Demographic and Genetic Attributes of Dispersing and Resident Individuals of an Enclosed *Microtus pennsylvanicus* Population

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ABSTRACT

A dispersal polymorphism may exist in emigrants from cyclic populations of *Microtus pennsylvanicus* biasing trap-revealed movements of unenclosed animals in favour of sedentary or colonizing individuals. The dispersal tendency of emigrants from an enclosed population was investigated by releasing animals via tubes into one of two adjacent enclosures, one vacant and one inhabited. Individuals from the enclosed population were monitored for age, sex, weight and electrophoretically detectable serum transferrin genotype in an intensive live-trapping program.

In 1973 the minimum number alive in the introduced enclosed study population reached approximately 167/ha when breeding stopped in October. In 1974 intensive breeding increased the population density to 333/ha by mid-July when a long decline in numbers and breeding intensity began without an intervening plateau. An adjacent unenclosed area had a much lower density and longer breeding season in 1974. The growth rate of young males in the enclosed population tended to be lowest during the decline period in 1974. Survival of the enclosed population was high throughout but was lowest during the decline phase in both sexes, especially males. Low transferrin heterozygote survival during the decline coincided with a significant heterozygote deficiency in females whereas in males genotype frequencies did not depart from Hardy-Weinberg equilibrium values throughout the study. Twenty-nine suitable animals were released during the decline in five periods from July to November 1974. The proportions of males and transferrin heterozygotes in the released group were generally greater than in the source population. In the test enclosures 21% of the released animals continued their movement through the vacant area while 41% (no significant difference) moved

through the inhabited enclosure. In the vacant test area, females had a greater tendency than males to continue dispersal whereas no difference was noted in the inhabited area. Low frequency of captures in the tubes, predator disturbances and cold weather forced the termination of the study.

The role of dispersal as a population regulating mechanism was further substantiated. The genetic differences between emigrant and resident animals lend support to Howard's hypothesis that a genetic polymorphism influences the tendency to disperse. Support is also given to Myers' and Krebs' contention that among dispersers an additional density dependent polymorphism influences the distance dispersed.

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INTRODUCTION

Voles and lemmings (Rodentia; Cricetidae) have long been known to exhibit periodic fluctuations in their population sizes. Throughout North America, where they have been studied, populations of these rodents reach high densities every three to four years, and the word cycle has been loosely used to describe the alternating sequence of high and low densities. Changes in the density of a local population can be quite remarkable, up to two orders of magnitude in less than a year (Krebs and Myers, 1974) and many theories have been proposed which seek to explain the factors controlling the oscillation. Four major problems have been identified by Krebs and Myers (1974) which must be solved before the forces controlling the fluctuation of the populations can be understood: 1) what prevents unlimited increase in the population, 2) what causes the cyclic periodicity of three to four years, 3) what produces synchrony of populations over large areas and 4) what determines the amplitude of the fluctuation? The answers to the last three questions are dependent upon the answer to the first. When the processes involved in producing a cycle of abundance are understood, then other aspects of population demography can be more adequately investigated experimentally.

Over the last fifty years a large number of theories have been hypothesized as controlling population fluctuations. Chitty (1967) pointed out that for a given factor to be the cause of the cycles, it must be both necessary and sufficient to produce the observed fluctuations. Population fluctuations must occur only in the presence of the factor and every time the factor is present. If the population

does not behave as predicted, the factor postulated as the critical controlling variable must be rejected as the cause and the apparent causal relationship taken as coincidental. Chitty (1960) discussed and rejected many of the early hypotheses, most of which relied on factors extrinsic to the population: infectious disease, predation, food supply, weather, shock disease and adrenopituitary exhaustion as unsatisfactory because they were either untestable or were not both necessary and sufficient to explain the observed oscillations.

In recent years emphasis has shifted from extrinsic to intrinsic factors which might operate to regulate rodent populations. Chitty (1960) proposed that deterioration of the "quality" (or viability) of the rodents in the face of increasing adversity in the biotic environment caused the cessation of population increase. Chitty was unable to suggest a mechanism for the changes in rodent viability but argued that the hypothesis was testable.

The regulation of population numbers may involve dispersal of surplus individuals from expanding populations, a process to which investigators have recently turned their attention. The possibility of a polymorphism in dispersal tendency which could be significant in population processes was suggested by Howard (1960) and Lidicker (1962). Lidicker proposed that if the tendency to emigrate had a genetic basis the expression of which was sensitive to changes in the population density, then dispersal might act to keep a population below its carrying capacity. Such a tendency would also be individually beneficial. Wandering individuals would come in contact with more individuals and possibly mate more frequently, they would breed with individuals from other demes producing offspring of greater heterozygosity and presumably fitness, and would have a great chance of survival by moving out of

dense areas before devastating crashes in population numbers occurred. It was also thought that such a dispersal polymorphism would be valuable to a species by reducing inbreeding, extending the range of the species, spreading new genes, facilitating the reinvasion of disturbed areas (Howard, 1960), and possibly serving as a mechanism in population regulation (Lidicker, 1962).

Murray (1967) provided a possible behavioural basis for a dispersal polymorphism although he was interested in demonstrating the relationship between dispersal and the advantage to the individual. Observation of the dispersal distances, which a maturing animal moves until he finds an area in which he can settle, show that some animals are long distance dispersers and some short distance dispersers. Dominant animals will breed and reproduce close to their birth site while increasingly subordinate animals will be more successful as they disperse further in search of a place to settle. If dispersal tendency and social behaviour were genetically related, a genetic-behavioural polymorphism might exist.

A different genetically-based behavioural mechanism was proposed by Chitty (1967). In his model, favourable selection shifts from slightly aggressive, fecund individuals during the early increase phase to very aggressive individuals who suffer reduced fitness and viability at the peak phase to docile, fecund individuals with high viability during the ensuing decline. During the increase phase selection will favour increasingly aggressive individuals who succeed by interfering with the reproduction of more docile individuals. However, it is hypothesized that selection for good viability and fecundity in these aggressive animals will be relaxed, resulting in a population which is very vulnerable to adverse conditions.

The selective loss of these hyper-aggressive individuals from peak populations leads to a rapid decline in numbers producing a phase of low numbers during which encounters between individuals will be sufficiently reduced so that selection will now favour fecund individuals. Krebs et al. (1973) modified Chitty's hypothesis to incorporate dispersal during the increase phase. These emigrants could become the colonizers of adjacent vacant areas or after a period in marginal habitats could immigrate into populations during the phase of low numbers.

Experimental work on the demography of Microtus spp. was initiated by Krebs and co-workers in 1965. They investigated the demographic changes over a population cycle of fenced and unfenced populations and defined the genetic, behavioural and reproductive attributes of resident and "dispersing" voles (Krebs et al., 1969; Tamarin and Krebs, 1969; Keller and Krebs, 1970; Krebs, 1970; Gaines et al., 1971; Myers and Krebs, 1971; Gaines and Krebs, 1971). A significant observation was that populations held in 0.8 ha enclosures rose to densities 3-4 times greater than adjacent unenclosed populations. Eventual declines in the enclosed populations were accompanied by severe overgrazing and destruction of the grassland habitat. The inescapable conclusion was that dispersal is necessary for microtine species to maintain their numbers below the carrying capacity of the environment. Emigration rather than increased mortality in situ must operate to reduce the rate of population increase in unfenced areas.

Previous attempts to measure dispersal from rodent populations have been indirect (Stickel, 1946; Calhoun and Webb, 1953; Van Vleck, 1968; Myers and Krebs. 1971). The general method of these workers was to monitor the movement of animals from live-trapped areas into unoccupied central areas maintained by removal-trapping. Dispersers

were defined as those individuals which were captured in the removal area.

Earlier workers did not deduce any relationship between the amount of dispersal and population density or rate of change of population density. However, Myers and Krebs (1971) found that the amount of dispersal and the proportion of losses from central populations attributable to dispersal were greater during the increase phase of a population cycle of Microtus pennsylvanicus than during the peak or decline phases. If their method for identifying dispersers was accurate, then large numbers of adult voles must be dying in situ rather then emigrating during the decline phase. Krebs et al. (1973) and Krebs and Myers (1974) suggested that peak population densities may produce a different type of dispersing animal so that during the decline phase, the observed large losses of adults may be due in part to "pathological" rather than "colonizing" dispersers. "Pathological" dispersers would not settle on the vacant areas where they would be detected by live-trapping but would emigrate over longer distances, probably suffering high mortality. Because of the biases inherent in the removal-trapping definition of dispersal, an accurate measure of dispersal over the population cycle can only be obtained by monitoring egress from semi-enclosed populations.

In my study an attempt was made to investigate dispersal in a M. pennsylvanicus population by monitoring departures from a semi-enclosed population rather than by recording immigration to an area of reduced density. Because of the problems associated with other definitions of dispersal, I felt that the identification of all individuals attempting to leave an enclosed population would give a more accurate assessment of dispersal during increase, peak and decline phases of the population cycle. By allowing dispersing animals

to move through either an enclosed area containing a resident population of low density or a vacant area maintained by removal-trapping, I hoped to distinguish "colonizing" dispersers from "pathological" dispersers. Colonizers would be expected to settle very readily in the vacant area, whereas a slightly smaller proportion of released animals would be expected to settle in the occupied experimental area because of interactions with the residents, (Sadlier, 1965; Healey, 1967; Watts, 1968). The longer distance pathological dispersers would be expected to move through both test areas rather quickly.

The substantial shifts in the behavioural and dispersal attributes of a population that are hypothesized by Chitty (1967) and Krebs et al. (1973) as essential to the regulation of its numbers require dramatic genetic changes due to fluctuating selection coefficients. Because it is impossible to investigate the actual genetic changes underlying behaviour, biochemical markers were chosen to follow overall genetic effects. Tamarin and Krebs (1969) and Myers and Krebs (1971) used two polymorphic blood serum proteins, transferrin and leucine aminopeptidase as markers. In my study the transferrin phenotype (and presumably genotype) was determined electrophoretically for as many individuals as possible. It was then possible to look for genetic differences between the dispersing and resident segments of the population and to follow changes in the genetic composition of the population through time.

This study was designed to compare the genetic and demographic characteristics of dispersing and resident animals from a semi-enclosed M. pennsylvanicus population during the increase, peak and decline phases of a population cycle.

MATERIALS AND METHODS

Study Area and Trapping

The study was carried out in an abandoned field (Plate 1) set aside for ecological study at Brock University in St. Catharines, Ontario. A 1.4 ha area of relatively uniform old-field habitat which had not been cultivated in 8 years was enclosed by a chain-link fence in 1972. In June 1973, when this project began, the area inside the fence was partitioned into four squares each 0.3 ha (Figure 1). Three of these areas were fenced with 26 gauge galvanized sheet steel extending 0.3 m into the ground and 0.6 m above. Downward metal deflectors were put on all posts and seams in the fence. Ground-level openings at the junction of some metal sheets caused by frequent alternation of freezing and thawing in the winter of 1973-74 accompanied by constant and occasionally strong winds were repaired as soon as possible after discovery.

Each of the four 0.3 ha areas was covered with a grid of trap points 8 m apart. The enclosed areas A, B and C had grids of 7 x 7, whereas the unenclosed area D had a grid of 6 x 8. In late June 1973, resident animals were removed from the three enclosed areas by continuous snap-trapping until three consecutive 24 hr capture-free periods occurred (126 animals in 14 days). A selected number (see below) of young Microtus individuals were introduced to enclosures B and C in early July, 1973. Longworth live-traps (Chitty and Kempson, 1949) baited with whole corn and oats and provided with dry non-absorbant cotton in the nest box were set near the trap points wherever sign of vole activity was found. At first capture each animal was given a number by clipping off toes according to a code. Each foot was assigned a place value and each of the four large toes on

PLATE 1 Seasonal changes in the old field habitat (*Solidago-Aster*-grass complex) A - spring, B - summer, C - fall, D - winter. The ruled scale is 1.5 m long (0.1 m divisions).

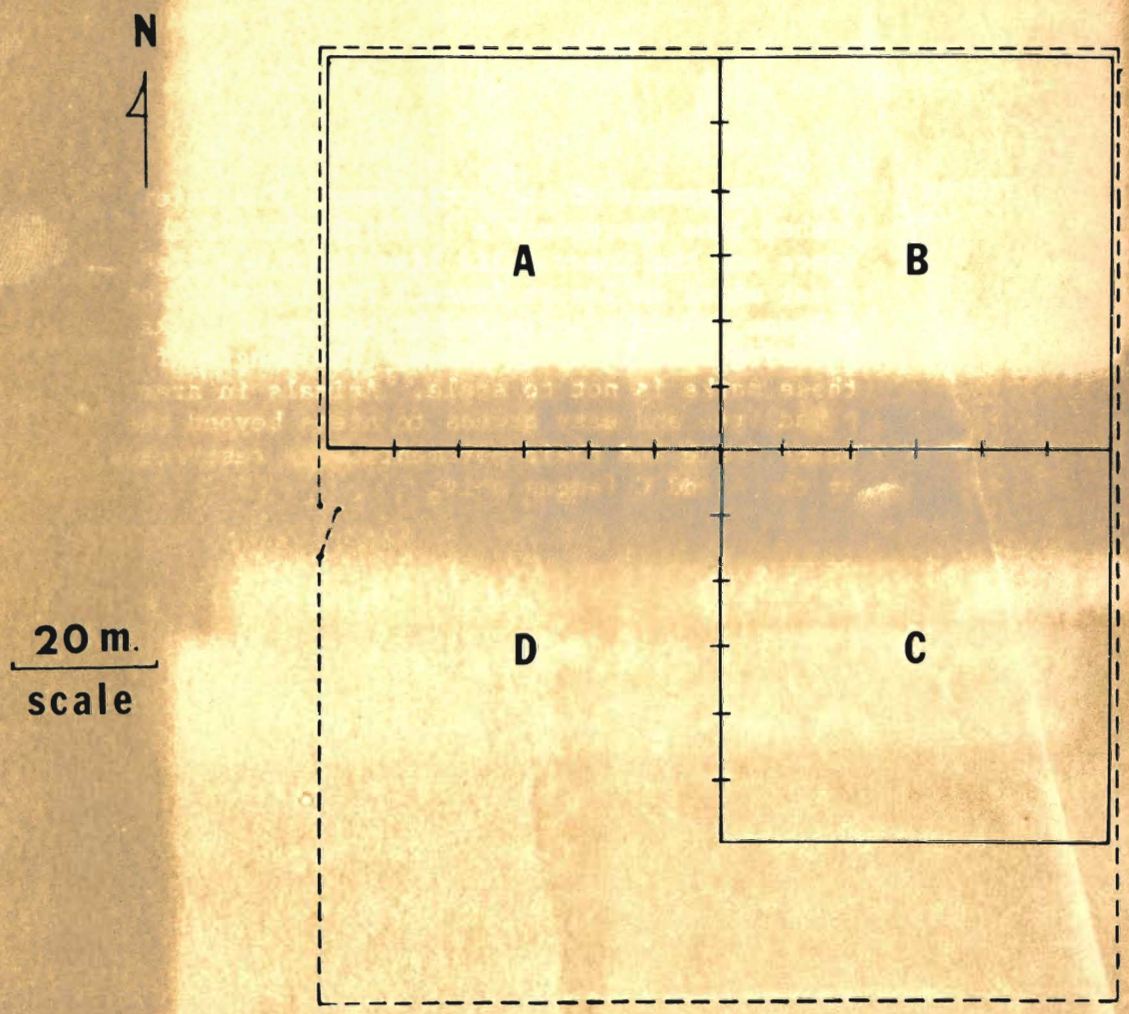

A


B

C

D

FIGURE 1 The arrangement of the enclosures. The outer dashed line represents a 2 m high chain-link fence and the inner solid line the 0.6 m high sheet metal enclosure fence. The locations of release tubes is indicated by the small marks crossing the enclosure lines. The length of these marks is not to scale. Animals in area D had free and easy access to areas beyond the outer fence and their movements were restricted by the A and C fences only.


