

Induction of Winter Breeding in Small Mammals by 6-MBOA

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ABSTRACT

A plant-derived cyclic carbamate, 6-methoxybenzoxazolinone (6-MBOA), is known to initiate reproductive activity in *Microtus montanus*. We studied overwintering populations of four other rodent species, and observed increased reproduction in experimental populations of two herbivorous species, *Microtus pennsylvanicus* and *Sigmodon hispidus*, but not in two omnivorous species, *Oryzomys palustris* and *Mus musculus*. These results suggest that low concentrations of 6-MBOA may trigger seasonal breeding primarily in herbivorous small mammals.

INTRODUCTION

Many species of herbivorous mammals appear to initiate breeding after ingesting green vegetation (e.g., Batzli and Pitelka 1971, Reynolds and Turkowski 1972, Van De Graaf and Balda 1973, Negus and Berger 1977). Later, Berger et al. (1981) demonstrated that reproduction in one small herbivore, *Microtus montanus*, the montane vole, is cued by 6-methoxybenzoxazolinone (6-MBOA) and/or its precursor 2, 4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one (DIMBOA), which is common in seedlings and in rapidly growing plant parts (Sanders et al. 1981). Berger et al. (1981) suggested that 6-MBOA might elicit reproductive responses in many mammals because its site of action is high in the neuroendocrine system. We sought to examine the generality of the effects of 6-MBOA in initiating breeding among seasonally reproducing mammals by using a phylogenetically diverse group of four rodents studied during the winter months, the usual period of non-breeding for many rodents. *Microtus pennsylvanicus* (meadow vole) and *Sigmodon hispidus* (hispid cotton rat), which are almost exclusively herbivorous (Zimmerman 1965, Fleharty and Olson 1969), responded to the stimulation of 6-MBOA by increasing their levels of reproduction in midwinter, but the omnivorous *Oryzomys palustris* (marsh rice rat) and *Mus musculus* (house mouse) did not respond (Negus et al. 1961, Rose and McGurk 2006, Miller and Webb 2001).

STUDY AREA AND METHODS

This experimental field study was conducted during the winter months of December to February. We set four 0.25-ha live-trapping grids at each of two sites in southeastern Virginia. At one site, with oldfields dominated by the perennial grass Fescue (*Festuca* spp.), pairs of grids were separated by a highway interchange in the Bowers Hill section

of Chesapeake, Virginia. The other study site was located in non-tidal marshes on Fisherman Island, a small barrier island 40 km north, at the entrance to the Chesapeake Bay, where American beach grass (*Ammophila breviligulata*) and wax myrtle (*Myrica cerifera*) were dominant. The tidal creek that separated the pairs of grids isolated at least the grid populations of house mice. At each location the Fitch live traps on two 5 by 5 grids, with 12.5-m intervals, received supplemental food while the other two grids received sham-treated food. (In all respects, the experimental protocol followed Berger et al. [1981].) The food was in the form of rolled oats bait coated with 6-MBOA dissolved in ether and ethanol (4:1) at a concentration of 40 µg 6-MBOA g⁻¹ oats (treatment) or solvent only (sham-treated control). Solvent was allowed to evaporate. The 6-MBOA- or sham-treated oats were placed inside the locked-open traps (and replenished every third day) between periods of active trapping, making the traps into feeding stations. Trapping was conducted during weeks 1, 3, and 5. Each rodent was marked with a numbered ear tag, and externally evaluated for sex and reproductive condition before its release. Traps were locked open between trapping periods, permitting free access to the bait. After 5 weeks, animals were live trapped and removed to the laboratory for necropsy. To determine baseline levels of winter breeding activity, samples of rodents were collected in habitat contiguous with the trapping grids at the start of the study in late December. At the end of the experiment, animals were removed from the trapping grids, euthanized, and necropsied for signs of reproductive activity. Testes were weighed to the nearest mg, and the cauda epididymides were examined for the convolutions that indicate the presence of mature sperm (Jameson 1950). Uteri were examined for visible signs of pregnancy, ovaries for evidence of ovulation (corpora lutea), and reproductive tracts were weighed to the nearest mg. Animals were necropsied without knowledge of whether they had been removed from control or experimental grids until all necropsies had been completed. Then, an animal's treatment (6-MBOA or sham) was determined by examining its field tag number.

