per session. By using a sample size of, say, 12 and assuming 100 percent discrimination accuracy, we can make probability estimates for the selection of drug or vehicle levers. Thus, no more than three rats could choose the vehicle lever for P to equal .05. If nine or more rats selected the drug lever, then the treatment condition was subjectively identical to the training dose of PCP, with $P < .05$. When four to eight of the 12 rats chose the PCP lever, responding was considered different from both training conditions (that is, not significantly different from 50 percent).
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12. A higher dose of D-PIA (3.2 mg/kg) blocks PCP cuing. This agrees with the correspondingly weaker ability of D-PIA to bind to adenosine receptors [R. F. Bruns, J. W. Daly, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5547 (1980); J. W. Daly, R. F. Bruns, S. H. Snyder, *Life Sci.* **28**, 2083 (1981)].
13. Observations of the animals given CHA or L-PIA in combination with PCP indicate that the adenosine analogs tested do not completely antagonize all the behavioral effects of PCP. Thus, doses of CHA which block PCP cuing did not antagonize the locomotor hyperactivity produced by PCP. Similarly, administration of adenosine analogs did not alter the median lethal dose of PCP.
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15. A reduction in the percentage of drug-associated responses to about 50 percent following various treatments is not uncommon in drug discrimination paradigms. We have observed a nonspecific reduction in PCP discriminability similar to that seen with haloperidol and diazepam (Table 2) when rats were treated with subtoxic doses of vasopressin, doxapram, and naloxone. In contrast, agents including physostigmine, scopolamine, tacrine, mecamylamine, diphenhydramine, methysergide, cyproheptadine, cinanserin, clonidine, prazosin, propranolol, baclofen, clozapine, sulpiride, phenytoin, Metrazole, and a number of purine derivatives all failed to reduce PCP-associated discrimination below the 75 percent level, even at doses high enough to frequently produce a significant disruption of responding.
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18. In studies with whole rat brain membranes in 50 m*M* tris (*p*H 7.7) we were not able to displace labeled CHA with PCP or labeled PCP with CHA. In the latter case, however, high levels of nonspecific binding may have obscured displacement.
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Female Control of Male Reproductive Function in a Mexican Snake

Abstract. *Male* Thamnophis melanogaster *court immediately when exposed to estrogen-treated, attractive females and continue courting for 6 to 8 days. Males exposed to estrogen-treated females will court both intact and ovariectomized females. These males undergo a period of testicular recrudescence, whereas males exposed only to ovariectomized females do not. Sexual attractivity can be induced in female* T. melanogaster *without estrogen treatment by heavy feeding, which results in significant increases in liver size and activity.*

The semiaquatic garter snake *Thamnophis melanogaster* is active throughout the year at Lago de Cuitzeo, Michoacan, Mexico (*1*). Several brief, synchronous breeding periods can occur each year, and the timing of these periods may vary within and between years. We report that female *T. melanogaster*, treated with estrogen, elicited a period of sexual activity in males both in the field and the laboratory. This period of sexual activity lasts 6 to 8 days, with males becoming less discriminatory and courting ovariectomized and intact untreated females as well as estrogen-treated females. Measures of testicular activity increased after sexual activity ceased in males exposed to estrogen-treated females both in the field and the laboratory. Female sexual attractivity increased after extensive feeding. The results indicate that (i) female *T. melanogaster* initiate the breeding periods and in turn stimulate testicular activity in males, and (ii) female *T. melanogaster* can become attractive and initiate the onset of a breeding period in response to nutritional condition.

Perception of a pheromone present on the skin of attractive female *Thamnophis* is necessary to elicit courtship behavior from male conspecifics (*2, 3*). This attractiveness pheromone is synthesized in the liver and is chemically related to vitellogenin, the circulating precursor of yolk (*3*). Sexual attractivity can be induced in female *Thamnophis* by treating them with exogenous estrogen (*4–7*), which stimulates the liver. Conversely, ovariectomy renders female *Thamnophis* unattractive (*4*).