N
A
B
D
C
20 m.
scale

each foot was assigned the value 1, 2, 4 or 7, providing 9,999 combinations when only 2 or less toes on each foot were cut. No more than two toes were removed from any foot and only the left fore and two hind feet were used. At each capture the following attributes were noted: sex; weight; trap station; in females, vagina perforate (open or closed) or imperforate, nipples large (lactating) or not large, pregnant or not pregnant; in males, testes scrotal or abdominal. The voles were weighed to the nearest gram by suspending them in a plastic bag from a small spring scale. Pregnancy in females was determined by palpation. This method can be used only in late pregnancy when the embryoes become large enough to be distinguished from intestinal contents. The determination of testes position in males was also equivocal since many males were observed to quickly retract the testes from the scrotum into the abdomen.

Enclosures B and C were live-trapped for three consecutive nights throughout the summer and fall of 1973 on a weekly basis. The traps were set in the early evening, checked early the next morning and locked open during the day. In 1973 the traps were locked shut between trap periods. After it was learned that the voles could open and get into the closed traps, the traps were locked open when not set. During the winter 1973-74, trapping was undertaken whenever the weather became mild enough to ensure survival of captured animals as no special equipment was used for winter trapping. In 1974, trapping was more flexible due to the release program to be described later.

While the removal program was proceeding in early July 1973, an adjacent unenclosed area of the field was live-trapped to secure animals for introduction to the enclosures. Five perforate, nulliparous females and 3 scrotal males each weighing less than 30 gm were

marked and introduced into each of enclosures B and C on July 7 and July 6, 1973 respectively. In enclosure C, one female died in a trap soon after introduction and a young unmarked male appeared, presumably missed in the removal trapping, so that the effective introduced population in enclosure C was 4 males and 4 females.

In the spring of 1974 work began on the study of dispersing animals. To allow dispersal from enclosure B, 1.2 m lengths of 4.1 cm internal diameter polyvinylchloride (PVC) tubing were inserted at regular intervals through the fences at ground level (Figure 1). Two live traps were connected via a PVC "Y" junction to the receiving end of the tubes so that animals using these exists could be captured and identified prior to release (Plate 2). The open ends of the tubes were capped when not in use. Five tubes (10 traps) opened from B into A along the A-B fence and 5 into C along the B-C fence. Five tubes led into area D from each of enclosures A and C.

Prior to the release of animals leaving B into either A or C enclosures the following procedures were carried out. Enclosure A was removal-trapped in late May 1974 before releases began so as to provide an uninhabited area. Although the fence enclosing A seemed to be secure, occasionally unmarked animals, juveniles and adults were captured in July. Throughout July and August there was very little sign of vole activity in A. For example, piles of cut stems of timothy (Phleum pratense) which voles cut to get at the seed head were common in B but rare or absent in A. Conversely, tall stems of timothy were very common in A but absent in B (Plate 3). Trapping of A in late September however revealed approximately 25 unmarked voles which were removed. The means of entry of these animals is unknown. Because vole sign was rare, it is believed that these animals had recently invaded the enclosure.

PLATE 2 The placement of a set of tube traps used in the identification of dispersing individuals from an enclosed population. Two live-traps (left) were connected via a "Y" junction to a 1.2 m length of PVC tubing which passed through the fence (right) where the end was opened during trapping periods.

PLATE 3 Divergence of habitats due to selective grazing. In the vacant enclosure A (left) beetle infestations reduced the *Solidago* spp. and *Aster* sp. densities while in the inhabited enclosure B (right) selective cutting of *Phleum pratense* (timothy) by *M. pennsylvanicus* have resulted in a marked difference in the appearance of the vegetation in August 1974.

In enclosure C an attempt was made to simulate a low population level with balanced age structure by cropping the over-wintered population. A population with equal numbers of males and females composed of 6 adults (> 35 g), 12 sub-adults (25-35 g) and 20 juveniles (< 25 g) was attempted. Cropping of the population was carried out on May 6-8 and July 11-13, 1974.

All exit tubes were first opened on June 3, 1974 and were frequently opened until early July. No releases into A and C were made during this period as the intention was essentially to insure that the voles would enter the tubes. Beginning in July 1974, animals from the enclosed B population which entered the dispersal tubes were released into A and C during 5 periods: July 13-26, Aug. 8-20, Sept. 5-13, Sept. 26 - Oct. 6, Oct. 16-31. The duration of the release period was modified by predator disturbance (see below) and occasional cold weather. An animal was released from B if, when it had been captured in a tube trap it was relatively young (< 30 g) and had not been previously caught at an adjacent point in the B trap grid. In this way animals whose activity ranges included the tube entrances were not released. An attempt was made to maintain a numerical balance in the releases into A and C. The sex of the animal was not considered as the number of animals entering the traps was too small to allow both a numerical and a sexual balance in releases into each enclosure. The animals were released at the mid-points of the A-B and B-C fences. Following each release period, enclosures A and C were live-trapped simultaneously to check on the presence of the released animals. Released animals which entered the tube traps in enclosures A and C were not released into D but put back into that enclosure (A or C). After an animal had been in enclosure A for a month or more it was removed along with any unmarked animals.

Released animals were not released from C and became part of its population. Recruitment of juveniles and sub-adults into the C population tended to be sporadic so that released animals from B helped maintain the desired age structure.

Racoons (Procyon lotor) and skunks (Mephitis mephitis) became very troublesome during the period June - October, 1974. Neither the chain-link fence nor the enclosure fences provided an effective barrier to these vole predators and they caused some disturbance to the dispersal experiments by pulling traps off the tubing (or opening Longworth traps) and extracting the captured voles. Because opened traps did not always show signs of blood, it was not possible to determine how many or which animals were predated or escaped through the open tubes. Because this predation was biasing my measurement of dispersal, trapping was interrupted when disturbances became evident and Havahart and Conibear traps were set until a predator was caught. Predation interference occurred approximately twice a month for two or three nights at a time. A maximum of 8 of 40 tube traps or 15 of 49 Longworth traps were disturbed in a night. Most often only one or two traps were dislodged.

Area D was live-trapped on three occasions in 1974: June 17-19, Sept. 10-12 and Oct. 5-7. No other trapping was done outside the enclosure fences.

Vegetation

A vegetation analysis (Table 1) was completed in the periods July 20 - Aug. 10, 1973 and Aug. 6-16, 1974. The number of stems and the percent cover for each species of vascular plant was determined for a randomly chosen square meter near trap points. In 1973, a count was made at each trap point in areas A, B, C and D. In 1974, 21 points were sampled in enclosures A, B and C (3 randomly

Table 1. Stem Densities[a,b] of Common Plants in Enclosures

Species	1973 Enclosures A	B	C	1974 Enclosures A	B	C
Poa compressa	177	199	269	214	99	157
Phleum pratense	51	66	30	59	63	25
Agrostis alba	24	-[c]	8	-	-	13
Solidago canadensis	60	39	62	17	62	25
Aster novae-angliae	13	13	9	6	13	7
Aster lateriflorus	1	3	5	-	18	12
Vicia cracca	7	8	6	5	2	3
Daucus carota	2	1	1	3	-	-
Circium vulgare	6	3	1	1	1	-
Barbarea vulgaris	2	1	-	-	-	-
Taraxacum officinale	2	1	-	-	-	-
Geum virginianum	-	2	-	-	2	1
Erigeron annuus	-	1	-	3	1	-

a no. stems m^{-2}

b species with less than 1 stem m^{-2} in all areas are not listed

c less than 1 stem m^{-2}

chosen points in each of 7 rows). The study area was found to be a Solidago-Aster complex with associated grasses, Poa compressa, and Phleum pratense.

Weather

The climate at St. Catharines is continental modified by the proximity of two of the Great Lakes. Summers are hot and occasionally dry while winters are much wetter with higher temperatures than observed north of Lake Ontario. It is sufficiently cold to support an intermittent snow cover throughout the winter. Snow may occur from mid-November until late March. Frequent warm air masses from the south produce transient warm periods during which the snow cover melts. The soil on the study area remained saturated until early May and occasional pools formed in the enclosures but did not seem to hinder the activity of the voles. In summer the soil became very hard and dry with wide fissures forming, especially on the paths. A summary of the weather changes over the study period is presented in Table 2. These values are not an accurate measure of the micro-climate at the study area but indicate the overall seasonal weather changes in the St. Catharines area.

Blood Sampling and Electrophoresis

Blood sampling of animals began on April 29, 1974. Previously unsampled animals in enclosures B and C were sampled at every trap period while animals in area D were blood sampled during two periods, June 17-19 and Oct. 5-7, 1974. Young animals weighing less than 20 g or pregnant females close to term were not blood sampled. Animals selected for blood sampling were removed to a field shed and held for approximately 1 hr or carried to the Glenridge Campus and held in standard animal cages for approximately 5 hr. Animals were released at their site of capture. Although many animals bled profusely for

Table 2 Monthly Averages of Selected Climatic Parameters[a]

Month 1973	Temperature (°C) Mean Maximum	Mean Minimum	Monthly Mean	Measurable Precipitation Total (cm)	Days With	Total Bright Sunshine (hr)
May	16.8	7.6	12.2	7.16	20	146.0
June	25.6	14.9	20.2	6.63	15	237.0
July	27.8	16.9	22.4	4.83	9	277.7
August	28.3	17.6	22.9	1.60	4	263.1
September	22.7	11.6	17.2	6.91	6	200.4
October	16.9	7.4	12.2	11.56	12	153.4
November	8.5	1.4	5.1	11.10	14	69.3
December	1.2	-4.7	-1.7	11.23	18	49.9
1974						
January	0.3	-7.4	-3.6	5.26	19	85.7
February	-1.5	-9.4	-5.4	5.61	16	90.9
March	4.7	-3.4	0.7	7.06	16	121.9
April	13.8	2.8	8.3	7.52	19	174.7
May	17.0	6.2	11.6	9.37	13	213.6
June	23.6	13.5	18.5	5.51	11	232.8
July	27.1	15.8	21.4	4.24	5	301.2
August	26.8	15.8	21.3	3.45	7	254.2
September	20.0	10.4	15.2	6.68	13	165.0
October	14.0	3.5	8.8	3.10	11	190.0
November	8.2	0.8	4.5	7.77	14	65.3

[a] All data except Total Bright Sunshine from Niagara Regional Weather Office. Total Bright Sunshine data from Vineland Agricultural Research Station.

a time, only one is known to have died in the trap immediately after blood sampling.

Blood was collected by clipping off the tip of the tail, removed as droplets formed with a 1 ml Tuberculin syringe (gauge 25 needle) (Pharmaseal; Brantford, Ont.), suspended in 500 µl of ice-cold saline solution (0.9% NaCl (W/V)) to prevent clotting and haemolysis and stored on ice until centrifuged. The blood samples were centrifuged at 4°C in an IEC clinical centrifuge (Model CL, International Equipment Co., Needham Hts., Mass., U.S.A.) at top speed for 8 min. The supernatant was removed and stored in labelled glass vials at -20°C.

Prior to electrophoresis, the total protein concentration of the serum-saline samples was determined spectrophotometrically using the method of Warburg and Christian (in Dawson et al., 1969). Electrophoresis of the serum samples on polyacrylamide gels followed the procedures of Davis (1964) except that Solution A was titrated to pH 9.0 rather than pH 8.7. The following stock solutions were prepared and stored at 4°C.

Solution A	36.6 g Tris (2-amino-2-(hydroxymethyl) propane-1,3-diol)
	0.23 ml Temed (N,N,N^1,N^1-Tetramethylethylene-diamine)
	approx. 48 ml 1N HCl
	distilled water to 100 ml
	pH adjusted to 9.0
Solution B	5.98 g Tris
	0.46 ml Temed
	approx. 48 ml 1 N HCl
	distilled water to 100 ml
	pH adjusted to 6.7

Solution C	28.0 g Acrylamide
	0.735 g Bis (N,N^1-Methylenebisacrylamide)
	distilled water to 100 ml
Solution D	10.0 g Acrylamide
	2.5 g Bis
	distilled water to 100 ml
Solution E	0.004 g Riboflavin
	distilled water to 100 ml
Solution F	40.0 g Sucrose
	distilled water to 100 ml

The following working solutions were made fresh daily:

Large Pore Gel Solution	1.25 ml B
	2.50 ml D
	1.25 ml E
	5.00 ml F
Large Pore Wash Solution	5.0 ml B
	5.0 ml E
Small Pore Gel Solution	3.5 ml A
	7.0 ml C
	3.5 ml distilled water
	14.0 ml .0014% ammonium persulphate (W/V)
Small Pore Wash Solution	3.5 ml A
	7.0 ml C
	3.5 ml distilled water.

The large pore gel solution was divided into 500 µl aliquots and a small amount of the serum-saline solution added in sufficient quantity that a 200 µl subsample of the large pore-serum-saline mixture contained 100-150 µg of protein. The sample gel (200 µl of the large pore-serum-saline mixture) was put in vertical, acid-washed glass tubes

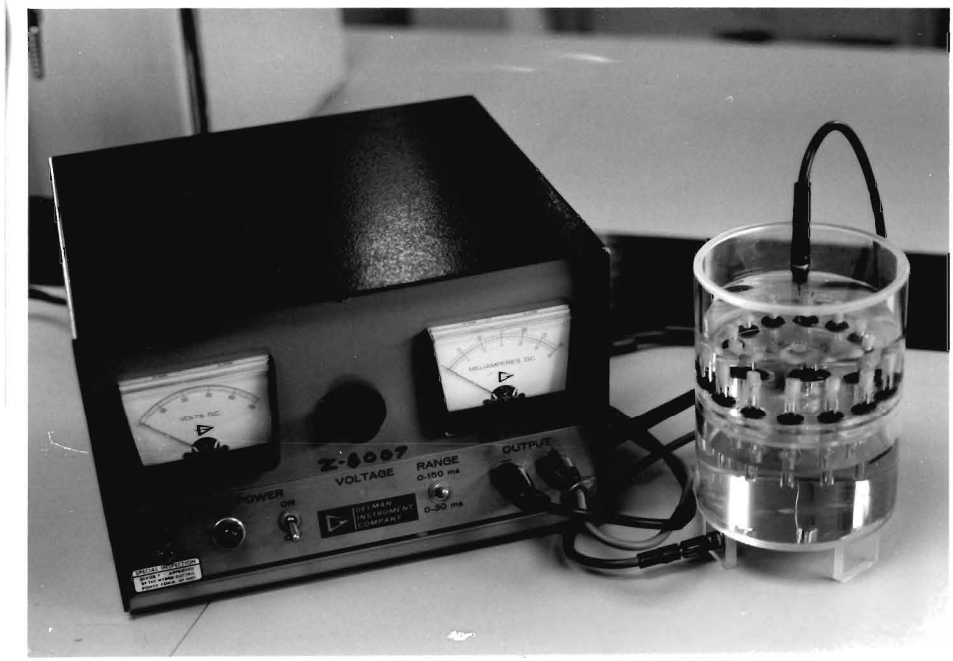
(5 mm ID x 75 mm long) which had been soaked in 0.5% photoflo (V/V) for 24 hr. A thin water layer was added to the gel to prevent the formation of a meniscus and the gel was then polymerized using intense light for 30 min. Next, 500 µl of large pore gel (the stacking gel) was polymerized on top of the sample gel in a similar fashion. The remainder of the glass tube was filled with small pore gel solution (the running gel), capped with saran wrap and polymerized in the dark for 45 min. The tube was washed twice with the appropriate wash solution before the addition of the stacking and running gels.

