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(*Microtus pennsylvanicus*)**

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HETEROZYGOSITY, AGGRESSION, AND POPULATION FLUCTUATIONS IN MEADOW VOLES (*MICROTUS PENNSYLVANICUS*)

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Abstract.—We tested whether variation in heterozygosity could produce cyclic changes in population size in meadow voles (*Microtus pennsylvanicus*). For this to occur, three conditions must be met: (1) populations are more outbred (heterozygotic) at high than low population density, (2) heterozygotic voles are more aggressive than relatively inbred individuals, and (3) heterozygotic voles have lower reproductive fitness, though being superior at defending resources. We found no evidence that heterozygosity varied with population size or that reproductive success varied with heterozygosity. However, the former test was indirect and relatively weak. We directly measured aggression and heterozygosity of individual voles. Aggression was significantly related to heterozygosity: higher heterozygosity correlated with more aggression in males and less aggression in females. The proportion of variance in aggression that could be explained by heterozygosity was small. These results suggest that changes in population size of meadow voles could not be driven by systematic changes in heterozygosity with population size.

Key words.—Aggression, heterozygosity, *Microtus pennsylvanicus*, population cycles, voles.

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In the last decade, there has been considerable interest in linking behavior and life-history variation among individuals with population dynamics (e.g., Sibly and Smith 1985; Komnicki 1988). One of the earliest connections of behavior and population dynamics was an attempt to explain cyclic changes in population size of small mammals (Chitty 1967) by behavioral and genetic changes in populations. Chitty proposed that different genetic types of small mammals were selected for at high and low population densities. He suggested that the animals favored at high population density were more aggressive but had lower rates of reproduction. Low reproductive rate and poor survival led to a rapid decline in population density. At low densities, more docile and fecund animals were favored by selection, resulting in an increase in population size. Chitty assumed that the genetic differences were the result of specific genes controlling aggression and associated life-history traits (body size and age at sexual maturity) and that selection altered the frequencies of these genes. Smith et al. (1975) proposed a modification of Chitty's hypothesis. Smith et al. suggested that there were no specific genes being selected as density varied

but rather than variation in heterozygosity with population density caused differences in levels of aggression. Smith et al.'s hypothesis (hereafter termed the "outbreeding hypothesis," following Tamarin and Sheridan 1987) requires that two independent assumptions are met.

First, the outbreeding hypothesis requires that population structure varies with density. When population density is low, the population is predicted to be structured into relatively small and independent breeding groups. Within each group, inbreeding will occur and heterozygosity will decrease. However, as population densities increase, dispersal is expected to increase, causing the breakdown of the small breeding groups. This dispersal results in outbreeding and an increase in heterozygosity at high population densities. This pattern would be observed only if one could follow a population through an entire cycle of increase and decline. If the population sampled is relatively stable, then one may not find a relationship between density and heterozygosity over the relatively small range of densities observed.

Second, the outbreeding hypothesis requires that as population size increases, a point is reached when some event causes a decline in fitness, leading to a decline in population size. The hypothesis is that more aggressive animals are better competitors for resources and hence presumably have higher survival but simultaneously have a lower reproductive success. This

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facet of the hypothesis can be tested directly, in two stages: (1) a test of whether more heterozygotic animals are more aggressive, and (2) a test of whether more heterozygotic animals have a lower rate of reproduction. The outbreeding hypothesis predicts that both of these patterns will be found. A further, indirect test of the outbreeding hypothesis can also be made. The hypothesis predicts that increases in average heterozygosity in a population will immediately precede, and not follow, a decline in population size.

Support for the outbreeding hypothesis is mixed. Rates of aggression were maximal in some vole species at peak densities (*Microtus ochrogaster* and *Microtus pennsylvanicus*, Krebs 1970; *Clethrionomys gapperi* females but not males, Mihok 1981), or when populations were growing rapidly (*Microtus townsendii*, Krebs 1985). The most aggressive populations of old-field mice (*Peromyscus polionotus*) on average were more heterozygous (Garten 1976). Finally, high density populations had higher average heterozygosity than low density populations in old-field mice (Smith et al. 1975) and pocket gophers (*Thomomys bottae*, Patton and Yang 1977; *Thomomys umbrinus*, Patton and Feder 1978). In contrast to the above supporting data are other studies. The level of aggression did not change systematically with the phase of the population cycle (Hofmann et al. 1982) for the same species studied by Krebs (1970), though Hofmann et al. confound seasonal and cyclic changes in population size in their analyses. In addition, studies have found that there is no relationship between heterozygosity and the phase of the population cycle (*M. ochrogaster*, Gaines 1981; *Microtus agrestis*, Nygren 1980).

Weaknesses exist with the empirical studies supporting the outbreeding hypothesis. The studies of old-field mice (Smith et al. 1975) were comparisons among populations; different processes may occur within than among populations. Often, few variable loci have been assessed for variation in heterozygosity (e.g., Berry and Jakobson 1975; LeDuc and Krebs 1975; Leigh Brown 1977; Smith et al. 1984). Behavioral observations and genetic data have been collected mainly on males (but see Mihok 1981; Krebs 1985); thus, the relationship between heterozygosity and aggression in females is unclear. Thus, there is need for tests of the outbreeding hypothesis that are conducted within single populations, looking at variation within both sexes, and assessing heterozygosity at several loci.

