19. R. Jones and G. Vrbová, *J. Physiol. (London)* **236**, 517 (1974); R. Jones and F. Vyskočil, *Brain Res.* **88**, 309 (1975).
20. T. Lømo and R. H. Westgaard, *Cold Spring Harb. Symp. quant. Biol.* **40**, 263 (1976).
21. Section of the radicular nerve was performed just outside the vertebral canal where it is easily accessible between the lateral vertebral processes, immediately cranial to the iliac spine. This is a simple procedure which avoids possible complications due to the opening of the dural space necessary to reach the ventral root.
22. The amount of denervation obtained in EDL muscles of different rats varied roughly between 40 and 90 percent, whereas there was much less variability between the two sides of the same rat. This was assessed by measuring tetanic tension following sciatic nerve stimulation at 100 pulses per second, 24 to 30 hours after root section. At this time practically all fibers innervated by the sectioned root must have been no longer responsive to indirect stimulation, because of the rapid degeneration of axon terminals consequent to axotomy (*23*). That this was the case also in our experimental condition, which was characterized by a long nerve stump, was demonstrated by sectioning all the radicular nerves giving rise to the sciatic nerve in several rats and measuring indirect tetanic tension 24 hours later. No tension was measurable in some cases whereas in others it was negligible. Accordingly, in a similar series of completely denervated EDL muscles we found no fibers, 20 hours after denervation, exhibiting MEPP's in experiments in vitro recording at the end-plate region (160 fibers tested in four muscles).
23. R. Miledi and C. R. Slater, *Proc. R. Soc. Ser.* B **169**, 289 (1968).
24. The physiological solution bathing the muscles was equilibrated with 95 percent O_2 and 5 percent CO_2 (*p*H 7.2) and maintained at 28° to 29°C. The amount of resistance to TTX of each fiber was characterized by the rate of rise of its action potential given by the amplitude of the electronically recorded first derivative of the action potential.
25. M. R. Bennett, E. M. McLachlan, R. S. Taylor, *J. Physiol. (London)* **233**, 481 (1973); J. J. McArdle and E. X. Albuquerque, *J. Gen. Physiol.* **61**, 1 (1973).
26. Average values were 2.86 ± 0.18 (standard error) for the junctions of normal control muscles and 2.71 ± 0.10 for the innervated junctions of the partially denervated muscles. In the latter a few fibers (19 out of 270) had indeed a MEPP's frequency that was lower (range, 0.08 to 0.43 second^{-1}) than in any of the normal fibers (see Fig. 2C) and they must represent freshly reinnervated fibers. This is also indicated by the fact that they had a resistance to TTX that was higher than that of the remaining population of innervated fibers (means, 276.8 ± 42.0 volt/second and 126.1 ± 6.1, respectively; $P < 0.001$) and even higher than that of the denervated fibers (mean, 183.5 ± 6.6; $P < .02$).
27. At this time new junctions cannot have formed, whereas MEPP's have disappeared in all denervated fibers (*22*). To minimize variability of innervation, most of the muscles (12) of this group were from one side of animals in which TTX resistance was measured later (that is, at 48 to 72 hours) on the opposite side.
28. This investigation was supported by research grants from the Muscular Dystrophy Association of America and from the Consiglio Nazionale delle Ricerche of Italy.

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Pheromonally Induced Sexual Maturation in Females: Regulation by the Social Environment of the Male

Abstract. *Social subordination, which suppresses gonadal function in juvenile and adult male house mice, also suppresses the activity of an androgen-dependent urinary pheromone that accelerates the rate of sexual maturation in juvenile females. Pheromone production may also be suppressed by the presence of pregnant or lactating females. This suggests that the social environment may influence the fertility of population females by altering urinary pheromone activity in the male.*

