THESIS

EFFECTS OF SOCIAL CONTACT AND GROUP SEXUAL COMPOSITION ON PERIODICITY, TIMING, AND DURATION OF ULTRADIAN ACTIVITY IN MONTANE VOLES (*Microtus montanus*)

Submitted by

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We hereby recommend that the thesis prepared under our supervision by Loretta Walters entitled "Effects of social contact and group sexual composition on periodicity, timing, and duration of ultradian activity in montane voles (*Microtus montanus*)" be accepted as fulfilling partial requirements for the Thesis in Zoology.

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Effects of social contact and group sexual composition on periodicity, timing, and duration of ultradian activity in montane voles (*Microtus montanus*).

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Many vole species exhibit regularly occurring bouts of activity approximately every two hours throughout the day. Montane voles (*Microtus montanus*) were observed under constant light and temperature, both individually and in same-sex and mixed-sex pairs, to examine the effects of social contact and sexual pairing on these activity patterns. All singly housed voles exhibited ultradian activity, with no significant differences in periodicity between individuals or between sexes, suggesting an intrinsic behavior. Significant differences in periodicity of activity existed between experimental pairings, with female pairs exhibiting the longest period and male pairs exhibiting the shortest period. In female/female pairs, the amount of either female's activity generally did not change from that of females housed alone. In male/male pairs, one or both males decreased their activity from that of individual males. In male/female pairs, one or both members of the pair generally decreased its activity from that of individually housed females. Members of mixed-sex pairs also tended to decrease activity. Significant differences between social pairings existed in timing of activity bouts, with the two members of female pairs being active at different times, and both paired males and members of mixed-sex pairs being active at the same times. These activity patterns may be interpreted within the context of the montane vole's social system.

Many species of voles have been shown to exhibit ultradian activity patterns, or bouts of activity that recur regularly several times each day (e.g., *Microtus agrestis*; Lehmann 1976; *Microtus arvalis*; Daan and Slopsema 1978, Gerkema and van der Leest 1991; *Microtus pennsylvanicus*; Webster and Brooks 1981). However, ultradian activity has never been described for montane voles (*Microtus montanus*), a species found throughout relatively moist areas of the North American west (Hoffmann and Koeppl 1985).

Some authors (Daan and Slopsema 1978, Madison 1985) have speculated that ultradian activity patterns may be intrinsic biological rhythms, similar to the well-known circadian rhythms but with a shorter period. These speculations were based on the fact that there is no known external cue with an ultradian periodicity which could stimulate the voles to become active every one to two hours. One study presented data that seem to support the idea that microtine ultradian rhythms are endogenous. *Microtus arvalis* continued to exhibit ultradian activity in the absence of food, water, rest, and photoperiod, following the surgical removal of the suprachiasmatic nuclei in the brain, which are thought to control circadian rhythms (Gerkema and

van der Leest 1991). However, animal behaviorists stipulate that in order for an observed activity pattern to be classified as a true biological rhythm, two requirements must be met: (1) the activity pattern must continue in the absence of environmental cues, and (2) the activity pattern must show a free-running phase (i.e., a period that gradually drifts out of synchronization with its entraining environmental cue) under constant environmental conditions (e.g., Alcock 1993). It has not been definitively shown that ultradian activity meets *both* of these requirements. Thus, I will simply refer to the activity patterns as activity bouts, while accepting that they may indeed be driven by true biological rhythms. Whether or not these activity bouts are indeed actual rhythms or patterns is not critical to this study.

Some authors have also suggested that social factors may alter the timing and duration, but not the existence, of microtine ultradian activity bouts (Daan and Slopsema 1978, Lehmann and Sommersberg 1980, Madison 1985). This idea has never been directly investigated in montane voles, which have different social systems than some other vole species (Dewsbury 1981, 1990). *Microtus montanus* maintains and defends territories and has a resource-defense polygynous mating system (Jannett 1980, 1982; Wolff 1985), while other vole species range from monogamous with intersexually shared territories to promiscuous with no stable territories. In conjunction with their distinctive social system, montane voles may show unique modifications of their daily activity in the presence of other individuals.

The objectives of this study were: (1) to determine whether montane voles exhibit regularly recurring ultradian activity bouts, and (2) to determine whether social contact with a conspecific of the same or opposite sex affected the periodicity, timing, or duration of these bouts. Periodicity refers to the cyclic time interval from the beginning of one activity bout to the beginning of the next activity bout. Timing is analogous to synchronization, meaning that members of a pair were active at the same or different times. Duration refers to how long a vole was active within an activity bout.