Our study was designed to test the assumptions required by the outbreeding hypothesis, using populations of the meadow vole (*M. pennsylvanicus*). Specifically, we asked four questions. (1) Does inbreeding (high homozygosity) occur more often at low than high population densities? (2) Are more heterozygotic voles more aggressive? (3) Does heterozygosity affect reproductive success? (4) Do changes in heterozygosity precede changes in population density? The principal aim of this paper is to examine the second of these questions. Whereas previous work has focused mainly on male microtines, this study considers both males and females. We will show that there is little or no evidence to support the hypothesis proposed by Smith et al. (1975) and refute some assumptions of their hypothesis.

MATERIALS AND METHODS

Field Data and Population Estimates

Four vole populations were used in this study. One population (Airport) located in metropolitan Toronto, Ontario was used in a previous study (Boonstra and Rodd 1983). The other sites (Amherst Island, Collins Bay, and Wolfe Island) were located near Kingston, Ontario (for detailed descriptions see Pavone 1992). At each site, a control grid, which comprised 49 trapping sites (7 × 7 array, each site with one Longworth trap) at 7.6-m intervals, was set up to monitor population demography. Except for Amherst Island, an additional trap was added to alternate trap positions to accommodate high densities. The remainder of each study site (at least 30 times larger than the control grid) was designated as a removal area from which animals were removed for behavioral and genetic study. Traps in the removal areas were at least 20 m away from the control grid and were seldom moved from their original positions at the start of each season. Occasionally, animals from the control grids were trapped in the removal areas, but this rarely occurred (nine times at Airport, three at Collins Bay, three at Amherst, and one at Wolfe). The control grids at the three Kingston sites were trapped approximately every 3–4 wk from mid-May 1982 until August 1984. The Airport site, because of its proximity, was trapped every 2–3 wk from May 1982 to November 1983. See figure 1 for a graphical presentation of the trapping dates. No trapping was done between December and mid-March of each year because voles were not breeding during this period. Voles on control

grids were tagged with numbered fish fingerling tags at first capture to allow mark-recapture estimates of population size.

One of four removal areas was trapped every 2 wk such that each removal area was trapped, on a rotating basis, every 2 mo. The exact schedule is presented in figure 1. A maximum of 40 animals of various ages and reproductive conditions were removed and brought back to the laboratory for behavioral and electrophoretic studies. The potential impact of removals was not measured but was considered to have negligible effects on population numbers. In the laboratory, vole mass and skull width were measured as indices of size. Reproductive status was assessed for males as either testes abdominal (nonreproductive), or testes scrotal; females were classified as not pregnant, pregnant, or lactating. Additionally, litter size of pregnant and lactating females was recorded and minimum numbers of pregnancies assessed from uterine scars.

Mammalian predators (*Mustela erminea*, *Blarina brevicauda*, *Procyon lotor*, and *Mephitis mephitis*) were removed from control and removal grids whenever caught. This study required changes in population size that could not be attributed to external factors such as predation; thus, predator removal was used to minimize one extrinsic source of variation in population size.

Population sizes (fig. 1) and rates of change of population size were calculated as Jolly-Seber mark-recapture estimates, except for first and last values in a series, which were estimated as minimum number alive. Calculation of population sizes was done using computer programs developed by C. J. Krebs.

Behavioral Data

Prior to and during testing, we attempted to keep conditions as standardized as possible. Voles were held captive at the field sites for from 6 h to 3 d before return to the laboratory. Prior to behavioral tests, voles were housed alone in opaque polypropylene cages for 18 to 20 h. The length of this isolation period was constant for

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FIG. 1. Population changes (Jolly-Seber estimates) for *Microtus pennsylvanicus* on four 0.4-ha trapping grids in southern Ontario. Closed circles (●) are females, open circles (○) are males. Along the top of each graph is located the time and number of voles removed from areas adjacent to these grids for analyses of aggression and heterozygosity.

all voles in a group that were tested with each other. With few exceptions, behavioral tests were conducted from 8:00 A.M. to 4:00 P.M.

Behavioral tests involved placing two voles in a neutral, circular (30 cm diameter) arena for 10-min bouts. Opponents differed no more than 5 g in mass and were the same sex and reproductive status as both of these variables are known to affect aggression (Reich et al. 1982). This testing procedure assumes that interactions with physically equal opponents are a good indicator of levels of aggression in encounters with all individuals in a population. Each vole was tested from one to six times with a different opponent in each trial; 90.5% (1036 of 1145) of voles took part in five trials.

Five behaviors were recorded for voles during trials. These behaviors were approach, amicable, avoid, threat, fight, and retaliation. See Pavone (1992) for detailed descriptions of these behaviors, which differed only slightly from the definitions used by Krebs (1970). Behaviors were recorded on a manual tally logger. L.P. made all behavioral observations.

We used principal-components analysis (PCA) to summarize the various behaviors into a single index of aggression. The PCA was based on a correlation matrix, and each trial of a vole was used as a separate data point. Inspection of factor loading scores (table 1) indicates that the first principal component is a good index of aggression: the highest loadings on this principal component were threat and fight, and amicable loaded opposite all other behaviors. Hence, we used factor scores from the first principal component as our index of aggression, and all reference to measures of "aggression" henceforth in this paper will refer to this index. Note, however, that the first principal component accounted for only 40% of all variance in behaviors; some of the variation in aggressive behavior is not accounted for by our index of aggression. Further, the raw indices of aggression had to be corrected for artifacts introduced by the experimental design and for variance introduced by dealing with voles of different sex and reproductive status.

