Demographic consequences of artificial selection at the LAP locus in voles (*Microtus townsendii*)

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A number of studies on small mammals have shown that changes in the frequency of alleles at polymorphic loci are correlated with population fluctuations. To determine whether the genetic composition of a population affected its density changes, we altered gene frequencies in two field populations of the vole *Microtus townsendii*. The fast allele, LAP^F, was present in a control population at a frequency of about 35% from July 1971 to July 1973. By removing homozygous LAP^S/LAP^S voles from one experimental population we maintained an LAP^F frequency of about 75%. Removal of LAP^F/LAP^F homozygotes from a second population resulted in an LAP^F frequency of about 25%. We monitored demographic variables of the populations while the selection was being applied. The populations went through increasing and peak phases and then declined sharply during the spring of 1973. Different genotypes had an advantage in survival and reproduction during different phases of population density on the control area, and the selection that maintained the polymorphism on the control area could be correlated with population density. The altered allelic frequencies on the experimental areas did not produce any consistent effects on demography.

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Un certain nombre d'études sur les petits mammifères ont démontré que les changements de fréquence d'allèles à des locus polymorphiques sont reliés à des fluctuations de populations. On a modifié les fréquences de gènes chez deux populations de campagnols, *Microtus townsendii*, afin de déterminer si la composition génétique d'une population joue un rôle dans les changements de densité. L'allèle rapide, LAP^F s'est avéré présent dans une proportion d'environ 35% de juillet 1971 à juillet 1973 chez une population-témoin. En retranchant les campagnols homozygotes LAP^S/LAP^S d'une population expérimentale, on a réussi à maintenir une fréquence de LAP^F d'environ 75%. Le retrait des homozygotes LAP^F/LAP^F d'une seconde population a produit une fréquence de LAP^F d'environ 25%. Les variables démographiques des populations ont été suivies tout au cours de la sélection. Les populations ont traversé des phases d'augmentation puis des sommets pour ensuite décliner brusquement au printemps de 1973. Dans la région témoin, chaque génotype connait des périodes avantageuses, du point de vue de la survie et de la reproduction, correspondant à une phase particulière de densité de la population et la sélection qui maintient le polymorphisme dans cette région est en corrélation avec la densité de population. La modification des fréquences des allèles dans les régions expérimentales n'a produit aucun effet défini sur la démographie.

[Traduit par le journal]

Introduction

Microtine rodents undergo periodic fluctuations in population density which are not directly related to known changes in the environment of the population (Chitty 1960; Krebs 1966; Krebs *et al.* 1969). The absence of obvious extrinsic regulation of such populations has led Chitty (1960, 1967) to suggest that genetic changes are a necessary part of the conditions leading to numerical fluctuations. During the period of low numbers and population increase, so the story goes, certain genotypes are favored by selection. As density increases, a genotype with strongly developed aggressive behavior gains the selective advantage through the elimination from the breeding population of those less able to withstand crowding. A decline in numbers will occur when the increasing selection for aggressive individuals has decreased the total population fitness with regard to forces other than intraspecific competition.

The above hypothesis assumes that the population contains genetic variation capable of responding to short-term selective pressures.

Electrophoretic studies of proteins have revealed large amounts of apparently persistent allelic variation in natural populations (e.g., Lewontin and Hubby 1966; Selander *et al.* 1971). Evidence that selection does maintain such variation and can be effective over short periods has been found (Birdsall 1974; Canham 1969; Gaines and Krebs 1971; Myers 1974). It is at least reasonable, then, to use electrophoretic variants as genetic markers when one is investigating the implications of Chitty's hypothesis of population regulation through genetic change.

Several authors have monitored electrophoretic markers during detailed demographic studies on fluctuating populations. Semeonoff and Robertson (1968) found that the allelic frequency of an esterase polymorphism changed during a population decline of *Microtus agrestis*. Canham (1969) reported that the relative fitnesses of the genotypes of a transferrin and of an albumin polymorphism were related to changes in density of *Peromyscus maniculatus* and *Clethrionomys gapperi* populations. Changes in allelic frequencies at a transferrin locus (Tamarin and Krebs 1969) and at both transferrin and leucine aminopeptidase loci (Gaines and Krebs 1971) have been correlated with changes in density in *Microtus pennsylvanicus* and *M. ochrogaster* populations. The results of these studies are compatible with Chitty's hypothesis that the changes influencing numerical fluctuations are genetic. They are also consistent with the view that genetic changes have no causal relationship with population fluctuations, but are only a side effect of the numerical changes (Charlesworth and Giesel 1972).

This paper describes an attempt to discover the relationship between the allelic frequency of an electrophoretic marker, leucine aminopeptidase (LAP),[1] and the demography of a field population of *Microtus townsendii*. We chose to work with LAP because the genotypes could be accurately typed in the lab, and we had the faith to suspect that the particular polymorphism was maintained by the same forces that operated on the LAP polymorphism of *Microtus pennsylvanicus* and *M. ochrogaster* studied by Gaines and Krebs (1971). On each of two experimental areas, we continuously selected for one allele of the LAP polymorphism by introducing voles homozygous for the chosen allele and removing voles homozygous for the alternate allele. Demographic variables were monitored while the selection was being applied. The object was to determine whether changing the allelic frequency of the electrophoretic marker would be sufficient to change the demographic patterns between the populations. We made no attempt to distinguish between effects due to the individual alleles and those due to possible linkage complexes. Unfortunately we were unable to monitor selection on other marker loci at the same time. Birdsall (personal communication) has searched for additional electrophoretic variation in *M. townsendii* but has been unable to find any other polymorphisms.

[1]Leucine aminopeptidase (EC 3.4.11.1.) = α-amino-acyl-peptide hydrolase (cytosol).

Methods

Study Area

The study area was on Westham Island in the Fraser River delta, 4 mi (ca. 6.4 km) west of Ladner, British Columbia, and about 25 road miles (ca. 40 km) from the University of British Columbia campus. Trapping was carried out from July 1971 to July 1973 at the Department of National Defence radio-receiving station on the island. The ground was generally flat, with an old, introduced pasture-mix vegetation. The land was protected from flooding by dikes and drainage ditches, but standing water covered some sections during the winter of both years.

Three sites were marked out with 100 stakes each, in a 10 by 10 pattern. Each stake was 25 ft from the next, and each grid covered 1.5 acres. The size and relative position of each grid are shown in Fig. 1. From November to March in 1971 and 1972, about 20% of grid G, about 30% of grid I, and about 40% of grid H were covered with standing water. About half these amounts was present on each grid through December and January of 1972 and 1973.

Trapping Schedule

Longworth live-traps were placed in *M. townsendii* runways near each stake on the grids. An attempt was made to have an excess of traps available to the voles at all times. Therefore, one or two traps, depending on the population density, were placed near each stake. Every 2nd week, traps were set Monday afternoon and checked Tuesday morning and afternoon and Wednesday morning. The traps were locked open in place to serve as prebait stations between trapping periods. During hot weather, trapping was done only over the two nights to avoid mortality in the traps during the heat of the day.