The gels were vertically electrophoresed in an apparatus (Plate 4) with two buffer reservoirs, upper and lower. Both reservoirs contained 500 ml of buffer (0.006% Tris (W/V) and 0.0288% glycine (W/V)) diluted 1:10. Bromophenol Blue (0.1 ml, 0.001% (W/V)) was added to the upper reservoir to act as a front marker during electrophoresis. The gels were electrophoresed cathodally at 5 mA/gel for approximately 40 min until the front marker was within 5-8 mm from the end of the tube.

The gels were removed from the tubes by rimming under water with a long needle. The distance from the beginning of the running gel to the marker front was measured to the nearest 0.01 in(0.25 mm). The gels were then fixed in 12.5% perchloric acid (V/V) for 90 min, stained with 10 ml of a 1:19 mixture of 0.0025% Coomassie Blue (W/V) and 12.5% trichloroacetic acid (W/V) for 12-14 hr and then destained in 6% perchloric acid (V/V) until the desired stain intensity was reached (approximately 3 hr). The distance from the start of the running gel to the transferrin bands (see below) was measured as above. The gels were stored in 7.5% acetic acid (V/V) in glass screwcap vials.

A transferrin-specific stain (2,4-dinitroso-1,3-napthalenediol) was used to identify the set of bands corresponding to this iron-transporting protein (Ornstein, no date). The possible confusing

PLATE 4 The electrophoresis chamber and powerpack. The chamber contains two buffer reservoirs which are connected via the electrophoresis gels held in vertical glass tubes. The anode is at the top of the apparatus and migration is downward.


Z-6007
VOLTAGE
RANGE
0-150 ma
0-30 ma
OUTPUT
POWER
ON
HEATH
INSTRUMENT
COMPANY

effects of cell lysis products were also determined. A suspension of blood cells was obtained by centrifuging a blood sample and resuspending the pellet in 0.9% saline for 5 repeated washings. The cells were lysed by resuspending the pellet in distilled water after the last wash. Gels were sliced longitudinally with a razor, one-half stained in Coomassie Blue and the other half in the transferrin stain. In three sets of gels (cells, cells + serum, serum) the green bands characteristic of the transferrin-specific stain were found only in those gels containing serum and were always distinct from the red haemoglobin bands. These green bands corresponded to distinct bands on the Coomassie Blue stained halves and were not close to other bands. (Plate 5)

The transferrin genotype of individuals was determined by inspection of the electrophoresis gels. Two bands S(slow) and F(fast) were found in three combinations: one slow (SS), one slow/one fast (SF) and one fast (FF). It was assumed that these bands represented the gene products of a locus with two co-dominant alleles (Myers and Krebs, 1971). For each gel the migration distance of each Tf band was divided by the front marker migration distance yielding an R_x value. Frequency distributions of the Arcsin transformed R_x values (Appendix I) of the two bands in heterozygous (SF) individuals were constructed. Overlap between the distributions for each band was slight. Homozygous individuals whose transformed R_x value was above the zone of overlap were designated FF and those with transformed R_x values below the overlap zone SS. Individuals whose transformed R_x value was intermediate were co-electrophoresed with homozygotes of known phenotype to determine their phenotype. Because of a malfunction in a pH meter, Solution A prepared after Dec. 19, 1974 had a slightly different pH than Solution A prepared before this date. An overall shift in the mobility of all proteins was noted. However, the relationship between the mobilities

PLATE 5 Disc electrophoresis of M. pennsylvanicus serum stained with Coomassie Blue. The origin is at left and migration to the right. The transferrin (Tf) bands are marked. A slight band was generally associated with the leading F (fast) band. A homozygous Tf-FF and heterozygous Tf-SF are shown. Homozygous Tf-SS were also frequently observed.


Tf-FF
Tf-SF

of the two transferrin bands did not change. The mobility shift was taken into account when the frequency distributions and designations of genotype were made.

To test the constancy of the transferrin phenotype, 24 randomly chosen animals were resampled at intervals of up to six months after the first sampling. In all cases the transferrin phenotypes of both samples were identical. Further, there were no observed changes in phenotypes in blood samples used many times as co-electrophoresis standards. The genotype designations can therefore be taken as accurate.

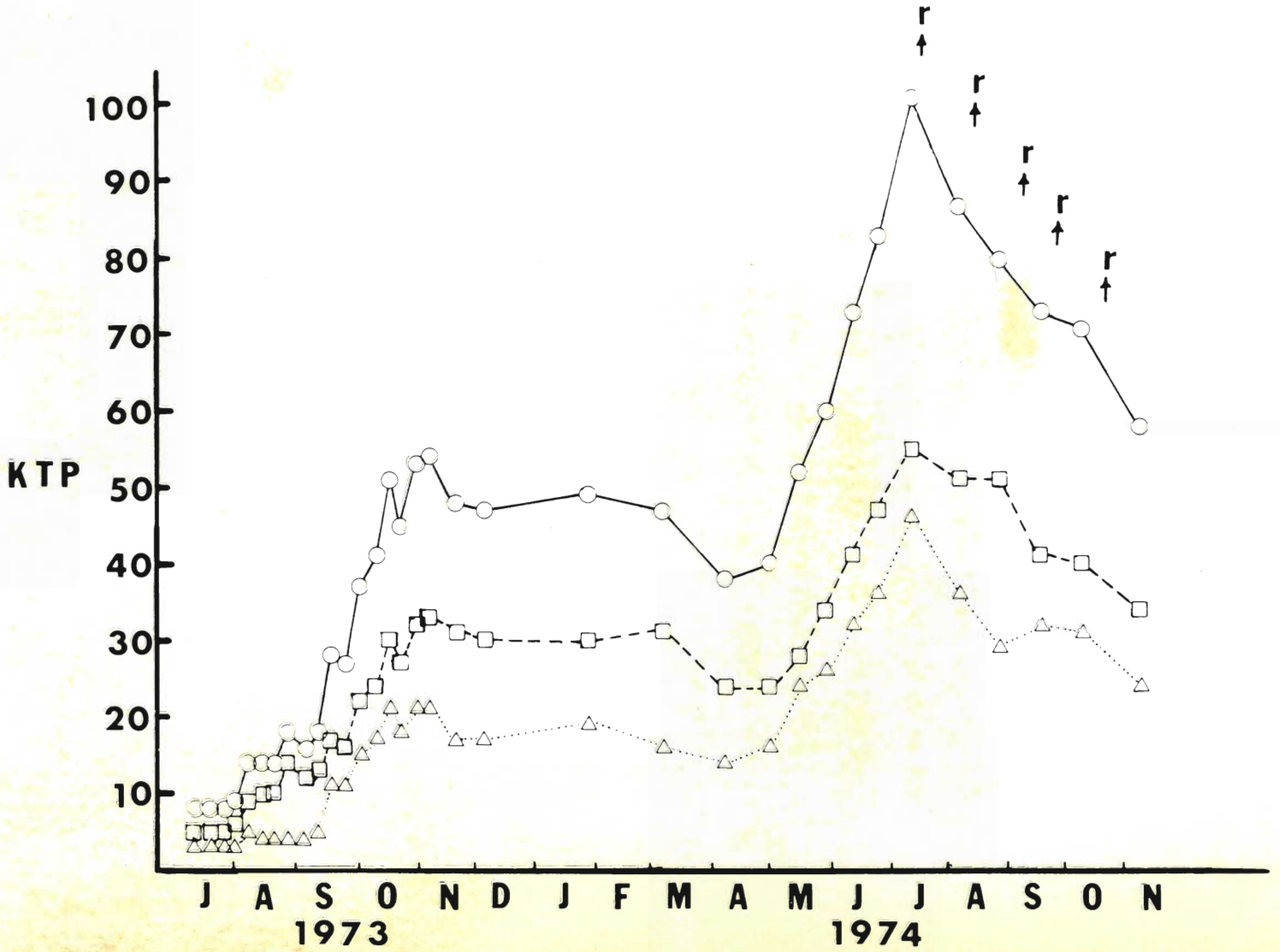
RESULTS

Population Growth

Changes in the size of the known trappable population (KTP) in enclosure B are shown in Figure 2. The first recruits entered the trappable population three weeks after the initial introduction. Numbers increased throughout the remainder of the summer at a rate of four individuals per week in a sigmoidal fashion until the beginning of November. The population size remained stable throughout the winter with a slight decline in March. During the period from May 1 to July 12, 1974, the population increased rapidly by six individuals per week. In July the rate of population increase took a sudden shift producing a continuous decrease in the population size until the end of the study. The number of males and females in the B population are also shown in Figure 2. The number of females always exceeded the number of males throughout the study. The sex ratio significantly differed from 1.00 on three occasions: late August 1973 ($\chi^2 = 4.500$; $p < .05$) early March 1974 ($\chi^2 = 4.170$; $p < .05$) and late August 1974 ($\chi^2 = 5.513$, $p < .02$). From Figure 2 it appears that these instances were due to increased male mortality rather than female natality.

Changes in numbers in the C population in 1973 closely followed those in the B population (Figure 3). Following a lag period of 48 days, the C population grew rapidly to approximately 40 animals by mid-October (5 individuals/week) and stayed between 35 and 45 until cropping was performed in early May 1974. Population numbers erupted in late June - early July 1974; juvenile mortality and the subsequent cropping returned the population size to 30. A second eruption was observed in late September 1974 but the numbers fell either because

FIGURE 2 Known trappable population (KTP) in enclosure B as determined by saturation live-trapping. The arrows indicate the periods during which animals were released into other enclosures. All individuals —○— , females -□- , males ····△···· .


r
r
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r
100
90
80
70
60
KTP
50
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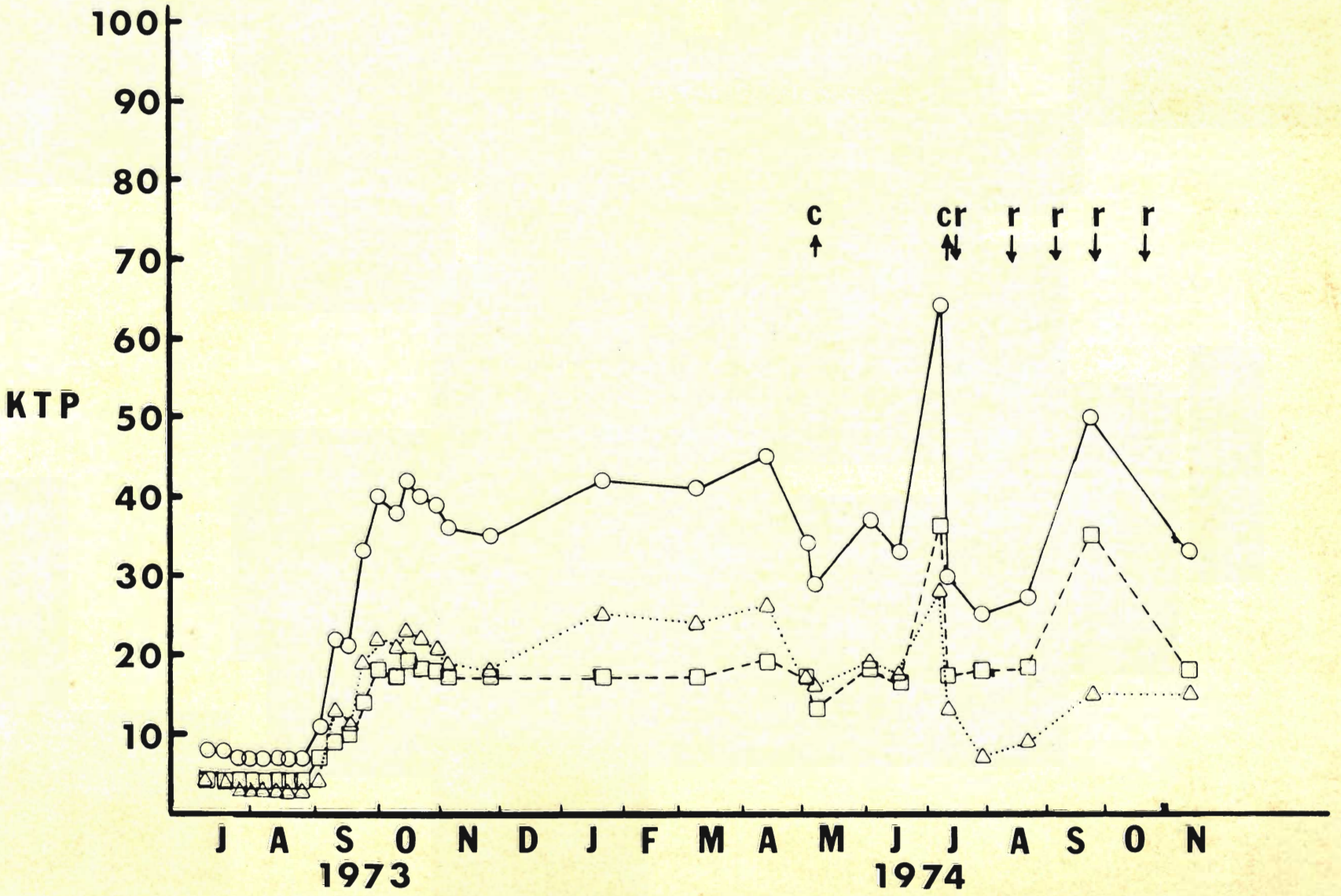
30
20
10
J A S O N D J F M A M J J A S O N
1973
1974

FIGURE 3 Known trappable population (KTP) in enclosure C as determined by saturation live-trapping. The downward arrows (C) indicate the periods when the population was cropped. The upward arrows (R) indicate the periods when individuals were released into enclosure C from enclosure B. All individuals —○— , females — —□— — , males ····△···· .


100
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KTP
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cr
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J A S O N D J F M A M J J A S O N
1973
1974

of fall mortality or low trappability. The sex ratio was close to 1.00 until late summer 1974 when an excess of females was noted. During trap periods July 28 - 31 and Sept. 22 - 24 a significantly greater number of females was observed ($\chi^2 = 4.00$, $P < .05$; $\chi^2 = 7.22$, $P < .01$).

The population in the unenclosed area D was sampled on three occasions. In mid-June 1974 28 individuals were captured, A significant number of these were female (19) ($\chi^2 = 4.0936$, $P < .05$). In mid-September 30 animals (13 males, 17 females) were captured and in early October 1974 the population size was 37, 14 males and 23 females. This sex ratio does not differ significantly from 1.00.

Breeding

Changes in the reproductive condition of the populations studied were principally determined by external characteristics of females, noticeable pregnancy and lactation (Table 3). Since the proportion of females visibly pregnant or lactating follows actual breeding activity (copulation) by two to three weeks, statements on breeding activity within a given period account for this delay. The first evidence of breeding activity in enclosure B was apparent 8 days after introduction of the original animals, indicating that at least two females were pregnant when introduced. Breeding continued throughout the summer and early fall, ending in early November 1973. In the spring of 1974 the onset of breeding occurred in mid-March. Throughout the early part of the summer at least 20% of the females were lactating at each trap period. In July 1974 the intensity of breeding began to decline, eventually ending in early November.

In enclosure C, pregnant or lactating females were captured two weeks after introduction suggesting the conception occurred just before or just after introduction. Breeding continued throughout

Table 3 Percent of total females captured during each month which were pregnant, or lactating. The number of pregnant or lactating females is given in parentheses.

