercept females as they move toward calling males. We released sexually responsive females of *Hyla cinerea* near associations of calling and satellite males and found that satellite males frequently intercepted females as they moved toward calling males. Our results thus support the second hypothesis.

We studied *Hyla cinerea* in two ponds near Savannah, Georgia. We censused the frogs in the larger pond on nine nights to determine the frequency of calling and satellite male associations. Males less than 50 cm apart were considered to be associated. We started each census at least 1 hour after the males began calling and observed each association for at least 1 minute. Some associations terminated when a female or another male moved into the vicinity. Others persisted for at least 2 hours. On the average, about 16 percent of the calling males in our study pond had at least one satellite male (Table 1). We observed two satellites per calling male on nine occasions and three satellites per male once.

We conducted 30 field experiments between 10 p.m. and 1 a.m. Eastern Daylight Time from 19 June to 12 July 1977 to determine whether satellite males compete directly with calling males for females. In the smaller pond, we marked males for identification by freeze branding (*7*). In the large pond, we identified individuals by color pattern and size differences. In only three of the 30 experiments was the calling male significantly larger than the satellite male. We observed each association for at least 5 minutes before beginning an experiment. We then released a gravid female, found in amplexus on the same or a previous night, at least 1 m from the two males. The calling male and satellite male were separated by a mean distance of 20.5 cm [standard deviation (S.D.) = 11.5] when the female was released. The release site of the female relative to the positions of the males often was dictated by physical features of the pond and emergent vegetation. In four experiments the satellite male was between the female and calling male, and in six experiments the calling male was closer to the female release site. In 20 experiments the calling and satellite males were approximately equidistant from the release point. In ten experiments we observed the behavior of the animals with a dim artificial light; in the other experiments no artificial light was used after the female was released and began to orient to the calling male. The mean time from release of the female to amplexus was 9.2 minutes (S.D. = 6.3) (*8*).

Table 1. Frequency of satellite males on nine nights.

Date in July 1977	Number of males		Percentage of calling males in a satellite association
	Calling	Satelite	
6	133	22	16
7	122	20	16
8	128	24*	18
10	150	27†	18
11	152	22	14
12	148	23*	15
13	145	25†	17
14	139	24‡	17
15	120	20§	16
Total	1237	207	16
Mean	137.44	23.00	
S.D.	12.21	2.29	

*One of these associations involved a calling male with two satellite males. †Two of these associations involved a calling male with two satellite males. ‡Three of these associations involved a calling male with two satellite males. §One of these associations involved a calling male with three satellite males.

Table 2. Mating success of calling and satellite males.

Condition	Amplexus achieved by	
	Calling male	Satellite male
Artificial light	5	5
No artificial light	12	8

The results show that satellite males were successful in sexually parasitizing vocalizing conspecifics (Table 2). The initial positions of the two males relative to the release point had little or no effect on the mating success of the two males because the female seldom took a direct route to the calling male. Furthermore, the satellite male often moved rapidly toward the female, presumably as soon as he detected her movements. Satellite males also pursued other males which moved into the vicinity of an association. A slightly higher percentage of satellite males intercepted females when the artificial light was used, but the difference was not significant (*G* test) (*9*) in comparison with their success rate when no light was used.

Since we used females which had been in amplexus at the time of capture, it is possible that they were unusually responsive not only to mating calls but also to advances of noncalling males (*10*). This could have artificially inflated the success rate of the satellite males. Nevertheless, it is clear that satellite males are not merely subordinate males passively waiting for a calling site. In the 17 experiments where the calling male successfully achieved amplexus with the female, we observed the satellite for 1 minute or more following amplexus. In three instances the satellite male began calling; in one instance the satellite male left the area; and in 13 instances the satellite male remained silent in the area. These results fail to support the hypothesis that satellite males are waiting for call sites.

We often observed calling males trying to repel or avoid noncalling males. On many occasions the male stopped vocalizing and pursued frogs which moved in his immediate vicinity. Other males were repelled by means of encounter calls (pulsed vocalizations), butting, and wrestling. On ten occasions, when the calling male changed positions, the satellite male oriented toward the calling male and followed it for up to 2 m. Evidently the frogs detect one another by vision and tactile stimuli (the movement of the emergent vegetation when the animals hop). Satellite males often remained motionless near the calling male until the female or another male moved into the vicinity. Some males employed the satellite strategy from night to night while others changed strategies from night to night or on the same night. Ecological factors, such as the density of the population, the physical nature of the breeding site, and the length of the breeding season are important considerations for a fuller understanding of these two forms of male sexual behavior.

Although the conditions thought to be important for the evolution of divergent male reproductive strategies [for example, a great differential in male reproductive success where females are not a controllable resource (*11*)] are common in anuran breeding systems; previous observations of anuran sexual behavior in the field usually have been anecdotal. Satellite male behavior has been described in fish, iguanid lizards, elephant seals (*6*), and ruffs (*11*); however, the success rates of these satellites in intercepting females are unknown. Our experimental approach not only shows that noncalling male tree frogs try to intercept females but also provides an estimate of their success. The male satellite behavior may be found in other species that utilize well-defined signals as a means of mate location and selection.

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References and Notes

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→ J. G. van Rhijn [*Behaviour* **47**, 153 (1973)] first used the term "satellite" to describe males with this alternative mating strategy.