A vole caught for the first time was bled from the suborbital sinus and given a numbered ear-tag. Tag number, location on the grid, and weight to the nearest gram were recorded for each animal caught during a trapping period. Breeding condition was assessed from testes position in the males and from vaginal perforation, nipple development, and pubic symphysis closure in the females. Litters in traps and noticeable pregnancies were recorded.

Electrophoresis

We used a horizontal starch-gel electrophoretic method with Connaught starch (Connaught Medical Research Laboratories, Toronto, Canada) and the buffer system used by Selander and Yang (1969) for plasma proteins. The gel molds, trays, and slicing procedure were adapted from those described by Tsuyuki *et al.* (1966). We applied the serum from the blood samples to the gel slots with filter-paper inserts, subjected the gels to 150 V for 3 h and then incubated them for 2 h in the staining solution of Brewer (1970) at pH 5.2.

Results

The Enzyme Variant LAP

The functional name leucine aminopeptidase has been applied to "various amino acid naphthylamidases" which reduce the substrate l-leucyl-β-naphthyl amide (Smith *et al.* 1965). Staining revealed two zones of LAP activity. The zone of slower mobility appeared to be monomorphic in all animals typed; the second zone was polymorphic, and the three staining patterns were labelled FF, FS, and SS. The polymorphism conformed to a two-allele autosomal inheritance model. A breeding colony was maintained to provide information on the genetic control of the polymorphism, but only 29 crosses were successful in the 2 years. The results of typing the 271 offspring for LAP showed no evidence against the assumed mode of inheritance (Table 1).

Based on these electrophoretically determined genotypes, a program of artificial selection was carried out in the field. Mice typed as SS on grid G were removed and released on grid H: those typed as FF on grid H were removed and released on grid G (see Fig. 1). The selection was carried on throughout the study as new mice entered the population. Grid I was the control grid; all new mice were typed for LAP but none were removed or added.

TABLE 1

LAP types for colony crosses of *M. townsendii* tested for chi-square goodness of fit

LAP cross	No. of breeding pairs	No. of offspring SS	FS	FF	*P*
SS × SS	2	40	0	0	
SS × FS	11	53	51	0	> .98
SS × FF	3	0	15	0	
FS × FS	6	11	15	10	> .50
FS × FF	5	0	20	22	> .90
FF × FF	2	0	0	34	

Transfers

During the study, a total of 66 animals, representing 8.6% of the total catch on grid H, were typed as FF and removed. These animals were released on grid G, but only 19 (27%) of these 66 stayed on grid G until at least the next trapping period (2 weeks). These additional 19 represented 1.7% of the total of 1110 animals caught on grid G during the study. Of the 104 (9.3%) animals typed as SS on grid G and removed, 61 (59%) were caught at least one trapping period after release on grid H. These represented 7.9% of the total of 765 animals caught on grid H. During both breeding and non-breeding seasons a greater proportion of the animals transferred to grid H took up residence than did those transferred to grid G (see Table 2).

For the presentation of results we will refer to breeding seasons defined by the percentages of voles in breeding condition on the grids. During the study, five periods of breeding performance could be distinguished as follows.

Summer 1971	July 1 to Nov. 1, 1971	Breeding
Winter 1971–1972	Nov. 15, 1971, to Feb. 7, 1972	Non-breeding
Summer 1972	Feb. 21 to Sep. 18, 1972	Breeding
Winter 1972–1973	Oct. 2, 1972, to Feb. 5, 1973	Non-breeding
Summer 1973	Feb. 18 to July 23, 1973	Breeding

Most tables will be presented with the data grouped over these breeding seasons.

Sources of Error

Mortality directly attributable to the experiment appeared to be low with the trapping program employed. The totals of animals found dead in traps not properly prebaited, accidently killed during handling, and found dead of unknown causes were 43 (4%) out of 1110 on grid G, 36 (5%) of 765 on grid H, and 35 (4%) of 959 on the control grid I. We found no evidence that this mortality was non-random with respect to sex or genotype. Within this study, we had no satisfactory method of establishing the effect that bleeding had on the survival of the individual voles. Of the total of 2834 animals caught, 2774 (98%) were bled, and the majority of these were bled at the time of first capture. We assume for the purpose of this discussion that the effects of bleeding were the same over both sexes and all genotypes.

As discussed by Krebs (1966) and Hilborn (1974), the trapping procedure was designed to