Month	Enclosure B			Enclosure C		
	% Pregnant	% Lactating	Number Females	% Pregnant	% Lactating	Number Females
1973						
July	20 (1)	40 (2)	5	75 (3)	25 (1)	4
August	11 (1)	22 (2)	9	50 (2)	50 (2)	4
September	6 (1)	12 (2)	17	11 (1)	44 (4)	9
October	25 (6)	29 (7)	24	0	6 (1)	18
November	0	0	33	0	0	17
December	0	0	30	no data	no data	
1974						
January	0	0	30	0	0	17
February	no data			no data		
March	0	0	31	0	0	17
April	8 (2)	29 (7)	24	0	16 (3)	19
May	15 (5)	26 (9)	34	18 (3)	41 (7)	17
June	27 (11)	37 (15)	41	28 (5)	33 (6)	18
July	9 (5)	29 (16)	55	11 (4)	14 (5)	36
August	10 (5)	14 (7)	51	17 (3)	11 (2)	18
September	2 (1)	15 (6)	41	9 (3)	17 (6)	35
October	3 (1)	5 (2)	40	no data		
November	0	3 (1)	34	0	0	18

the summer, ending in early October 1973. In the spring of 1974 breeding resumed in late March and continued vigorously until early July when cropping of the population occurred. Breeding continued throughout the remainder of the summer at a low intensity until it ended in October. No evidence of breeding was found during the winter of 1973-74 in either population B or C.

In June 1974, 26% of the females in area D were visibly pregnant and/or lactating. In October, 1974, 35% of the females were in this condition.

Electrophoresis:

Coomassie Blue staining of the electrophoresis gels revealed two sharp, distinct bands separated by approximately 1.3 mm which corresponded to the transferrin-specific stained zone (Plate 5). No interference or confusion with haemoglobin or other blood cell contents was encountered. Prior to December 19, 1974, the mean transformed R_x values $\pm$ 1 SD for samples from heterozygous animals were: S- 36.31 $\pm$ 0.81; F - 37.99 $\pm$ 0.85. After December 19, 1974 these values shifted to: S- 37.24 $\pm$ 0.56; F- 39.03 $\pm$ 0.56. Despite the change in mobilities caused by the pH change in solution A (see Methods) the separation of bands was not altered.

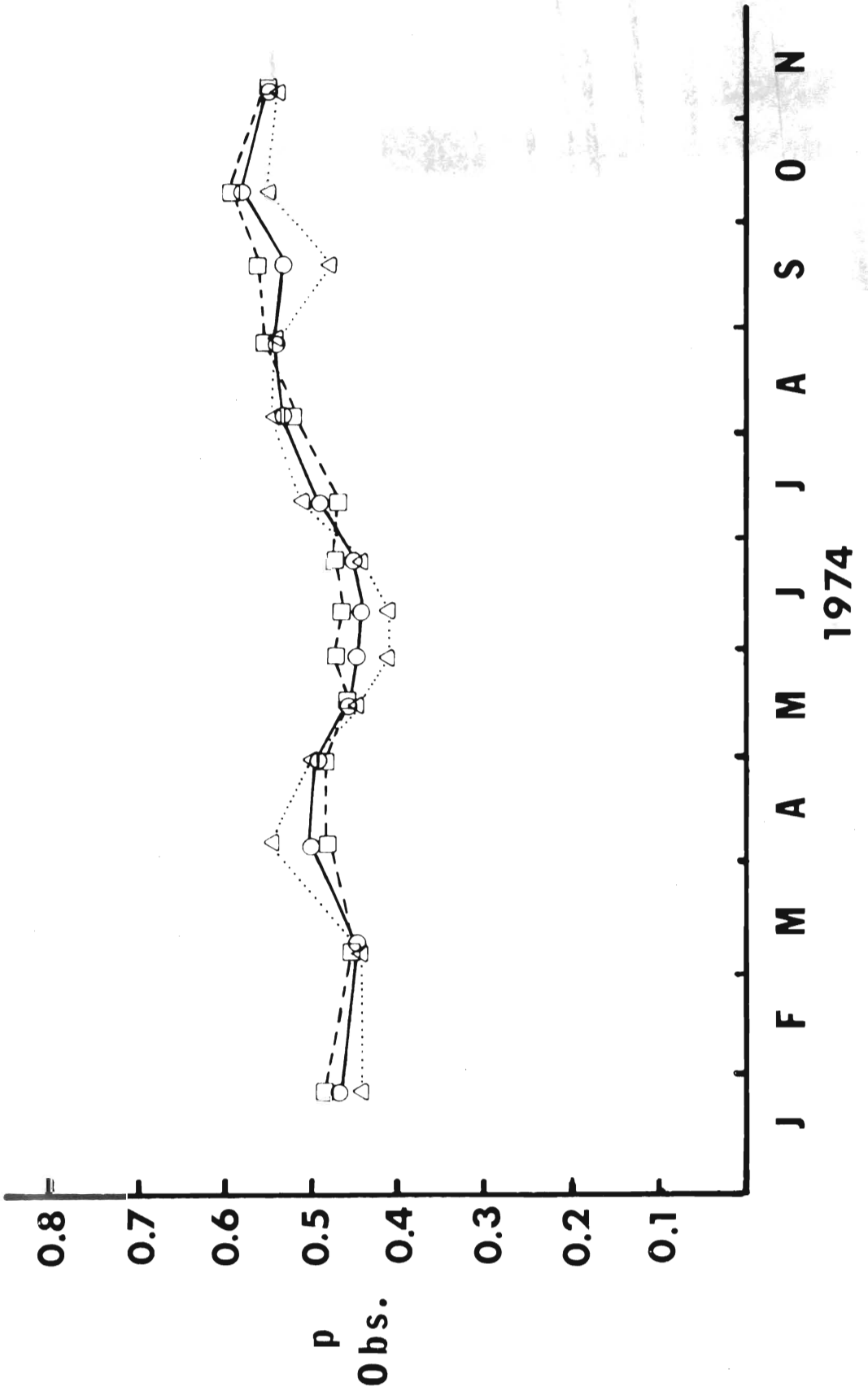
There was no evidence that the transferrin phenotype of individual animals changed during the course of the experiment. None of the 24 animals resampled at time intervals ranging from 8 to 160 days changed their transferrin phenotype. The check sample contained 15 males and 9 females, which represented 6 SS, 13 SF and 5 FF individuals. Further, the phenotype of individual blood samples re-electrophoresed as standards remained constant.

Genotype Frequencies:

All individuals captured within each trapping period were classified according to sex, genotype, (if known) and capture status (new recruit or recapture). Since the number of recruits in each sex-genotype category was always less than 10 and usually less than 2 or 3 in each trap period, their numbers are included with recaptured animals in the subsequent analysis. Figure 4 shows changes in the frequency of the S allele (p) in the experimental population in enclosure B for 1974. As blood sampling began in April, allele frequencies were estimated for earlier periods from the genotypes of surviving individuals. These estimates are strongly biased in favour of long-surviving genotypes and are therefore less accurate than frequencies calculated for the population after April. The frequency of the S allele varied little over the sampling period, ranging from 0.44 to 0.58 for the total population. There was little difference in allele frequency between the sexes over the sample period.

A different situation was observed in the cropped population in enclosure C where the total population, and thus the sample sizes for a given period were smaller (Figure 5). Throughout 1974 the observed frequency of the S allele (p) was greater in males than in females. A decrease in p from 0.61 to 0.44 occurred in males in June-July 1974 because of increased recruitment of FF homozygotes (Table 4). The subsequent increase in p during August resulted from recruitment of SS homozygotes. In females, the two abrupt changes in p were associated with population cropping (Table 5). At the first cropping (May) all the known SS homozygous females were removed while at the second cropping (July) an excess of FF homozygotes were killed. Although only a few individuals from any sex-genotype category were removed, since the population was not very large, dramatic changes in p were

FIGURE 4 The frequency (p) in the enclosed B population of the S allele of serum transferrin is indicated by disc electrophoresis. Throughout this period the genotype of 60% to 98% of all captured individuals was determined. All individuals —○— , females—□— , males ····△···· .


0.8
0.7
0.6
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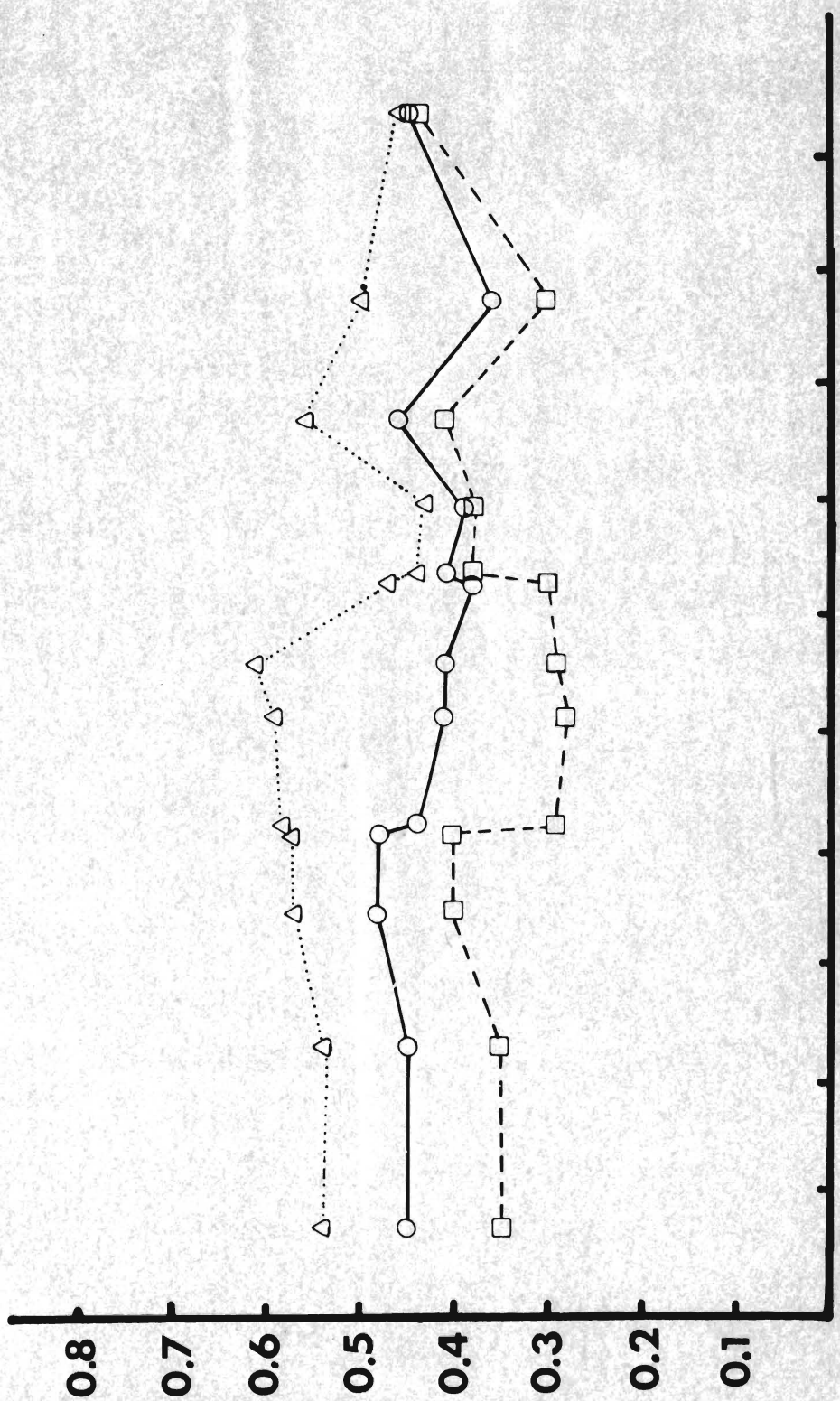
0.2
0.1
p
Obs.
J F M A M J J A S O N
1974

Figure 5 The frequency (p) in the enclosed C population of the S allele of serum transferrin as indicated by disc electrophoresis. Throughout this period the genotype of 52% to 96% of all captured individuals was determined. All individuals released into the C population are included. All individuals —○—, females – –□– – , males ····△···· .


0.8
0.7
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0.4
0.3
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p
Obs.
J F M A M J J A S O N
1974

Table 4 Transferrin Genotype Frequencies of Recruits into the Enclosed C Population June - August, 1974.

	Males				Females			
Trap Period	unk	SS	SF	FF	unk	SS	SF	FF
June 3 - 5	7	-	-	-	2	-	2	3
June 17 - 18	2	-	-	1	-	-	-	1
July 8 - 10	6	1	3	5	11	1	8	9
July 11 - 13	1	1	-	1	-	-	-	-
July 29 - 31	-	0	2	1	-	-	2	1
Aug. 21 - 23	-	3	1	-	-	1	1	-

Table 5. Transferrin Genotype Frequency Changes in Enclosure C Due to Cropping.

	Males				Females			
Trap Period	unk	SS	SF	FF	unk	SS	SF	FF
May 3-5, 1974 (before cropping)	3	3	10	1	2	2	8	5
Number Removed	-	1	4	-	-	2	2	-
May 6-8, 1974 (after cropping	4	3	8	1	1	-	7	5
July 8-10, 1974 (before cropping	9	5	8	6	11	1	13	11
Number Removed	-	3	3	4	-	1	4	6
July 11-13, 1974 (after cropping)	4	2	4	3	4	1	8	4

observed. For the population as a whole, the frequency of the S allele fluctuated between 0.48 and 0.36 for the study period.

The frequency of the three transferrin genotypes in the total enclosed B population, in the males of enclosure B, in the females of enclosure B and in the total enclosed C population are presented in Tables 6, 7, 8 and 9 respectively. Expected frequencies for Hardy-Weinberg equilibrium conditions were calculated using Levene's (1948) method for small sample sizes (Appendix II - sample calculations). In the total enclosed B population the genotype frequencies were at Hardy-Weinberg equilibrium until August 1974 when a deficiency of heterozygotes was first noted (Table 6). The observed χ^2 values were continuously less than 1.0 from January until the first of August when they jumped to 1.4 or greater. In two trap periods, September 17 - 19 and October 8 - 10, the observed χ^2 value was significant. In two other late 1974 trap periods the observed χ^2 value approached significance ($P \leq .1$). Males in enclosure B exhibited genotype frequencies which did not significantly differ from Hardy-Weinberg expectations (Table 7). A slight deficiency of heterozygotes was observed from mid-July 1974 until the end of the study. Females (Table 8) also exhibited heterozygote deficiencies in late 1974. Significant and nearly significant χ^2 values were observed in September and October. Genotype frequencies in enclosure C (Table 9) did not differ significantly from Hardy-Weinberg expectations throughout the study.

