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INTERPLAY BETWEEN SOCIAL ECOLOGY AND PHYSIOLOGY, GENETICS AND POPULATION DYNAMICS OF MICE

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ABSTRACT: Interplay Between Social Ecology and Physiology, Genetics, and Population Dynamics of Mice.

The interplay between socioecological and biological processes manifests itself at the level of individuals, populations, and species. The biology of individuals is deeply modified when they are groups; many of the attributes of populations such as size, distribution, composition, etc. are related to social interactions, and at the level of species, patterns of social relations within groups tend to be structured in ways that influence survival, reproduction, and exchange among populations.

In one experimental approach to these problems, the social ecology of freely growing populations of mice in large enclosures was related to behavioral, physiological, and health changes of individuals, to demographic changes and to changes of gene frequencies. Another experiment examined the process and effects of artificial selection for the same trait in different social environments.

Population Experiment.

The population enclosures were octagonal structures subdivided into central and peripheral sections with a total surface area of 13.3 square feet. From a founder group of mice of known genetic (progeny of a four-way cross among inbred mouse strains C57L/J, SWR/J, C3HeB/FeJ, 129/J) and environmental background, three equivalent samples of mice were distributed into replicate population enclosures (Pop A and B) and into standard laboratory cages as randomly mated male-female pairs—the control group (Pop C).

During the first year of study, daily observations of the enclosures were made, and several censuses were performed. Identifiable cohorts, animals born during each census interval, were established to provide an additional way of analyzing changes in the populations.

In Pop C, reproduction remained constant and mortality was negligible. Marked changes occurred in Pop A and B. The sizes (1000—A and 800—B mice) and densities (85—A and 60—B mice per square foot) are several times greater than those of any previously reported population of small mammals. However, there would have been 100,000 mice in each enclosure at the end of a year had the populations continued to grow as they did at first. Changes of reproductive physiology constituted prominent aspects of self-

regulation in the enclosures. Peak demographic input rates occurred during the third month, but were already associated with decreased productivity per adult female. Analysis of maturation and reproduction pointed to inhibition of reproduction in sexually mature females as the most important factor in the decline of productivity. Pregnancy rates fell steadily and inhibition of full-term gestation occurred. Gonads and reproductive cells of males were adult, but a large proportion of males showed little sexual activity.

Neonatal mortality was particularly striking in Pop B, where 30% of females showed advanced pregnancy during the last 5½ months with no newborns surviving. About 25% of the mice in the enclosures died during the year. Highest weekly death rates occurred during the first half of the year before peak numbers were present. Autopsies of mice of Pop A revealed little in the way of abnormal findings.

Biomass either paralleled or increased more rapidly than numbers in both enclosures, contrasting with some other population studies in which growth was impaired with crowding.

Changes of behavior included: 1) disappearance of circadian activity peaks, 2) decline in frequency of fighting per male but an increase in unusual aggressiveness, 3) aberrations of sexual behavior, 4) deterioration of maternal care, 5) cannibalism, 6) striking decrease in social responsiveness.

Cohorts in the populations were biologically distinguishable subunits in contrast to control cohorts, which showed no such differentiation. Cohorts in Pop A and B differed with respect to reproduction physiology, mortality, and behavior, and intercohort differences persisted at all levels of population density.

Many of the properties of Pop A and B mice changes when the mice were placed in different social environments, attesting to the specificity of the influence of social factors. For example, mice of Pop A, randomly paired in control cages, showed a marked rise in reproduction, and cohorts reproductively inhibited before were most productive in the new social environment. Bahvioral tests performed outside the enclosure environment revealed: 1) intercohort differences among Pop A mice contrasted with stereotyped behavior of Pop C mice, and 2) changes in behavior of Pop A mice both immediately after removal from the population and after six weeks in new social conditions. Pop B mice changed their social environment by emigrating into the empty interconnected enclosure of Pop A. Two distinctive subpopulations formed. Greater changes in reproduction, mortality, and behavior occurred

in the emigrant subpopulation, which underwent more extensive social reorganization. Immediately following reunion of the two subpopulations, a population crash occurred, possibly related to the sudden changes of social conditions.

Use of genetically defined animals made feasible the study of gene frequency changes. Polymorphism of alleles at the C locus affecting coat color differed between Pop A and B on the one hand and Pop C on the other. Although the magnitude of the upward change of recessive c in Pop A and B was not large, the consistency and similarity of the change in Pop A and B and lack of change in Pop C suggested the action of systematic processes and the probable adaptiveness of the changes. There was little evidence of differential adult reproduction or mortality among the phenotypes but there were suggestions of differential neonatal survival. The relatively slow rate of change of the alleles after the first generation suggested the establishment of a state of balanced polymorphism at the C locus. Hemoglobin allele and genotype frequencies of mice of Pop A alive at the end of the year did not deviate from what might have been predicted on the basis of panmixia.

Selection Experiment.

Selection for the same trait in varied environments tends to involve genetic and phsyiological differences. The question of adaptability to different social environments was studied; heavy body weight at sexual maturity was chosen as the trait for selection; groups of different sizes—pairs or groups of 20-30 mice— were the environmental variables. Sexes were kept separate between weaning and sexual maturity. A within-litter selection method was used.

Crowding depressed weight at sexual maturity but equal improvement with selection occurred in both social environments. Heritability was also equal in crowded and uncrowded groups. Environmental exchange carried out in the sixth and seventh generation suggested that mice selected in crowded environments performed slightly better in both crowded and uncrowded environments.

The large sizes and unusual degree of crowding attained by the freely growing populations in this study compared with previous studies may be related to the types of animals used, to the number of individuals in the founder nuclei, and to the physical structure of the enclosures. Extreme crowding was compatible with general physical health. The decline of fertility and fecundity, the decreased survival of newborns, and the appearance of behavioral aberrations — rather than disease or an increase in adult mortality— repre-

sented the major self-regulatory mechanisms that eventually limited population growth. The growth of individuals was not inhibited. Social withdrawal and the decline of social interaction rather than a rise of interaction characterized the populations. Such findings cast doubt about the generality of the so-called "Stress" theory of social ecology that emphasizes increased interaction and pituitary-adrenal hyperactivity as the principal mechanisms involved in self-regulation of vertebrate populations.

Other formulations of mammalian social ecology, such as those that focus on the importance of early development, of spatial requirements, of neurophysiological reactivity, and of communications, constitute additional explanations of the interplay of social and biological processes in crowded populations.

Although man's potential reactions are more complex and variable than those of lower vertebrates and give prominence to the role of symbols and culture, his social environment is even more fundamental to his entire existence. This, if anything, increases the importance of the interplay of socio-ecological and biological processes for man.

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INTRODUCTION

Biology has always concerned itself with the nature of relationships between living things and their environment (1). Environment, difficult as it may be to define in detail, includes "the substances and conditions with which organisms interact and maintain dynamic equilibria" (2). Many ecological studies (3) examine the characteristics and effects of interactions between vertebrates and such environmental components as weather (2,4-8), nutritional resources (9-11), habitat (12-15), and the "hazards" of predators and microorganisms (16-19).

Fewer studies, however, concern themselves with the profound biological changes that may result when vertebrates interact with members of their own species, with what can be called the SOCIAL component of environment (20-25). Yet, such interaction influences not only behavior, but all aspects of biology. The interplay between social ecology and biological change is expressed at the level of individual, group, and population—and even species.

Vertebrates come together in a great variety of aggregations or groups (26-31). The spectrum of groupings reflects both inter-species differences and differences in grouping constellations within species. A group may consist of two, a small number, or even the entire population, which may be large or small depending on the species. Members of groups may be of the same or different sexes, of one or many ages. The previous experiences of the group members (32-34), their behavior (35-37), and physiological state (38-40), all influence the nature of the group and the interactions that take place within it.

Animals may aggregate once or many times in their lives for transient or long periods, or relationships may be lifelong (41). Groups may result from the coming together of individuals to carry out specific activities which may or may not entail overt interaction among the participants. Where the focus of the group is the performance of a specific function—mating, feeding, fighting, traveling, care of young, etc.—interaction is usually apparent. But in other groups, each individual seems to carry out its activities simply in proximity of other members of its own species (42).

No vertebrate in nature spends its lifetime in social isolation, and, on the whole, intra-specific aggregations of one form or another, different socio-ecological situations, become more and more important for survival of individual, population, and species as one goes up in phylogeny

(43-46). Organisms of the same species thus constitute an important aspect of environment with which vertebrates interact dynamically.

The social environment possesses certain distinctive attributes compared with other environmental categories. For example, social units—organisms and groups of organisms—have more variable reactions from moment to moment than other environmental substances; social units actively receive, exchange or repel contact. Such reactions, repeated and modified, are integrated into sequences and patterns of biological activity that define the experiences of their participants.

The evolutionary forces that "produce" organisms also produce their social environment, other organisms, and groups and populations of the species. In a direct sense, here, the environment evolves along with the organisms (47). Like every aspect of environment, the social milieu presents challenges at all levels of biological organization. Response to challenge may or may not be successful. The social component of environment, however, also encompasses the distinctive "solutions" for survival and reproduction of individual, population and species, although biosocial solutions at one level may at times appear to conflict with solutions at other levels (48).

A growing number of studies of small and large groups of vertebrates, and even of entire populations of vertebrates both in the field and within the confines of laboratories, are beginning to delineate some of the dynamic interplay between social ecology and other biological processes (49-57).

At the level of the individual, changes in almost all aspects of development (58-64), physiology (65-70), and health and disease (71-74) result from social exchanges. The modifications of the individual's biology will, of course, vary with the particular organism and the particular group. Age (75-79), sex (80-83) and physiological states (84-86), past experiences, on the one hand, and the timing and durations of social interaction on the other (88-90), are important determinants of specifically how biology may be modified. Many attributes of vertebrates can develop normally only in the presence of other organisms of their own kind (91-92); such attributes are really potentials whose differentiation and actualization takes place only within the social environment of groups or populations.

Groups and populations of vertebrates are clearly more than collections of isolated organisms. They have emerged as functionally organized entities, and several of the mechanisms which act to integrate them are being studied (93-107). Some mechanisms seem to involve specific forms of inter-

action, whereas others appear to be related to rates and intensities of social stimulation (108-9). In any case, many of the properties of groups and populations, such as size, density, composition, spatial distribution, birth and mortality rates, sex ratios, group performance, appear to be influenced by modes of social interchange intrinsic to the populations or groups (110-14). Thus, in addition to such long recognized extrinsic factors as food supply, physical conditions, predators, etc., intrinsic forces generated by social life appear important in explaining population processes (48).

Lastly, patterns of social interrelations within groups and populations of vertebrates tend to be structured in ways that potentially influence the evolution of vertebrate species. Survival to sexual maturity and opportunities for reproduction in vertbrate populations are related to social patterns (115-17). At the same time, the social organization of vertebrate populations greatly influences the possibilities of reproductive exchange among different groups or populations (118-22).

Multiple factors always operate simultaneously in social situations. These variables can only be manipulated partially and never with complete unambiguity by experimentalist or field biologist. Practical difficulties arise in setting up sufficiently complex but manageable experiments or programs of field research. A variety of experimental approaches are available.

In the experiments to be presented, effects of different social environments on biological properties of mice were studied by following behavioral, demographic, physiological, and genetic parameters. In one series of experiments, the social ecology of freely growing populations of mice in large enclosures was related to behavioral, physiological and health changes of individuals, to demographic changes of the populations, and to gene frequency changes as measures of effects at the level of species. The other approach is concerned with much the same questions, but involves experiments that examine the Process and the Effects of artificial selection for the same trait in different social environments. The environment in which selection is carried out affects the properties called into play in the evolution of selected traits (121-130), and the question in this selection experiment revolves around the results of selection when the nature of the social group is the environmental variable.

POPULATION EXPERIMENTS

Population Experiments

Populations of vertebrates allowed to grow freely in the laboratory provide unusual opportunities for the study of complex living systems. The ability to control environmental factors such as climate and availability of food and water, and to eliminate predators, makes it possible to focus directly on the socio-ecological factors and to observe the development of populations over the course of time. Freely growing laboratory populations represent demographic, physiological, and genetic approaches to the study of social interaction, as well as a socio-ecological approach to the study of numbers, behavior, physiology, and evolution.

The present studies focus on both the dynamic interdependence of some of the processes that operate in vertebrate populations, and their effects on the biology of individual, group, population, and species. The aim was to clarify as many relationships as possible among such properties as social organization, numbers, densities, behavior, physiology, health, and gene frequencies.

A four-way cross between unrelated inbred mouse strains yielded a founder population used to initiate three study groups: two replicate freely growing populations and a control group. Equal samples of founder males and females were placed initially in two large population enclosures (Pop A and B), and in a series of standard small laboratory cages as breeding pairs (Pop C— the control group). The enclosures were provided with food, water, and nesting material ad lib., and the mice disturbed as little as possible (Fig. 1).

The studies are divided into two phases (Fig. 2). The initial phase lasted a year and consisted of continuous observations of behavior, social organization, and spatial distribution of mice, and of several population censuses that made possible measurements of genetic, physiological, and demographic parameters.

Animals born during each census interval, constituting birth cohorts, were tagged with characteristic marks so as to permit identification at all subsequent censuses. The purpose of establishing identifiable cohorts is to provide an additional way of analyzing changes in the populations. In addition to looking at what happens to the population as a whole, it is possible to examine what happens to parts of the populations, that is, to the

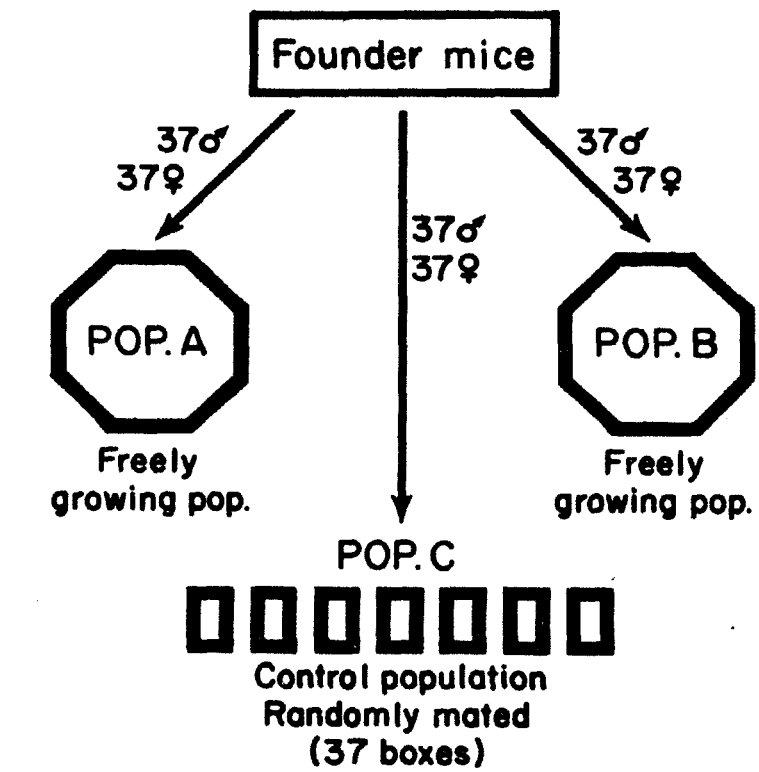

Outline of Population Studies
Crosses between 4 inbred strains of mice
Founder mice
37♂ 37♀
37♂ 37♀
37♂ 37♀
POP. A
POP. B
Freely growing pop.
Freely growing pop.
POP. C
Control population
Randomly mated
(37 boxes)

Figure 1

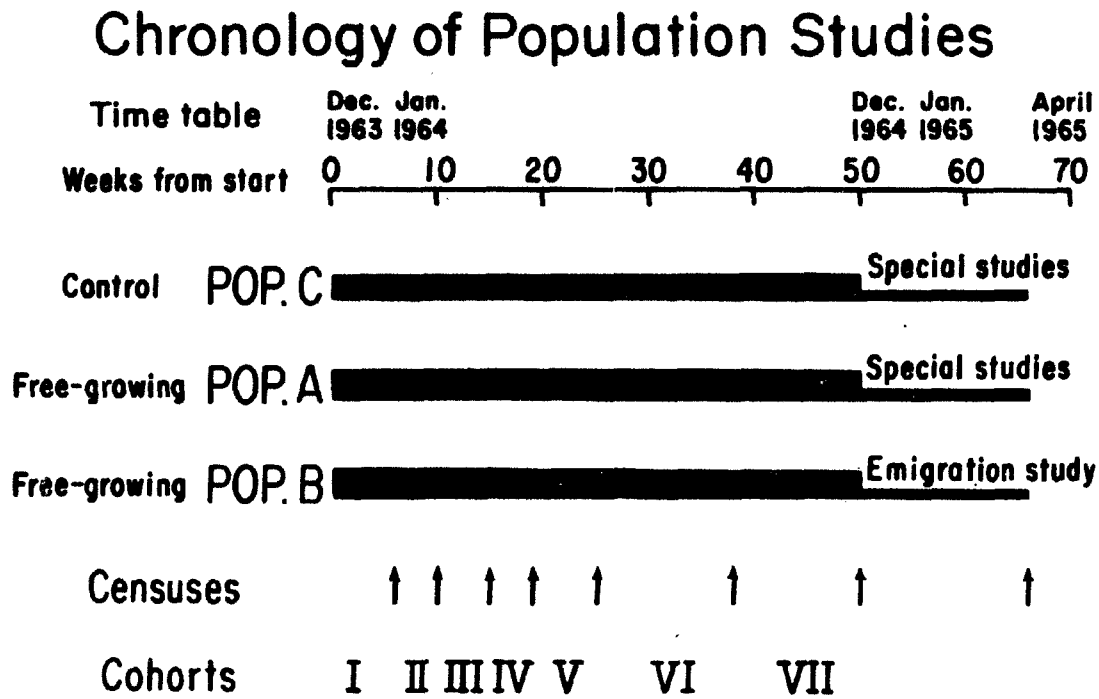

Chronology of Population Studies
Time table
Dec. 1963
Jan. 1964
Dec. 1964
Jan. 1965
April 1965
Weeks from start
0
10
20
30
40
50
60
70
Control
POP. C
Special studies
Free-growing
POP. A
Special studies
Free-growing
POP. B
Emigration study
Censuses
Cohorts
I
II
III
IV
V
VI
VII

Figure 2

cohort groups and to observe if these groups become differentiated from one another. The differentiation of such cohorts in terms of vital statistics, physiology, and behavior, it turned out, was a distinct phenomenon of the enclosure populations and did not occur in the control group.

The second phase lasted three months and consisted of studies of specific behavioral, physiological, and pathological properties. One population, Pop A, was discontinued and samples of its mice were compared with samples of control mice in a series of special studies. The other population, Pop B, was given access to the empty enclosure of Pop A so that the process and socio-ecological effects of emigration could be studied.

Materials

1. Location of study: the experiment was carried out in a modern general laboratory building in a room used exclusively for this purpose. The room had its own ventilator, temperature and humidity controlling units. The temperature was kept at $78 \pm 1^{\circ}F$, the humidity at about 50%. Sound insulation was sufficient to keep out major city noises.

2. Cages:

a. Populations: A modified version of enclosures used by J. Calhoun (131) for rat and mouse populations was designed. Figures 3 and 4 show the floor plan and side view of the octagonal structure, and give locations and dimensions of subareas and objects. Figure 5 is a photograph of one of the enclosures taken two months after the start. All partitions consisted of galvanized sheets of steel 1/16" thick. The distance between opposite parallel outer walls is four feet, and the entire surface area is 13.33 square feet. Food and water are located in the center. Ramps lead to the fountain platform above the feeder. The symmetrical arrangement of 16 periperally located retreats aims to avoid behavioral and physiological differentiation based upon distance from the food source.

An opening provides access to and from the central area from each of the retreats. All sixteen openings could be sealed simultaneously by lowering a removable octagonal panel snugly inside the inner wall.

Each of the sixteen retreats contains a covered cylindrical nest box, a two-pound coffee can with a single opening at first, and a teflon container of similar dimensions later.

Stainless steel mesh (3/8") welded to a steel frame formed the floor,

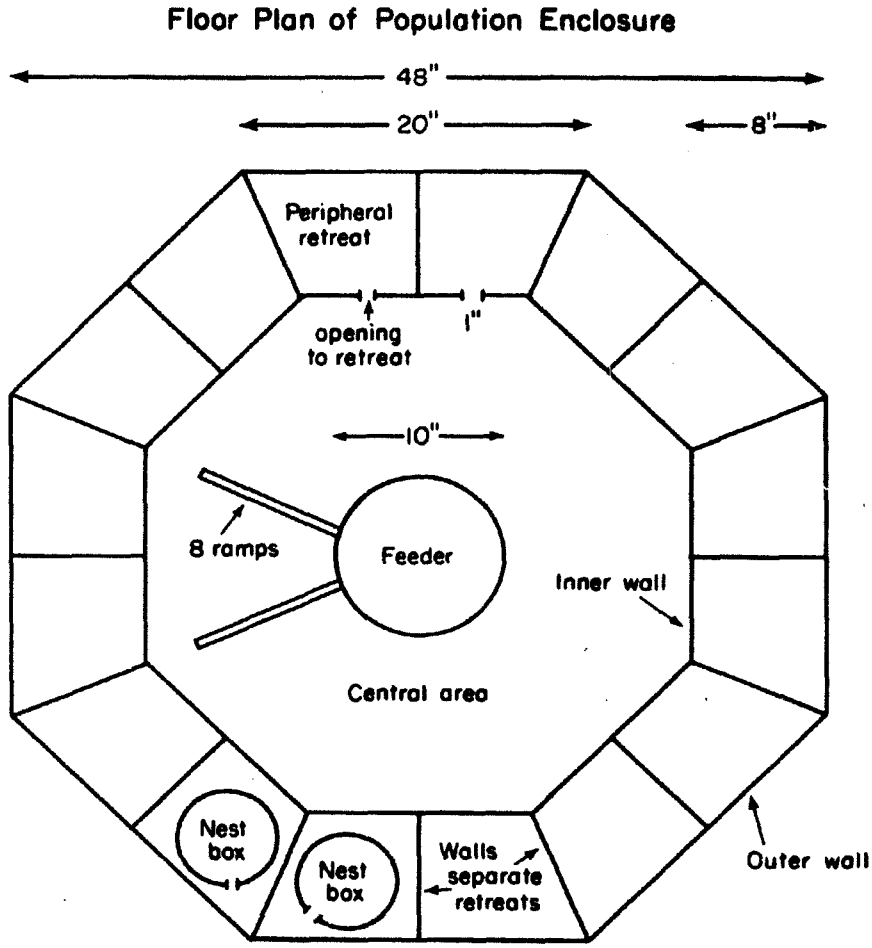

Floor Plan of Population Enclosure
48"
20"
8"
Peripheral retreat
opening to retreat
1"
10"
8 ramps
Feeder
Inner wall
Central area
Nest box
Nest box
Walls separate retreats
Outer wall

Figure 3

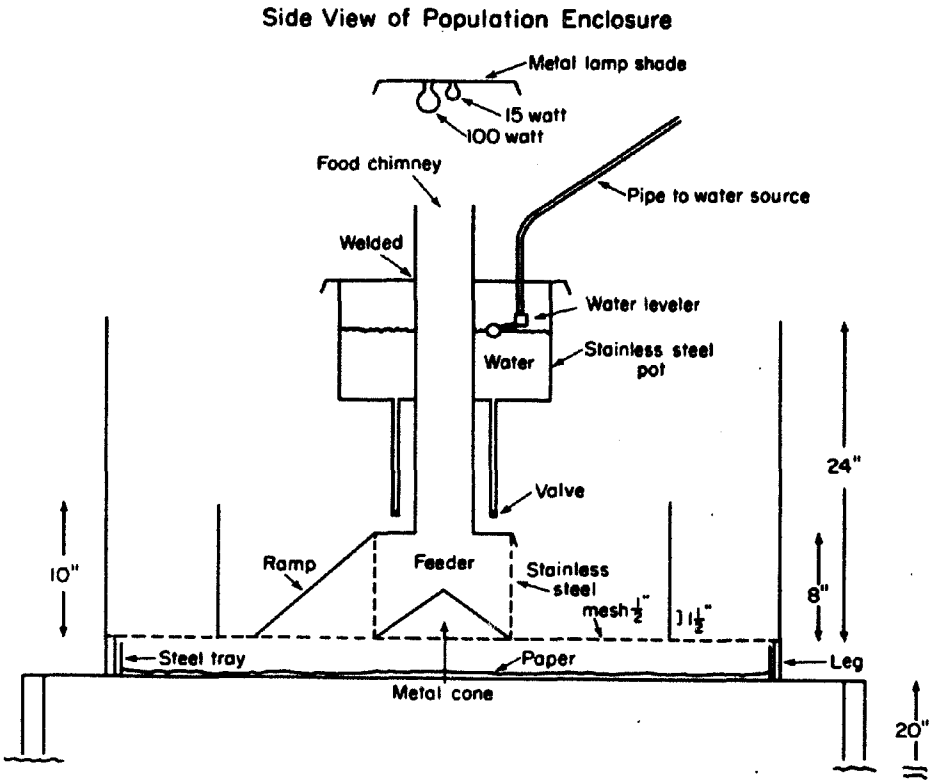

Side View of Population Enclosure
Metal lamp shade
15 watt
100 watt
Food chimney
Pipe to water source
Welded
Water leveler
Water
Stainless steel pot
24"
Valve
10"
Ramp
Feeder
Stainless steel mesh ½"
1½"
8"
Steel tray
Paper
Leg
Metal cone
20"

Figure 4

Figure 5 Population enclosure.

and fecal pellets and urine dropped to sheets of wrapping paper in trays below (changed three times each week).

Each enclosure rested on a level platform two feet above the floor of the laboratory. The two cages stood about two feet away from each other and were equidistant from the door and windows of the room.

Throughout the experiment, a 15-watt bulb provided light from 10AM to 10PM, and a 100-watt bulb at other times. Both bulbs were mounted in a shade of galvanized steel (12"x12") suspended from the ceiling to six feet above the floor of the enclosure. The lighting schedule was regulated by an automatic timer.

Nesting material, supplied ad lib., consisted of strips cut from folded paper towels (1-1½" wide by 8" long). About two inches of pine wood shavings and twenty to thirty strips were put in each nest box after cleaning. General cleaning and removal of wet clumps pf paper and piles of excreta with long forceps were performed as needed.

b. Controls. Figure 6 shows the dimensions of the stainless steel cage, water bottle, and removable stainless steel feeder (½" mesh, two inches high). The cage top was made of steel mesh (3/8"). The control cages stood on racks in the same room as the population enclosures. Nesting material was pine wood shavings. Cages were changed about once every two and one-half weeks and cleaned by autoclaving.

3. Food and Water: All animals received pellets of D and G and ordinary New York City drinking water.

Procedures

1. Observations. It was possible to observe both population enclosures by standing on a stepladder situated between them. Observations were recorded both in the form of notations and as brief descriptive statements. The cohort markings of individual mice could often be identified from the observation post and a few mice were recognizable as individuals because of some peculiarity, although on the whole the large numbers of mice precluded individual identification. Each enclosure was observed for at least two 10-minute periods daily (or about 20 hours a month), but often for longer periods. Over the course of each 8-9 days, some observations were made each hour from 8AM to 12 midnight. A small amount of photography and cinematography was done.

2. Censuses: Seven population censuses were carried out during the year for each of the three study groups. Pop B was censused an eighth time at

Control Cage

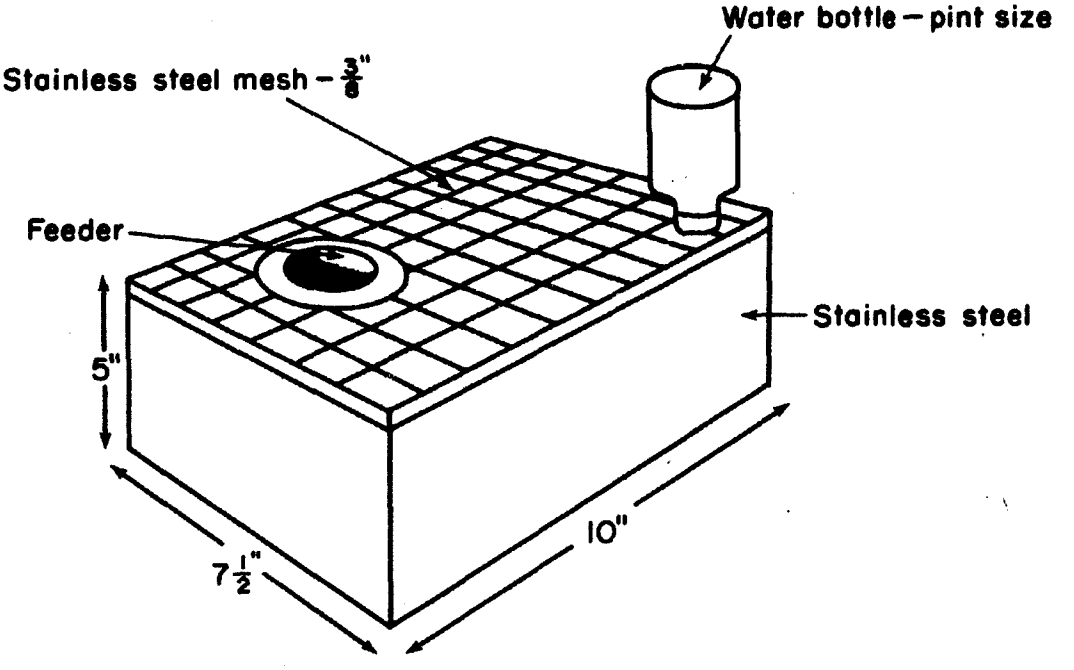


Figure 6

the end of the special migration study.

All mice born in a census interval still alive at census time and weighing at least 4 grams constituted a birth cohort and received an identifying ear punch. The founder mice make up Cohort I and subsequent cohorts are labeled with consecutive Roman numerals. Census dates, census intervals for specific cohorts, and mean ages for the mice of each cohort are displayed in Table 1. Exact birth dates were known for the founder mice; the birth date of other cohorts was estimated as the mid-point of the census interval during which they were born (except for Cohort II where the timing of births was more accurate).

At the time of each census, all mice were transferred into 11x12x5-inch plastic cages provided with pine wood shavings, food pellets, and water. Data recorded on each mouse included cohort, sex, weight, coat color, state of fur, evidence of pregnancy, wounding, gross pathology, and unusual behavior. The mice were weighed on a Kilomat scale (Sartorius). Censuses upset the population for about five to six hours. All census data was punched on paper tape and processed on a Control Data 160-A computer.

3. Breeding and mating control animals: Each control cage was inspected three times a week. Young were weaned when a second litter appeared in a box or when the mice were about 27-28 days old. Litter size, sex, and coat color of young were recorded. Weaned males and females were separated and kept in groups of six in metal cages (12x12x6 inches). All mice born in a census interval belonged to the control cohort corresponding to the population cohort of that interval.

Since all control mice could not be kept, every twelve weeks a random sample of 37 males and 37 females was picked from all the mice (300-500) of the previous period. The selected mice were randomly paired.

Materials and methods for special studies of behavior, reproduction, bacteriology, and pathology are described separately in the appropriate sections.

Initiation of Study

1. Constitution of founder population. The founder mice were derived from a four-way cross among unrelated inbred strains of mice (Table 2). Crosses between SWR/J females and C57L/J males and between C3HeB/FeJ females and 129/J* males were made at the R. B. Jackson Laboratory.

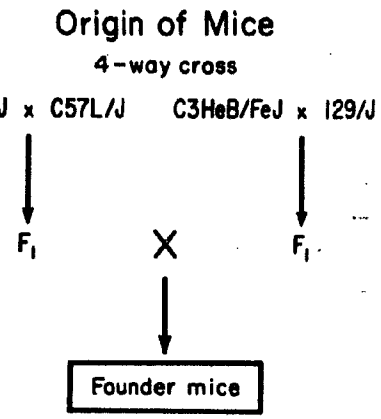
*Committee on Standardized Genetic Nomenclature for Mice (Cancer Research 20, 145-169, 1960).

Table 1 Census Dates and Intervals, Cohorts and Cohort Ages

	Year	1963	1964						1964	1965
	Census month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov	Mar
Cohort birth	Weeks after onset	2	6	10	15	19	25	38	50	68
Nov-Mar (1964-65)	Cohort VIII									2
Aug-Nov (1964)	Cohort VII								7	-
Jun-Aug	Cohort VI							6	18	-
Apr-Jun	Cohort V						3	16	28	52
Mar-Apr	Cohort IV					2	8	21	33	58
Feb-Mar	Cohort III				2	6	12	25	37	63
Jan-Feb (1964)	Cohort II			1	6	10	16	29	41	57
5 Dec (1963)	Cohort I	2	6	10	15	19	25	38	50	68

Each horizontal line shows the census interval during which the mice of a cohort were born, the designation of the cohort, the mean age in weeks of the cohort at the time when the cohort was first noted, and the mean age in weeks during subsequent censuses. The founder cohort (Cohort I) occupies the bottom line and subsequent cohorts are noted successively above.

Table 2



*Abbreviations are in accordance with Committee on Standardized Genetic Nomenclature for Mice (Cancer Research 20, 145-169, 1960)

Thirty matings of each reciprocal cross between the two F_1's were made at The Rockefeller University (60 matings in all). The fathers had been removed 15 days after each F_1 male-female pair was brought together. 125 young of 17 F_1 females (SWR x C57L females) and 113 young of 16 F_1 females (C3HeB x 129 females) born on the 22nd and 23rd days post pairing were used for the studies.

2. Cross-fostering. Behavioral difference between the two types of F_1 mothers, such as jumpiness, tendency to evade pickup by forceps, squeaking on being picked up, retrieving babies scattered on cage floor, made cross-fostering of the young advisable. Young were, therefore, removed from their mothers at four days of age and randomized among fifteen mothers of each type in litters of eight.

Ten cross-fostered litters, five from each F_1 female, were randomly assigned to each of three study groups: 1) Pop A-enclosure; 2) Pop B-enclosure; 3) Pop C-control cages.

3. Transfer to enclosures and weaning. The founder litters of the three study groups lived in standard laboratory cages until they were 2 weeks old, when appropriate litters were transferred to nest boxes of the enclosures or to fresh control cages. Each enclosure litter was confined to its own peripheral retreat area temporarily provided with its own food and water.*

One week later when the founder mice were of weaning age, all the mothers were removed from the enclosures and control cages. The confining partitions were removed from the enclosures. Control males and females were random-paired.

*Openings from retreats to the central area of the enclosure were sealed, and metal strips placed across the tops of retreats effectively prevented mothers from climbing out.

RESULTS: Population Dynamics

The enclosure populations attained large sizes and densities (Tables 3,4, Figure 7). Pop A grew to 1000 mice within 9 months and Pop B reached its peak of about 800 mice within 5½ months. The densities corresponding to these population peaks are 85 and 60 mice per square foot, respectively.

Population growth

The growth curves of the population can be divided into three stages: an initial period before births, a period of rapid growth in which the two populations showed similar patterns of change, and a final phase where growth leveled off but in divergent ways for the two populations.

Initial period: six weeks from birth to sexual maturity of founder mice

One hundered twenty males and 115 females counted at four days of age comprise the founder population (Table 5). Six males and four females died during the ten days that followed cross-fostering. These deaths may have included the seven underweight "puny-looking babies" noted at the time of cross-fostering, or may have been the result of disturbances that sometimes accompany cross-fostering.

The mice of founder cohorts were just reaching sexual maturity at the first census. Three additional mice had died in Pop B. Pop A contained the same number of males and females, 37 of each, as the control group, while Pop B had one more male and two fewer females.

Period of rapid population growth

The total numbers of mice increased rapidly during the next 4½ months in both populations. The over-all similarity of their growth patterns during this period of expansion is noteworthy because of the subsequent divergence in their population dynamics.

Period of leveling off

No newborn mice survived in Pop B during the next six months and the population declined in size by about 50 mice every three months. Pop A on the other hand continued to increase in size until there were more than 1000 adult mice at its last census in November 1964.

Total numbers — balance between recruitment and mortality

Changes in the numbers of mice represent the combined effects of recruitment and mortality. There was no opportunity for migration.

Table 3 Population Dynamics: Pop A - Numbers

	Year	1963	1964						1964
	Census month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov
	Weeks after onset	2	6	10	15	19	25	38	50
Aug-Nov (1964)	Cohort VII								113
Jun-Aug	Cohort VI							321	276
Apr-Jun	Cohort V						240	183	175
Mar-Apr	Cohort IV					245	(185)*	175	121
Feb-Mar	Cohort III				165	158	(142)*	132	135
Jan-Feb (1964)	Cohort II			126	120	122	(122)*	124	115
5 Dec (1963)	Cohort I	74	74	71	64	66	(63)*	61	55
	Total	74	74	197	349	591	753	996	1020

*At the time of the June 1964 census, the mice of cohorts I-IV were not distinguished by cohorts. The figures in parentheses are interpolations between the numbers of the previous and following censuses.

Table 4 Population Dynamics: Pop B - Numbers

	Year	1963	1964						1964	1965
	Census month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov	Mar
	Weeks after onset	2	6	10	15	19	25	38	50	78
Nov-Mar (1964-5)	Cohort VI									108
Aug-Nov (1964)	-								-	-
Jun-Aug	-							-	-	-
Apr-Jun	Cohort V						257	231	202	192
Mar-Apr	Cohort IV					256	(190)	188	184	164
Feb-Mar	Cohort III				198	172	(156)	140	132	134
Jan-Feb (1964)	Cohort II			143	121	119	(116)	115	112	95
5 Dec (1963)	Cohort I	76	73	72	70	68	(65)	61	56	51
	Total	76	73	215	389	615	784	735	686	744

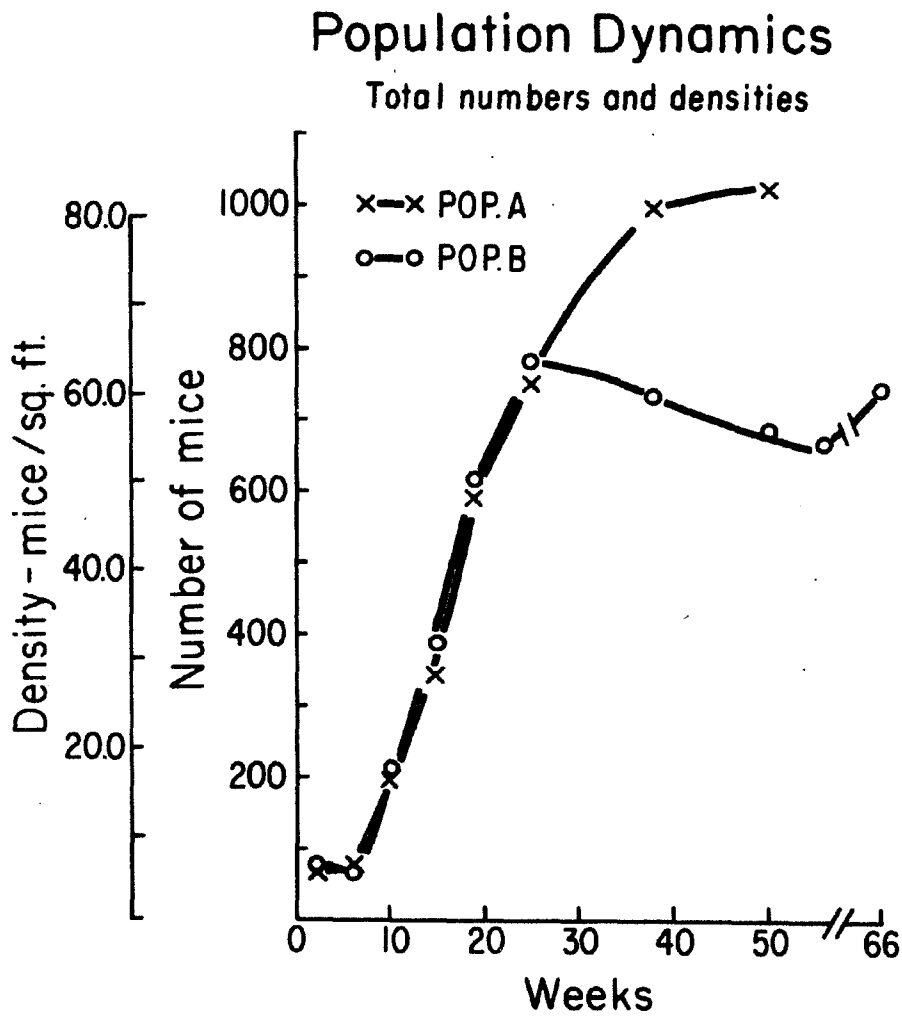

Population Dynamics
Total numbers and densities
Density - mice / sq. ft.
80.0
60.0
40.0
20.0
Number of mice
1000
800
600
400
200
×—× POP. A
o—o POP. B
0
10
20
30
40
50
66
Weeks

Figure 7

Table 5 Population Dynamics: Foundation Population

Date		Freely Growing Populations		Control Group	Totals
		Pop A	Pop B	Pop C	
10 Dec. 1963	Males	40	40	40	120
	Females	39	38	38	115
	Total	79	78	78	235
19 Dec. 1963	Males	37	39	38	114
	Females	37	37	37	111
	Total	74	76	75	225
17 Jan. 1964	Males	37	38	38	113
	Females	37	35	37	109
	Total	74	73	75	222

Recruitment — the input into the populations

The numbers of new mice counted at each census, of course, represent only those mice still alive on the day of census. The presence of dead newborns in the cages indicated that a larger number had been born during all census intervals. Records of dead litters were kept but these incomplete figures are excluded from census data.

