Experimental Triggering of Reproduction in a Natural Population of *Microtus montanus*

Abstract. *A nonbreeding winter population of* Microtus montanus *was given limited supplements of fresh green wheatgrass over a 2-week period. The population was sampled at the end of the feeding period and compared to an adjacent population receiving no grass supplements. All of the experimental females were pregnant, while animals from the control area remained in nonbreeding condition. Replication of the experiment during the next winter produced similar results. The data suggest that* Microtus montanus *cues reproductive effort from chemical signals in the plant food resources.*

Population cycles of microtine rodents (lemmings and voles) have been intensively studied for more than 50 years (*1*). Numerous theories have been proposed to explain the remarkable 3- to 4-year fluctuations in density that have been observed in various species in diverse areas of the Northern Hemisphere. Peak densities may reach several hundred or more of these rodents per acre, and densities in the trough of the cycle may be as low as two or three animals per acre. Considerable changes in reproductive and survival rates seem to occur during the increasing and decreasing phases of the cycle. Nevertheless, few investigators have attempted to define and experimentally test the environmental factors influencing reproductive rates in microtine rodents.

In laboratory studies of the reproductive physiology of *Microtus montanus* we have demonstrated the presence of nonestrogenic compounds in growing wheatgrass that is highly stimulating to reproductive activity (*2*). Observational field data from Wyoming and Utah support the concept that *M. montanus* cues reproductive effort on the basis of chemical signals in the food plants (*3*). The initiation of breeding in the spring may vary by a month or more from year to year and is closely correlated with the onset of vegetative growth. We now present experimental evidence from a natural population of *M. montanus* of a reproductive triggering stimulus in the plant food resource.

Field experiments were undertaken during midwinter on a population of *M. montanus* inhabiting salt grass (*Distichlis stricta*) meadows at Timpie Springs, Utah (*4*). The first experimental regime was initiated in January 1975. An initial sample of 27 animals (15 females and 12 males), taken in late January before any manipulation, indicated that the population was in a typical nonbreeding winter condition (Table 1). A small island approximately 400 m^2 in area was selected as the experimental site, and an area on the adjacent mainland was designated as the control. Both areas were homogeneous habitats, vegetated by dense, almost pure stands of salt grass. The island was also identical in soil and topographic conditions to the adjacent mainland control area. For 2 weeks after the initial sampling period, approximately 500 g of sprouted wheatgrass (*5*) cut into 100-mm squares were placed every other day beside *Microtus* runways on the island. A total of about 3000 g of grass was placed on the island during the feeding period. Less than 50 percent of the grass was consumed. Thus, we estimate the average daily consumption of the grass by each animal on the island to have been 3 to 5 g, with a total average consumption of 45 to 75 g. The mainland control area was not treated. The control area was located approximately 140 m from the island and separated by 10 m of ice and open water. It comprised an area of approximately 0.2 ha. Following the 2-week feeding regime, we sampled animals from both the island and the mainland. The testes of males were removed, weighed, fixed, and sectioned for histological examination. The uteri of females were removed, cleaned, and weighed. Ovaries were fixed and sectioned for examination.

On the experimental island, all of the females were in early stages of pregnancy (Table 1). None of the females from the untreated mainland were pregnant. The samples from the two areas were of comparable age structure as evidenced by body weight and total length. The mean body weight of males and females from the island [grass supplement (GS) treatment] was 37.0 g (range, 31 to 42 g); mean total length was 167 mm (range, 154 to 178 mm). For the mainland sample [no grass supplement (NGS)], mean body weight was 32.0 g (range, 26 to 41 g); mean total length was 157 mm (range, 150 to 171 mm). Histological examination of the ovaries from mainland females revealed no large developing follicles. Ovaries from the island females contained corpora lutea of pregnancy as well as numerous large follicles. Males in both areas had enlarging testes in the mid-February sample. In the NGS mainland area the mean paired testicular weight was 250.2 ± 40.7 mg (N = 21), which did not differ significantly from the mean testicular weight of 218.6 ± 64.2 mg (N = 9) for animals from the GS island population. Histological examination of the testes revealed all animals to be in breeding condition.

Continued sampling of the mainland area was undertaken to determine the time females began their reproductive effort. On 10 to 12 April, of a sample of 23

Table 1. Response of female *M. montanus* from a natural population to fresh wheatgrass supplements (GS) during January and February 1975, compared with responses of control animals receiving no grass supplements (NGS).