The first correction involved taking the results from multiple trials for each vole and combining them into a single index of aggression for that vole. We tested for three potential artifacts introduced by the behavioral testing procedure: changes in behavior between trials caused by prior experience in the test arena (Lagerspetz 1964), variation caused by the time of day of trials, and

TABLE 1. Results of principal-components analysis (PCA) on behavioral data. Factor loadings scores are given for the first three principal components. The final row of the table is the percentage of total variance accounted for by each of the principal components.

Behavior	PC1	PC2	PC3
Approach	0.534	-0.567	0.245
Amicable	-0.516	0.209	0.530
Avoid	0.632	-0.308	-0.432
Threat	0.820	0.248	0.237
Fight	0.781	0.176	0.459
Retaliate	0.386	0.686	-0.357
Variance (%)	39.7	17.1	15.4

effects of the slight differences in size between opponents. Effects of the number of previous trials were tested with repeated measures ANOVA and profile analysis (Winer 1971, pp. 296–300; Wilkinson 1988, pp. 541–552). Only data from those voles undergoing five behavioral trials were included in this analysis. A statistically significant, nonlinear decrease in aggression of each vole appeared through its series of trials (within vole variation $F = 46.5$; $df = 4, 4216$; $P < 0.001$). This effect of previous experience was removed by calculating the mean level of aggression for each trial (1–6) over all voles and subtracting these averages from the raw indices for each vole. Next, we used a polynomial regression to determine if the average level of aggression varied with time of day. A statistically significant ($F = 16.6$; $df = 2, 5576$, $P \ll 0.001$; only linear and cubic terms were statistically significant) and biologically trivial ($r^2 = 0.006$) trend occurred for aggression to be higher in midday trials than early morning and late evening trials. We eliminated effects of time of day by subtracting the index of aggression predicted by the regression from the observed index. Finally, we tested for effects of three differences between opponents: number of previous bouts, mass, and head width (a measure of skeletal size). We calculated the difference between opponents for these three parameters and tested whether indices of aggression were correlated with the calculated differences (both signed and absolute values). None of the six correlations was statistically significant (all $r \leq 0.02$; all $P \geq 0.14$), even with sample sizes of 5578. Thus, we ignored any slight artifacts owing to variation between voles.

After variation caused by bout number and time of day were statistically removed from indices of aggression, we assessed the consistency

of aggression within voles by calculating the repeatability (Sokal and Rohlf 1981, p. 216; Lessells and Boag 1987). Repeatability was 34.9%, indicating that about 65% of all variance in aggression was found within individual voles. Although large, the amount of variation in behavior of individual voles was consistent with that found by Krebs (1970) for *Microtus pennsylvanicus* and *Microtus ochrogaster*. As a result of the analyses conducted above, we calculated our indices of aggression for each vole as the average of the values measured for all (from 1–6) of a vole's trials, after correcting these values for variation caused by the number of previous bouts and the time of day of the bouts.

It was necessary to look for sources of variation in aggression among voles that would prevent direct comparisons in aggression level among voles. The potential confounding variables examined were sex (Mihok 1981; Krebs 1985), reproductive status (Gleason et al. 1980; Boonstra 1984), sampling location (a difference in average aggression among the four study areas), and time of year (Turner and Iverson 1973). Preliminary analyses (nested ANOVA with reproductive status nested within sex) showed that aggression varied with both sex ($F = 9.4$; $df = 1, 1140$; $P = 0.002$) and reproductive status ($F = 156.2$, $df = 3, 1140$; $P < 0.001$). Reproductive status has a different hormonal basis in the two sexes (Rosenblatt and Seigel 1981; Van del Poll et al. 1981) and probably has different behavioral consequences (Boonstra and Rodd 1983). Hence, data from the two sexes cannot be combined within categories of reproductive status.

Next, we examined whether the average level of aggression varied among the four study sites by conducting two-way ANOVAs with reproductive status and study site as the main effects. Analyses were conducted separately for each sex. For both sexes, the average difference in aggression between sites was affected by reproductive status (reproductive status $\times$ site interactions males: $F = 2.98$; $df = 3, 571$; $P = 0.03$; females: $F = 2.06$; $df = 6, 554$; $P = 0.056$). In general, reproductive males were more aggressive than nonreproductive males, and lactating females were more aggressive than pregnant females, which were more aggressive than nonreproductive females; just the degree of difference varied among study sites. The statistically significant interactions mean that differences in reproductive status had to be corrected for separately for each study site. These corrections were made by

obtaining estimates of the mean level of aggression for each combination of study site and reproductive status, and subtracting the index of aggression of each vole from the appropriate average.

Finally, we detected variation in average aggression with calendar date for both sexes: in spring/summer voles were more aggressive than fall/winter individuals. This seasonal effect was accounted for in the analyses presented in the Results.