Daily surveys of nest boxes for births were avoided because they disturb the normal activities of mice and disrupt nest structures. The term "recruitment" refers to new mice assigned membership to the population and ear punched to indicate cohort membership.

Cohorts II and III are the progeny of the founder mice (Cohort I). Cohort III is larger than Cohort II in both populations and in the control group (Table 6, lines 1-3). An increase in the number of females producing litters as well as an increase in mean litter size from 6.7 to 8.0 mice during the census interval in which Cohort III was produced explains this increase in cohort size for the control population and presumably might also be the explanation in the populations (Table 6, line 4). Both Cohorts II and III of Pop B are slightly larger than those of Pop A but smaller than the corresponding cohorts born in the control environment.

Table 6 Population Dynamics: Number of Mice in Cohorts II and III

		Study Group	Cohort II	Census interval Jan-Feb	Cohort III	Census interval Feb-Mar
line	1	Pop A	Cohort II	126	Cohort III	165
	2	Pop B	Cohort II	143	Cohort III	198
	3	Pop C	Cohort II	229	Cohort III	289
	4	Pop C	Cohort II 34 out of 37 females had litters Mean litter size = 6.7		Cohort III 36 out of 37 females had litters Mean litter size = 8.0	

The mice of Cohort IV of Pop A and B included third litters of the founder mice, and some first litters of the mice of Cohort II, by this time old enough to have young. Potential "contributors" to the other cohort (V) born during the period of rapid growth included founder mice, and mice of Cohorts II and III.

Mice of all previous cohorts were potential parents of the 321 mice (Cohort VI) censused at the end of August 1964 in Pop A. During the following three months, 113 mice (Cohort VII) survived to be censused.

Although there was no recruitment in Pop B during the last six months, mice were born throughout this period and dead newborns were seen almost daily. For example, 16 and 18 live newborns were present in the enclosure of Pop B on the day of the August and November 1964 censuses, respectively.

Recruitment rate

Table 7 summarizes both the numbers of new mice recruited and the weekly recruitment rates for each census interval; rates are valuable for comparisons since the census intervals are not of equal length.

Table 7 Population Dynamics: Recruitment

Numbers of mice recruited and recruitment rates for each census interval

Census interval months	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Census interval in weeks	6	4	5	4	6	12	13
Pop A							
No. of mice recruited	-	126	165	245	240	321	113
Recruitment rate per week	-	31.5	33.0	61.3	40.0	24.7	9.4
Pop B							
No. of mice recruited	-	143	198	256	257	0	0
Recruitment rate per week	-	35.8	39.6	64.0	44.5	0	0

The recruitment rates rise at first in both populations to peak levels of over 60 mice per week during the March-April census interval (Fig. 8). During the following census interval, the weekly recruitment rates have already dropped to 40 mice per week. There is no recruitment at all in Pop B during the last 6 months, as mentioned. In Pop A, the weekly recruitment rate falls steadily to fewer than 10 mice per week during the last three months.

Recruitment—physiological aspects

The number of sexually mature females is, of course, the limiting factor for recruitment during any census interval. Sexual maturity of males matters much less since mice are polygamous and sexually mature males were always present during these studies.

Normal rates of sexual maturation (six to seven weeks of age for the attainment of sexual maturity, nine to ten weeks of age for the production of first litters) were assumed in estimating the number of females capable of contributing young. Table 8 and Figure 8 show that the numbers of potentially breeding females estimated in this way were about equal in Pop A and Pop B, except during the last census interval when Pop A had about 100 more potential breeders. Roughly, a ten-time increase in the number of potentially breeding females occurred during the course of the year in both populations.

Table 8 Population Dynamics: Recruitment

Numbers of potentially breeding females in the experimental cages.

Year	1963-64	1964					1964
Census interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Pop A	0	34	33	94	149	332	463
Pop B	0	35	34	90	150	363	351

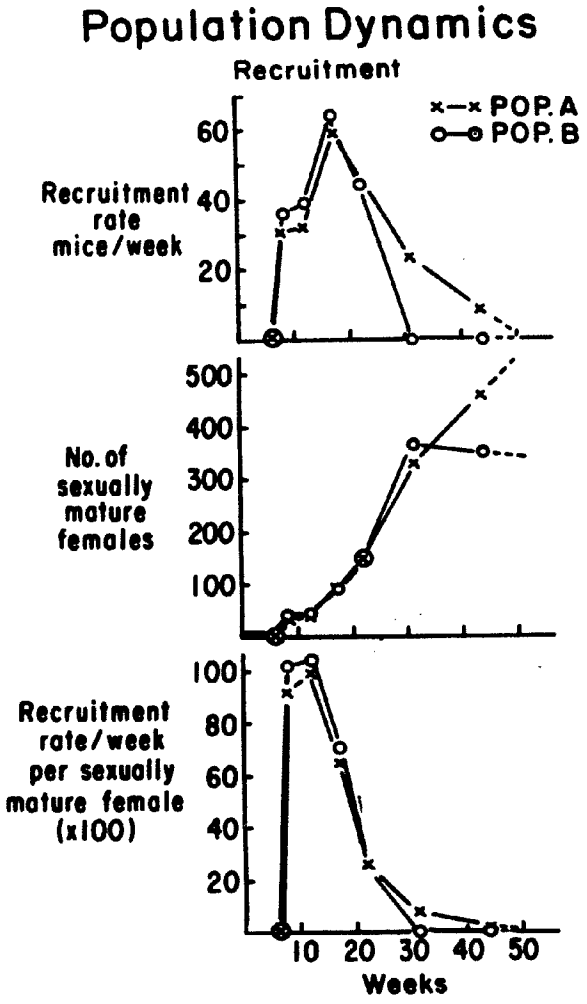

Population Dynamics
Recruitment
×—× POP. A
○—○ POP. B
Recruitment rate mice/week
60
40
20
No. of sexually mature females
500
400
300
200
100
Recruitment rate/week per sexually mature female (x100)
100
80
60
40
20
10
20
30
40
50
Weeks

Figure 8

The weekly recruitment rate for each census interval (Table 9) can now be expressed in terms of the number of potentially contributing female.

Table 9 Population Dynamics: Recruitment

Weekly recruitment rates per female of breeding age

Census interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Pop A							
Recruitment rate/week	0	31.5	33.0	63.1	40.0	24.7	9.4
$\frac{\text{Recruitment rate/week}}{\text{breeding female}} \times 100$	0	92	100	65	26	7	2
Pop B							
Recruitment rate/week	0	35.8	39.6	64.0	44.5	0	0
$\frac{\text{Recruitment rate/week}}{\text{breeding female}} \times 100$	0	102	104	70	24	0	0

The peak weekly recruitment rate of the March to April census interval is associated already with a declining recruitment rate per potentially contributing female This more meaningful index of recruitment continues to fall throughout the lifetime of the populations even more rapidly than the weekly recruitment rates. For example, for the 312 new mice recruited during the June to August census interval in Pop A, the recruitment rate per potentially contributing female had fallen to less than 10% of its level during the first two recruitment periods, whereas the weekly recruitment rate had fallen only to 75% of the corresponding level*

*To obtain an even more accurate index of the productivity of females during the different census intervals the number of potentially contributing females of each cohort should be multiplied by the number of potential litters they could have during any time interval. Such products are particularly important for the last two 3-month census intervals during which the females of most cohorts, theoretically, could each have two to three litters.

The weekly recruitment rates divided by the total number of potential litters for each census interval would show recruitment trends similar to those noted when the ratios were derived by dividing recruitment rates by potentially contributing females, but the productivity would be down by a factor of two to three during the last two census intervals in Pop A.

These relationships, graphically illustrated in Figure 8, suggest several possible explanations. First, delayed sexual maturation may have produced fewer breeding females than estimated. Second, the reproductive physiology of sexually mature, potentially breeding females may have been inhibited. This could result in the production of fewer or smaller litters than expected or in no litters at all. Finally, newborn mortality may have been very high. Of course, one or more of these processes may have been operative during any census interval. Observations of actual pregnancies provide partial answers to some of these alternatives. Visible (advanced) pregnancy in females of the most recent cohort was looked for and recorded as a part of daily observations of the enclosures. Of course, the presence and frequency of advanced pregnancy was assessed at all censuses.

At least a portion of the females of each cohort was maturing at the expected rate, inasmuch as some pregnant animals were noted among the females of each cohort when they were assumed to be at risk for pregnancy on the basis of a normal rate of sexual maturation (Table 10). However, the percentages of pregnant females, in certain cohorts especially, are low. It is, thus, still not possible to distinguish between two possibilities: delayed sexual maturation in all but a small fraction of the females, or inhibition of pregnancy of females that had matured at a normal rate. Assuming sexual maturity parallels growth, the growth curves of the females favor the idea of pregnancy inhibition (see below— biomass results). The mean weights of the females at the time when pregnancies were first noted are consistent with weights associated with sexual maturity. The only exception occurred on Cohort VI of Pop A, where the mean weights at 6 weeks of age are lower than expected for sexually mature females. Either the growth and sexual maturation of these mice were truly inhibited or their age was incorrectly estimated.

Only the females of the first cohort born in the population cages matured more rapidly in both enclosures than did their parents. Almost one-half of these females were pregnant at six weeks of age; none of the females of the founder cohorts had been pregnant at this age.*

The percentages of pregnant females are slightly higher in Pop A

*No control exists for this phenomenon since the progeny of the control founders were mated only at the end of twelve weeks.

Table 10 Population Dynamics; Recruitment. Pregnant females in each cohort at each census

Census month (1964)	Jan		Feb		Mar		Apr		Jun*		Aug		Nov	
Weeks after onset	6		10		15		19		25		38		50	
	P.	A.R.	P.	A.R.	P.	A.R.	P.	A.R.	P.	A.R.	P.	A.R.	P.	A.R.
Pop A														
Cohort VII													3	24
VI											0	74	7	132
V									0	0	20	93	10	89
IV							0	0	30	85	30	80	8	74
III					0	0	8	74	25	63	17	57	6	63
II			0	0	27	59	25	59	24	59	22	59	12	54
I	0	0	30	34	30	33	35	35	27	32	17	29	3	26
Total	0	0	30	34	57	92	68	168	106	239	106	392	49	463
% P. of A.R.	-		88.2		62.0		48.5		44*		27.0		10.6	
Pop B														
Cohort V									0	0	29	128	27	114
IV							0	0	21	98	17	97	22	94
III					0	0	4	70	24	71	22	70	15	62
II			0	0	19	57	28	57	25	52	17	50	17	51
I	0	0	26	35	27	33	27	33	18	29	12	25	12	22
Total	0	0	26	35	46	90	59	160	88	250	97	370	93	343
% P. of A.R.	-		74.3		51.1		36.9		35*		26.2		27.1	

P. = pregnant
A.R. = at risk for pregnancy (numbers in these columns are estimated on the assumption of a "normal" rate of sexual maturation)
*Estimated— from cohort pregnancy curves, Fig. 10

than in Pop B at the first five censuses, but the reverse is true for the last two censuses (Table 11, Figure 9). Over 90 females were pregnant at each of the last two censuses in Pop B, but in spite of this not a single mouse was recruited into this population during the last six months. Subsequent studies proved that much larger percentages of the females of both population could become pregnant and raise litters when placed in different social environments.

Identification of the cohort memberships at each census permitted comparisons of the incidences of pregnancy among the different cohorts. The significantly and consistently highest percentages are those of the females of the founder cohort (I) in both populations; the only exception occurs at the last census of Pop A when the females of Cohort II have a slightly higher percentage (Figure 10). Comparisons of pregnancy rates at the same ages among any two cohorts generally reveal higher rates for the females of any cohort born at an earlier stage in the history of the populations.

Perhaps, the same group of females in each cohort had many litters, while other females had none at all. The numbers of actually pregnant females at each census may be taken to be representative of such a hypothetical group of littering females. It is possible to recalculate weekly recruitment ratios in terms of pregnant females (Table 12). Such recruitment ratios are impressively higher for Pop B than for Pop A during the first four census intervals both because the weekly recruitment rates are higher and the number of pregnant females are lower in Pop B during this period.

The recruitment rates per pregnant female fall less rapidly and never to as low levels as those calculated on the basis of potentially mature females because the number of pregnant females is smaller. In Pop A the number of pregnant females during the last four months of the study was very low. In Pop B, though the numbers of pregnant females are high during the last six months, there was no recruitment and the ratios fall to zero.

If the number of pregnant females for each cohort are representative of the number of litters contributed by that cohort, it becomes possible to estimate the theoretical contributions of the females of each cohort to the production of other cohorts and to the population as a whole (Table 13, Figure 11). It would seem that the original cohort produced about 60% of the total number of mice of the new cohorts of Pop B and about 45% of those of Pop A. Cohort II produced slightly more than 20% of the mice of subsequent cohorts in both enclosures, and Cohort III between 10 and 15% of the mice.

Table 11 Population Dynamics: Recruitment. Pregnancies expressed as percentages of females at risk in each cohort at each census.

	Year	1964						1964	1965
	Census month	Jan	Feb	Mar	Apr	Jun*	Aug	Nov	Mar
	Weeks after onset	6	10	15	19	25	38	50	
Pop A	Cohort VII							4.0	-
	VI						0.0	5.3	-
	V						21.5	11.2	-
	IV					35*	37.5	10.8	-
	III				10.8	40*	29.8	9.5	-
	II			45.8	42.3	40*	37.3	22.2	-
	I	0.0	88.2	90.9	100.0	85*	58.6	11.5	-
	Total	0.0	88.2	62.0	48.5	44*	27.0	10.6	-
Pop B	Cohort V						22.7	23.7	20.7
	IV					18*	17.6	23.4	29.8
	III				5.7	35*	34.9	24.2	45.9
	II			33.3	49.1	45*	34.0	33.3	35.6
	I	0.0	74.3	79.4	81.8	70*	48.0	54.6	41.7
	Total	0.0	74.3	51.1	36.9	35*	26.2	27.1	31.4

*These values are estimated from cohort pregnancy curves in Fig. 10

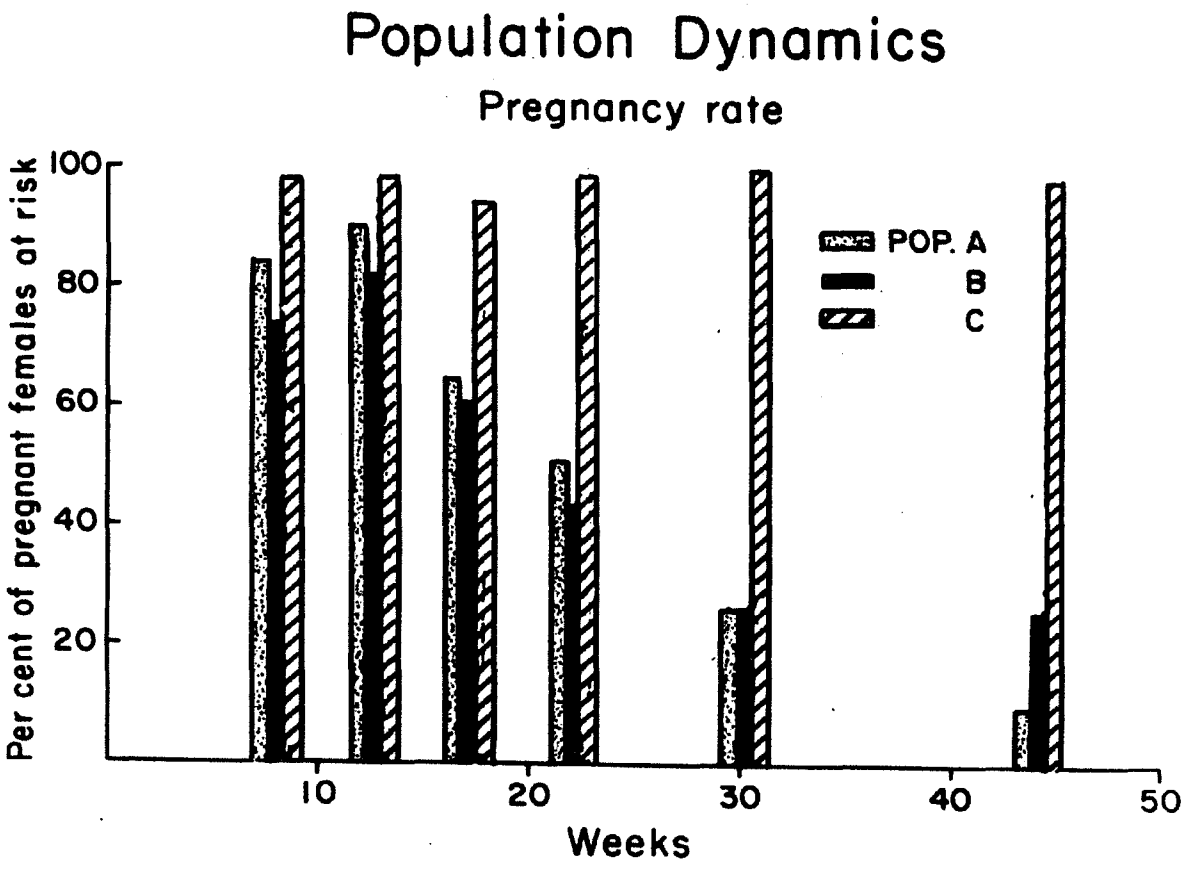

Population Dynamics
Pregnancy rate
Per cent of pregnant females at risk
100
80
60
40
20
10
20
30
40
50
Weeks
POP. A
B
C

Figure 9

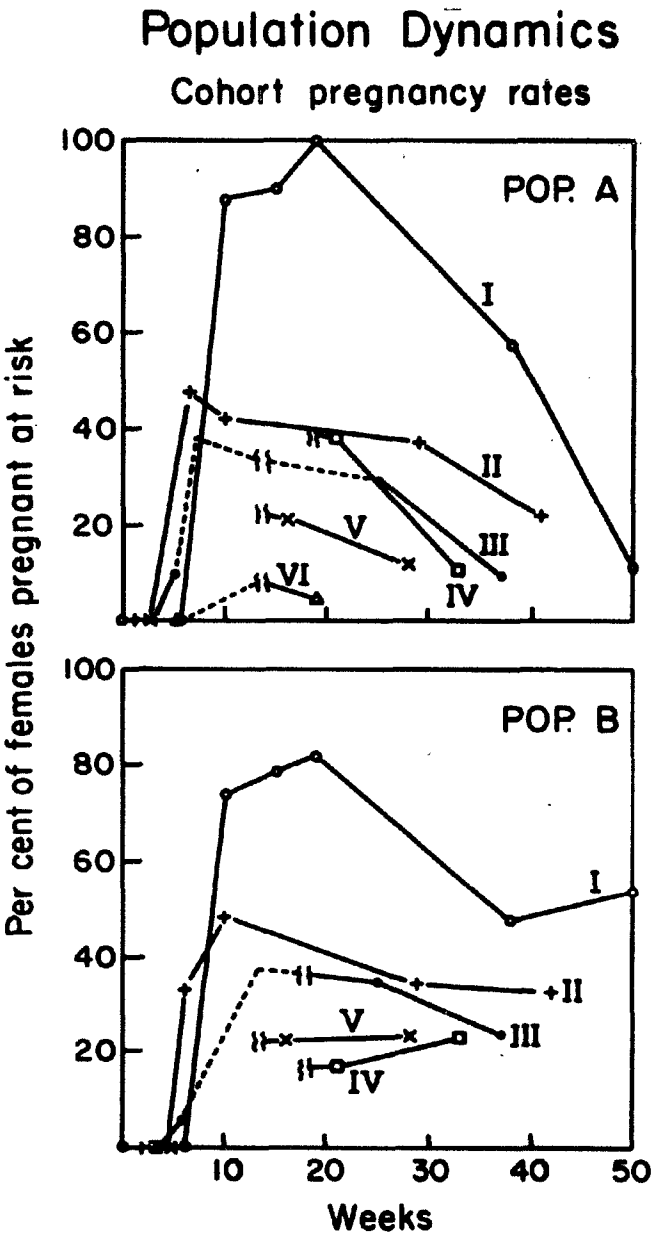

Population Dynamics
Cohort pregnancy rates
POP. A
POP. B
I
II
III
IV
V
VI
Per cent of females pregnant at risk
100
80
60
40
20
10
20
30
40
50
Weeks

Figure 10

Table 12 Population Dynamics: Recruitment related to the number of pregnant females.

	Census interval	Weekly recruitment rates per pregnant females Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Pop A	Weekly recruitment rates	0	31.5	33.0	63.0	40.0	24.7	9.4
	No. of pregnant females	0	30	30#	68	106*	106	47
	$\frac{\text{Weekly recruit. rate}}{\text{No. of pregnant females}} \times 100$	0	105.0	110.0	92.6	37*	23.3	20.0
Pop B	Weekly recruitment rates	0	35.8	39.6	64.0	44.5	0	0
	No. of pregnant females	0	26	27#	59	88*	97	93
	$\frac{\text{Weekly recruit. rate}}{\text{No. of pregnant females}} \times 100$	0	137.7	146.6	108.4	50*	0	0

#A number of the females of Cohort II were pregnant at the time of the Feb-Mar census; however, none of these females could have possibly contributed animals born during the census interval.

*Estimated

Table 13 Population Dynamics: Recruitment.
Contributions of females of each cohort to other cohorts and to the populations as a whole on the basis of pregnancy data

	Year	1964						1964
	Census interval	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov	Jan-Nov
Pop A	Cohort produced	II	III	IV	V	VI	VII	All cohorts
	Size of cohort	126	165	245	240	321	113	1210
	Contributing cohorts	%	%	%	%	%	%	%
	Cohort VII							
	VI						15.9	1.5
	V					18.9	22.7	7.1
	IV					28.3	18.2	9.2
	III			11.8	28	16.0	13.6	11.8
	II			36.8	33	20.8	27.3	22.0
	I	100.0	100.0	51.8	39	16.0	6.8	45.6
	Total	100.0	100.0	100.0	100	100.0	100.0	100.0
Pop B	Cohort produced	II	III	IV	V			All cohorts
	Size of cohort	143	198	256	257			854
	Contributing cohorts	%	%	%	%			%
	Cohort V							
	IV							
	III			6.8	42			14.7
	II			47.5	31			23.6
	I	100.0	100.0	45.8	27			61.7
	Total	100.0	100.0	100.0	100			100.0

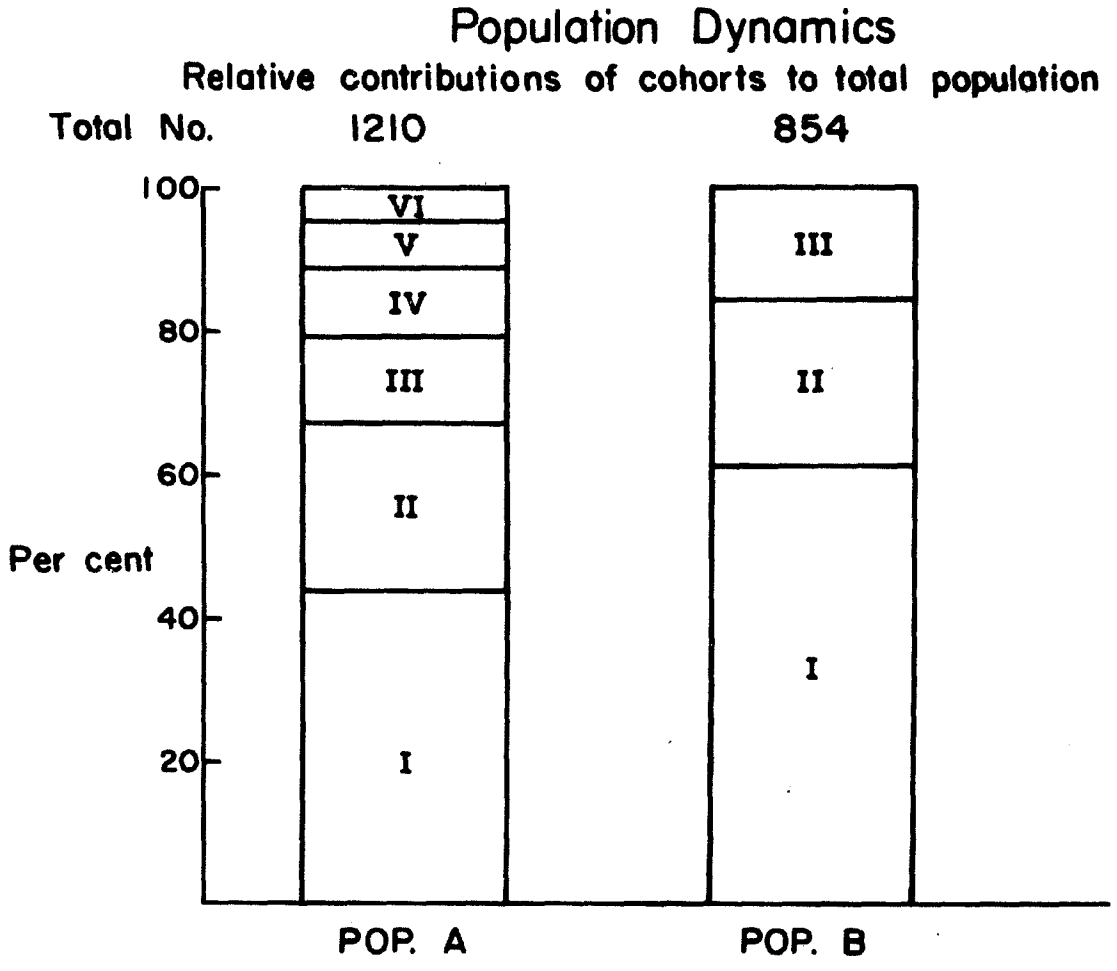

Population Dynamics
Relative contributions of cohorts to total population
Total No.
1210
854
100
80
60
40
20
Per cent
VI
V
IV
III
II
I
III
II
I
POP. A
POP. B

Figure 11

Cohorts III and IV each produced about 20% of each of the last two cohorts born in Pop A or 8% of the total population. It is impossible, however, to determine what fraction of these potential contributions gave rise to the actual numbers of individuals recruited into each cohort and into the population as a whole.

Crude Recruitment Rates

Recruitment rates calculted in terms of total numbers of individuals present are generally of little value. Such so-called crude rates do not discriminate between reproducing and non-reproducing individuals. In the context of this study in which the total available space did not change from beginning to end, the crude recruitment rates become measures of the effects of population density on recruitment (Table 14, Figure 12).

The mean crude recruitment rates per week diminish by 50% as population density rises from 10 to 20 mice per square foot, levels off between densities of 20 and 40, and again decreases by 50% as density reaches 60 mice per week. The further rate of decline of the crude recruitment rate in Pop A is somewhat more gradual as density approaches the peak level of 80 mice per square foot.

The Control Population—Pop C

One of the functions of Pop C was to provide a baseline to evaluate the possible effects of other environmental variables common to all three study groups, such as season, temperature, reversed day-night light cycle, type of food, etc. The census intervals of the control population correspond to those of the freely growing ones. The numbers of females bearing litters and the sizes of litters were known exactly for Pop C, permitting exact calculations of demographic indices (Table 15).*

Recruitment and weekly rates of recruitment into Pop C in each of the census intervals are displayed in lines 1 and 2 of Table 15.

The apparent fall in recruitment during the fourth census interval (production of Cohort IV) disappears when recruitment is converted into a weekly rate per breeding female (lines 3-4). Ages of "breeding" females were estimated according to the cohort membership of the females involved. Some of the females, of course, were not mature enough to produce litters during the first few weeks of each 12-week period.

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*Control group matings, it will be recalled, were set up by breeding a random sample 37-male-female pairs from the total pool of animals available each twelve weeks.

Table 14 Population Dynamics: Crude Recruitment

	Year	1963-1964	1964					1964
	Census Interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Pop A	Mean Pop size	74	136	273	470	672	875	1008
	Pop density (mice/ft^2)	5.5	10.2	20.5	35.3	50.5	65.7	75.8
	Recruitment/wk	-	31.5	33.0	63.0	40.0	24.7	9.4
	$\frac{\text{Recruitment/wk}}{\text{Pop size}}$	-	23.6	12.1	13.4	6.0	2.8	0.009
Pop B	Mean Pop Size	74	144	302	502	700	760	710
	Pop density	5.5	10.8	23.4	37.7	52.6	57.1	53.4
	Recruitment/wk	-	35.8	39.6	64.0	44.5	0	0
	$\frac{\text{Recruitment/wk}}{\text{Pop size}}$	-	24.9	13.1	12.8	6.4	0	0

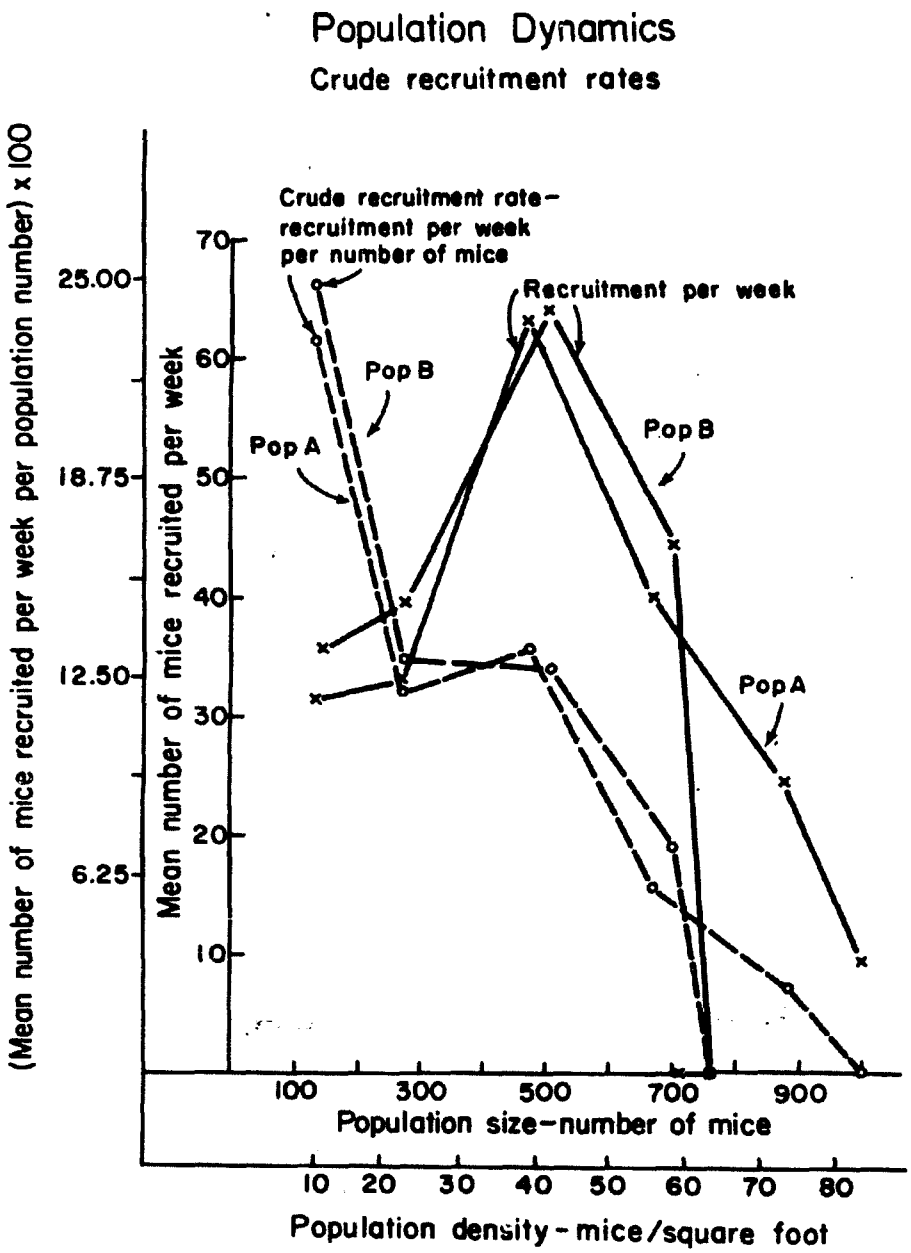

Population Dynamics
Crude recruitment rates
(Mean number of mice recruited per week per population number) x 100
25.00
18.75
12.50
6.25
Mean number of mice recruited per week
70
60
50
40
30
20
10
Crude recruitment rate-
recruitment per week
per number of mice
Recruitment per week
Pop B
Pop A
Pop B
Pop A
100
300
500
700
900
Population size-number of mice
10
20
30
40
50
60
70
80
Population density - mice/square foot

Figure 12

Table 15 Population Dynamics: Recruitment - Control Group - Pop C

	Year	1963-1964	1964					1964
	Census interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Line	Weeks recruited	6	10	15	19	25	35	58
	Cohort	-	II	III	IV	V	VI	VII
1	No. mice	-	229	289	100	294	387	546
2	Recruitment/wk	-	57.3	57.8	25.0	49.0	43.0	61.0
3	Females at risk	-	37	37	16	37	37	37
4	$\frac{\text{Recruitment/wk}}{\text{Female at risk}} \times 100$	-	154.7	156.2	156.3	132.4	116.2	164.8

Recruitment rates per breeding females do not change during the first three census intervals. There is a slight drop off at the next census and the lowest level of recruitment occurs for the summer census interval when Cohort VI was born. The decline of productivity in Pop C during this period suggests that the corresponding decline in the freely growing populations during this census interval may in part have been due to factors extraneous to the populations, e.g., seasonal decline in productivity. The recruitment rate of the last census interval exceeds that of the founder cohort, evidence for the temporary nature of the fall of productivity during the summer. The control mice did not lose their reproductive "vigor" during the year.

The level of productivity in the control population was higher than that of either of the experimental enclosures throughout the year (Table 16). The mean number of mice recruited per week per breeding female is a suitable parameter for making comparisons. During the first two intervals of population growth, the ratio of recruitment in control and enclosure environments is 3:2. The ratio rises to 2.1 : 1, then to 5.2 : 1, and finally to 16.1 : 1 and 82:1 (last two census period for Pop A only) (Figure 13).

Table 16 Population Dynamics: Recruitment

Comparison of recruitment in freely growing populations and control population

	Mean number of mice recruited per week per 100 females at risk*						
Year Census interval	1963-64 Dec-Jan	1964 Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	1964 Aug-Nov
Pop A	0	92	100	65	26	7	2
Pop B	0	102	104	70	24	0	0
Pop C	0	155	156	156	132	116	164

*All values multiplied by 100 and rounded off to nearest integer

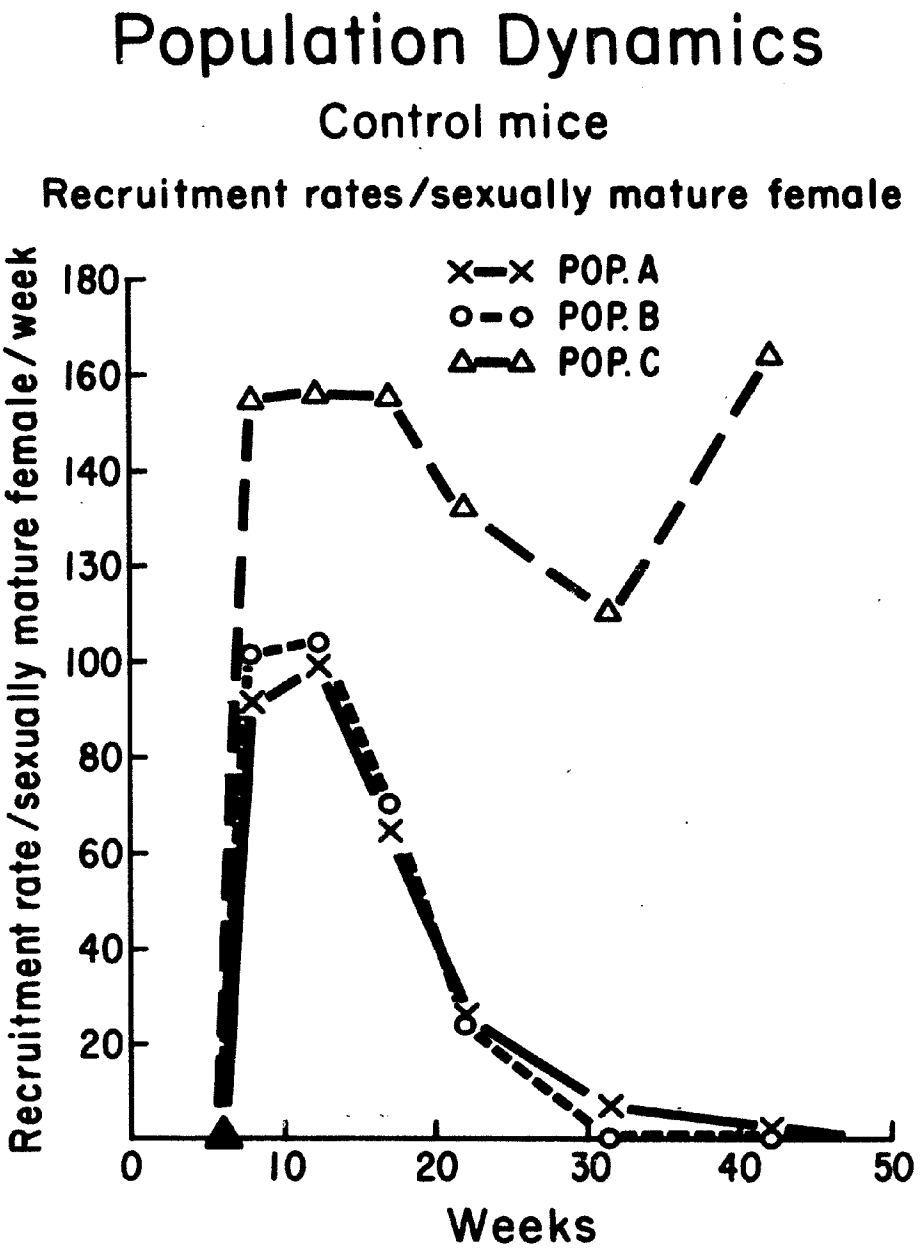

Population Dynamics
Control mice
Recruitment rates/sexually mature female
POP. A
POP. B
POP. C
Recruitment rate/sexually mature female/week
180
160
140
130
100
80
60
40
20
0
10
20
30
40
50
Weeks

Figure 13

Mortality

During the fifty weeks of study, 262 mice of the 1,176* mice of Pop A died and 246 of the 932 mice of Pop B died (Table 17).

Table 17

Population Dynamics: Mortality

Over-all Mortality

	Pop A	Pop B
Mice at risk	1,176	932
Number dead	262	246
% dead of at risk	22.28	26.39

This refers to mice either present initially or recruited into the population. The mortality rate of Pop A is slightly lower, 22.28% vs. 26.39% in Pop B. Expressed as survivorship, 77.72% of the mice ever present in Pop B survived to the last census (Figure 14).

The cumulative mortality curves show an initial lag phase, shorter in Pop B than in Pop A, a rapid rise in mortality during the April-June census interval, and a constant though less steep rise during the last six months in both populations (Table 18, Figure 15). Pop B suffered greater mortality during the first six months, but only 60% as many animals died in Pop B during the last six months as in Pop A.

The most meaningful measure of mortality for any census interval is the weekly death rate expressed in terms of numbers of mice at risk (Table 19). Mortality rises sharply in Pop B during the third and fourth census intervals (Figure 16) to a rate that is two to four times higher than that of Pop A during this time. The mortality rate of Pop A becomes comparable to that of Pop B (20 mice/1000 at risk/week) only during the fifth census interval.

In both populations the highest mortality rates occur before peak population size and density are reached. As a matter of fact, the weekly mortality rate of mice at risk falls to less than one-third of the previous

*The number of mice at risk for death in Pop A was 1,176 rather than 1,289 since the 113 mice recruited in Pop A during the last census interval do not enter mortality statistics.

