A STUDY OF SOME EFFECTS OF INCREASED POPULATION DENSITY ON REPRODUCTION IN TWO INBRED LINES OF MICE

A thesis presented in partial fulfilment of the requirements for the Degree of Master of Agricultural Science

by

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March, 1965.

ACKNOWLEDGEMENTS

The author is indebted to his supervisors, Dr. M.F. McDonald and Dr. R.E. Munford, for their guidance, advice and criticism in the presentation of this thesis; and to Dr. D.S. Flux for his advice and comments.

Special thanks are due to Miss M.G. Campbell and the staff of the library for their promptness in obtaining literature; to Messrs. C. Fairless and E. Ormsby, staff of the small animal house; to Mr. C. Muir for preparing figures reproduced in this thesis; and to the typist, Miss M. Marriott.

Finally, the author wishes to thank persons, not mentioned above, who have contributed in any way to this work.

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Chapter I

INTRODUCTION

The Theory of Regulation of Reproductive Rate within Populations

The original hypothesis considered that sociopsychological pressure might act as a stressing stimulus in some proportion to population density, which would activate the complex hypothalamo-hypophyseal-adrenocortical-gonadal system (Selye, 1939, 1954; Harris, 1956). This proposal was based on field data reported in the literature and on the results of experiments in the laboratory with the pituitary-adrenocortical system.

A relationship between reproductive endocrine function and population density was foreshadowed by Pearl and Surface (1909), who demonstrated decreased egg production by domestic fowl with increased population size even though the area per bird was kept constant. Crew and Mirskaia (1931) and Retzlaff (1938) demonstrated a similar inverse relationship between population size and reproductive performance in albino mice. These authors recognized the importance of behavioural factors in producing these effects. Retzlaff observed that socially dominant female mice gave the best reproductive performance.

Selye (1939) demonstrated that stimuli evoking a response of the pituitary-adrenocortical system also suppressed the reproductive function of female rats. He postulated that the decreased reproductive activity might result from a shift in pituitary function to produce an increased amount of adrenocorticotrophin (ACTH) at the expense of the production of gonadotrophins although an actual decrease in the production of gonadotrophins was not shown.

More recently, increased activity of the pituitary-adrenocortical system in response to increased population density, accompanied by decreased

reproductive activity, has been demonstrated in populations of house mice (Christian, 1955a, b, 1956a, 1960), rats (Christian and Davis, 1956), voles (Louch, 1956), deer mice (Helmreich, 1960) and chickens (Flickinger, 1961).

Christian (1959a) has postulated that the mechanisms regulating reproductive rate within populations are evolved from sociopsychological pressures which act as stressors to the individual in some relation to population density. These pressures presumably will stimulate the release of ACTH from the anterior pituitary via pathways involving the higher brain centres and the hypothalamus with a resultant increase in adrenal glucocorticoids (Harris, 1956). Furthermore, it has been suggested that the secretion of gonadotrophins and growth hormone may be suppressed simultaneously, with a resultant decline in the output of sex steroids (Selye, 1939). Assuming these hypotheses are correct, then there should be progressively increased adrenocortical activity and decreased reproductive activity with increasing population density.

Studies on Innate Control of the Reproductive Rate of Populations

(a) Laboratory populations of fixed size

Adrenocortical and reproductive responses were related to the number of mice comprising the populations when male albino or wild-stock house mice were placed in groups of various sizes following three weeks of isolation (Christian, 1955a, b, 1956b). The weights of the adrenal glands increased with increasing population size. The increases in weight were due to an increased amount of cortical tissue, primarily of the zona fasciculata, as shown by measurements of the zonal widths (Christian, 1955b, 1956b). There is excellent evidence that the fasciculata is the zone primarily responsible for the production of glucocorticoids as opposed to mineralocorticoids (Eisenstein and Hartroft, 1957; Hartroft and Eisenstein, 1957). The weights of the adrenal glands of wild-stock house mice increased with population size

at three times the rate of albino mice. The wild mice were much more aggressive than the albino mice, and the differences in adrenal reactivity probably reflected such behavioural differences (Christian, 1956b).

Involution of the thymus gland, in the absence of an increase in circulating oestrogens or androgens, is an index of adrenal glucocorticoid activity (Weaver, 1955). Therefore, increases in adrenocortical secretion in some proportion to the changes in adrenal weight were indicated by the declines in weight of the thymus glands to below the isolated control weights. The thymic weights were regulated in a roughly inverse fashion to the adrenal weights (Christian, 1955a, b, 1956b).

The preputial glands and seminal vesicles, as indicators of androgen activity (Burrows, 1949; Rennels, Hess and Finerty, 1953), declined in size with increasing population size in both varieties of mice (Christian, 1955a, b, 1956b).

A significant decline in the weight of the testes due to a decrease in seminiferous tubule diameter with increasing population size was eliminated by adjusting the weights for body weight (Christian, 1955a). Body weight decreased significantly with increasing population size (Christian, 1955a). Evidently the testes and body weight responded to population density to the same relative degree.

