Levels of Female Aggressiveness in Fluctuating Populations of *Microtus ochrogaster* and *M. pennsylvanicus*

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Abstract : Levels of aggressiveness of females from free-living populations of *Microtus ochrogaster* and *M. pennsylvanicus* were compared during phases of the population cycle. *M. ochrogaster* displayed no significant difference in the proportion of aggressive females or in the means of five behavioral variables (approaches , threats , attacks , retreats , attacks/ approaches) among phases of the population cycle. None of these variables showed a significant linear regression against population density. Neither did the proportions of aggressive *M. pennsylvanicus* females differ among phases. The mean number of threats in this species was significantly higher during the low than the increase and decline phases and the mean number of attacks was significantly higher during the low than all other phases. The results of this study are similar to those obtained for males and do not support the polymorphic behavior hypothesis regarding generation of population cycles in arvicoline rodents

Key words : Aggressive behavior ; Chitty hypothesis ; Prairie vole *(Microtus ochrogaster)* ; Meadow vole *(Microtus pennsylvanicus)* ;

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　　The polymorphic behavior hypothesis[1,2] continues to be considered in discussions of factors involved in cyclic fluctuations in population density of arvicoline (microtine) rodents , as well as other species of small mammals[3 - 6]. This hypothesis is based on the existence of genetically determined behavioral polymorphisms within populations. Selection for aggressive genotypes during the increase phase of the population cycle and for non-aggressive genotypes during the decline phase are presumed to be involved in generation of the population cycle. If so , a greater proportion of the population would be composed of aggressive animals during the latter part of the increase and during the peak than other phases. When a large proportion of the population is aggressive , a population decline could be triggered by diversion of energy away from reproduction by the highly aggressive animals during the peak phase , by increased mortality and by reduced reproduction associated with social stress phenomena[7,8] or by increased emigration[9,10].

　　Levels of aggression of males in fluctuating arvicoline

populations have been assessed by observation of agonistic behavior during dyadic encounters in neutral arenas for four species: *Microtus pennsylvanicus*[11 - 13], *M. ochrogaster*[13], *Clethrionomys gapperi*[14], and *M. townsendii*[15]. Only Krebs[11] found evidence of selection for aggressive morphs during the increase phase of the population cycle. Conley[16] and Mihok[14] have provided data related to changes in aggressiveness of females. Since neither of these studies included a complete population cycle, evidence for involvement of selection for aggressive behavior was not conclusive.

Rose and Gains[17] found increased wounding, an indication of level of aggressiveness, of male, but not female *M. ochrogaster* during winter in comparison to other seasons, while Rose[18] observed increased wounding of both male and female *M. pennsylvanicus* during winter. Such seasonal variation in aggression may mask variation in aggressiveness resulting from selection during phases of the population cycle.

During the course of a study of aggressiveness of males from fluctuating populations of *M. ochrogaster* and *M. pennsylvanicus*[13], we obtained data for females of these two species. Owing to small sample sizes and similarity in the results for both sexes, we did not present data for females at that time. Because the polymorphic behavior hypothesis continues to be included in discussions about the generation of population cycles of arvicoline rodents we present herein the data for females.

1 METHODS

The study sites were located in the University of Illinois Biological Research Area ("Phillips Tract"), 6 km NE of Urbana, Illinois USA (40°15 N, 88°28 W). We obtained data for *M. ochrogaster* in alfalfa, Medicago sativa, and bluegrass, Poa pratensis, habitats and for *M. pennsylvanicus* in bluegrass. We have described the study sites in considerable detail elsewhere[19 - 21].

A grid system with 10 m intervals was established in both study sites, with one wooden multiple-capture live-trap at each station. Each month we pre-baited for 2 days and then trapped for 3 days; cracked corn was used for prebaiting and as bait in the traps. We set traps in the af-ternoon and checked then at approximately 08:00 h and 15:00 h for the following 3 days.