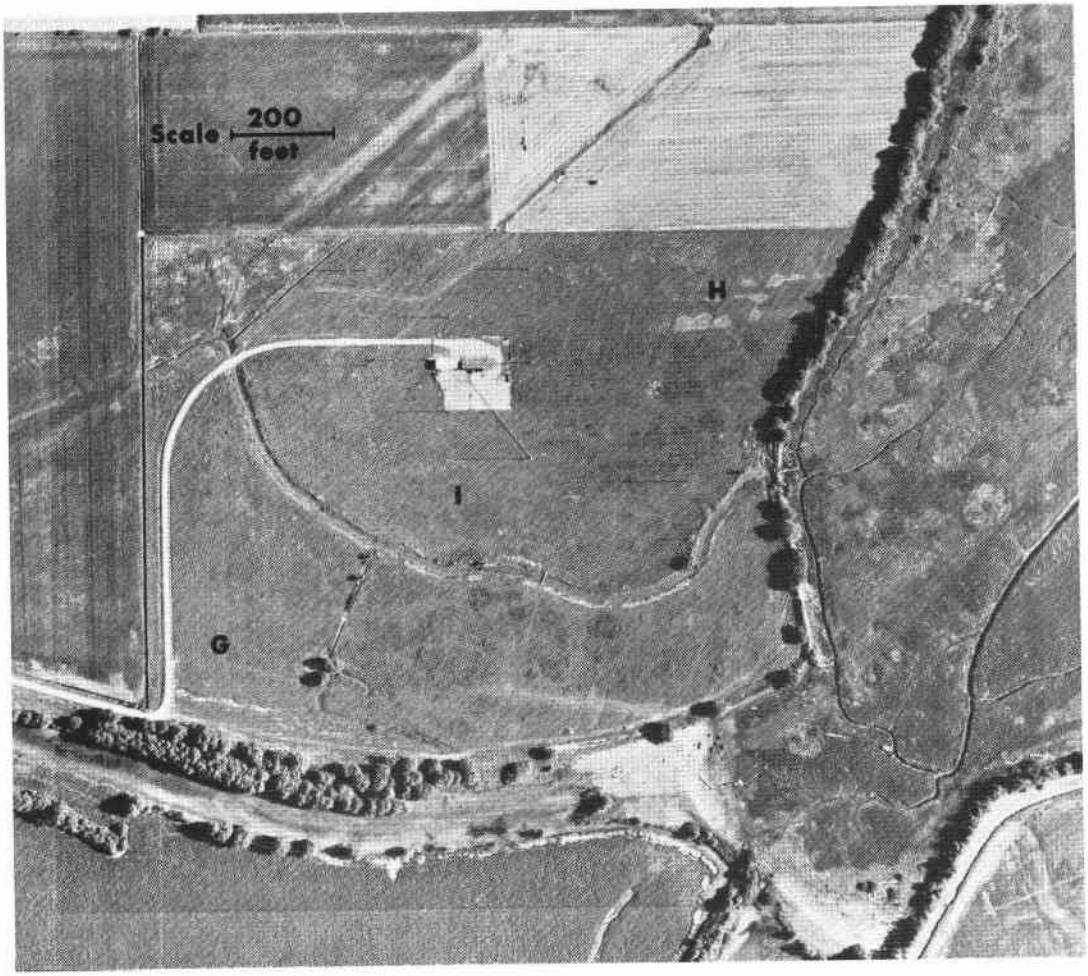

FIG. 1. Aerial photograph of the Westham Island receiving station. This 37-ac site is surrounded by farmland and marshes. The central grid, I, was the control. Homozygous FF voles were removed from grid H and transferred to grid G, and homozygous SS voles were moved in the opposite direction.

enumerate all animals present in the trappable population to avoid the necessity of assuming random sampling when estimating population parameters. An index of trappability was calculated to determine the efficiency of this enumeration. We estimated the trappability of *M. townsendii* by comparing the actual number caught during one trapping period with the minimum number known to be alive at that trapping. The minimum number known to be alive contains those previously tagged mice that are missed during the trapping period but caught at some later trapping. Between 81% and 98% of all animals in the trappable population present on a grid were caught during each trapping period. We found no difference between the trappabilities of males and females during any time of year on any grid, nor were there any differences between grids. During the late winter on all grids, trappability was uniformly good (above 90%), but during the summer, trappability was lower (80–90%), probably owing to the altered trapping program mentioned above.

Very small voles were rarely caught in Longworth traps, but this would affect our analysis only if the probability of capture was different between grids or genotypes. We considered mean body weight at the time of first capture as an index of relative trappability (Table 3). We compared the different genotypes on the control grid

TABLE 2

Number of transferred *M. townsendii* remaining on the experimental grids until at least the next trapping session. Total number transferred in parentheses

	Transferred to grid G		Transferred to grid H	
	Males	Females	Males	Females
Summer 1971	0 (5)	1 (3)	8 (9)	4 (8)
Winter 1971–1972	6 (9)	3 (3)	6 (11)	9 (10)
Summer 1972	3 (16)	3 (9)	6 (16)	7 (13)
Winter 1972–1973	0 (2)	2 (10)	12 (17)	5 (13)
Summer 1973	0 (4)	1 (4)	1 (3)	3 (3)
Totals	9 (36)	10 (29)	33 (56)	28 (47)

TABLE 3

Mean body weight at the time of first capture of *M. townsendii* on the control grid I. Number of voles in parentheses

Season	LAP genotype	Mean wt. ± SE	
		Males	Females
Summer 1971	FF	46.00±4.94 (7)	40.50±6.07 (6)
	FS	43.31±2.47 (26)	38.93±2.15 (29)
	SS	47.94±3.80 (17)	41.14±2.87 (21)
Winter 1971–1972	FF	37.80±5.05 (5)	38.27±2.11 (11)
	FS	42.48±1.84 (42)	35.24±1.00 (46)
	SS	42.05±2.53 (22)	33.04±1.59 (26)
Summer 1972	FF	57.69±1.95 (13)	41.06±2.77 (17)
	FS	51.15±1.42 (72)	40.28±1.68 (57)
	SS	45.45±1.57 (64)	40.22±1.44 (68)
Winter 1972–1973	FF	39.67±3.31 (18)	34.06±2.15 (17)
	FS	41.96±1.57 (52)	37.12±1.07 (77)
	SS	39.79±1.71 (43)	38.72±1.73 (39)
Summer 1973	FF	51.00±4.52 (8)	43.00±6.03 (3)
	FS	44.15±2.36 (26)	38.00±2.20 (26)
	SS	43.52±2.71 (25)	35.83±2.07 (29)

with a Kruskal-Wallis one-way ANOVA (Siegel 1956). We found no significant differences in mean body weight at the time of first capture between individuals of different genotypes. Mean body weight at first capture was lower for the males during summer 1971 than during the summer of 1972 or 1973, and was lower for females than for males at all times. We concluded from these data that the individuals of the different genotypes were entering the trapped population at comparable sizes, and assumed that they were equally susceptible to trapping throughout their adult life.

Population Density

The minimum numbers of males and females known to be alive are graphed for each grid (Fig. 2). Grid H had a consistently lower density

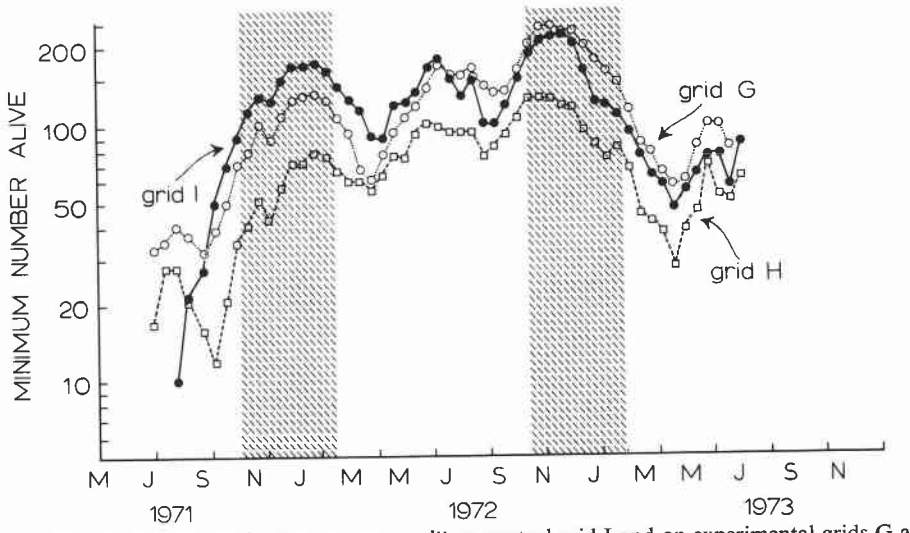

FIG. 2. Population density of *Microtus townsendii* on control grid I and on experimental grids G and H. ● = grid I, ○ = grid G, □ = grid H.

than that on either grid G or grid I, but the three populations underwent similar numerical changes. Densities on grid H were about 60% those on grid G and grid I. Numbers dropped on grid H during the autumn of 1971. On all grids, vole density increased throughout the winter of 1971–1972, dropped slightly in the early spring of 1972, and increased throughout the summer. After a short decline in the fall of 1972, densities reached their highest on all grids during December 1972 (maximum 300/ha, or 120/ac). The population declined until May 1973, then increased until the end of the study in August 1973.