Social stresses resulting from high population densities may be important factors regulating mammalian populations under natural conditions. Christian (*1*) proposed that ecological and social stresses associated with high or increasing populations would, by increasing adrenal function and reducing gonadal function, interfere with an organism's growth, reproduction, and general physiological homeostasis. Although the initial emphasis in this regulatory system was on the role of intensified male-male social interactions and the associated pathologies resulting from such stress (*2*), recent studies have emphasized the inhibition of reproductive function in both juvenile and adult females as primary correlates of increasing density in a population (*3*). The social environment of the female, however, may consist of two antagonistic influences affecting fertility. On the one hand, stimuli from other females act to reduce reproductive potential by inhibiting sexual maturation in juvenile females (*4*) or by inhibiting estrus in grouped adults (*5*). In contrast, adult males produce androgen-dependent urinary priming pheromones that accelerate sexual development in juvenile females and induce estrus in grouped, anestrous adults (*6*). From a theoretical perspective the interactions between male stimulation and female inhibition of reproductive function could be important determinants of female fertility in a population.

The capacity of the urine from male mice to stimulate puberty in females is quantitatively regulated by the androgen state of the male (*7*). The chemical stimulus (pheromone) responsible for this effect is associated with the protein fraction of urine and has a molecular weight of approximately 860 (*8*). We demonstrate here how social factors that alter gonadal function in the male also alter the activity of the male's urinary pheromone and thereby may supply a mechanism for the density-dependent regulation of female fertility by the males of a population. To do this we used two experimental conditions known to cause changes in testicular function: cohabitation of dominant and subordinate males (*9*) and cohabitation of juvenile with adult males (*10*). We hypothesized that urine from adult males subordinate to their cagemates or from juvenile males exposed to adult males would be less effective in stimulating female maturation than urine from either dominant males or urine from juvenile males housed with females of the same age.

The ability of urine from male mice to accelerate the onset of puberty in females was assayed by measuring the uterine weight of prepubertal females after application of 0.03 to 0.05 ml of male urine to the oronasal groove daily for 8 days. Mean uterine weight was used as an index of pheromonal activity (*11*).

To determine if social subordination could influence pheromone activity, 34 male mice were caged individually at 21 days of age. At 70 days, 17 of these males were randomly chosen and trained as fighters to create the dominant subjects (*12*). The remaining 17 males were paired continuously with one of the trained animals. Five pairs were eliminated from the study because dominance was reversed during the experimental period. After 1 week under these conditions, urine was collected daily for 8 days from each male, pooled by group, and delivered to corresponding groups of females for assay. A control group of females received water.

Urinary pheromonal activity of subordinate animals was significantly less than that of dominant animals (Table 1). There were essentially no differences in uterine weight between those females receiving urine from subordinate males and those receiving water. We killed the remaining pairs of males and weighed their adrenal glands, testes, and seminal vesicles. Subordinate animals had significantly heavier adrenals (5.94 ± 0.46 mg compared to 4.92 ± 0.29 mg; $P < .01$, *t*-test) and significantly lighter testes (211.9 ± 15.5 mg

Table 1. Mean body weight and uterine weight (± standard error) of juvenile female mice ($N = 15$ per treatment) exposed for 8 days to urine from dominant or subordinate males that had been paired for 1 week; N.S., not significant.

Treatment	Body weight (g)	Uterine weight (mg)
Water control	17.5 ± 0.3	45.8 ± 6.8
Urine, subordinate male	18.0 ± 0.4	47.6 ± 6.7
Urine, dominant male	17.9 ± 0.3	73.4 ± 7.8*
Analysis of variance (d.f. = 2, 42)		
	$F = 0.79$ N.S.	$F = 4.72$ $P < .025$

*Mean significantly different from those for controls and urine-subordinate males [$P < .05$, least significant difference method (*17*)]

Table 2. Mean uterine weights (± standard error) with corresponding analysis of variance for juvenile female mice exposed to urine from young males that were paired either with identically aged, nonsibling females or with adult males. Uterine weights for assay controls were 21.2 ± 2.7 mg for water-treated animals and 44.0 ± 8.4 mg for animals receiving urine from isolated, adult males ($P < .01$).