At first capture, we toe-clipped all animals (< 2 toes on each foot) for individual identification. At each capture we recorded species, grid station, individual identification, sex, reproductive condition (males: testes abdominal or descended; females: vulva open or closed, pregnant, as determined by palpation, and nipple size), and body mass to the nearest 1 g.

During each trapping session females with body mass 20 g that were judged to be non-reproductive (i.e., closed vulva, no embryos detected by palpation and nipples not enlarged) were removed to a nearby field laboratory for behavioral trials. The purpose of this study was to test for selection for genetically discreet behavioral morphs in response to population density. Because we were interested in determining if selection for genetically discreet behavioral morphs occurred in response to population density, only non-reproductive females (as defined above) were used in the behavioral trials. This avoided the confounding effects of short-term variation in aggressiveness related to reproductive state. Female voles display varying levels of aggressiveness because of changing hormonal levels associated with reproductive condition, e.g., pregnancy or lactation[22].

To compare our results with those of our study of males[13] and other studies, we utilized a neutral arena for the behavioral observations. Although Ostfeld[23] predicted removal of animals from their home range may alter their aggressive state, Harper and Batzli[24] have shown that data obtained in neutral arenas are representative of levels of aggressiveness displayed by individuals in their home ranges. The observational arena consisted of a 40 cm × 20 cm × 20 cm wooden enclosure with a Plexiglas front and a removable opaque central partition. Approximately 1 cm of wood shavings covered the floor; the shavings were changed after every third trial. Although current protocol calls for changing bedding and sanitizing a chamber following each trial, we noticed no difference in behavior of the voles in the second and third trials from that in the first trial. The arena was located behind a black plastic curtain with a small opening though which observations

were made. A 40 w fluorescent light was suspended 1 m above the arena.

Non-aggressive adult female *M. ochrogaster*, of similar body mass, from a laboratory colony were used as unfamiliar, standard opponents in the behavioral trials. The non-aggressive females were selected through a series of behavioral trials (see below) among laboratory-reared females. Use of standard non-aggressive animals ensured that aggressiveness in the trials was initiated by the field animal. A standard female was used in no more than two trials a day. We observed few instances of a standard animal initiating aggressiveness during any of the trials. When such behavior was observed the animal was removed from the experiment. All standards were replaced every 3 - 4 months.

Because *M. pennsylvanicus* were difficult to breed in the laboratory, standard non-aggressive animals were unavailable. As for our study of males[13], we tested two field-captured female *M. pennsylvanicus* against each other. Pairs were matched for body mass. Distance between points of capture of the two females was maximized to ensure low probability of familiarity. Behavioral data normally were recorded for both animals in a trial. However, in order to test as many *M. pennsylvanicus* as possible during afternoon trap checks, it was sometimes necessary to use a female that had already been tested in the morning. In these instances, only data for the new animal were analyzed.

Each trial was preceded by a 2 min period during which the voles were separated by the central partition. The partition was then removed and interactions recorded for 10 min with a keyboard attached to a 20 channel Esterline - Angus event recorder.

Seven agonistic postures and acts were recorded during the dyadic encounters: threat, upright, lunge, boxing, wrestle, chase, and retreat/avoid. See Hofmann *et al*.[13] for a description of each category. For the purpose of determining aggressive behavioral scores, threats and uprights were lumped as threats, while lunges and boxing strikes were counted as attacks[13]. The number of approaches (within 5 cm of the other animal) made by the test animal was also recorded.

1.1 Data analysis

To determine if the proportion of individuals displaying aggressive behavior varied during phases of the population cycle and seasons, each female was categorized as non-aggressive or aggressive. Aggressive females were those that performed threats, attacks, or chases during dyadic encounters. Chi-square analysis of contingency tables was used to test the null hypotheses that the numbers of non-aggressive and aggressive females were independent of phase or season.