Area D, which was not intensively studied, was sampled twice to obtain an estimate of allele and genotype frequencies in an unenclosed population. Table 10 presents a summary of the genetic data obtained. The frequency of the S allele (p) was the same (0.48 - 0.49) in both June and October. Also, the genotype frequencies were at Hardy-Weinberg

Table 6 Distribution of Transferrin Genotypes in Total Enclosure B Population 1974

Trap Period	SS		SF		FF		χ^2_b	P_c
	O	E_a	O	E	O	E		
Jan. 26 - 28	7	6.16	13	14.68	9	8.16	0.128	0.70
Mar. 4 - 6	7	6.20	14	15.60	10	9.20	0.102	0.75
Apr. 5 - 6	10	8.87	16	18.25	10	8.87	0.257	0.60
Apr. 29 - May 1	10	8.88	17	19.24	11	9.88	0.248	0.60
May 14 - 16	10	9.71	23	23.68	14	13.71	0.008	0.95
May 27 - 29	13	10.79	23	27.42	19	16.79	1.006	0.30
June 10 - 12	14	12.62	30	32.76	22	20.62	0.255	0.60
June 24 - 26	17	15.54	35	37.92	24	22.54	0.255	0.60
July 11 - 13	24	21.89	42	46.22	26	23.89	0.526	0.50
Aug. 5 - 7	28	23.70	34	42.60	23	18.70	2.922	0.09
Aug. 26 - 28	26	23.03	33	38.94	19	16.03	1.412	0.25
Sept. 17 - 19	24	19.18	25	34.64	20	15.18	4.631	0.03
Oct. 8 - 10	26	21.76	24	32.48	16	11.76	3.793	0.05
Nov. 7 - 9	19	15.74	20	26.52	14	10.74	2.560	0.10

a calculated according to Levene (1949)
b continuity correction included
c df = 1, probability associated with χ^2
O = observed frequency
E = frequency expected at Hardy-Weinberg equilibrium

Table 7 Distribution of Transferrin Genotypes in Males
Enclosure B 1974

Trap Period	SS		SF		FF		χ^2_b	P_c
	O	E_a	O	E	O	E		
Jan. 26 - 28.	2		4		3		-d	
Mar. 4 - 6	2		4		3		-	
Apr. 5 - 6	4	3.64	6	6.72	3	2.64	-	
Apr. 29 - May 1	4	3.62	7	7.76	4	3.62	-	
May 14 - 16	4	3.92	10	10.16	6	5.92	-	
May 27 - 29	4	3.56	10	10.88	8	7.56	-	
June 10 - 12	5	4.60	13	13.80	10	9.60	0.010	0.90
June 24 - 26	6	6.00	16	16.00	10	10.00	0.000	1.00
July 11 - 13	12	10.63	18	20.74	11	9.63	0.392	0.60
Aug. 5 - 7	12	10.19	14	17.62	9	7.19	0.937	0.40
Aug. 26 - 28	9	7.91	12	14.18	7	5.91	0.302	0.60
Sept. 17 - 19	8	6.88	13	15.24	9	7.88	0.303	0.60
Oct. 8 --10	10	8.70	12	14.60	7	4.70	0.488	0.50
Nov. 7 - 9	8	6.91	10	12.18	6	4.91	0.353	0.60

a calculated according to Levene (1949)
b continuity correction included
c df = 1, probability associated with χ^2
d expectations too small, χ^2 inappropriate.
O = observed frequency
E = frequency expected at Hardy-Weinberg equilibrium

Table 8 Distribution of Transferrin Genotypes in Females
Enclosure B 1974

Trap Period	SS		SF		FF		χ^2_b	P_c
	O	E_a	O	E	O	E		
Jan. 26 - 28	5	4.38	9	10.24	6	5.38	0.059	0.80
Mar. 4 - 6	5	4.42	10	11.16	7	6.42	0.041	0.85
Apr. 5 - 6	6	5.13	10	11.74	7	6.13	0.180	0.70
Apr. 29 - May 1	6	5.13	10	11.74	7	6.13	0.180	0.70
May 14 - 16	6	5.66	13	13.68	8	7.66	0.010	0.90
May 27 - 29	9	7.15	13	16.70	11	9.15	1.007	0.30
June 10 - 12	9	7.93	17	19.14	12	10.93	0.211	0.60
June 24 - 26	11	9.43	19	22.14	24	12.43	0.528	0.50
July 11 - 13	12	11.17	24	25.66	15	14.17	0.070	0.80
Aug. 5 - 7	16	13.39	20	25.22	14	11.39	1.607	0.20
Aug. 26 - 28	17	15.00	21	25.00	12	10.00	0.865	0.40
Sept. 17 - 19	16	12.29	12	19.42	11	7.29	4.718	0.03
Oct. 8 - 10	16	12.92	12	18.11	9	5.96	3.331	0.07
Nov. 7 - 9	11	8.70	10	14.60	8	5.70	2.092	0.15

a calculated according to Levene (1949)
b continuity correction included
c df = 1, probability associated with χ^2
O = observed frequency
E = frequency expected at Hardy-Weinberg equilibrium

Table 9 Distribution of Transferrin Genotypes in Total Enclosure C Population 1974.

Trap Period	SS		SF		FF		χ^2_b	P_c
	O	E_a	O	E	O	E		
Jan. 20 - 23	3	4.42	14	11.16	5	6.42	0.814	0.40
Mar. 7 - 8	3	4.42	14	11.16	5	6.42	0.814	0.40
Apr. 12 - 15	5	6.63	18	14.74	6	7.63	0.796	0.40
May 3 - 5	5	6.63	18	14.74	6	7.63	0.796	0.40
May 6 - 8	3	4.47	15	12.06	6	7.47	0.830	0.40
June 3 - 5	3	4.36	16	13.28	8	9.36	0.620	0.40
June 17 - 19	3	3.80	13	11.40	7	7.80	0.141	0.70
July 8 - 10	6	6.07	21	20.86	17	17.07	0.051	0.80
July 11 - 13	3	3.56	12	10.88	7	7.56	0.037	0.85
July 29 - 31	2	3.40	14	11.20	7	8.40	0.807	0.40
Aug. 21 - 23	5	5.41	14	13.18	7	7.41	0.010	0.90
Sept. 22 - 24	7	5.47	17	20.06	19	17.47	0.722	0.40
Nov. 11 - 13	7	6.20	14	15.60	10	9.20	0.102	0.70

a calculated according to Levene (1949)
b continuity corrections included
c df = 1, probability associated with χ^2
O = observed frequency
E = frequency expected at Hardy-Weinberg equilibrium

Table 10 Genetic Summary of Area D Population Unenclosed 1974

Genotype/Trap Period		June 17 - 19	Oct. 5 - 7
SS	O	6	6
	E[a]	5.62	7.63
SF	O	11	20
	E	11.76	16.74
FF	O	6	7
	E	5.62	8.63
χ^2		0.011	0.770
P		0.90	0.40
p		0.48	0.49
q		0.52	0.51

[a] calculated according to Levene (1949)

O = observed frequency
E = frequency expected at Hardy -Weinberg equilibrium
P = probability associated with χ^2, df = 1

equilibrium at both sampling periods.

Genotype frequencies in enclosure B and area D were compared for trap periods June 17 - 19 (D) and June 24 - 26 (B), and October 5 - 7 (D) and October 8 - 10 (B) with 2 x 3 contingency χ^2 . The genotype frequencies did not differ in June ($\chi^2 = 0.294$, $P = .85$) but in October the proportion of heterozygotes was higher in D, yielding a significant difference ($\chi^2 = 6.059$, $P = .05$).

Survival

Survival in live-trapping programs is defined as recapture of an individual at a specified time period following first capture, or as the proportion of individuals captured during one trap period which are recaptured or known to be alive during the next trap period. Since in this study the trap periods were not at equal time intervals, any index of survival must be corrected to account for this. Unequal or widely spaced trap periods also add biases due to animals being missed entirely due to no or low trap exposure. These problems cannot be corrected without modifying the trap program.

Survival rates were calculated for the B population for the periods July - November 1973 and April - November, 1974 when adequate trap records were available. Survival was calculated as the proportion of individuals captured in trap period t_i which survived for seven days. The proportion of individuals which were known to be alive in trap period t_i and the next trap period t_{i+1} was designated S_i. The calculated survival rate per day depends on the time interval between periods, Δt_i given by $t_{i+1} - t_i$, and is given by $S_i^{1/\Delta t_i}$. Survival per 7 days, S_{i7}, is given by $S_i^{7/\Delta t_i}$. Survivorship was calculated for the total enclosed B population for both 1973 and 1974 (Table 11) and by genotype for females (Table 12), males (Table 13) and the whole population (Table 14) for 1974. Because the loss of some individuals from the B

Table 11 Proportion of Animals Surviving Seven-Days S_{i7} Enclosure B

1973		1974		
Trap Period (t_i)	S_{i7}	Trap Period (t_i)	S_{i7}	S_{i7}^*
July 10 - 11	1.0	Apr. 5 - 6	0.992	0.992
July 16 - 18	1.0	Apr. 29 - May 1	0.940	0.951
July 23 - 25	1.0	May 14 - 16	0.980	0.980
July 30 - Aug. 1	1.0	May 27 - 29	0.975	0.975
Aug. 6 - 8	0.929	June 10 - 12	0.908	0.920
Aug. 13 - 15	1.0	June 24 -26	0.904	0.913
Aug. 19 - 21	1.0	July 11 - 13	0.891	0.921
Aug. 26 - 28	0.823	Aug. 5 - 7	0.938	0.957
Sept. 3 = 5	1.0	Aug. 26 - 28	0.886	0.907
Sept. 10 - 12	1.0	Sept. 17 - 19	0.914	0.932
Sept. 17 - 19	0.929	Oct. 8 - 10	0.907	0.926
Sept. 24 - 26	1.0			
Oct. 1 - 3	0.936			
Oct. 10 - 12	0.943			
Oct. 16 - 18	0.887			
Oct. 22 - 24	0.965			
Oct. 29 - Nov. 1	0.957			

* excludes all animals which died in traps, were released, removed or escaped

Table 12 Seven-Day Survival of Females in Enclosure B, 1974

Trap Period	Genotype				
	unknown	SS	SF	FF	Total
Apr 5 - 6	0.888	1.0	1.0	1.0	0.988
Apr. 29 - May 1	1.0	0.918	0.952	0.855	0.922
May 14 - 16	1.0	1.0	1.0	0.935	0.982
May 27 - 29	1.0	1.0	0.961	0.953	0.971
June 10 - 12	0.700	1.0	0.939	1.0	0.952
June 24 - 26	0.888	0.877	0.955	0.970	0.937
July 11 - 13	0.774	0.976	0.842	0.961	0.904
Aug. 5 - 7	1.0	1.0	9.447	0.950	0.967*
Aug. 26 - 28	1.0	0.895	0.837	0.913	0.882
Sept. 17 - 19	0.874	0.979	0.874	0.899	0.923
Oct. 8 - 10	0.774	0.896	0.910	0.943	0.910

* significant difference between sexes in overall survival, $P < .05$

Table 13 Seven-Day Survival of Males in Enclosure B, 1974

Trap Period	Genotype				
	unknown	SS	SF	FF	Total
Apr. 5 - 6	-	1.0	1.0	1.0	1.0
Apr. 29 - May 1	-	0.874	1.0	1.0	0.968
May 14 - 16	1.0	1.0	0.947	1.0	0.978
May 27 - 29	0.816	1.0	1.0	1.0	0.980
June 10 - 12	0.0	0.775	0.920	0.894	0.842
June 24 - 26	0.0	1.0	0.824	0.912	0.856
July 11 - 13	0.0	0.976	0.848	0.881	0.876
Aug. 5 - 7	-	0.909	0.863	0.874	0.882*
Aug. 26 - 28	-	0.923	0.869	0.944	0.902
Sept. 17 - 19	1.0	0.956	0.885	0.855	0.902
Oct. 8 - 10	0.0	0.943	0.818	0.910	0.910

* significant difference between sexes in overall survival, $P < .05$

Table 14 Seven Day Survival of Genotypes in Enclosure B, 1974

Trap Period	Genotype			
	Unknown	SS	SF	FF
Apr. 5 -6	0.888	1.0	1.0	1.0
Apr. 29 - May 1	1.0	0.901	0.972	0.911
May 14 - 16	1.0	1.0	0.977	0.964
May 27 - 29	0.894	1.0	0.978	0.973
June 10 - 12	0.535	0.926	0.931	0.956
June 24 - 26	0.705	0.923	0.899	0.947
July 11 - 13	0.656	0.987	0.845	0.929*
Aug. 5 - 7	1.0	0.963	0.914	0.922
Aug. 26 - 28	1.0	0.905	0.850	0.923
Sept. 17 - 19	0.909	0.971	0.879	0.881
Oct. 8 - 10	0.687	0.914	0.914	0.930

* significant difference among genotypes, $P < .02$

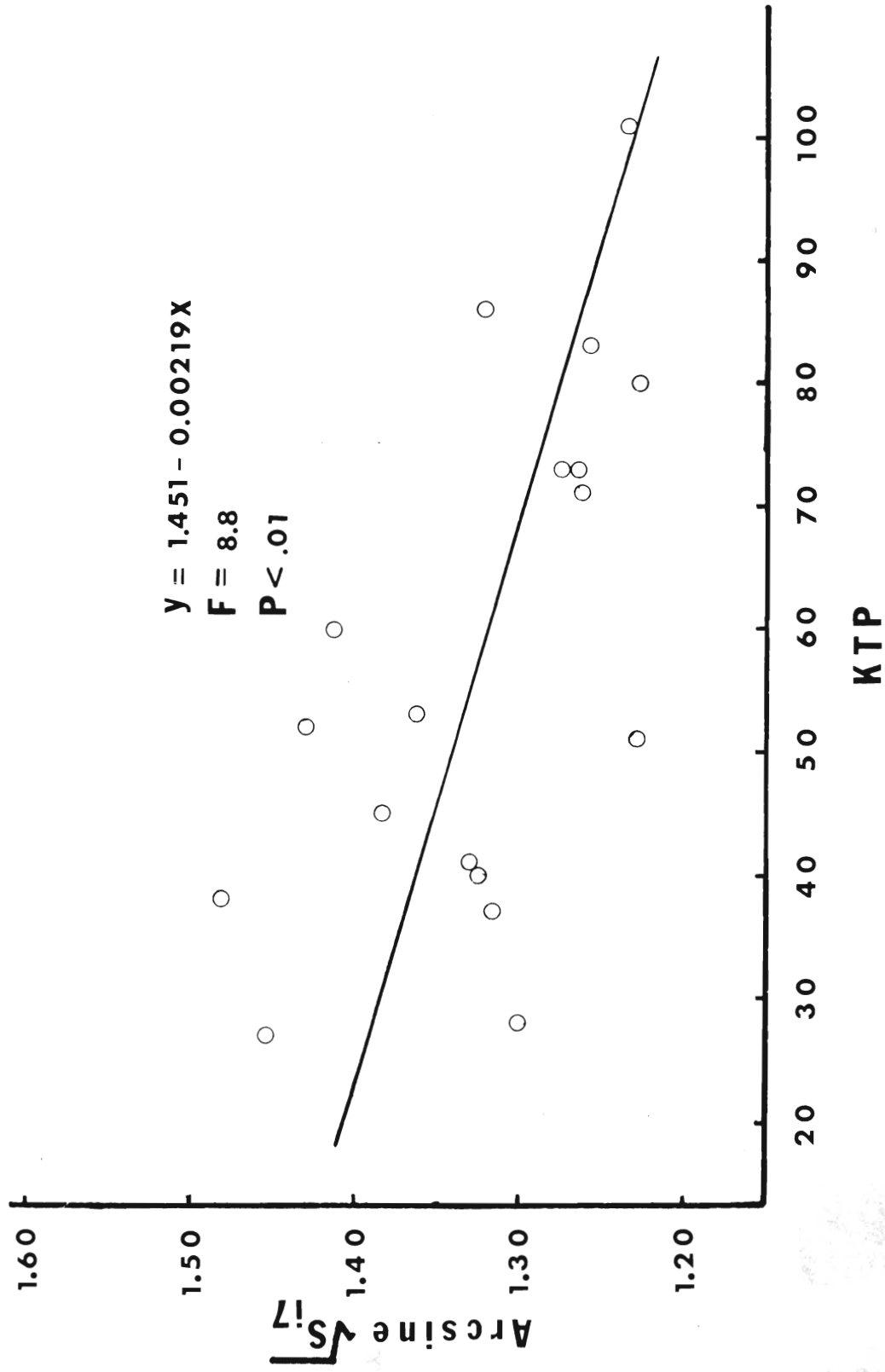
population was due to experimental error (trap mortality, escapes) or experimental manipulation (releases) for the 1974 trap periods survival was recalculated excluding all animals whose cause of exit from the population is known. The values are presented in the last column of Table 11.