Cohabitation condition of young males (treatment)	Age of males (days)			
	30	44	58	72
Young females	32.5 ± 7.7	42.4 ± 10.1	44.8 ± 8.5	22.9 ± 3.8
Adult males	18.3 ± 3.2	26.1 ± 4.7	37.2 ± 7.1	46.7 ± 11.0
	$P < .05$*	$P < .01$*	N.S.*	$P < .01$*

Source	Analysis of variance			
	Days 30 to 58		Days 30 to 72	
	d.f.	F	d.f.	F
Treatment	1	5.20†	1	0.94
Age of males	2	4.40†	3	2.77‡
Treatment × age	2	0.34	3	2.73‡
Error	89		119	

*Least significant difference method (*17*). †$P < .02$. ‡$P < .05$.

compared to 245.5 ± 9.4 mg; $P < .05$) than dominant males, a result consistent with previous reports (*9*). There was, however, no significant difference in seminal vesicle weights between the two groups. Since the production of the urinary pheromone responds rapidly to changes in androgen levels (*7*), the lack of a correlative difference in seminal vesicle weight may have resulted from a lower sensitivity or a slower rate of response to changes in androgen levels of seminal vesicles than of urinary pheromone.

In the second experiment we determined if cohabitation with adult males would alter the onset of pheromone production in young males. Forty 21-day-old males were divided randomly into two equal groups. Mice from one group were paired with adult males and the others with identically aged, nonsibling juvenile females. The female and adult male stimulus animals were rotated every 5 days to avoid habituation and thereby maintain high levels of aggression by the adult males. We collected and stored (*13*) urine from juvenile males exposed to adult males and to juvenile females at 30, 44, 58, and 72 days of age. These eight urine samples were assayed for pheromonal activity in eight groups of 15 to 16 juvenile females. As assay controls, two additional groups of 16 females received either water or urine from isolated, adult males (*14*).

Pheromonal activity in maturing males increased with age under both cohabitation conditions between 30 and 58 days of age (Table 2). By 72 days, however, there was a significant suppression of pheromonal activity in the urine from the female-exposed animals. This resulted in a significant treatment × age interaction in the analysis of variance for days 30 to 72 (Table 2). By excluding the day-72 uterine weight data from the analysis, a significant treatment effect was found between days 30 and 58. This indicated a significant delay in the appearance of pheromonal activity in the urine of animals housed with adult males relative to that in males housed with comparably aged females. Coincident with this day-72 suppression of pheromonal activity was the production of litters by the stimulus females, beginning on day 58 and continuing throughout the experiment. These results suggest that male pheromone production is depressed not only by the presence of other males but also may be suppressed by pregnant or lactating females. In total, our results show that social factors capable of suppressing gonadal function in male mice also reduce their capacity to stimulate pheromonally sexual maturation in females.

Clarifying the role of priming pheromones in rodent population dynamics has posed an elusive problem. A purely stimulatory system as suggested by Whitten and Bronson (*15*) is inconsistent with laboratory and field data indicating that fertility is suppressed in spite of abundant male stimuli (*2, 3*). Our data suggest that intense male-male social interactions occurring under high density populations, social subordination, and possibly the presence of strange, pregnant, or lactating females (*16*) may suppress pheromonal activity in the male by suppressing gonadal function. The suppression of female maturation by other adult or juvenile females (*4*) in combination with the reduced capacity of stressed young and low-ranking adult males to overcome this inhibition may be used as a model to explain, in part, suppressed fertility in populations where males are abundant. Androgen-dependent priming pheromones of the male may play an integrative role in controlling fertility in a population as a function of population density.