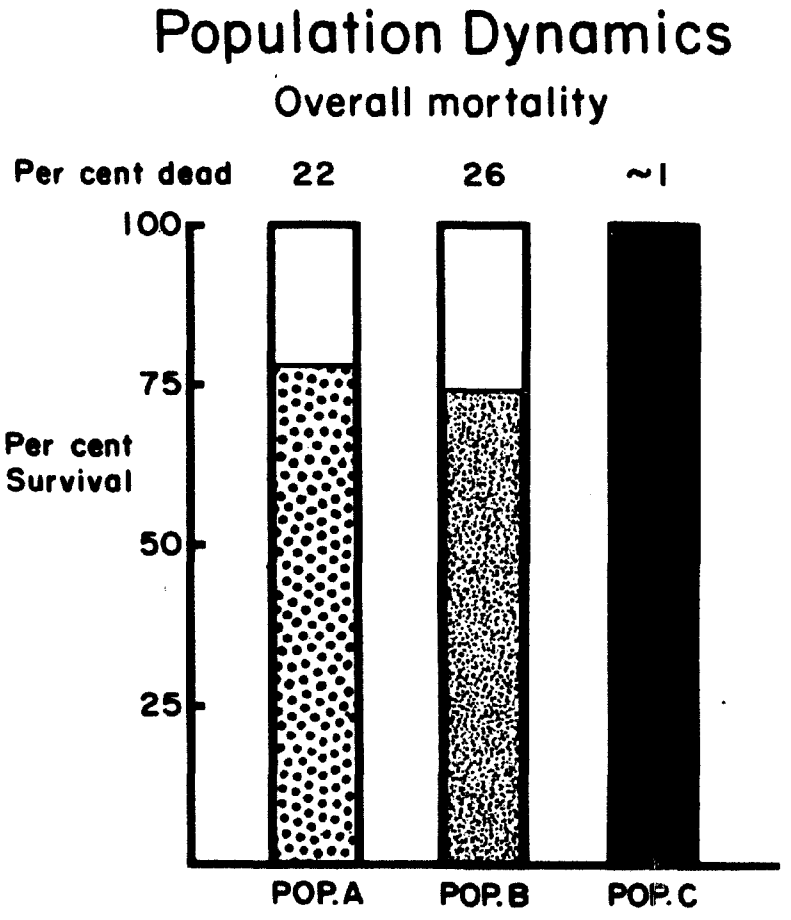

Population Dynamics
Overall mortality
Per cent dead
22
26
~1
100
75
Per cent
Survival
50
25
POP. A
POP. B
POP. C

Figure 14

Table 18 Population Dynamics: Mortality

Mortality during each census interval

	Census interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Pop A	At risk	79	74	197	349	591	753	996
	Dead	5	3	13	7	78	78	78
	(Dead/Risk) x 100	6	3	6	2	13	10	8
Pop B	At risk	78	73	215	389	615	784	735
	Dead	5	1	24	30	88	49	49
	(Dead/Risk) x 100	6	4	11	8	14	7	7
				Cumulative Mortality				
	Pop A	5	8	14	28	106	184	262
	Pop B	5	6	30	60	148	197	246

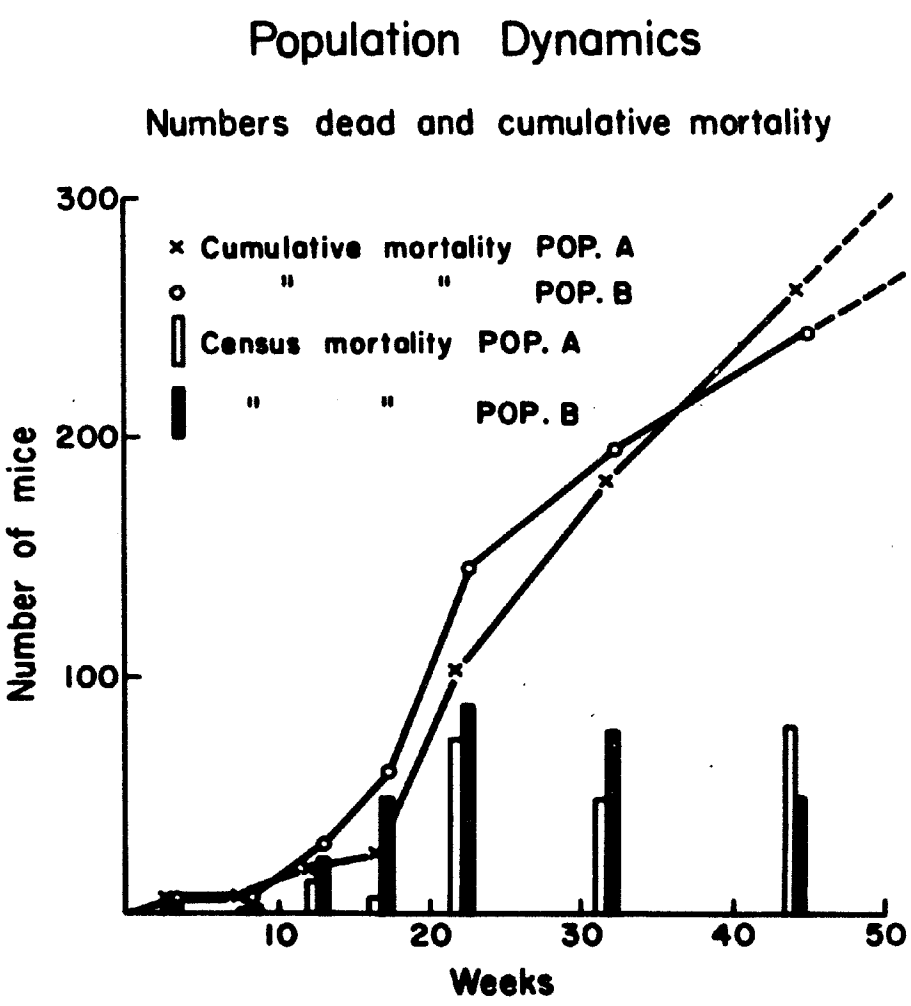

Population Dynamics
Numbers dead and cumulative mortality
300
200
100
Number of mice
× Cumulative mortality POP. A
o " " POP. B
Census mortality POP. A
" " POP. B
10
20
30
40
50
Weeks

Figure 15

Table 19 Population Dynamics: Mortality

Weekly Death Rate

Census	interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Pop A	Dead	5	3	13	7	78	78	79
	Weeks	5	4	5	4	6	13	12
	Dead/wk	1	1	2.6	1.8	13.0	6.0	6.4
Pop B	Dead	5	1	24	30	88	49	49
	Weeks	5	4	5	4	6	13	12
	Dead/wk	1	1	4.8	7.5	14.3	3.6	4.1
Weekly death rate/mice at risk (Deaths/mice at risk/week) x 1000								
	Pop A	10.5	7.5	12.0	5.0	21.7	7.7	6.7
	Pop B	10.7	10.0	22.8	20.0	23.3	5.4	5.9

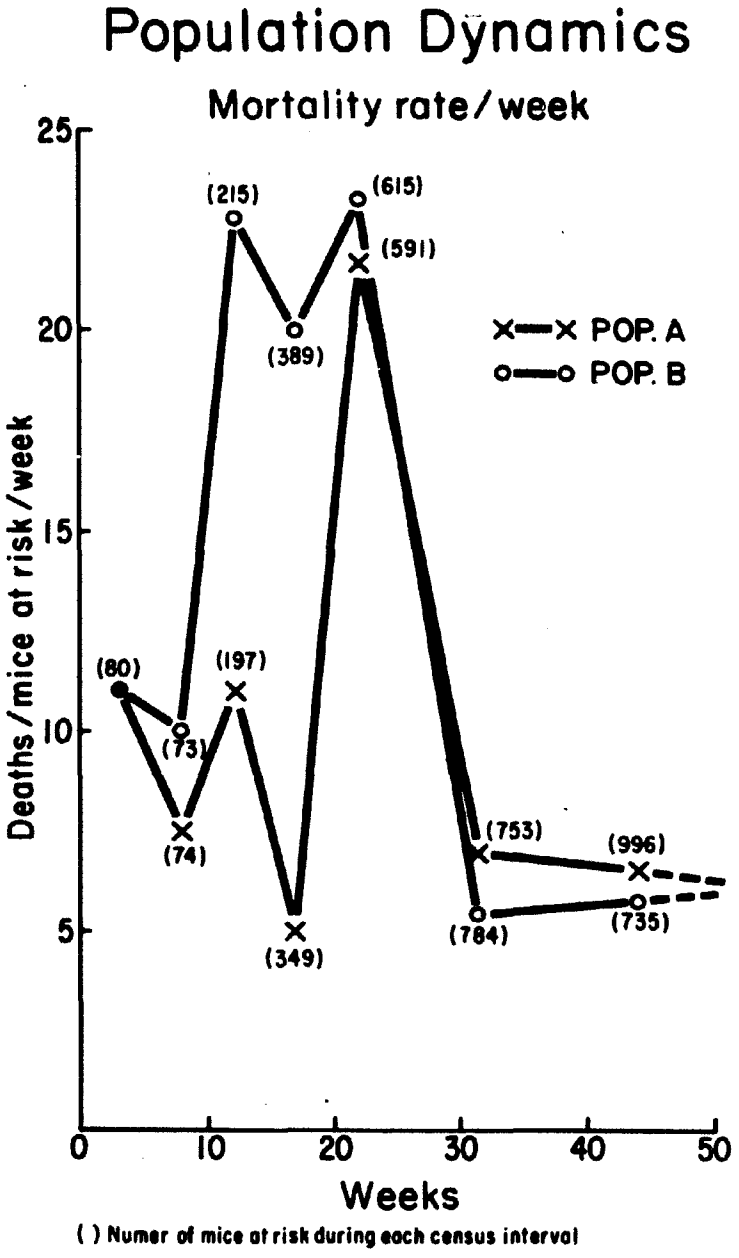

Population Dynamics
Mortality rate/week
Deaths/mice at risk/week
25
20
15
10
5
0
10
20
30
40
50
Weeks
POP. A
POP. B
(80)
(73)
(74)
(197)
(215)
(389)
(349)
(615)
(591)
(753)
(784)
(996)
(735)
( ) Numer of mice at risk during each census interval

Figure 16

peak values during the last six months in both populations. During this time, the numbers of mice at risk in Pop A actually rose by about 400 mice.

The pattern of mortality or survivorship varies among the different cohorts (Table 20). The founder cohort (I) was followed from birth and all mortality could, therefore, be recorded. Subsequent cohorts were slightly older when first censused; the mice of Cohort II were about one week old, Cohorts III and IV about two weeks old, Cohort V about three weeks old, and Cohorts VI and VII about six weeks old. Thus, a portion of their mortality was missed. Despite this, certain distinct features stand out when cohort patterns of mortality are compared.

First, certain cohorts, though younger, nevertheless have lower survivorships than older cohorts; that is, though exposed to the risk of death for shorter times, have suffered greater mortality. The cohorts to which this applies are listed in Table 21. By this measure, for example, Cohort II in both populations is a successful cohort since all four of the cohorts younger than itself in Pop A and two of three cohorts younger than itself in Pop B have greater mortalities. Cohort III in Pop A has a higher survivorship than two of the three younger cohorts. Thus, the first two cohorts born in the enclosure in Pop A and the first cohort in Pop B, all progeny of the founder mice, show superior survivorship.

Differences in patterns of mortality among different cohorts stand out when survivorship is compared for similar ages (Figure 17). Survivorship values for 6, 12, 18, 24, 30, 36, and 41 weeks of age are tabulated in Table 22 and Figure 17.

The high survivorship pattern of Cohort II and the low survivorship pattern of Cohort IV are almost identical in the two populations. In Pop A, the pattern of high survivorship of Cohort II is distinct from that of all other cohorts. The patterns of survivorship of Cohorts I and III are almost identical and show higher survivorship at all ages than those of Cohorts III and IV.

In Pop B, the patterns of survivorship of the founder (I) and of the last cohort (V) are almost identical for the four ages they have in common. The survivorships of Cohorts II, III and IV do not differ significantly at 6 and 12 weeks of age, though they are all significantly lower than the corresponding survivorship percentages of Cohort I. At 30, 36, and 41 weeks the survivorship of Cohort II is like that of Cohort I and their values are

Table 20 Population Dynamics: Survivorships of different cohorts

	Year	1963	1964						1964	1965
	Census Month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov	Mar
	Interval in weeks	0	6	4	5	4	6	13	12	16
Pop A	Cohort VII								100.0	-
	VI							100.0	86.0	-
	V						100.0	76.3	73.0	-
	IV					100.0	75.6	71.4	61.3	-
	III				100.0	95.8	86.1	80.0	81.8	-
	II			100.0	95.2	96.8	97.6	98.4	91.3	-
	I	100.0	93.7	89.9	81.0	83.5	79.8	77.2	68.5	-
Pop B	Cohort V						100.0	89.8	78.5	74.7
	IV					100.0	78.1	73.4	71.9	64.0
	III				100.0	86.8	78.8	70.7	67.7	67.7
	II			100.0	84.6	83.2	81.1	80.4	77.7	66.5
	I	100.0	93.5	92.4	88.4	85.9	82.0	78.0	71.8	65.4

Table 21

Population Dynamics:
Survivorships of older vs. younger cohorts

Pop A	Older Cohort	Younger Cohorts with less survivorship
	Cohort I	Cohort IV
	II	III, IV, V, VI
	III	IV, V
Pop B	Cohort I	Cohort III, IV
	II	III, IV

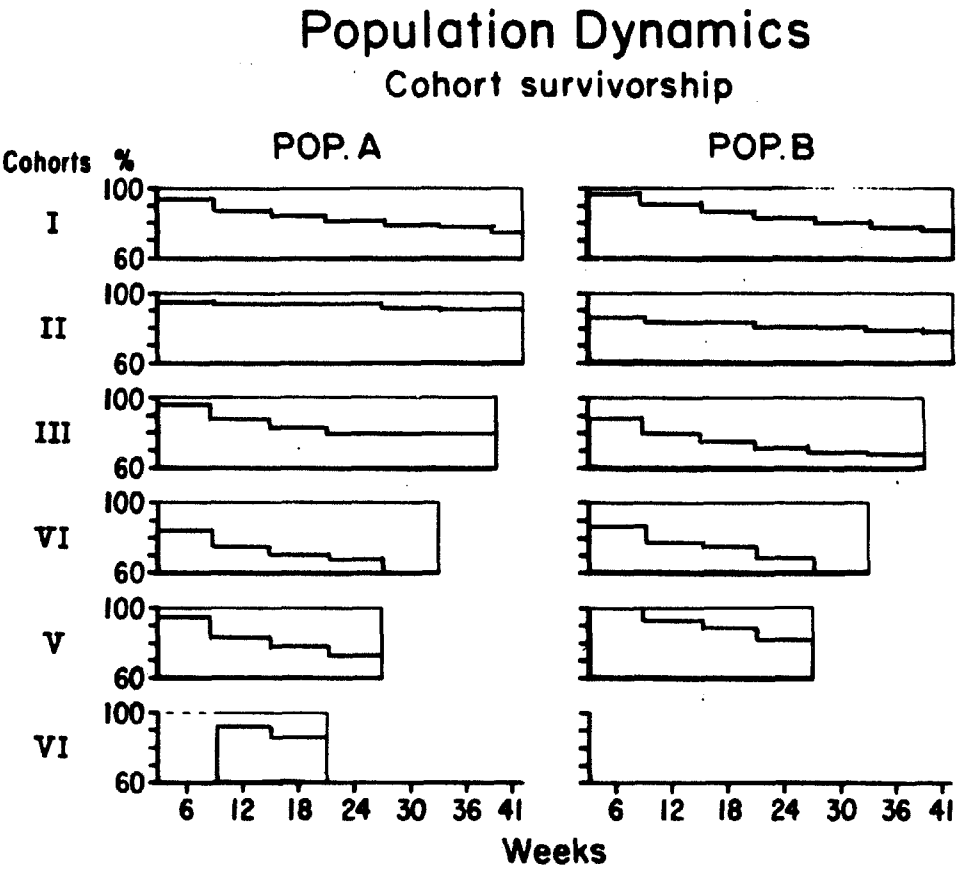

Population Dynamics
Cohort survivorship
POP. A
POP. B
Cohorts %
I
II
III
VI
V
VI
100
60
6 12 18 24 30 36 41
Weeks

Figure 17

Table 22

Population Dynamics: Mortality

survivorship of different cohorts at specific ages

	Cohort	I	II	III	IV	V	VI	VII
	Age (wks)							
Pop A	6	94	95	96	83	94	-	-
	12	88	94	88	74	83	93	-
	18	84	94	83	71	76	86	-
	24	81	93	80	68	73	-	-
	30	79	92	80	62	-	-	-
	36	78	92	80	-	-	-	-
	41	75	91	-	-	-	-	-
Pop B	6	97	85	88	86	98	-	-
	12	91	82	79	77	93	-	-
	18	87	81	75	75	88	-	-
	24	83	81	71	69	82	-	-
	30	80	80	69	62	-	-	-
	36	79	79	68	-	-	-	-
	41	76	78	-	-	-	-	-

significantly higher than those of Cohorts III and IV.

The mortality of males is greater than that of females. In Pop A, the death rates are 15.56/100 males and 10.18/100 females. If the original mice are left out of the calculations (since they were not born in the population), the rates become 14.74/100 males and 8.41/100 females, approaching a 2:1 ratio. In Pop B, where the numbers of males and females were equal, the death rate noted is 20.05/100 males and 14.25/100 females and when the original mice are excluded, 19.78/100 males and 12.24/100 females

The death rates per 100 sexed adults are lower than those calculated for total cohorts which include a certain number of unsexed juvenile animals, especially in Pop B (Table 23). This suggests that the mortality of juvenile mice was relatively higher than that of adult mice.

Table 23. Population Dynamics : Mortality

Mortality rates of adults and juvenile mice (per hundred)

	Sexed adults	All recruited mice
POP A	13.05	22.05
POP B	17.15	26.05

The Sex Ratio

There were 1991 males and 1933 females among the total of 3924 mice of the three study groups combined. This represents a percentage of males of 50.74, certainly within the limits of a theoretical 50:50 male-female ratio (Table 24).

The proportion of males and females among the 2001 mice of the two freely growing populations combined is 1037 males and 964 females compared with 983 males and 940 females of the total of 1923 mice of the control group. The percentages of males of Pop A plus Pop B of 51.80% and of Pop C of 49.60% are not significantly different from one another and both percentages fall within a theoretical 50:50 distribution.

Table 24

Sex Ratio of Mice Recruited
into Pop A, Pop B, Pop C

Including Cohort I				
	Males	Females	Total	% Males
Pop A + B + C	1991	1933	3924	50.74

	Pop A	Pop B	Pop A + B	Pop C
Males	623	414	1037	954
Females	550	414	964	969
Total	1173	828	2001	1923
% Males	53.1	50.0	51.8	49.6

Excluding Cohort I				
	Males	Females	Total	% Males
Pop A + B + C	1871	1818	3689	50.72

	Pop A	Pop B	Pop A + B	Pop C
Males	583	374	957	914
Females	511	376	887	931
Total	1094	750	1844	1845
% Males	53.3	49.9	51.9	49.5

But this comparison is misleading, since the percentage of males of Pop A and Pop B are dissimilar. The percentage of males in Pop A is 53.11, whereas Pop B has exactly a 50.00% ratio. The difference between the percentages of males of Pop A and Pop B is not statistically significant. But the difference between the percentage of males in Pop A alone and a theoretical 50:50 sex ratio is statistically significant. The difference between the percentages of males of Pop A and of Pop C also now shows statistical significance.

These differences are further accentuated when the mice of the founder cohorts, whose sex ratios were fixed at 50:50, are subtracted from the three groups. As a result, the ratio of males rises further in Pop A and falls in Pop B and C.

The proportion of males in Pop A as a whole fluctuates around 50% during the first four censuses, rises to a peak level of 53.58% at the end of six months, and declines slowly thereafter. This is due to greater male than female attrition in Cohorts III-VI, for Cohorts I and II show a steady rise in the proportion of males during this period. (Table 25, Figures 18, 19, 20).

Table 25 Pop A: Numbers of males and females

Census interval		Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov
Weeks after start		6	10	14	19	25	38	50
					MALES			
	Cohort							
1964	VII							64
	VI						173	137
	V					100	90	86
	IV				80	(100)	95	77
	III			52	84	(79)	75	72
1964	II		58	62	63	(64)	65	61
1963-64	I	37	37	31	31	(31)	32	29
	All cohorts	37	95	145	258	374	530	526
					FEMALES			
	Cohort							
1964	VII							49
	VI						148	139
	V					85	93	89
	IV				89	(85)	80	74
	III			61	74	(63)	57	63
1964	II		56	58	59	(59)	59	54
1963-64	I	37	34	33	35	(32)	29	26
	All cohorts	37	90	152	257	324	466	494
					Pop A: Percentage of Males (% Males)			
	Cohort							
1964	VII							56.6
	VI						53.9	49.6
	V					54.1	49.2	49.1
	IV				47.3	54.1	54.3	51.0
	III			46.0	53.1	55.6	56.8	53.3
1964	II		50.9	51.7	51.6	52.0	52.4	53.0
1963-64	I	50.0	52.1	48.4	47.0	49.2	52.5	52.4
All cohorts	M/total	20.0	51.3	48.8	50.1	53.6	53.2	51.6

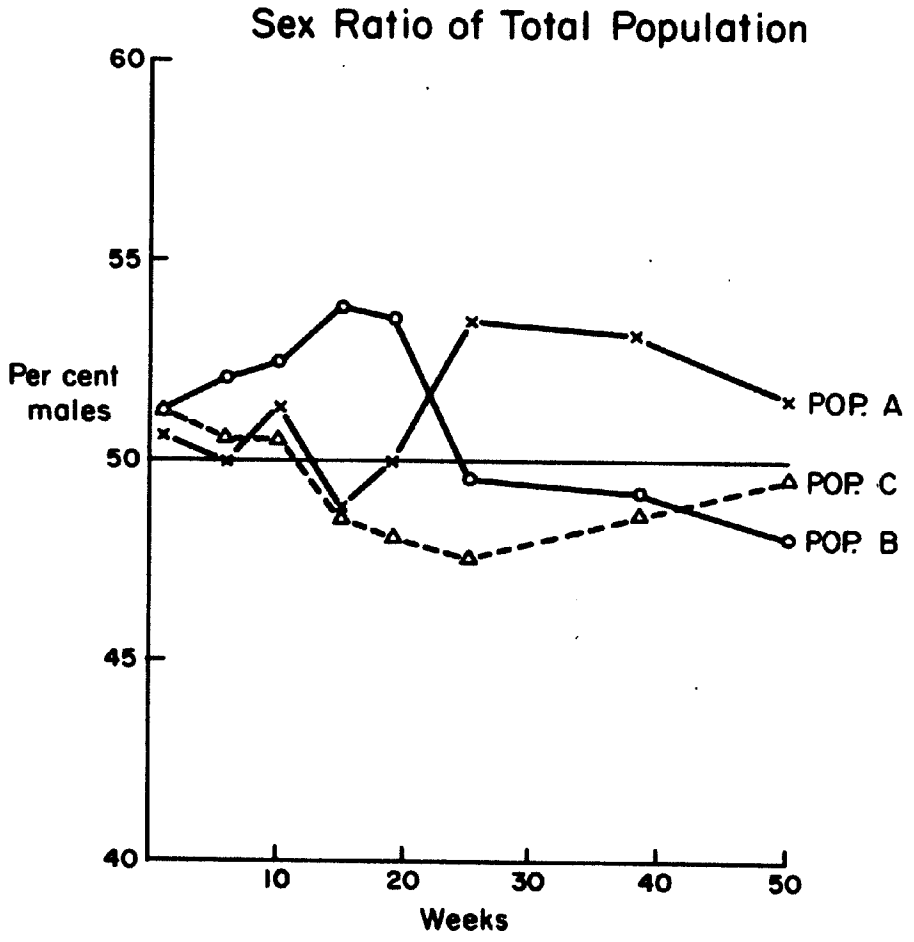

Sex Ratio of Total Population
60
55
50
45
40
Per cent
males
10
20
30
40
50
Weeks
POP. A
POP. C
POP. B

Figure 18

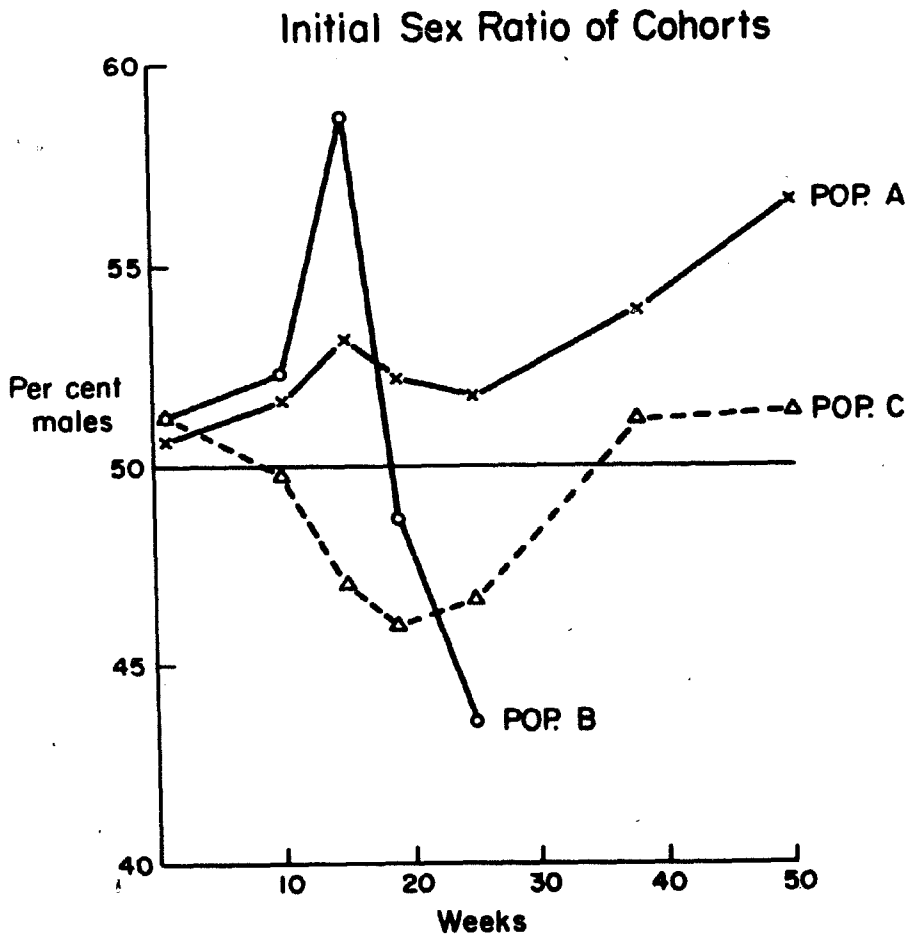

Initial Sex Ratio of Cohorts
60
55
50
45
40
Per cent
males
POP. A
POP. C
POP. B
10
20
30
40
50
Weeks

Figure 19

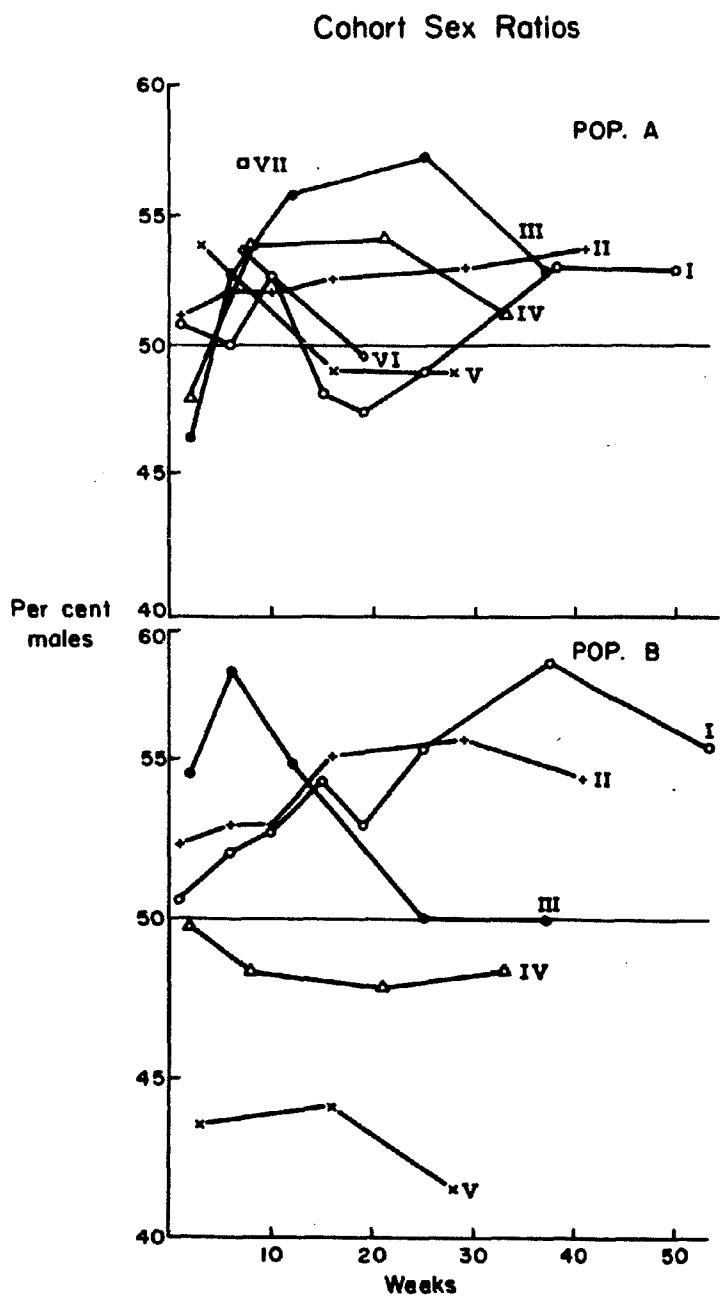

Cohort Sex Ratios
POP. A
POP. B
VII
III
II
I
IV
VI
V
Per cent males
60
55
50
45
40
10
20
30
40
50
Weeks

Figure 20

The sex ratios of the cohorts of Pop B show much greater heterogeneity than those of Pop A. The initial percentage of males rises successively in each of the first three cohorts, reaching a peak of 58.72% males in Cohort III. The percentage of males rose further in Cohorts I and II, but fell to 50% in Cohort III. The initial percentage of males in Cohort III and IV are both below 50% (49.73% and 43.58%, respectively) and both decline further. The changes in the proportion of males in Pop B as a whole of course reflects the changing sex ratio in cohorts—a steady rise during the first 4 months, a sudden fall at 6 months, and a gradual decline thereafter. The relatively large sizes of Cohorts IV and V explain the fall of the male ratio to below 50%. During the last six months, the declining proportion of males represents greater male than female deaths, since no new mice were recruited. (Table 26, Figures 18, 19, 20).

The sex ratios for Pop C as a whole were calculated assuming 100% survival of all cohorts throughout the study (Tables 27, 28). The sex ratio of Pop C parallels that of Pop A during the first four months but differs from both experimental populations thereafter, especially during the last six months when the proportion of males rises steadily.

Biomass

The biomass of the population as a whole, of males and females, of mice of various cohorts, and of certain groups of individuals differing by given traits, were calculated from the weights obtained at each census.

Population Biomass

The sum of the weights of the 74 mice of the founder cohort of each population was about 120 to 150 grams shortly after birth on December 6, 1963. Four and one-half months later (mid-April census) the total weight of mouse "protoplasm" was over 10,000 grams in each enclosure (Tables 29, 30). One year after the start of the study, the biomass of Pop A was 28,038 grams and that of Pop B was 22,032 grams.

Population Biomass — first six months

Mouse protoplasm increased at an exponential rate in both enclosures in the five months that followed the attainment of sexual maturity of the mice of the founder cohorts. The increase of protoplasm runs parallel to the increase of numbers during this period (Figure 21). The parallelism between biomass and numbers applies to each population as a whole, as well as to males and females separately in each enclosure. Female and male biomasses differ

Table 26 Pop B: Numbers of males and females

Census interval	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov	Mar
Weeks after start	6	10	15	19	25	38	50	67
Cohort								
			MALES					
VI								unsexed
-							-	-
-						-	-	-
V					112	102	84	80
IV				93	(92)	90	89	75
III			60	101	(85)	70	60	67
II		68	64	63	(64)	64	61	50
I	38	38	38	36	(38)	36	31	'28
All cohorts	38	106	162	293	389	362	331	301
			FEMALES					
VI								unsexed
-							-	-
-						-	-	-
V					145	129	118	112
IV				94	(98)	98	95	89
III			50	71	(71)	66	66	67
II		62	57	56	(52)	49	51	45
I	35	34	32	32	(29)	25	25	24
All cohorts	35	96	139	253	395	373	355	337
			Pop B: Percentage of Males (% Males)					
VI								unsexed
-							-	-
-						-	-	-
V					43.6	44.2	41.6	41.7
IV				49.7	48.4	47.9	48.4	45.7
III			54.6	58.7	54.9	50.0	50.0	50.0
II		52.3	52.9	52.9	55.2	55.7	54.5	52.6
I	52.1	52.8	54.3	52.9	55.4	59.0	55.4	53.9
All cohorts M/total	52.1	52.5	53.8	53.6	49.6	49.3	48.1	47.2

Table 27 Control Population: Number of Mice

Year	1963	1964						1964
Census month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov
Weeks after onset	2	6	10	15	19	25	38	50
Cohort VII								546
VI							387	387
V						294	294	294
IV					100	100	100	100
III				289	289	289	289	289
II			229	229	229	229	229	229
I	75	75	75	75	75	75	75	75
Total	75	75	304	593	693	987	1374	1920

The underlined numbers indicate the initial sizes of each cohort. Only samples of mice of each cohort were kept, either as breeders or until the end of the study for special studies. There were no deaths among the control mice saved; hence, the initial number of mice in each cohort is repeated for each of the subsequent censuses as though all the mice had been kept and had remained alive.

Table 28

Numbers of Males and Females,
and % of Males in Pop C

	Males	Females	Total	% Males
Dec 1963	40	38	78	51.3
Jan 1964	38	37	75	50.7
Feb	152	152	304	50.0
Mar	288	305	593	48.6
Apr	334	359	693	48.1
Jun	471	516	987	47.6
Aug	609	705	1374	48.7
Nov 1964	952	968	1920	49.6

Table 29 Biomass: Pop A

	Year	1963 Dec	1964 Jan	Feb	Mar	Apr	Jun	Aug	1964 Nov
	Interval weeks		6	10	15	19	25	38	50
Cohorts	Cohort VII								2393
	VI							3270	6644
	V						3597	5116	5117
	IV					1554	-	5364	4499
	III				1668	3358	-	4421	4217
	II			855	2890	3394	-	4099	3735
	Cohort I		1711	2283	2239	2432	-	2200	1833
	Total		1711	3138	6797	10736	-	24475	28438
Males	Cohort VII								1454
	VI							1724	3610
	V						2024	2510	2699
	IV					725	-	2874	2457
	III				790	1802	-	2556	2408
	II			436	1405	1810	-	2141	2138
	Cohort I		912	1074	991	1039	-	1107	1001
	Total		912	1510	3186	5176	-	12912	15767
Females	Cohort VII								939
	VI							1546	3034
	V						1584	2606	2418
	IV					829	-	2495	2042
	III				878	1554	-	1865	1809
	II			419	1485	1784	-	1942	1597
	Cohort I		799	1209	1248	1393	-	1093	832
	Total		799	1628	3611	5560	-	11547	12671

Table 30 Biomass: Pop B

	Year	1963	1964						1964	1965
	Census month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov	Mar
	Interval in weeks		6	10	15	19	25	38	50	78
Cohorts										
	Cohort V						4559	6539	6070	-
	IV					2059	-	5706	5845	-
	III				1572	3585	-	4387	4326	-
	II			982	2823	3350	-	3666	3813	-
	I		1650	2373	2527	2460	-	2157	2078	-
	Total		1650	3355	6922	11457	-	20455	22032	21317
Males										
	Cohort V						2109	2961	2702	2784
	IV					999	-	2874	2943	2558
	III				866	2146	-	2353	2218	2416
	II			509	1414	1652	-	2012	2108	1571
	I		922	1126	1208	1141	-	1226	1137	953
	Total		922	1635	3488	5938	-	9426	11108	10282
Females										
	Cohort V						2450	3578	3368	3532
	IV					1060	-	2832	2902	2862
	III				706	1440	-	2034	2008	2136
	II			473	1409	1698	-	1654	1705	1588
	I		728	1247	1319	1319	-	931	941	917
	Total		728	1720	3434	5517	-	11029	10924	11035

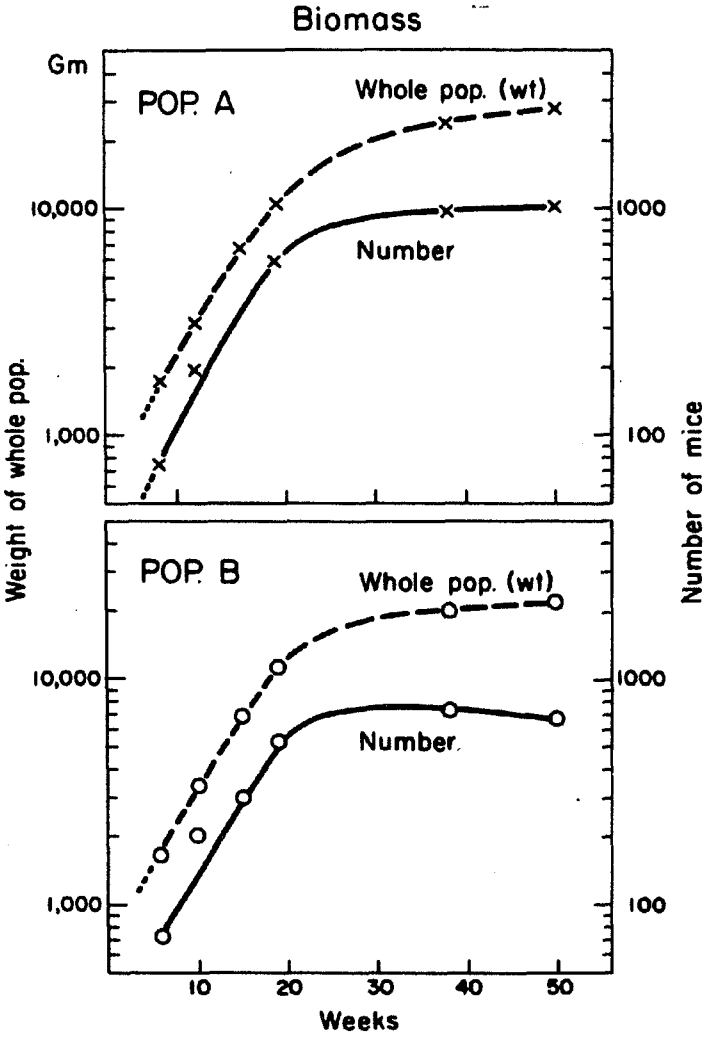

Biomass
Gm
POP. A
Whole pop. (wt)
10,000
1,000
Number
1000
100
Weight of whole pop.
Number of mice
POP. B
Whole pop. (wt)
10,000
1,000
Number
1000
100
10
20
30
40
50
Weeks

Figure 21

slightly but not significantly from one another. The biomass of females starts out at a lower level than that of males in both enclosures. The biomass of non-pregnant female mice tends to be lower than that of males throughout life and the near-equality of female and male biomass in the two populations is due to the large number of pregnant females during this period of rapid population growth.

Total Biomass

The total biomass of both populations increases much more slowly during the last six months. Pop A has a greater total and a more rapid rate of increase of biomass than Pop B but rates of increase of biomass have fallen in both populations from an average of 900 gm/week in the first six months to 360 and 300 gm/week in Pop A and B, respectively. Changes in population dynamics are basic to these changes in biomass. In this period, recruitment declined (Pop A) or stopped altogether (Pop B), and mortality rose. As a result, also, the age composition of the populations, especially of Pop B, shifted toward a greater proportion of older animals compared with the greater proportions of younger animals during the first six months; thus, relatively fewer mice in both enclosures had the rapid kind of birth rate typical for mice between birth and sexual maturity.

In Pop A, the biomass of males exceeds that of females, due to the greater number of males and to the diminishing number of pregnant females. In Pop B, on the other hand, the biomass of females is greater at first and later equal to that of males, due to the relative increase in the proportion of females and to the moderately high pregnancy rates.

Growth

Males. The pattern of growth of males of all cohorts consists of an initial period of rapid growth lasting about 10 weeks; this levels off but the mean weights of males continue to rise slowly thereafter.

Except for one cohort in Pop A, there are no significant intercohort differences in the mean weights of males of the same ages (Figure 22). This applies both to comparisons of cohorts within and between the two populations.

Cohort VI of Pop A (June-August, 1964) is the exceptional cohort and the mean weights of its males (and females) at six and sixteen weeks of age are significantly lower than those of other cohorts. Growth of the mice of Cohort VI may have been inhibited, or the mice may all have been born during the last six weeks of the census interval rather than uniformly throughout

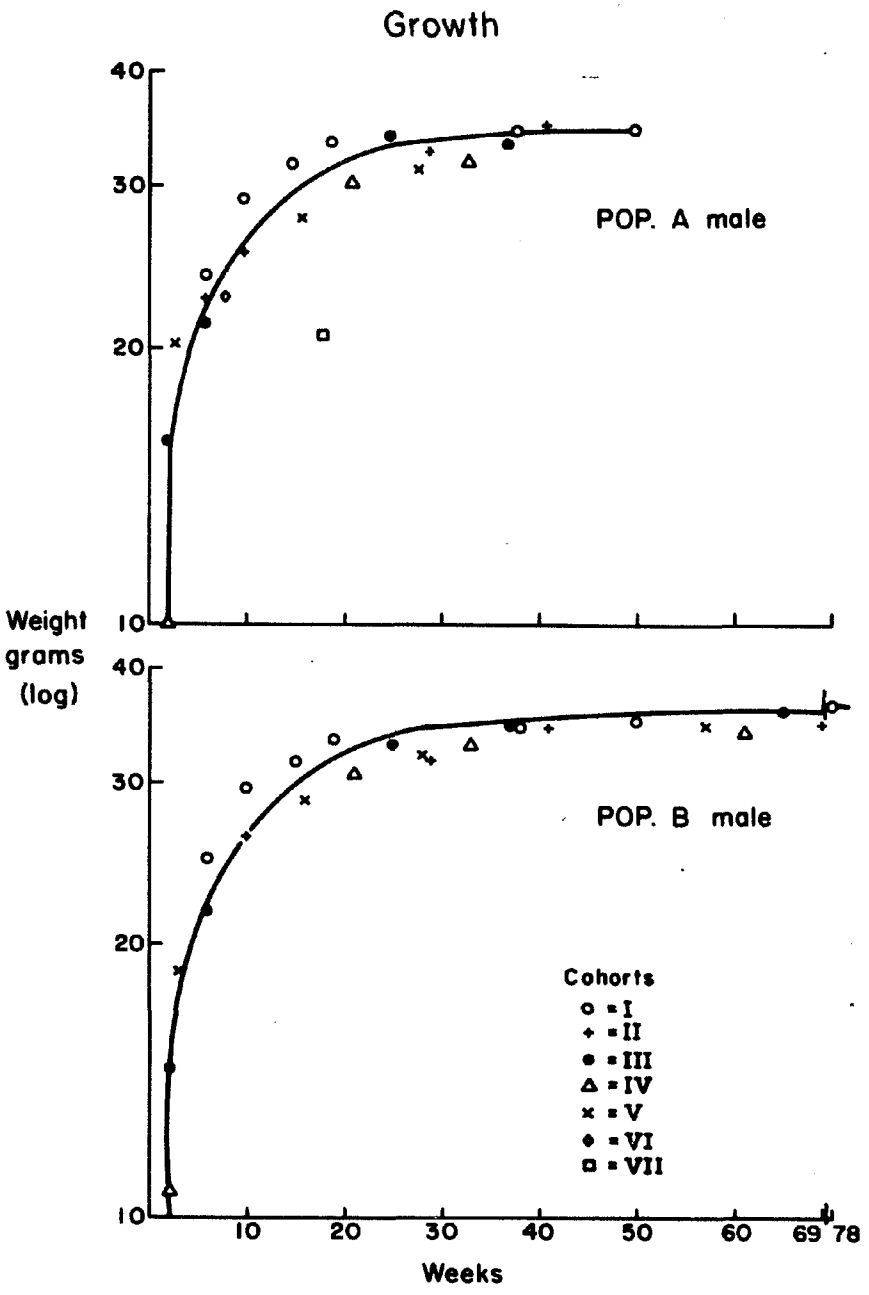

Growth
40
30
20
10
POP. A male
Weight
grams
(log)
40
30
20
10
POP. B male
Cohorts
o = I
+ = II
● = III
△ = IV
× = V
◆ = VI
□ = VII
10
20
30
40
50
60
69
78
Weeks

Figure 22

the census interval and hence be younger than assumed. The weights fall right in line with weights of other cohorts if the two mean weights available are each shifted six weeks backwards.