One complication exists in correcting for variation in aggression between sites that requires comment. If differences in average aggression among study sites were caused by differences in the average level of heterozygosity among sites, then correction for differences among sites would be eliminating the effect of heterozygosity on aggression. However, this is not a major problem. Repeatabilities (Sokal and Rohlf 1981; Lessells and Boag 1987) were calculated for both aggression and heterozygosity to determine whether variance in these measures was mainly among study sites or among sampling periods within an individual site. Only about 5% of variance in heterozygosity and 11% of variance in aggression occurred among study sites. Our corrections would, at worst, remove only a small proportion of variance in aggression that could not be ascribed exclusively to heterozygosity or to other intrinsic differences among study sites.

In summary, our measure of aggression of voles is based on from one to six trials (mostly five) for each individual. The index is a summary of several agonistic behaviors that were produced by PCA. Indices of aggression have been corrected for effects of variation in aggression within individual voles (effects of previous experience and time of day) and variation among voles (reproductive status and study site). The analyses of behavior presented in the Results were done separately for each sex and took into account seasonal variation in aggression.

Genetic Data

After behavioral tests were conducted, voles were sacrificed for analysis of heterozygosity. For all individuals, starch-gel electrophoresis (methods slightly modified from Selander et al. 1971) was used to assess variation at 13 loci. The proteins examined were transferrin, aminopeptidase (cytosol), and albumin all from blood plasma; malate dehydrogenase-2, isocitrate dehydrogenase-1, -2, fumarate hydratase, aspartate ami-

notransferase-1, phosphoglucomutase-1, -2, -3, lactate dehydrogenase, and mannosephosphate isomerase all from kidney. In addition, from 1983 onward cellulose-acetate electrophoresis (as described by Sheridan and Tamarin 1985) was used to examine salivary amylase-1 and -2. These 15 loci were chosen from a survey of 38 loci, six buffers, and the following tissues; brain, hemolysate, kidney, liver, muscle, and heart. The loci initially screened were essentially all known at the time to be polymorphic in some species of small mammal. The 15 loci finally chosen were all those that produced consistent results and could be scored unambiguously in our laboratory. The tissues finally chosen were those that could be rapidly prepared for analysis, because of logistic constraints on time. The number of alleles per locus varied from one to six ($\bar{x} = 3.1$), and the most abundant allele of each of the 13 polymorphic loci represented an average of 87.5% of all alleles at a locus. Although polymorphism was not a criterion for choice of loci to be screened in our study, the proportion of polymorphic loci was higher than that found for a large selection of loci from *Microtus californicus* (15/32; Bowen and Yang 1978).

Heterozygosity was calculated (see Ayala 1982) using a program written specifically for our data set. This program was capable of calculating observed and expected heterozygosity for subsets of the population (i.e., sex, study site, and sampling period) and observed heterozygosity for individual voles. Heterozygosities of individual voles varied from 0 to 0.47, with a mean of 0.13 ± 0.08 SD. We examined the consequences of five potential confounding influences on the heterozygosities calculated for the separate study sites and sampling periods: effects of using different numbers of loci before and after 1983, the potential of insufficient sampling of individuals to obtain representative heterozygosities, correlation in heterozygosity between one sampling period and the next (autocorrelation), differences in heterozygosity between sexes, and seasonal variation in average heterozygosity.

We faced a problem in combining data from 1982, when only 13 loci were analyzed, and 1983–1984 when 15 loci were analyzed. We are interested in determining whether there are differences in heterozygosity and behavior between sampling periods. Calculating heterozygosities based on different numbers of loci may produce a systematic bias in the heterozygosities from the 1982 data. Simply discarding the amylase data

did not seem appropriate, as the majority (18 of 32) of samples included these data, and the amylases were both moderately variable loci. Instead, we chose to determine the size of the bias introduced by using variable numbers of loci. This determination was done by using the sampling periods from which we had data from all 15 loci to calculate heterozygosities both with and without data from the two amylases. The average heterozygosities were then compared with a paired *t*-test, which showed that there was a statistically lower heterozygosity when data from the amylases were omitted ($t = 1.96$, $df = 17$, $P = 0.067$). However, the average heterozygosity was smaller by only 0.006. In contrast, the standard deviation in heterozygosity among sampling periods was 0.028. The bias produced was only 20% of a standard deviation, insufficient to be a major source of variance. Hence, we chose to ignore this slight bias in our analyses.

The second potential problem is sampling an insufficient number of voles to obtain a representative measure of heterozygosity for each sampling period. This would be manifested by (though not the exclusive cause of) a low repeatability in heterozygosity from one sampling period to the next, within each study site. The correlation in heterozygosity from one sampling period to the next was low and not statistically significant ($r = 0.13$, $df = 26$, $P = 0.52$). We had sufficient statistical power (Cohen 1988) to detect a correlation of 0.5 with 80% probability, and a correlation of 0.57 with 90% probability. Hence, our inability to detect a statistically significant correlation appears not to be the result of low sample sizes. Heterozygosity simply changed unpredictably between successive sampling periods. Hence, we may have collected an insufficient number of voles at each sampling period. We tested for this possibility with a form of cross-validation: we calculated heterozygosity separately for males and females, within each sampling period, and correlated the values. The average heterozygosity of males and females varied in parallel ($r = 0.58$, $df = 30$, $P < 0.001$). We concluded from these results that although average heterozygosities of populations varied greatly through time, this effect was not an artifact of undersampling or of sampling biases associated with a single sex. We also concluded from these findings that autocorrelation is a relatively minor problem when we are examining links between heterozygosity and population dynamics: because heterozygosity changed so much between

sampling periods, any correlation between heterozygosity and population changes is likely not to have resulted from long-term but functionally uncorrelated patterns of variation in heterozygosity and population size.

