vitellogenic growth, as reported by R. W. Gwadz and A. Spielman [*ibid.* **19**, 1441 (1973)].
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19. *Aedes aegypti* larvae, Segemaganga strain, were fed increasing daily portions of yeast, lactalbumin, and laboratory rat chow. "Unfed" adults were allowed 5 percent sucrose, maintained at 27°C, and were given the injections in the abdomen 2 to 5 days after emergence. While follicle separation started approximately 24 hours after the first injection, it was generally slower than after blood feeding; therefore we routinely examined the germariums 2 to 3 days after the last injection. The sheath of the ovariole was removed to confirm follicle separation. Females were scored positively after three or more follicles were identified as separated from the germariums. Generally, more than half of the ovarioles of positively scored females contained newly separated secondary follicles.
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Puberty Delay by a Urinary Cue from Female House Mice in Feral Populations

Abstract. *Urine produced by wild female house mice, living in high- and low-density populations and confined to areas within a highway cloverleaf, was tested for its ability to delay puberty in juvenile female mice. Only urine collected from females in the dense population at its maximum density delayed puberty in test females. Urine collected when the population was less dense, or from a population that remained sparse, failed to delay puberty. These results suggest that a urinary factor present at high densities may delay puberty and thus help to slow further population growth.*

In confined populations of house mice (*Mus musculus*), population growth decreases as density increases through decreased reproductive rate, increased neonatal mortality, or both (*1*). A delay in the onset of puberty is a common cause of reduced reproductive rate in these populations. Delayed female puberty in albino mice (*M. musculus*) can result from the presence of grouped females or their urine (*2*). Similarly, soiled bedding from the cages of grouped wild female house mice retards puberty in females derived from wild *M. musculus* stocks (*3*). Chemical cues contained in urine may therefore play an important role in regulating natural rodent populations through controlling the rate of sexual maturation. This report provides, to our knowledge, the first evidence that wild female house mice living under natural conditions produce a urinary component that delays the onset of puberty in juvenile female mice coincident with density increases.

We observed populations of house mice living on "highway islands" (cloverleaf sections of Interstate 40, a major highway near Raleigh, North Carolina) over a 2-year period. Live trapping revealed restricted emigration and immigration (*4*), with relatively isolated rodent populations of varying densities and species compositions on highway islands at different successional stages (*5*). Because of this diversity, we removed all small mammals from two highway islands by snap trapping (*6*). Eight sexual pairs of second-generation laboratory-born house mice were introduced to each island in the spring of 1978 (*7*). Detailed floristic and faunistic descriptions of the cloverleafs, as well as data on house mouse emigration rates and population dynamics will be published elsewhere (*8*). Growth of the mouse populations was monitored for a year by trapping for 6 to 8 days within a 2-week period at least every 2 months. A grid of 51 live traps (Sherman) (6.5 by 16.5 by 5 cm) placed 10 m apart and lined with three pieces of filter paper (Whatman No. 1) (4 by 8 cm) to collect voided urine was used. On capture, we individually marked mice by toe clipping, recorded animals' weights and reproductive conditions, and removed the filter paper from the traps and stored it at −40°C. Schnabel (*9*) population estimates from individual mark-recapture data reveal changes in the population numbers over time (Fig. 1). Both populations peaked in December, but the number of individuals on each highway island varied considerably. The population estimate for population 1 in December was 16.0; that for population 2 was 73.7.

So that the puberty delaying potency of the urine of female mice from these populations could be assayed, we first determined whether urine from laboratory-housed wild female mice affects the onset of puberty in juvenile female albino mice (*10*). We housed four laboratory-born wild adult female mice (*11*) in 18 by 28 by 11 cm polypropylene cages with freely available food and water under a 14:10 light-dark cycle for 4 weeks. Individual females were removed and placed overnight in a Sherman trap lined with filter paper to absorb voided urine. A 2-cm^2 piece of filter paper impregnated with urine (*12*) was placed in the cage of a 25-day-old female albino mouse for 6 days (*13*). A second group of 25-day-old females receiving 2 cm^2 of clean filter paper for 6 days served as controls. After vaginal perforation, we lavaged the females' vaginas daily to determine the age of first estrus, which is designated by a completely cornified smear (*14*). Juvenile females exposed to the urine of grouped female wild mice attained puberty significantly later than control females (Table 1).