The changes, with increasing population size, in size of the adrenal, thymus, and preputial glands, the body and the seminal vesicles, indicate increases in ACTH production and decreases in the effective activity of gonadotrophins and growth hormone. However, what appears to be the effect of a decrease in gonadotrophin activity of the pituitary may reflect mechanisms other than a simple reduction in production or secretion. There may be direct effects of ACTH or adrenal hormones by altering the sensitivity of the reproductive organs to the gonadotrophins, or ACTH may have increased the renal secretion of gonadotrophins (Smith, 1951). Possibly all of these

mechanisms were operating simultaneously (Christian, 1959a). This work supports Selye's concept of a decrease in gonadotrophic activity due to stress (1939) but does not establish that there was an actual decrease in gonadotrophic hormone production by the pituitary. However, the changes noted are consistent with an effective reduction in gonadotrophic activity irrespective of the mechanism producing the reduction. Social pressures are evidently the factors responsible for producing such changes in organ weight and function with changes in population size, as food and water were supplied in excess of utilization and from multiple sources, and the other environmental conditions were maintained constant for all populations. Fighting per se cannot be responsible for the changes, as fighting essentially ceased after the first few hours except for occasional sporadic encounters. Occasionally there was no fighting in a group and yet the organ responses were still evident. In addition, the dominant animals fought the most, yet had the smallest adrenals (Christian, 1959a).

If the pressures causing the observed physiological changes are truly sociopsychological in nature, reserpine should at least partially block the pituitary-adrenal response to grouping by its suppressive action on aggressive behaviour (Plummer, Earl, Schneider, Trapold and Barrett, 1954). Fighting between mice was reduced markedly by treatment with reserpine, and the usual increase in adrenal weight and decreases in thymus and seminal vesicle weight, associated with grouping, were largely prevented (Christian, 1956b). Reserpine did exert somewhat comparable, but less marked, effects on the organ weights in isolated mice, but these were not significant. These experiments with reserpine supported the belief that increased population density acts through sociopsychological mechanisms to produce stress with its attendant physiological reactions.

Comparison of the behaviour of mice at two different population density levels suggests that territorialism and social rank are two poles of a

behaviour continuum related to density. At low densities individuals of this species are territorial, but at high densities they may become associated in groups which have a social rank (Davis, 1958).

In a series of experiments with 18 groups of 6 wild-stock house mice each (Davis and Christian, 1957) the mean adrenal weight increased progressively with a decreasing dominance rank. The preputial glands decreased in weight with decreasing rank, but a relationship between the weights of the seminal vesicles and rank could not be demonstrated (Christian, Davis and Hilton, unpublished - cited by Christian, 1959a).

In albino male mice, from groups of 4, 5 or 6 each, the mean adrenal weight decreased with increasing number of injuries, but this was paralleled by a decline in body weight (Christian, 1959b). The data was interpreted to indicate a tendency for adrenal weight to increase with decreasing social rank and not to reflect fighting per se. Barnett (1955) has shown a comparable relationship between adrenal function and dominance or subordinance in rats. Flickinger (1961) reports that, after sexual maturity, the adrenal weights of cocks correlated reciprocally with social rank. Subordinate grouped cocks were found to have a slower testes weight gain and delayed onset of spermatogenesis compared to dominant grouped males.

Nulliparous and parous female mice of albino and wild-stock strains were placed in groups and compared to isolated controls (Christian, 1960). Mild hyperplasia of the zonae fasciculata-reticularis, increased involution of the adrenal X-zone and significantly lower uterine weight were associated with grouping. Adrenal weight was not changed, as the alterations in the zona fasciculata and X-zone evidently offset each other. The author suggests that an increase of adrenal androgens may account for accelerated involution of the X-zone and inhibition of reproductive function. The X-zone is involuted by androgens (Howard, 1927, 1959; McPhail and Read, 1942a, b; Jones, 1952). Degeneration of the adrenal X-zone of inbred house mice, in

response to grouping, appeared to be due to increased androgenic-progestational hormones (Mody and Christian, 1962).

Evidence for androgen production by hyperplastic adrenal glands of mice is given by Christy, Hofmann and Huseby (1962). Spayed female BALB/C mice developed adrenocortical hyperplasia in 4 to 6 months; the salivary glands then became masculinized and seminal vesicular grafts were stimulated.

Adrenal androgens were studied in immature female mice at non-virilizing doses thought to be within the limits of endogenous secretion (Varon and Christian, 1963). Certain androgenic steroids produced delay in maturation of the reproductive tract. Increased follicular atresia and absence of pre-ovulatory follicles and corpora lutea in the ovary indicated inhibition of gonadotrophins, possibly of luteinizing hormone. In addition, direct action of these steroids on the gonads was suggested. However, some steroids produced the reverse effects.

The authors suggested that involution of the adrenal X-zone, produced by some of these steroids, was similarly caused by an effect on pituitary gonadotrophin activity and by direct action on the adrenal gland.

In female mice subjected to increased population density, the degree of cortical hyperplasia does not account for the magnitude of response to increased density seen in populations of mixed sex (Christian, 1960). There appears to be an additional effect on reproductive function due to increased secretion of adrenal androgens.

(b) Freely growing confined laboratory populations

Freely growing populations of wild-stock house mice into which the animals were born and in which they remained were studied by Christian. The populations and experimental design were similar to those of Brown (1953), Strecker and Emlen (1953), Southwick (1955a) and Clarke (1955), all of whom used freely growing confined populations of house mice or voles.