Tests for differences ($P < 0.05$) in body and gonadal weights of pretrial, control, and treatment animals were made using one-way analysis of variance (ANOVA), and identification of differing group means was made by using Least Significance Difference tests. Student's *t*- and Chi-square tests also were used.

RESULTS

Only *Microtus* showed evidence of reproduction in late December at the start of the study (Table 1), with one of eight females being pregnant and one-third of males judged to be fertile. The other three species showed the typical pattern of temperate rodents in the wintertime: no embryos or corpora lutea in females and regressed testes and looped epididymides in males.

The 6-MBOA appeared to have had a variable effect on the four rodent species. Reproductive organ weights were significantly larger in both sexes of *M. pennsylvanicus* and in male *Sigmodon* (Table 1). This pattern was consistent with other evidence of reproductive activity. For example, a significantly greater proportion of the adult female *Microtus* was pregnant on the experimental grids (10/22) than on the control grids (3/20, $\chi^2 = 4.55$, $P < 0.05$) and the mean weights of litters were significantly heavier (4761.6 ± 1162.77 SE mg, treatment; 896.7 ± 564.07 SE mg, control; $t = 2.92$, $P < 0.025$), suggesting they had become pregnant earlier and were

TABLE 1. Effect of 6-MBOA on standardized reproductive organ weights (♀ = ovary + uteri weight/body weight (mg/g); (♂ = paired testes weight/body weight (mg/g) of four species of rodents (Treatment) compared to those receiving sham-treated supplemental food (Control) and to baseline reproduction at the start of the experiment (Pretrial). Measurements are given as mean weight ± standard error of the mean. Pregnant females were excluded from the analysis of organ/body weight ratios. Percent breeding for females refers to the percent pregnant and for males to the percent with convoluted epididymides. For *Mus*, B and F refer to the Bowers Hill (B) and Fisherman (F) study sites.

Species	N	Pretrial	% Breeding	N	Control	% Breeding	N	Treatment	% Breeding
<i>Mus musculus</i> (B) ♀	27	0.57 ± 0.08	0.0	10	1.45 ± 0.29	0.0	3	3.89 ± 1.25	0.0
<i>Mus musculus</i> (F) ♀	33	1.19 ± 0.21	0.0	25	2.92 ± 0.38	12.0	18	2.10 ± 0.27	0.0
<i>Microtus pennsylvanicus</i> ♀	8	1.08 ± 0.24	12.5	15	2.79 ± 0.46	15.0	12	4.66 ± 0.56	45.5
<i>Sigmodon hispidus</i> ♀	6	0.47 ± 0.12	0.0	4	0.72 ± 0.35	0.0	16	0.74 ± 0.01	0.0
<i>Oryzomys palustris</i> ♀	2	0.52 ± 0.04	0.0	7	0.98 ± 0.20	0.0	5	1.16 ± 0.22	0.0
<i>Mus musculus</i> (B) ♂	25	1.61 ± 0.24	0.0	16	4.07 ± 0.29	60.0	7	4.20 ± 0.31	57.1
<i>Mus musculus</i> (F) ♂	35	2.75 ± 0.08	4.0	29	4.63 ± 0.22	62.0	18	4.53 ± 0.20	58.8
<i>Microtus pennsylvanicus</i> ♂	9	9.35 ± 1.38	33.3	35	14.42 ± 2.70	37.5	21	19.40 ± 0.70	65.0
<i>Sigmodon hispidus</i> ♂	3	1.22 ± 0.69	0.0	7	2.93 ± 0.73	0.0	14	5.22 ± 0.45	42.9
<i>Oryzomys palustris</i> ♂	14	1.22 ± 0.22	0.0	4	5.29 ± 0.58	0.0	5	5.90 ± 0.52	0.0

nearer term. There were no significant differences in the numbers of embryos per female (3.1 ± 0.28 SE, treatment; 2.7 ± 0.88 SE, control). A greater proportion of male *Microtus* exhibited convoluted cauda epididymides on the experimental grids than on the control grids ($X^2 = 3.93$, $P < 0.05$). Male *Sigmodon* showed a similar pattern ($X^2 = 4.20$, $P < 0.05$). No differences in breeding activity between animals on control and experimental grids were noted for the remaining groups (*Mus*, *Oryzomys*, and female *Sigmodon*; Table 1).