Changes in population density occurred in synchrony on the three study areas. The direction of density change between trapping periods (+, 0, −) was compared between the grids. Density changed in the same direction on all three grids between 34 of 50 trapping periods for males and between 38 of 50 periods for females. Large fluctuations in density occurred on each grid during the study, but all grids changed with similar rates at the same time.

Variability among study sites is traditionally dealt with through replication of control and experimental areas, but in this study we had no replicates. The control differed from grid G in allele frequency (see below) and from grid H in *M. townsendii* population density. Peak densities reached on grids I and G were about 240 voles, but on grid H were about 130 voles. Grids I and G were less subject to winter flooding than grid H, and reduction of available habitat is the simplest explanation for the lower density on grid H.

Allelic Frequency

From July to September 1971 we monitored LAP^F frequency on all three grids before we started experimental manipulations. Unfortunately the three grids did not have the same allelic frequencies before the experiment began. In August 1971 the frequency of LAP^F was 59% on grid G, 60% on grid H, but only 27% on control grid I. We interpret these differences as random effects due to the small population present on control grid I at the start of our study.

In September 1971 we began selecting against the LAP^F allele on grid H and in favor of this allele on grid G. Figure 3 illustrates the changes in allelic frequency on the three areas throughout the experiment. The two experimental grids G and H were very quickly driven to significantly different allelic frequencies and maintained apart for the 2 years of study. Control grid I maintained frequencies closer to grid H than to grid G. The experimental technique was thus successful in that we were able to drive allelic frequencies apart in two experimental populations and keep them apart for the entire study.

Allelic Frequency and Density

We now inquire whether there is an association between allelic frequency changes and population density. This question is relevant only for

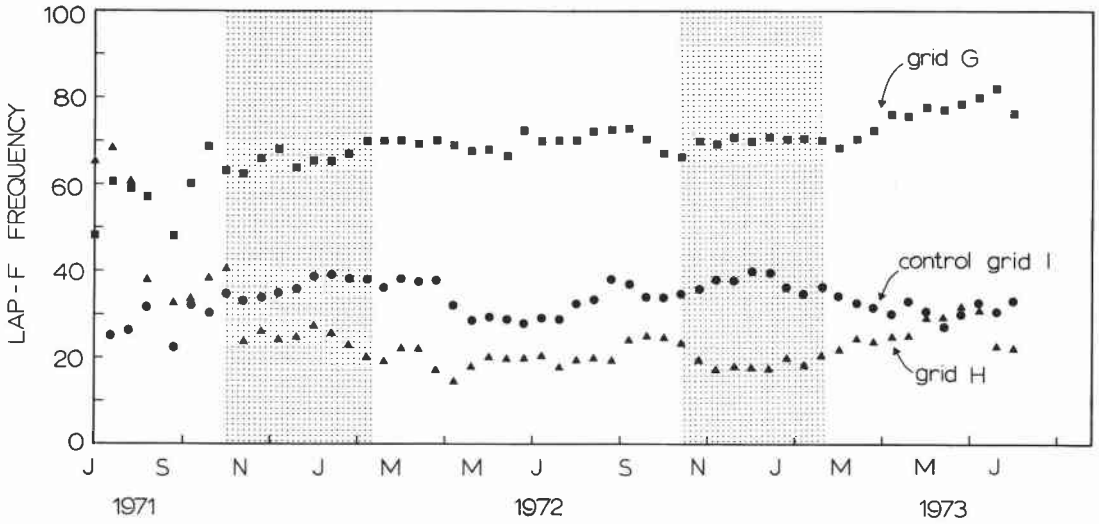

FIG. 3. Allelic frequency of LAP^F on grid G (all SS voles removed), grid H (all FF voles removed), and control grid I. Artificial selection was applied from September 1971 to August 1973.

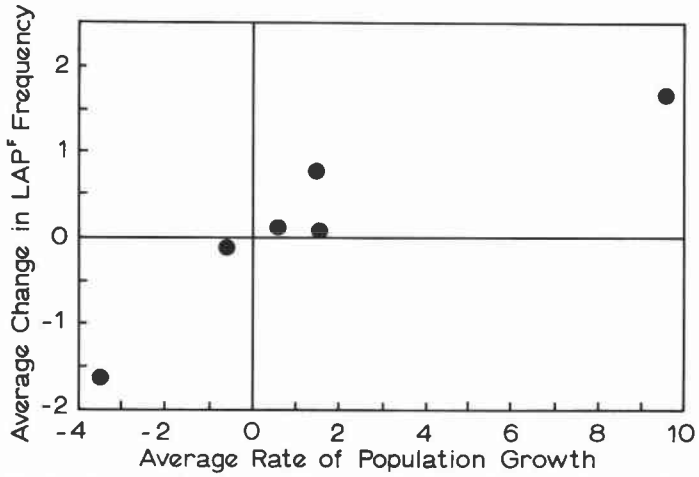

FIG. 4. Relationship between rate of population growth (percent per week) and changes in LAP^F frequency (percent per 2 weeks) for the control grid I population of *Microtus townsendii*. Each point represents an average for a summer or winter period, 1971–1973.

the control grid I, since the experimental grids are affected by our artificial selection.

In control populations of *Microtus townsendii*, LAP^F frequency tracks population density. In increasing populations the frequency of LAP^F rises, and in declining populations the frequency of LAP^F falls. This effect can be seen by comparing Figs. 2 and 3, but is illustrated more directly in Fig. 4. We have found this result in other populations of *M. townsendii* and Birdsall (1974) has reported on it. The results shown in Fig. 4 vindicate our choice of LAP as a marker gene for this experiment.

Demography and Genotypes

If the genotypes of the LAP polymorphism have different selective advantages depending on the population changes, then one should be able to discover this from demographic information. We considered survival rates, reproductive performance, and body weights in order to discover any such relationship.

We wished to separate genetic effects on demography from area effects. The logical method was to consider the demographic information available for each genotype on each grid. However, all SS homozygotes were removed

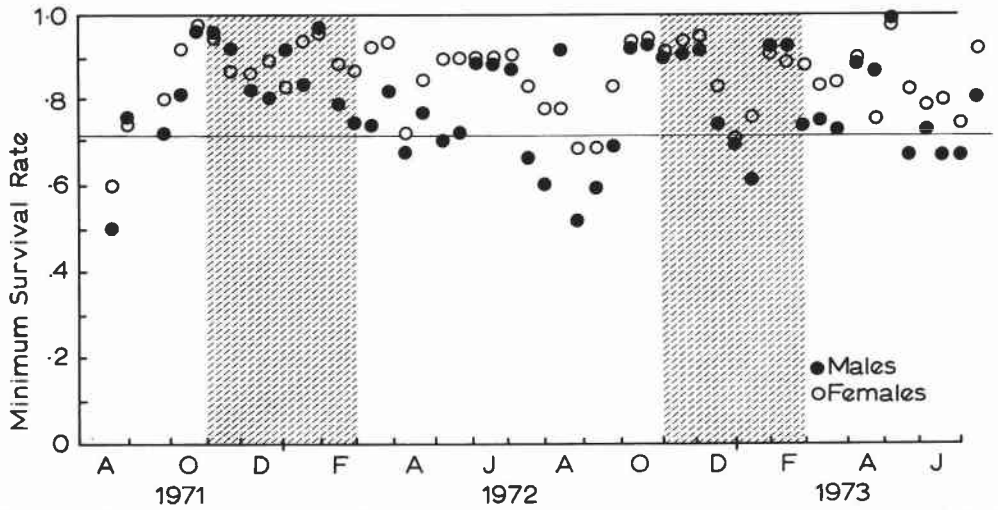

FIG. 5. Minimum survival rates per 14 days for *Microtus townsendii* males and females on control grid I. Winter months are shaded. Horizontal line separates poor survival (more than half of the population disappearing per month) from good survival.