A few pairs of mice were introduced into a large cage and the population

allowed to develop from these (Christian, 1956a). Food and water were available ad libitum from several sources, and nesting space was in excess of the usage rate. Temperature and light were kept constant. No competition for food or water was observed, and there appeared to be no serious competition for nest space. Eight of these populations were used. Six were allowed to approach or actually reach upper asymptotic levels and two were stopped at an estimated one half of the asymptotic value (intermediate populations). The growth form of these populations of mice (like those of Strecker and Emlen, 1953; and Southwick, 1955a) was sigmoid, and growth was self-limited. The number of mice in the asymptotic populations varied from 21 to 130 with five of them between 60 and 130. Litter survival was so low at these levels that recruitment into the adult population had ceased.

The changes in the adrenal glands and reproductive organs of mice from the freely growing populations paralleled those from earlier experiments with populations of fixed size. Adrenal weight increased approximately 25 per cent in the male and 14 per cent in the female mice from asymptotic populations, mainly as a result of an increase in adrenocortical tissue.

The increased amount of adrenocortical tissue largely resulted from cellular hypertrophy and hyperplasia of the zona fasciculata, but in young mice it was also due in part to a delayed involution of the X-zone (Christian, 1956a). The X-zone of mice is involuted at puberty in the male and at first pregnancy in the female (Howard, 1927; Jones, 1949).

There was a suppression of reproductive activity in both sexes coincident with the increase in adrenal weight. Unlike the adrenals, however, the thymus and reproductive organs of the younger mice were affected more than those of the older members of the population. The onset of puberty was delayed in the younger mice, and this delay was reflected by a delayed involution of the thymus and adrenal X-zone as well as by the reproductive organs. Spermatogenesis was depressed in all but the heaviest males as

determined by histologic evaluation of the testes of each mouse (Christian, 1956a).

Reproductive performance was depressed in female mice. The number of young born per female in the population declined as the population size increased (Christian, 1956a). There was a decreased number of viable embryos, an increase in the number of resorbing embryos, a decreased total litter size, and a decrease in the prevalence of pregnancy in females from the experimental populations.

Increased resorption of implanted embryos, resulting in a 60 per cent reduction in effective natality, was observed when deer mice were subjected to crowded conditions (Helmreich, 1960). Significantly increased resorption of embryos was recorded in the highest density classes of house mice living in corn ricks (Southwick, 1958).

A regular decline in the rate of infant survival until weaning paralleled the decline in birth-rate (Christian, 1956a). The decline was essentially linear with the logarithm of the population size. The young were also forced from the nest at an earlier age and appeared unthrifty. Their stomachs contained no milk at autopsy, suggesting that lactation might have been depressed along with the other reproductive functions (Christian, 1956a).

Wide variations in the asymptotic values for several populations in the same amount of space, other things being equal, (see above) have been reported for house mice (Brown, 1953; Strecker and Emlen, 1953; and Southwick, 1955a) and voles (Clarke, 1955). Such variations in the maximal population size appear to be due to the behavioural differences between populations in terms of social structure, and especially the aggressiveness of particular individuals (Brown, 1953; Southwick, 1955b). It is note-worthy that in Christian's experiments the physiological alterations were qualitatively similar for asymptotic populations irrespective of the actual numbers of

animals involved. These factors led to the definition of population density in terms of "units of social pressure" with a varying number of animals comprising a unit of social pressure (Christian, 1959a).

It is of interest that Louch (1956) found a similar increase in adrenocortical function associated with increased population density in three confined populations of voles (Microtus pennsylvanicus) using counts of circulating eosinophiles as the indicator of cortical function, instead of adrenal and thymus weights. There was also increased litter mortality with increased population density.

(c) Natural populations

The same relationship between the adrenal cortex, reproduction and population density, as shown in laboratory experiments, was found in natural populations of Norway rats from the street-blocks of Baltimore city (Christian and Davis, 1956). Rats were taken from 19 populations, usually more than once from each population, and each time the adrenal weights were obtained and the population categorized in terms of population status. There was a fundamental difference with respect to sex in the responses of the adrenal cortices to low stationary populations. In most circumstances, however, the adrenal weight of Norway rats was directly related to population density. These results were supported by those obtained by monthly trapping of populations of farm rats over a period of two years. The adrenal weights of these rats varied with the population density as measured by trapping success (Christian, 1959c).

Three populations of rats were reduced to a constant 32 per cent below the initial level of high density by trapping (Christian and Davis, 1955). The adrenal weights for both sexes combined were reduced 28 per cent by the initial reduction in population density.

Davis (1951) demonstrated that reproduction is suppressed in rats from high stationary (dense) populations, which have a decreased prevalence of

pregnancy compared to animals from populations increasing in size. The differences were more striking in the main spring breeding season. Davis was unable to find any changes in litter size associated with population status, so that the effect of increased population density on rats appears to be entirely one of decreased fecundity or increased litter mortality. He concluded that, although there was an increased prevalence of pregnancy, mortality at parturition must be greater in increasing (low density) populations, as the prevalence of lactation was the same for all categories, but that there must also be a greater survival of young at low densities in order to produce the increase in population size.

Natural populations of voles, Microtus pennsylvanicus (Louch, 1958) and M. montanus (Christian, 1959c), showed similar adrenal responses to changes in population density. In the latter case, reproductive performance was inversely related to density. Southwick (1958) has studied house mice living in corn ricks and found that reproductive disturbances at high densities were limited because of the high quality of the habitat.