In 1973, survival of the B population (Table 11) was generally high. From July to the beginning of October, 1973, survival was almost uniformly 100%. As the population reached its overwinter size, the survival dropped to 95% or less per seven days. In 1974, seven-day survival declined from approximately 98% in the late spring to 90% during the summer and early fall. For the non-winter periods September 17 - November 1, 1973 and April 5 - November 9, 1974, seven-day survival was found to be significantly related to population density (Arcsin ($\sqrt{S_{i7}}$) = 1.451 - 0.00219 (KTP), F = 8.8, P < .01) (Figure 6).

The seven-day survival of females (Table 12) and males (Table 13) were found to be different. Survival in females was generally above 90%. On only one occasion (August 26 - 28, 1974) did it fall to 88%. Male survival dropped below 90% in late May and stayed between 84 and 88% until August when it rose to 90%. Differences in survival between the two sexes were tested using 2 x 2 contingency χ^2 (died, survived) x (male, female). Low probability χ^2 values due to higher female survival were observed for the June 10 - 12 (χ^2 = 3.576, P < 0.1) and June 24 - 26 (χ^2 = 2.463, P <0.2) trap periods. A more significant difference was observed for the August 5 - 7 trap period (χ^2 = 5.258, P < .05).

The relationship between transferrin genotype and survival in the B population in 1974 revealed that the seven-day survival of heterozygotes was significantly less than for homozygotes only in the July 11 - 13,1974

FIGURE 6 Regression of seven-day survival (S_{i7}) using the angular transformation on the known trappable population (KTP) in enclosure B for the non-winter months of 1973 and 1974.

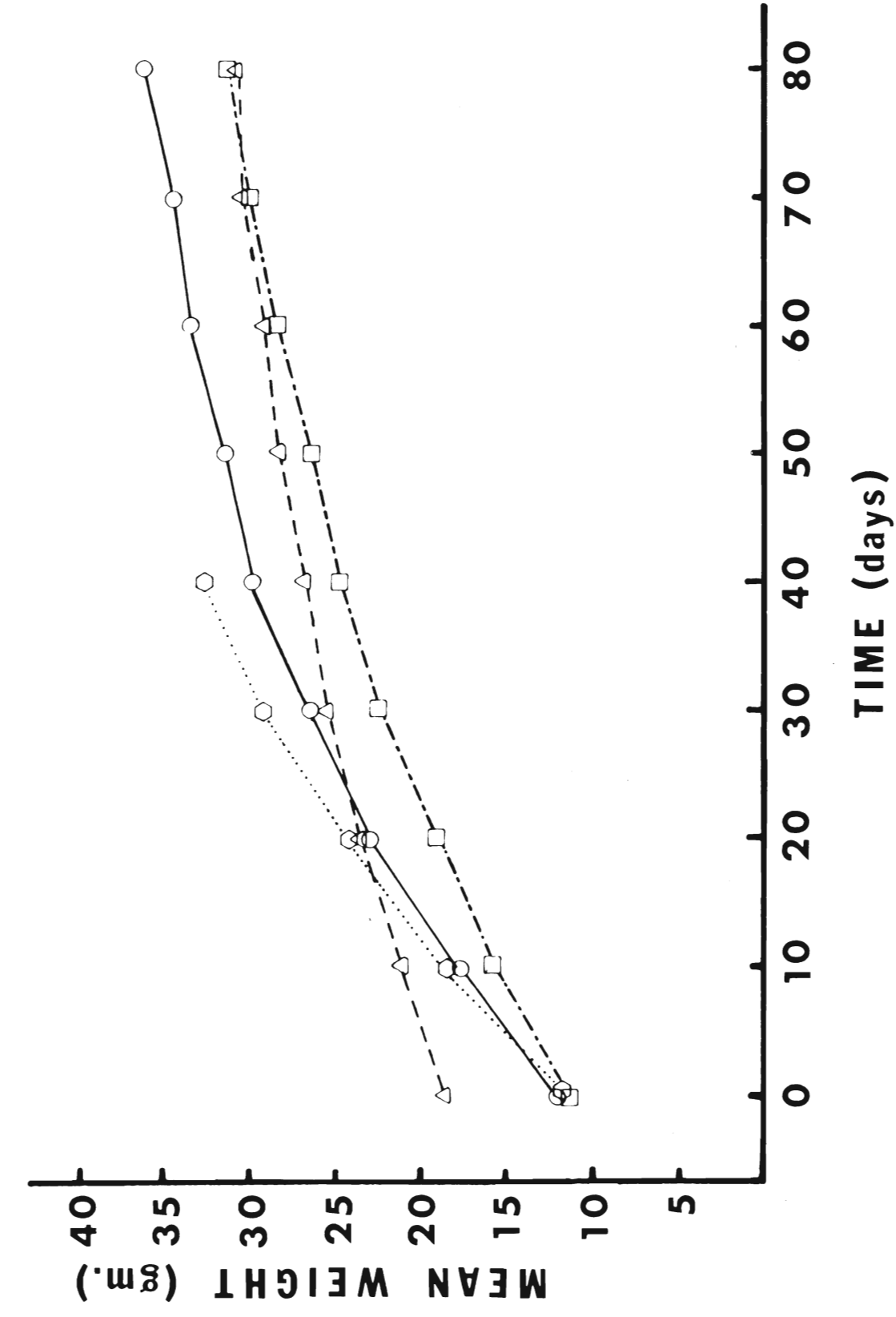

y = 1.451 - 0.00219X
F = 8.8
P < .01
Arcsine √S_i7
1.60
1.50
1.40
1.30
1.20
20
30
40
50
60
70
80
90
100
KTP

trap period ($\chi^2 = 8.782$, $P < .02$) (Table 14). In all other trap periods survival did not differ among genotypes. The survival of animals with unknown genotype was not considered in these analyses. Sex-genotype interactions were not adequately studied because of small expectations produced when the sample was partitioned into sex and genotype categories. However, the order of genotypes with decreasing survival was the same in males and females in four of the eleven trap periods in 1974 (Tables 12, 13). Three of these trap periods were consecutive: July 11 - 13, August 5 - 7 and August 26 - 28. In these periods survival was lowest for heterozygotes.

Growth Rates

Changes in body weights of animals in the enclosed B population were investigated for four periods: I July 10 - October 1, 1973; II October 1 - December 5, 1973; III May 5 - July 11, 1974; IV July 11 - November 5, 1974. These periods correspond to the early increase (I) and late increase (II) phases of 1973 and the increase (III) and decrease (IV) periods of 1974 (Figure 2). The weights of selected young males with captures in at least three trap periods prior to the attainment of adult weight were plotted versus time since first capture. Females were not used because of large fluctuations in weight due to pregnancy. The weight of each animal was interpolated at 10 day intervals and then, for a given time since first capture the interpolated weights were averaged and the averages plotted versus time. This produced a smooth curve which asymptotically rose to the adult weight (Figure 7). Groups I, II and III showed average weights of first capture of 11-12 gm while group IV animals were approximately 19 gm at first capture. Although group IV had a higher weight at first capture, the asymptotic weight (30 gm) was close to that of the other groups.

FIGURE 7 The mean weight of groups of selected young males versus time since first capture. The weight of each individual was plotted independently versus time and then interpolated at 10 day intervals. These weights were then averaged and plotted here. The time period and sample size in parentheses for each line is —○— July 10 - Oct. 1, 1973.(7); —·—□—·— Oct. 1 - Dec. 5, 1973 (9); ·····○····· May 5 - July 11, 1974 (9); — —△— — July 11 - Nov. 5, 1974 (10).


MEAN WEIGHT (gm.)
40
35
30
25
20
15
10
5
0
10
20
30
40
50
60
70
80
TIME (days)

The percent increase in body weight per day was calculated at ten day intervals and plotted versus body weight (Figure 8). Throughout the growth period, the relationship between percent change in body weight per day and body weight was essentially linear. The analyses of regression lines fitted to the data (Figure 8) are summarized in Table 15.

The 95% confidence intervals (Table 15) were calculated for the y-intercept, the maximum growth rate. The confidence intervals for groups I, II and III overlapped one another's intercepts, indicating equal growth rates. The confidence interval for group IV did not overlap with either the intercept or confidence interval of any other group. The slopes of pairs of regression lines (the rate of change in body weight with increasing body size) were compared in all combinations by t-tests. Both groups I and III had significantly greater slopes than group IV. All other comparisons showed no significant differences. Therefore groups I, II and III exhibited the same maximum growth rate and same change in growth rates with increasing size. Group IV had a lower maximum growth rate than the other groups and a slower decline in growth than groups I and III.

Releases: Colonizers and Dispersers

From July 14 to October 24, 1974 a total of 29 animals was released from enclosure B into enclosures A or C. This included 3 individuals which escaped through open tubes (see predator disturbance, Methods), were captured in A or C, and since they conformed to the release criteria were released at their point of capture. Table 16 summarizes the releases made. More males than females were released in four of the five release periods. A total of 9 males and 5 females were released into enclosure A, 8 males and 7 females into enclosure C. Three of the animals released into each of the enclosures were not

FIGURE 8 Percent change in weight per day versus body weight for the groups shown in Figure 8. In each group the growth rate for each point was calculated for the period from one point to the next in Figure 7. The lines are calculated regressions (Table 15). Symbols as in Figure 7.

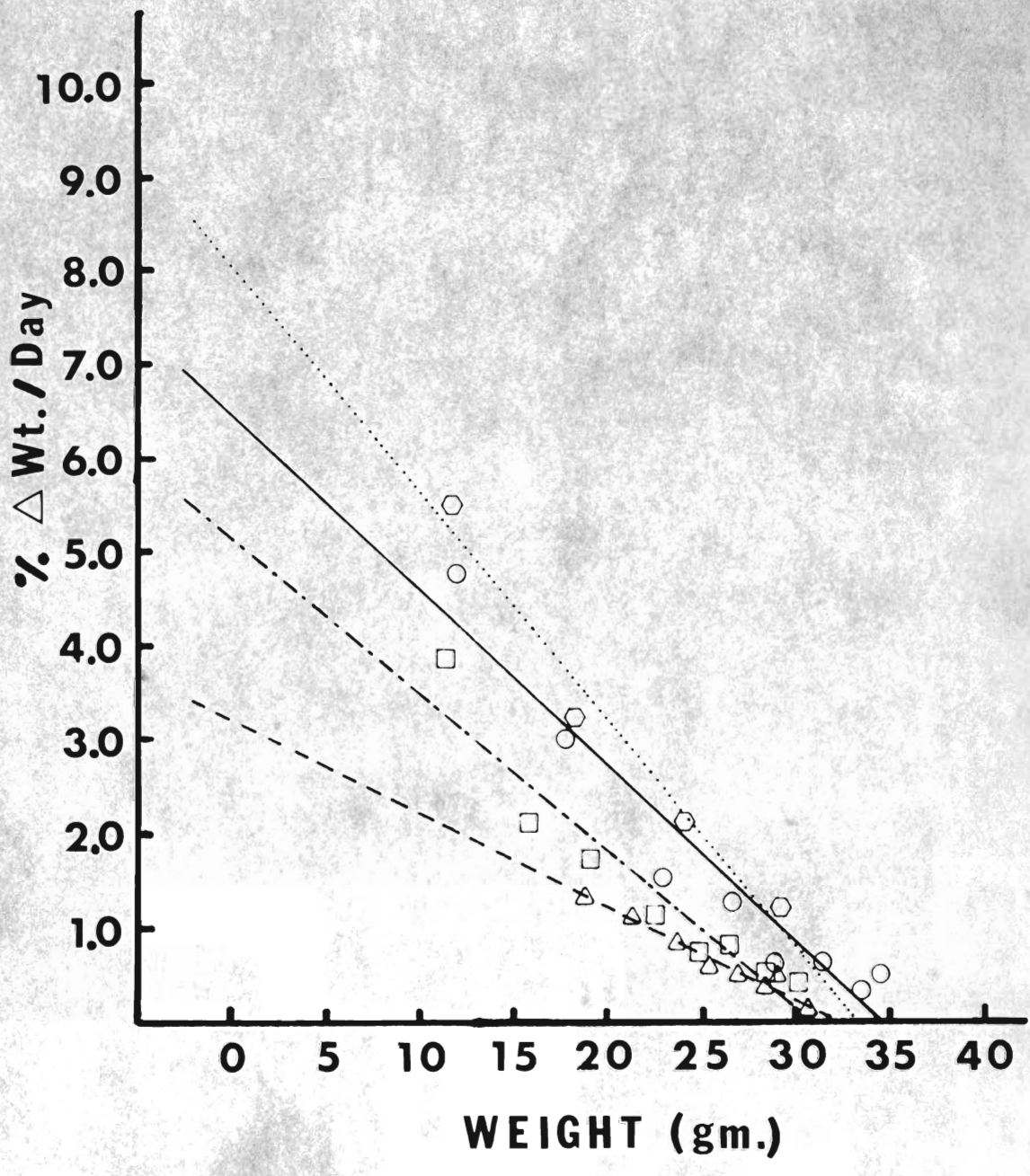

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
% △ Wt. / Day
0
5
10
15
20
25
30
35
40
WEIGHT (gm.)

Table 15 Regression Analysis of Growth Rates vs Body Weight

Period[a]	Regression Equation	F	P	95% CI about Y-intercept
I	y = 6.46 - 0.19 x	84.0	<.001	6.69 - 6.22
II	y = 5.17 - 0.17 x	55.0	<.001	6.47 - 3.88
III	y = 8.04 - 0.24 x	52.5	<.025	11.18 - 4.91
IV	y = 3.22 - 0.10	6.0	<.05	3.24 - 3.20

[a] see text for definitions

Table 16 Summary of Animals Released

Release Period	Sex		Released Into		Transferrin Genotype			
	M	F	A	C	unknown	SS	SF	FF
July 13 - 26	6	4	5	5	1	0	7	2†
Aug. 8 - 20	4	2*	3	3	0	2	3	1
Sept. 5 - 13	2	3	2	3	0	3	2	0
Sept. 26 - Oct. 6	2	1	1	2	0	0	3	0†
Oct. 16 - 31	3	2	3	2	1	2	0	2
Total	17	12	14	15	2	7	15	5

* $P < .02$, higher prop. o→o→ than encl. B (see text)

† $P < .05$, heterozygote excess

recaptured, giving trap histories for 11 animals in A and 12 in C.

Comparisons of the sex ratio and genotype frequencies were made between the released animals and the total enclosed B population for each release period and the trap period in B which immediately preceded the release period. These comparisons were performed using the Fisher Exact Probability Test (Siegel, 1956) which calculates the 1-tailed probability of the observed or more extreme frequencies for a 2 x 2 contingency table when the marginal totals are regarded as fixed, using the hypergeometric distribution. Animals released during the August 8 - 20 period had a significantly higher proportion of males than the B population ($P = .02$). During all other release periods the proportion of males in the release group, although high, was not significantly higher than the proportion of males in the B population. The sex ratio of the total released sample was not significantly different from the overall sex ratio of the B population for the period July 11 - October 10 ($\chi^2 = 2.568$, $P = .15$). Genotype frequencies were compared by combining homozygous animals (SS +FF) since the expectations were otherwise too small for a χ^2 analysis. On two occasions (July 13 - 26 and September 26 - October 6) a significant excess of heterozygotes was observed in the release sample. During the other three release periods, the proportion of heterozygotes (or homozygotes) did not differ significantly from the proportion observed in the B population. When the total numbers of homozygotes and heterozygotes released were compared with the totals in the B population for the same time period, it was found that the high proportion of heterozygotes in the released population approached significance ($\chi^2 = 3.41$, $.10 > P > .05$). In summary, the sex ratio of released animals differed on only one occasion from the sex ratio of B population; an excess of males was observed on this occasion. A significant excess

of heterozygous animals was observed in the released population on two occasions. Overall, the high proportion of heterozygous animals in the released group approached significance.