Females. The mean weights of females of all cohorts also increase rapidly during the first 10 weeks of life (Figure 23). The mean weights of the females of Cohort II lie above those of the females of the founder cohort at six weeks of age because of the occurrence of early pregnancy in Cohort II. The mean weights of the males of Cohort II by contrast lie slightly below those of the males of the founder cohort at six weeks.

The mean weights of females of corresponding cohorts of Pop A and B at corresponding ages do not differ significantly except for the weights obtained at the last census. In Pop A, the mean weight of females of all cohorts falls at the last census mainly because of the marked decrease of pregnancies. In Pop B, the situation is different; females of the founder cohort showed a slight fall in weight at the August census but the mean weights of the females of all other cohorts continue to rise due to the persistence of moderate pregnancy rates in this population. (The additional rise in the mean weight of females of all cohorts at the census of March 1965 is associated with a further increase of pergnancies at that time).

The mean weights of females of different cohorts within each population differ more from each other than male weights of corresponding cohorts. Plotted according to age, the curves of mean weights of cohorts lie one above the other—the founder cohort on top and successively younger cohorts below. These differences are correlated with pregnancy rates, heavier mean weights being, of course associated with higher pregnancy rates.

The Control Group

The mean weights of the control mice of the founder cohort were lower than those of the corresponding cohorts of Pop A and B at six weeks of age (Table 31). Samples of mice of Pop C of each sex and of each cohort were weighed at the last census (Table 32). All but the mice of the youngest cohort had been housed throughout as male-female breeding pairs; the mice of the youngest cohort had not been mated yet at the time of the last census. The mean weights of all control males except those of Cohort VI are significantly lower than those of the population mice. Control males of Cohort VI are significantly heavier than their population counterparts.

The control females, on the other hand, with the exception of the youngest cohort, were significantly heavier than the mice of the populations

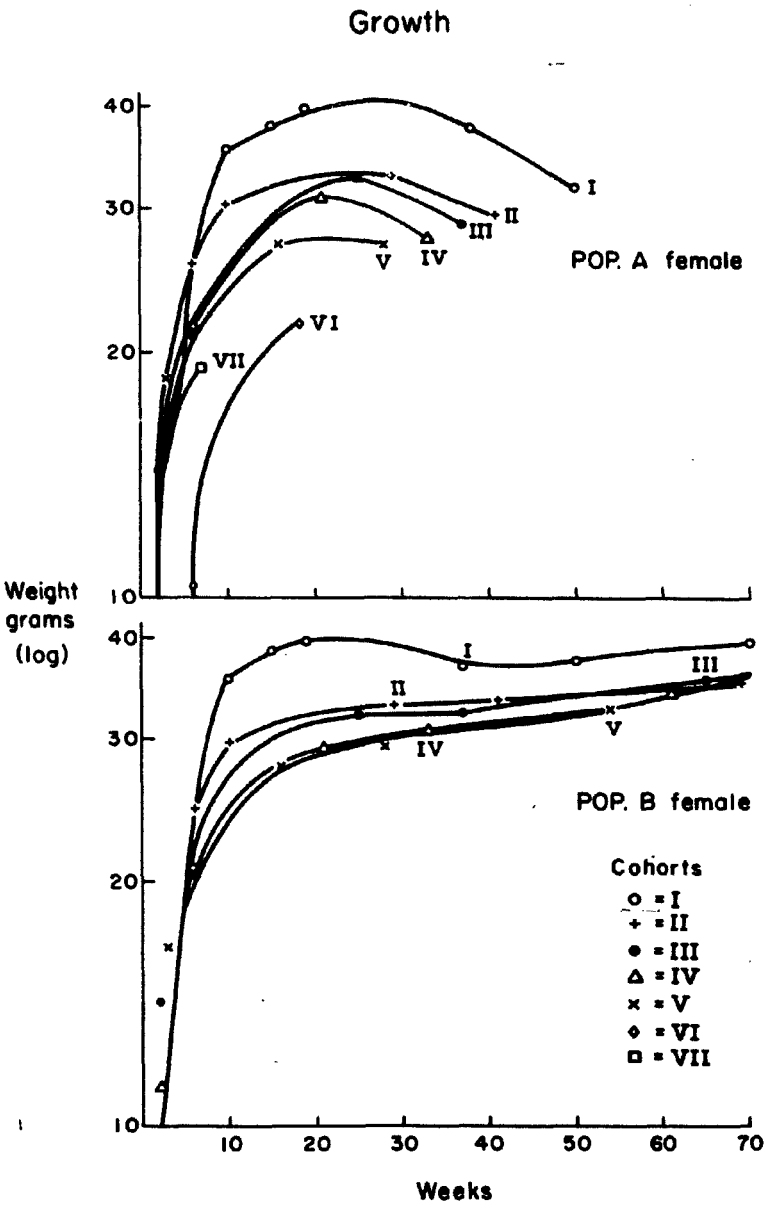

Growth
40
30
20
10
I
II
III
IV
V
VI
VII
POP. A female
Weight grams (log)
40
30
20
10
I
II
III
IV
V
POP. B female
Cohorts
o = I
+ = II
● = III
Δ = IV
× = V
◇ = VI
□ = VII
10
20
30
40
50
60
70
Weeks

Figure 23

Table 31

Weights of Mice of Founder Cohorts
of Pop A, B, and C at 6 Weeks of Age

	Pop A*		Pop B*		Pop C*	
	mean	±S.E.	mean	±S.E.	mean	±S.E.
Males	24.00	0.62	24.92	0.48	21.27	0.33
Females	21.59	0.34	20.80	0.33	18.72	0.34

*Pop A and C—37 males and females; Pop B—39 males, 35 females

Table 32 Weights of Mice of Pop C at Last Census (Nov. 1964)

Pop	Cohort		Males				Females			
			mean	±S.E.	N		mean	±S.E.	N	
C	II & III		30.85	0.91	20	*	39.26	1.52	20	
A	II	*	35.05	0.34	61		29.57	0.54	54	
A	III	n.s.	33.44	0.49	72		28.71	0.43	63	
C	IV & V		30.47	0.65	20	*	39.35	2.66	20	
A	IV	*	31.91	0.33	77		27.59	0.19	74	
A	V	n.s.	31.38	0.28	86		27.16	0.39	89	
C	VI	n.s.	26.21	0.47	20	*	38.24	2.33	20	
A	VI		26.35	0.22	137		21.82	0.27	139	
C	VII		20.64	0.35	20		17.46	0.05	20	
A	VII	*	22.72	0.46	64	*	19.16	0.45	49	

* = statistically significant at $p \leq 0.05$ and placed next to higher mean

n.s. = not statistically significant

at the last census. The females of the youngest cohort had not been mated at the time of the last census, and weighed about the same as the population mice; most of the other control females were heavy because they were pregnant.

Observations of Behavior

December 10-26, 1963. (60 observation periods per enclosure)

The tops of nest boxes in the population cages were kept open; both population and control litters were examined daily. There were no detectable differences in the behavior of young mice of the three study groups. Movement out of nest boxes in the enclosures or nest areas of control cages first occurred when young mice attempted to continue nursing as the adult female left the nest. Soon the young too moved in and out of nest boxes on their own, and spent longer periods of time away from the nest area where they returned to nurse

Activities of young mice included: sniffing the sides of nest boxes, attempts to climb up the sides of cages, sniffing each other and the adult females, and increasing self-grooming. In both control cages and population environments, young mice had sudden outbursts of running and hopping, a type of behavior characteristic for mice of this age. Young began to eat solid food pellets and to drink from water bottles.

December 26, 1963 to mid-January, 1964. (62 observation periods)

All mothers were removed from the population and control cages on December 26, 1963, and the sealed entrances to the central area were opened. The weaning mice went to the central food hopper within a day after the mothers had gone. To orient mice to the food source, pellets were strewn on the floor in trails leading to the central food hopper for two days. The young mice used the water fountain above the food hopper, climbing on the ramps leading to the platform or going straight up the sides of the food hopper. Large numbers of mice, 20 to 40, came to the central feeding area in the early morning (7 and 10AM) and late afternoon hours (6 and 9PM); between these times, rarely more than one or two mice were ever seen in the feeding area. Mice left the peripheral area only to eat and drink.

The mice were able to climb the pertitions separating peripheral retreats and gain access to the roofs (placed back on December 26, 1963) of the nest boxes. Frequently, mice, almost exclusively males, crossed from

retreat roof top to retreat roof top all around the enclosure. The average time for one "revolution" around the roof tops of the enclosures was 15 seconds and some mice kept going around and around as long as 10 minutes at a time. Often, several mice were engaged in this activity at the same time.

Checks of occupancy of nest boxes, carried out before 7AM or after 10PM, revealed non-random distribution of mice. Often, three or four boxes of mice contained 10-15 mice each with 5-6 more mice in the retreat area, while other boxes and retreats contained no mice at all. In the large groups, about three-fourths of the mice were females.

Social behavior displayed by mice at this time was quite characteristic of the species. Animals stopped as they approached one another, directed the head or stretched the entire forepart of the body towards the other individual, sniffed, approached closer, stopped, approached again, touched noses, sniffed about the head or ano-genital area and moved closer together. Individuals stood quietly together for awhile, groomed each other, mated, or began to fight; or they moved away from each other, went to the food hopper, or back to a retreat, etc...

Aggressive interaction between mice began at about the same time in both enclosures when the animals were five weeks old. At first these were really competitions for a food pellet that an individual mouse had pried out of the food hopper. One to three mice would be involved in these "competitions," pulling the pellet away from the mouse holding it; the mice pushed each other or chased each other around the food hopper and around the roof tops of the nest boxes. Towards the end of the census period, mice began to "wrestle" with each other after a few minutes of the chase, to bite each other in the flanks, tumbling over as they held each other.

A few more typical mouse fights unrelated to competition for food also occurred. Males approached one another sideways, circled, rattled their tails and trembled, held on and bit each other, and stopped as one mouse reared up on its hind legs in the submission posture, or froze or fled. An aggressive type of grooming, often preceding fights, was noted; a mouse, vigorously, would "lick-bite" another mouse that crouched without movement.

Brief sniffing about the ano-genital area was a frequent component of encounters between mice as early as the fourth week of age. During the fifth week of age, such sniffing, especially by males of females, became more prolonged. Some females stopped and raised their hindquarters on such occasions. Abortive attempts to mount were first recorded during the fifth week

and characteristic prolonged mounts with copulatory thrusts and intromissions during the sixth week.

Mid-January to mid-February 1964. (62 observation periods)

Marked changes occurred in aggressive and reproductive behavior in the population enclosures during this period. Aggressive interaction reached a peak frequency during the first ten days (Table 33). Competition over food pellets continued and sometimes "spilled over" into full blown fights, but, in general, fights were unrelated to food. There were many long fights, up to a minute of uninterrupted fighting between two males, during the early part of the month. Later in the census interval, fights tended to consist of 10-15 second flurries. Some of the males had bloody wounds about the hindquarters and tails after the fighting.

Table 33

Social behavior in freely growing populations of mice
Aggressive interactions in the freely growing populations:
mid-January to mid-February 1964

	Numbers of A.I. per male*			Total adult males
	Days 1-10	Days 11-20	Days 21-30	Days 1-30
Minutes observed	200	200	200	
Pop A	36	22	14	37
Pop B	41	24	12	38

Each enclosure was observed for two 10-minute periods each day and the total number of aggressive encounters for each 10-day period multiplied by 3/10. The specific times for observations were rotated randomly so that observations were made during hours from 8 AM to midnight at least once a week.

*The number of post-weaning males in each enclosure remained constant throughout the census interval— 37 males in Pop A, 38 males in Pop B.

"Chain" reactions involved several males in fights. At times, females were attacked during such "chain" fights but the attacking male would stop immediately and move away. The briefer duration and decreasing frequency of fights towards the end of the census interval was related to the increasing numbers of males who instantaneously retreated when threatened.

Exceptional males could be identified because of their unusual locations in the enclosures and by distinguishing features, such as pattern of wounds, coat color, etc. Some of these males spent most of the day and night lying in specific parts of the central area; although they appeared never to attack, they fought back vigorously when attacked, and then returned almost immediately to their usual spot.

A rise in the frequency of successful mating occurred at the beginning of the census period. Although the "queuing" type of sexual activity seen later was not yet noted— chases and repeated brief mounts of a female by packs of six to ten males— a second male would often mate with a female that had just completed copulation, mounting during the period of grooming that characteristically follows mating.

Females showed variable sexual receptivity, ranging from those that made no attempt to avoid being mounted and did not move during copulation, to those that assumed the upright defense posture of fighting males and then fled. Whether this variability was related to the stage of the estrus cycle of particular females or reflected intrinsic behavioral variability was not investigated.

Most females in the population enclosures were visibly pregnant during the last 10-12 days of this census period and many gave birth. Concomittantly, mating activity increased.

In both cages, females often carried newborns from one nest box to another. A single female might carry 15 babies in succession from one to another specific nest box, but some females took newborns to several different nest boxes. No babies were left in the central area. Females often attempted to take babies from each other's mouths; this did not appear to injure the babies.

The constant movement of young animals was a prominent feature of the large nurseries of the population enclosures. The motion of newborns in these nurseries lasted all day as 15 to 40 babies pushed each other to start or continue nursing from the one or two females in the nest box. By contrast,

the much smaller number of babies in control litters would lie quietly for long periods during the day.

By the end of the census interval, there were nurseries in most nest boxes (14 in Pop A, 15 in Pop B). The nests consisted of paper strips arranged in interlacing nets around a central depression in the sawdust; in some nests, the paper was packed loosely up to the roof of the box.

In general, most mice still came into the central area of the enclosures only to eat or drink— mainly during the early morning or late afternoon hours. Many males now spent considerable periods of time on the roofs of the nest boxes and even slept there. Some males also slept in nest boxes, often straddling babies in the typical position usuallly assumed by females.

Mid-February to mid-March 1964. (62 observation periods)

A new crop of babies was born in the enclosures during the third week of this period. Although some of the mice born during the previous census interval were already eating at the food hopper, many did not stop nursing, and the nurseries were crowded with two unequally "matched" and competing cohorts. The movement of young animals in the nurseries was incessant. Newborns were often carried from nursery to nursery as before and occasionally males participated in this activity.

Nurseries were present in all nest boxes except for one or two boxes in each enclosure used predominantly as "toilets." Each peripheral retreat area had accumulations of feces and urine in a particular site, e.g., a corner, but in addition large piles of excreta gradually accumulated and filled certain nest boxes— the "toilets."

The form and frequency of aggressive interactions changed little until the last week of the census interval, when males of the founder cohort began attacking males of the first cohort born in the enclosures. This took place seemingly by chance during "chain-reaction" fights among older mice at first, but soon young males were attacked without relation to "chain reactions." Such attacks were slightly more frequent and longer-lasting in Pop B than in Pop A; the attackers were more persistent in chasing and repeatedly attacking the younger mice. In both enclosures, some males of Cohort II, characteristically larger than other males of the cohort, fought back vigorously when attacked.

During the last week of the census interval, several instances of mating between males of the founder cohort (I) and females of Cohort II were

seen. The partners of such matings were disproportionate in size; copulation tended to last longer (longer than one minute on each of four occasions) than that between males and females of the same age (less than a minute on four occasions).

Mid-March to mid-April 1964. (58 observation periods)

The amount of "traffic" and activity in the central area was still greatest in the early morning and late afternoon, but was now also considerable between these activity peaks. During the early morning and evening hours the mice at the feeding box were primarily females, old males, and the youngest mice. Other males were observed eating throughout the day and night. Mice in control cages continued to eat during the usual times of peak activity.

A few groups of males, old and young, huddled for long periods of the day in the corners of the central area in both enclosures, often surrounding themselves with shreds of paper; many ran to the peripheral retreats during fights and returned later. Large aggressive and older, non-attacking but fighting males also slept on the floor of the central area. Very few females remained in the central area after eating or drinking.

Fighting tended to occur in concentrated bouts lasting up to 2-3 minutes, but during many of the observation periods there was no fighting. Fights usually involved males of the founder cohort, but also included males of the first two cohorts born in the enclosure. Attacks, on several occasions, were not preceded by any of the usual threat movements; a male simply pounced on another male from behind. In Pop A, five pregnant females were attacked, one repeatedly. Females did not fight back.

On four occasions in Pop B only, an adult male in a peripheral retreat attacked most mice that entered or attempted to enter the retreat area. This kind of territorial behavior lasted between one and four days. In each case, certain males, females and babies remained in the particular retreat and were not attacked.

Many of the pregnant females of Cohorts I and II gave birth in both enclosures. Sexual "queuing" by packs of males involving small females was noted twice in Pop B. Females were chased by males into nest boxes, and nurseries were disrupted as mating took place in the nests.

Some nurseries were located outside of nest boxes in the peripheral retreats. An impression that the number of albino females present or nursing

in nurseries was out of proportion to the total number of albino females, and that many nurseries had only albino females was confirmed by careful nest box surveys (Table 34).

Table 34

Social behavior in freely growing populations of mice

Coat color of adult females in nurseries

		Nurseries				Population as whole		
Pop	Surveys	No. nurseries	Albino females	Non-Alb. females	% Alb.	Alb. females	Non-Alb.	% Alb.
Pop A	#1	12	13	4	77	32	61	34
	#2	10	15	7	68	32	61	34
	#3	17	20	8	71	32	61	34
	Total	39	48	19	72	32	61	34
Pop B	#1	12	16	6	73	38	51	43
	#2	16	11	9	55	38	51	43
	#3	14	13	7	65	38	51	43
	Total	42	40	22	65	38	51	43

The number of albino and non-albino females present or nursing in nurseries was checked in surveys of nest boxes taken at 8 AM, 7 PM, and 9 AM, six, four, and two days before the April census.

Mid-April to beginning June 1964. (80 observation periods)

The number of adult mice increased rapidly in both enclosures. Many more mice now remained in the central area at all times (Table 35).

Table 35

Social behavior in freely growing populations of mice
Numbers of adult mice in central area of enclosures

		CENTRAL AREA					
	Total adults	8 AM	Noon	4 PM	8 PM	Mean	% in center
Pop A	513#	76	70	89	68	76*	15*
Pop B	527#	96	84	48	112	85*	16*

The numbers of mice in four diagonally opposite sections of the central octagon were counted and their sums doubled to obtain the tabulated values. Mice eating or drinking were excluded from the counts. The counts give approximations of the proportion of mice in the central area

#Approximate number of adult mice

*Rounded off to nearest integer

In many parts of the central area and in the peripheral retreats around the nests, mice piled up paper strips in stacks three to four inches high and huddled in tunnels and spaces under the paper. Although the upper surface of the paper was covered with moisture and feces, the paper tended to be completely dry and clean below, where the mice huddled.

Many of the mice, mostly males, often in small clusters in the central area, were conspicuous by their virtual immobility for long periods of time, e.g., half and hour. Many males of all cohorts remained predominantly on the roofs of the nest boxes and slept there. Other mice, mostly males but also some females, began to position themselves on the thin metallic partitions which divided peripheral retreats from each other and from the central areas. These animals even slept in this position and looked like roosting chickens, the fore and hind parts of their bodies straddling either side of the partition.

The majority of mice in the peripheral retreats and nests—females, babies, and young males— remained either on or under the surface of stacks of paper there (Table 36).

Table 36

Social behavior in freely growing populations of mice

Sex and cohort membership of adult mice in peripheral retreats

	Cohort	MALES No. Males*	% of retreat	% of total	FEMALES No. females*	% of retreat	% of total
Pop A	I	0	0	11.3	3	4.9	12.3
	II	1	4.3	23.4	12	19.7	25.7
	III	2	8.7	28.8	19	31.1	26.4
	IV	20	87.0	36.5	27	44.3	35.6
	Total	23	27.4	53.4	61	72.7	46.6
Pop B	I	0	0	13.0	6	11.5	11.6
	II	0	0	23.1	4	7.7	20.8
	III	1	5.6	30.7	23	44.2	28.4
	IV	17	94.4	33.2	19	36.6	39.2
	Total	18	22.5	52.6	52	77.5	47.4

*The numbers represent the sums of mice counted in four separate retreats— one on each of four successive days preceding the June census. There were about 500 adult mice in each population on the day of the census.

All forms of activity—eating, fighting, mating, sleeping, carrying of babies, etc.—occurred at almost any time of the day or night in the central area now. Mice no longer ran over roof tops. Two mice in Pop A and one in Pop B showed "circling" behavior, continuously "revolving" in circles of about 2-4 inches in diameter for the rest of the year.*

The "unusual" behavior exhibited by mice as individuals approach each other (stopping, sniffing, stretching of the forepart of the body, touching of noses, as previously described) often was not displayed now. Instead "social" encounters were characterized increasingly either by immediate "engagement" into an activity such as grooming, fighting, mating, etc., without the "fore" behavior, or mice would pass very close to one another or actually bump into one another without showing any reaction to each other. A series of such "unreactive" encounters by a specific individual would often suddenly be followed by the recurrence of interaction.

The frequency of aggressive interaction changed little during the course of these six weeks (Table 37).

Table 37

Social behavior in freely growing Populations of Mice.
Aggressive interactions, mid-April; beginning June 1964.

	Mean no. adult males	Aggressive interactions per hour Week 1	2	3	4	5	6	Mean
Pop A	266	24	20	16	19	2	24	17.5
Pop B	285	18	30	26	14	20	12	20.0

Numbers of aggressive interactions per hour represent the sums of aggressive interactions observed during four 15-minute periods at 8 AM, 12 noon, 4 PM, and 8 PM of each Wednesday during the six weeks.

*Mice showing "circling" behavior have been noted in other populations originally derived from the same four-way cross used in the present study—personal communication.

The number of fights per hour was lower than at the end of January 1964 and the number of fights decreased from one per hour per adult male to about 6 per hour per 100 adult males. During certain fights in both enclosures, aggressive males repeatedly attacked smaller or wounded males, continuing their attacks despite the upright position of submission assumed by the victim.

Obvious instances of territorial defense of a retreat now took place in both enclosures. One "territory" was maintained for six days in Pop B, the others lasted for periods ranging from half a day to three days.

Many females of all cohorts were pregnant, and litters were born daily; littering set off series of "queuing" chases by packs of males which lasted up to a half an hour. Such chases led, for instance, to more than fifty brief mounts as males repeatedly pushed each other off the same female. Vaginal bleeding was often noted afterwards. Attempts of males to mate with small females and other small males, and assumption of unusual mating positions with older females occurred in both enclosures.

For example, males mounted the front end or side of the female, or copulated in a ventroventral position, or two or more males clutched different parts of the female's body and simultaneously attempted to copulate.

Collections of squirming babies of various sizes piled up in nurseries; some nurseries were now in the central area in both enclosures. Young mice frequently were dropped in the central area or in corners away from nurseries.

Beginning June to end August 1964 (110 observation periods)

By this time, the entire floor of the enclosure was covered with stacks of paper 5-10 inches high. Groups of mice were observed in networks of tunnels and spaces at the bottom of the paper. Most of the nest boxes were either empty or occupied by only one or two mice; there were only two nurseries inside nest boxes in Pop A and none at all in Pop B; other nurseries were located in the retreats around the nest boxes. Three to four nest boxes in each enclosure were used as "toilets" by the mice. Observations of several prolonged fights, as well as episodes of prolonged "queuing"—mounting by packs of males in the nest boxes—suggested that adults might be remaining out of them because of difficulties of escape. Alternate nest boxes were removed from the enclosures and when the space provided was promptly occupied by mice, all the nest boxes were taken out (third week in August).

The enclosures of Pop A contained visibly greater numbers of new mice than that of Pop B. This impression was borne out by the censuses, which revealed that not a single newborn had survived in Pop B. Dead litters of newborns were seen in both cages and were more numerous in Pop B. Adult mice in both cages looked well-fed, had smooth fur; many males, especially older ones, had wounds or scars.

Activity in both cages had further decreased—in Pop A more than in Pop B. Many of the mice in the central area remained still for long stretches of time and could be identified during observations made several hours apart. The early morning and late afternoon peaks of activity were no longer distinguishable.

Differential distribution of males and females and of mice of different cohorts in the central and peripheral areas persisted. The high concentration of females in peripheral retreats and the presence of smooth-furred, entirely unscarred, fat, and usually young males with the females in these locations was striking. The occasional older male in these aggregates typically had well-healed, old scars, and was fatter than other males of the same age. By contrast, a large proportion of the animals found on the surface of the paper in the central area were males of older cohorts, especially scarred individuals. Lines of "roosting mice" slept on the partitions of the enclosures at all times.

Many instances of social interaction between mice now lacked "preliminary behavior." Another change noted was that mice often did not respond with expected, characteristic behavior in specific social situations. For example, a female, eating at the food hopper and suddenly mounted by a male, continued to eat while the male continued mating. Or again, a male attacked by another male failed to show patterns and postures either of attack or of submission.

Fighting was infrequent; there were fewer than ten fights per hour and often none at all during many of the 15-minute observation periods. Aggressive males at times went around the enclosure attacking and briefly biting a succession of other mice. "Chain reactions" of fighting often involved females and young animals.

Males sometimes terminated attempts to mate, or successful copulation with a female, or an episode of homosexual mounting, by a sudden attack (eleven such incidents in Pop B and one in Pop A during the 110 observation periods).

Chases and sexual "queuing" by several packs of males simultaneously were noted frequently and resulted in vaginal bleeding.

Males of Cohorts I and II were involved in episodes of territoriality 10 times in Pop B and twice in Pop A. Defended peripheral retreats were usually surrounded by a dozen or more mice, some of which repeatedly started to climb into the retreat only to be attacked and forced to flee.

There were pregnant females in both enclosures but nurseries with mice older than newborns only in Pop A.

Beginning September to end November 1964. (140 observation periods)

General features of the physical environment changed little during this census interval. The distribution of mice of different cohorts in the central and in the peripheral areas also remained similar to that noted at the end of the previous census interval.

Almost every inch of the surface of the enclosure of Pop A was covered with mice. Mice slept on the fountain platform and even on the ramps leading to it. In many of the peripheral retreats of Pop A, mice were packed so tightly that they often slept standing on their hind legs. Mice could scarcely move without bumping into others; the majority of contacts were not accompanied by visible social interaction.

Two mice in Pop A were found dead on the metal "roosting" partitions in the periphery. These animals had apparently died while asleep and rigor mortis had preserved the characteristic half-moon posture of "roosting" animals.

Many of the behavioral changes noted previously became accentuated, especially in Pop A. Even the posture assumed by many inactive mice often remained unchanged for long periods of time. Homosexual activity was observed between males of equal size, as well as between larger and smaller males, and at times included the full sequence of postures and movements of mating, even lasting as long as matings with females. Unusual postures during attempts to copulate with females and males were more frequent than before, especially during queuing episodes. For the first time, males copulated with pregnant females (noted 42 times in Pop A, 7 times in Pop B).

Fighting was recorded in Pop A during about one-half of the observation periods and slightly less often in Pop B. Females were attacked in both enclosures, especially when aggressive males were engaged in "making the rounds," i.e., briefly attacking many mice in quick succession. Pregnant

females were attacked about twice as often in Pop B as in Pop A.

Dead litters of newborn mice were seen in both enclosures almost every day. In Pop A, some mice were surviving beyond the first or second day of life, but no juvenile mice were visible in Pop B. The nurseries in Pop A were frequently disturbed by packs of males chasing females. Nursing mothers were attacked and forced away from nurseries. Again, a relatively large proportion of albino females was noted giving care to young. Frank cannibalism of live newborns by both females and males was observed for the first time in September 1964 in Pop B and recorded 32 times subsequently in this enclosure.

A copy of motion pictures of the experimental setting for these studies, of control cages and of the freely growing populations, is permanently stored in the Illustration Department of The Rockefeller University. Pop A (Figure 24) was disbanded after the November 1964 census, 50 weeks after the start of the experiment, and samples of mice were obtained for special studies of reproduction, behavior and pathology, which will be described. The remaining mice were sacrificed.

Figure 24 Pop A in November 1964. There were about 1,000 mice in the enclosure.

Genetics

Use of mice from crosses between inbread strains made it possible to constitute populations with known gene frequencies. Changes of allele frequencies at two gene loci associated with polymorphic traits were studied, namely, alleles at the "C" locus affecting coat pigment production, and alleles at the "Hb" locus determining hemoglobin protein structure.

The genetic basis, mode of inheritance, and relationship between phenotype and allele of both traits have been established clearly (132-137).

The phenotypes. C locus. Figure 25 shows genotypes of the inbred strains, F_1's and founder mice at the C locus. All mice were classified according to coat color at each census. Mice with black agouti, brown agouti, solid black, solid brown, dilute brown, and grey coats have either one or two of the dominant alleles at the "C" locus. Both albino (c) and chinchilla (c^{ch}) are recessive alleles and all three homozygous, recessive phenotypes (and genotypes) could be identified, i.e., cc, $c^{ch}c$, $c^{ch}c^{ch}$. The polymorphism at the "C" locus is discussed in terms of the dominant allele versus the recessive alleles combined.*

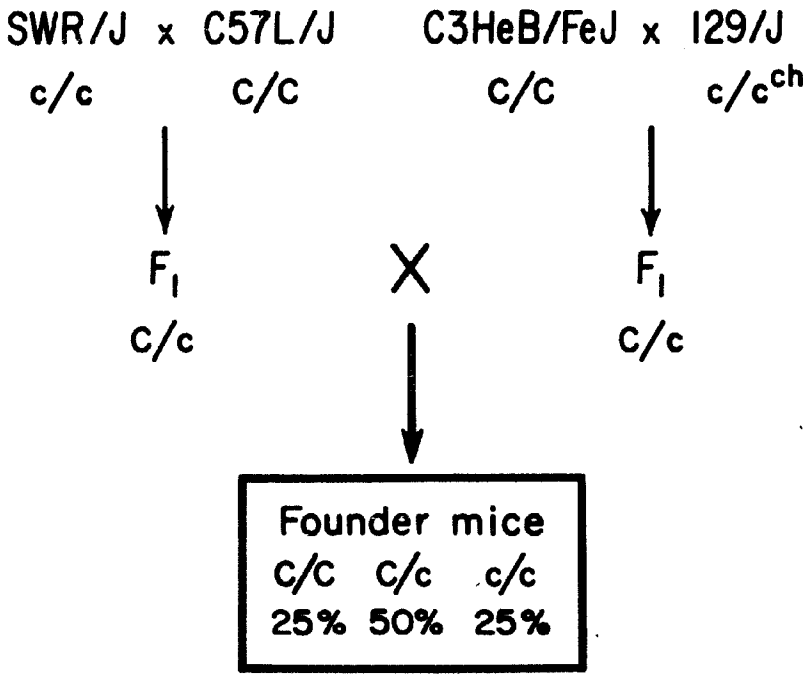
Hb locus. All mice of the founder population are heterozygous at the Hb locus (Figure 26). The three potential hemoglobin genotypes are: Hb^1/Hb^1, Hb^1/Hb^2, and Hb^2/Hb^2. They are distinguished both by the solubilities of their carbon monoxide derivatives and by their crystalline or amorphous characteristics after salting out (139-141). These methods permit the identification of all three genotypes.

Blood was obtained after the last census (November 1964) by cardiac puncture after anesthetizing the animals with chloroform and opening the thoracic cavity. About 1 cc of blood was collected from the first 200 mice using heparinized disposable Pasteur pipettes. The blood of each mouse was expelled into small plastic test tubes and centrifuged at 5000 rpm for 3 minutes. The plasma was removed, the red cells washed with 2 cc of isotonic sterile saline and centrifuged again. The supernatant was discarded and red cells and plasma were frozen and saved.

*A few homozygous chinchilla animals may have been missed since, in brown non-agouti genotypes, C/- and c^{ch}/c^{ch} are indistinguishable, but this was an unimportant source of error as there were so few non-agouti brown animals in any of the populations. The homozygous chinchilla genotype in black non-agouti mice can be separated from black animals having one or two dominant C alleles (138). Several mice classified as chinchilla were crossed with mice of inbred strains and in all cases the presumptive assignment of genotype was confirmed.

Genotypes of Founder Mice

"C" locus



Note: The symbol "c" is used to denote both recessive alleles, "albino"-c and "chinchillo"-c^{ch}

Figure 25

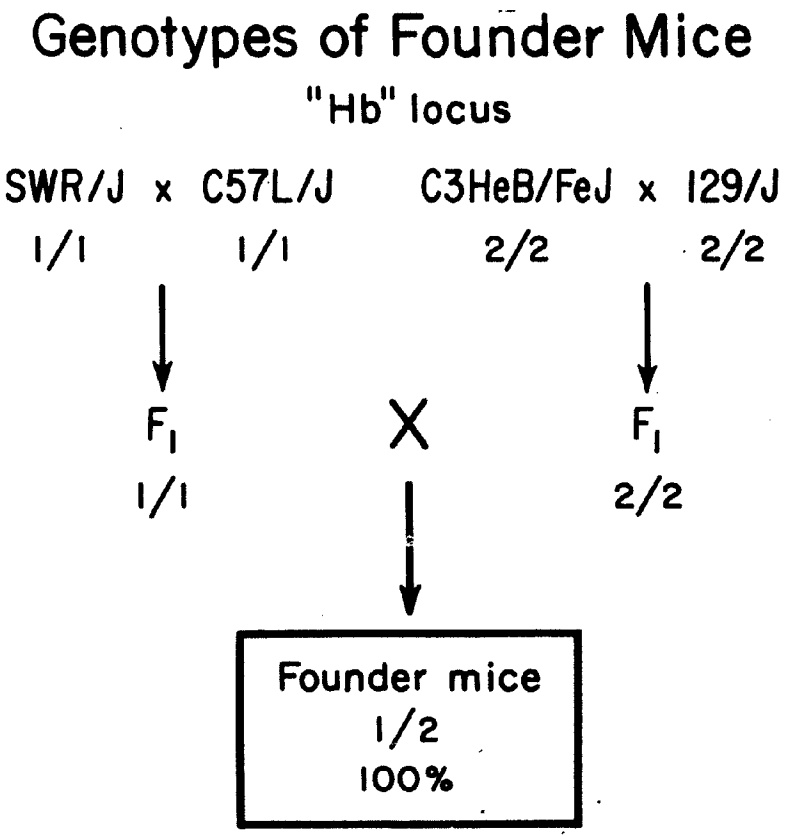

Genotypes of Founder Mice
"Hb" locus
SWR/J x C57L/J
1/1
1/1
C3HeB/FeJ x 129/J
2/2
2/2
F1
1/1
X
F1
2/2
Founder mice
1/2
100%

Figure 26

Blood from an additional 769 mice of Pop A, from those of Pop C, and from all mice used as standards, was collected directly from the heart into preheparinized microblood capillaries (Micro Blood Collecting tubes—Heparinized, Natelson type, Aloe Scientific Company, St. Louis, Mo.). The tip of each capillary is sealed with plasticene and the capillaries are centrifuged at 10,000 rpm for 5 minutes.

Red cells separate clearly from plasma and more than 95% of the tubes showed no hemolysis. Individually labeled tubes are placed vertically in the freezer immediately after centrifugation.

Pure red cells and plasma can be obtained at any later time by making appropriate nicks in the capillary tubes and breaking off the columns of the frozen red cells or plasma.

To 0.80 cc of deionized water, 0.02 cc of red blood cells are added and allowed to stand for ten minutes. Carbon monoxide is bubbled into the lysed red cell preparation for about 15 seconds. The solubility of the carbon monoxide hemoglobin is determined in K_2HPO_4-KH_2PO_4 buffer. The stock solution of buffer is prepared with deionized water to prevent crystallization and is 3.5 Molar with respect to phosphate (pH 6.8). Four ml of buffer are added to the solution of HbCO, changing the concentration of phosphate to 2.86 Molar. The tubes are inverted to mix and shaken gently to form a moderate head of foam to reduce evaporation and exchange with oxygen. The tubes are left in a water bath at 30°C for 21 hours; salting out may be incomplete at shorter durations and denaturation takes place at longer durations. The solution is filtered through 1 Whatman filter paper, and the optical density of the clear filtrate read immediately at 575 mμ in a spectrophotometer (Coleman Junior). The appearance of the hemoglobin which has been salted out is determined by examining a few drops of unfiltered solution on a slide in an ordinary microscope. The optical density and appearance of standard hemoglobins were checked routinely.

The mean optical density, the range of optical densities and the characteristics of the crystal or amorphous material examined in the microscope for each of the hemoglobin genotypes are displayed in Table 38.

Alleles at a different locus, the so-called solubility or Sol locus, also affect the physicochemical properties of hemoglobin determined by "Hb" alleles (142). Different combinations of Sol and Hb alleles affect the appearance of salted out protein and produce small shifts of solubility of HbCO (within the range indicated).

Table 38

Population genetics: Characteristics of mouse hemoglobins

Genotype	Solubility (O.D. at 575μ)		Physical appearance of hemoglobins
	Mean	Range	
Hb1/Hb1	0.12	0.02-0.20	Crystals shaped as large hexagonal plates, about 150-200μ in diameter in the higher part of the O.D. range. The sizes of the hexagonal plates tend to decrease as optical density decreases. The plates measure about 40-50μ in diameter at O.D.'s of 0.02 to 0.04; crystal needles often pass perpendicularly through the center of these small plates or lie freely in the solvent.
Hb1/Hb2	0.36	0.30-0.55	Clumps of small (15-20μ in diameter) irregularly shaped crystals; sizes of crystal aggregates vary widely; amorphous material in many of the preparations.
Hb2/Hb2	0.25	0.20-0.30	Amorphous material in varying amount.

Combinations of alleles at the Hb locus with alleles Sol 1 and Sol 2 have characteristic solubilities and appearances. The genotypes of the Sol locus of two of the inbred strains used in the original four-way cross, C57L/J and 129/J, are Sol 1/Sol 1. The alleles at the Sol locus of the two other strains differ from Sol 1 and Sol 2 and from each other. Attempts to devise conditions that would permit simultaneous identification of all combinations of alleles at the Hb and Sol loci of the genotypes used were only partly successful. Only the clear-cut polymorphism at the Hb locus is reported.

Estimation of allele frequencies. C locus. Dominance complicates the estimation of allele frequencies at the C locus. Only homozygous recessive individuals can be identified. The estimate of the frequency of recessive c is the square root of the proportion of homozygous recessive individuals

$$q_{(c)} = \sqrt{\frac{R}{G}}$$

R = number of albino plus chincilla mice;
G = total number of mice with variance

$V_{(g)} = \frac{1-q^2}{4G}$, and the frequency of the dominant allele is the difference between $q_{(c)}$ and one (143).

An assumption basic to estimation of recessive allele frequencies from the square root of homozygous recessive genotypes is the existence of so-called "Hardy-Weinberg conditions"- a large population, random mating and the absence of mutation, migration, and selection. The applicability of mathematical methods provided by the Hardy-Weinberg principle depends on the degree of correspondence between biological actuality and theoretical concept. However, provided changes of allele frequency are estimated for a single generation at a time, and provided the changes are not large— conditions that are met in this study— use of Hardy-Weinberg mathematics gives reasonable approximations even where one or more of the theoretical requirements are not strictly met.

The other coat colors for which mice were scored also can be related to alleles at specific gene loci. Polymporphism at these loci is also complicated by dominance, and only homozygous recessive individuals can be recognized. The initial frequencies of these other recessive alleles in the founder cohorts is only 0.2500; the inaccuracy of the estimate of allele frequency from the proportion of homozygous individuals is much greater at such lower frequencies. Also, of course, it is impossible to identify color genes that albino mice carry. Thus, the determination

of polymorphism of other coat color loci is restricted to subsamples of the total population.

Since all three hemoglobin genotypes can be distinguished, estimates of the frequencies of the two Hb alleles and their variances are:

$$P(Hb_1) = \frac{a + \frac{1}{2}b}{2G} \; ; \; q_{(Hb_2)} = \frac{\frac{1}{2}b + c}{2G} \; ; \; V_{(p \text{ or } q)} = \frac{pq}{2G}$$

where a, b, and c are the number of individuals of genotype Hb^1/Hb^1, Hb^1/Hb^2, and Hb^2/Hb^2, respectively, and G represents the total number of individuals (143).