from grid G as they entered the population. Any estimates of demographic variables for that genotype on grid G were based on a small sample of the new animals in the population, and could not be considered representative. A similar bias existed on grid H, where FF homozygotes were removed. On the control grid I, all three genotypes could be considered for comparisons of demographic variables. Therefore, we followed a standard procedure of analysis. When testing for effects due to grids we compared the heterozygotes between all three grids, the FF homozygotes between grid G and grid I, and the SS homozygotes between grid H and grid I. When looking for genetic effects we compared the genotypes within each grid: FF homozygotes, heterozygotes, and SS homozygotes on grid I; FF homozygotes with heterozygotes on grid G; and SS homozygotes with heterozygotes on grid H.

Survival and Genotype

Survival rates fluctuated considerably in the control population. Figure 5 illustrates changes in minimum survival rates for males and females on grid I. The average survival rate over the entire study was 0.80 per 2 weeks for males and 0.86 for females on the control area. Superimposed on times of normal high survival are periods of poor survival. We arbitrarily define poor survival to be any rates below 0.707 per 2 weeks (half of the population disappearing per 4 weeks). For the control population, periods of poor survival occurred at the following times for males: July–August 1971, April–May 1972, July–September 1972, January 1973, and May–June 1973.

There are two major periods of poor survival each year. During the late summer there are severe infestations of botflies in our *Microtus townsendii* populations, and individuals can carry up to 10 bots. These infestations are severe enough to weaken and probably kill some individuals. Thus high mortality can be present in both sexes in late summer. The second period of low survival occurs in the spring and is often associated with the beginning of the breeding season. The spring period of low survival is typically confined to males, although females can be affected for short time periods in some years.

Survival rates on experimental grids G and H were similar in pattern to those of the control grid (Fig. 5) and are not presented here. We illustrate only the concordance of periods of poor survival for the three areas (Fig. 6). There is broad agreement between the three populations in the timing of the spring and summer mortality with, however, a few clear differences. First, the summer mortality of 1971 was much more prolonged on the experimental grids G and H than on the control. We cannot attribute much of the late September and October losses to botflies, and we note that this period of loss coincided with the start of our transfer experiment and the intensive selection which separated the allelic

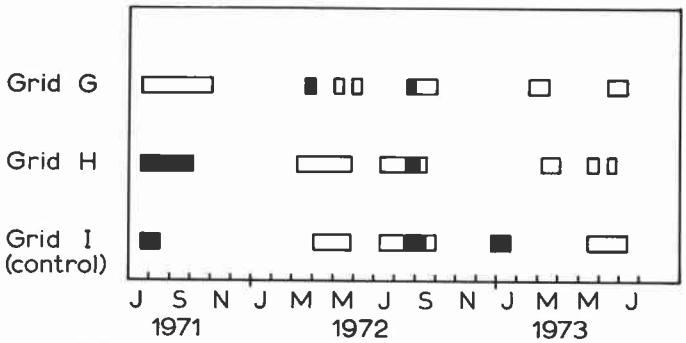

FIG. 6. Concordance of time periods of poor survival in *Microtus townsendii*. Solid rectangles indicate poor survival in both males and females, open rectangles indicate poor survival in males only.

frequencies on grids G and H. Second, the episode of heavy mortality in January 1973 was found on the control but not on the experimental grids. We believe this episode to be caused by very heavy rains and local flooding of the control grid while the experimental grids were less affected because of small differences in microtopography. Third, the timing of the spring losses in 1973 was not as discrepant as Fig. 6 indicates. The control grid, for example, had reduced male survival in February and March 1973 (Fig. 5) but not quite severe enough to classify as "poor survival" as defined above. Nevertheless, there is more variation among the three populations in the spring losses of 1973 than there was in 1972.

To determine if the different LAP genotypes had different survival rates as adults, we compared the minimum survival rates for genotypes for each grid. Transferred animals were not included in the calculation of survival rates on the experimental grids. There is virtually no variation in survival rates among genotypes on the control grid I, except for male heterozygotes which survived poorly in the summer of 1973 (complete data in LeDuc 1974). We conclude that there is no selection at the LAP locus acting on viability of adults in the control population.

On the experimental grids there is again little variation in survival of the different genotypes. The single exception is poor survival of male heterozygotes in the summer of 1971 on both grid G and grid H. Thus the poor survival associated with the start of this experiment was particularly severe on males heterozygous at the LAP locus. The poor survival of male heterozygotes found on the control grid during the summer of 1973 was not found on either grid G or on grid H at that time. Conversely, the poor survival of male heterozygotes found on grids G and H in summer 1971 was not found on the control grid.

Breeding Condition and Genotype

There are four major components of reproductive fitness, but we are able to measure only three of them. We have a measure of (1) fraction of animals breeding, (2) length of breeding season, and (3) age at sexual maturity. We are unable to measure the fourth component, size of litters.

Figure 7 shows the fraction of animals breeding and the length of the breeding season for control grid I. We use the position and size of the testes as an index of breeding for males and the size of the nipples as an index of lactation for females. Two major effects are shown in Fig. 7. First, breeding continued throughout the winter of 1971–1972 but stopped for 4 months during the winter of 1972–1973. Second, breeding was curtailed by late July in the high-density year of 1972.

The breeding seasons on grids G and H were identical to those shown in Fig. 7, and hence are not presented here (data in LeDuc 1974). Thus, by altering the genetic composition of these populations we have not changed the fraction of animals breeding or the length of the breeding season.