We need to test whether average heterozygosity differed between sexes, because we are analyzing the relationship between aggression and heterozygosity separately for the two sexes. By indicating that a statistically significant correlation exists between male and female heterozygosity, the previous paragraph partially addresses these concerns. However, we still need to know if the absolute value of heterozygosity differs between sexes. This was determined in a paired *t*-test between males and females from the same sampling period and study site. Females had an average heterozygosity that was higher by 0.002, but this difference was not statistically significant ($t = 0.05$, $df = 31$, $P = 0.62$). We had sufficient statistical power (Cohen 1988) to detect a difference of 0.012 with an 80% probability, and a difference of 0.014 with a 90% probability. If there is a biologically real difference in heterozygosity between sexes, the difference is trivial. Hence, when using population average heterozygosities we used values calculated for males and females combined. The average was used under the assumption that the larger sample sizes were more likely to approximate the population's real heterozygosity.

Finally, it is possible that average heterozygosity is simply a function of season (Berry and Murphy 1970; Berry and Jakobson 1975). We tested this by entering the average heterozygosities for each sampling period into a quadratic regression against time of year (week, in which the first week in January was designated "1"). No statistically significant relationship existed ($r^2 = 0.03$; $F = 0.47$; $df = 2, 29$; $P = 0.63$). An effect of season this small, if real, had only about a 1.8% probability of being detected; sample sizes were sufficient to have detected an r^2 of 0.35 with 80% probability, and of 0.40 with 90% probability. Hence, we cannot rule out the possibility of an undetectably small variation in average heterozygosity through the year. However, we did not control for seasonal variation in heterozygosity in our analyses.

Statistical Analyses

Conventional statistical analyses were conducted using versions of SAS (SAS Institute 1982), BMDP (Dixon et al. 1983), and SYSTAT (Wil-

kinson 1988). Nonstandard, randomization tests (e.g., Manly 1991) were used in two cases in which no conventional statistical test was appropriate. These randomization tests were conducted using C programs written specifically for these analyses by W.M.H. Because biologically real relationships might be too small to be detected statistically given the samples sizes that we have, we conducted power analyses for nonsignificant statistical tests whenever an appropriate power analysis was found in Cohen (1988). For each power analysis, three calculations were made. First, we calculated the probability of being able to detect a biologically real effect of the size actually observed with $\alpha = 0.05$. Second, we calculated the size of effect that could have been detected with an 80% probability; third, the effect detectable with a 90% chance was calculated. Statistical tests were considered "significant" if $P \leq 0.05$ or less.

RESULTS

Population Size and Inbreeding

Vole populations are predicted to be subdivided into smaller, relatively unmixing demes at low population densities (Smith et al. 1975). This population structure would be evidenced by a higher proportion of homozygotic individuals than expected by chance (Wahlund Effect). The prediction would be demonstrated statistically as a positive correlation between the degree of outbreeding and population size. We measured degree of outbreeding as the difference between observed and expected average heterozygosity for each sampling period and study site (larger positive values indicating a more outbred population); separate estimates were made for each study site and sampling period. Population density at the time a cohort of voles is born, not density at trapping, should be related to the degree of inbreeding. Most young voles will enter traps at about 4–6 wk of age (Boonstra et al. 1987; unpubl. data). Hence, we chose to correlate deviation from expected heterozygosity with population density roughly 6 wk previous (range 4–9 wk, mean = 6.0 weeks).

Conventional statistical techniques are not appropriate for the analysis. The first reason is that each of the study sites has only a series of three to seven data points with which to correlate outbreeding and population density. If data from each site were analyzed separately, the statistical power to detect weak correlations would be very

TABLE 2. Relationship between heterozygosity and aggression. Positive slopes indicate that aggression is greater in more heterozygous voles. The final column of the table gives partial coefficients of determination from the multiple-regression analysis, after variation in aggression through the season has been controlled.

Site	Sex	Slope	df	P	Partial r^2
All	male	0.699	1, 565	0.050	0.007
	female	-0.606	1, 557	0.054	0.007
Airport	male	0.723	1, 157	0.263	0.008
	female	-0.057	1, 152	0.902	0.001
Amherst	male	-1.306	1, 126	0.082	0.024
	female	0.018	1, 102	0.985	≥ 0.001
Collins	male	1.213	1, 135	0.081	0.023
	female	-0.705	1, 187	0.207	0.009
Wolfe	male	2.397	1, 135	0.004	0.060
	female	-1.690	1, 104	0.030	0.045

low, and biologically real patterns would likely be overlooked. Combining data from different sites is also inappropriate for a standard correlation analysis, as the data do not come from the same population, and each population may show a quantitatively different pattern. We would be combining data from four within-population relationships, and each population may show a different maximum and minimum heterozygosity and rate of change in population size. The result of combining data from four independent patterns could be that the actual probability of any correlation coefficient may not be that provided in standard tables of statistical significance. These potential problems meant that there was no suitable, conventional statistical test appropriate for our data. For this reason, we opted to use a randomization test (e.g., Manly 1991). The randomization procedure was as follows: at each randomization, the pairings of heterozygosity and population density were rearranged within each study site. In essence, we were asking whether the observed associations between heterozygosity and rate of change in population size were greater than would have occurred if the observed pairings were random. The pairings were conducted separately for each study site, because each site had an independent series of data. The test statistic used was a conventional product-moment correlation, and data from all four sites were combined for the calculation of the statistic. A program was written specifically for these analyses. The random number generator used was RAN1 from Press et al. (1988) and the algorithm for shuffling pairs of data was found in Manly (1991). For each of the two analyses, 9999 randomizations (plus the real correlation) were conducted. This number of randomizations

should be sufficient to calculate a null distribution (Manly 1991).