Ten males, maintained in isolation cages, were offered two females daily in behavioral tests (*7*). Ovariectomized females were offered for the first 5 days, and no courtship activity was observed. Beginning on day 6, estrogen-treated intact females (*5–7*) were offered and were immediately courted. After day 13 no additional courtship activity was observed even though estrogen-treated females were changed every 5 days. However, ovariectomized females, when offered to males in daily alternation with estrogen-treated females, were courted after day 3, although not to the extent that estrogen-treated females were. We thus hypothesized that breeding periods could be initiated in nature by some females becoming attractive in a cyclic fashion. Males exposed to those females would then court other females, and a synchronous breeding period could result.

At Lago de Cuitzeo, two adjacent populations of approximately 300 snakes each were monitored for 7 days; no sexual activity was observed in either population. Twenty percent of the adult females of the test population ($N = 28$) were collected, marked, treated with estradiol (*5–7*), and released. After 1 hour and 15 minutes, three males were observed courting a treated female. Sexual activity continued for 7 days in the test population; no sexual activity was observed in the control population. Males collected after courtship activity had ceased were more reproductively active than males collected before the breeding period, as judged by measures of testicular activity (Table 1). In the laboratory, groups of males, killed at intervals during a period of controlled daily exposure to estrogen-treated females, showed increases in serum androgens and spermio-

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Table 1. Response of male *T. melanogaster* to the presence of estrogen-treated conspecifics. Five males were collected immediately before the release of 28 estrogen-treated intact females into a natural population of 300 snakes (before exposure). Males were collected while courting or copulating (sexually active) and on day 8, when sexual activity in the population had ceased (five snakes per sample). All snakes were killed by Nembutal overdose within 1 hour of capture and weighed and measured; blood was collected from the heart. The animals were then fixed in 10 percent neutral buffered Formalin. Males in all groups had similar body length (average, 54.6 ± 1.3 cm; $N = 15$) and body weight (average, 100.5 ± 7.3 g; $N = 15$). Serum androgens were assayed by specific radioimmunoassay (*10*). Relative testes weight of males exposed to estrogen-treated females increased, but not significantly. Both seminiferous tubule diameter and serum androgens increased significantly in the exposed groups [$F(2,12) = 14.44$, $P < .001$, and $F(2,12) = 5.73$, $P < .025$, respectively). Spermiogenesis was graded on a scale from few to many transforming sperm.

Treatment group ($N = 5$)	Testes weight (percent of body weight)	Seminiferous tubule diamenter (μm)	Serum androgens (pg/ml)	Spermiogenesis
Before exposure	0.69 ± 0.08	161.1 ± 12.7	889 ± 123	Few to many
Sexually active	1.03 ± 0.16	248.4 ± 14.9	3143 ± 585	Many
Sexually inactive	0.86 ± 0.07	282.4 ± 20.7	3818 ± 935	Many

genic activity after sexual behavior ceased (Fig. 1).

Female *T. melanogaster* can initiate a breeding period and alter the reproductive state of males. Because of the strong connection between nutritional status and reproduction (*8*), we maintained three groups ($N = 4$ per group) of females on different dietary regimens in the laboratory (*9*). Only well-fed females became sexually attractive, but there was no statistically significant change in ovarian weight between the three groups. Relative liver weight of well-fed females was nearly twice that of the starved group (well fed, 3.9 ± 0.4 percent of body weight; low fed, 2.2 ± 0.2 percent; starved, 2.2 ± 0.1 percent; $F(2,9) = 12.74$, $P < .005$). Further, serum calcium (*10*) was highest in the well-fed group (well fed, 17.0 ± 0.4 mg per decaliter of serum; low fed, 15.4 ± 1.4 mg; starved, 12.7 ± 1.1 mg; $F(2,9) = 4.33$, $P < .05$). Liver size and serum calcium in female *T. melanogaster* are both increased by estrogen treatment (*11*). However, serum estradiol of well-fed females was low (well fed, 10 ± 1 pg per milliliter of serum; low fed, 507 ± 99 pg; starved, 512 ± 68 pg; $F(2,9) = 17.4$, $P < .001$). These findings suggest that feeding may promote estrogen binding to the liver (*12*). The disappearance of estradiol from the circulation of well-fed females may reflect increased binding. Thus, the liver may be stimulated to produce vitellogenin (that is, pheromone) in well-fed females without increasing steroid synthesis.