Methods

Study Animals and Housing Conditions

Montane voles were obtained from a large laboratory colony founded several years ago at the University of Utah from wild-caught stock near Green River, Wyoming, USA. The colony received new injections of wild-caught individuals about once a year; all of the voles used in this study were born in the University of Utah colony. At the time this study began, all were approximately one year old adults. When transferred to Weber State University, they were housed individually in opaque fiberglass cages (approximately 65 x 20 x 20 cm) with wire mesh tops and wood shavings plus cotton-batting nesting material. The voles were kept in a windowless laboratory under a 16L:8D photoperiod, and were given alfalfa-based rabbit pellets and water *ad libitum*. A total of six voles, three of each sex, from the Weber State University colony were involved in the following tests. These six individuals were selected randomly by drawing their cage-identification letters out of a hat.

Experimental Procedures

Single Voles

Observations of singly housed voles were performed to determine whether they would show ultradian activity bouts in the absence of environmental or social cues. These individuals also served as their own controls for the paired portion of the experiment.

For the single-vole treatment, I placed one vole at a time in an environmentally controlled chamber (Sherer, a division of Kysor; Marshall, Michigan). The chamber is a rubber-sealed, windowless structure with precisely adjustable environmental conditions. Tests were done under constant light and 18^{o}C temperature. The chamber's constant, insular environment allowed me to test the voles' activity in the absence of environmental cues. Distance from the rest of the vole colony and the sealed nature of the test chamber made it impossible for the tested voles to receive any social cues.

When in the environmental chamber, voles were placed in clear plastic cages for filming. Cage size was reduced by a wood-framed, wire mesh divider to approximate that of the home cage. I included wood shavings and cotton batting nesting material from the vole's home cage to minimize disturbance. I set up a video camera on a tripod opposite the filming cage but allowed the vole to acclimatize to the new cage and chamber, and to the camera's presence for approximately 24 hours. I continued to provide food and water *ad libitum* throughout all experimental procedures.

Next, I filmed the vole in the chamber for two 10-h sessions, in which the camera was set to record, in time lapse, one second out of every minute. Approximately 24 hours elapsed between each of the filming sessions. I repeated this cage transfer, acclimatizing, and filming procedure for each of six voles (three males and three females). Replications were done to ensure a substantial sample size and to test for any intrinsic sex differences in ultradian activity.

I viewed the resulting videotapes on a videocassette recorder (VCR), timing each vole's filmed activity bouts with a stopwatch. I began timing activity as soon as the camera began recording, and recorded each time the vole began any activity and the time when it ended that activity bout, within a one-second shot. I then converted these collapsed times into real-time values. I also counted the number of consecutive one-second recording shots during which each vole was active. Times were converted from minutes and seconds to minutes and tenths of minutes to facilitate analysis and plotting of activity (see Appendix). These plots allowed me to quickly assess whether the voles were exhibiting periodic rather than just random activity.

To analyze these data, I arbitrarily designated discrete activity bouts as those periods of activity separated by more than 30 seconds of non-activity on the time-lapse videotape, which would account for 30 non-active minutes in real time. It seems logical to surmise that with a half-hour of non-activity, the vole was not in the midst of an activity bout, but rather was resting between two bouts. Rarely, a vole would make a brief movement without initiating any

prolonged activity; such movements were not considered part of an activity bout. I recorded these movements, but discarded them from analysis if they were isolated by more than 30 real-time minutes of non-activity before *and* after their occurrence.

For singly housed voles, I measured only the periodicity (amount of time between the beginning of one activity bout and the beginning of the next activity bout) of their activity to determine whether an ultradian cycle was present. Mean periodicity for each vole was obtained by averaging all times between its activity bouts. I compared mean periodicity of the activity patterns among individual voles of the same sex with a Kruskal-Wallis test (Ambrose and Ambrose 1987). This test is particularly suited to this type of data because it is intended for more than two samples that may contain unequal numbers of measurements, such as the intervals between activity bouts of three individual voles. Differences between the sexes when housed singly were analyzed with the rank sum test, a modification of the Wilcoxon rank sum test (e.g., Ambrose and Ambrose 1987).

Social pairings

To determine if contact with a conspecific of the same or opposite sex affected timing or duration of activity bouts in montane voles, I followed the same procedure used for single voles, except that I put another vole and its nest material on the other side of the filming cage divider. The divider was necessary to maintain the familiar cage size, and for separation of the two voles to prevent agonistic interactions. These wire-mesh dividers allowed the voles to see, smell, and hear each other, while preventing fighting.