Once we established that urine from laboratory-housed wild females delays puberty in albino mice in a manner comparable to albino mouse female urine, we

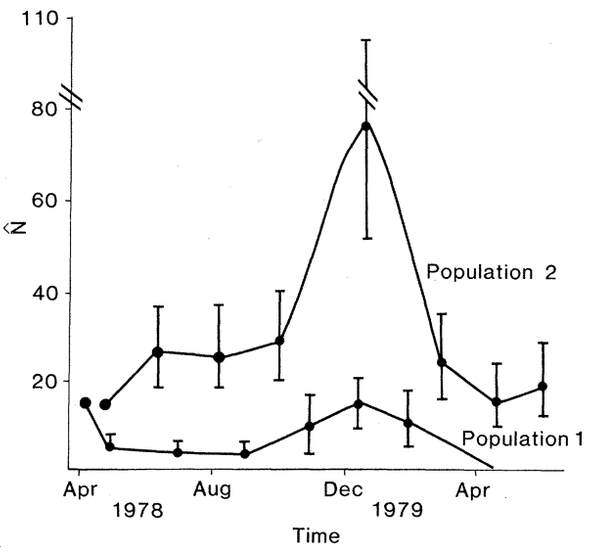

Fig. 1. Schnabel population estimates and their 95 percent confidence limits for two populations of house mice confined to highway islands. Mice were introduced to the highway islands in the spring of 1978, and the growth of the populations was monitored for 1 year (*7*).

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Table 1. Age in days (mean ± standard error) at first estrus of juvenile female laboratory mice exposed to the urine of wild female house mice.

Treatment	Living conditions of urine donor females				
	Laboratory population ($N = 4$ per cage)	Natural populations			
		Low density (spring)		High density (winter)	
		Population 1	Population 2	Population 1	Population 2
Female urine	45.4 ± 1.4 ($N = 20$)	39.7 ± 1.2 ($N = 19$)	41.4 ± 1.3 ($N = 20$)	39.9 ± 1.5 ($N = 19$)	46.8 ± 1.7 ($N = 20$)
Control	38.7 ± 1.1 ($N = 19$)	39.2 ± 1.5 ($N = 20$)	39.9 ± 1.5 ($N = 20$)	38.1 ± 1.2 ($N = 19$)	39.2 ± 1.6 ($N = 20$)
	$P < .05$*				$P < .05$*

*Mann-Whitney U test.

were then able to use juvenile albino females to assay urine from wild mice living under natural conditions. The experimental conditions described above were used in a series of experiments designed to establish the presence or absence of puberty-delaying components in the urine of females from the two populations on highway islands. Urine collected in the spring (*15*), when population densities were relatively low, had no effect on the age of first vaginal estrus of laboratory-housed juvenile females (Table 1). In December, each population was approximately three times as large as their spring population estimates, although the density of population 2 was much greater than that of population 1 (Fig. 1). Only urine taken in December from population 2 females delayed first estrus in juvenile females when compared with controls (Table 1); population 1 female urine collected at the same time had no effect on first estrus.

The change in the ability of the urine from the females in population 2 to delay puberty coincided with both (i) changes in population density and (ii) seasonal changes such as decreasing photoperiod and temperature and the varying presence and abundance of plants and animals. Since the suppressive effects of the urine of females from population 1 did not change from spring to winter, decreasing photoperiod and temperature may be ruled out as agents responsible for the observed urinary changes. We believe that increasing density is the factor responsible for changes in urinary potency. Yet, because the plant compositions of the islands are not identical (*6*), we may not eliminate seasonal changes in vegetation as a possible cause for the change in the female urine.