Population genetics of alleles at C locus. The initial frequencies of dominant and recessive alleles at the "C" locus according to the mating scheme were 0.500, and the proportion of albino mice among the founders, 0.259, support the expected segregation of alleles. The initial frequency of recessive c is 0.509 $\pm$ 0.028, and of dominant C, 0.491.

In the original distribution of founder mice among the three study groups, homozygous recessive mice, males and females separately, were randomized first assuring the equitable distribution of at least 50% of all recessive alleles (Table 39) and the remaining mice, heterozygous or homozygous for dominant "C" alleles, were randomized similarly.

The first two cohorts born in Pop A, B, and C were the progeny of the founder mice exclusively. A rough estimate of the allocation of recessive c alleles hidden in heterozygous founder parents in the three study groups, it was felt, might be obtained from the number of homozygous recessive progeny in each group provided that systematic mechanisms tending to change gene frequencies would be weak during the first generation. The precision of such an estimate depends in part on the extent to which females participated in reproduction during this period. Advanced pregnancies in over 85% of the females of the founder mice in Pop A, B, and C during the first two breeding periods indicated that the number of pregnant females was large enough to give a satisfactory estimate.

Since each control cage was a family unit, pedigree analysis permitted a more accurate estimate of the frequency of recessive c in Pop C, especially as more than one litter and large litters were available.*

*The detection of heterozygotes would have improved further had albino mice been matched only with non-albino mice but the matings were always set up by a randomizing scheme. No extra matings were set up to test pairs that had produced no homozygous recessive young.

Table 39

Population genetics:

Numbers of mice homozygous recessive
at C locus in founder cohorts of Pop A, Pop B, and Pop C

	Pop A		Pop B		Pop C		Totals	
	M/F	Total	M/F	Total	M/F	Total	M/F	Total
10 Dec 1963 (4 days old)								
H.R.	7/14	21	8/12	20	8/12	20	23/38	61
Other	33/25	58	32/26	58	32/26	58	97/77	174
Total	40/39	79	40/38	78	40/38	78	120/115	235
17 Jan 1964 (6 wk old)								
H.R.	5/14	19	8/12	20	7/12	19	20/38	58
Other	32/23	55	30/23	53	30/25	55	92/71	164
Total	37/37	74	38/35	73	37/37	74	112/109	222

M/F = male/female

H.R. = homozygous recessive at C locus

Other = heterozygous or homozygous dominant at C locus

It seemed reasonable to attribute gross difference between the proportions of homozygous recessive mice in the first two cohorts born in the two enclosures to differences in the original allocation of heterozygous genotypes among the founders of Pop A and Pop B rather than to differences in reproduction or survival during the initial period. After all, social and other environmental conditions in the two enclosures during this period were as similar as they were ever likely to be.

The frequency of recessive c in the founder cohort of Pop C is 0.506 when calculated from the square root of the proportion of homozygous recessive founder mice, and 0.527 from pedigree analysis. The numbers of each type of mating with respect to "C" among the founder mice of Pop C are shown in Table 40. The presence of homozygous recessive progeny from the two types of matings that include non-albino parents indicates the presence of heterozygosis.

Table 40

Population Genetics:
Frequency of allele recessive-c in founder cohort from pedigree analysis

Pop C

Type of mating	No.		cc parents x no. matings	Cc parents x no. matings	c alleles	Total c+C alleles
cc x cc	1	1	2 x 1 = 2	-	4	4
cc x C-	17	12	1 x 17 = 17	1 x 12 = 12	46	68
C- x C-	19	14	-	2 x 14 = 28	28	76
				Total	78	148

frequency of c = $\frac{78}{148}$ = 0.527 ± 0.041 — pedigree analysis

cc = homozygous recessive genotype and phenotype

C- = heterozygous or homozygous dominant genotype; same phenotype

Thus, the gene pool of the founder mice of the control group, at least, contained approximately the desired 0.50:0.50 proportions of "C" alleles (if anything, slightly favoring recessive alleles). This suggests that the combined gene pools of the founder cohorts of Pop A and Pop B also contain approximately 0.50:0.50 proportion of "C" alleles.

The frequency of recessive c in each of the two progeny cohorts of

the founder mice of Pop C is slightly below 0.500, but the deviations from 0.500 have no statistical significance. The frequencies of recessive c in both progeny cohorts of the founder mice of Pop A and Pop B, however, were significantly higher than the estimated frequencies of recessive c in their parental cohorts, and in the corresponding progeny cohorts of the control group (Table 41). The magnitude and unidirectional character of the changes in both populations suggest the action of systematic processes, invalidating the possibility of making inferences of allele frequencies in founder mice from allele frequencies in their progeny. Perhaps the founder cohort of Pop B had a slightly higher frequency of recessive c than that of Pop A, since the first two progeny cohorts in Pop B have slightly higher frequencies of recessive c than the corresponding cohorts of Pop A.

The frequencies of recessive c in the total population at successive censuses in Pop A, B, and C are listed in Table 42A and presented in Figure 27. The differences in the over-all pattern of change of recessive c between the two large populations on the one hand and the control group on the other, stand out clearly. In both Pop A and B, the mean frequency rises sharply at first, remains elevated, and is greater than 0.6000 at the end of the year. The greatest change occurs during the early period of population growth in both populations. The frequencies of recessive c at each census differ significantly from 0.500 (with the exception of the February census in Pop A), from corresponding frequencies of the control population, but not from one another after the initial rise. The mean frequencies of recessive c in Pop C fluctuate around 0.500, though most are slightly below 0.500, but none differs significantly from 0.500.*

Of course, the mean frequency of recessive c in the total population at any time represents the combined action of "addition" of new alleles as a result of birth and recruitment of new individuals, and of "subtraction" of old alleles due to mortality.

The initial frequency of recessive c in each of the new cohorts of Pop A, B, and C are displayed in Table 42B and Figure 28. The frequencies in all of the cohorts of Pop A and B are above 0.500 and in six of the ten cohorts they are above 0.6000. In Pop A, the mean frequencies in suc-

*The mean frequency of recessive c at each census in the control population presented in Table 42A and Figure 27 was obtained by adding the number of mice with homozygous recessive phenotype at each census to those of all previous censuses, dividing each sum by the total number of mice ever present in the control population up to that time, and taking the square root as usual. This is reasonable since mortality was negligible in the control population throughout the year.

Table 41 Population Genetics:
Frequency of allele c in founder cohorts and in their progeny

Date	Pop C			Pop A			Pop B		
	Freq. c	± S.E.	No. of mice	Freq. c	± S.E.	No. of mice	Freq. c	± S.E.	No. of mice
Founder cohort									
10 Dec 1963	0.506	0.047	78	0.515	0.047	79	0.506	0.047	78
19 Dec 1963	0.503	0.047	75	0.506	0.047	74	0.512	0.048	76
17 Jan 1964	0.506	0.048	74	0.506	0.048	74	0.523	0.049	73
19 Feb 1964	0.506	0.050	74	0.517	0.050	71	0.528	0.049	72
20 Mar 1964	0.506	0.050	74	0.522	0.051	66	0.534	0.050	70
Progeny of founder									
Feb Cohort II	0.489	0.028	229	0.504	0.036	122	0.626	0.033	130
Mar Cohort III	0.484	0.024	289	0.616	0.030	158	0.623	0.028	174

Table 42A Population Genetics: Frequency of recessive-c in Pop C, Pop A, and Pop B at censuses

	Pop C			Pop A			Pop B		
	Freq. c	± S.E.	No. mice	Freq. c	± S.E.	No. mice	Freq. c	± S.E.	No. mice
10 Dec 1963	0.506	0.047	78	0.515	0.047	79	0.506	0.047	78
19 Dec 1963	0.503	0.047	75	0.506	0.047	74	0.512	0.041	76
17 Jan 1964	0.506	0.048	74	0.506	0.048	74	0.523	0.049	73
Feb	0.494	0.024	303	0.568	0.030	186	0.593	0.028	202
Mar	0.488	0.017	596	0.583	0.022	300	0.612	0.022	301
Apr	0.490	0.014	696	0.574	0.017	515	0.613	0.016	548
Jun	0.501	0.014	990	0.578	0.014	698	0.608	0.014	784
Aug	0.488	0.010	1377	0.587	0.013	993	0.598	0.014	726
Nov 1964	0.481	0.009	1923	0.614	0.013	1019	0.601	0.014	680
Mar 1965	-	-	-	-	-	-	0.610	0.014	729

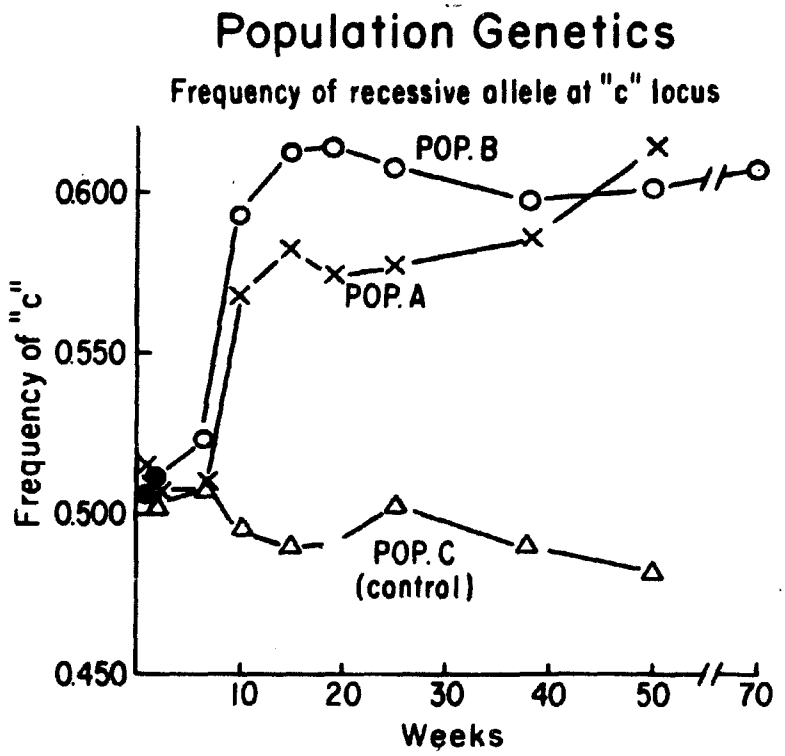

Population Genetics
Frequency of recessive allele at "c" locus
POP. B
POP. A
POP. C
(control)
0.600
0.550
0.500
0.450
Frequency of "c"
10
20
30
40
50
70
Weeks

Figure 27

Table 42B Population Genetics: Initial frequency of recessive-c in each cohort of Pop A, Pop B, and Pop C

		Pop C			Pop A			Pop B		
Founders		0.506	0.047	78	0.515	0.047	79	0.506	0.047	78
Feb 1964	Coh.II	0.489	0.028	229	0.586	0.036	122	0.626	0.033	130
Mar	III	0.484	0.024	289	0.616	0.030	158	0.623	0.028	174
Apr	IV	0.500	0.042	100	0.580	0.030	175	0.611	0.028	187
Jun	V	0.528	0.024	294	0.616	0.028	184	0.564	0.026	257
Aug	VI	0.450	0.022	387	0.575	0.022	320	-	-	-
Nov	VII	0.464	0.018	546	0.668	0.034	114	-	-	-
Mar 1965	VIII	-	-	-	-	-	-	0.637	0.037	108

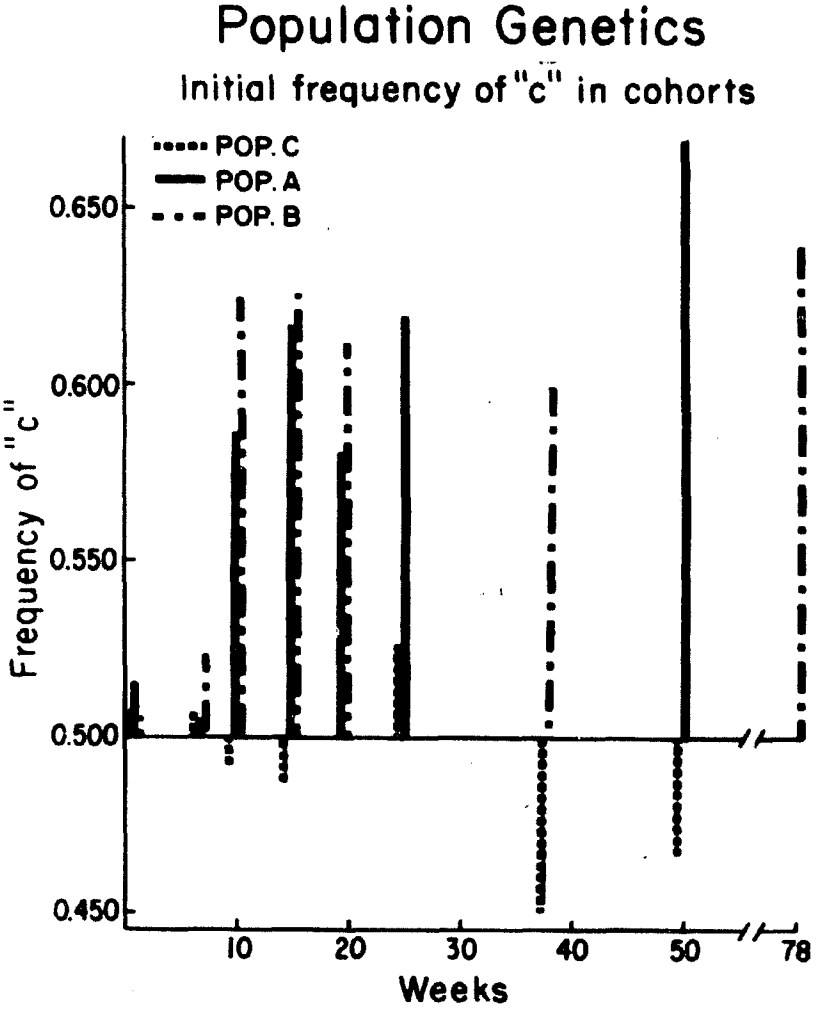

Population Genetics
Initial frequency of "c" in cohorts
POP. C
POP. A
POP. B
0.650
0.600
0.550
0.500
0.450
Frequency of "c"
10
20
30
40
50
78
Weeks

Figure 28

cessive cohorts follows a kind of picket fence pattern; the highest level of 0.6685 occurs in the last cohort—November 1964 census. In Pop B, the highest mean frequencies occur in the first two cohorts born in the enclosure; the following two cohorts have slightly lower frequencies, with a fall to 0.5650 in Cohort V (June 1964). When the next new cohort of mice was recruited into Pop B ten months later (March 1965), the frequency of recessive c again was significantly higher than 0,500, namely 0.6379.

The initial frequencies of recessive c in cohorts of Pop C are all lower than those of corresponding cohorts of Pop A and B. The frequencies of recessive c in the gene pool of each set of parents in Pop C does not differ significantly either from the frequency of c in the gene pool from which it was chosen or from 0.500 (Table 43). The frequencies of recessive c in each of the cohorts produced by each set of parents in Pop C also does not differ significantly from the frequencies of the parents; some cohorts have slightly higher, others slightly lower frequencies than their parents.

Table 43

Population Genetics

Pop C: Frequency of recessive c in parents and progeny

1. Parental cohort— founders
   c = 0.527 ± 0.041 (pedigree analysis)
   c = 0.506 ± 0.047 (square root recessive c)

   Progeny: Cohort II c = 0.489 ± 0.028 (N=229)
   Cohort III c = 0.484 ± 0.024 (N=289)

2. Parental cohort— random sample from Cohorts II & III
   c = 0.474 ± 0.046 (pedigree analysis)
   c = 0.442 ± 0.051 (square root recessive c)

   Progeny: Cohort IV c = 0.500 ± 0.042 (N=100)
   Cohort V c = 0.528 ± 0.024 (N=294)

3. Parental cohort— random sample from Cohorts IV & V
   c = 0.452 ± 0.046 (pedigree analysis)
   c = 0.506 ± 0.047 (square root recessive c)

   Progeny: Cohort VI c = 0.450 ± 0.022 (N=387)

4. Parental cohort— random sample from Cohort VI
   c = 0.459 ± 0.046 (pedigree analysis)
   c = 0.447 ± 0.051 (square root recessive c)

   Progeny: Cohort VII c = 0.465 ± 0.018 (N=547)

The frequencies of recessive c change little with time within any of the cohorts of Pop A or B (Table 44, Figure 29). In Pop A, the greatest change upward occurs in Cohort VI (August 1964); this may have been part of a trend, since the frequency of c in Cohort VII (November 1964) was 0.6685, the peak value for all cohorts, but, of course, at this time the population was discontinued.

In Pop B, the frequencies of c in the founder cohort rise steadily throughout the year, but those of Cohorts II, III and IV changed little with time; the frequency of c of Cohort V falls, then rises during the last third of the lifetime of the cohort. The last determination of c was carried out in March 1965, about 3½ months after the termination of Pop A and C. For each cohort of Pop B this last frequency is higher than the previous one and the new cohort recruited at this time has a frequency of 0.6380—the highest initial value for any cohort in this population.

Hb locus. The unexpectedly large number of mice in the populations made it impossible to analyze hemoglobin genotypes at each census. The hemoglobin genotypes of 932 animals of Pop A were identified at the end of the study, representing slightly more than 96% of the hemoglobins obtained and 91% of the animals of the total population at the last census. Of course, the genotypes of 25% of the total mice of Pop A that died during the course of the year were not examined and this makes the hemoglobin study less satisfactory.

Identical numbers of each of the two homozygous genotypes and a nearly perfect 1:2:1 Mendelian ratio of the three genotypes were present (Table 45). Thus, the frequency of each of the two Hb alleles was 0.500, just as it had been in the founder cohort.

Chi square tests were applied to the distribution of Hb alleles and hemoglobin genotypes among males and females, among mice of different cohorts, and among mice classified as to coat color at the "C" locus (Table 45). Three null hypotheses were tested: 1) the three genotypes are present in 1:2:1 ratios; 2) the frequency of Hb^1 equals the frequency of Hb^2; and 3) given the gene frequencies, the three genotypes are present in Hardy-Weinberg ratios.

Statistical significance was attained in only two of the eleven groups in which comparisons were made. In Cohort V (June 1964), there is an excess of Hb^2, the genotypes are not in Hardy-Weinberg, or in 1:2:1

Table 44 Population Genetics: Frequencies of recessive c in each cohort throughout life of cohort

	Year	1963	1964						1964	1965
	Census month	Dec	Jan	Feb	Mar	Apr	Jun	Aug	Nov	Mar
	Weeks after onset	2	6	10	15	19	25	38	50	78
Pop A Cohort births										
Aug-Nov 1964	Cohort VII								0.668	-
Jun-Aug	Cohort VI							0.573	0.637	-
Apr-Jun	Cohort V						0.616	0.604	0.632	-
Mar-Apr	Cohort IV					0.540	-	0.580	0.563	-
Feb-Mar	Cohort III				0.612	0.616	-	0.638	0.616	-
Jan-Feb 1964	Cohort II			0.597	0.586	0.594	-	0.582	0.582	-
Dec 1963	Cohort I	0.515	0.506	0.517	0.522	0.518	-	0.511	0.494	-
Pop B										
Nov-Mar 1965	Cohort IV									0.637
Apr-Jun 1964	Cohort V						0.564	0.573	0.571	0.576
Mar-Apr	Cohort IV					0.611	-	0.601	0.601	0.588
Feb-Mar	Cohort III				0.625	0.623	-	0.615	0.623	0.630
Jan-Feb 1964	Cohort II			0.626	0.652	0.648	-	0.638	0.634	0.646
Dec 1963	Cohort I	0.506	0.523	0.528	0.534	0.542	-	0.543	0.566	0.583

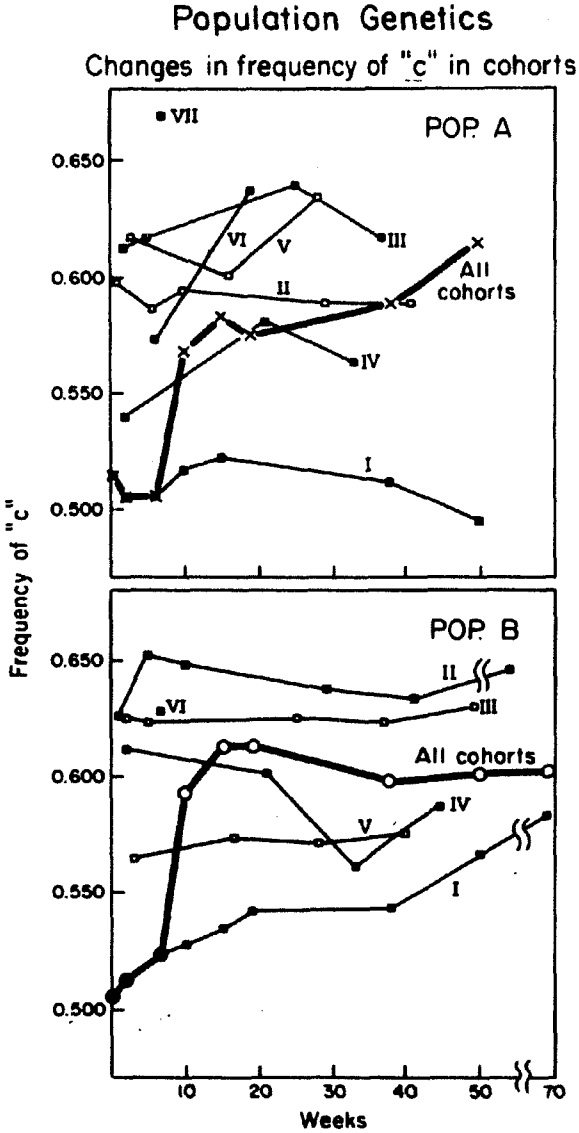

Population Genetics
Changes in frequency of "c" in cohorts
POP. A
VII
VI
V
III
II
All cohorts
IV
I
0.650
0.600
0.550
0.500
POP. B
II
VI
III
All cohorts
IV
V
I
0.650
0.600
0.550
0.500
Frequency of "c"
10
20
30
40
50
70
Weeks

Figure 29

Table 15 Population Genetics: Frequencies of Hb genotypes in Pop A at time of last census (Nov 1964)

	Numbers of mice			Genotype Frequencies			Gene Frequencies		Null Hypotheses*		
	Hb1/1	Hb1/2	Hb2/2	Hb1/1	Hb1/2	Hb2/2	Hb1	Hb2	H_o1	H_o2	H_o3
Pop A	234	464	234	0.251	0.498	0.251	0.500	0.500	ns	ns	ns
Males	125	224	109	0.273	0.489	0.238	0.529	0.471	ns	ns	ns
Females	109	240	125	0.230	0.506	0.264	0.477	0.523	ns	ns	ns
Cohorts											
II	32	60	27	0.269	0.504	0.227	0.521	0.479	ns	ns	ns
III	29	84	31	0.201	0.583	0.216	0.493	0.507	ns	ns	ns
IV	39	70	40	0.262	0.470	0.268	0.497	0.503	ns	ns	ns
V	36	56	54	0.246	0.384	0.370	0.443	0.557	###	#	##
VI	69	136	57	0.263	0.519	0.217	0.523	0.477	ns	ns	ns
VII	29	58	25	0.259	0.518	0.223	0.517	0.483	ns	ns	ns
C/- mice**	135	278	154	0.238	0.490	0.272	0.483	0.517	ns	ns	ns
c/c mice**	99	186	70	0.279	0.524	0.197	0.541	0.459	ns	#	ns

*H_o1: genotypes 1/1, 1/2, 2/2 are present in 1:2:1 ratio
H_o2: the frequency of allele Hb1 = frequency of allele Hb2
H_o3: given the gene frequencies, the genotypes are present in Hardy-Weiberg proportions

All chi-squares have 1 degree of freedom
#p=0.05 ##p=0.01 ###p=0.001
ns: statistically non-significant p>0.05

**C/- mice: heterozygous or homozygous dominant at C locus
c/c mice: homozygous recessive at C locus

ratios. The frequency of Hb^1 among albino mice is slightly higher than expected by the null hypothesis.

Special Studies

Gross examination of organs and tissues of a random sample of 200 mice was carried out immediately after the last census (November 1964) and tissue sections were prepared for microscopic studies. At the time of autopsies, reproductive status was assessed directly by inspection of reproductive organs. The microbiota of the intestinal tract of a random subsample of autopsied mice were cultured.

The interplay between socio-ecological and other biological processes was studied further by observing the mice of the population enclosures after changing their social environments. This was done in two ways. First, samples of mice were taken from Pop A at the November census, and reproductive function and selected aspects of behavior were studied.* Reproduction was evaluated after the mice were placed in male-female pairs in control type cages. A series of tests was performed with the animals to elicit their behavioral responses in a variety of situations. These were concerned with: 1) social interaction in small groups of males, 2) sexual behavior of males, 3) maternal behavior of females, 4) agonistic behavior between pairs of males and 5) motor and autonomic reactivity in a "novel situation." Similar tests were performed with control animals.

For Pop B, environmental change was achieved by letting the mice emigrate into the empty enclosure of Pop A, which was interconnected with that of Pop B.

Special Study: Gross and microscopic examinations of organs and tissues of enclosures and control mice. The organs of samples of 180 and 40 mice of Pop A and C, respectively, were examined immediately after the animals were anesthetized with chloroform and bled from the heart. No abnormalities were noted.

The organs were fixed in unbuffered 10% formaldehyde. Miss Lillian R. Gregg (Section Cutting Service of The Rockefeller University) prepared

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*In many of the special studies to reduce the number of social groups tested, cohorts were combined into age groups:

Group I - Cohort II and III	Group III - Cohort VI
Group II - Cohort IV and V	Group IV - Cohort VII

sections of heart, lung, liver, kidney, spleen, adrenal, gonad, thymus and colon of each mouse for histological study. The organs were dehydrated in alcohol, cleared with methyl salicylate, embedded in paraffin, and sections, 6-7 micra in thickness, were stained with hematoxylin and eosin.

Tissue sections of 81 and 29 mice of Pop A and C, respectively, prepared to date reveal the following pathological diagnoses:

Pop C: Cohort II - Male - carcinoma of caecum

Pop A: Cohort I and IV - male - perivascular lymphocytic infiltration of kidney.

There were no remarkable differences either in the size of cross sections of adrenal glands or of relative proportions of adrenal cortex and medulla between mice of Pop A and C.

Special Study: Bacteriology of intestinal microbiota of mice of Pop A and C

Microbiota present in the gastrointestinal tract of mice may be beneficial or harmful to their hosts, depending upon the specific kinds and relative proportions of microbiota present, and the age, physiological state, and environmental situation of the host (144-5). The contents of the caecum of samples of mice of the different cohorts of Pop A and Pop C were cultured. Techniques that permit the quantitative enumeration of several species of bacteria were used.

Procedure

Different media were used for the culture of the various intestinal bacteria (146).

The entire caecum, obtained within a few minutes after the mice were anesthetized and sacrificed with chloroform, was cut open and, together with its contents, agitated in 5 ml of charcoal water for 5 minutes on a mechanical shaker. Calibrated loopfuls of undiluted suspensions were spread on the surface of the culture media and the numbers of colonies obtained were counted (Table 46).

Results

The caecum of 17 of 40, or 42.5%, of the mice of Pop A contained species of Clostridia compared with 3 of 28, or 10.7%, of the mice of Pop C. Escherichia coli grew from the caecal cultures of 6 of 40, or 15%, of the mice of Pop A but only from one of 28, or 3.6%, of control mice. These are the outstanding differences between the two groups.

Table 46

Special Studies:

Microbiota of Cecum— Pop A and C

	Age Group	Cohorts	Sex	No. mice	E. Coli	Entero-cocci	Lacto-bacilli	Clos-tridia	Bacter-oides
Pop A	I	II, III	M	3	3/B*	3/B	3/F	0	3/E
			F	6	0	6/A	5/G	5/A	6/E
	II	IV, V	M	7	0	5/B	6/G	4/A	5/D
			F	4	0	4/A	4/7	3/A	4/F
	III	VI	M	5	2/A	5/B	5/G	4/B	5/E
			F	5	0	4/A	5/C	1/C	3/B
	IV	VII	M	5	0	3/B	5/D	3/B	4/D
			F	5	1/B	2/B	5/D	3/A	4/C
Pop C	I	II, III	M	5	0	2/A	5/F	1/A	4/E
			F	5	1/A	1/A	5/F	0	5/F
	II	IV, V	M	4	0	0	4/D	0	1/F
			F	4	0	1/A	4/D	0	2/E
	III	VI	M	5	0	3/A	5/E	1/A	4/E
			F	5	0	5/B	5/G	1/A	5/G

Fractions = number of mice with positive culture/number of colonies

Numbers of colonies are indicated thus: 0 = no colonies
A = 1-29 "
B = 30-99 "
C = 100-199 "
D = 200-299 "
E = 300-399 "
F = 400-499 "
G = 500 or more colonies

The numbers of enterococci were slightly higher in Pop A than in Pop C mice, though they were low in both groups. The males of Groups I, II, and III of Pop A have consistently though only slightly higher numbers of enterococci than the females in these groups. The numbers of lactobacilli and bacteroides are high in all age groups of mice of Pop A and C, with the exception of the females of Groups III and IV of Pop A in which they are slightly depressed. Salmonella and proteus were not detected.

The flora of the caecum of other mouse colonies has been studied in the same way. For example, the so-called NCS colony of "Swiss" mice have large numbers of lactobacilli, bacteroides and anaerobic strep in their intestinal tract, no E. coli, Clostridia, and Proteus, and only small numbers of other coliform organisms. These mice tend to have large litters, a rapid growth rate, and fewer respiratory diseases than other colonies of "Swiss" mice, such as the CFW or SS Lynch colonies that have higher numbers of E. coli and in which Clostridia and Proteus can be detected (144, 147).

The mice of Pop A thus resemble the NCS mice with respect to lactobacilli, bacteroides and enterococci, but differ from these mice in that they do harbor Clostridia. Many mice of Pop A, like NCS mice, had no E. coli, but some mice did, like CFW mice and SS mice. The mice of Pop C, having a significantly smaller incidence of cultures with E. coli and Clostridia and with smaller numbers of enterococci than the mice of Pop A, are even more like NCS mice.

Of course, the environmental conditions of the mice of the NCS colony are radically different from those of the mice of Pop A. The NCS mice were derived by Caesarian section from mice of the SSL colony, and have been maintained since then in highly protected conditions (148).

Special Study: The direct assessment of fecundity and fertility

The index of fertility of females at the time of censuses was the presence of visible and, hence, fairly advanced pregnancy; the index for males was the presence of descended testicles. Autopsies were performed on 200 mice of Pop A on the day following the November census. The ovaries and uterus of females and testes of males were examined to assess fecundity and fertility more directly than possible during the censuses (Table 47).

At autopsy, the criterion of female fecundity was the presence of corpora lutea in the ovaries (149). Female fertility was assessed by the presence of embryos or of placental sites in the uterine horns. Tubules of

Table 47

Special Studies:
Direct assessment of reproduction

Evidence of fecundity and fertility from examination of reproductive organs

FEMALES

	Cohort	No.	Fecundity: Non-fecund	Fecundity: fecund	Fecundity: % fecund	Fertility: fertile early preg.	Fertility: late preg.* (embryos)	Externally visible preg.	Very small uterus
Pop A	I	12	4	8	66.7	1	2	1	0
	II	12	4	8	66.7	0	5	3	0
	III	19	8	11	57.9	3	2	2	0
	IV	10	5	5	50.0	2	3	2	1
	V	14	6	8	42.9	3	3	1	1
	VI	15	4	11	26.7	1	1	1	8
	VII	15	3	12	20.0	0	0	0	7
	Total	97	34	63	65.0	10	16	10	17
Control**									
	II,III	5	0	5	100.0	2	3	2	0
	IV,V	5	0	5	100.0	2	2	2	0
	VI	10	0	10	100.0	4	5	5	0
	Total	20	0	20	100.0	8	10	9	0

*Late pregnancy = embryos at least 1/8 inch long
**Early pregnancy = placental sites only

MALES

Pop A males— 87 examined (Cohort I: 10, II: 11, III: 16, IV: 7, V: 13, VI: 15, VII: 15); all but 2 of last cohort fecund.

Control males— 30 examined (Cohort II,III: 5, IV,V: 5, VI: 10, VII: 10); all fecund.

the cauda epididymis visible to the naked eye indicated male fecundity. Where any doubt arose, the cauda was teased out on a slide and examined under the low power of a microscope and the presence or absence of sperm became the index of fecundity. All males of Pop A, with the exception of two males of the last cohort (November 1964), were fecund (Table 47). All control males examined were also fecund.

Ten females of the random sample of 97 females selected from Pop A for autopsy studies were judged to be pregnant when examined before autopsy; by this criterion, at least, the sample was representative, since the frequency of pregnancy of 10.3% is similar to the 9.3% pregnancies in the whole population at the November census. One third of the females did not have visible corpora lutea and were judged to be non-fecund. Approximately one-half of the females in Cohorts VI and VII, the two youngest cohorts, had a threadlike uterus.

About a third of the females with intrauterine embryos had not been judged to be pregnant by external signs prior to autopsy, but almost all of these had very small embryos. Ten females with intrauterine spots or placental sites had not been judged to be pregnant either. Combined, these two groups of early pregnancies make up about 40% of the pregnancies at autopsy. Thus, the direct assessment of pregnancy was about $2\frac{1}{2}$ times higher than that determined "clinically" at the time of the November census.

Special Study: Reproductive function of Pop A mice in control cages

Reproduction was depressed in Pop A during the last six months. Were the reproductive capabilities of mice of cohorts known to have been productive during earlier stages irreversibly depressed, or would these mice start to reproduce again in a behaviorally less abnormal and less crowded environment? Were the mice of cohorts born during the last six months capable of normal reproduction? If the mice of Pop A did reproduce in the different environment, how long would it take for normal reproduction to begin?

Samples of mice of each cohort were bred as male-female pairs in standard laboratory cages, the environment of the control mice. Ideally, all possible combinations of males and females of Pop A and C should have been set up as breeding pairs to reveal the possible contributions of males and females of different cohorts of Pop A to the decline of reproduction and recruitment in the population enclosure, but insufficient space made this im-

possible.

Procedure

Fifteen males and females of each age group of Pop A were set up as breeding pairs. Fifteen male-female pairs of Pop C of corresponding age groups had been saved and kept throughout the year for this study in the room housing the population enclosures. There were only nine pairs of mice of age Group IV of Pop C.

All cages were inspected between 8 and 9AM and 8 and 9PM daily for new litters and the number of young counted immediately. Twenty-one days after the birth of a litter the numbers of weanling males and females were counted and the young removed from the cage. Cages were changed once a week.

Results

All young (3 Pop A litters and 24 Pop C litters) born during the 21 days following the setting up of breeding pairs were discarded to make control and population groups comparable. During the subsequent 42 days—two periods of 21 days each — a total of 83 litters were born to control pairs, or 77% of the theoretical maximum of 108 litters for two 21-day periods. (Table 48).

Table 48

Special studies: Reproductive function

Number of litters produced by Pop A and C mice

	Age group	Cohort	No. of breeding pairs	Number of litters			
				Days 22-42	Days 43-64	Total	% Max.
Pop A	I	II, III	15	4	4	8	26.6
	II	IV, V	15	6	6	12	43.3
	III	VI	15	6	8	14	46.6
	IV	VII	15	6	8	14	46.6
	Total		60	22	26	48	40.0
Pop C	I	II, III	15	11	6	17	56.6
	II	IV, V	15	14	10	24	80.0
	III	VI	15	14	11	25	83.3
	IV	VII	9	8	9	17	95.0
	Total		54	47	36	83	76.9

The mice of Pop A produced 48% of a possible 120 litters, or 40% of maximum. Though this is only slightly more than half of control productivity, 34 out of 60 females of Pop A, or close to 60%, had at least one or more litters in the control environment compared with the 10% showing advanced pregnancy at the November census when the samples were taken. These 34 females produced 382 young in two months compared with 108 mice recruited into Pop A during the three months from August to November 1964 from more than 400 potentially breeding females.

The specificity of the influence of social factors was demonstrated by changes of reproductive function among mice of different age cohorts. The reproductive abilities of the mice of the two youngest cohorts of Pop A had been inhibited in the population enclosure. These mice had contributed few, if any, mice to the population. But substantial numbers of mice of these two cohorts were fully capable of producing litters. Thus, 28 of the 48 litters of Pop A mice were born to females of Cohorts VI and VII (age Groups III and IV) (Table 48). The oldest age group of Pop A, Group I, consisting of females of the first two cohorts born in the enclosure, produced the smallest number of litters in the new environment, though they had been more productive than Cohorts VI and VII in the enclosure. Moreover, mice of both Group I and II of Pop A failed to show an increase in the number of litters produced during the second time interval (Table 49).

Although Pop A mice produced a smaller total number of litters than Pop C mice, the mean size of the litters of Pop A mice was larger than that of control mice during each of the two 21-day periods and for the two periods combined, and the largest of all litter means — 10 young — were born to mice of Cohort VI (August 1964) of Pop A. This is another indication that the previously depressed reproduction of mice of this cohort was not permanent (Table 50).

Another measure of reproductive function is the time interval between mating and the birth of a litter (Table 51). It took longer for Pop A mice to have litters than for control mice. Medians for these time intervals were computed for each age group of Pop A and control mice. For first litters, even the shortest median among the four age groups of Pop A was longer than the longest median of the four control age groups. Among the mice of Pop A, the shortest median occurred for the oldest mice and rose

Table 49

Special studies: Reproductive function

Number of females having litters

	Age group	Females with litters: Interval 1 only	Interval 2 only	Interval 1 and 2	At least 1 litter	Females without litters
Pop A	I	2	2	2	6	9
	II	2	3	4	9	6
	III	1	3	5	9	6
	IV	1	5	4	10	5
	All	6	13	15	24	26
Pop C	I	5	0	6	11	4
	II	5	1	9	15	0
	III	3	0	11	14	0
	IV	1	0	8	9	0
	All	14	1	34	49	4

Some females had litters only during interval 1 (days 22-42), others only during interval 2 (days 43-64), others during both intervals, and others during neither interval.

Table 50

Special Studies:

Reproductive function and litter sizes of mice of Pop A and C

Pop	Age group	Period 1 m.l.s.*	n.	Period 2 m.l.s.*	n.	Periods 1 & 2 m.l.s.*	n.
A	I	6.0	4	5.5	4	5.8	8
	II	6.8	6	7.4	7	7.1	13
	III	8.2	6	10.0	8	9.2	14
	IV	7.2	5	8.7	9	8.2	14
	Total	7.1	21	8.3	28	7.8	49
C	I	5.0	11	7.2	6	5.8	17
	II	8.4	14	6.7	10	7.4	24
	III	7.7	14	8.9	11	8.2	25
	IV	4.6	8	7.1	9	5.9	17
	Total	6.7	47	7.7	36	7.1	83

*m.l.s. = mean litter size

Table 51

Special Studies:
Reproductive function

Interval from mating to birth of litter

	Group	To 1st litter Range in days	Median in days	No. of litters	To 2nd litter* Range in days	Median in days	No. of litters
Pop A	I	20-61	29.0	6	26-28	27.0	2
	II	23-60	35.0	9	22-29	24.0	4
	III	22-57	35.0	9	25-38	27.0	5
	IV	24-65	41.0	10	25-40	27.5	4
Pop C	I	20-35	21.0	11	23-27	24.5	6
	II	19-42	22.0	15	21-42	28.0	9
	III	17-28	21.0	14	21-30	27.0	11
	IV	23-50	24.5	9	22-32	26.0	8

*Applies only to females having two litters

progressively so that the longest median occurred among the youngest mice. However, for mice of Pop A having two litters the median time interval between potential mating and birth of a second litter became shorter, no longer differed among the four age groups of Pop A, and did not differ from that of control mice. Although the youngest mice of Pop A took longer to begin reproducing in the control environment, they rapidly caught up with the older mice once they began to reproduce.

The survival of newborns of Pop A mice in the new environment was high. Of the young born to Pop A mice, 4.7% died before weaning during the first 21-days interval and this percentage fell to 2.1% during the second 21-day period. The corresponding percentages for the control mice were 3.9% and 4.0%, respectively.

The sex ratio of young born to Pop A mice in the new environment is not significantly different from a theoretical 50% ratio male:female. The proportion of males was lower than that of each of the last four cohorts of Pop A at the time those cohorts were first censused in the enclosure. The sex ratios of young born to control mice were less than 50%, although the differences from 50% are not significant.

In summary, reproduction of mice of all cohorts of Pop A increased substantially when the animals were placed as breeding pairs in control cages as judged by the number of pregnancies and litters, by the decreasing interval between potential mating and birth of litters, and by newborn survival. The enhanced reproductive activities of mice of previously unreproductive cohorts when in the population enclosures showed that their reproductive physiology too was not permanently depressed. However, since about 40% of the females of Pop A had no litters at all during the 66 days of confinement in control cages, the possibility remains that, at least for certain groups of individuals, inhibition of reproduction may be a more long-lasting or even irreversible process.

Special Study: Maternal behavior

A rough gauge of one aspect of maternal behavior was obtained by observing the reactions of females to the presence of eight 4-day old babies scattered on the floor of a small cage.

Testing procedure: A female was placed in a 10"x7½"x4" lucite cage (mesh top) that was placed in the center of a 20" square enclosure, surrounded by 20" high masonite wall. A 100-watt bulb in an 8"x8" square

metal shade was hung just above the upper edge of the enclosure. Five minutes later, 8 babies were distributed evenly on the floor of the cage (the floor was subdivided into eight equal sections).