Kalela (1957) made an intensive three year study of populations of another species of vole, Clethrionomys rufocanus, which has rooted molars permitting classification of the animals into age groups. During the period of peak densities there was a marked failure of juvenile voles, especially males, to reach sexual maturity by late summer, compared to the preceding and succeeding seasons of lower densities. In the area of extreme density, juvenile females also failed to reach sexual maturity. The prevalence of pregnancy in mature females in late summer was less in populations of high densities than in populations of low densities. Kalela found no significant changes in litter size associated with population density in those females which became pregnant. It was also noted that the size of these voles decreased during the period of declining population density, suggesting adverse effects on the young during periods of maximum density.

Thus, results for natural rodent populations are similar to those from experimental populations of rodents in the laboratory.

(d) Effects on later generations

Christian (1956a) autopsied nestling mice from populations of high density. His observations suggested a failure of lactation coincident with the depression of other reproductive functions. However, there was no way of distinguishing between diminished lactation and a failure to nurse properly as a result of interference by other mice, a loss of maternal behaviour or other factors which result in infrequent nursing or total litter abandonment (Brown, 1953; Southwick, 1955b). A failure of lactation is compatible with the known effects of stress on the reproductive system (Selye, 1939) and lactation in particular (Selye, 1954). Furthermore, Chitty (1955) showed diminished lactation in voles due to crowding as measured by the weights of the offspring in a limited series of experiments. Further experiments (Christian and LeMunyan, 1958) were designed similarly to determine whether the effect of population density on the offspring was due to a failure of lactation or to some other factor. The experiments involved cross-nursing of pups born to isolated and control mothers. The crowded mothers were isolated following 6 weeks in groups of 20 pairs per cage. Litters born after separation of the groups into isolated pairs were divided and half the litter exchanged with half that of a litter of equal size and age born to a permanently isolated female.

The weights of the young raised by the previously crowded mother averaged approximately 15 per cent less than those raised by the isolated mother. The important factor determining the weights of the young at birth or any subsequent time was whether the mother which nurtured them had been crowded or isolated. The proportion of infant mice which survived to weaning was the same for both crowded and isolated mothers. The effect on the young is, therefore, clearly a failure of maternal lactation and not an inherited defect

or one brought about by intra-uterine factors (Christian and LeMunyan, 1958). The young of crowded mice probably suffer from inanition to a degree, depending on the degree of stress in the mothers, from birth onwards. There is evidence, however, that adrenocortical hormones may be passed to the offspring via the milk and thus may exert a direct suppressive effect on the growth of the young (Glaubach, 1952). It is more than likely, then, that the physiological defects noted by Chitty (1954), in young voles from populations of high density, are due to lactational deficiencies (Christian, 1959a).

It was of particular interest to find that the effects of crowding persisted in mice for several months after they had been removed from the crowded conditions and placed in isolation (Christian and LeMunyan, 1958). There appeared to be some permanent effect on fertility and pregnancy as the first litters of several of the mice were stillborn (births actually observed) before they delivered living young (Christian, 1959a). Only 77 per cent of the females surviving the period of crowding subsequently bore litters and only 82 per cent of these litters survived to weaning.

These results emphasized the long-lasting effects of high population density on reproduction in mice.

(e) Disease resistance

Experiments with mice on induced granuloma formation (Christian and Williamson, 1958), and on resistance to infection by Trichinella larvae (Davis and Read, 1958), suggest that grouping of mice may increase the production of glucocorticoids sufficiently to depress the inflammatory response. Thus, the hypothesis that resistance to disease decreases with increasing population density seems to be supported by experimental evidence (Christian, 1959a).

Purpose and Scope of the Study

The literature reports the effects of increased population density on the reproductive organs of male and female mice in laboratory populations of fixed size, and on the reproductive rates of freely-growing confined populations in the laboratory. However, no reports have been found on the effects of increased population density on reproductive rates of laboratory populations of fixed size, although, by inference from other studies, the nature of such effects may be indicated.

The following study was undertaken to demonstrate some effects, if any, of increasing the female population size per unit area, male population size being maintained at unity, on the reproductive mechanisms and reproductive rate of inbred house mice.

The reproductive mechanisms investigated were those primarily associated with the female and information on male reproductive mechanisms was obtained only at stages where males were necessary concomitants in determination of female criteria.

TABLE 1

Some details of lines of C57BL, 101 and CBA strains of mice held in stock, as at March, 1963.

Strain	Line	Number of Generations of Full-sib Mating [1]	Mean Size of First Litters [2]	Coat Colour
C57BL	1 **	27	5.6 ± 0.44	Black
	2	27 *	5.5 ± 0.37	"
	3	2	—	"
	4	1	—	"
101	1	6	6.1 ± 0.55	Light-bellied agouti
	2 **	33	5.4 ± 0.31	"
	3	18	5.6 ± 0.39	"
CBA	1	29	6.2 ± 0.46	Black
	2	2	—	Agouti
	3	32	5.4 ± 0.47	"
	4	2	—	"

[1] As from initiation of the line.

[2] For latest 20 generations of full-sib mating, where applicable.

* Back-crossed to the previous generation twice during the course of inbreeding.

** These lines were selected.

Chapter II

MATERIALS and METHODS

Mice

The two lines of mice to be used in these studies were required to have a high level of inbreeding, a high reproductive rate and, in view of the possibility of conducting experiments involving ovum transplantation, a genetic marker such as coat colour.