A tendency appeared for populations to be more outbred at higher population densities, although this tendency was not statistically significant. The observed correlation between outbreeding and density was $r = 0.27$. However, more than 2900 of the 9999 random reorderings of data yielded correlation coefficients larger than 0.27 and the resulting probability that the observed pattern was biologically real was $P = 0.59$ (two-tailed).

Heterozygosity and Aggression

For the outbreeding hypothesis to hold, there must be an increase in aggression with increased heterozygosity. We tested for this pattern separately for each sex. As noted in the Materials and Methods, variation in aggression with season (a curvilinear relationship) should be statistically controlled in these analyses. We did so with a multiple regression of aggression against heterozygosity, including week (first week in January = "1") and week² as dependent variables to remove any effects of season. The only other consideration was whether to use the population-wide estimates of heterozygosity and aggression for each sampling site and period, or whether to use individual voles as the data points. Other studies (Smith et al. 1975; Garten 1976) have chosen the former, with the stated justification that heterozygosities calculated for individuals are likely to have greater error than estimates for an entire sample. However, we chose to present analyses using individual voles as data points because (1) errors in estimating individual heterozygosity are unlikely to affect the general pattern given the large sample sizes, and (2) the

TABLE 3. Effects of heterozygosity on reproduction. "Male potential" is the probability that a male will have scrotal testes, and "Female potential" is the probability that a female will have a litter on capture. "Litter size" and "Number litters" are for females only. "Season" is a test for seasonal variation (quadratic), and the probability is the lower of either the linear or quadratic term; "Mass" is the vole's mass at capture; "Stage" distinguishes whether females were pregnant or weaning; and "Heterozygosity" is the heterozygosity of individuals. Probabilities associated with the first two dependent variables were calculated from logistic regression, and for the last two variables by ANCOVA. Squared semipartial correlations are the percentages of total variance in the dependent variable that were accounted for by variance in heterozygosity alone. "Power" is the probability of statistically detecting a semipartial r^2 of the observed size. The final two columns of that table indicate the semipartial r^2 s that could have been detected with power of 80% and 90%.

	Study site	Season	Mass	Stage	Heterozygosity	Semipartial r^2	Power	r^2 for $(1 - \beta)$	
								0.8	0.9
Male potential	$P < 0.001$	$P < 0.001$	$P < 0.001$	N/A	$P = 0.95$	N/A	N/A	N/A	N/A
Female potential	$P < 0.001$	$P < 0.001$	$P < 0.001$	N/A	$P = 0.95$	N/A	N/A	N/A	N/A
Litter size	$P = 0.005$	$P > 0.21$	$P < 0.001$	$P < 0.001$	$P = 0.69$	0.001	0.04	0.04	0.05
Number litters	$P = 0.96$	$P > 0.70$	$P = 0.064$	N/A	$P = 0.97$	≈ 0	≈ 0	0.04	0.05

strength of the relationship (as measured by the coefficient of determination) will give an indication of how much of the variability in aggression of individuals is related to their heterozygosity. In nature, vole populations are not homogeneous but are collections of variable, independent individuals; thus, our analyses seem appropriate. Still, it must be remembered that the strength of the relationship between aggression and heterozygosity is a minimal estimate, because part of the variance in estimated heterozygosity is caused by misestimating heterozygosity of individuals. As a final note, we found the same patterns as described below, when population average heterozygosities were analyzed.

For both sexes, there was a statistically significant relationship between heterozygosity and aggression, after a statistical correction was made for season (table 2). More heterozygous males were more aggressive, as expected; however, more heterozygous females were less aggressive. Although heterozygosity is statistically related to aggression, less than 1% of all variance in aggression was explained by differences in heterozygosity among voles (table 2).

Potentially, aggression was not affected by heterozygosity at all of the study sites, or the relationship between heterozygosity and aggression was observed only because different sites varied both in average aggression and heterozygosity. Combining data from all four sites would not reveal these trends. Therefore, we conducted the multiple regressions separately for each study site (table 2). At one of four sites (Amherst), patterns were reversed with aggression decreasing with

increased heterozygosity of males and very little relationship among females. The separate sites, as with the combined sites, showed very little of the variance in aggression related to differences in heterozygosity ($\leq 6\%$ in all cases).