DISCUSSION

Our results indicated that 6-MBOA stimulates breeding in two species of small mammals in the grassland habitat. As with montane voles (Berger et al. 1981), meadow voles of both sexes showed a rapid reproductive response following exposure to 6-MBOA. Gonadal weights of male and female meadow voles, under stimulation of 6-MBOA, increased significantly compared to controls and pregnancy rates were significantly higher too. Such synchronous responses by both sexes may be an adaptation of microtine rodents to quickly exploit food resources for secondary production when they become available. Using one male and two female reproductive indices in *Microtus ochrogaster*, the prairie vole, from eastern Kansas, Rose and Gaines (1978) demonstrated synchrony of males and females in their breeding activity: adjusted testes weights, pregnancy rates, and litter sizes showed significant seasonal concordance over a population cycle. Although other studies of reproduction in *Microtus* (Keller and Krebs 1970, Negus et al. 1977, among others) have sought to identify the length of the breeding season, most have not examined the differences between males and females in the onset or cessation of breeding. Nevertheless, it is clear from reproductive studies that *Microtus* sometimes continues breeding throughout the winter months, even in arctic locations where long dark winters prevail (Krebs and Myers 1974). Whether 6-MBOA and related plant compounds are involved in the breeding synchrony of the sexes in initiating winter breeding at northern latitudes remains to be determined, but 6-MBOA did enhance the low level of winter breeding among the meadow voles in our study by significantly increasing the proportions of breeding males and females compared to contemporaneous animals on control grids.

By contrast, in a winter study conducted near Vancouver, Canada, Korn and Taitt (1987) found that only female *Microtus townsendii* (Townsend's vole) responded to the same 6-MBOA treatment that we used (Berger et al. 1981) in our study. Although males on the experimental grid became significantly heavier (but did not have longer body lengths) than control males, all males had similar proportions of descended testes, a valid measure of fertility (McCravy and Rose, 1992). Korn and Taitt (1987) speculate that males respond more strongly to photoperiod and females to new plant growth as the determining factors in the reproductive cycle of Townsend's vole.

Except for control male *Sigmodon*, pretrial animals had significantly lower gonadal weights than either treatment or control animals that were collected five weeks later (Table 1), a pattern also observed in *M. montanus* (Berger et al. 1981). This result might be due either to the effects of providing supplemental food or may represent the seasonal pattern of gonadal changes leading up to regaining fertility and the resumption of breeding. The latter possibility seems more likely because control and treatment animals were not significantly heavier or longer than pretrial animals, as would be expected if supplemental food *per se* were a significant factor in the weight differences.

Several authors have suggested that green vegetation or their extracts put males in a state of reproductive readiness: Negus and Pinter 1966, Berger and Negus 1974 for *Microtus montanus*; Chew and Butterworth 1964, and Van De Graaf and Balda 1973 for *Dipodomys merriami*, a desert kangaroo rat. The latter authors evaluated levels of reproduction of *D. merriami* from two desert areas, one receiving three times more rainfall in autumn and winter than the other. By mid-February, the animals on the wetter area were significantly heavier, and were breeding at high levels, 92% for females and 95% for males, using reproductive features determined by necropsy, whereas kangaroo rats from the drier site showed 7% levels of reproduction (Van De Graaf and Balda 1973). Later, McClenaghan (1987) administered a range of concentrations of 6-MBOA to 40 wild-caught female *D. merriami* in the laboratory. Because neither ovaries nor uteri increased in weight following injections of 6-MBOA, McClenaghan suggested that factors other than 6-MBOA were responsible for the reproductive differences observed by Van De Graaf and Balda (1973) on *D. merriami*.