Date	Treatment	N	Pregnant (P)
23 to 25 January*	NGS	15	0.0
15 to 18 February	NGS	11	0.0
	GS	11	1.0
12 to 14 March	NGS	20	0.05
6 April	NGS	23	0.04
10 to 12 April	NGS	23	0.96

*This first sample was taken before any manipulation.

Table 2. Response of female *M. montanus* from a natural population to fresh wheatgrass supplements (GS) during January 1976, as compared with responses of control animals receiving no grass supplements (NGS). The results represent the combined data from the two experimental and control areas; ovarian activity is given as the proportion of the females exhibiting different types of ovarian activity. Cycling data exclude pregnant animals.

Date	Treatment	N	Ovarian activity			Uterine weight (mg)
			Pregnant	Cycling	Quiescent	
24 to 27 December*	NGS	22	0.0	0.0	1.0	11.5 ± 6.9
18 to 31 January	NGS	10	0.0	0.0	1.0	12.3 ± 5.6
	GS	15	0.33	0.60	0.07	30.5 ± 18.3†
22 February	NGS	12	0.0			
1 April	NGS	12	0.17			
30 April	NGS	7	1.0			

*The first sample was taken before any manipulation. †These data differ significantly (P < .001) from corresponding values from control (NGS) animals.

females, 22 were pregnant (Table 1). This coincided with the sprouting of salt grass rhizomes in the habitat. Thus, the control population initiated reproductive activity approximately 6 weeks later than the treated island population.

We replicated the regime in the winter of 1975–1976 but began the experiment earlier in the winter, on 24 December. The basis for this procedure was our hypothesis that males in the previous experiment had responded in both areas to increasing photoperiod. We hypothesized that beginning the experimental regime closer to the winter solstice might more clearly demonstrate male responses to the wheatgrass supplement. In addition, we used two experimental areas, the island and another area on the mainland several hundred yards away. Two mainland control areas were also used. One of the control areas was located directly adjacent to the mainland experimental area but was separated from it by a channel of water and ice approximately 10 m wide. The treatment with sprouted wheat began on 27 December 1975 and ended on 10 January 1976.

The results (Table 2) were similar to those of the earlier experiment, although the incidence of pregnancies was lower. This was not unexpected, as the animals would not as yet have experienced the stimulating influence of increasing photoperiod. Hence, a somewhat greater time lag could be expected in response to the wheatgrass stimulus. Further, heavy snowfall during the feeding period resulted in about 50 percent less consumption of wheatgrass than in the previous experiment. Nonetheless, all but one of the females from the experimental areas (Table 2) were either pregnant or cycling with large developing follicles in the ovaries. Again, none of the females from the control areas were pregnant, and the ovaries from these animals lacked developing follicles. Similarly, male gonadal development was significantly affected in those populations receiving the grass supplements. Males from the experimental areas had a mean paired testicular weight of 89.9 ± 38.0 mg ($N = 10$) as compared to a mean testicular weight of 28.3 ± 11.2 mg ($N = 9$) in the control areas ($P < .01$, t-test). Histological examination of the testes demonstrated that 70 percent of the treated animals had begun active spermatogenesis as compared to no spermatogenesis in animals from the control areas. Continued sampling from the control areas demonstrated that the normal onset of breeding in the untreated populations did not occur until April 1976.

These results are unequivocal experimental evidence that wild populations of *M. montanus* cue their reproductive effort from compounds present in actively growing plant tissue. The results are noteworthy in view of the fact that the low temperature regimes of midwinter and the suboptimal food resources are unfavorable for reproduction and survival of young.

Few investigators have attempted to experimentally manipulate food resources in natural populations of microtine rodents. Krebs and DeLong (*6*) supplemented crimped oats or corn and chick starter mash in areas populated by *Microtus californicus* but observed no increases in reproductive activity, perhaps because of the absence of stimulating compounds present in growing plant tissues. However, there is abundant observational evidence that various species of mammals cue their reproductive efforts on the basis of chemical signals present in the plant food resources (*7*). Experimental manipulations of the sort described here should be undertaken with other species of herbivores to test the generality of this cueing mechanism.