We compared the fraction of individuals of the three genotypes which were in breeding condition during the five breeding periods. Table 4 gives these data for the control population. Only one time period showed significant genotypic variation. During summer 1971, when the population was increasing rapidly, the LAP^S/LAP^S

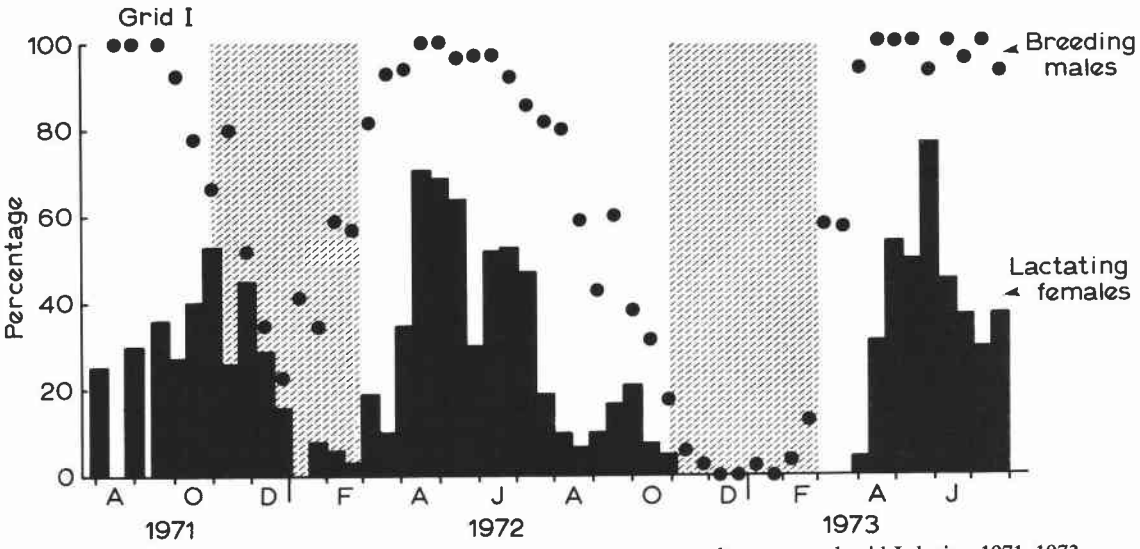

FIG. 7. Breeding condition of male and female *Microtus townsendii* on control grid I during 1971–1973.

TABLE 4

Percentage of adult *M. townsendii* males with scrotal testes and lactating females on control grid I. Sample size in parentheses

	% breeding males			% lactating females		
Grid I	FF	FS	SS	FF	FS	SS
Summer 1971	100 (10)	94 (32)	69** (32)	75 (4)	48 (48)	27** (59)
Winter 1971–1972	26 (30)	46 (160)	51 (84)	4 (24)	21 (112)	20 (101)
Summer 1972	87 (46)	83 (313)	77 (255)	35 (85)	34 (470)	32 (387)
Winter 1972–1973	17 (53)	10 (146)	9 (150)	6 (32)	5 (182)	5 (151)
Summer 1973	88 (44)	74 (77)	78 (122)	33 (21)	33 (132)	39 (139)

**$P < .01$.

homozygote was breeding less frequently than the other genotypes. Throughout the rest of the study we could detect no differences among genotypes in the fraction of individuals in breeding condition.

From the results on the control grid we would predict that grid H, with a high proportion of LAP^S/LAP^S voles, would have a low reproductive rate during the increase phase of summer 1971, and conversely that grid G with almost no LAP^S/LAP^S voles would have a higher reproductive rate. These predictions were in fact verified. In summer 1971, grid H had only 3 of 27 LAP^S/LAP^S females lactating, and only 22% of all females were recorded as lactating, compared with 38% for the control population. On grid G, by contrast, 45% of adult females were lactating. These results tend to support Birdsall's (1974) contention that selection favors LAP heterozygotes when the population is increasing rapidly. We could not detect any other differences among genotypes in breeding intensity for the other time periods.

Age at Sexual Maturity

Age at sexual maturity is an important demographic parameter but most difficult to estimate. We attempt to deal with it here by estimating

TABLE 5

Weight at sexual maturity for LAP genotypes of *Microtus townsendii* on the control grid I, 1971–1973. Parentheses enclose 95% confidence limits

	LAP phenotype			
Grid I	FF	FS	SS	Total
Summer 1971	43 (40–46)	42 (40–44)	43 (40–47)	43 (41–45)
Winter 1971–1972	66 (50–87)	54 (51–57)	52 (48–56)	55 (52–57)
Spring 1972	—	38 (33–44)	41 (37–46)	38 (35–42)
Summer 1972	53 (50–56)	51 (49–52)	50 (49–52)	51 (50–52)
Winter 1972–1973	—	69 (58–82)	79 (58–108)	71 (63–81)
Summer 1973	47 (44–50)	44 (42–47)	44 (42–46)	47 (46–48)

TABLE 6

Average growth rates (% per day ± SE), adjusted by regression to a standard 35-g vole, of *M. townsendii* males on control grid I. Sample size in parentheses

Grid I	FF	FS	SS
Summer 1971	0.84 ± .34 (11)	1.25 ± .19 (42)	1.64 ± .21 (32)
Winter 1971–1972	0.75 ± .21 (30)	0.66 ± .09 (178)	0.71 ± .10 (99)
Summer 1972	0.41 ± .40 (34)	0.54 ± .10 (241)	1.29 ± .12 (213)
Winter 1972–1973	0.19 ± .13 (68)	0.18 ± .08 (210)	0.29 ± .07 (202)
Summer 1973	0.79 ± .40 (34)	1.59 ± .30 (49)	1.32 ± .19 (99)

two components of age at sexual maturity—weight at maturity and body growth rate. If weights at maturity are identical and one genotype grows faster than the others, we will have presumptive evidence that this genotype has a lower age at sexual maturity.

Table 5 gives the median weights at sexual maturity for LAP genotypes on the control grid. Males and females were combined for this analysis, since we could find no differences between them, and the techniques of Leslie *et al.* (1945) were used to compute the median weight at sexual maturity. There are no significant differences among genotypes in the weight at maturity, in spite of strong seasonal variation in size at sexual maturity. We concluded that weight at maturity was effectively constant over all genotypes and that any variations in growth rate would be a direct reflection on the age at sexual maturity.

Table 6 gives the average growth rates in body weight for male voles from control grid I. We have adjusted growth rates to a standard 35-g vole with the same technique used in previous work (Krebs *et al.* 1969). Growth rates are typically low during the winter months, and were lower in winter 1972–1973 than in winter 1971–1972, but we have found no differences among LAP genotypes in winter growth. Hence we concentrate our analysis on the summer months. LAP^S/LAP^S homozygote males grow more rapidly than the other genotypes in the control population. We tested this conclusion by covariance analysis for each summer and found significant

TABLE 7

Average growth rates (% per day ± SE), adjusted by regression to a standard 35-g vole, of *M. townsendii* males on grid H. Sample size in parenthesis

Grid H	FS	SS
Summer 1971	0.75 ± .35 (17)	1.19 ± .36 (13)
Winter 1971–1972	0.76 ± .18 (82)	0.79 ± .12 (43)
Summer 1972	0.56 ± .16 (181)	1.70 ± .11 (123)
Winter 1972–1973	0.41 ± .09 (249)	0.31 ± .08 (141)
Summer 1973	1.30 ± .22 (55)	1.65 ± .23 (71)

differences in every case. Female growth rates on the control grid seem to follow the same pattern as in the males, but they are more variable and confounded with pregnancies so we have not analyzed female growth rates statistically.