Three strains (C57BL, 101 and CBA) comprising eleven lines of mice were available in the small animal house at Massey University. (For the method of naming strains see Committee on Standardised Genetic Nomenclature of Mice, 1960.) From the breeding records of each line, data were obtained on the level of inbreeding (number of generations of full-sib mating) and reproductive performance (mean size of the first litter of the latest 20 generations). These data for each line are summarised in Table 1.

On the basis of these data the lines selected were C57BL line 1 and 101 line 2 (hereafter referred to as C57BL and 101, respectively), both having a high coefficient of inbreeding (>99.5 per cent) and similar mean first litter size. Two of the CBA agouti lines had an irregular breeding history and the third CBA agouti line showed considerable variation in first litter size. The CBA black line had a higher reproductive rate than lines of other strains. The particular 101 line was selected on the basis of number of generations of full-sib mating and the particular C57BL line on the basis of breeding history.

Design of the Experiments

To examine the effects of increased population density on these mice, two levels of density were selected. The control density was one pair per box and the experimental (grouped) density was five females and one male per

box (hereafter referred to as groups), thus producing a three-fold increase in population density between control and grouped mice.

Studies on the general reproductive processes of the mice were replicated. Results of the first study (experiment A), on a limited number of animals, were augmented by the results of a second study (experiment B) on a larger number of mice. Experiment B began at completion of experiment A. As there was evidence of certain quantitative differences between the results of the two experiments, it was not possible to combine these results. Thus, data presented in Chapter III pertain to three variables, namely, experiments, lines and levels of population density.

Pre-natal mortality was also studied. The treatments were the same as in experiments A and B. Because of the necessity of having a large number of animals, virgin females were accumulated over a period of some months. This introduced variation due to the ages of mice. Thus, data presented in Chapter IV also pertain to three variables, namely, ages of mice, lines and levels of population density.

Housing and Feeding

The mice were housed in a room 10' x 12' x 9' high maintained at 21°C. by a fan-type heater operating through a thermostat. Lighting was by natural daylight via a window (3' 8" square) facing south-west.

The experimental mice and breeding stock were kept in plastic boxes ($5\frac{1}{2}$" x $11\frac{1}{2}$" x $4\frac{1}{2}$") held on wall-mounted racks. Mice not currently involved in experiments (surplus males, spayed females, virgin females) were stored in wire cages (12" x 18" x 6" high) placed on metal trays and capable of holding 20 to 40 animals. Boxes and trays contained $\frac{1}{2}$"-1" of sawdust and wood-wool was provided for nesting material.

Changes of all boxes, cages and trays were made once a week, fresh sawdust and nesting material being used. Soiled containers were washed and sterilised in Zephiran solution (cages and trays were steam sterilised in the

later stages of the experiments).

The mice were fed a diet of pellets prepared by a local milling company to the following formula:-

Wheat meal	80 parts
Barley meal	20
Ground oats	8
Buttermilk powder . .	64
Wheat germ meal . . .	10
Lime	2
Salt	1
Total	185

Food and water were provided ad libitum; special care was taken with high densities of mice to ensure that food and water containers were kept at a high level.

Breeding Methods

(a) Stock mice — The mice originated from twenty individuals of each line selected from the University stock and were randomly mated to increase their numbers.

As the number of males available was limited, one male was mated to two females per box. The productivity of the females did not appear to be adversely affected by doing this.

The breeding colony was expanded to, and maintained at, approximately twenty females of each line.

All pups were recorded at birth, earmarked and weaned at 28-35 days of age. This weaning age was selected after consideration of the growth rate of the C57BL pups which weighed approximately 7 grammes at 21 days, but 9 to 10 grammes at 28 days old, the latter being a more desirable weaning weight. The 101 pups grew faster and might have been weaned satisfactorily at 21 days of age.

(b) Experimental mice — The weaned female mice to be used in experiments A and B were transferred to new boxes, whereas those to be used in the studies on pre-natal mortality were placed temporarily in cages. The females studied in experiments A and B were randomly distributed to the control and grouped treatments. In this manner, females from one litter were normally dispersed between the two treatments and a box of grouped mice rarely contained females from one litter only. In making up a group, females weaned on the same day were used. The males were placed in cages at weaning and were removed as required to be introduced singly (i.e. one male per box) to the females as soon as the latter were assigned to the separate treatments. Males at least two weeks older than the females were used whenever possible in experiment A. All males were mature (3+ months old) in experiment B.

Control of Disease and Parasites

Young mice occasionally suffered from infantile diarrhoea which was treated by administration of aureomycin in the drinking water for up to one week. When not effective at the end of this time the treatment was replaced by one consisting of a loose feed mixture of 1 part buttermilk powder to 2 parts barley meal containing penicillin (Vetspen), placed within the box. This mixture was given for up to one week during which time the normal feed was reduced to a bare minimum.

Mid-way through the study period, tapeworms (Hymenolepis nana) were discovered in other mice in the small animal house. To guard against infection the floor of the room was washed down with chloride of lime once a week. Despite these precautions, tapeworms were found, at autopsy, in some experimental mice. The incidence of tapeworms is given in Appendix I.

Lice were controlled by dusting with malathion powder.

On several occasions the external genitalia of some male C57BL mice became inflamed. To prevent transmission of any organisms involved in what appeared to be an infectious disease, hands and the instrument for detecting

vaginal plugs were washed in Zephiran after contact with the animals.

Detection of Vaginal Opening

In immature female mice the mouth of the vagina is closed by a membrane which ruptures at puberty. Thus detection of vaginal opening presents a convenient method for determining age at puberty.