The fate of animals subsequent to release into enclosure A or C was also followed. The movements of the animals in these enclosures were used to classify the released animals as either dispersers or colonizers. If a released animal entered a dispersal tube trap in either A or C within 30 days following its introduction, it was classified as a disperser. All other animals were classified as colonizers. As mentioned in the methods, all animals were removed from enclosure A approximately one month after introduction so as to provide an "empty" habitat for subsequent releases.

The numbers of dispersers and colonizers are presented in Table 17. A larger proportion of dispersers was found in the C enclosure (7/15 compared to 3/14). This difference was found by the Fisher Test (Siegel, 1956) to be non-significant (1-tailed P = .15). The proportion of male dispersers was significantly lower than the proportion of female dispersers in enclosure A (1-tailed P = .027) but not in C (1-tailed P = .22). Comparisons of genotype frequencies could not be made adequately due to small sample sizes. All genotypes were represented in dispersers in both enclosures.

Vegetation

The vegetation analyses are summarized in Table 1. In both years the numerically dominant species were Poa compressa L., Phleum pratense L., Solidago canadensis L., Aster novae-angliae L., and Vicia cracca L. For each species, densities were compared within enclosures between years and within years between enclosures by the Kruskal-Wallis One-Way Analysis of Variance (Table 18). In 1973, enclosure A had higher densities of both Phleum and Aster than enclosure C , enclosure B had

Table 17 Fate of Released Animals: Colonizers and Dispersers

Enclosure	Males			Females			Total		
	R	D	C	R	D	C	R	D	C
A	9	0	9	5	3	2*	14	3	11
C	8	5	3	7	2	5	15	7	8

R = released
D = disperser
C = colonizer

* prop ♂ D < prop ♀ D, P = .05 (see text)

Table 18 Differences in Stem Densities of Dominant Species[a]

Species	Overall	Comparison								
		A-B	A-C 1973	B-C	A-B	A-C 1974	B-C	A	B 1973/1974	C
Poa compressa	**	-	-	-	-	-	-	-	-	-
Phleum pratense	**	-	*	**	-	**	**	-	-	-
Solidago canadensis	**	*	-	**	**	**	-	**	-	-
Aster novae-angliae	**	-	**	-	-	-	-	**	-	-
Vicia cracca	**	-	-	-	-	-	-	-	**	-

[a] Heterogeneity among groups tested using Kruskal-Wallis One-Way Analysis of Variance (Siegel, 1956; Javor and Rothstein, 1975)

* $P < 0.10$

** $P < 0.05$

a denser growth of Phleum and sparcer Solidago than C whereas A and B differed only in that Solidago was denser in A. In 1974, very local eruptions of the phytophagous striped cucumber bettle, Diabrotica vittata Fab. in A caused a significant decrease in stem density of Solidago and Aster from the 1973 densities. Further the release of A from significant grazing pressure and the continuance of grazing in B and C changed the appearance (Plate 3) and vegetation structure of the enclosures. Poa was least dense in B (not significantly so) while Solidago provided a dense cover. In A, the Solidago and Aster populations declined while the major grass species remained the same. Enclosure A can be characterized in 1973 as having a dense growth of both grass and composite species while in 1974 it was largely grassy. Enclosure B was similar to A in 1973 but became less grassy in 1974, while enclosure C went from quite grassy with a good growth of composites in 1973 to a more sparcely vegetated area in 1974.

DISCUSSION

Although studies of rodent population fluctuations were initiated fifty years ago, it was not until recent years that accurate and detailed demographic data were obtained. This long history of descriptive studies has revealed little of the demographic changes associated with the cycle of abundance. Most studies have been plagued by long intervals between trapping periods, inappropriate application of various census techniques due to invalid assumptions and little attention to demographic details. Removal and mark and recapture census methods require certain assumptions of randomness and uniformity which have never been satisfied in natural populations (Krebs and Myers, 1974). To circumvent these difficulties a program of saturation live-trapping at frequent intervals to enumerate the population was adopted by Krebs et al. (1969 et seq.). Hopefully the errors involved in this method are small.

The methods used in this study closely followed those of Krebs et al (1969 et seq.) because I wished to compare my results with the findings of the Indiana studies and because I felt that the general methods used by Krebs provided the most accurate demographic data. In these studies close attention was paid to many demographic factors including population density, reproductive performance and dispersal. An experimental rather than an observational approach was adopted because previous studies had demonstrated that many aspects of the population cycle could not be measured without manipulating the population. For example, the importance of dispersal in the regulation of rodent population size was not fully realized until populations were enclosed and dispersal inhibited.

My methods differed from those of Krebs and his students in the use of release tubes for identifying dispersers. This approach had been suggested by Myers and Krebs (1971) after they realized the inadequacy of their measure of dispersal as immigration to a vacant area. The remainder of the methods closely followed Krebs' previous work. Saturation live-trapping at short time intervals and blood sampling of almost the entire adult and sub-adult population were used to provide detailed demographic and genetic data.

Demographic and Genetic Characteristics

Population Density

For many years field workers have relied on mark-recapture indices for estimating the densities of rodent populations. However the work of Tanaka (1963) has shown that considerable heterogeneity in trap response exists among individuals of a species and that among species a large variation in the trappability occurs. Hence the densities obtained from trapping programs with short, widely spaced trap periods are unreliable. In this study an enclosed population was exposed to 147 trap-nights per trap period or approximately 5000 trap-nights over a one and a half year period. Since Krebs *et al.* (1969) found that the trappability of *M. pennsylvanicus* declined in the summer, the season when I observed all population increases and since the probability of an individual escaping capture was slight, I feel that the changes in the known trappable population (see below) are accurate measures of the changes in population density.

The changes observed in the size of the population in enclosure B (Fig. 2) are similar to those observed by Krebs *et al.* (1969) for their fenced population on grid B. Numbers increased exponentially through the first summer, levelled off in winter and increased again the second summer. In the summer of 1974 numbers in enclosure B far

exceeded the density of animals found in the adjacent unenclosed but similar habitat of area D. Even the rapid drop in numbers beginning in July 1974 did not bring the population size to a level comparable to the unenclosed D population. Because the D population was trapped on only three widely spaced occasions the numbers obtained cannot be taken as extremely accurate estimates of the population density. However, from the daily capture records and field observations I believe that the total resident population of area D was much less than twice the number observed. Even if the extreme estimate that only one-half of the unenclosed population was captured is accepted, the enclosed B population still rose to a much greater density in 1974.

The abnormally high population density of enclosure B is undoubtably due to the fence enclosing the population. Krebs (1969) suggested that this "fence-effect" may operate in two ways. First, the fence reduces predation pressure on the enclosed population by inhibiting predator movement into the enclosures. Second, the fence prohibits dispersal of the voles,presumably a significant factor in the regulation of unenclosed populations. During my study Red-tailed Hawks (Buteo jamaicensis G melin), American Kestrels (Falco sparverius L.) raccoons (Procyon lotor) skunks (Mephitis mephitis), feral cats (Felis domesticus L.) and dogs (Canis familiaris L) had frequent and easy access to the enclosures and predation was occasionally evident throughout indicating that no appreciable difference in predation pressure occurred between areas B and D. The data support the contention that the prevention of dispersal results in an abnormal demography. The importance of in situ mortality in reducing population increase appears to be slight as very high survival was consistently observed in enclosed populations by Krebs et al. (1969) and myself. In unenclosed populations, however, survival was erratically poor and generally less than in enclosed

populations (Krebs et al., 1969). Myers and Krebs (1971) have shown that over half of the losses from an increasing unenclosed population can be attributed to dispersal. It seems reasonable therefore to conclude that the extremely high population density in enclosure B was due to the prevention of potential dispersers from leaving the enclosed area.

Breeding Intensity

Unlike other workers studying rodents farther north or south, I found no evidence of winter breeding during the increase phase (Table 3). In 1973 breeding in the B and C populations remained fairly constant until it ceased in November and October respectively. In 1974, breeding in the C population ended in October whereas the unenclosed D population was still reproductively active. However, in the much denser B population the intensity of breeding began a marked decline in July, eventually reaching zero in November. Whereas breeding in the less dense populations abruptly ended with the coming of cold weather, in the crowded population the percent of breeding females began to decline long before the weather changed.

Krebs and Myers (1974) found no evidence in the literature that the intensity of breeding as measured by the pregnancy rate changed with the population cycle. They did note many studies which found that breeding terminated earlier in the fall in the peak and decline phases for many different species. The drop in breeding observed here may be due to the abnormally high density achieved and may not reflect normal changes in breeding associated with the population cycle.

Growth Rates

The growth of individual animals has significance in the processes of population regulation since the dispersal, reproduction and death

of individuals are probably linked to their body size or maturation. Most workers have used changes in body weight as a measure of growth although changes in body length may be a better index because length is a measure of skeletal development (Krebs and Myers, 1974). The analysis of individual growth rates is still a difficult task because of the multiple confusing influences of a variety of factors on body growth. Changes in the growth rates of individuals through time may be influenced both by seasonal changes and by changes in the phase of the population cycle. In a three year live-trap study of M. pennsylvanicus population in Minnesota, Brown (1973) noted that young born in the spring and early summer reached adult size within 3 months and then experienced a weight loss in the fall whereas animals born in mid to late summer stopped growth in the fall. All animals maintained their full weight throughout the winter and resumed body growth in the spring. Krebs et al. (1973) calculated regression lines for growth rate per day versus body weight for increasing, peak and declining phases of the population cycle. In both sexes of M. pennsylvanicus growth rates were higher in increasing than in peak populations and higher in peak than declining populations. Krebs and Myers (1974) found no evidence that these differences in growth rates can be explained by seasonal growth patterns associated with spring-born or fall-born young. Neither Brown (1973) nor Krebs et al. (1973) attempted to determine the possible interaction between seasonal and population phase effects on body weight.

In my study, the growth rates of young males from the enclosed B population indicate that growth during the period July 11 - November 5, 1974 was lower than during all other periods (Figure 8, Table 15). If this period represents the peak or early decline phase then the lower growth rates are in agreement with Krebs et al. (1973). The growth rate

regression line which most closely paralleled for that period July - November 1974 was the one for October - December 1973 (Figure 8). Lower calculated growth rates in the fall in both rates are supported by field observations and concur with Brown (1973). From these data, it seems likely therefore that the effects of season and phase in the population cycle interact to yield the observed body weight dynamics.

Survival Rates

Measures of "mortality" in live-trapping programs are actually measures of general losses because emigration and mortality *in situ* cannot usually be distinguished. With the use of mouse-proof fences however, one can attribute almost all losses to mortality or to changes in the trappability of individuals. This minimum estimate of survival will be quite accurate if changes in the trappability of individuals are slight.

Continued high survival rates in both sexes were observed by Krebs *et al.* (1969) in fenced populations of *M. pennsylvanicus* and *M. ochrogaster*. Adjacent unfenced populations had lower survival with sporadic periods of very low survival in males. Higher survival in enclosed populations than in open populations might be expected if the dispersal which the fences prohibited was of considerable importance to the regulation of numbers. If true mortality were not significantly affected by the fences then the differences in survival noted indicate the rate of loss of individuals by dispersal from unenclosed populations.

Overall the enclosed B population exhibited survival rates comparable to those recorded by Krebs *et al.* (1969)for fenced populations. The seven-day survival rate of the enclosed B population was usually above 0.9 (14-day survival rate above 0.81) (Table 11). During 1973 and until late May 1974 seven-day survival was very high, 0.95 or

greater (14-day survival rate above 0.90) but it declined to approximately 0.90 in early June 1974 as the high population density was achieved, and remained constant until the end of the study.

When the released animals are removed from the analysis, the data indicate that the change in population growth observed in July-August 1974 (Figure 2) was associated with a shift in survival (Table 11). Adding the cumulative number of animals released (Table 16) to the known population in enclosure B for each trap period in late 1974 does not eliminate the decline in numbers but just reduces the rate. Seven-day survival for the period July-November 1974 calculated with released and accidentally killed animals removed is still slightly lower than for April and May. A general physiological change may have occurred in the population in July as the breeding rate also began a noticeable decline. The mechanism at the heart of these changes is of prime importance to population regulation but we can only speculate on its nature at present.

A significant negative regression was found between population density and survival in the B population for the non-winter months of the study (Figure 6). Because survival is calculated from the trap revealed loss rates, a complicating behavioural factor which contributed to trap competition among the enclosed animals may have occurred. When the population was at high densities daily trap success rates were very high indicating that trap competition was occurring. Kikkawa (1964) has shown that considerable social interaction took place at the trap mouth in Clethrionomys glareolus and Apodemus sylvaticus populations with the socially dominant individuals captured most readily. Watts (1970) was able to decrease the age of first capture of young C. gapperi by selectively removing large resident adult males from his study areas. These studies suggest therefore

that competition for the traps via social interactions could lead to a reduced probability of recapture of subordinate animals, effectively dropping the survival rate. While I recognize the possibility that the negative regression between population density and survival in the enclosed B population may be partly an artifact of agonistic behavioural interactions leading to trap competition, I do not believe it to be solely responsible for the demonstrated survival changes.

The observed lower survival rates at high population densities may also reflect increased mortality *in situ*. The frequency of interactions or contacts among individuals probably increased with population density and since losses from the population are only partially accounted for by dispersal, increased mortality as a result of social interaction is possible. Physio-behavioural syndromes such as the adrenal-pituitary stress hypothesis of Christian (1950) have been proposed but the research has involved laboratory studies of populations maintained at abnormally high densities leading Chitty (1960, 1967) to reject such hypotheses because of their lack of universality.

Genetic changes

The genetic-behavioural hypothesis (Chitty, 1967; Krebs *et al.* 1973) predicts that selection will produce allele frequency changes over the population cycle for loci associated with aggression and reproduction. Because the exact nature of these genes is unknown, in this study as in others these changes were indirectly monitored by electrophoretically detectable biochemical markers. Frequency changes in the marker loci are thought to reflect selection at linked gene complexes which act to regulate population numbers. Semeonoff and Robertson (1968) found a change in the frequency of an esterase allele during a population decline in *Microtus agrestis* in Scotland.

Tamarin and Krebs (1969) found changes in the frequency of transferrin alleles over a population cycle in enclosed and unenclosed populations of M. pennsylvanicus and M. ochrogaster in Indiana. In some of their populations the frequency of the Tf^E allele was inversely correlated to population density while a more complex relationship was found in the remaining populations. Survival rate and Tf^E frequency were usually correlated during the decline phase. Gaines and Krebs (1971) extended this work finding more correlations between changes in density and changes in allele frequencies. They were, however, unable to distinguish whether the demography or changes in gene frequency was the causative agent of the numerical changes observed.