Table 7 gives the average growth rates for males from experimental grid H, and illustrates the same pattern of high growth rates for LAP^S/LAP^S homozygotes during the summer breeding season. Experimental grid G had no LAP^S/LAP^S homozygotes, and there were no significant differences in growth among the genotypes on this grid (LeDuc 1974).

We conclude that in summer the age at sexual maturity is significantly lower among LAP^S/LAP^S homozygotes than among the other LAP genotypes.

Body Weights

Peak populations of voles are characterized by individuals of larger than average size (Chitty 1960) and we have evidence for this effect in *Microtus townsendii*. Figure 8 gives the body-weight distributions for males from control grid I. Adult male voles were larger during the spring and summer of 1972, the year of highest population density. Males were also larger in the winter of 1971–1972, when winter breeding occurred, than they were in the non-breeding winter of 1972–1973.

The heavier than average males during 1972 tended to be disproportionately more the LAP^F/LAP^F genotype. Table 8 gives the mean values for control grid I. The difference in body weight between genotypes was most marked in the spring months but was also significant during the summer. It was present in 1973 as well as in 1972.

The experimental populations on grids G and H also show the heavier body weights of the LAP^F/LAP^F homozygote (Table 8), and indicate that heterozygous males may be equal in size to the LAP^F/LAP^F genotype. We expect from these results that the overall body-weight distribution would be reduced on grid H, from which all FF phenotypes were removed, and increased on grid G, from which all SS phenotypes were removed. If we compare the average body weights for March 1972, we find only part of these predictions verified: grid I, 61 g ± 2 (SE); grid H, 53 g ± 3; grid G, 59 g ± 2. Males on grid H were on the average smaller throughout the summer of 1972, compared with control grid I. But males on grid G were not larger on the average, than grid I males, but equal in size to the control males.

Discussion

Vole populations typically undergo fluctuations in numbers with specific characteristics first elaborated by Chitty (1960) and further reviewed by Krebs and Myers (1974). Many of these characteristics are found in populations of *Microtus townsendii* near Vancouver. Specifically we have described a period of increase with winter breeding, individuals with high body weights in the peak year, a shortened summer breeding season in the year of high density, and no breeding during the winter after the peak year. We will assume that *Microtus townsendii* is a more or less typical microtine rodent undergoing periodic oscillations in density.

If natural selection is an integral part of the mechanism which generates periodic oscillations, as claimed by Chitty (1967), we ought to be able to manipulate natural populations by appropriate kinds of artificial selection. This experiment is the first artificial selection experiment done on a field population of small rodents. We thought that the genetic markers among the blood proteins were a good starting point since they have been found to be associated with density changes in other *Microtus* species (Gaines and Krebs 1971; Tamarin and Krebs 1969). Furthermore we needed to do this experiment to check the hypothesis of Charlesworth and Giesel (1972) that genetic changes in blood-protein-

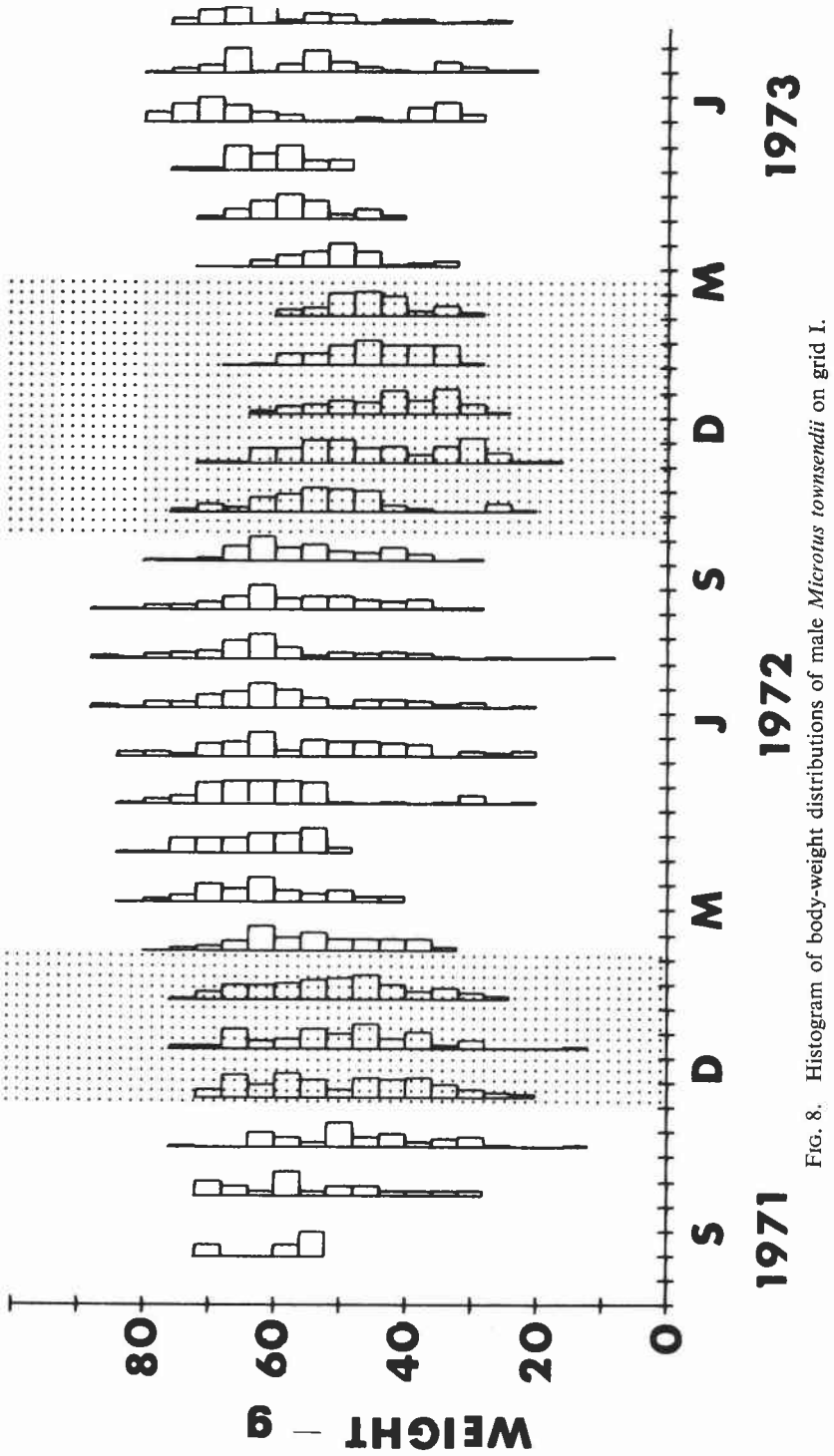

FIG. 8. Histogram of body-weight distributions of male *Microtus townsendii* on grid I.