We are currently working on the chemical isolation and identification of the plant compounds that stimulate reproductive activity in *M. montanus*. In the course of these studies we have identified a group of phenolic compounds that inhibit reproduction in both male and female *M. montanus* (*8*). These compounds are reported to increase in concentration in grasses as they approach flowering and fruiting (*9*). Simultaneously the stimulator compounds disappear in the matured plants. It thus seems probable that both initiation and termination of reproductive activity in *M. montanus* may be cued by chemical signals in the plant food resources, the inhibitors being effective only when the stimulators are absent. Further investigation of such plant-herbivore interactions may elucidate the problems of microtine population dynamics.

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References and Notes

1. For example, W. J. Hamilton, Jr., *J. Agric. Res.* **54**, 779 (1937); C. Elton, *Voles, Mice and Lemmings: Problems in Population Dynamics* (Oxford, London, 1942); F. A. Pitelka, *Cold Spring Harbor Symp. Quant. Biol.* **22**, 237 (1958); D. Chitty, *Can. J. Zool.* **38**, 99 (1960); G. O. Batzli and F. A. Pitelka, *J. Mammal.* **52**, 141 (1971); C. J. Krebs, M. S. Gaines, B. L. Keller, J. H. Myers, R. H. Tamarin, *Science* **179**, 35 (1973).
2. A. J. Pinter and N. C. Negus, *Am. J. Physiol.* **208**, 633 (1965); N. C. Negus and A. J. Pinter, *J. Mammal.* **47**, 596 (19 → R. Hinkley, Jr., *ibid.*, p. → P. J. Berger and N. C. Negus, *ibid.* **55**, 747 (1974); N. C. Negus and P. J. Berger, *Experientia* **27**, 215 (1971).
3. N. C. Negus *et al.*, *J. Mammal.*, in press.
4. Timpie Springs, Utah, is located 80 km west of Salt Lake City on Interstate 80.
5. Winter wheat was sprouted from seed in the greenhouse at the University of Utah. The wheat was sprouted in a vermiculite substrate to a height of 7 cm.
6. C. J. Krebs and K. T. DeLong, *J. Mammal.* **46**, 566 (1965).
7. W. E. Poole, *CSIRO Wildl. Res.* **5**, 21 (1960); A. E. Newsome, *ibid.* **11**, 187 (1966); J. C. Beatley, *Ecology* **50**, 721 (1969); N. C. Negus and P. J. Berger, in *Biology of Reproduction*, J. T. Velardo and B. A. Kasprow, Eds. (Third Pan American Congress of Anatomy, New Orleans, 1972), p. 89; H. G. Reynolds and F. Turkowski, *J. Mammal.* **53**, 893 (1972); K. M. Van de Graaf and R. P. Balda, *ibid.* **54**, 509 (1973).
8. P. J. Berger, E. H. Sanders, P. D. Gardner, N. C. Negus, *Science* **195**, 575 (1977).
9. S. Z. El-Basouni and G. H. N. Towers, *Can. J. Biochem.* **42**, 203 (1964); S. Kuwatsuka and H. Shindo, *Soil Sci. Plant Nutr. (Tokyo)* **19**, 219 (1973).
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On Explosive Cenozoic Volcanism and Climatic Implications

We disagree with several of the major conclusions proposed by Ninkovich and Donn (*1*) concerning the Cenozoic history of explosive volcanism as determined from the analysis of volcanic ash in Deep Sea Drilling Project (DSDP) sites. In our previous discussion on the history of Neogene explosive volcanicity, we concluded that the distribution of volcanic ash reported in DSDP sites throughout the oceans is consistent with a much higher rate of explosive volcanism during the last 2 million years (*2*). Volcanism on a global basis and in various regions has *not* been uniform throughout the Neogene, but rather shows maxima in activity occurring during the Quaternary, at various times during the Pliocene, and during the Middle Miocene. These episodes are separated by intervals of much lower inferred intensity, although there is variability from region to region. The distribution of volcanic ash in deep-sea sequences is clearly the result of a number of factors other than the volcanism itself. These include the effects of plate motion, wind direction, wind speed, and diagenetic alteration of the ash, but these are unlikely to be the primary controls of the globally compiled Neogene record. Increased explosive volcanism during the last 2 million years coincides approximately with that episode of the Cenozoic marked by major and rapidly fluctuating climate.

Ninkovich and Donn's conclusions based on volcanic ash in western Pacific and Indonesian region DSDP sites of Ne-