Animals: Five females from each age group were selcted for this study. The females of Pop A were tested immediately after removal from the population environment. Five weeks later, the same females were tested again; during this time each female had been housed with a male as breeding pairs in small cages. The five females of each control group had been housed with a single male throughout the year, of course. The 4-day old babies used for the tests came from reciprocal matings between individuals of the two types of F_1 mice of the original crosses used to produce the founder mice.

Observations: 1) the time in seconds until a baby was picked up and carried for a short distance; 2) whether all babies were retrieved and piled into a single area; and 3) other persistent behavior, such as digging and burrowing in sawdust, exploration of the cage, and retrieval of young but to different areas of the cage, scattering. Ten minutes of observations were allowed, but the period of observation was discontinued as soon as all the young were retrieved into one nest area.

Results: During the tests done immediately after the November census (Table 52), there were clear-cut differences between the mothers of Pop A and C. All control mothers handled the babies, whereas three of the mothers of Pop A failed to do so during the ten minutes allotted. Twelve of the 15 control mothers retrieved all eight young to a single nest, whereas only four of 20 females of Pop A did so. The median for the number of seconds elapsed before a female handled any baby was 17 seconds for control mice vs. 121 seconds for Pop A females. The types of behavior displayed by the two types of mothers also differed. Seven of the control mothers showed prominent digging behavior, producing nest-like depressions into which young were retreived. On the other hand, about twice as many females of Pop A as of Pop C persistently explored the cage. Retrieval of young to more than one spot in the cage was also more common among females of Pop A.

The behavior of control females was unchanged in the second round of tests five weeks later. The behavior of Pop A females had changed markedly since the first test. All the females now handled at least one baby. Fourteen out of 20 females retrieved all the young to a single nest. The median for the number of seconds elapsed before handling of a baby was 15 seconds — similar

Table 52 Special Studies: Maternal Behavior - Summary table

Date	Group	No. females	Not handling babies*/handling babies	Sec. elapsed to handling** range	median	Females retreiving young to single nest	Persistent behavior		
							Explore	Burrow	Scatter
11/64	Control I-III	15	0/15	6-41	19	13	5	7	1
	Pop A I-IV	20	3/17	9-450	121	4	12	0	4
1/65	Control I-IV	20	0/20	5-34	15	18	5	9	1
	Pop A I-IV	20	0/20	5-280	14	14	9	6	2
11/64	Control I-II	10	0/10	4-41	20.5	9	5	5	0
	Control III	5	0/5	14-31	17	4	0	2	1
	Pop A I-II	10	1/9	9-450	91	2	4	0	4
	Pop A III-IV	10	2/8	22-295	137	2	8	0	0
1/65	Control I-II	10	0/10	5-34	16	9	1	2	1
	Control III-IV	10	0/10	5-32	13.5	9	4	7	0
	Pop A I-II	10	0/10	5-240	12.5	7	3	3	2
	Pop A III-IV	10	0/10	5-280	15.0	7	6	3	0

*Observations discontinued after 10 minutes
**Applies only to females handling young

to the median of 16 seconds of the control group, but the range for this parameter was 5 to 280 seconds for Pop A females compared with 5-32 seconds for control mice. Pop A females still showed more exploratory behavior than control mothers, but now also displayed digging or nest-making activity shown by control females.

No differences of behavior were noted among females of different age groups of Pop C, either during the first or second set of tests. The median time before handling a baby was shorter for older than younger females of Pop A during the first series of tests. Older mice showed more scattering behavior than younger ones. During the second round of tests, there were no differences between older and younger mice of Pop A.

Special Study: Sexual behavior of males

Sexual behavior was assessed by observing the response of males to the presence of a female of an inbred strain (BALB/c3) known to be highly receptive sexually.

Testing procedure: The enclosure for the test was a lucite box 10"x7½"x4". This was placed in the middle of a square enclosure 20"x20", surrounded by 20" high brown masonite walls. A 100-watt bulb in a 10" square metal shade was placed just above the upper edge of the enclosure.

The female was confined in a cylindrical compartment made of ½" hardware cloth attached to the wire mesh top of the cage. Ten minutes after the male was placed in the cage, the top of the cage was lifted, freeing the females, and another top was placed on the lucite cage

Animals: Each female used in a test had given birth to a litter in the preceding 12 hours. The same female was used for five males of Pop C and Pop A of the same age group. The order of testing was randomized. After being tested, each Pop A male was kept in isolation in a standard laboratory cage for 12 hours and then observed again in the presence of the same female (above procedure). One month later, the same experimental prodecure was followed with five males from each age group of Pop A that had been paired with a female in a standard laboratory cage on the day of census.

Observation: 1) minutes to first attempt at mounting (recorded to the closest minute); 2) successful copulation defined as mounting plus two or more thrusts (observations were discontinued when this took place but were continued otherwise for 15 minutes); and 3) any other persistent activity of

the male, such as sniffing of the female, self-grooming, lying still, attacking of the female.

Results: There were marked differences of sexual behavior by the above criteria between males from Pop A and Pop C during the first set of tests (Table 53). Six out of 20 males attempted to mount the females, and two males mated successfully when tested immediately after transfer from Pop A. The intensity or quality of sexual behavior changed little after twelve hours of isolation. By contrast, only two of the 20 control males did not attempt mounting and only one male that mounted failed to display successful mating.

Table 53

Sexual behavior of males of Pop A and C

	Pop	Time	Mounts	Complete Mating*	Other activities** sn	gr	ly	at
1st test#	A	AM	6/20	2/6	9/20	5/20	5/20	1/20
		PM	7/20	2/7	7/17	3/17	6/21	1/21
	C	AM	18/20	17/18	1/2	-	-	1/2
2nd test##	A	AM	15/20	12/15	5/5	-	-	-

#1st test - immediately after November census
##2nd test - one month after males were caged as breeding pairs

*number of individuals with successful copulation/males mounting
**number of individuals showing persistent activity/all observed persistent activities

sn=sniffing; gr=grooming; ly=lying down; at=attacking female

When sexual behavior of Pop A males is related to age groups during the first test (Table 54), it is apparent that males of older cohorts were different from those of younger ones, though the latter were sexually mature. Many of the mice of Pop A exhibited other persistent behavioral activities during the first series of tests; about half of them sniffed the female, and about a third simply lay down in the cage or spent most of the time in self-grooming.

One month later, the sexual behavior of males of Pop A that had been paired with a single female in a control cage was essentially the same as that of control males.

Table 54

Sexual behavior of males of Pop A
Age group comparisons

1st Test: Pop A

Age group	Mounts	Complete mating	Other activities sn	gr	ly	at
Group 1	4/5	1/4	4/5	1/5	-	-
Group 2	2/5	1/2	1/4	2/4	-	1/4
Group 3	0/5	-	2/6	1/6	3/6	-
Group 4	0/5	-	3/6	1/6	2/6	-
Groups 1 & 2	6/10	2/6	5/9	3/9	-	1/4
Groups 3 & 4	0/10	-	5/12	2/12	5/12	-

Special Study: Social interaction in small groups of males

The object of this study was to observe social interaction and possibly the development of social organization in small groups of males.

Procedure: Groups of males were observed in a special population cage made up of transparent plastic (lucite). This consisted of an octagonal central area (24" between parallel sides, 8" high) with a central food hopper; two cylindrical nest box retreats (6" in diameter and 5" high) were each connected by a tube (4" long, 1½" in diameter) to each side of the central octagon. Each male was confined in a separate nest box for ten minutes, after which a partition blocking the entrance to the tubes and restricting the mice to the nest boxes was removed.

Animals: Sixteen males of each age group of Pop A were observed. Males of Group I and IV of Pop C were tested in similar fashion; there were only 8 males in control Group I. The order of testing the six groups was randomized.

Observations: The animals were observed for four 15-minute periods: period I immediately after removal of partitions; period II—3 hours later; period III—6 hours later; period IV—21 hours later.

Particular attention was paid to 1) the initial behavior displayed by the mice; 2) the type of interactions between animals; 3) the distribution of mice in the nests and central area of the cage.

Results: Mice of Pop A

Group I: Period I - Eleven of the 16 animals had scars at the start. Three males were in the central area by the time the partitions had been removed from all 16 retreats— about 30 seconds— and 13 mice had left the peripheral retreats in the next minute. During the next three minutes, the mice went in and out of the peripheral retreats and explored the central area. They showed the same kind of unreactivity to each other exhibited in the population enclosure. A fight occurred during the fifth minute of observation. The fight lasted 10-15 seconds and one of the mice attacked three other mice in rapid succession during the next minute. There were brief fights in the cage throughout the remainder of the observation period. Mice continued to explore, eat and drink.

Period II - Eight retreats were occupied by mice, five of them by a single mouse. Four mice fought intermittently in the central area.

Other mice coming out of retreats were attacked briefly but went to the food hopper. Four episodes of mounting occurred in the central area.

Period III - Three retreats were occupied by three to four mice each. Prolonged fights of 30-60 seconds occurred between two specific males. These two also attacked most of the other mice that came into the center and chased them back into the retreats. Sexual mounting occurred repeatedly in one of the retreats.

Period IV - One aggressive mouse attacked and chased all other mice that entered the central area. All the mice were huddled in two retreats. The observation period was prolonged for 15 minutes, during which time no mice remained in the center for more than a few seconds (except for the aggressive male).

Group II: Period I - Six mice had scars. Two mice came out of the retreats during the first minute. Eight more mice slowly stepped into the central area during the next four minutes. Mice explored the cage in a slow-stretch, sniff, stop, stretch fashion. Six mice stayed in connecting tubes throughout the fifteen minutes, their heads protruding into the center. Some of the mice that had entered the center also returned to peripheral nest boxes and stationed themselves in the connecting tubes.

The first fight occurred between two unscarred mice at the eleventh minute following a preliminary period of body shaking, tail rattling, circling and threat postures. Each of the two fighters then attacked other mice. Attackers did not follow mice into retreats, and withdrew on contact with mice blocking connecting tubes. There were single mice in each of nine enclosures at the end of the period and three mice in one enclosure.

Period II - The mice were dispersed in ten peripheral retreats. Not a single mouse was observed eating during this period. Many fights involved at least five different males. Half of the mice showed fresh red wounds.

Period III - Most of the mice were in two retreats. In one retreat, a male repeatedly attempted to mount other males. Three aggressive males in the center attacked each other and all other mice that entered the center.

Period IV - Not a single animal came out of the two re-

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Period IV - Not a single animal came out of the two re-

treats that now contained most of the mice. Four aggresive mice in the central area fought in pairs, threes and fours. One of these mice rushed over and attacked any individual coming from the peripheral retreats.

Group III: Period I - Not a single mouse had scars at the start. Three animals came out of peripheral retreats during the first five minutes and began to move slowly around the cage, stopping to encounter each other, followed by prolonged sniffing. They entered other peripheral retreats, sniffing the mice in them. The latter typically came out of the retreats following such encounters. Little else occurred during the 15 minutes. Most of the mice had come out of retreats at the end of an additional ten minutes of observation.

Period II - Several mice were running around and around the central area, hopping up to the top of the enclosure, stopping and starting, hopping again, etc. There were many very brief fights accompanied by squealing, with often little more than threat motions. Submissive mice stayed in the center of the cage and did not retreat to nest boxes. There was a dead mouse without evidence of external injury in one peripheral retreat.

Period III - Fighting continued in flurries during this period. About half of the mice were actually fighting, while the others were hopping and running around. Mice stopped to eat briefly.

Period IV - There were paper nests in nine of the sixteen retreats. Two mice were fighting.

Group IV: Period I - None of the mice had scars. About half of the mice came into the central area within a minute. These animals began to scurry back and forth, crossing rapidly from the center into and out of peripheral retreats throughout the 15-minute period.

Periods II and III - The behavior of the mice was similar during both periods. The majority of the mice were still active and running, but they stopped and sniffed each other and the cage much more frequently than during the first period of observation. Some mice were eating, drinking, and a few sleeping.

Period IV - The mice now moved much more slowly. There were paper nests in six retreats. No fighting was observed.

Mice of Pop C

Groups I and IV: Mice of both groups displayed almost identical behavior in each of the four periods of observation and the two studies are described together.

Period I - Mice came out of nest boxes immediately after the removal of the partitions. On reaching the central area, most mice "froze," stood still, and showed visible generalized piloerection. Some of the mice ran back into nest boxes. Approach by an animal to within 6-8 inches of another mouse, either in the central area or at the mouth of a connecting tube, again led to "freezing" and piloerection, and, for some mice, to shaking of the entire body. After a few such encounters, tail rattling, circling, and violent fighting broke out involving at first two, and then more individuals. The first few fights were brief (10-15 seconds), but bouts of fighting became prolonged. Mice began to bite into and hold onto each other's backs. Some mice remained in nest boxes and engaged in vigorous fighting with any mouse entering their retreats. One mouse in Group I and two mice in Group IV that entered the central area of the enclosure after fighting had started and that were attacked ran rapidly around the center, stopped, and had generalized seizures and died.

Period II - A fight occurred almost every time any mouse got within twelve inches of another mouse. Fighting lasted from a few seconds to more than a minute, and ended as one mouse assumed the upright posture of submission. Some mice ran immediately into retreats when attacked, then turned around and faced the center of the enclosure, blocking the opening of the tube leading into the retreat. Fierce fights occurred when a second male tried to enter the retreat.

Periods III and IV - The patterns of behavioral interactions were unchanged. Many males remained in retreats during Periods III and IV, and fighting took place whenever two males came into contact. There was one dead mouse in the center of the enclosure in the study of age Group I and two dead mice in Group IV.

Special Study: Agonistic behavior of paired males

The purpose of this study was to make observations of the behavior of males taken out of the enclosures and paired in situations that generally elicit agonistic behavior.

Procedure: A single male was confined by a removable partition in one of two compartments (6"x5"x4") at opposite ends of a 24"-long box. The box had a steel mesh bottom and the top was covered by a glass plate. The box was placed inside a 30"x30"x30" masonite enclosure; a 100-watt bulb in a ten-inch square steel shade was hung over the rim of the enclosure. Fifteen minutes later the partition confining the mice to their compartments was removed.

Animals: Samples of males of all age groups of Pop A and of Groups I and IV of Pop C were tested. Where animals participated in more than one test, the second test was begun as soon after the first one as possible and the animal was kept in a standard laboratory cage meanwhile.

Observations: 1) Time in minutes before the onset of fighting, so-called latency; 2) the character of fighting; and 3) winners and losers. The period of observation was 15 minutes long, but some pairs were separated before this.

Pairings: 1) Males of the same age group; 2) males of different age groups, i.e., winners vs. winners., losers vs. winners, winners against mice showing no agonistic behavior; 3) Pop A vs. Pop C males, i.e., winners vs. winners, non-experienced Pop C vs. non-aggressive Pop A animals.

Results (Table 55): a) Males of same age group— Pop A. Aggressive interactions occurred in 4 out of 5 pairs in each of the older age groups of Pop A, in 1 out of 10 pairs in the younger age groups. Fights were brief (10-30 seconds) and consisted in almost all instances of a single attack. Many of the males spent the fifteen minutes exploring the cage, sniffing each other or huddling.

b) Winners against non-aggressive males— Pop A. Eight winners of aggressive interactions in the two older age groups of Pop A were paired with non-fighting males of the two younger age groups. Only two winners attacked the younger mice and on each occasion this consisted of only one attack.

c) Winners against winners, losers against losers —Pop A. There were fights in four sets of paired winners and three out of four sets of paired losers. The latencies were briefer in fights between paired winners. In all cases fights were brief.

d) Pop C vs. Pop C; Pop C vs. Pop A males. The pattern of interaction in all situations involving control males was strikingly similar. Control males showed a sequence of "freezing" immobility, piloerection, tail rattling and body shaking and a sudden onset of fighting;

Table 55

Special studies: Agonistic behavior of males

Pop	Group	No. pairs	Fights	Latency (minutes)	Description
Pairs of same age group.					
Pop A	I	5	4	7,9,9,11	Very brief fights (10-20 seconds); threat and one or two contacts elicit submission.
	II	5	4	4,9,12,14	
	III	5	1	12	Fight consists of single attack. Other pairs explored cage and each other.
	IV	5	0	-	Mice explore, huddle.
Pop C	I	1	1	7	All mice show freezing, piloerection, tail rattling, body shaking; fights start suddenly; bouts are long lasting; wounds are produced. Mice separated after 1½ minutes.
	IV	2	2	3,14	
Pop A: Winners of Groups I and II paired with non-fighters of Groups III and IV.					
	I or II vs. III or IV	8	2	5,7	Two fights each consisting of single attack and won by males of I and II. Other encounters marked by sniffing and huddling.
Pop A: Winners of Group I vs. winners of Group II.					
	I vs. II	4	4	4,6,7,11	Two fights initiated by I, two by II. Three fights won by I. Three fights brief, one persistent.
Pop A: Losers of Group I vs. losers of Group II.					
	I vs. II	4	3	9,12,14	Two fights initiated and won by II. Single attack only.
Double winners Pop A vs. winners Pop C.					
	I vs. I	1	1	1	Control mice showed same behavior as in Pop C vs. C fights. Attacks by Pop C mice are sudden and repeated; Pop A mice stop fighting, back and retreat.
	I vs. IV	1	1	6	
	II vs. IV	1	1	2	
Naive Pop C (IV) vs. non-fighter Pop A (IV).					
	IV vs. IV	4	4	2,4,6,7	Control mice show same behavior as in Pop C vs. C fights. Pop A mice in upright submission posture.

fighting was fierce with repeated attacks, biting and visible wounding. Fights involving Pop C males were discontinued before the 15 minutes had elapsed because of the fierceness of the fights. Control males won all three fights when matched against males of Pop A that had won in two previous bouts. Naive males (that had not fought before) of age Group IV of Pop C attacked Pop A males in stereotyped fashion.

Special Study: Motor and autonomic reactivity in "novel" environments

The reactions of mice of Pop A and C in environments with which they did not have a previous experience were studied. The environmental situations were: 1) an entirely empty and flat surface, a so-called arena; and 2) a flat surface broken by vertical solid barriers, called an open field. Both motor and autonomic reactivity of mice placed individually in each of these situations were observed. These standard tests have been found valuable in studying reaction of small mammals to novel situations (150-152). Animals with different social experiences react differently in these test situations (153-157).

Procedure: 1) The arena was a 20"x20" stainless steel plate; the outline of a square was drawn on this plate such that the areas outside and inside the square were equal. 2) The open field was a 20"x20" stainless steel plate divided into 25 identical squares with 12 barriers (5"x4½"x1/16") erected on alternate square borders. The two plates could be interchangeably placed at the bottom of a 20" high square enclosure. A 100-watt bulb and a 10" square steel shade were hung above the upper border of the enclosure.

In carrying out a test, a mouse was picked up by the tail with padded forceps and placed in a box with removable top; the box was inverted and placed in one corner of the enclosure; the top was removed and 15 seconds later the box was lifted up and stop watch started for observation.

Animals: Ten males from each of the four age groups of Pop A were tested immediately after the November census (test time one). Nine weeks later similar numbers of males of each age group were tested again (test time two). These males had been housed as breeding pairs in control type cages during the 9-week interval. Ten males of each age group of Pop C were also tested at this time.

Mice were all tested first in the arena and then in the open field (5 days later). The order of testing mice from different age groups and backgrounds was randomized.

Observations: Arena. The number of seconds spent in the middle square were counted for a two-minute period.

Open field. The number of squares entered during each of two consecutive minutes were counted.

The number of animals showing defecation and urination in each test situation were counted.

Results (Table 56):

Arena. The mean time spent by Pop A mice in the central square was equal at both times and was lower than that of Pop C mice. Pop A mice defecated and urinated less at time one than at time two, when their scores were similar to those of Pop C mice.

Open field. The mean number of squares entered by all mice was lower in the first than in the second minute. The scores for the first minute were equal for Pop A mice at both times and lower than those of Pop C mice. The score of Pop A mice during the second minute increased more than twice as much at time two than at time one, and the amount of increase was about equal to that shown by Pop C mice during the second minute. Again, defecation and urination was lower for Pop A mice at time one than at time two, and the scores at time two equal those of Pop C mice.

Special Study: Changes of social environment resulting from emigration; Pop B — End November 1964 - mid-March 1965

Pop B had been somewhat of a puzzle. It had reached its peak size of about 800 mice six months previously and during these six months not a single new mouse had been recruited. The animals, however, looked healthy and about one-third of the females, or about 100 mice, showed signs of advanced pregnancy throughout this time. Adult mortality had been relatively low. Even after two additional months of observations (end November 1964-mid-January 1965) no newborns survived.

The mice of Pop B were given the opportunity to change their own social environment by emigrating into the enclosures off Pop A, which was now empty and interconnected with that of Pop B. This situation provided an opportunity to study the process of emigration, and the effects of emigration on behavior, physiology, social organization and demographic processes.

Table 56 Special studies: Motor and autonomic reactivity in "novel" environments

		OPEN FIELD						ARENA			
		Squares Entered						Time in cen. square			
		Minute 1		Minute 2							
		range	mean*	range	mean*	D#	U##	range	mean*	D#	U##
Pop A (Nov 1964)	Group I	16-36	25.7	13-39	31.3	0	0	0-10	4.3	0	0
	II	10-28	15.7	12-24	18.0	2	1	0-13	4.6	1	1
	III	1-28	11.6	0-27	12.6	0	0	1-9	5.2	4	3
	IV	11-39	22.9	11-40	26.2	6	3	1-5	2.7	6	2
	All Groups	1-39	19.0	0-40	22.0	2.0	1.0	0-13	4.2	2.8	1.5
Pop A (Jan 1965)	Group I	4-31	15.6	4-46	23.7	10	9	1-8	3.8	10	8
	II	4-26	15.9	9-44	28.0	1	9	0-16	3.1	9	6
	III	11-36	20.8	16-46	34.6	4	1	1-14	5.1	10	8
	IV	9-28	16.4	21-38	27.2	9	5	2-20	7.5	8	7
	All Groups	4-36	17.2	9-46	28.4	6.0	6.0	0-16	4.9	9.0	7.1
Pop C (Jan 1965)	Group I	13-62	32.4	19-70	40.2	9	11	1-14	6.9	8	4
	II	11-38	19.8	1-40	29.9	7	10	0-13	4.4	9	6
	III	14-44	26.0	8-55	31.8	7	5	2-15	8.4	10	4
	IV	8-34	20.4	19-68	32.4	8	6	2-16	8.9	10	6
	All Groups	8-62	24.1	1-68	33.6	7.8	8.0	0-16	7.2	9.0	5.0

*Means do not differ significantly from medians

#D = animals defecating
U = animals urinating

After two months, the animals were to be reunited in the original enclosure and, later, again offered the opportunity to emigrate. Of particular interest would be to determine whether the patterns of emigration, the same changes of social structure, etc. would occur during such a second manipulation of the environment.

The two enclosures were connected January 19, 1965 (Figure 30). Twenty-eight mice entered the empty cage during the first hour and went into one of its peripheral retreats. Sixteen additional mice also entered, but returned almost at once to their original cage. The majority of mice that entered enclosure A subsequently behaved in one of two ways. One group moved into the new enclosure almost immediately, began to build paper nests, ate or drank, fell asleep, and "settled" there. The other group came into the enclosure slowly, explored the new environment, returned to the original enclosure, re-entered the new cage, moved back and forth repeatedly. Typically these mice rushed back to their original cage when disturbances or fights occurred in enclosure A. Ten days elapsed before the distribution of mice between the two cages became stabilized. After this, little movement between the two cages was observed.

The impression that the numbers of mice were roughly equal in the two enclosures was confirmed by the census seven weeks later. The emigrant population became Sub-Pop BA and the other, Sub-Pop BB. The physical structure of the two cages, such as the arrangement of paper, was similar after about two weeks.

Behavioral differences between the two subpopulations were obvious. During the first month, fighting was more frequent in the emigrant population (BA). Fighting occurred during almost every observation period in Sub-Pop BA, or about three time more often than in Sub-Pop BB. Intensity of aggressiveness was also higher in Sub-Pop BA, where attackers were more persistent and fights longer. Cohort memberships of aggressive mice differed in the two subpopulations. Observations during the first month revealed that forty out of fifty consecutive attacking mice in Pop A belonged to Cohorts II and IV: many of these males showed minimal evidence of previous scarring. By contrast, the attackers in Sub-Pop BB were mainly scarred males of Cohorts I, II and III. After a month, the frequency of fighting decreased to about one third of its previous level in Sub-Pop BA and changed little in Sub-Pop BB.

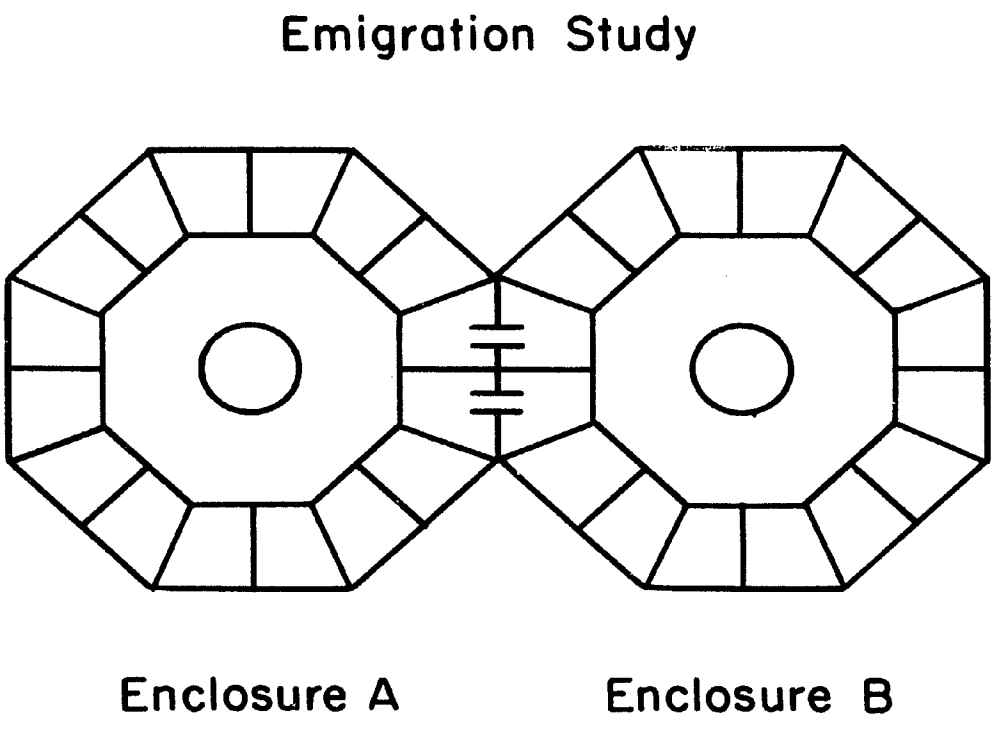

Figure 30 Interconnected enclosures.

Attacks on females in Sub-Pop BA were recorded only one-third as often as in Sub-Pop BB. Attacks of pregnant females were rare in both subpopulations. Episodes of "territoriality" in Sub-Pop BB were noted four times and twice in Sub-Pop BA.

Mating frequency, especially of normal matings, increased, although "queuing" by packs of males continued to occur in both cages. Males of the two youngest cohorts mated frequently in Sub-Pop BA but rarely in Sub-Pop BB. The frequency of homosexual matings decreased in both cages.

Five and a half weeks after the enclosures were connected, an "outburst" of births occurred, about a week earlier in Sub-Pop BA than in Sub-Pop BB. The number of newborns in Sub-Pop BA was greater. Scattering of newborns away from nurseries and cannibalism of newborns continued in enclosure B, but was uncommon in Sub-Pop BA. For the first time in almost a year, mice of Pop B survived the newborn stage. Large groups of babies were born in both cages just before the census.

Census: March 12, 1965

The mice of both subpopulations looked healthy, had smooth fur, normal amounts of body fat and included many pregnant females.

Numbers:

The number of old mice in Sub-Pop BB was slightly greater than that in Sub-Pop BA (Table 57, line 1). If the new mice (new cohort) are included in the count, the size of Sub-Pop BA exceeds that of Sub-Pop BB (Table 57, lines 2 and 3).

Table 57

Special studies: Migration.
Live mice in subpopulations at March 1965 census

		SubPop BA	SubPop BB
Line 1	Adults	296	324
Line 2	New Cohort	88	20
Line 3	Total	384	344
	Newborn	74	64
	Grand total	458	408

The presence of a new cohort of mice and of a large number of babies (Table 58) represents the outstanding feature of the census. Eighty mice between 5 and 10 grams, and twenty-eight mice between 2 and 5 grams that looked older than two or three days were counted. Production of a cohort of 108 mice represents a recruitment rate of about 15 mice per week. If the first three weeks during which the mice "settled down" are omitted from the calculations, the recruitment rate of about 30 mice per week approaches those of the previous year (Table 59). In addition to the new cohort, 138 newborns were counted in the two cages.

Table 58

Special studies: Migration.

New mice alive at the census of March 1965

		Sub-Pop BA	Sub-Pop BB	Total
New Cohort	5 - 10 gm	68	12	80
	*2 - 5 gm	20	8	28
	Total	88	20	108
Babies		74	64	138
Total new mice		162	84	246

*Older than newborn

Table 59

Special studies: Migration.

Mean number of mice recruited per week compared with earlier rates in Pop B.

Year	1964					1964	1964-65	1965
Census interval	Jan-Feb	Feb-Mar	Mar-Apr	Apr-Jun	Jun-Aug	Aug-Nov	Nov-Feb	Feb-Mar
Recruitment*/week	36	40	64	45	0	0	0	30

*Rounded off to the nearest integer

The emigrant subpopulation produced five times as many mice in the five- to ten-gram weight class (juvenile mice) as Sub-Pop BB. The difference in the number of live babies was small, 74 (A) and 64 (B). For the first time in almost a year, Pop B had increased in size. Males from all cohorts are distributed evenly between the two enclosures, except for those of Cohort II (Table 60). A greater proportion of females of Cohorts I and V were in enclosure B and of Cohort III in enclosure A.

Table 60

Special studies: Migration.

Distribution of males and females of different cohorts between the two subpopulations

	Cohort I		Cohort II		Cohort III		Cohort IV		Cohort V	
SubPop	BA	BB	BA	BB	BA	BB	BA	BB	BA	BB
Males	13	13	15	29	33	34	35	40	39	41
Females	8	16	23	22	39	22	42	45	50	61
Total	21	29	38	51	72	56	77	85	89	102

The percentage of pregnant females in both subpopulations combined was only slightly greater than that of Pop B at the November 1964 census (Table 61). The percentages of pregnant females in each cohort in Sub-Pop BA, however, were higher than those of corresponding cohorts that had remained in Sub-Pop BB, particularly in Cohorts I and II.

Table 61

Special Studies: Migration.

Pregnancy rates (per hundred females at risk for pregnancy at March census)

Cohorts	Nov 1964	Mar 1965		
	Pop B	Pop B	SubPop BA	SubPop BB
I	54.6	41.7	62.5	31.2
II	33.3	35.6	47.8	22.7
III	24.2	45.9	48.7	40.8
IV	23.4	29.8	33.3	26.7
V	23.7	20.7	22.0	19.2
Total	27.1	31.4	37.0	25.9

Forty-two dead mice were recovered between the last census of Pop B and first census of the subpopulations (Table 62, sec. 1). This represents a mortality rate of about 3 mice per week— insignificantly lower than the mortality rate in Pop B before emigration. The mortality rate was three times higher after the opening of the second enclosure than before (Table 62, sec. 2).

Twice as many mice, both males and females died in Sub-Pop BA as in Sub-Pop BB. Male mortality exceeded that of females as it had during the previous six months; about twice as many males died as females in each subpopulation (Table 62, sec. 3).

Three-fourths of the dead animals came from Cohort II and III, i.e., 19 of 22 or 86% in Sub-Pop BA and 6 of 10 or 60% in Sub-Pop BB. Three times as many dead babies were found in Sub-Pop BB as in Sub-Pop BA on the day of census.

Table 62

Special studies: Migration

Deaths of adult mice— 29 November 1964-12 March 1965

Section 1	Male	Female	Total
Total dead bodies recovered in cages	27	15	42
Total deaths from differences between two censuses	29	16	45
Number of mice not recovered	2	1	3

Section 2			
Cohort I	1	1	2
II	0	2	2
III	0	0	0
IV	1	0	1
V	0	1	1
Cohort not identified	0	1	1
Total	2	5	7

Section 3	SubPop BA			SubPop BB			Pop B (BA + BB)		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Cohort I	0	0	0	1	0	1	1	0	1
II	7	2	9	3	1	4	10	3	13
III	0	0	0	0	0	0	0	0	0
IV	7	2	9	2	0	2	9	2	11
V	0	2	2	1	1	2	1	3	4
Cohort not identified	2	0	2	0	1	1	2	1	3
Total	16	6	22	7	3	10	23	9	32

Genetics:

Polymorphism at "C" locus in subpopulations at census. The frequency of recessive c was higher in the new cohort than in the adult population, though the difference is not statistically significant (Table 63). The frequency in the new cohort of 0.638 differs significantly from 0.500.

Table 63

Special studies: Migration

Number of adult mice homozygous recessive at C locus at census of 12 March 1965.

SubPop BA			SubPop BB			Total		
c/c	C/-	Total	c/c	C/-	Total	c/c	C/-	Total
98	199	297	129	194	323	227	393	620

c/c = homozygous recessive at C locus

C/- = heterozygous or homozygous dominant

The babies were scored for alleles at the "C" locus by examining the eyes, which are unpigmented in homozygous recessive mice. Pigment was present in 90 babies and absent in 45 babies. This gives a frequency of 0.571 (N = 138) for c, a frequency lower than that of the juvenile mice.

A greater number of adult homozygous recessive mice were present in Sub-Pop BB than in the emigrant subpopulation (Table 64). The frequency of c among the adults of Sub-Pop BB was 0.632, compared with a frequency of 0.577 for those in Sub-Pop BA. In both subpopulations the frequency of c is lower in the babies than in the juvenile mice.

Reunion of Population:

Both subpopulations were returned to enclosure B after the census (7:30PM, March 12, 1965) and the passageways connecting enclosure B with enclosure A were closed. During the next five hours, the mice were very active as they usually were following a census.

However, 13½ hours after the mice had been replaced in the enclosure, the intensity of activity had changed little from the previous evening. Most of the animals were continuously on the move and remained that way throughout the next 24 hours. About half of the paper shreds placed in the enclosure were untouched; usually, following a census,

Table 64

Special Studies:
Migration

Frequency of recessive c in Pop B at November 1964 and March 1965 censuses

November 1964		c	±S.E.	N
Pop B		0.601	0.030	680
March 1965				
Pop B—old cohorts		0.605	0.031	620
New Cohort		0.637	0.074	108
Babies		0.571	0.069	138
New mice (new cohort + babies)		0.611	0.050	246
Pop B (including new cohort)		0.610	0.028	728
SubPop BA	adults	0.576	0.046	297
	new cohort	0.621	0.083	88
	babies	0.592	0.093	74
SubPop BB	adults	0.632	0.043	323
	new cohort	0.745	0.148	20
	babies	0.544	0.104	64

mice used large amounts of paper to build nests and other structures in their environment. Most of the nurseries had no adult females and many babies and young mice were scattered in peripheral retreats and in the central area. Constant fighting persisted in one or more areas of the enclosure. Eighteen newborn babies, six mice of the new cohort (4 males and 2 females) and nine older mice (Cohort I - 2 males and 1 female; III - 3 males; IV - 2 males; and V - 1 female) were dead.

At 9PM on March 13, 1965, slightly more than 24 hours after the census, there were four additional dead babies, and 11 dead adults (Cohort I - 1 female; II - 1 male and 2 females: III - 2 males: IV - 3 males; V - 2 males and 1 female). Autopsies of gross organs of these animals revealed abnormal lungs in two animals; there were darkened areas of consolidated tissue in all lobes.

The next morning, 36 hours after the census, the hyperactivity and amount of fighting had diminished. Two more adults (Cohort IV - 1 male and 1 female) and many newborns had died and parts of the incompletely cannibalized bodies of babies were present in six peripheral retreats.

On March 15, 1965, 48 hours after the census, a "chattering"-like sound was audible on entering the room housing the population enclosure. In marked contrast to the previous hyperactivity, most of the mice were standing still, head down, body drawn together. Many of the mice had ruffled coats. There were more than 40 dead mice. Of 500 mice, 292 showed one or more gross signs of disease before they were sacrificed with chloroform. Twenty-two mice had purulent conjunctivitis, eighteen had visible subcutaneous abcesses (in the pelvic area and over the shoulders). Autopsies were performed on fifty randomly selected mice and gross evidence of disease found in 27 of them (Table 65).

Microorganisms were recovered from the homogenized organs (liver, spleen, kidney, lung) of 6 out of 15 randomly selected mice and were identified by Dr. Rose Mushin as <u>Proteus mirabilis</u>. The same microorganisms were found in fecal specimen of each of 10 other animals.

Table 65

Special Studies: Pop B— Migration and subpopulations

Gross organ autopsies of sample of mice of Pop B— 16 March 1965

	Lung	Spleen	Liver	Kidney	Heart	Peritoneal fluid	Total
Number of mice with pathologic findings	19	12	2	1	0	6	40

Abnormalities noted:

Lungs: areas of consolidation, "rubbery" lungs

Spleen: enlargement— two to three times normal size

Liver: hemorrhagic areas

Kidney: hemorrhagic areas

Peritoneal fluid: hemorrhagic— 4; purulent— 2

Mice with

1) one pathological organ— 19

2) two pathological organs— 4

3) three " " 3

4) four " " 1

SELECTION EXPERIMENT

Table 65

Special Studies: Pop B— Migration and subpopulations

Gross organ autopsies of sample of mice of Pop B— 16 March 1965

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Peritoneal fluid: hemorrhagic— 4; purulent— 2

Mice with

1) one pathological organ— 19

2) two pathological organs— 4

3) three " " 3

4) four " " 1

SELECTION EXPERIMENT

Selection for large body weight in different social environments.

Selection for performance for a variety of traits has been carried out with many vertebrate species, both in agriculture (158-160) and for experimental purposes (161-166).

That the expression of all traits depends on the interaction of heredity and environment has been demonstrated, among other, by the fact that when a trait has been selected for in one type of environment, its expression may turn out to be quite different in another environment. Conversely, selection for the same trait in different environments tends to involve differences in heredity and physiology. For example, selection for large body size in Drosophila kept on different diets showed that the developmental changes basic to the response for the same character were deeply influenced by the nature of the different environments (129, 167-71). Selection for large body size in mice given high vs. low protein diets produced different large "mouse genotypes," mice having different physiological and biochemical properties (130). Such evidence suggested that similar principles might also apply to selection for a given trait when different types of social environment were used as environmental variables.

Attempts to select for components of Darwinian fitness in different social situations met with experimental difficulties. For simplicity and to see what the possibilities were to use this approach as a model to study adaptability to crowding, large body weight at sexual maturity was finally chosen as the character for selection, and groups of different sizes as the social environmental variables. Selection was carried out in uncrowded groups, animals kept as pairs, and in crowded groups consisting of 20 to 30 animals in a single cage.

The three types of problems posed by this series of experiments were: 1) what is the response to selection in each environment; 2) how would the different selection lines perform when placed in the opposite environment, that is, animals selected in uncrowded environments tested in crowded groups and vice versa; and 3) what kinds of characters are correlated with the trait selected in the different environments.

Procedure: The same kinds of crosses between the same four strains of mice used to produce the founder population for the population studies were used to initiate the selection lines, i.e., the founder mice of the selection study were progeny of reciprocal crosses between the two F_1's derived from SWR/J x C57L/J and C3HeB/FeJ x 129/J matings.

Thirty-two litters born on each of two successive days were used to setup replicate uncrowded and crowded lines. An uncrowded line consisted of mice randomized in pairs in standard laboratory cages (stainless steel, 10"x7"x5"; wire mesh top, 3/8"; circular feeder, 2" in diameter, 3" in height, 3/8" mesh). Mice of all litters of each crowded line were placed together in a single larger cage (stainless steel, 12"x12"x8"; wire mesh top, 3/8"; central circular feeder, 5" in diameter, 5" in height, 3/8" mesh). In the last two generations, mice of both crowded lines were placed in a common cage because of small numbers of animals. Sexes were separate. Food as pellets (D and G) and tap water were always present in excess. All cages were changed and autoclaved weekly and provided with pine wood shavings. The temperature of the room was kept between 77 and 79 degrees Fahrenheit through the year.

Mice were weighed, sexed, and earpunched and placed in these social environments at 22 days of age, weaning time for mice, and remained in them for 22 days until sexual maturity, when they were weighed again.

Selection for all lines was a within-litter procedure, a scheme that has the advantage of eliminating maternal effects common to litter mates, which are particularly important for traits such as weight (172-4). The heaviest male and female of each litter at sexual maturity were selected each generation to be parents of the next generation; thus, eight males and eight females were selected in each of the four (replicate crowded and uncrowded) lines each generation.

A mating scheme suggested by Dr. Allan Robertson* and communicated to me by Dr. D. Falconer* was used for all lines as outlined below:

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*The Institute of Animal Genetics, University Department of Animal Genetics, MARC Unit of Animal Genetics and MRC Induced Mutagenesis Group, Edinburgh, Scotland.

Mating Pair Number		Number of Males	x	Number of Females
1	=	1	x	2
2	=	3	x	4
3	=	5	x	6
4	=	7	x	8
5	=	2	x	1
6	=	4	x	3
7	=	6	x	5
8	=	8	x	7

Mice with the same number are full sibs and not the same individual. Matings 5-8 in this experiment were reciprocals of matings 1-4.