Voles were divided into three experimental treatment groups: female/female pairs, male/male pairs, and male/female pairs. I ran three replications of each pairing using different voles for each replication. All other methods of acclimatizing, filming, and recording of data, as well as environmental chamber parameters, were identical to those used for single voles. I plotted the activities of each pair on one graph so that they were superimposed (see Appendix), allowing the determination of periodicity and duration of activity of each pair

member, as well as whether the members of the pair were active at the same or different times. I analyzed differences in mean periodicity among the three types of experimental pairings with a Kruskall-Wallis test (Ambrose and Ambrose 1987). I compared timing and duration of activity using a chi-square contingency table.

I observed three aspects of activity within pairs: periodicity (cyclic time intervals from the beginning of one activity bout to the beginning of the next), timing (synchronization--whether or not the two members of a pair were active at the same time), and duration--how long each member of a pair was active within a bout.

Results

Presence of Ultradian Activity and Periodicity

All single voles showed ultradian activity bouts (see Appendix) with no significant differences in periodicity among singly housed males (Kruskal-Wallis test, H= -8.88, p>0.05), nor among singly housed females (H=2.46, p>0.05). Periodicity for singly housed males ranged from 1.4 h to 1.8 h. Periodicity for singly housed females ranged from 1.6 h to 2.3 h. The periodicity did not differ significantly between singly housed males as a group and singly housed females as a group (rank sum test, Z=0.87, p>0.05). Median periodicity for all singly housed males was 1.7 h, and for all singly housed females was 2.1 h.

All vole pairs also showed ultradian activity patterns with periods of approximately 1.5-2.0 hours (see Appendix). Highly significant differences in mean periodicity existed between the three experimental treatments of male/male pairs, female/female pairs, and male/female pairs (Fig. 1; H=533.10, p<0.05). Male/male pairs exhibited the shortest periods (about 1 h) between activity bouts, which were noticeably shorter than those of singly housed males. Female/female pairs exhibited the longest periods (about 2 h), and their periodicity was similar to that seen in singly housed females. Mixed sex pairs showed intermediate period lengths (about 1.5 h).

Timing and duration of activity

Among paired voles, no statistically significant differences were found in the duration of activity of female/female, male/male, and male/female pairs (Table 1, $\chi^2=3.4$, $p>0.05$). However, significant differences existed between the social pairings in timing of activity bouts, with members of female/female pairs being active at different times, and members of both male/male pairs and mixed-sex pairs being active at the same times (Table 2, $\chi^2=9.2$, $p<0.05$)

Discussion

These results show that both individual and paired montane voles do indeed show what appear to be ultradian bouts of activity. This is the first time that such activity patterns have been reported in *Microtus montanus*. Previous studies on montane vole behavior have tended to focus on their reproductive physiology and copulatory behaviors (Jannett 1980, 1981, 1982; Negus and Berger 1988). However, activity patterns have the potential to influence mating and sociality among montane voles and should therefore be investigated further. For example, if members of a vole population synchronize their activity bouts at a time when mates are being selected, direct competition for mates may be increased. Alternatively, intraspecific aggression may be avoided by altering periodicity or timing of activity so that individuals contact each other less often.

The fact that all single montane voles of each sex showed activity bouts with similar periodicity suggests that the ultradian activity pattern may be an evolutionarily conserved behavior. Indeed, many other vole species show ultradian activity (e.g., *Microtus agrestis*; Lehmann 1976; *Microtus arvalis*; Daan and Slopsema 1978, Gerkema and van der Leest 1991; *Microtus pennsylvanicus*; Webster and Brooks 1981). However, the significant differences in periodicity and timing observed when the voles were in contact with each other indicates that this behavior can be modified when necessary to mediate social interactions.

Singly housed male and female voles displayed no differences in the periodicity of their activity bouts. Thus, differences observed in the activity of the various sexual pairings must be due to differences in the way members of each sex reacted to each other, rather than from any intrinsic differences in male and female activity. Also, singly housed males and females were virtually indistinguishable in specific behaviors, which included cage climbing, eating, and drinking.

The fact that only non-significant differences in duration of activity were observed in the different pairings of voles (Table 1) may be due to a small sample size. Alternatively, perhaps duration of activity is less important than its timing in social interactions, and therefore remains consistent between sexes.

Female/female pairs

Both members of most female/female pairs were active at different times. Periodicity was longest in female/female pairs, and most similar to that observed for singly housed female voles. In addition, specific behaviors appeared similar to those observed in females housed alone. These consisted of climbing on the cage, eating, and drinking. Some characteristics of montane voles observed in natural populations may help to explain these findings, although the usual precautions must be kept in mind when generalizing findings to field populations.