Only slight changes in allele frequency were observed in the enclosed B population (Figure 4) while greater changes were noted in the cropped enclosed C population (Figure 5). Throughout 1974 the frequency of the S allele was very similar in both sexes in the B population and no relationship was found between the S allele frequency and any of the demographic parameters. The large changes in S allele frequency in the C population can be attributed to unequal cropping or recruitment of genotypes. Because the population in enclosure C was small, the removal or addition of a few individuals of one genotype significantly altered the allele and genotype frequencies. Despite the marked changes in allele frequency in each sex, the allele frequencies for the whole population remained relatively constant. In summary, the S allele frequencies in both enclosures B and C varied little and were not clearly related to any of the demographic parameters.

Genotype fitness differences have been found for marker loci in various species of rodents. Canham (1969) showed a correlation between density and heterozygote fitness at albumin and transferrin

loci in Clethrionomys rutilus and C. gapperi populations in northern Canada. In the Indiana populations of Microtus ochrogaster and M. pennsylvanicus Tamarin and Krebs (1969) and Gaines and Krebs (1971) found changes in genotype survival over population cycles. In M. ochrogaster one transferrin genotype (Tf^E/Tf^E) had a selective advantage during the increase phase while the heterozygote (Tf^E/Tf^F) had an advantage during the decline phase. In M. pennsylvanicus the transferrin genotypes Tf^E/Tf^E homozygotes and Tf^C/Tf^E heterozygotes had generally greater survival than Tf^C/Tf^C homozygotes throughout the cycle of abundance.

In my study, changes were noted in the frequencies of the different transferrin genotypes in the enclosed B population in 1974. The distribution of genotypes was very close to Hardy-Weinberg equilibrium expectations until August when a deficiency of heterozygotes (Tf-SF) developed (Table 6) corresponding with the beginning of the decline in population density (Figure 2). When the genotype frequencies were compared between the enclosed B population and the unenclosed D population, the heterozygote deficiency was found only in the enclosed population. The deficiency of heterozygotes occurred in females (Table 8) while males remained at Hardy-Weinberg equilibrium (Table 7). Although the proportion of heterozygotes in the sample released from the B population was nearly significantly greater than the proportion in the B population, the majority of released animals were male. Hence, because the loss of female heterozygotes due to releases was slight, the deficiency of female heterozygotes during the decline must be due to other causes.

The calculated seven-day survival rates show the same trends as genotype frequencies in the enclosed population (Tables 12, 13, 14).

Survival was uniformly high until late June 1974 when heterozygote survival dropped below 0.90. For the remainder of the study heterozygote survival although not significantly different, was the lowest of the three genotypes for males, females and the entire population.

These observations of heterozygote disadvantage at the transferrin locus do not concur with the observations of Tamarin and Krebs (1969) and Gaines and Krebs (1971). A different pattern of genotype and allele dynamics in the southern Ontario population compared with the Indiana population may be due to one of three causes. First, if the transferrin locus is able to mark the presumed behavioural gene complex because of linkage disequilibrium in the Indiana population, then the difference noted in the Ontario population may be due to a different disequilibrium relationship. Because of the strong founder effect in my experiment there may be a different combination of Tf "allele" and behavioural "alleles" in the introduced genotypes than in the Indiana populations. Second, the transferrin locus in my population may be on a different linkage group as a result of a translocation. Regardless of whether the Chitty hypothesis were correct, the transferrin locus would show different behaviour in the two populations. The third possibility is that the Chitty hypothesis is incorrect and that the transferrin allele frequencies fluctuate according to random processes. It has been assumed throughout that the transferrin alleles have equal fitness and are functionally identical.

It is possible by experimentation to distinguish the cause of the difference in genotype frequency dynamics that I observed. To demonstrate the existence of selection at the transferrin locus or at a closely linked locus one could start a number of enclosed populations at the same transferrin allele frequency. Selection would

produce great homogeneity in changes in density and allele frequency among the populations. This experiment has been performed with positive results by Gaines et al. (1971) using M. ochrogaster from Indiana but should be repeated elsewhere using other species to test the reproducibility of their results. If selection were indicated by this experiment then the linkage relationship of the transferrin locus to a presumed super-gene responsible in part for the regulation of numbers could be investigated. Chitty's hypothesis requires polymorphism at loci responsible for spacing behaviour, viability and fecundity. If populations were initiated with populations homozygous and monomorphic at the transferrin locus, then if the super-gene complex were closely linked an aberrant demography would result due to homozygosity at the super-gene locus.

The experiment just described is of secondary importance because the real problem has to do with the presumed super-genes and not transferrins. The basic requirement of the Chitty hypothesis, a high degree of polymorphism, has yet to be tested. This could be done by producing populations of microtines which were monomorphic for a large segment of their genome. An aberrant demography would indicate definite genetic effects. It may also be possible to assign to a linkage group some genes which influence the demographic attributes previously mentioned by testing populations monomorphic for only one chromosome. Admittedly the science of Karyology is still quite young with respect to mammals and very extensive breeding programs would be required to produce the strains but such a direct approach is needed. Such a genetic dissection of population processes is probably beyond the capabilities of modern science but the complete understanding of small mammal demography will require it.

Rodent-Vegetation Interaction

Freeland (1974) has recently proposed that changes in the floristic composition of old-field habitat as a result of selective microtine grazing act to produce a cycle of abundance in the rodents. He suggests that the selective consumption of preferred plant species by an increasing population of herbivores causes a greater proportion both of the plant community and of the voles' diet to be composed of non-preferred species many of which contain plant toxins. The toxins produced by these unpalatable species act in a variety of ways to reduce the viability of the individual rodents and the density of the population, releasing the preferred species from grazing pressure and restarting the cycle.

Microtus spp. show strong preferences for grass species and such forbs as clover (Trifolium spp.), dandelion (Taraxacum officinale) and plantain (Plantago lanceolata) (Thompson, 1965; Zimmerman, 1965; Batzli and Pitelka, 1970). The plant species which Freeland lists as containing toxins, Carex spp., Solidago spp., Ranunculus sp., etc. have very low preference ratings in the diets of microtines. By his model, overgrazing of the grasses releases these forbs, increasing their contribution to the voles diet.

In my study some noticeable changes in the vegetation occurred; in 1974 enclosure A was released from M. pennsylvanicus grazing but suffered heavy infestations of Diabrotica vittata while enclosures B and C had continued vole grazing and sporadic insect damage (Table 1, Table 18). No significant changes in the density of the grass species from 1973 to 1974 were noted. In enclosures B and C the density of Poa compressa declined slightly while it increased in A from 1973 to 1974. Although no change in the stem density of Phleum pratense was found, in 1974 most of the tall seed heads of this species were

still standing in A whereas many had been cut down by voles in the other enclosures. Although the changes in grass species densities are in the directions predicted by Freeland they are very small and a concomitant increase in non-preferred species did not occur. Insect damage significantly reduced the stem densities of Solidago sp. and Aster sp. in enclosure A in 1974. These species did not change in either of the other enclosures. We may therefore conclude that floristic changes were not responsible for the decline in population density observed in 1974. However, this does not preclude the possibility that the mechanism proposed by Freedland is operating in unenclosed populations.

Releases

Most demographic studies of unenclosed populations of small mammals have been unable to quantify movements of animals into and out of the study area. It is usually assumed then, that immigration is balanced by emigration and that changes in the population density can be attributed solely to natality and mortality. The measurement of dispersal and the definition of "disperser" have been difficult problems for investigators. However, since recent work (eg. Myers and Krebs, 1971) indicates that dispersal is a significant factor in population regulation, investigators can no longer ignore it because it is difficult to quantify.

Dispersal has most frequently been measured by trapping out individuals which move into a vacant area. Stickel (1946) found that twice as many male as female Peromyscus leucopus moved into a central snap-trap region from a surrounding live-trapped area. The order of capture of these emigrants corresponded to the proximity of their original home ranges to the centre of the study area. Van Vleck (1968) also found a greater percentage of males when he snap-trapped a

population of microtines than when he enumerated it by live-trapping. Further, he found a correlation between the density of the surrounding area and the amount of dispersal of marked animals into the central area. Myers and Krebs (1971) studied the relationship between the movement of individuals from unenclosed populations of Microtus to adjacent vacant areas and the phase of the population cycle. Dispersal (by this definition) was most prevalent during the increase phase and during fall and winter. The proportion of losses from the control populations attributable to movement onto the removal area declined from approximately 60% during the increase phase to less than 20% during the decline phase. Young females were found to disperse in high numbers during all phases of the cycle, especially the decline.

The use of removal trapping to measure dispersal is known to be inadequate for at least two reasons. As pointed out by Stickel (1946) and Calhoun and Webb (1953) a removal-trapped area tends to act as a "biological vacuum" into which local residents will move. As resident animals adjacent to the removal area are removed, residents farther away tend to move towards the vacant area, giving the impression of dispersal. In removal trap programs such as Myers and Krebs (1971) it is necessary for dispersing individuals to remain on the study area for up to 12 days before they can be captured. Such programs will miss individuals which pass through the removal area without stopping. Although the use of dispersal tubes and enclosed populations to study dispersal was suggested by Myers and Krebs (1971) no other study to my knowledge has yet implemented this technique. Garten and Smith (1974) used pitfall traps with snow drift fences to measure the movement from semi-enclosed populations but as their investigation did not cover a complete cycle of abundance, only seasonal differences were noted.

The original intention of this study was to quantify dispersal from an enclosed Microtus pennsylvanicus population during increase, peak and decline phases. I wished to see if the "disperser" segment of the population was a random sample with respect to sex ratio and transferrin genotype. This "leaking" experiment met with two major difficulties; the enclosed population did not increase as much as anticipated and then declined unexpectedly in late summer 1974 with no peak or plateau phase, and predator disturbances interferred with the release program.

As a result of these unexpected events, this study investigated dispersal during the early decline phase. The sample that entered the release tubes in enclosure B had a greater proportion of males and of transferrin heterozygotes than the enclosed population. Of these released animals one-third subsequently entered release tubes in test enclosures A and C and were hence classified as dispersers. The remainder were termed colonizers. When the behaviour of the released animals in enclosures A (vacant) and C (inhabited) was scrutinized, I found that the proportion of dispersers was slightly greater in enclosure C (0.41) than in enclosure A (0.21). In enclosure A, females had a greater tendency then males to disperse rather than colonize whereas no difference was found between the sexes in animals released into C. Because of small samples the data could not be partitioned by both sex and genotype.

General Conclusions

Although this study has been limited in scope by unforeseen events, some statements about dispersal and population regulation can be made. The conclusions made by Krebs et al. (1969, 1973) as to the importance of dispersal in the maintainance of microtine population regulation are supported. An enclosed population grew to densities

much higher than an adjacent unenclosed population and exhibited very high survival rates over the entire study. In the later part of the study changes in transferrin genotype frequencies related to changes in genotype survival rates were noted. The selective losses of female heterozygotes during this period could not be entirely accounted for by the release program indicating a selective force of unknown nature. These genetic changes do not conflict with the hypothesis of Chitty (1967). The release program indicated that emigrants are qualitatively different from the source population and that within this group of animals a behavioural polymorphism of some sort exists confirming the ideas of Myers and Krebs (1971). Some of the animals which left the area had a tendency to keep moving while others settled readily in less crowded areas. The manifestation of this behavioural polymorphism seemed to be density dependent as more dispersers were found in the sample released into the inhabited area than in the sample released into the vacant area.

The development of a comprehensive model to explain small mammal cycles of abundance still requires many years of research. Despite the apparent complexity of the models of Chitty (1967) and Krebs et al. (1973) involving fluctuating selective forces and changes in the behavioural and genetic make up of populations it is an over simplification of the real world. The intricate dynamics of demographic and physiological parameters such as body growth rate, reproductive performance and biochemical marker genotype have yet to be woven into the model.

The findings of this study give support to various aspects of the behavioural-genetic model but provide little new information. The observed selective mortality and dispersal in a declining population is in agreement with the earlier ideas of Howard (1960) and Lidicker

(1962) as well as later theorists. Support was also found for individual differences in dispersal behaviour among dispersers as suggested first by Murray (1967) and later by Myers and Krebs (1971). Some unexpected differences in dispersal behaviour were found between males and females but sample sizes were too small for conclusive statements to be made.

This study represents a first attempt at the use of leaking tubes to measure dispersal from an enclosed population and as such was a qualified success. For meaningful information to be obtained in this field it will be necessary for investigators to continue the use of an experimental approach by attempting to relate the genetic and behavioural changes of controlled experimental populations to the demography. Because dispersal studies in unenclosed populations are unmanageable, the continued use of release tubes or exit doors from enclosed populations is necessary.

The field of small mammal population regulation is still in a mild state of confusion because of the plethora of theories which have yet to be adequately tested and discarded (see Krebs and Myers, 1974). As yet no comprehensive model has been developed which provides a satisfactory mechanism for any species' demography or for the general cyclic pattern of small mammals. Hopefully continued experimentation will uncover the underlying mechanism of density regulation in cyclic populations of small mammals.

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Appendix I Arcsine Transformation

Although the major effect of applying an arcsine transformation to binomial data is to make the variance independent of the mean, I transformed R_x values in order to increase the variance[1]. R_x, the relative mobility of the transferrin bands, was replaced by the angle (in degrees) whose sine was the square root of R_x, or

$$\text{angle} = \text{Arcsin } (R_x)^{1/2}$$

eg. Front migration = 1.80 in

Band migration = 0.63 in

R_x = 0.63/1.80 = 0.35

angle = Arcsin $(0.35)^{1/2}$ = 36.27°

A similar transformation was performed on calculated survival rates so that parametric statistical methods could be applied.

[1] Snedecor, G. W. and W. G. Cochran. 1967. Statistical methods 6th ed. Iowa State Univ. Press. Ames. Iowa. 593 pp.

Appendix II Hardy-Weinberg equilibrium expectations for genotype frequencies

Levene[2] deduced that for small samples the exact expected frequencies of different genotypes that would occur at Hardy-Weinberg equilibrium could be calculated as follows. If the observed frequencies of genotypes AB, AB and BB are a, b and c respectively then the expected frequencies of these genotypes are:

$\frac{g_1(g_1-1)}{2(2G-1)}$, $\frac{g_1 g_2}{2G-1}$, and $\frac{g_2(g_2-1)}{2(2G-1)}$ respectively where

$$G = a + b + c$$

$$g_1 = 2a + b$$

$$g_2 = b + 2c$$

for example:

genotype	SS	SF	FF
number observed	16	20	14

Hence G = 50, g_1 = 52 g_2 = 48

number expected SS 13.39, SF 25.22, FF 11.39

[2] Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20: 91 - 94.

Appendix III Reproductive Biology of *Microtus pennsylvanicus*

So that the potential for population increase can be appreciated by the reader, a general discussion of the reproductive biology of *M. pennsylvanicus* is included here. The following is largely a summary of Banfield's [3] discussion of the topic. Breeding can occur year-round under optimal conditions but most frequently the breeding season extends from April until October. Females are polyoestrous and apparently mate promiscuously. The gestation period is twenty-one days long, and post-partum oestrus occurs immediately after parturition. Litter size averages 6.3 but much variation occurs. Females may be simultaneously pregnant and nursing a litter and in the field each female has an average of 3.5 litters per year. Young females may become sexually mature at 25 days of age and have their first litter 45 days after birth whereas males become sexually mature at approximately 45 days of age. Age of maturity in this species changes with population density and season and may be associated with body weight. It has been estimated that less than 20% of juvenile *M. pennsylvanicus* survive the first month of life so that a great potential for selection exists[4].

[3] Banfield, A.W.F. 1974. The Mammals of Canada. Univ. Toronto Press, Toronto. 438 pp.

[4] Krebs, C.J., B.L. Keller, and R.H. Tamarin. 1969. *Microtus* population biology: demographic changes in fluctuating populations of *M. ochrogaster* and *M. pennsylvanicus* in southern Indiana. Ecology 50:587-607.