6. K. D. Wells, *Ecology* **58**, 750 (1977).
7. C. H. Daugherty, *Copeia* **4**, 836 (1976).
8. Ambient light intensities were usually sufficient to ascertain that the female had been clasped.
9. R. R. Sokal and F. J. Rohlf, *Introduction to Biostatistics* (Freeman, San Francisco, 1973), p. 299.
10. R. S. Oldham and H. C. Gerhardt [*Copeia* **2**, 223 (1975)] showed that females found in amplexus are more responsive to mating calls of another species (*Hyla gratiosa*) in single stimulus experiments than are females found unclasped and induced to a responsive state by pituitary injections. B. S. Martof and E. F. Thompson, Jr. [*Behaviour* **13**, 243 (1958)] described the precision with which a female *Pseudacris triseriata* avoids noncalling males during her approach to the calling male. As stated, our experimental animals usually took indirect routes, but noncalling males actively pursued them. We do not know whether or not a female which is in the early stages of responsiveness to mating calls could avoid amplexus with a noncalling male. Also, females could have rejected the males later since we did not watch the pairs to the end of oviposition.
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Adrenocorticotropin in Rat Brain: Immunocytochemical Localization in Cells and Axons

Abstract. *By means of antiserum (purified by affinity chromatography) directed against adrenocorticotropin (ACTH) 11-24, cell bodies and beaded axons were visualized in rat brain. The ACTH-like immunoreactivity (ACTH-LI) was primarily located in the hypothalamus (cells and axons). Fibers were scattered throughout thalamus, amygdala, periaqueductal gray area, and reticular formation. There was no change in the distribution of ACTH-LI in rats that had been subjected to hypophysectomy. This distribution of ACTH-LI parallels that of β-lipotropin and is altered by specific lesions in a similar fashion. The presence of ACTH-LI in cells and beaded axons in brain raises the possibility that it is a neuroregulator functioning as a neurotransmitter, neuromodulator, or neurohormone.*

Adrenocorticotropin (ACTH) is a peptide synthesized in the anterior and intermediate lobes of the pituitary gland (*1*). In addition to its role in regulation of the adrenal cortex, ACTH is thought to have a role in stress, motivation, learning, and memory (*2*), and has been reported to bind to opiate receptors in rat brain,

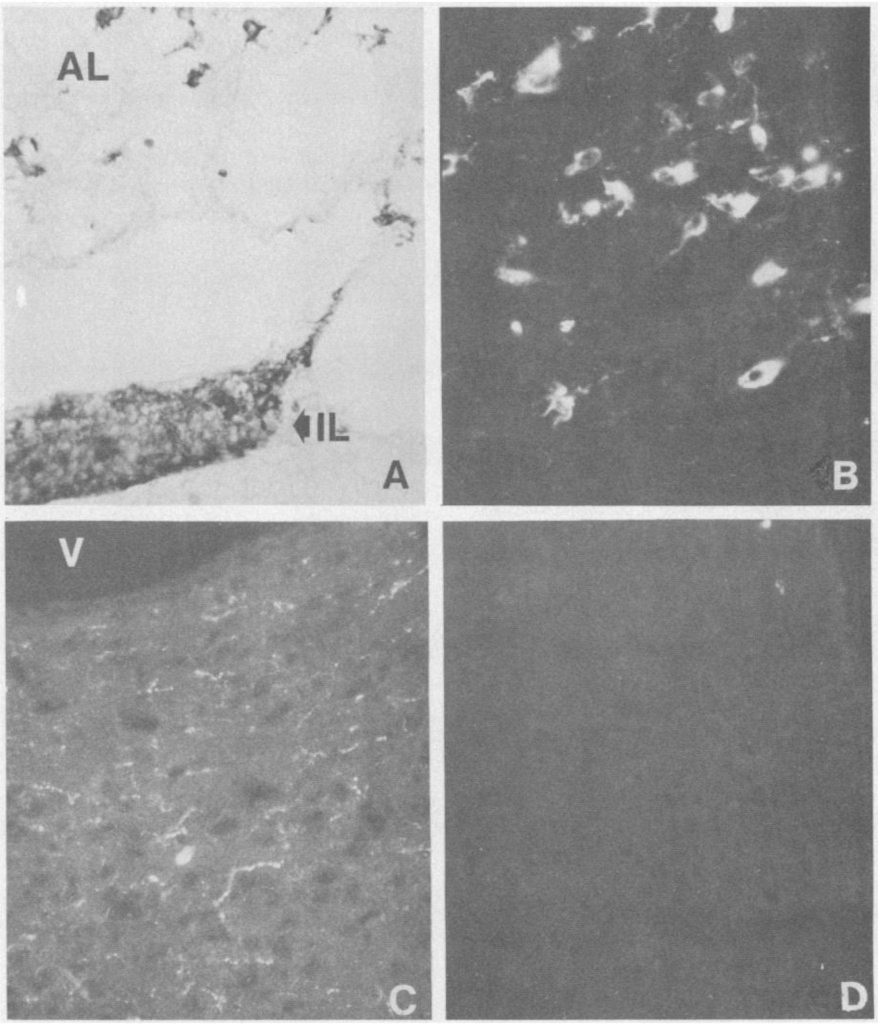

Fig. 1. (A) Adrenocorticotropin-positive cells in the anterior lobe (*AL*) and intermediate lobe (*IL*) of a normal rat pituitary showing unstained nuclei (×270; horseradish peroxidase stain). (B) Adrenocorticotropin-positive cells in the basal hypothalamus (arcuate region) of a colchicine-treated (50 μg, intracerebroventricularly) rat showing unstained nuclei (×270; fluorescein stain). (C) Adrenocorticotropin-positive fibers in the dorsomedial hypothalamic nucleus of a colchicine-treated rat. Note the fine beaded appearance (*V*, third ventricle) (×270; fluorescein stain). (D) Control for (B). Antiserum to ACTH and 100 μ*M* ACTH 1-24 was incubated on a serial section adjacent to that seen in (B). The photograph was taken of the arcuate region similiar to that in (B). There were no ACTH-positive structures in any such slides. (Colchicine-treated rat; section ×270; fluorescein stain.)