The introduction of ovariectomized female rhesus monkeys, which had been continually treated with estrogen, into a feral troop was shown to accelerate the normal breeding season by 1 month (*13*). Serum testosterone concentrations increased in male rhesus monkeys (*14*), wallabies (*Macropus eugenii*) (*15*), and ringdoves (*16*) that were exposed to sexually mature intact females. Chronically estrogen-treated female sparrows can prolong the breeding season and raise serum testosterone in males (*17*). However, our data show that female *T. melanogaster* can elicit male reproductive behavior and alter male reproductive state after sexual activity has ceased. Furthermore, nutritional status of the female, communicated by a pheromonal cue, appears to be the proximate factor driving the reproductive cycle in this species and may also explain the variability in timing of breeding periods in nature of this snake as well as many tropical and subtropical animal species.

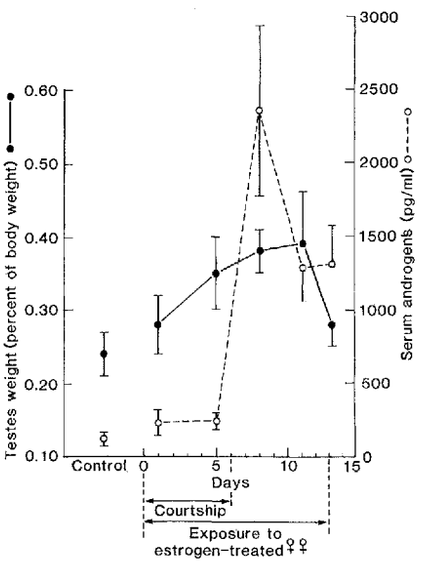

Fig. 1. Reproductive state of male *T. melanogaster* in the laboratory was altered by exposure to attractive females. Males were offered two estrogen-treated intact females daily for 2 minutes each, and behavior towards the females was scored (*10*). Pairs of stimulus females were changed every 5 days. Groups of five males each were killed on days 1, 5, 8, and 11; on day 13, ten males were killed. Males were sexually active through day 6. A control group ($N = 5$) was exposed to ovariectomized females in a similar testing regimen, and these were killed on day 5; none were sexually active. Males did not differ significantly in body length (average, 49.9 ± 1.1 cm) or body weight (average, 68.6 ± 4.8 g) between groups. Serum androgens increased tenfold [$F(4,25) = 9.80$, $P < .001$] after the courtship period. Relative testes weight increased but not significantly [$F(4,25) = 1.99$, $P > .10$]. Spermiogenic activity was greatest in the groups killed on days 8 and 11.

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9. Well-fed females received 50 g each of a chopped-fish diet once a week for 5 weeks; low-fed females received 5 g each of the same diet once a week; and the starved group was not fed. The females were initially of similar body length (average, 53.5 ± 4.5 cm; $N = 12$) and body weight (average, 138.8 ± 18.6 g; $N = 12$). The starved group lost 7.2 ± 2.1 percent of body weight, the low-fed group lost 3.2 ± 0.8 percent, and the well-fed group gained 22.3 ± 7.8 percent.
10. Calcium is bound to vitellogenin in snake serum and thus serves as an index of vitellogenin concentration [H. Dessauer, W. Fox, N. Gilbert, *Proc. Soc. Exp. Biol. Med.* **92**, 299 (1956)].
11. Liver: untreated ($N = 5$), 2.2 ± 0.1 percent of body weight; treated ($N = 8$), 4.1 ± 0.2 percent; $t = 4.78$, $P < .001$; calcium: untreated ($N = 5$), 12.7 ± 0.8 mg/dl; treated ($N = 8$), 232.5 ± 25.9 mg/dl; $z = 71.36$, $P < .001$.
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