The scheme keeps inbreeding to a minimum. Least related males or females were chosen to make up breeders for unfertile matings when necessary. Matings were set up when all litters had reached 44 days of age. Only one litter was raised in each generation, except when cross-tests were done in generation six and seven when an additional litter was required.

Results

Response to selection. The response of males and females to selection was different. The generation means for weights at 44 days of age together with standard errors calculated from the variances of litter means are included in Table 66. The generation means plotted in Figure 31 show that the mean weights of mice crowded in unisexual groups between weaning and sexual maturity are depressed compared to those of uncrowded pairs. Response curves of replicate lines are highly similar. Progress with selection occurred in both environments. Response is slow but steady during the first 4-5 generations, but becomes irregular thereafter in all lines. Fertility declined during the last two generations for all lines, and prevented the experiment from being continued beyond seven generations.

The slopes of straight lines fitted to response curves provide approximations to mean progress. The rate of progress turned out to be comparable for animals of each sex in crowded and uncrowded environments. Progress of females was lower than that of males (Table 66).

The selection differential for each generation is the mean deviation of selected parents from their litter means weighted by the number of offspring in the next generation, and successive selection

Table 66

Selection: Response and selection differentials in crowded and uncrowded environments for weight at 44 days of age

Generation	Replicate 1 Response Means (g)	Replicate 1 ±S.E.	Replicate 1 Selection Differential (g)	Replicate 1 No. Litters	Replicate 1 No. Mice	Replicate 2 Response Means (g)	Replicate 2 ±S.E.	Replicate 2 Selection Differential (g)	Replicate 2 No. Litters	Replicate 2 No. Mice
					Uncrowded Males					
1	22.9	0.12	3.2	8	32	22.5	0.25	3.7	8	32
2	22.7	0.15	1.5	8	29	22.2	0.17	2.1	8	31
3	24.2	0.46	2.2	7	25	24.0	0.37	2.1	8	27
4	25.9	0.45	1.2	7	25	25.2	0.25	1.0	8	28
5	23.4	0.24	1.7	7	22	23.6	0.46	1.4	7	28
6	30.7	0.90	1.6	6	18	29.4	0.73	2.6	7	22
7	32.3	1.46	0.8	4	15	30.6	0.69	0.8	6	21
					Crowded Males					
1	20.3	0.17	2.3	8	30	20.0	0.20	2.6	8	34
2	20.5	0.51	1.0	8	26	20.8	0.22	1.1	8	21
3	22.2	0.56	2.1	7	23	22.8	0.43	2.0	7	28
4	22.0	0.75	1.3	7	19	21.3	0.56	1.6	6	15
5	22.5	0.36	2.1	6	19	24.1	0.65	2.2	6	22
6	24.6	0.29	2.4	6	23	24.2	0.49	3.4	6	16
7	28.1	0.42	0.0	3	11	29.8	1.44	0.2	3	11
					Uncrowded Females					
1	19.1	0.17	1.2	8	30	19.1	0.21	1.6	8	30
2	19.2	0.44	1.0	8	29	19.2	0.27	1.2	8	27
3	19.6	0.32	1.0	7	21	19.6	0.28	1.3	8	19
4	19.9	0.49	1.1	6	24	20.7	0.28	0.6	8	26
5	20.0	0.37	2.0	7	30	21.2	0.45	0.6	7	31
6	24.2	0.50	0.3	6	22	25.1	0.29	1.7	7	19
7	26.3	0.91	2.2	4	7	25.1	0.89	0.6	6	19
					Crowded Females					
1	17.3	0.10	1.1	8	29	17.1	0.10	1.4	8	26
2	16.6	0.42	1.1	8	27	16.9	0.14	1.0	8	31
3	19.1	0.53	0.7	7	22	19.5	0.22	1.3	7	18
4	18.6	0.69	1.3	7	26	18.3	0.75	2.1	6	25
5	17.3	0.23	1.1	6	23	18.8	0.38	1.1	6	17
6	21.5	0.14	0.3	6	14	20.0	0.22	1.7	6	18
7	21.6	0.53	1.6	3	10	23.0	0.61	2.8	3	11

The response to selection is the mean of litter means in grams for weights at 44 days of age in each generation.
The selection differential is the mean deviation in grams of selected parents from their litter means weighted by the number of offspring in the next generation.

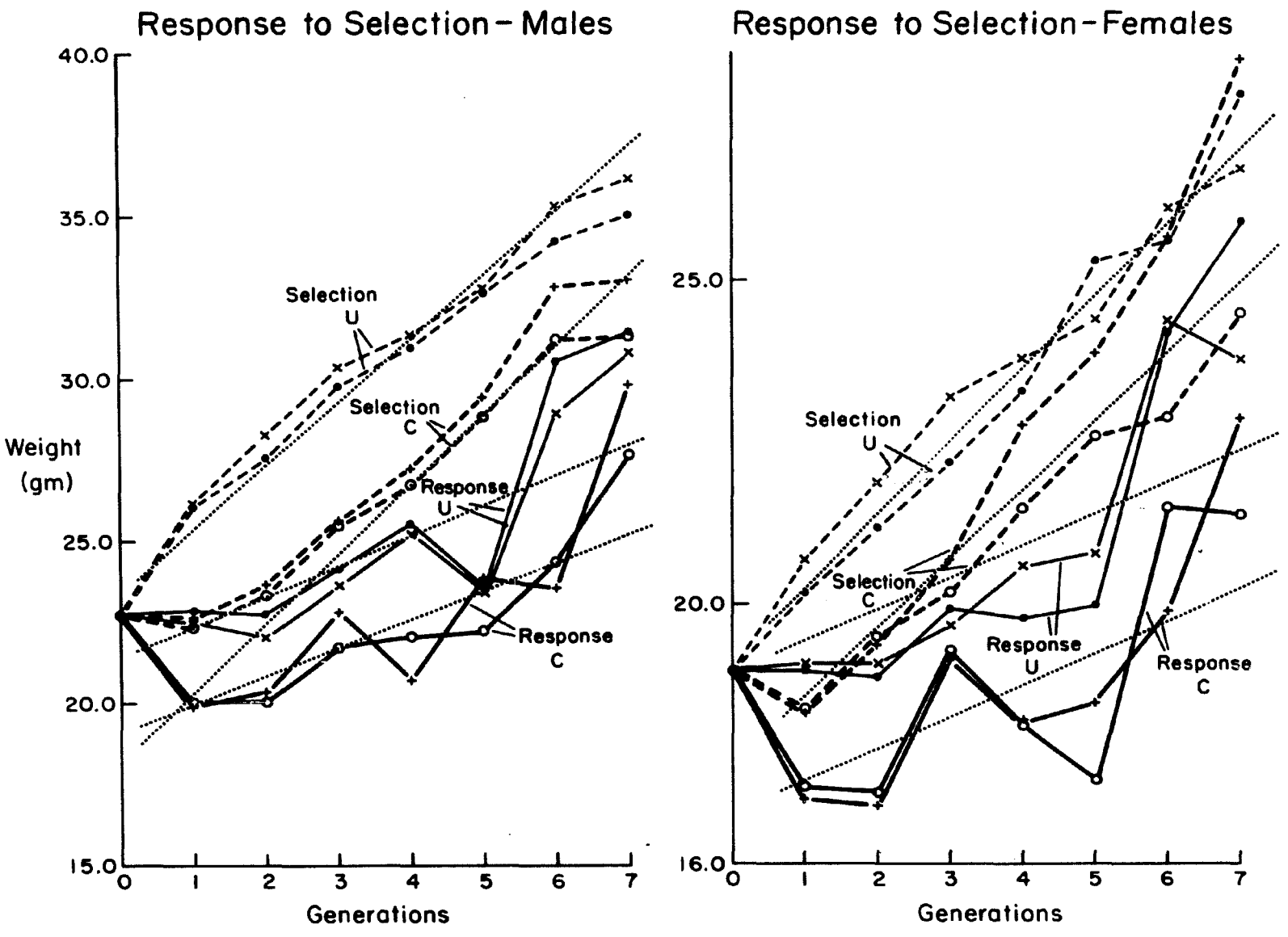

Figure 31 Selection for heavy weight at 44 days in uncrowded and crowded groups: response and cumulative selection differential.

The response curves— solid lines— of uncrowded "U" and crowded "C" lines represent the mean of litter means for weights at 44 days of age. The selection curves— cross-hatched— of uncrowded "U" and crowded "C" lines represent cumulative selection differentials, that is, the sum of successive weighted selection differentials of each generation. The ratio of the slopes of the straight lines—dotted lines— fitted to the response and selection curves provides approximations to the heritabilities of six-week weights in uncrowded and crowded groups.

differentials added together make up the cumulative selection differential. The cumulative selection differentials represent the mean weight at sexual maturity that mice of different lines should have had, had the weights of offspring in each generation equaled those of their selected parents. The slopes of cumulative selection curves are constant and replicate lines are highly comparable, except for the crowded female lines which diverged slightly. The slopes of straight lines fitted to the selection curves show similar rates of change for uncrowded and crowded animals of the same sex, and females again had smaller rates of gain than males (Table 66).

The heritabilities of six-week weight, the ratio of response to selection differential, are about equal for both sexes in the two environments. The magnitude of the heritabilities are large; the value of 50% suggests that the fraction of within-litter variances that is heritable may be about equal to the fraction attributable to environmental components but the lack of contemporaneous non-selected lines makes the exact values of the heritabilities not absolutely reliable. This is particularly important with respect to the irregular pattern of response during the last two generations (Table 67).

Table 67

Selection: Mean response,
Selection differential, and Heritability for weight at 44 days of age

		Response g./gen. (R)	Selection Differential g./gen. (S)	Heritability R/S in %
Uncrowded:	Males	1.0	2.0	50
	Females	0.7	1.3	50
Crowded:	Males	1.0	2.0	50
	Females	0.7	1.3	50

Phenotypic variances (within line, between litter), expressed as coefficients of variability to permit comparisons between lines having different generation means, fluctuate in all lines (Figure 32). The coefficients of variability lie between 4 and 8% during the first generation. In the uncrowded lines and in one crowded male line, the coefficients of variability range between 10 and 18% thereafter. In three out of four crowded lines, values of over 20% are attained during the first four generations but decline to 4-8% during the last two generations.

Environmental exchange. Mice selected in crowded environments weighed less than those selected in uncrowded environments at the end of six and of seven generations. Table 68 shows what happened when crowded lines were placed in uncrowded conditions at weaning time and vice versa when uncrowded lines were subjected to crowding. Cross-testing was performed in generations six and seven.

Males selected in crowded environments weighed more than those selected in uncrowded environments when tested in both uncrowded and crowded conditions at high levels of statistical probability— 4 of 8 comparisons. One line of uncrowded males outperformed crowded males in crowded conditions, and in three comparisons the weights of the lines did not differ significantly. The cross-tests with females revealed an equal number of tests in which mice of each type of line weighed more than those of the opposite line in each environment, but the differences in cross-tests showing superiority of crowded lines attain higher levels of statistical significance. The weights of cross-tested animals of all lines and both sexes were greater than the initial or unselected weights.

Correlated traits. Changes in several biological characters were noted along with the response for the selected trait (Table 69). The number of fertile matings declined in all lines but there were no differences between uncrowded and crowded mice. Mean litter size was slightly larger in uncrowded lines but here too the difference was not significant. Mice of uncrowded lines mated more promptly than those of crowded lines, and the difference of the mean interval between potential mating and birth of a litter for all generations was significant at the 5% level (Figure 33). Weaning weights did not differ between uncrowded

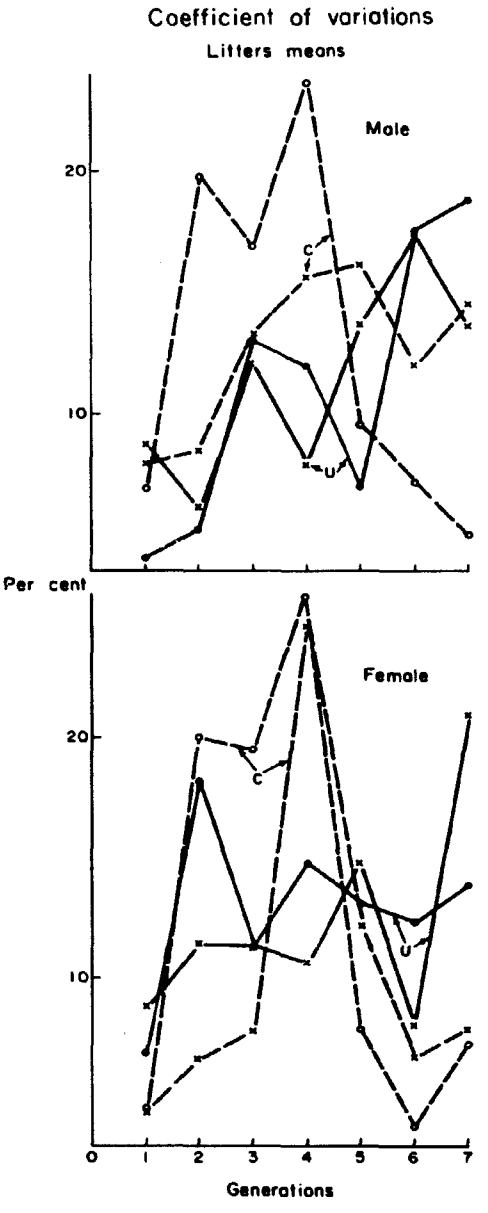

Figure 32 Selection experiment. Coefficients of variability of 44-day weights.
Since uncrowded and crowded lines have different means, phenotypic variances have been plotted as coefficients of variability.
"U"—uncrowded replicate lines—solid curves
"C"—crowded replicate lines—cross-hatched curves

TABLE 68. Selection: Environmental Exchange

MALES

	Generation 6				Generation 7		
	Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1 & 2
Tested in Uncrowded Environment							
	U_1	C_1X^*	U_2	C_2X^*	U_1	U_2	$C_{1,2}X^*$
Mean Wt.	30.62	31.08	29.03	30.13	31.53	30.92	31.49
± S.E.	0.221	0.102	0.176	0.121	0.189	0.123	0.111
n	18	20	22	20	16	21	18
Δ(g)	+0.46		+1.10		-0.04	+0.57	
t	1.42		3.90		--	2.43	
p	n.s.		*0.001 C>U		n.s.	0.05 C>U	
Tested in Crowded Environment							
	C_1	U_1X^*	C_2	U_2X^*	C_1	C_2	$U_{1,2}X^*$
Mean Wt.	24.45	23.68	23.58	24.46	27.80	29.89	28.36
± S.E.	0.104	0.113	0.274	0.191	0.142	0.246	0.100
n	22	21	16	18	12	11	20
Δ(g)	-0.77		-0.88		+0.56	-1.53	
t	3.50		1.87		+2.33	-4.37	
p	0.002 C>U		n.s		0.02 U>C	≪0.001 C>U	

*UX and CX--cross-tested

TABLE 68. (continued)

FEMALES

	Generation 6				Generation 7		
	Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1 & 2
	Tested in Uncrowded Environment						
	U_1	C_1X^*	U_2	C_2X^*	U_1	U_2	$C_{1,2}X^*$
Mean Wt.	24.24	23.95	24.94	23.96	25.92	23.85	25.22
± S.E.	0.079	0.116	0.116	0.106	0.594	0.171	0.087
n	22	21	19	18	7	19	18
Δ(g)	-0.29		-0.98		-0.72	+1.37	
t	1.51		4.41		1.10	5.3	
p	n.s.		<0.001 U>C		n.3.	≪0.001 C>U	
	Tested in Crowded Environment						
	C_1	U_1X^*	C_2	U_2X^*	C_1	C_2	$U_{1,2}X^*$
Mean Wt.	21.55	19.84	19.95	20.17	21.47	22.99	22.29
± S.E.	0.078	0.102	0.113	0.104	0.131	0.187	0.159
n	14	18	18	19	10	11	16
Δ(g)	-1.71		+0.22		+0.82	-0.70	
t	9.5		1.0		2.83	2.02	
p	≪0.001 C>U		n.s.		0.01 U>C	n.s.	

*UX and CX-- cross-tested

Table 69

Selection: Correlated traits

	Uncrowded		Crowded	
	Repl. 1	Repl. 2	Repl. 1	Repl. 2
1. Fertile matings (means)	6.7	7.5	6.5	6.3
2. Mean litter size	7.0	7.0	6.7	6.7
3. Promptness of mating (means)	21.6	21.7	23.2	22.9
4. Mortality bet. 22-44 days	2/329	14/360	6/302	8/293
5. Runts	0	1	2	6
6. Frequency allele recessive c				
Progeny	0.279 ± 0.017		0.192 ± 0.017	
Parents	0.351 ± 0.022		0.352 ± 0.022	
7. Proportion of males (%)	50.1	52.6	50.0	50.1

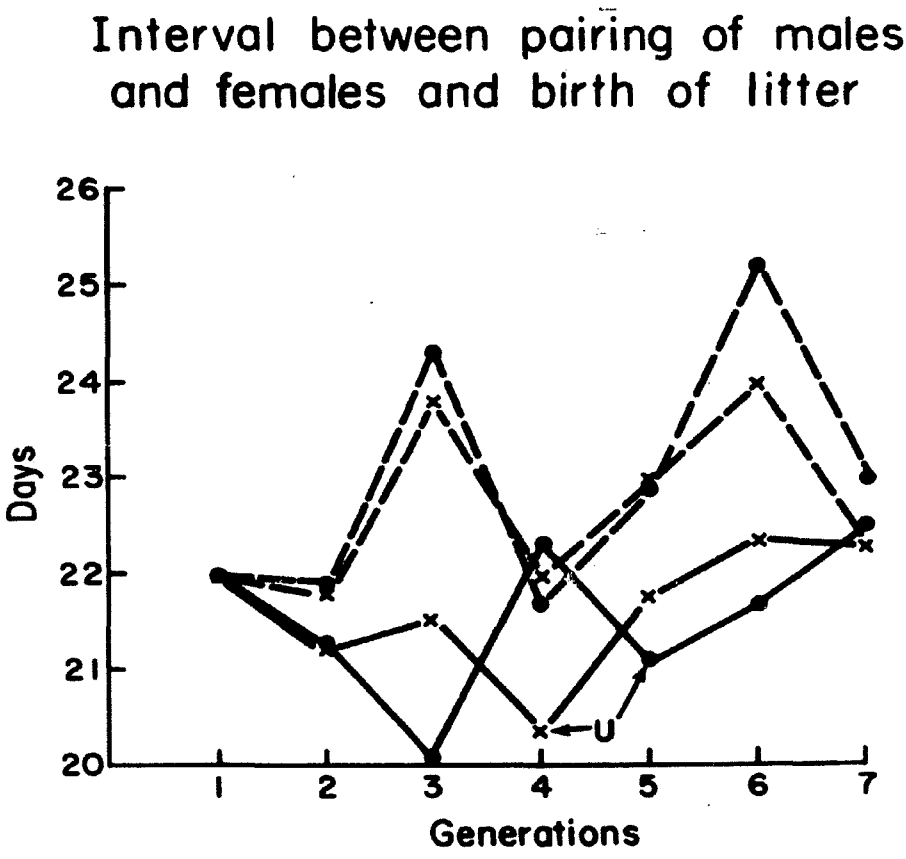

Figure 33 Selection experiment.

and crowded mice throughout the seven generations in spite of the differences of mean weights at sexual maturity in the two types of lines. The weights at weaning time rose above those of the original or unselected mice in the sixth generation in the uncrowded lines and in the seventh generation in the crowded lines (Figure 34).

Mortality between weaning and sexual maturity was low and similar in the two types of environment. There was one runt (a mouse with low weight falling outside the normal distribution of the rest of its litter) among the 689 uncrowded mice and six runts among the 595 crowded ones. The frequency of recessive alleles at the "C" locus was equal in parents selected in uncrowded and crowded environments by pedigree analysis, but uncrowded progeny had a significantly higher frequency of recessive c than those of crowded ones. The sex ratio of all four lines did not differ significantly from 50%.

Autopsies of gross organs of mice of infertile matings in the seventh generation revealed no abnormalities.

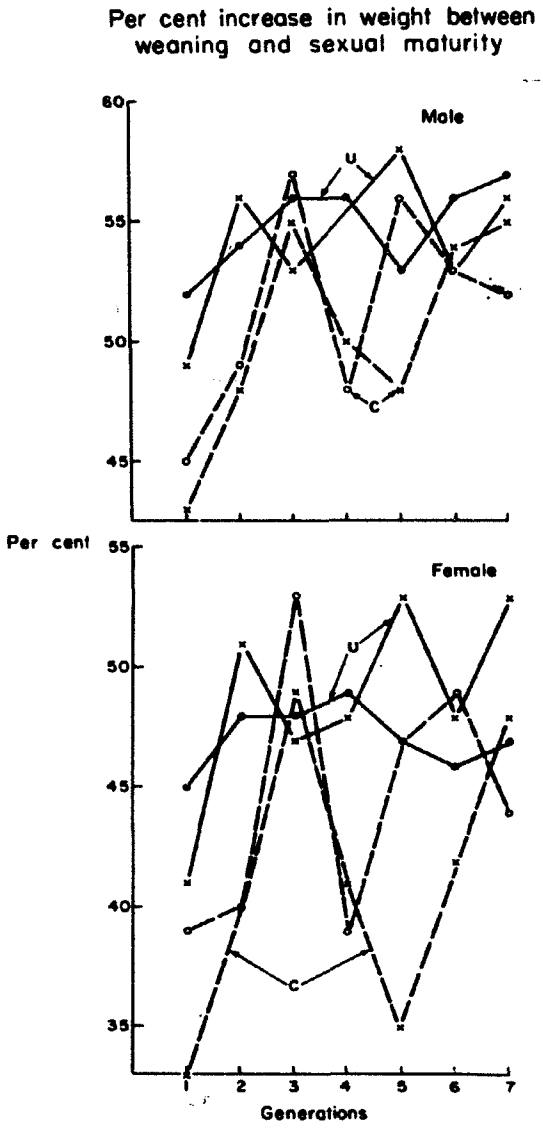

Figure 34 Selection experiment.

DISCUSSION

DISCUSSION

The studies demonstrate the range of interplay between socio-ecological and biological processes in experimental populations of infra-human vertebrates. Three general lines of evidence highlight the simultaneous changes and dynamic interaction between biology and socio-ecology in the populations of mice. First, distinct changes accompanied the socio-ecological transformations in the enclosures in contrast with the constancy of physiological, behavioral, genetic and demographic processes in the control environment. Second, cohorts in the populations were biologically distinguishable subunits in contrast to control cohorts, which showed no such differentiation, and the differences of attributes and life histories of cohorts reflect interrelationships between social and biological factors. Third, changes occurred in many of the biological properties of animals of the enclosure populations when the animals were placed in different social environments, attesting to the specificity of the influence of social factors.

Changes in enclosures

Population Dynamics

Many things occurred in the enclosures that did not occur in the control group. Most dramatic of these changes were those of population dynamics. One of the unusual aspects of the population studies was the large sizes and high densities attained by the freely growing populations. Both populations grew to sizes that are several times larger than those of any previously reported laboratory populations of small mammals (175-182). Other populations of Mus musculus followed for equivalent periods of time reached their upper plateaus with 25 to 150 mice, even where the amount of space was one to seven times greater than that of the present study. In no other study do densities approach those of 60-80 mice per square foot attained in Pop B and Pop A. An exceptional population of Mus musculus of slightly more than 200 mice was produced only when "tranquilizer" drugs were present in the drinking water (183-4).

Under natural conditions, infestations of fields or of storage depots by small mammals have reached large sizes (185-88). An example of physically delimited, natural "enclosures" in which this occurs are grain ricks, where more than 1500 wild mice have been known to accumulate in a

year's time (189). Of course, the space available in such ricks is 25 to 50 times greater than that of enclosures A and B and thus the densities are never as high.

The types of animals, the number of individuals used as founder nuclei, and the physical structure of the environment are some of the factors that may underlie the differences of size and density of the populations of the present study and those of other studies.

The aggressiveness of feral and wild caught animals may be one explanation for the lower population sizes attained in studies using them as subjects (190-92). However, even in a long-term freely growing population of albino, presumably domestic, laboratory mice, population sizes and densities remained very low (193). A few studies of populations of inbred strains of mice and rats also produced small populations (194-5).

The extent to which the large sizes attained by Pop A and B were related to the particular genetic backgrounds of the mice cannot be answered until the entire study is repeated with mice of other four-way crosses in similar enclosures. The behavioral and physiological properties of young and adults of the present crosses may have been important factors promoting survival in the type of environment that developed in the enclosures.

The findings of a pilot population study, preliminary to the one reported here, suggests that the particular type of mice used is not the only factor at the root of the population sizes attained. This population, initiated with 10 pairs of C57BL/J mice and 10 pairs of BALB/CJ mice, grew to a size of over 300 in an octagonal enclosure of similar design but with only one-half the surface area of the enclosures of Pop A and Pop B, and maintained this size over an 18-month period (196).

Most freely growing populations have been initiated with small numbers of animals, one to four pairs of mice. Such strategy is dictated usually by the desire to enhance control over genetic and environmental variability of the animals. Both these goals were obtained in the present studies without recourse to such small numbers of founders.

It is possible that the relatively large number of mice used to initiate the two freely growing populations was related to the large population sizes attained. The initial rapidity of growth of the populations resulting from reproductive activities of about 35 females may have delayed

the behavioral and physiological mechanisms that ordinarily inhibit recruitment and enhance mortality at much lower population sizes and densities.

Such mechanisms, indeed, did come into operation in the populations but only when 800-1000 mice had accumulated within the enclosures. It would be of interest to initiate other replicate populations with only one or two pairs of animals from the same four-way cross.

Another way to examine this problem would be to compare the asymptotic sizes and mechanisms of self-regulation of populations with rapid vs. slow initial growth rates. Different strains, or combinations of strains, of mice with appropriate constellation of behavioral and physiological properties would be particularly suitable for such studies. Population growth rates also could be controlled by means of artificial predation.

The physical structure of the environment can play an important role in the development of social groupings (197). The spatial layout of physical barriers, the distribution of objects in the environment, and the location of food and water "modify the frequencies and patterns of contact between individuals, which in turn are important variables in determining the size and stability of groups..." (198).

For example, the complexity and richness or, on the other hand, bareness of the physical environment may influence the development of social behavior of individuals and populations (199-200). Manipulation of the physical structure of the environment can produce strikingly different social constellations of individuals and different population dynamics in freely growing populations. There are marked differences between the population dynamics of deer mice released into identical enclosures after a period of confinement in enclosures that differ in physical layout (201). Dissimilar social and population dynamics were produced in enclosures having single versus multiple sources of food (202), or when the relative position of a cage in a series of connecting units was altered (203).

The physical structure of the enclosures of the present study is only moderately complex; the mice provided additional environmental variety by subdividing the floor area of the enclosure into surface and subsurface areas with nesting material.

Individuals of different age, sex and social "status" tended to aggregate in different areas of the enclosures. These social subunits, how-

ever, were continuously broken down because all individuals had to come to the center of the enclosure for food and water. To what extent this continuous breakdown of socially differentiated subunits, and the ensuing obligatory social interaction, influenced the large sizes and densities of the populations is unclear. Sizes reported in other mouse populations were slightly smaller when single rather than multiple food and water sources were provided (178).

Whether changing the complexity of the physical environment would lead to altered population dynamics can be subjected to systematic experimentation. For example, the amount of space and degree of environmental complexity could be increased by using higher outer walls with shelves for nest boxes, with access to the ground via ramps in the enclosures.

There is another way of looking at the population dynamics. The control population would have yielded 100,000 mice in 35 weeks had it continued to grow as it did at first (Figure 35). For the particular genotypes used, the intrinsic rate of natural increase— the constant "r" of the equation $N_t = N_o e^{rt}$ (where N_o = the number of animals at time zero, N_t = number of animals at time t, and r = the infinitesimal rate of increase) — would not have changed during the year of study since reproduction remained essentially constant and mortality was nggligible in the control environment. In the enclosures there would have been 100,000 mice at the end of one year had the initial growth rates persisted. But the physiological and behavioral mechanisms for rapid and opportunistic build-up of populations of mice became liabilties as space and other resources are restricted. Changes in the social component of environment appeared to influence behavior, physiology, and mortality, providing some control over potentially runaway demographic inflations. The self-regulatory processes called into play were closely related to changes in social interaction affecting reproduction and, to a lesser extent, mortality, especially of newborns.

Changes of reproductive physiology

Changes of reproductive physiology constitute prominent aspects of self-regulation in the enclosures. Fertility and fecundity declined. Demographic input peaked early in the year and fell steadily thereafter, despite the increase in the number of adult females potentially able to contribute to recruitment. The fall of input per breeding females, in fact, even pre-

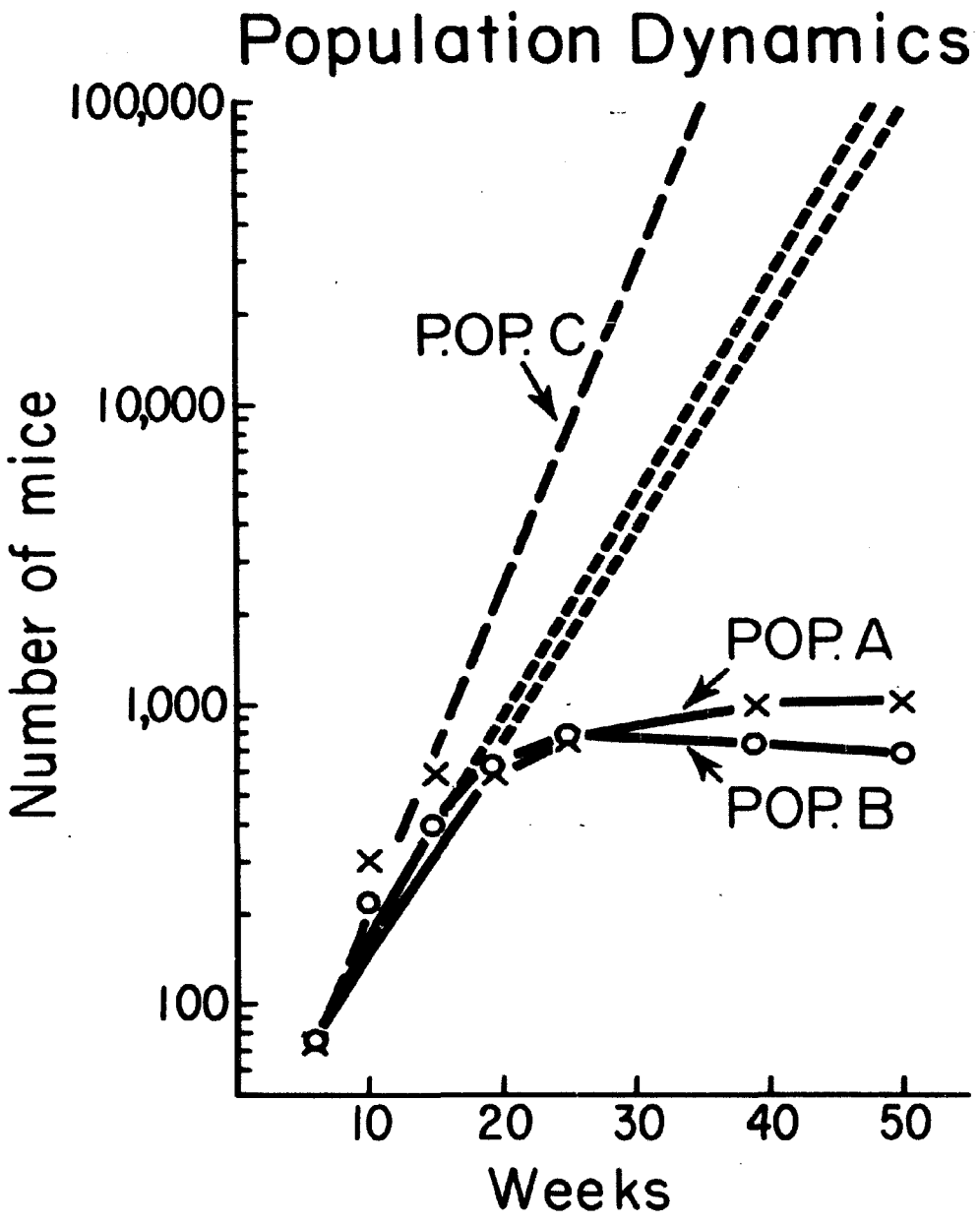

Figure 35. Population dynamics. Population growth was exponential in the enclosures during the first 15-20 weeks. The projection of such growth rates beyond this period (cross-hatched lines—Pop A and B) reveal how large the populations might have gotten in the absence of the self-regulatory processes that appeared. By contrast, population growth remained constant in Pop C; however, this population was not allowed to attain the sizes indicated, since only samples of its mice were saved every 12 weeks to become breeders for the next period.

ceded peak recruitment and declined rapidly thereafter.

The analysis of maturation and reproduction pointed to inhibition of reproduction in sexually mature females as the most important factor in the decline of productivity per adult female. Delay or inhibition of sexual maturation of females in conditions of high population density has been documented in certain populations of laboratory and wild mammals (204-208), but there was little evidence that this occurred here.

The percentages of pregnant females in the enclosures fell steadily in contrast to control females that showed steady 100% pregnancy rates. Yet the nonpregnant females of Pop A had sexually mature adult gonads when autopsied at the end of the year and, indeed, promptly resumed normal reproduction when taken outside the enclosures. Inhibition of full-term gestation or abortions probably occurred, since more than twice as many females showed evidence of early pregnancy compared with the 10% with advanced pregnancies at the time of autopsies.

The usual 4-6 day estrus cycle of mice may be disturbed by grouping (209-10) and spontaneous pseudopregnancy (212-13) and anovulatory anestrus (211) are frequent under conditions of crowding. In a number of studies, pregnancy block or abortion brought about by the exposure of recently inseminated females to males (214-16) other than the specific mate has been noted to diminish when females congregate (205, 217). Perhaps this protective mechanism was related to the differential distribution of females in the periphery of the enclosures, although its effectiveness may have been diminished by the necessity of going to the center of the enclosures for food.

Behavioral and social abnormalities observed in the enclosures were often directly related to reproduction. The extent to which behavioral anomalies, such as bizarre mating behavior, attacks before and during pregnancy, rapid succession of mounts by many males, etc., contributed to the inhibition of female reproduction and to interruptions of full-term gestation is not known (199, 202).

Reproductive physiology of males as judged by the size of gonads and the presence of sperm in the cauda epididymis did not appear to be affected by the profound social and demographic transformations that took place in the enclosures. Almost all males were fecund. This differs from

other studies of rodents in which male fecundity was reduced at lower levels of population density, and in situations characterized by lesser degrees of socio-behavioral abnormalities than those of the present study (68, 176, 218-19).

Reproductive ability of males appeared and was affected predominantly at the behavioral level. Large numbers of males were essentially asexual during the last 4-5 months. Indeed, not a single male of the last two cohorts of Pop A even attempted to mount sexually receptive female subjects at the end of the year. Large numbers of males of Cohorts V (June 1964) and VI (August 1964) were hardly ever observed mating; their behavior and distribution in the enclosures were more like those of females and, indeed, older males in the enclosures often displayed sexual behavior towards them as though they were females.

Mortality

All three populations illustrate a phenomenon common to most mammals, namely, that beyond the newborn period, chances for survival improve greatly (220-24). The experiments were not continued long enough to determine physiological longevity (longevity under "optimal" or control conditions) of the genotypes of Mus musculus used. There were no deaths among adult control mice during the fifteen months of study. As far as could be determined, there was no newborn or juvenile mortality in the control environment either.

Newborn mortality was an important mechanism of demographic control in the population enclosures. The importance of neonatal mortality was evident from observations of dead newborns. It was particularly striking in Pop B, where 30% of the females showed advanced pregnancy during the last 5½ months, although no recruitment at all was taking place. Social and behavioral factors underlying newborn mortality include crowded nurseries, nurseries containing young of different ages and sizes preventing normal nourishment of newborns, continuous activity of young in nurseries, inadequate, disturbed and abnormal maternal behavior, disruption of nurseries by males, cannibalism, interference with nests, etc. Females also may have lacked adequate milk supplies but this was not studied. Such breakdown of patterns of care of young has been noted in other crowded and socially unstable populations (175, 194, 202).

The pattern of mortality in the two enclosures during the year was unusual. The noteworthy feature is that the highest weekly death rates occurred during the first half of the year, before peak numbers were present in the populations.

Pathology - Causes of Death

The causes of juvenile and adult deaths were difficult to document. Gross autopsies done during the course of the year were unsatisfactory. Little gross and microscopic evidence of pathology was found in the sample of almost 200 mice of Pop A autopsied at the end of the year. Hypoglycemia has been implicated as a cause of death in crowded and socially unstable populations of certain mammals (225), though hyperglycemia has also been found to occur in grouped situations of rats (226). The histologic appearance of the liver in the present study, in so far as this reflects carbohydrate metabolism, suggests normal function. There was no evidence of splenic hypertrophy and increased erythropoiesis, as has been noted in certain studies of crowded and aggressive groups of mice (228) and voles (227). Brief descriptions of vaginal and peritoneal hemorrhage and of abnormal dilitation of the uterus with extensive inflammation throughout the reproductive organs have been reported in crowded populations of rats (199). Although such pathology was not found in the present study, focal inflammatory changes were noted in the kidneys of a small group of mice, similar to those described in rats of the study referred to above, and found in a crowded population of woodchucks (229).

Bacteriology

The caecal cultures of samples of mice of Pop A grew an essentially normal spectrum of microbiota (144). Certainly, specific disease cannot account for the changes noted in the populations. Perhaps autopsies of samples of mice at the time of peak mortality rates that occurred early in the year would have been more rewarding.

Growth

For many strains and species of domestic and wild mammals, growth varies inversely with increasing numbers of interacting individuals, even where density is low (99, 230). In one study, crowding of pregnant mice produced weanlings with stunted weights that, in turn, reared growth-

deficient young (232). Biomass and mean growth rate as indices of complexes of physiological processes were little affected by the social and population density changes in the present study and similar exceptions to an inverse relationship between density and growth have been found in other populations (222, 406); maximum mean weights have even coincided with peak densities in populations of wild voles (234).

In numerous studies, social status is associated with better growth, at times because of differential access to food sources (236-37), but in other cases even when food is readily available to subordinates (235). In certain populations of rats, however, subordinate animals weighed more, though it appeared that this might be due to altered body composition with a greater proportion of fat (194). No studies of body composition were made in the present study to assess the possibility that the similar weights of members of different cohorts might be due to different body constituents.

Dynamics of replicate populations

Distinctive differences in the patterns of population dynamics of the two experimental populations occurred, though they were maintained throughout in identical physical environments. Variability of population growth curves of replicate laboratory populations in other studies has been attributed in part to the uncertainties and diversities of the genetic and social background of the founding mice (99, 178). These confounding factors were eliminated in the present study. Differences of dynamics between the two populations thus should presumably reflect the evolution in each population of distinctive physiological, behavioral and social processes.

Even during the period of rapid population growth when the rates of increase in Pop A and B were almost identical, the relative contributions of birth and death rates differed in the two populations. Both the rate of recruitment and the mortality rates of Pop B were greater than those of Pop A. During the second half of the year, the two populations had altogether dissimilar dynamics: one population increased in size when the other had stopped growing and continued to recruit more than four hundred additional mice after the other one had stopped growing.

Prominent behavioral differences between the two populations included the greater incidence of cannibalism and territorial defense of peripheral retreats in Pop B.

Behavior

The studies do illustrate the influence of different forms of social experience on the expression of behavior. The behavior of control mice was strikingly uniform. Sexual and maternal reactions, patterns of aggression, and motor and autonomic reactivity of control animals were stereotyped. The intensity of aggressiveness of control males did increase following fights, but did not diminish in group situations where such decline appeared necessary for social organization. Control males showed no discrimination in their interactions with other males. Whereas control males attacked unaggressive males of young cohorts of Pop A in stereotyped fashion, only some of the aggressive males of older cohorts of Pop A that attacked males of their own age attacked the young males when paired with them.

Networks of specific relationships among members of groups or populations of vertebrates are continuously reinforced and maintained by frequent encounters (238). Animals in groups may react differently to classes of individuals, such as older males, subordinate animals, etc., and in certain species recognition appears to depend on specific inter-individual experiences (239-242). The experiences of the control animals, like those of most laboratory animals, were highly restricted: socially, to contact with littermates and an adult female and male until weaning, and to a mate and recurrent litters afterwards, and, physically, to a bare cage provided with basic necessities. The development of social behavior is understandably limited in such conditions (198).

Social interaction in the milieu of the freely growing populations had profound effects on most behavioral modalities. Animals from the populations behaved differently from control animals, although some of the differences between the two groups disappeared after the mice of Pop A had spent a month in control cages as breeding pairs.

The development of behavioral abnormalities characterized both freely growing populations. Gross aberrations of specific types of behavior, such as repetitive running, inappropriate maternal care, cannibalism, and hyper-, homo-, and a-sexuality, have been noted for other species, especially under conditions of captivity and crowding (194, 243-46).

Although certain abnormal forms of aggressiveness, such as aggressiveness toward females (including pregnant ones) and young mice, increased during the course of the year, the frequency of fighting per individual per

unit time decreased markedly as the population grew in size. This has been noted in other group situations (108). Decline in fighting is generally attributed to progressive social organization (247) and, conversely, social disorganization, as occurred following emigration in Pop B, is generally accompanied by an intensification of aggressiveness (248).