Heterozygosity and Reproductive Success

Heterozygosity may affect reproductive success of voles (Smith et al. 1975), and changes in reproductive success may affect population dynamics. All animals that were trapped were assessed for reproductive condition. Additionally, for pregnant or lactating females we know the number of offspring that they had, and estimated the total number of litters that they bore. Thus, for both sexes we have a measure of the potential to produce offspring, and additionally for females we have measures of offspring actually produced. In examining relationships between heterozygosity and reproductive success we had to control for variation in our measure of reproduction with time of year, vole age (indexed by mass), study site, and (for number of offspring in litter) whether females were pregnant or lactating. Effects of heterozygosity on reproductive potential (i.e., scrotal testes for males, and pregnant/lactating for females) were tested with logistic regression, whereas variation in female litter sizes and numbers of litters were tested with ANCOVA. Effect of heterozygosity on numbers of litters was examined only for females that had produced at least one litter. The measure of heterozygosity used was individual heterozygosity.

No indication existed that heterozygosity strongly affected reproductive success (table 3).

For neither sex did reproductive potential vary with heterozygosity, after differences between study sites, vole ages, and season were controlled. Additionally, for females there was no relationship between heterozygosity and litter size or number of litters produced (table 3). For these latter two variables, if the effects detected were biologically real, we had little statistical power to detect such small effects. However, even relatively small effects of heterozygosity could have been detected with high statistical power.

Heterozygosity and Population Dynamics

Smith et al. (1975) predicted that high heterozygosity will cause declines in population size. Testing this prediction is difficult with the data that we have. The data are descriptive, and there are reasons to believe that changes in heterozygosity either cause or result from changes in population size (Smith et al. 1975; Gaines and Whitam 1980). Our approach was to test whether heterozygosity predicted subsequent changes in population size and did so better than a change in population size predicted subsequent heterozygosity. The former would suggest that heterozygosity causes population dynamics, and the latter would suggest the converse. For our measures of heterozygosity, we used mean heterozygosity for all voles in one sampling period. Our measure of change in population size was the rate of change in population size, as calculated from Jolly-Seber estimates (e.g., Krebs 1989). At small population sizes, extreme estimates of change in population size may occur (i.e., a density change from one vole/unit area to three voles/unit area would be an extremely large rate of change). To smooth out extreme estimates of change in population size, we used three-point running averages of rate of change of population size, with the rate in the time period in question being averaged with rates in the trapping periods immediately before and afterward. Running averages were not calculated when sequential trapping periods fell across the winter nontrapping season (fig. 1); population estimates from immediately before and after the nontrapping period were not used in our analyses. As with correlating degree of outbreeding and population size, it was not appropriate to conduct the current analyses with a conventional correlation analysis. As above, we used randomization tests to determine the statistical probability of our correlations.

The randomization tests indicate that there is

TABLE 4. Magnitude of variation in population sizes of meadow voles. Data for noncyclic and cyclic populations are averages ($N = 10$ and 16 , respectively) derived from Taitt and Krebs (1985). All densities are given in voles/ha.

	Minimum density	Maximum density	Ratio
Noncyclic	57.2	171.6	1:3.3
Cyclic	22.5	155.5	1:6.8
Airport	180.5	310.4	1:1.72
Amherst	13.5	167.6	1:12.4
Collins	32.3	401.2	1:12.4
Wolfe	2.7	338.2	1:125.5

no relationship between heterozygosity and the rate of change in population size. A tendency existed for high heterozygosity to precede an increase in population size ($r = 0.15$), but the randomization test indicated that a correlation this large had a reasonable chance of occurring randomly ($P = 0.296$, two-tailed). Likewise, although an increase in population size tended to be followed by a lower heterozygosity ($r = -0.16$), the randomization test suggested that a correlation of this magnitude was likely by chance alone ($P = 0.53$, two-tailed).

DISCUSSION

We found no statistically significant evidence that was entirely consistent with the hypothesis that variation in population structure with density can drive changes in population size of meadow voles. However, the tests of the four assumptions varied in their directness and robustness. The strongest tests were those looking for a relationship between heterozygosity and aggression (table 2). In these tests we could directly relate heterozygosity of individual voles to their level of aggressiveness. The link between heterozygosity and reproductive success (table 3) was more weakly tested for males, as we could not directly measure reproductive success but only the potential of voles to reproduce. Numbers of offspring produced by female voles were known, and, thus, the lack of relationship between heterozygosity and female fecundity is a strong test. However, it should be noted that effects of female heterozygosity on postparturition survival of offspring were not examined in this study. The weakest tests we conducted involved the effects of population size and changes therein. The outbreeding hypothesis describes genetic changes over an entire population cycle, whereas three of

FIG. 2. Relative magnitudes of influences on aggression. Variation in average aggression (uncorrected indices of aggression) is plotted as a function of actual range of heterozygosity, reproductive status, and season. Lines are predicted values from least-squares regressions in which only the factor in question was entered into the regression (i.e., we assume no interaction between heterozygosity, reproductive status, and season). Values on the x-axis are plotted from minimum to maximum value for heterozygosity, reproductive status (minimum, not reproductive; maximum, lactating), and season (minimum, week 16, maximum, week 37). Values are not plotted on the axes as the general patterns and not the exact values are important. Variation in aggression is plotted to the same scale for all regressions. Data are for female voles from the Wolfe Island study site, the population with the strongest relationship between heterozygosity and aggression.

our four populations were not observed to undergo a crash in population size that is central to the hypothesis, and for the fourth population we were unable to obtain sufficient voles for analysis of heterozygosity after the crash (fig. 1). Thus, we cannot reject the predictions completely. Nevertheless, three of four populations (Amherst, Collins, and Wolfe; table 4) showed variation in population size of magnitudes similar to that observed in meadow vole populations that exhibit multiannual cycles (Taitt and Krebs 1985). Even our large fluctuations in population size were not associated with changes in average heterozygosity.