Females were examined daily, care being taken to prevent physical breaking of the membrane during examination, and puberty was assumed to have been reached when the membrane had separated to the full width of the vulva.

Detection of First Oestrus

At the onset of first oestrus marked changes were visible in the external genitalia: the vulva tended to be swollen; there was reddening of the vulva and vagina; and the vaginal orifice was enlarged. With advance to metoestrus, considerable desquamation of the vaginal epithelium was observed, unless mating had occurred and a vaginal plug was present.

Detection of Mating

Detection of a vaginal plug gives evidence of mating.

To detect plugs a finely-sharpened, smooth-pointed pencil was used initially. Because the diameter of the pencil was greater than that of the vagina, deep penetration was not possible without causing vaginal distension and possibly the occurrence of pseudopregnancy. In later studies a fine-pointed, ball point pen refill was used and allowed maximum penetration with a minimum of irritation and distension. The instrument was washed frequently, after use, in Zephiran solution to avoid infection.

Vaginal plugs persisted normally for 12 to 24 hours in 101 females and for 12 up to 48 hours or more in C57BL females.

In 101 females the vaginal plug was always visible at the vulva, whereas in C57BL females the situation of the plug was variable, frequently being placed deep in the vagina, and careful probing was necessary for detection.

Observations at Birth of Litters

Pregnant females were examined daily for birth of litters.

The pups were sexed at birth. Males were distinguished from females by a larger genital papilla placed further from the anus, and a blackish spot between the two regions was also observed in males.

As pups could be born alive yet die immediately after birth, great care was taken in attempting to identify stillborn pups. Such pups were arbitrarily regarded as those very pale in colour, and in a prostrate position, with hind legs extended posteriorly as at birth.

Records

Ages and weights of the females were recorded at:-

Weaning
Vaginal opening
First oestrus
Mating
Conception
Birth of litters.

Data were collected on mating, conception and parturition for the first three full-term pregnancies, but only data for first litters have been presented in the results (Chapter III).

As litters were born, the litter size, sex ratio and number of stillborn pups were recorded. The litters were examined daily and all deaths up to weaning (at 28 days post partum) recorded, the live pups being weighed on the first two days post partum and again at 21 and 28 days. Pups were killed at 28 days of age and the sex ratio checked.

As a precautionary measure against failure to detect vaginal plugs, daily records were kept of all females approaching, or at, oestrus as indicated by the external appearance of the vagina. Females were recorded and weighed the day following the appearance of an oestrous vagina. These data were used in the event of pregnancy occurring without the detection of a vaginal plug.

Slaughter of Mice for Autopsy

For studies on pre-natal mortality, mice, selected at random, were killed at either $3\frac{1}{2}$ or $18\frac{1}{2}$ days post coitum. From each box of grouped females, some were killed at $3\frac{1}{2}$ days post coitum, and the remainder at $18\frac{1}{2}$ days of pregnancy. On each occasion the female removed was replaced by a spayed mouse to keep the number in each group constant. Thirty females of each line were spayed and available for this purpose.

In animals destined for slaughter at $3\frac{1}{2}$ days post coitum the vagina was closely examined for signs of approaching oestrus. When these were present the mouse was not killed. Likewise the decision to kill a female at $18\frac{1}{2}$ days post coitum was made only if pregnancy was indicated.

The females were killed by placing them in a jar containing ether absorbed into cotton wool.

Examination of the Reproductive Tract

(a) Observations at $3\frac{1}{2}$ days post coitum — A mid-ventral incision was made to expose the reproductive tract.

The ovaries were examined macroscopically in situ and the corpora lutea counted after tearing open the bursa ovarica, care being taken to avoid rupture of blood vessels and resultant fading of the corpora lutea.

The reproductive tract was removed by separating it from the broad ligament and cutting through the utero-cervical junction. The ovaries were removed and examined under a low-power (17.5x) binocular microscope to check the number of corpora lutea.

The lower end of the Fallopian tube was cut just above the utero-tubal junction and the tip of the uterine horn teased out. The uterine horns were separated at the cervical end.

The ova were flushed out with 0.6 ml. of 0.9 per cent saline solution using a 1 ml. syringe fitted with a blunted intradermal needle. This was inserted at the cervical end of the uterine horn and the uterus held firmly

around the needle with tweezers.

The washings, collected in watch glasses, were examined at 17.5x and 70x magnification and the numbers of ova counted. Normally cleaved ova were regarded as fertilized.

Uterine contractility was an initial problem in flushing out ova from the uterus. It was found that flushing of the uteri of C57BL mice could be done successfully within half an hour of killing, the uterus being fully relaxed. With 101 females, however, the uterus was extremely contractile and recovery of ova was poor at that time. An improvement in ovum recovery rate in 101 mice was obtained by allowing an interval of 2 to 4 hours between slaughter and flushing.

(b) Observations at $18\frac{1}{2}$ days post coitum — Corpora lutea were counted as previously described.

On immediate removal of the reproductive tract, live foetuses (which were still active) were counted in each uterine horn. Dead foetuses were very pale in colour, did not respond to stimulation and, when closely examined, showed no evidence of a heart beat. Foetuses dead for a longer period were discoloured and small in size.

The uterine horns were slit longitudinally, the foetuses removed and the implantation sites carefully identified and counted. The presence of deciduomata, indicating implantation sites where resorption had occurred, was also noted.