Females of most vole species generally tend to show less agonistic behavior toward each other than do males (Wolff 1985). Further, wild female montane and meadow voles (*Microtus pennsylvanicus*, a socially similar species in terms of male aggression levels and mating system, Colvin 1973, Dewsbury 1990) tend to overlap home ranges and form extended maternal families, especially at high population densities (Ambrose 1973, Jannett 1980). This may indicate that females of these vole species tend to be tolerant of each other's presence, and so do not readily change the periodicity of their activity when placed together. Members of female/female pairs were active at different times, i.e., their activity bouts were not synchronized to occur at the same time. These attributes of female activity may serve to reduce direct confrontations, which

would be valuable for females with overlapping home ranges and extended families. Related females may also be more tolerant of each other's presence than unrelated females, an associated issue that was not investigated in this study. With paired females it is also possible that each individual was indifferent to the presence of the other female, and simply ignored each other. In this case, they may not have altered the timing of their original movements, resulting in independently active females whose individual activity bouts just did not happen to coincide (in most pairings). It is not possible to determine from these data whether these females were purposely avoiding being active at the same times, or merely exhibiting individual, non-synchronized activity patterns.

Male/male pairs

Members of male/male pairs were most often active at the same time, and exhibited periodicity that was shortened from that of singly housed males. Specific behaviors of paired males were noticeably different from singly housed males. I observed much more cage climbing and rearing (standing up on hind legs) against cage walls in male/male pairs than in individually housed males. These may be stereotypical locomotor behaviors that voles exhibit under social stress (Cooper and Nicol 1994).

Again, studies of natural vole populations provide possible explanations for these results. Adult male montane voles hold and defend stable territories, either driving away or killing any other males in the territory (Jannett 1980, 1981). In fact, male montane voles are one of the most intrasexuallly aggressive (Colvin 1973) and least social vole species (Dewsbury 1990), and as would be expected, these males have more direct agonistic conflicts than do female voles (Wolff 1985). *Microtus montanus* males remain on their territory even when females leave it, indicating that territories are probably based on resources rather than the location of possible mates (Jannett 1982). Both high levels of intrasexual aggression and defense of a territory are probably related to the montane voles' highly polygynous mating system (Dewsbury 1981, Wolff 1985).

In such a social environment, males may benefit from synchronizing activity bouts to occur at the same time as those of potential intruders. The costs associated with the finding and establishment of a new territory could make it profitable to be fully vigilant of competitors, and thus be ready to defend one's territory or oneself from attack. Hence, a male should benefit from being active at the same times as another male. Perhaps the shortened periodicity observed in male/male pairs also reflects increased agitation and a need to frequently check on an opponent's activities. In summary, male voles may be exhibiting an activity pattern that maximizes awareness of a territory and its potential intruders, and minimizes the possibility of surprise attacks from such intruders.

Male/female pairs

Both members of male/female pairs were generally active at the same time. Specific behaviors differed markedly from those of individually housed males and females; both members of the mixed-sex pairs concentrated their movements around the cage divider, closely following each other back and forth along the wire mesh.

Populations of montane voles normally have one male's territory overlapping those of several females, where his presence induces the females' estrus and other reproductive behaviors, as long as nutritional resources are adequate (Jannett 1982, Negus and Berger 1988). It has been observed that a territorial male concentrates his activity around the nests of females in his territory, and the male may even forage together with one of these females. However, males and females never inhabit the same nest, nor does a male shift the location of his territory when a female leaves it. Further, males and females from different territories often will either avoid interacting with each other or will fight (Jannett 1982). Considering these aspects of natural male/female interactions, it seems possible that the members of the male/female pairs were active at the same time because they sensed mating possibilities, but decreased overall activity because they were initially wary of interacting with a stranger of the opposite sex. This explanation is further supported by the activity plots, which show the activity of males and females increasing

and synchronizing further as the individuals became more familiar with each other toward the end of most of these mixed-sex pairings.

The intermediate periodicity shown by male/female pairs was slightly shorter than periodicities of voles of each sex housed individually. This could be because mixed-sex pairs increased their monitoring of each other's activity for mating purposes, thus shortening activity periods, but did not feel threatened enough to shorten these periods as dramatically as did members of male/male pairs.