To compare the behavior displayed by different groups of females without arbitrarily choosing one parameter as an index of individual aggressiveness, multivariate analysis of variance (MANOVA) was performed on five behavioral variables for each trial. This data analysis was comparable to those of Krebs[11] and Hofmann *et al*.[13]. Variables were the numbers of approaches, threats, attacks, and retreat/avoidances, and the adjusted frequency of attacks (the number of attacks divided by the total number of approaches made by both voles during the 10-min trial, hereafter referred to as attacks per encounter). The number of chases was excluded from the analysis because of a lack of variation (chases almost never occurred during the dyadic encounters). Because of their non-normal distribution, the first four variables were converted by the square-root transformation. Multivariate analysis of variance was performed using the MGLH procedure of SYSTAT[24] and revealed the proportion of the variation in the behavioral scores that could be explained by phase or season. In addition, a linear regression[25] was run for each of the five behavioral variables against population density.

1.2 Population cycles and seasons

Population densities representing low, increase, peak, and decline phases, were arbitrarily defined, based on configuration of each of the population cycles[13] (Fig.1). The upper limits for the low phase of *M. ochrogaster* populations in the bluegrass and alfalfa areas were 20/ha and 28.5/ha, respectively. The peak phase included trapping periods with densities of >45/ha in the bluegrass area and >70/ha in the alfalfa field. Periods of intermediate densities were classified as part of

the increase or decline phase , depending on the population trend. There was an increase in population density in the alfalfa area during spring 1976 , but analysis of reproduction and survival indicated that this period represented a type H decline[19].
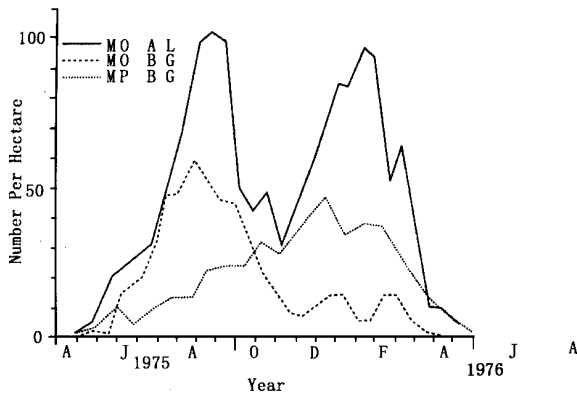

Fig. 1 Population densities of *Microtus ochrogaster* (MO) and *M. pennsylvanicus* (M P) in alfalfa (AL) and bluegrass (BG) study sites in the University of Illinos Biological Research Area , 1975 - 1976.

The following periods were included in each phase of the population cycle of *M. ochrogaster* in the alfalfa field : low , June - early August 1975 , May - December 1976 ; increase , late August - September 1975 ; peak , October - November 1975 ; decline , January - April 1976. The next population cycle in alfalfa was in 1981[21]. Phases of the *M. ochrogaster* cycle in the bluegrass area included the following periods : low , March - December 1976 ; incr-

ease , June - August 1975 ; peak , September - December 1975 ; decline , January - February 1976. The next cycle in bluegrass was in 1982 - 3.

For *M. pennsylvanicus* the delimiting densities for low and peak phases were 20/ ha and 42/ ha , respectively. Phases of the *M. pennsylvanicus* cycle included the following periods : low , June - November 1975 , December 1976 ; increase , December 1975 - April 1976 ; peak , May - early July 1976 ; decline , late July - December 1976. The next cycle began in February 1977.

Because sample sizes of *M. ochrogaster* females from some months were very small , seasonal analysis was performed on data from two seasonal periods : spring - early autumn (15 March - 14 October) and late autumn - winter (15 October - 14 March). For *M. pennsylvanicus* seasons were defined as follows : spring , 15 March - 31 May ; summer , 1 June - 14 September ; autumn , 15 September - 30 November ; winter , 1 December - 14 March.