Statistical Analysis and Presentation of Results

The data have been examined using, where appropriate, the methods of analysis of variance, analysis of covariance and "Student's" t-Test (Snedecor, 1956). The analyses were based on the n-fold hierarchal classification (Kempthorne, 1952, p. 107). In one analysis, where the variance of the data was proportional to the mean, a logarithmic transformation was made of the original data (Kempthorne, 1952, p. 154). In certain cases enumeration data have been transformed by taking square roots before analysis (Snedecor, 1956, p. 315).

Chi Square and Fisher's Exact Test were used as tests of independence for enumeration data (Goulden, 1952, pp. 369-374).

Results are presented in tabular form, where appropriate, with means, standard errors of the mean and variances quoted for each variable. The reported means have been taken to the first decimal place and the differences between means to the second decimal place, except that, where means were small, the means and differences between means have been taken to the second and third decimal places, respectively.

Where comparisons of the variables have been made, the probabilities of the observed differences arising by chance are quoted.

The types of analysis used are reported in each table.

TABLE 3

Weight (gms) at weaning of C57BL and 101 females weaned at 28 days of age.
(Data for levels of density pooled)

Experiment	Line	Number of Mice	Weight	
			Mean ± S.E.	Variance
A	C57BL	15	8.8 ± 0.45	3.04
	101	14	10.8 ± 0.36	1.79
B	C57BL	34	10.8 ± 0.30	3.13
	101	46	12.5 ± 0.21	2.11

Comparison of Lines	Experiment	Difference in Weights (gms)	Significance of Difference*
101 v C57BL	A	1.97	$0.01 < P < 0.05$
	B	1.66	$0.01 < P < 0.05$

Comparison of Experiments	Line		
B v A	C57BL	1.98	$0.01 < P < 0.05$
	101	1.67	$0.01 < P < 0.05$

* Probabilities from t-Tests following analysis of variance.

Chapter III

REPRODUCTIVE PERFORMANCE OF MICE IN EXPERIMENTS A and B

Age and Weight at Weaning

The ages at weaning of all females studied in experiments A and B are shown in Table 2.

TABLE 2

Ages (days) at weaning of all C57BL and 101 females studied in experiments A and B.

Experiment	Line	Total Number of Mice	Numbers of Mice per Age Range at Weaning					
			25-27	28	29-30	31-33	34-36	37-46
A	C57BL	37	4	15	0	3	8	7
	101	43	10	14	11	0	2	6
B	C57BL	58	0	37	1	11	9	0
	101	54	0	47	0	7	0	0

An analysis of variance was done on the weights of females weaned at 28 days of age. (No analyses were done on the weight of females weaned at other ages.) There were no significant differences between weights at the two levels of population density; hence data on control and grouped mice within lines have been pooled.

The variance of weaning weight was consistently higher in C57BL compared to 101 females (except line 101 controls in experiment B). Considering also that C57BL females were lighter at weaning (see Table 3) than 101 females, it is suggested that some of the former were stunted in growth at weaning (notably in experiment A). This may be a reflection of maternal performance in the C57BL line, or may be due to causes of genetic origin.

In both experiments, C57BL females were lighter than 101 females at weaning. By altering the hierarchy of the analysis of variance, to test for

TABLE 4

Age (days) at puberty as shown by vaginal opening in C57BL and 101 lines of mice. (Data for levels of density pooled)

Experiment	Line	Number of Mice	Age	
			Mean ± S.E.	Variance
A	C57BL	21	60.4 ± 2.49	130.15
	101	28	46.0 ± 0.89	21.96
B	C57BL	38	40.8 ± 0.96	35.24
	101	42	36.6 ± 0.42	7.56

Comparison of Lines	Experiment	Difference in Ages (days)	Significance of Difference*
C57BL v 101	A	14.3	$P < 0.001$
	B	4.2	$0.01 < P < 0.05$

Comparison of Experiments	Line		
A v B	C57BL	19.6	$P < 0.001$
	101	9.4	$P < 0.001$

* Probabilities from t-Tests following analysis of variance.

differences between experiments within lines, females of both lines were found to be heavier at weaning in experiment B.

Age and Weight at Vaginal Opening

There was some doubt as to the accuracy of detecting vaginal opening, especially in experiment A, and more so in C57BL mice. The ability to detect vaginal opening accurately improved with practice. Moreover, with the greater period for examination (from weaning) in experiment A, there was increased possibility of vaginal opening being caused by physical means. Therefore, more confidence is placed in the results of experiment B.

The analysis of variance revealed no significant difference between the ages at vaginal opening of control and grouped mice. These data have, therefore, been pooled (Table 4).

As indicated by vaginal opening, 101 females reached puberty before C57BL females. The difference was more marked in experiment A, probably due to the low weight at weaning of C57BL females. Females of both lines reached puberty much earlier in experiment B than in experiment A.

The variance of age at vaginal opening is considerably higher for C57BL females than 101 females, which may be a reflection of similar variances in weaning weight (see Table 3).

The weights of mice at vaginal opening are shown in Table 5. Weights of control and grouped females at vaginal opening showed a significant difference only in line 101, experiment B; control females were heavier than grouped females ($P < 0.001$). Such a result was not predicted by the corresponding values of line 101 in experiment A. This result may not indicate a greater growth rate of the 101 control females in experiment B, as the mean age of these at vaginal opening was 2.1 days greater than the mean age of the corresponding grouped females. In the absence of data on growth rate, no conclusions can be drawn.