The onset of puberty in female house mice and several other rodent species is modified by urinary cues acting as priming pheromones (*16*). Acceleration of puberty in juvenile females results from exposure to urine of adult male mice. The male's potency is directly influenced by his androgen level and social status (*17*). The active component of male urine has been characterized chemically (*18*). The urinary component that delays maturation of female mice has received less attention, although it may be an important factor controlling reproduction in natural mouse populations.

We have shown that an increase in population density is correlated with the production of a substance or substances in female urine that inhibits the onset of puberty of other female mice. It now remains to be discovered whether females in the wild respond to density-correlated urinary changes in a manner similar to laboratory mice and whether a male-produced puberty-accelerating compound acts in concert with the inhibitory effects from the female to regulate the rate of sexual maturation of females in wild populations of house mice (*19*). If so, isolation and identification of urinary compounds influencing puberty might yield substances useful in the development of programs designed to control rodent pest populations.

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4. From February 1978 to September 1979, 6658 trap nights were devoted to capturing mice on three highway islands and 5877 trap nights to the areas surrounding these islands. All *M. musculus* captured were individually marked and released. Of 201 mice caught and marked, only three (1.5 percent) migrated either off the island to the surrounding areas or from the surrounding areas onto the islands.
5. Both *M. musculus* and *Sigmodon hispidus* are found in abundance in broom sedge stands (*Andropogon* spp.) that typify the third to fifth year secondary succession in the North Carolina piedmont. As the pines (*Pinus* spp.) begin to take over, *Sigmodon* densities decrease and *Mus* captures are rare. On cloverleafs seeded with fescue (*Festuca* spp.) *Microtus pennsylvanicus* and *S. hispidus* predominate.
6. A 7 by 12 grid of snap traps (Victor) (trap distance, 10 m) was run nightly until three consecutive nights with no captures. On highway island 1 (unmown area, 0.35 ha), 536 trap nights in April 1978 yielded five *M. musculus*, seven *S. hispidus*, and one *Blarina brevicauda*. On highway island 2 (unmown area, 0.32 ha), 602 trap nights in May 1978 yielded 11 *M. musculus*, 3 *Peromyscus leucopus*, 1 *M. pennsylvanicus*, and 24 *S. hispidus*. During the course of the study, rodent species other than *M. musculus* were continuously removed. On highway island 2, broom sedge (*Andropogon* spp.) predominates, while highway island 1 contains many small pine (*Pinus* spp.) trees and much less broom sedge than island 2. Yet 60 of the 66 plant species found on island 2 are also found among the 68 species on island 1.
7. Eight pairs of wild mice captured at three locations in Raleigh produced the 32 mice that served as parents for the mice introduced to the two highway islands. To control for genetic variation between populations as much as possible, one member of a sibling pair was introduced to one highway island, the second sibling to the other highway island. None of the mice introduced to an island were from the same litter. All introduced animals were individually marked.
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10. Juvenile female albino mice rather than juvenile female wild mice were chosen as test animals because (i) wild female mice in the laboratory reach puberty at greater than twice the age of albino mice and, (ii) the stress experienced by wild mice from being handled might delay puberty by itself.
11. Soiled bedding from albino females grouped four to a cage delays puberty in albino juvenile females [L. C. Drickamer, *Dev. Psychobiol.* **7**, 257 (1974)].
12. Urine from males and females fluoresces under ultraviolet light. The portion of the filter paper impregnated with urine can therefore be identified.
13. Juvenile female mice derived from the Swiss Webster strain were maintained in rooms with controlled temperature (24° ± 2°C) and humidity (35 to 70 percent) under a 14:10 light-dark schedule. We culled litters 2 days old to six to eight pups containing at least one male. Litters were weaned at 21 days, and those females weighing between 8.5 and 12.0 g were isolated into 18 by 28 by 11 cm polypropylene cages containing bedding (Sanicell). Females were placed in treatment groups by the split-litter technique. Food and water were freely available.
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