Conclusions

Montane voles may commonly exhibit ultradian activity patterns in the laboratory. It would be difficult to prove whether this pattern exists in field populations since these voles are very secretive (Dewsbury 1981, Wolff 1985). Trapping data do, however, suggest that ultradian activity is present in natural populations of *Microtus arvalis* (Daan and Slopsema 1978). It seems likely that the patterns observed in this study are intrinsic given the fact that all of the voles I observed exhibited them, regardless of social stimuli, and in the absence of environmental cues.

Considering the significant variations shown by the voles in different social groupings, it appears that aspects of the voles' social behavior modify, but do not eliminate, the activity patterns of these animals. Again, I emphasize that since females and males housed singly did not differ in their periodicities, the observed significant differences between experimental pairings must be due to differences in how the sexes respond to each other rather than from any inherent sex differences in activity patterns.

The polygynous mating system and intrasexual male aggression of montane voles may drive the differences in periodicity and timing of ultradian activity patterns observed among the gender pairings. Behavior that is advantageous in the social context of montane vole populations probably regulates daily activity of individuals. Since all other variables were held constant

in this study, adjusted social behavior is likely responsible for the observed changes in activity when voles were placed in social contact with each other.

Future research possibilities

Future research could directly test my explanations for differences between social groupings. A topic needing further study is whether these ultradian activity patterns are under the control of an innate biological rhythm or are merely physiological or metabolic responses. This would require monitoring microtine ultradian activity in the absence of all external factors, and then determining (1) whether these rhythms persist and (2) whether they show a free-running phase. Even if both conditions were met, it would be difficult to say with certainty that these rhythms are under the control of the vole nervous system and not some other internal physiological regulator. Another possible research topic is whether vole population cycles (e.g., Krebs 1970) are in any way connected to ultradian activity as it may influence social and reproductive behaviors.

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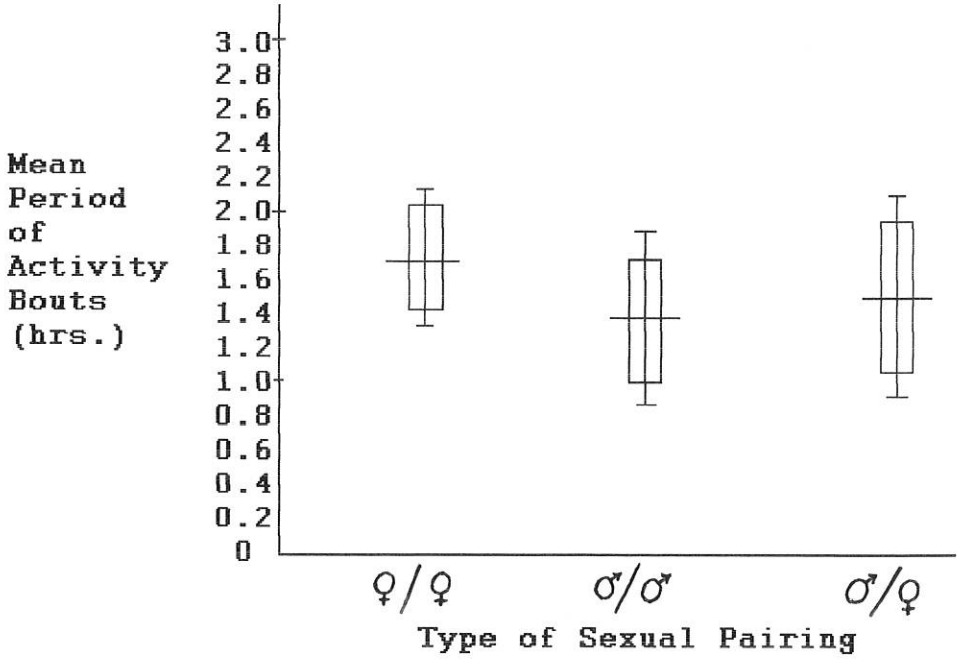

Figure 1. Periodicities of different sexual pairings of montane voles. Horizontal lines represent means, vertical lines represent ranges, and boxes represent standard deviations.

Table 1. Comparative duration of activity bouts of paired voles. Tabular values represent numbers of occurrences out of six trials per sexual pairing.

Type of sexual pairing	♀/♀	♂/♂	♂/♀
One or both members of pair decreased their activity	2	6	6
One or both members of pair increased their activity	0	0	3

Table 2. Comparative timing (synchronization) of activity bouts of montane voles. Tabular values represent numbers of occurrences out of six trials per sexual pairing.

Type of sexual pairing	♀/♀	♂/♂	♂/♀
Members of pair were active at same times	2	4	5
Members of pair were active at different times	4	2	1