Note that the correlations between heterozygosity and other parameters are minimum estimates. Our estimates of heterozygosity are based on only 13 or 15 loci. The variance in estimates of heterozygosity is inversely proportional to the number of loci examined (Nei and Roychoudhury 1974), and the few loci assessed in our study results in a large but unbiased error variance in our estimates. The result is relatively low statistical power to detect relationships of other vari-

ables with heterozygosity. The statistically significant relationships that we tested are unlikely to be the effect of sampling error, but biologically real relationships may have been missed in our analyses.

Our finding of a lack of correlation between population fluctuations and degree of heterozygosity is not completely inconsistent with previous studies. There exist both aspects of the social system of meadow voles that will cause heterozygosity to vary with population density and aspects that serve to dampen variation in heterozygosity with population fluctuations. Breeding females are highly territorial (Madison 1980; Webster and Brooks 1981; Boonstra and Rodd 1983) and show a high degree of natal philopatry (Boonstra et al. 1987), such that clusters of related females are formed. This clustering would tend to promote a demic structuring of the population at low densities. This result has been shown in a recent study of mitochondrial DNA (Plante et al. 1989), which found that genetic structuring of female transmitted DNA was highest at a low population density (similar to the relationship in *Microtus californicus* found by Bowen 1982). However, it is not clear whether demic structuring was present only for females, as neither study differentiated between sexes in their analyses. Also, Lambin and Krebs (1993) note that female demes can be maintained at high population densities, thus precluding variation in population structure with density. The second factor that could promote homozygosity at low population density is inbreeding through non-random matings, but Pugh and Tamarin (1988) found that inbreeding was at a level predicted by random mating (although with a small sample size). One factor that may serve to lower inbreeding and break up genetic demes is the high degree of promiscuity of male meadow voles, which should serve to increase genetic variability in demic groups. Boonstra et al. (1993) conservatively estimated that at least 33.1% of all litters are multiply sired.

We question whether variation in aggression with heterozygosity has important consequences for population dynamics of meadow voles. As with previous work (e.g., Garten 1976), we found that more heterozygotic males were generally more aggressive. However, the amount of variation in aggression that was explained by heterozygosity was small (table 2). Note also that the partial coefficients of determination in table 2 were calculated for residual variance after sea-

sonal, reproductive, and site specific variation in aggression was statistically eliminated! Further, males and females were not affected by differences in heterozygosity in the same manner. Finally, not all populations showed the same relationship between aggression and heterozygosity (table 2). Combined, these observations suggest that although there is a genetic basis for levels of aggression, this genetic variation may potentially be masked by variation in environment. This is illustrated in figure 2, where the magnitude of variation in aggression of female voles is shown as a function of season, reproductive status, and heterozygosity. The greatest variation in average aggression is caused by differences in heterozygosity; however, variation in reproductive status produces changes in average aggression that are more than half as great.

Variation in population dynamics and genetics between sexes requires more attention by researchers. The qualitatively different relationships between heterozygosity and aggression for the two sexes found in this study would make it difficult for the outbreeding hypothesis to work. The hypothesis would require modification such that inbreeding decreases for males and increases for females as population density increases. Data suggest that spacing behavior in microtines is sex specific, with males competing for access to breeding females and females competing for access to food and space to rear offspring (Boonstra 1977; Redfield et al. 1978; Boonstra and Rodd 1983; Ostfeld 1985). Lambin and Krebs (1993) suggest that such sexual differences will result in neighboring females being more closely related as population density increases, counter to the expectation for males. However, as long as one sex (males) disperses more widely as population density increases, offspring should still become more heterozygotic as population density increases. Additionally, there should not be any difference in average heterozygosity with offspring's sex, a result found in our study. Although independence of spacing behavior does not appear to have implications for genetic structure of populations, there are implications for population dynamics. Any variation in aggression that causes declines in males would have no effect on females, though a decline in female density should directly affect males (Boonstra and Rodd 1983). Though declines in adult survival are often sex specific (Krebs et al. 1969; Krebs and Boonstra 1978), there are no reported cases of cyclic populations (Krebs and Myers 1974; Taitt and Krebs

1985) in which both sexes do not eventually reach similar low numbers. Similarly, the correlation between densities of males and females in our study populations was high ($r = 0.69$).

In conclusion, we suggest that changes in aggression with changing heterozygosity cannot drive fluctuations in population size of meadow voles. Heterozygosity may control population size via other mechanisms, as our tests of variation in male reproductive success with heterozygosity and changes in heterozygosity with population size are weak. Nevertheless, we did not find any indication that reproductive rate (table 3) or fluctuations in population size were related to changes in average heterozygosity in a population. These results are consistent with the conclusions of Gaines (1985) and of Boonstra and Boag (1987), who showed that most of the variability in life-history traits of meadow voles are of maternal, not genetic, origin. Further studies following small mammals through complete population cycles are still required, and attention should be paid to potential differences between sexes in population structure (e.g., Lambin and Krebs 1993) and genetic response as populations fluctuate in size.

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