2 RESULTS

2. 1 *Microtus ochrogaster*

Phase analysis. The proportion of aggressive females did not differ significantly in the alfalfa and bluegrass populations during the low ($x^2 = 0.36$; $df = 1$; $P > 0.50$) , increase ($x^2 = 1.17$; $df = 1$; $P > 0.20$) , and peak ($x^2 = 0.74$; $df = 1$; $P > 0.30$) ; there were no data from the decline for the bluegrass population (Table 1).

Table 1 **Proportion of aggressive female** Microtus ochrogaster **in respect to phase of the population cycle and season in alfalfa and bluegrass study sites. N = sample size**

Phase/ season	N	Alfalfa Aggressive	N	Bluegrass Aggressive	N	Total Aggressive
Phase						
Low	1 5	0. 357	1 4	0. 467	29	0. 414
Increase	9	0. 333	2 6	0. 538	35	0. 486
Peak	1 7	0. 588	2 0	0. 450	37	0. 514
Decline	1 9	0. 264			19	0. 264
Season						
Spring-early autumn	2 1	0. 523	4 8	0. 437	69	0. 464
Late autumn-winter	3 8	0. 316	1 3	0. 547	51	0. 372

Accordingly, data from the two populations were combined for the three former phases in order to achieve larger sample sizes. The proportion of aggressive females did not differ significantly among the low, increase, and peak phases ($x^2 = 0.68$; $df = 2$; P 0.70). The proportion of aggressive females was lower during the decline phase for the alfalfa population than during the other three phases of the cycle (Table 1). The proportion of aggressive females during the decline phase, however, did not differ from expected values calculated from the overall proportions of aggressive females for the other three phases (0.475; $x^2 = 3.38$; $df = 1$; $P > 0.05$).

Similarly, MANOVA for the five behavioral variables for females from the alfalfa and bluegrass areas revealed no statistically significant difference during the low (Wilk s = 0.854; $F = 0.79$; $df = 5$, 23; $P = 0.57$), increase (Wilk s = 0.847; $F = 1.02$; $df = 5$, 28; $P = 0.43$), and peak (Wilk s = 0.787; $F = 1.63$; $df = 5$, 30; $P = 0.18$) phases. When data for the two populations were combined, there was no significant difference among the three phases (Wilk s = 0.941; $F = 0.57$; $df = 10$, 184; $P = 0.84$). Phase of cycle accounted for only 5.9% of the variation in the behavioral data.

Seasonal Analysis. Because the sample from late autumn - winter for the bluegrass area was small (Table 1), data for females from the alfalfa and bluegrass areas were compared to determine if they could be combined for the seasonal analysis. The proportion aggressive females did not differ significantly between populations during either spring - early autumn ($x^2 = 0.47$; $df = 1$; $P > 0.40$) or late autumn - winter ($x^2 = 2.14$; $df = 1$; $P > 0.10$). When data from the two populations were combined there was no significant difference between the proportion aggressive females during the two seasonal periods ($x^2 = 1.02$; $df = 1$; P 0.30).

Means of the five behavioral variables did not differ significantly between the two populations during spring - early autumn (Wilk s = 0.953; $F = 0.68$; $df = 5$, 68; $P = 0.64$) or late autumn - winter (Wilk s = 0.969; $F = 0.24$; $df = 5$, 37; $P = 0.94$). When data for females from the two populations were combined,

MANOVA revealed no seasonal heterogeneity (Wilk s = 0.948; $F = 1.22$; $df = 5$, 111; $P = 0.31$). Only 5.2% of the variation in the behavioral data was attributable to season.

Density analysis. None of the five behavioral variables for all *M. ochrogaster* females, both populations combined, showed a significant linear regression against population density (approaches: $r = 0.11$; $P = 0.25$; threats: $r = 0.03$; $P = 0.73$; attacks: $r = 0.0$; $P = 0.70$; retreat/ avoidances: $r = 0.10$; $P = 0.27$; attacks per encounter: $r = 0.11$; $P = 0.25$).