However, in a field study of another desert rodent, *Gerbillus harwoodii*, in which 6-MBOA was administered according to Berger et al. (1981), both sexes increased gonadal weights on experimental grids but only testes weights were significantly greater than for control males (Alibhai, 1986). Thus, desert rodents tested with 6-MBOA also show a variable response similar to those of grassland small mammals.

In many mammals (Sadleir 1969), including *Sigmodon* (Glass and Slade 1980a, 1980b, McClenaghan and Gaines 1978, Rose and Mitchell 1990, Bergstrom and Rose 2004), males are reproductively active two to four weeks before and after the reproductive seasons of females. For these species, two possible stimuli could induce females to become reproductively active: the presence of mature males or their pheromones are required to trigger neuroendocrine pathways in females, or males are necessary only after food, weather, and daylength factors bring females into the proper physiological state for reproduction. Experimental evidence indicates that for some species at least, male rodents influence the maturation rates of females, often through chemical signals in urine (e.g., Vandenberg 1967, 1969, 1976, Lombardi and Vandenberg 1977, Drickamer 1979). Although these studies have evaluated the effects of adult males on the rates of maturation of juvenile and subadult females, the same rules probably apply to stimulating gonadal recrudescence in adult females that had been reproductively quiescent, e.g., during the winter months. Thus, it seems likely that in the species in which males mature first the presence of reproductively ready males may be required to stimulate females to initiate the ovarian cycle and become reproductively mature. In *Sigmodon hispidus*, the males have a large perineal gland that recrudesces and regresses in synchrony with the seminal vesicles, both apparently being regulated by androgen levels produced by the testes (Rose and Winchell, unpublished). Although the role of the perineal gland is unknown, its cyclicity with both testes and seminal vesicles suggests that the perineal gland is an apocrine gland, secreting pheromones placed by the male's urine into the environment, and signaling the presence of reproductive males to females.

Because male cotton rats mature about one month earlier than females in Virginia (Rose and Mitchell 1990, Bergstrom and Rose 2004), the differential responses of male and female *Sigmodon* to 6-MBOA are not so difficult to explain. Males showed a clear response, both in testes weight/body weight ratios and in having sperm in the cauda epididymides. Whether the mechanism to stimulate females to breed comes directly

through food (but requiring a different dose or duration of 6-MBOA stimulation than for males) or indirectly through an association with reproductively mature males, we interpret *Sigmodon* to be a species that responds to the stimulation of 6-MBOA.

Due to their mixed diets, the initiation of breeding in omnivores may be less closely linked to primary production (and to such cues as 6-MBOA) than in herbivores. Thus, omnivorous species such as *Mus* and *Oryzomys* might be expected to rely less on chemical cues present in new growth of vegetation and more on other environmental factors for initiating reproduction. It should be kept in mind, however, that because only a single concentration of 6-MBOA was provided during our field study, the reasons for the failure of these species to respond remain unclear. Studies under controlled laboratory conditions will be required to determine whether there is a real dichotomy between herbivorous and omnivorous small mammals in their responses to 6-MBOA.

In summary, low concentrations of the plant-derived hormone, 6-MBOA, initiated or enhanced breeding in winter populations of herbivorous *Microtus* and *Sigmodon* but not in the omnivorous-to-carnivorous *Mus* and *Oryzomys* living in the same grassland communities. When spring arrives earlier than normal in cold environments, or in geographic locations in which winters are relatively mild, cues from newly growing vegetation may be superior to photoperiod in permitting a population to expand quickly and by exploiting the available resources. If the timing of the breeding season is a life-history strategy (Stearns 1976), natural selection should favor the retention of such adaptations.

ACKNOWLEDGMENTS

We thank Drs. C. N. Rowsemit and N. C. Negus for their technical support and cooperation in this study. Drs. Negus and P. J. Berger kindly provided the 6-MBOA. We thank R. Dueser, E. Conner, N. Slade, R. Holt, N. Negus, and P. Berger for comments on an earlier draft of this paper. All authors participated equally in the field and lab studies.

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