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References and Notes

1. J. J. Christian, *J. Mammal.* **31**, 247 (1950).
2. ——— and D. E. Davis, *Science* **146**, 1550 (1964).
3. J. J. Christian, *Biol. Reprod.* **4**, 248 (1971); *Horm. Correl. Behav.* **1**, 205 (1975); R. C. Terman, *Ecology* **49**, 1169 (1968); *J. Reprod. Fertil. Suppl.* **19**, 457 (1973); J. A. Lloyd and J. J. Christian, *J. Mannal.* **50**, 49 (1969).
4. L. C. Drickamer, *Dev. Psychobiol.* **7**, 257 (1974); J. G. Vandenbergh, *J. Reprod. Fertil. Suppl.* **19**, 411 (1973).
5. W. K. Whitten, *J. Endocrinol.* **18**, 102 (1959).
6. F. H. Bronson and W. K. Whitten, *J. Reprod. Fertil.* **15**, 131 (1968); D. R. Colby and J. G. Vandenbergh, *Biol. Reprod.* **11**, 268 (1974).
7. J. R. Lombardi, J. G. Vandenbergh, J. M. Whitsett, *Biol. Reprod.* **15**, 179 (1976).
8. J. G. Vandenbergh, J. S. Finlayson, W. J. Dobrogosz, S. S. Dills, T. A. Kost, *ibid.*, p. 260.
9. F. H. Bronson and B. E. Eleftheriou, *Gen. Comp. Endocrinol.* **4**, 9 (1964); J. A. Lloyd, *Proc. Soc. Exp. Biol. Med.* **137**, 19 (1971); T. D. McKinney and J. N. Pasley, *Gen. Comp. Endocrinol.* **20**, 579 (1973).
10. T. D. McKinney and C. Desjardins, *Biol. Reprod.* **9**, 370 (1973); J. G. Vandenbergh, *J. Reprod. Fertil.* **24**, 383 (1971).
11. House mice originally derived from the Swiss-Webster strain were maintained in rooms with a 14 : 10 light-dark cycle and controlled temperature and humidity (22° ± 2°C and 30 to 70 percent relative humidity, respectively). Females were born in a room containing no adult males. At 2 days of age litters were culled to a maximum of eight pups; males were discarded with the exception that at least one male was represented in each litter. When weaned at 21 days, females between 8.5 and 11.5 g were housed individually on clean Sanicel bedding in 18 by 28 by 11 cm polypropylene cages, moved to an animal room containing only these females, and divided into groups by the split-litter technique. Experimental treatments were then randomly assigned to these groups. The uterine weight bioassay yields results comparable to those in which pheromonally accelerated maturation is measured by the first occurrence of a cornified vaginal epithelium. [J. G. Vandenbergh, J. M. Whitsett, J. R. Lombardi, *J. Reprod. Fertil.* **43**, 515 (1975)]. Also, a dose-dependent relationship exists between urinary pheromone activity as measured by uterine weight and the androgen state of the donor males (*7*).
12. We used the "dangling" method of J. P. Scott [*Comp. Psychol.* **39**, 379 (1946)]. Training lasted 5 minutes per day for 1 week before the introduction of the naive (subordinate) animals.
13. After centrifugation fresh urine was sterilized through a 0.45-μm Swinnex Millipore filter and frozen at −14°C until used. Urine can be stored in this way for several months with no apparent effect on pheromonal activity (*7*).
14. Seasonal differences in the rate of sexual maturation of female mice occur under controlled laboratory conditions, but the effect of pheromonally active stimuli can be detected year-round [see Vandenbergh *et al.* in (*11*)]. This seasonal effect resulted in the lower uterine weights in both the water control and active groups in experiment 2.
15. W. K. Whitten and F. H. Bronson, *Adv. Chemoreception* **1**, 309 (1970).
16. Since our experiments were completed it has been demonstrated that pregnant and lactating females become highly aggressive toward strange male intruders [E. J. Noirot, J. Goyans, M. C. Buhot, *Horm. Behav.* **6**, 9 (1975); B. Svare and R. Gandelman, *Physiol. Behav.* **14**, 31 (1975)].
17. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969).
18. We thank C. Bradley for technical assistance and R. Oppenheim and J. M. Whitsett for critically reviewing the manuscript. Supported by NIMH grant 16870 to J.G.V.

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