TABLE 8

Mean body weights (±SE) for LAP genotypes of *Microtus townsendii*

Grid	Time	LAP genotype FF	FS	SS
Grid I	Spring 1972	67.3±1.6	62.5±0.8	59.0±0.8
Grid I	Summer 1972	56.9±1.6	55.4±0.7	52.2±0.7
Grid H	Summer 1972	56.3±1.7*	56.1±0.7	49.4±1.0
Grid G	Summer 1972	54.5±0.7	55.9±0.6	50.7±1.7*

*This genotype was selected against in this population

marker frequencies were side effects of the basic changes in population density.

There was clear evidence of selection acting on LAP genotypes in *Microtus townsendii*. The LAP^F allele was selected for during population increases. We have not been able to measure all the components of this selection. There seems to be no differential selection operating on adult mortality. In reproductive performance, LAP^S/LAP^S voles seemed to breed less frequently than the other genotypes during the population increase, but this effect was counterbalanced by a lower age at sexual maturity for the SS phenotype. Most of the selection acting on the LAP locus must be focused on juvenile survival, a part of the life cycle on which we have no direct data.

In spite of the differences in fitness we have been able to detect, our overall conclusion must be that the altered allelic frequencies on the experimental grids did not produce any striking effects on demography. The three populations increased, peaked, and declined in almost exactly the same manner. The one major difference between grids—the reduced average density on grid H—could not be associated with a reduced LAP^F frequency because the control population was also relatively low in LAP^F frequency. The demographic events associated with the population cycle seemed to occur on all three grids without any distinction.

Owing to possible interference with reproduction and social structure, the transferring of animals throughout the study may have been a major error in experimental design. One might postulate that the introduction of alien *M. townsendii* onto the experimental grids produced some social disturbance in the populations. Davis and Christian (1956) claimed that the introduction of animals into expanding populations of rats would stop the increase, and introductions into stationary populations would result in declines. On grid H in this study, introductions formed 7.9% of the total population, and this level of disturbance may have been a factor in keeping population densities low. Introductions to grid G formed only 1.7% of the total population, and densities were certainly no different from those on the control grid. In general, few were transferred during any one trapping period, and whatever social effects were produced by the strange voles would presumably have dissipated by the next trapping. Terman (1962) reported that the introduction of alien *Peromyscus* would inhibit homing behavior in residents for only the first 2 days after introduction.

The population that the voles were released into appeared to have an effect on recruitment of the transfers. Orr (1966) found that removing residents before releasing alien *Peromyscus* resulted in 40% recruitment of the new mice. In this study, 27% of the mice transferred to grid G stayed until the next trapping period, and 59% of those transferred to grid H stayed until at least the next trapping period. The relatively high population density on grid G was probably responsible for the poor recruitment of the transfers. The voles transferred to grid H were suddenly exposed to a less crowded area and this change in their environment might account for the relatively high rate of recruitment.

Experimental attempts at testing Chitty's hypothesis have involved the cropping of adult residents from populations of microtines (Krebs 1966; Smyth 1968). Their experiments were undertaken expressly to have an effect on the social processes in the populations. While the cropping of animals was done on a much larger scale by those authors, we did not consider the possibilities of social disruption through the removal of residents in this experiment. A more informative design would be to alter the allelic

frequencies only at the beginning of the study rather than to continue the selection process throughout. As well as minimizing the social disturbance, such an experiment would retain all genotypes on the grids. One could therefore compare grids directly using total values for demographic variables. The return to original allelic frequencies, if it occurred, could indicate specific selection pressures on the polymorphism.

A number of authors have shown that the allelic frequency of an electrophoretic marker can determine the fitness of an individual in a population. Yarbrough and Kojima (1967) found the selective advantage among the genotypes of an esterase-6 locus in *Drosophila melanogaster* cage populations to depend upon the frequency of the alleles in the population. Kojima and Tobari (1969) found that either homozygote of an alcohol dehydrogenase locus in *Drosophila melanogaster* had an enhanced viability if present at a low frequency, but had reduced viability if present in a high frequency. There were indications in *M. townsendii* that different genotypes had the relative advantage at different periods, but such differences apparently had little effect on the demographic events of the populations.

When we began this experiment, we had no evidence that the leucine aminopeptidase polymorphism influenced demography. Other studies have shown that the allelic frequencies of electrophoretic markers chosen essentially at random are correlated with demographic events. Redfield (1972), from a study on colonizing blue grouse, found that both age of habitat and the density of the population were correlated with the frequency of the heterozygotes at the Ng locus. Tamarin and Krebs (1969) found that a definable 'increase' genotype existed in both *Microtus ochrogaster* and *M. pennsylvanicus*. This transferrin genotype was selected for during increasing and peak periods of density, and selected against during the decline. These 'increase' and 'decline' genotypes were again defined by Gaines and Krebs (1971) for transferrin and leucine aminopeptidase. The above studies indicated that a selective advantage can be detected for a particular electrophoretic genotype although the biochemical basis for the advantage is not known. However, in an experimental analysis of the relative fitnesses of transferrin genotypes in *Microtus ochrogaster*, Gaines *et al.* (1971) found no significant effect of transferrin genotype on rate of population increase, percentage of lactating adults, recruitment index, or survival rate of voles in fenced enclosures. The results of our study indicate that the allelic frequency of leucine aminopeptidase in the *M. townsendii* population had no effect on the population processes during the experiment. Therefore, any associations of allelic frequency and numbers were probably an effect of numbers on the polymorphism rather than the converse (Charlesworth and Giesel 1972).

Apparently, selection does act on the various genotypes of electrophoretic markers, yet these genotypes do not have an absolute fitness. Sved *et al.* (1966) were the first to suggest that great numbers of genetic polymorphisms in a population would tend to obscure the optimal genotype. With some hundreds of independently segregating loci, there would be thousands of individuals in a population with essentially the same fitness. King (1967) makes the point that the loss of fitness sufficient to maintain a polymorphism need not significantly reduce the fitness of a population. Therefore changes in allelic frequency at such loci during numerical fluctuations would be determined by the degree of favorability of the environment, and the polymorphism itself would have no effect on the demographic processes of the population.

Our work with LAP was essentially a 'shot-in-the-dark' approach, although it was a logical next step in investigating the correlations of allelic frequency with demography. In the light of this study, an explanation that allelic frequency change causes demographic change cannot be accepted. However, this does not necessarily indicate a general relationship since LAP may have been a bad choice of marker. LAP was chosen for the same reason that other polymorphisms have been studied: the facility of typing it in the lab. Therefore, the studies to date which have invoked electrophoretic polymorphisms as markers for demographic change contain no information on genetic influences on demography. The individual locus may not be the appropriate unit of observation. An effort must be made to determine the genetical basis of population processes which are thought to be directly involved in numerical fluctuations before the hypothesis that genetic change influences numerical fluctuations in microtines can be evaluated.

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