2.2   *Microtus pennsylvanicus*

Phase analysis. The proportion aggressive females did not differ significantly among the four phases of the population cycle ($x^2 = 1.43$; $df = 3$; P 0.70; Table 2).

Table 2   Proportion of aggressive female Microtus pennsylvanicus **in respect to phase of the population cycle and season in bluegrass.   N = sample size**

Phase/ season	N	Aggressive
Phase		
Low	23	0.739
Increase	44	0.659
Peak	54	0.741
Decline	64	0.656
Season		
Spring	58	0.759
Summer	89	0.640
Autumn	34	0.735
Winter *	4	0.500

＊Winter data not included in statistical analyses (see text)

MANOVA, however, revealed a significant difference in the behavioral variables among phases (Wilk s = 0.853; $F = 1.92$; $df = 15$, 483; $P = 0.02$). The major contributors to this difference (as indicated by squared multiple correlations) were the mean numbers of threats and attacks. The mean number of threats was significantly higher during the low phase than during the increase ($P = 0.03$) and decline ($P < 0.01$) and the mean number of attacks was significantly higher during the low phase than during the other three phases (increase: $P = 0.02$; peak: $P = 0.04$; decline: $P = 0.02$). Phase of the cycle explained 14.7% of the variation in behavioral data.

Seasonal analysis. The number of trials during winter was too small to be included in the statistical analysis (Table 2). There was no significant difference in the proportion of aggressive females among spring, summer and autumn ($x^2 = 2.62$; $df = 2$; $P > 0.20$; Table 2). There also was no significant difference for the behavioral variables among these three seasons (Wilk s $= 0.933$; $F = 1.21$; $df = 10$, 344; $P = 0.28$). Only 6.7 % of the variation in the data was attributable to season.

Density analysis. Linear regression analysis for each of the behavioral variables against population density revealed significant negative relationships for both the number of threats ($r = -0.19$; $P = 0.01$) and attacks ($r = -0.15$; $P = 0.04$). It should be noted, however, that population density accounted for a relatively small proportion of the variation in the data.

3 DISCUSSION

Proportion of aggressive *Microtus ochrogaster* females and means of five behavioral variables did not vary significantly in respect to phase of the population cycle. Linear regression analysis revealed no significant correlation of the behavioral variables against population density.

The proportion of aggressive female *M. pennsylvanicus* also did not differ significantly among phases. However, means for two aggressive behaviors, threats and attacks, were significantly higher during the low phase than the increase. The mean number of attacks during the low phase also was significantly higher than that for the peak. A significant negative correlation was found between mean numbers of threats and attacks and population density. These results are opposite of what would be predicted by the polymorphic behavior hypothesis.

Variation in aggression for female *M. ochrogaster* and *M. pennsylvanicus* did not differ seasonally. Thus, seasonal effects did not mask any phase effects that might have existed.

That sample sizes for females were limited, reduces the power of statistical analyses. Thus, combining data from alfalfa and bluegrass may not have been entirely justified. However, the proportions of aggressive females a-

mong phases in each habitat tended to be approximately the same as for the grouped data. Further, the results of this study agree with the conclusions regarding aggressive behavior of male *M. ochrogaster* and *M. pennsylvanicus*, for which substantive data were available[13]. From analyses of data for both males and females we suggest no simple relationship between aggressiveness and phase of the population cycle in these two species. As such, our results do not agree with those of Krebs[11] who found selection for aggressiveness in males of both species during the increase and peak. The results do agree with those of Turner and Iverson[12] who found no significant difference in frequency of aggressive acts by male *M. pennsylvanicus* during periods of increasing and high densities. We conclude that selection for aggressive females was not a major factor driving cyclic fluctuations in abundance in our study populations.

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