Even more pathological, perhaps, were the sequential changes that transformed the basic format of social interaction, starting with the disappearance of behavioral reactions that precede social contact and terminating with total lack of social responsiveness, the absence of sequences of activities expected for specific types of social behavior. The unreactive immobility of many mice in the enclosures towards the end of the year represents another extreme form of behavioral abnormality. Obese, immobile and unreactive individuals have been identified in socially unstable laboratory populations of rats (194). Overcrowding may be associated with overactivity, on the other hand, as noted in wild populations of muskrats and lemmings (249-50).

Sex ratio:

The literature concerning factors that influence the sex ratio of mammals is conflicting (251-54). Of course, the sex ratios of the present study were determined in all three study groups at censuses, when the mice were from one to six weeks old, and are thus so-called tertiary sex ratios. The data are interesting, though difficult to interpret.

The proportions of males in both freely growing populations were higher than in the control group at a number of points. Both Pop A and B, however, showed a continuous decline in the over-all proportion of males during the last six months, during which recruitment had ceased in Pop B and had fallen markedly in Pop A. This suggests higher male than female mortality, a finding noted in other crowded populations of rodents (255-56).

Cohorts with lowest mortality had, as might be expected, the highest proportion of males, again supporting differential male mortality. The suggestion that "there seems if anything a tendency for stressed animals in the lab to produce more males than is the case with the isolated controls" (254) appears to be confirmed in Pop A but does not hold true for Pop B. Actually the proportion of males is significantly higher than 50% in Cohort III of Pop B but the trend in the next two cohorts shows a marked decline.

Cohort Differences

A cohort comprises individuals born during the same census interval. Members of different cohorts in the enclosures were born and developed in essentially different sociobiological environments, since the populations were undergoing both quantitative and qualitative changes throughout the year and, indeed, cohorts differed decidedly with regard to physiological and behavioral properties. There were differences between cohorts of comparable age in the two enclosures, differences among cohorts within each enclosure, and changes with time within single cohorts.

Reproduction

Pregnancy rates were, in general, inversely related to the period of entry of a cohort into the enclosures. Also, the older the female, the more likely she was to be fecund at the end of the year and substantial numbers of females in each cohort were nonfecund at this time. The presence of a normal-sized uterus among most of the nonfecund females of the older cohorts suggests that they had only recently ceased to ovulate. Among the younger cohorts, about 50% of the nonfecund females had uterine atrophy; anovulatory anestrus was probably of longer standing in these mice.

The accelerated sexual maturation of the females of the first cohort born in the enclosure is puzzling, but the observation was substantiated by the accurate timing of their births and the occurrence of the phenomenon in both enclosures. Sexual maturation did not appear to be delayed in any of the cohorts, though the criterion used to judge this—pregnancy in some members of a cohort—assumes homogeneity of entire cohorts with respect to maturational physiology. Examination of reproductive organs and testing of reproductive function of samples of females removed from population enclosures throughout the course of a comparable study should provide more exact answers to these questions.

Mortality

Cohort mortality rates present a somewhat more complicated picture, though once more there are significant intercohort differences. The pattern of survivorship at comparable ages varied among several cohorts. Cohort II showed the highest survivorship of any cohort born in the enclosures proper, especially in Pop A. This cohort, consisting of the first mice produced by the founders in the enclosures was exposed to

more rigorous early environmental conditions—behavioral, physiological, physical, etc.—than its parent cohort. Those mice that survived may represent a selected group, which may in part account for their enhanced survivorship compared with the mice of the founder cohort. On the other hand, this first cohort born in the enclosures faced milder "stresses," especially during early life, than mice of subsequent cohorts. Also, the mice of Cohort II have only one cohort older than themselves, compared with the increasing numbers of older cohorts that subsequent cohorts are in contact with. Age, and previous social experience, certainly are important variables that influence chances of survival among small mammals such as mice. There is no ready explanation for the high patterns of survival of Cohort V in Pop B and of Cohort VI of Pop A.

Behavior

Behaviorally, also, cohorts differed. This was more apparent among males than females. Males of Cohort V to VII, for example, tended to remain in peripheral retreats together with females, in contrast to males of earlier cohorts found in the center. Males of Cohorts V to VII were generally unaggressive, especially in Pop A; they rarely attacked, and had a low incidence of scarring. In both populations, males of Cohorts V to VII were rarely seen mating.

Although the trend in both enclosures was towards increasing physiological and behavioral change with increasing population density, the fact that intercohort differences persisted at all levels of population density emphasizes that density alone is not the only or primary causative factor of biological change. Of course, each cohort itself by its presence contributes to changing the social environment.

Changes of Social Environment

Removal from the population

The interplay of given social and other biological processes was highlighted also by changes that took place when the social environment of the population mice was altered "artificially." A large proportion of nonpregnant females promptly resumed normal reproduction when caged as male-female pairs in control-type cages at the end of the year. Females of cohorts that showed negligible evidence of reproduction within the population setting had high reproductive rates when allowed to breed outside the population environment. As a matter of fact, the reproductive rates of early

and late cohorts were reversed in the two types of social situations. The reproductively inhibited females of younger cohorts produced the largest litters when bred outside the population enclosure, exceeding even the productivity of control females of comparable age.

The tests of sexual, maternal and aggressive behavior, of motor and autonomic reactivity and of responses to grouping carried out immediately after the mice were removed from the enclosure, emphasized the differences in behavior between control and enclosure animals.

They also emphasized the existence of intercohort differences among population enclosure animals in contrast to intercohort uniformity among the controls.

Intercohort behavioral differentiation was also demonstrated by changes of behavior produced immediately following the removal of mice from the enclosure. For example, males of Cohort VI (August 1964) had never been seen to fight in the enclosure, showed no physical evidence of previous fighting, and did not fight at first when grouped in the special enclosure. Three hours later, however, most of these mice were fighting. The males of Cohort VII, on the other hand, also nonagressive in the population enclosure, did not fight at all when grouped in the same type of special enclosure; they remained in small aggregates and, unlike all other males, actively built paper nests.

Intercohort differences tended to decrease after the mice were housed as breeding pairs in control cages, and the behavior of the animals approached that of control mice.

Changes as a result of emigration

The simultaneous changes of biology and socio-ecology that followed emigration and the disruption of the well-established social structure of Pop B also illustrate the importance of interaction between such phenomena. Two distinctive subpopulations formed that differed from each other and to varying extents from the previous population. Changes were much more marked in the emigrant subpopulation, though both populations showed a decrease of aberrant behavior and an increase in recruitment. In the emigrant subpopulation, fighting was more frequent, mortality was greater, and recruitment, which began about five weeks after emigration, was six-fold greater than in the other subpopulation.

Changes in the social environment that followed emigration had differential effects on mice of different cohorts. All subcohorts in the new environment except V had higher pregnancy rates than those in the original enclosure and greatest changes were in the oldest two cohorts, where the emigrant females had markedly elevated rates. By contrast, three subcohorts in the old enclosure had lower pregnancy rates, and in only one cohort was there a significant rise of pregnant females. Fighting in the emigrant subpopulation was shown by males of cohorts that had not been aggressive previously.

The greater changes that occurred in the emigrant subpopulation which had undergone more extensive social reorganization again point to the influence of social conditions on biology. Although social disorganization is associated often with population decline (194, 248, 257), the present study and another study of small freely growing groups of mice (258) indicate that population growth may also follow social disorganization.

The outbreak of the massive infectious epidemic caused by Proteus mirabilis three and a half days after the reunion of the two subpopulations in the enclosure they had occupied for over a year previously was unexpected. Proteus is found in the intestinal tract of certain colonies of mice (145). No bacteriological studies had been done on the mice of Pop B for five months prior to the March epidemic. Proteus was not found at that time, nor was it present in the mice of Pop A at the November census.

Diminished resistance to infectious disease has been demonstrated in grouped mice compared with controls left in individual cages and experimentally inoculated with a parasite, Trichinella spiralis, that is not transferrable from mouse to mouse (259). Antibody response to injected antigens is depressed in grouped vs. isolated animals and tissue reactions of grouped animals may be similarly inhibited (260). Pathologic studies of many species of mammals in zoos show an increased incidence of degenerative diseases with changes of tissue distribution of lesions following the establishment and maintenance of generally more crowded cage populations (261).

The sequence of events in Pop B suggested that there might be a causal relationship between the sudden change of social environmental conditions to which the population was subjected and disease. The appearance

of the mice of both subpopulations prior to their enforced reunion was perfectly normal. The many births during the preceding four weeks, the normal appearance of babies, and the survival of newborns to juvenile ages all indicate previous good health.

Slow or chronic adjustment to difficult social environment as had occurred previously, though it had taken its toll in behavioral and physiological morbidity, had been compatible with life. Perhaps the rapidity of social changes following reunion may have exceeded the adjustive capacities of the organisms. This amounts to what might be called a social environmental catastrophe.

Death following sudden changes of social conditions has been described for individual mammals (262-63) and was observed in the males of Pop C placed in groups after prolonged existence as male-female pairs in small cages. Hypoglycemia (225), massive discharge of hormones of the adrenal medulla (264) and sudden release of norepinephrine from the brain (265) have all been implicated as mediating mechanisms, but the pathogenesis of such deaths is far from understood. The speed with which groups of mammals can adapt to sudden changes of social environmental conditions is a problem open to direct experimental analysis.

Of course, the disruption of the social organization of Pop B that followed emigration and the social instability of the emigrant subpopulation, especially at first, may have altered the physiology of the mice and made them more susceptible to any form of environmental stress.

Other aspects of self-regulation in populations of mammals

Even where the social structure of populations cannot be observed directly, as is the case of small, nocturnal or burrowing animals, long-term censuses and observations suggest that over any stretch of time, factors such as bad weather, predators, disease and lack of food are insufficient alone or in combination to explain the dynamics of many wild vertebrate populations (266-67). These populations are integrated units, largely self-regulating, and socio-behavioral processes seem to play important roles.

For example, several populations of voles living in an area near Oxford were studied simultaneously (268-69). These populations, though clearly separate, lived in relatively closely adjoining areas. Such adjoining populations were exposed to the same climatic conditions and preyed

upon by the same number of larger predators that roamed freely over their closely adjoining territories. Examinations of trapped animals showed that such populations often had a similar incidence of infections, such as tuberculosis. Yet, such adjoining populations time and again showed totally different dynamics; one population was growing, its neighbor shrinking, the third was stable. These populations were behaving as units much as experimental populations of small mammals in laboratories. The answer to their dynamic perperties lay beyond weather, disease, predation, and even food, which was usually abundant. Each of these populations was being regulated by forces which appeared to reside in the populations themselves. Individual animals captured from given populations tended to have physiological and behavioral attributes that could be distinguished from thoseof animals of other populations.

Populations of muskrats were classically thought to be limited by the predatory activity of minks, but the distinction of availability to predators and the mere presence of prey animals was shown by the fact that large and dense populations of muskrats could live securely despite the presence of considerable numbers of "muskrat-hungry" minks, as long as the muskrats behaved in a "socially tolerant manner" to each other. The individuals that succumbed to predation were proven to be those evicted from the territory of the muskrat population by members of their own species. Exactly what triggered off socially intolerant behavior that at times affects muskrat populations was not always clear. But the animals able to maintain their territorial holdings "lived securely with respect to their ancient predatory enemies." (272).

The findings noted in comprehensive studies of populations of the notorious lemmings (270-71), also fit clearly into this general picture. The complete asynchrony that often exists between predators and lemming population cycles and the lack of consistent changes in the quantity and quality of lemming foods with population dynamics discredited these two types of regulatory processes. Moreover, the members of different lemming populations often show distinctive social and migratory behavior and one population may crash while another remains at peak density a relatively short distance away. Lemmings, always antisocial and antagonistic to each other, appear to be more so during population declines. These and other studies suggest that "the dominant limiting factor of...the population is still its own sociology." (272).

Such viewpoints, of course, are not meant to imply that external environmental factors can never affect populations. "Calamitous environ-

mental changes can overcome the stability effects of social structure, and can cause local or regional extinction. Conversely extremely favorable conditions may cause the breakdown of population mechanisms" (179). But under commonly encountered environmental conditions, field studies suggest that social behavior and the social ecology of natural populations are prime forces that unify populations and govern their dynamics.

Genetics

Knowledge of the genetics of vertebrates is largely confined to that of species commonly used in laboratory research or of agricultural value (273-77). Much of it deals with traits of physiological, medical, and economic importance, or of traits that reflect the validity of fundamental genetic principles, e.g., Mendelian laws, gene interactions, linkage and recombination, chromosome mapping, etc.

A much smaller amount of genetic information is available about wild species of vertebrates (279, 282). Differences in gene frequencies have been described in a few instances for a few species in neighboring though geographically distinct terrains (278, 280-1). Much less is known about "intra"-populational genetics of vertebrate species (283-88). There is a minimum of quantitative data dealing with the selective advantage or disadvantage of given genotypes in natural populations of vertebrates. The frequencies of most genes studied in populations of man have not been followed long enough yet to indicate specific patterns of changes or their evolutionary significance.

Previous studies of laboratory populations of vertebrates have not concerned themselves with genetic parameters. Yet, such populations provide convenient settings for the study of the interplay between social and genetic processes and of interrelationships between population dynamics and genetics. Changes of gene frequencies in populations are fundamentals and expressions of evolutionary change (289-294). The study of polymorphic traits determined at single gene loci provides a convenient approach to population genetics.* (295-96).

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*Considerable debate about the significance of genes that find expression in the visible phenotype need not concern this discussion (296).

The four-way cross between standard strains of inbred mice made possible the study of gene changes.*

Alleles at the "C" locus

Although the magnitude of the changes in the frequencies of recessive alleles at the "C" locus in the freely growing populations were not large, the consistency of the changes, the similarity of the changes in the two populations, and the lack of change in the control group all suggest the action of systenatic processes and the probable adaptiveness of the changes in the population environment.

Migration was not possible; the magnitude and speed of change of the allele frequencies in both populations and the absence of change in the control population essentially eliminate mutation and genetic drift as causative processes. Most likely, the differential perpetuation of genotypes in the freely growing population was due to differential reproduction or viability (or both) in the population environment, in other words, to natural selection. The fluctuation of allele frequencies at the "C" locus and the lack of significant differences between parental and progeny cohorts at any time during the year suggest that what changes did occur in the control environment probably resulted from random sampling processes.

There is little reason to think that the visible phenotype determined by the recessive allele at the "C" locus has, itself, any definite selective value. Of course, the same allele that determines a phenotypically recognizable polymorphic trait often has other effects on the whole phenotype (302-304): "every gene that has been studied intensively has been found to be pleotropic to a greater or lesser degree" (296). Many coat color

*Other advantages of such animals include the possibility of duplicating the whole study by repeating the initial crosses and of comparing results found by using mice from one four-way cross to those obtained by crossing different sets of strains without confounding effects of different degrees of genetic heterogeneity.

Disadvantages, on the other hand, are that inbred animals are not random samples of all theoretically possible combinations of genes, since sub-vital recessive, semi-lethal alleles have been eliminated in the process of inbreeding and, even then, only certain combinations of homozygous genes appear to produce viable animals. Also, the genetic variability provided by a four-way cross is not contained in a coadapted system of genetic material and in the type of four-way cross performed, the existence of linkage restricts the random sampling of alleles (297-98).

alleles of Mus musculus have effects on body size as well (301, 306-11). Actually, the "survival" value of an allele may be due neither to a particular trait nor to its pleiotropic action, but may reflect the selective value of other closely linked alleles (312-13).

The question arises as to the nature of the association between recessiveness at the "C" locus and total fitness, or some specific component of fitness in the sociobiological context of the freely growing populations and the genetic system available.

Reproduction certainly was not a random process in the populations. Behavior and pregnancy data showed that every female and every male did not have equal opportunities either of mating or of giving birth to litters. No evidence emerged, however, to relate this non-randomness to homozygous recessiveness at the "C" locus or to indicate any reproductive superiority of homozygous recessive individuals.

Observations and measurements made during the first two gestation periods of the founder mice in which two cohorts having elevated frequencies of recessive c were produced are consistent with this interpretation. Observations revealed no differences between the sexual behavior of homozygous recessive and of other males, although this point was not studied in detail. The proportions of pregnant homozygous recessive and other females did not differ significantly (Table 70). The mean weights of pregnant albino females are slightly higher than those of non-albino females during both gestation periods in Pop A and during the second gestation period of Pop B, but the standard deviations are large and the differences between the means not statistically significant. Mean litter size of C/- and cc females presumably did not differ.

During the rest of the year, the over-all incidence of advanced pregnancy is slightly higher, if anything, in non-albino than in albino females in both populations, although the differences again are not statistically significant (Table 71). The same applied to the mean pregnancy weights throughout the year.

The increase of homozygous recessive alleles in the two populations could, of course, also be due to the relative reproductive superiority of heterozygous compared with homozygous dominant mice. This would explain directly the increased frequency of recessive allele in the progeny cohorts of the founder mice, for example, but since the heterozygous and homozygous

Table 70

Genetics

Pregnancy rates of c/c and C/- females of founder cohorts in Pop A and Pop B during first two gestation periods.

Pop	Genotype	Non-Preg. No.	Pregnant No.	Pregnant Mean wt.	±S.D.
1st Gestation period - production of Cohort I					
Pop B	C-	5	17	37.35	5.243
	cc	4	9	36.77	6.553
Pop A	C-	1	19	35.68	4.619
	cc	3	11	37.36	3.500
2nd Gestation period - production of Cohort II					
Pop B	C-	5	15	35.54	3.925
	cc	2	11	42.66	6.184
Pop A	C-	3	18	37.77	4.222
	cc	0	12	39.15	3.700

Table 71

Genetics

Frequency of advanced pregnancy among C/- and c/c females of Pop A and Pop B for all censuses combined.

	Genotype	Pregnant	Non-Preg.	Total	Frequency pt.	S.D.
Pop A	C/-	201	434	635	0.3165	0.019
	c/c	103	249	352	0.2924	0.024
Pop B	C/-	202	392	594	0.3400	0.019
	c/c	119	262	381	0.3124	0.023

dominant mice could not be distinguished this possibility can not be resolved.

Mortality also was a non-random process in the population environment, but here some evidence suggests the possibility of enhanced survival of newborn mice bearing recessive c alleles in the environment of the enclosures. The numbers of homozygous recessive females displaying maternal behavior were consistently out of proportion to their total numbers in the enclosures. Although such maternal behavior was bestowed on other newborns besides their own, since nurseries in the enclosures usually consisted of one to four dozen young, it seems plausible that litters of homozygous recessive females had relatively better chances of receiving good care necessary for survival.

Another observation that points to differential newborn survival is the relative difference of recessive c alleles among newborn and juvenile mice. There were relatively more homozygous recessive juvenile mice than newborns in both subpopulations of Pop B and this may have been based on differential survival.

There is no evidence of differential mortality between adult homozygous recessive and other mice in either Pop A or Pop B, since the frequencies of recessive c alleles changes little throughout the lifetimes of most cohorts.

Other possible reflections of fitness in the enclosures are the degreees of wounding of males as an index of social status, and the mean weights of animals. The proportions of homozygous recessive males with wounds of different severity (wounds were classified on a scale of four, ranging from no wound to deep flesh wound) did not differ from those of other males. There were small but statistically non-significant differences between the mean weights of homozygous recessive and black agouti males and females in all cohorts at every census and in both populations.

In spite of the popularity and universal use of albino animals in scientific research, only a few studies examine the association between recessiveness at the "C" locus and components of fitness. No clear relationships have been demonstrated. A few isolated studies point to both decreased and increased fitness of albino mice.

For example, albino mice were more radiosensitive than their non-albino littermates in crosses in which the genetic background for albinism was provided by mice of the A/J strain (314). The albino mutant that oc-

curred in a C57BL/J (Black) strain of mice does not breed as well as the black parent, but it does nothing else as well either: "It looks as though the general unthriftiness is due to something other than the c gene. It would be easy here to build up a case of guilt by association" (315).

Albinism appears to have a definite influence on a type of "laboratory" behavior studied by experimental psychologists, so-called escape behavior. This complex behavior is significantly slowed in albino mice. The "contribution of this single genetic locus is sufficient to be discernably expressed even in the presence of hybrid vigor resulting from heterozygosity at each loci" (316-17).

A series of studies of competitive fighting and mating, matching an albino with a non-albino strain of mice showed that the albino strain was superior under certain conditions and the non-albino strain superior in others (318-20). The studies outline the influence of hereditary factors and the dependence of behavior on the situation in which the behavior is elicited. The studies say littler about albinism, since they were restricted to a specific strain of albino mice and have yet to be extended to others. The longevity of inbred albino strains does not differ significantly from that of non-albino strains (321).

Behavioral and physiological attributes required for laboratory living include unaggresiveness, placidity, adaptability to varied situations, and the ability to live and reproduce in crowded conditions. The choice of "albino" animals by the scientific community may have been dictated by the fact that albino animals possess these traits as well as aesthetically pleasing appearances. Of course, the same attributes may favor success in the type of environment that developed in the experimental populations.

It would be of great interest to clarify the contributions of behavior to Darwinian fitness in different kinds of socio-ecological situations. If such correlations were established, behavior would acquire the potential of becoming a cause of evolution as well as one of its consequences (322-23).

Experimental evidence suggests that certain genotypes survive poorly in complex social contexts. The animals used in one study of freely growing populations were mice of two strains that had been through extensive inbreeding and that had been reared in small cages for many generations with

restricted social environments. The social behavior and adjustments of the two strains were quite different; "without exception mice of one strain exhibited a marked tendency either to avoid all situations requiring a response not previously expressed or to develop a more stereotyped behavior ...mice of the other strain exhibited marked plasticity and rapidity of adjustmant..." (195). Socialization even at low population density proved to be impossible for the mice of one strain that seemed unable to establish a stable behavioral organization and the members of their population died rapidly.

The potential contribution of heredity to "social success" is also suggested by demonstrable differences of behavior that relate to the care of young (325-27), to sexual activities (328-31), to fighting (336-38), etc., among inbred strains of animals (339).

There are some observations relating social status to reproduction in natural populations (340-44), but little is known about the extent that progeny of socially successful individuals themselves attain social success and, if so, to what extent they owe their success to genetic endowment. For example, on an island colony of rhesus monkeys, sons of high-ranking mothers while still sub-adult gained precedence over older and larger males. Such precocious males attained their status without ever engaging in serious fighting. But whether their success was due to genetic factors or accorded tham because of their previous relationship with high-ranking females was not determined (345).

The present experiments were not carried out really long enough to ascertain if a stable gene frequency equilibrium at the "C" locus had been reached. The relatively slow rates of change of the frequency of the recessive allele after the first generation represent conditions that suggest possible establishment of a state of balance genetic polymorphism, a condition in which both alleles at the "C" locus might be expected to be maintained in the populations (347).

A two-allele system in stable genetic equilibrium results when the heterozygous genotype has a higher fitness than either homozygous genotypes (289-90). The relationships between fitness of different genotypes required for stable genetic equilibrium may be represented as follows:

	Genotypes		
	CC	Cc	cc
fitness	$1 - s_{(C)}$	1	$1 - s_{(c)}$

"s" stands for the selective coefficient. The fitness of the heterozygote is arbitrarily assigned a value of 1 (selective coefficient = 0), whereas the fitness of the two homozygotes are less than one by some amount equal to the selective coefficient of each.

When the frequencies of the two alleles are at equilibrium, the magnitudes of the selective coefficients can be estimated from the relationships

$$\hat{q}_{(c)} = \frac{s_{(C)}}{s_{(C)} + s_{(c)}} , \qquad \hat{p}_{(C)} = \frac{s_{(c)}}{s_{(c)} + s_{(C)}}$$

where $\hat{q}$ and $\hat{p}$ are the equilibrium frequencies of the alleles (348). The selection coefficient of the recessive allele is about 2/3 as large as that of the dominant allele if 0.6 and 0.4 are substituted for the equilibrium values of the two alleles respectively. Of course, fitness and selective coefficients are highly sensitive to changes of environment and genetic system (349-50). It is possible that the slow upward trend of the frequency of recessive alleles in Pop A during the last three months represent the beginning of renewed change.

Alleles at the Hb locus

The determination of Hb polymorphism in Pop A represents a first step of a program of investigation of other polymorphisms of blood constituents in populations of small vertebrates. The genetic basis of the phenotypic polymorphism of several constituents of mouse blood, including beta globulins (351), a gamma globulin isoantigen (352), a transferin globulin (353), a prealbumin protein (354), and a serum esterase (355), are known.

The decision to start with polymorphism of hemoglobin was prompted 1) by the suggestion of differences in the ratio of different types of hemoglobins in wild populations of mice (356) (admittedly based on studies of very small samples of animals); 2) because the hemoglobin locus is in the same linkage groups as the "C" locus, the recombination frequency between c and Hb being 2.49 ± 0.52 in males and 5.1 ± 0.58 in females (357); 3)be-

cause of reported change of red cell physiology with changes of population dynamics, e.g., amounts of hemoglobin in populations of rabbits (358), hemolysis during population crashes of voles (359); 4) by the relative ease of analyzing Hb compared with the other constituents mentioned; and 5) by the fact that for another vertebrate besides man, namely sheep, there appears to be an association between a type of hemoglobin protein and a component of fitness (360).

The data of the present study do not reveal any superiority for any one of the three "Hb" genotypes. Allele and genotype frequencies at the end of the year are those that might have been predicted on the basis of panmixia. "Hb" allele and genotype frequencies differed from expected at generally accepted levels of probability in only two of the eleven sets of comparisons of subgroups of the population. Most intriguing was the higher than expected association of allele Hb^1 with recessiveness at the "C" locus. "c" was introduced into the gene pool of the founder cohort in linkage with Hb^1 in strain SWR/J and with Hb^2 in strain 129/J. The association of Hb^1 with homozygous recessiveness at "C" suggests that the adaptive value of recessiveness at the "C" locus may be based on a whole group of linked genes derived from the SWR/J strain.

The study of Hb polymorphism in Pop A was far from satisfactory, unfortunately. Only two points in time were sampled, the beginning and the end, and the hemoglobins of more than a fifth of the mice recruited into the population — the mice that died — were not studied.

Artificial selection for adaptiveness to crowding

The social changes that occurred in the freely growing populations were the result of the activities and interactions of the mice themselves. Although the structures of the enclosures and the laboratory setting of the experiments were not natural situations, both the physiological and genetic changes that took place were the results of natural processes intrinsic to the populations themselves. The selection experiment is an attempt to see whether adaptiveness to different types of social situations could be studied by classical techniques of animal breeding.

Artificial selection for a trait related to Darwinian fitness, such as social dominance for mice, would have made the specific orientation of the selection experiment more akin to that of the freely growing popula-

tion, but this was not possible. Large body weight at 44 days of age, the attribute selected for in the mice kept in uncrowded and crowded groups, may or may not be related to a component of fitness; nevertheless, it was felt that different types or degrees of behavioral, physiological, or metabolic attributes would probably contribute to growth between weaning and sexual maturity in the two different kinds of social settings. Comparisons of such differences after their intensification by artificial selection, in turn, might provide another model for the study of adaptiveness to different social environments. The data showed that it is possible to increase mean weight at 44 days of age by artificial selection in mice subjected either to uncrowded or crowded environments. The rate at which mean weight rose per generation was about equal in both types of social groups. The mean superiority of selected individuals with respect to weight was also highly comparable for mice in both type lines over the course of the seven generations of study. The proportion of phenotypic variance relative to 44-day weight that was heritable for crowded individuals was essentially equal to that heritable for uncrowded ones.

The equality of heritability existed in spite of the fact that growth and weight between 22 and 44 days of age were depressed for the crowded mice. Mean weights at weaning age (22 days), it will be recalled, were highly similar for mice of both types of lines. The lower weights of crowded mice at 44 days of age could not be explained on the basis of long-term effects of low weights at birth or during infancy; thus the lower paternal and maternal weights of crowded mice had no effect on their progeny during the pre-weaning period of development.

The depression of growth between weaning and 44 days of age indicates further how specific the interaction between different types of social groups and biological properties can be. Mice crowded in the unisexual groups of the selection experiment apparently constitute unfavorable influences for weight gain between 22 and 44 days of age. By contrast, the weight and growth curves of the animals in the freely growing populations of the enclosures, mice of fundamentally similar genotypes, were not affected by much greater degrees of crowding, e.g., differences in the age and sex distributions of crowded animals, as well as differences in the physical enclosure, it appears, may have dissimilar biological repercussions. It is known, for example, that the presence of females may alter the tendency of males to fight (361).

The decline of fertility that caused the premature termination of the experiment made it impossible to determine specifically how the various selected lines differed from each other. It was not possible, for example, to estimate the environmental and genetic bases of certain correlated traits noted, such as litter size and promptness of mating. The frequency of recessive c alleles in progeny of crowded parents differs from what was found in the population studies, as did also the effect on weight.

Nevertheless, the findings on environmental exchange suggest that selection in crowded conditions may have been producing mice that were more "adaptable" with respect to weight at sexual maturity than those selected in uncrowded conditions. The superiority of crowded males in four out of eight cross-tests compared with that of uncrowded males in one cross-test only, and the slight superiority of crowded females imply that selection in the unfavorable environment was, if anything, more successful. That is, mice of the selected crowded lines were superior in both types of social environments. This is analogous to results of selection of mice for body weight on low compared with high protein diets (130). But the lack of significant difference in more than half the comparisons make the observation tentative. At the same time, the response obtained in uncrowded lines held up when the animals were crowded, that is, the weights were greater than those of unselected animals and, conversely, adaptability to crowded conditions did not reduce the possibility of weight increase at 44 days in mice subsequently placed in uncrowded environments. Further experimentation will be necessary to identify the biological, physiological and metabolic traits required for success with respect to weight at 44 days of age in crowded and uncrowded groups and to evaluate their relativeness, if any, to Darwinian fitness in more complex social environments.

The nature of genetic changes that might explain the simultaneous enhancement of response to selection for weight and infertility have been discussed extensively in the literature (262-65). "A naturally existing phenotype is the product of a genotype that has a long history of selection for maximum fitness. Any selection for a new phenotype will force the abandonment of the previously integrated genotype and will thus lead to lowered fitness due to either an accumulation of homozygous recessives or a disharmony between the newly favored genes and the remainder of the genotype" (366). It is somewhat surprising that a limit to selection based on genetic factors should have occurred so soon. Neither the speed of selection nor the degree of inbreeding—about 40% at the end of seven generations—had reached levels

usually associated with selection limits (367-71, 373). The fact that it occurred in all four lines suggests the possibility of environmental causes (372). Aside from the gross autopsies, which were not revealing, this point was not investigated.

Formulations of Mammalian Socio-ecology

The empirical evidence that attests to the interplay of socio-ecological and other biological processes raises questions about mechanisms that mediate this interaction. No single theoretical framework encompasses the variety of phenomena of mammalian socio-ecology, but several formulations are relevant to the phenomena noted in the experiments.

Early experience

The prolongation of preadult dependency among vertebrates, especially among mammals, has focused attention on the importance of the period of early life (374-77). Fundamental to this approach is the principle of development, namely, "once a system becomes organised whether it is the cells or the embryo that are multiplying and differentiating or the behavior patterns of a young animal that are becoming organised...it becomes progressively more difficult to reorganise the system, that is organisation inhibits reorganisation. Further organisation can be strongly modified only when active processes of organisation are going on..." (377). The processes of organization that determine the development of the social individual begin after birth and are most active during the early life of the organism. Current studies are even beginning to outline the effects that social experiences of pregnant females can have on the subsequent behavior and physiology of their young (378-82).

Prolongation of dependency imposes demands for prenatal care and is increasingly related to the development of family ecology (383). The helplessness of the young may even put behavioral constraints on the group as a whole; mother and young have become the center of attention in many primate groups (384-85). Of course, the great protection afforded during the early periods of life enhances opportunities for the learning of skills and traditions and for the establishment of specific inter-relationships within given groups and populations (29, 119). This, in turn, augments the importance of the group as the nexus of health, survival and evolution. The increasing emphasis on learning and social life among higher vertebrates promotes increasing independence of individual, population, species from the physical environment (386).

The immature mammal is particularly susceptible to environmental events, and social contacts during this period are highly influential in

permitting and promoting the emergence of phylogenetically determined patterns of behavior and of physiological reactivity (387-88). The importance of critical periods for species recognition (389-90), for the formation of social bonds (377, 391, 118), and during which the reactivity of several physiological systems may be conditioned for life (192) has been delineated for several species.

Many of the changes that occurred in the populations had particular relevance to the early period of development. The crowded and disrupted nurseries, the lack of maternal care, early exposure to abnormally behaving adults, etc., may well have influenced subsequent adaptability.

Space

The importance of space as an organizing element in vertebrate social life has also been recognized (393-95). The spatial requirements of individuals vary among different species (21, 101), but for all mammals, space seems to be an organizing element in regulating both behavioral interactions and their physiological concomitants. Observations of wild and domestic mammals indicate that each animal surrounds itself by an area of varying size which, in a sense, it treats as its private space (244).

In certain studies that emphasize the central importance of space, particular types of ecological phenomena, such as different forms of social organization, are first related to spatial parameters; once such relationships are established, only the spatial parameters and configurations are considered. Individuals are treated as particles and interactions as forces. The problems are transformed into a kind of social physics, as the movements, the velocities, the concentrations and associations of particles become basic variables (23). This permits a certain amount of abstract conceptualization that lends itself to mathematical manipulation. This, in turn, hopefully leads to new hypotheses about socio-ecological phenomena which can then be tested empirically.

For example, photographs of groups of domestic animals taken at regular intervals over the course of many hours revealed striking spatial relationships. There was a highly significant tendency towards non-random spacing when individuals were considered as points. In fact, this non-random distribution bore a distinct relationship to social status. Dominant animals were surrounded by significantly more space than subordinate

ones. Subordinate individuals tended to move away from the area surrounding the dominant animal (396).

Disregard of normal spatial requirements can lead to behavioral and physiological pathology, as occurs so often in zoo situations (244). The spatial constraints imposed on the mice of the crowded populations were severe. Studies of rat populations suggest that small mammals may react to conditions of abnormal density by social withdrawal, characterized by what is interpreted as decreased perception and what is observed as decreased response to other individuals (131). The sequences of changes in the basic patterns of social interaction in the population are consistent with such mechanisms of social inhibition and social withdrawal

Stress

A much more specific example of possible mechanisms mediating social and physiological processes is put forward by the so-called "Stress Theory". Under the rubric of stress, the organism reacts to a wide variety of of potentially injurious external agents, not only by specific responses but also in a variety of non-specific ways involving changes in the activities of several endocrine organs. Social stimuli are viewed much the same way as physical stimuli, that is, as tending to disturb the internal constancy of the organism, and the organism reacts to social stimuli with the same systemic nonspecific neuro-endocrinological syndrome that characterizes its responses to physical stimuli (397). Social stimuli are, of course, mediated via the central nervous system, whose relationships with neuro-endocrinological mechanisms have been established (399-400).

The strss approach to social ecology among mammals has been elaborated in detail with respect to population processes (401). Crowding, competition, and fighting produce increasingly intense physiological reactions that act to oppose further increases in density and competition, that is, reproduction may be inhibited, individual growth depressed, resistance to disease lowered, and pathological conditions increased. The stress syndrome at the level of populations amounts essentially to a negative feedback mechanism.

Although many studies indicate an increase in adreno-cortical activity with increasing animal density in small groups (402-404), laboratory populations (99), and wild populations of several vertebrate species (398, 405),

other studies have failed to confirm density related adreno-cortical changes (406-408). In part, this is due to disagreements about questions that affect interpretations of results, to methodological differences, and to the frequent disregard of other variables that also alter adreno-cortical function, such as species, season, pregnancy status, sex, age, etc. (409). It has been suggested that aggressive interaction in group situations may be more important than density itself in causing adrenal reactivity (410-14). But aggressive interactions declined in frequency in the present as well as other studies as population size increased.

Thus, considerable question exists about the generality of this formulation and there are many exceptions to the hypothetical density-behavior-endocrine relationships central to the approach.

Social withdrawal and restriction of interaction which may occur in groups of vertebrates may be accompanied by other evidences of non-specific physiological reactions. However, these appear to involve the adrenal-medullary axis, rather than the pituitary-adrenal axis (108, 415). This was not considered, however, in the present studies.

A criticism of many studies that emphasize the importance of stress physiology is their lack of proof linking increased endocrine activity with abnormal processes, i.e., many of the studies demonstrate the existence of physiological hyperactivity but fail to prove its causal relationship with altered population processes (416).

A more general criticism of stress physiology as a unifying principle in social ecology of vertebrates is the exclusive emphasis of this approach on the negative side of social interaction and its neglect of the positive effect of social life. By equating all external stimuli, social and others, as primarily injurious stresses, the stress approach neglects the need of social stimulation for normal development and survival in many species of vertebrates. The importance of positive feedback for many of the vital changes that take place in biological systems differs sharply from the required constancy of non-biological systems from which basic notions of negative feedback inherent in the stress approach have been derived.

The population environments certainly were stressful situations. The only direct index of adrenal activity measured in the population was the histology of cross sections of adrenal glands and these showed little difference from controls. Moreover, there was no inhibition of growth of

individuals in the populations, a fact which speaks against the prominence of "stress physiology."

Neurophysiological reactivity

Another approach to sociobiological processes focuses on neurophysiological reactivity. Every stimulus from the environment in addition to the specific message it carries contributes to what has been called the basal level of neurophysiological activity (417). The intensity, the duration, and the variability of stimuli, whatever their source may be, appear to contribute in nonspecific ways to the activation of parts of the brain stem and basal ganglia (418). These areas receive and send out fibres to many parts of the brain, to the autonomic nervous system, and to endocrine glands (108). Activated by sensory stimuli from the environment, these areas, in turn, participate in activating the entire nervous system and play an important role in the maintenance and modification of body states such as wakefulness and emotional arousal (419). A decrease or an overload of sensory input into these parts of the brain stam and thalamus alter their relations with the rest of the brain autonomic system and endocrine organs, and this may produce behavioral and physiological changes (420).

Although the evidence for these principles has been largely accumulated in studies that made use of physical stimuli, the principles derived appear to be just as applicable to social situations. After all, the importance of social life among mammals makes social factors increasingly significant sources of environmental stimulation for them (421).

With increases in population densities such as occurred in Pop A and B, certainly, social stimuli became even more prominent. They may well have amounted to a chronic overload of sensory input.

Communication

Lastly, social behavior among mammals, of course, involves the existence of very specific sequences of interactive behavior (422-25). These sequences not only communicate specific "messages" between participants, but also initiate highly specific physiological mechanisms. Sounds, smells, postures, sequences of movements synchronize the activities and physiologies of individuals (426-27). The specificity of such behavioral-physiological sequences have been especially well outlined for activities such as mating, fighting, following, and dominance-subordinance relationships.

Among many species of vertebrates, repeated stimulation between individuals during early parts of courtship may be required for the synchronization of gonadal cycles or the maturation of gametes (428). The signals involved here tend to be species restricted. There are examples of such "social or partner control of reproductive physiology among fish, birds, and mammals and it may well be that the sequence of external stimulus, brain activity, pituitary secretion, gonadal and mating behavior is of considerable evolutionary antiquity" (429). Interaction among members of groups or populations of vertebrates may become so defined that animals come to "respond to the species social releasors only when furnished by certain individuals which they know personally" (37, 430).

Whatever their etiology, abnormalities of behavior of certain individuals as occurred in the populations may constitute the presentation of abnormal cues which, in turn, may trigger off abnormal responses. It is, of course, equally possible that abnormal perceptions occur, resulting in abnormal responses to normal cues. This kind of process may lie at the basis of the rapidly increasing psycho-pathology of crowded populations of small mammals.

CONCLUDING REMARKS

The question, of course, arises as to what relevance this kind of work has to man. Clearly both the social environment of man and his potential reactions are infinitely more complex, variable, non-species specific, and give prominence to the use of symbols and culture.

At the same time, however, man's social environment is even more fundamental to his entire existence, thus possibly increasing the importance of the interplay of socio-ecological and biological processes.

The profound biological effects of diminished social interaction have been demonstrated in socially deprived infants (431-35). Poor growth occurs where nutritional deficiency can be ruled out and delay in bone maturation may continue long after the period of social deprivation (436). The use of telemetering has begun to make possible the on-going measurements of the effects of social interaction on physiological processes.

Sociological research of small groups has been little concerned with physiological accompaniments of grouping (437-43). Measurements of physiological parameters, such as respiratory and heart rate, galvanic skin resistance, and of metabolic processes of individuals in social situations are promising (444), but only a few reports have considered the simultaneous physiological responses of several individuals in social situations (445).

There is much discussion at present of the vast increases in populations and of the new urban revolution, in which the majority of the peoples of many nations live in cities with little access to the countryside and free space. Little is known about the effects of crowding and density on human life. One of the few careful studies of this problem suggests that there may be correlations between emotional disturbances in children and lack of apartment space. There have been some suggestions that a whole gamut of metabolic and degenerative diseases may at least in part reflect biological repercussions of social phenomena (446). Of course, density must always be correlated with specific social and cultural practices. The deleterious effects of crowding may well be due to the sudden congregation of individuals from rural regions into cities, with its accompanying social upheaval just as occurred during the industrial revolution (447-48). Perhaps the greater rate of social interaction today and

the constant stimulation between unrelated individuals in big cities may represent a form of overload to man's physiological systems.

It has been suggested frequently in recent years that human evolution in the biological sense is drawing to a close, to be replaced with cultural and social evolution. This view, it would seem, neglects the importance of the interplay of socio-ecological and biological phenomena. Though, indeed, there seems to have been little change in man's gross characteristics in the past thousands of years, changes may have and may be continuing to occur as man adapts to his ever-changing social environment. At a time when the human species is faced with a variety of critical population problems, the experimental and comparative approach to population may provide some insights in relatively short periods of time.

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