101 females were heavier at vaginal opening than C57BL females in

TABLE 5

Weight (gms) at puberty, as shown by vaginal opening, in C57BL and 101 lines of mice. (Data for levels of density pooled)

Experiment	Line	Number of Mice	Weight	
			Mean ± S.E.	Variance
A	C57BL	20	16.5 ± 0.23	1.03
	101	26	15.9 ± 0.17	0.79
B	C57BL	35	14.8 ± 0.17	0.96
	101	39	16.3 ± 0.17	1.19

Comparison of Lines	Experiment	Difference in Weights (gms)	Significance of Difference*
C57BL v 101	A	0.59	$0.3 < P < 0.4$
101 v C57BL	B	1.46	$0.01 < P < 0.05$

Comparison of Experiments	Line		
A v B	C57BL	1.66	$0.01 < P < 0.05$
B v A	101	0.39	$0.4 < P < 0.5$

* Probabilities from t-Tests following analysis of variance.

TABLE 6

Age (days) at first oestrus in C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Mice	Age	
				Mean ± S.E.	Variance
C57BL	A	Control	14	73.1 ± 2.66	98.99
		Grouped	19	72.3 ± 3.10	182.09
	B	Control	14	50.2 ± 1.24	21.41
		Grouped	32	57.2 ± 1.55	77.32
101	A	Control	19	54.0 ± 1.58	47.67
		Grouped	10	67.2 ± 6.10	371.51
	B	Control	17	41.6 ± 0.94	14.88
		Grouped	17	50.1 ± 3.23	177.06

Comparison of Density Levels	Line	Experiment	Difference in Ages (days)	Significance of Difference*
Grouped v Control	C57BL	A	- 0.81	0.8 < P < 0.9
		B	6.97	0.01 < P < 0.05
	101	A	13.20	0.001 < P < 0.01
		B	8.47	0.01 < P < 0.05

Comparison of Experiments	Line		
A v B	C57BL	17.54	0.01 < P < 0.05
	101	12.73	0.05 < P < 0.1

* Probabilities from t-Tests following analysis of variance.

experiment B, but not in experiment A. C57BL females were heavier at vaginal opening in experiment A than in experiment B, but 101 females did not differ significantly in weight between the experiments. Lack of conformity in these results is largely due to line C57BL in experiment A.

Age and Weight at First Oestrus

The ability to regularly predict mating by the externally visible macroscopic changes in the vulva and vagina indicated that detection of oestrus by the same method could be done with considerable accuracy.

The ages of mice at first oestrus are shown in Table 6. Grouped females showed first oestrus later than control females in all comparisons except within line C57BL in experiment A.

The variance was consistently higher for grouped females and it was noted that in line C57BL, experiment A, the same trend existed, indicating a differential response between mice at the two levels of population density regardless of the lack of difference in mean age. As the variance was proportional to the mean (Table 6) the original data for age at first oestrus were transformed by taking logarithms (Table 6a). This transformation did not alter the statistical significance of main differences as revealed by analysis of variance. Thus conclusions based on an analysis of variance were not invalidated because of non-normality of the original data.

No differences between the lines were found, but females in experiment B showed first oestrus earlier than in experiment A, the difference being significant only for line C57BL.

Analysis of the weights of female mice at first oestrus revealed no statistically significant differences among any of the comparisons. The overall mean weight was 17.5 $\pm$ 0.13 gms.

TABLE 6a

Age (days) at first oestrus in C57BL and 101 lines of mice at two levels of population density. (Data transformed by taking logarithms)

Line	Experiment	Density	Number of Mice	Age *	
				Mean	Variance
C57BL	A	Control	14	72.5	1.04
		Grouped	19	71.2	1.04
	B	Control	14	50.0	1.03
		Grouped	32	56.6	1.03
101	A	Control	19	53.6	1.03
		Grouped	10	64.9	1.09
	B	Control	17	41.4	1.02
		Grouped	17	48.7	1.06

* Mean and variance derived from logarithms.

TABLE 7

Interval (days) between first oestrus and first mating in C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Mice	Interval Mean ± S.E.	Interval Variance
C57BL	A	Control	14	12.4 ± 3.19	142.71
		Grouped	19	4.2 ± 1.70	54.92
	B	Control	15	5.4 ± 2.71	110.26
		Grouped	29	14.1 ± 4.73	648.19
101	A	Control	19	5.5 ± 1.88	66.82
		Grouped	10	23.3 ± 5.75	331.12
	B	Control	17	1.5 ± 0.92	14.39
		Grouped	11	28.6 ± 11.15	1367.85

Comparison of Density Levels	Line	Experiment	Difference in Interval (days)	Significance of Difference*
Grouped v Control	C57BL	A	- 8.2	$0.1 < P < 0.2$
		B	8.7	$0.1 < P < 0.2$
	101	A	17.8	$0.01 < P < 0.05$
		B	27.1	$P < 0.001$
Comparison of Controls				
C57BL v 101		A	6.8	$0.2 < P < 0.3$
		B	3.9	$0.5 < P < 0.6$

* Probabilities from t-Tests following analysis of variance.

Interval from First Oestrus to First Mating

Table 7 shows the interval from first oestrus to first mating for mice in the various treatments. There was a significant delay in the occurrence of first mating in grouped 101 mice compared to their isolated controls. With C57BL mice there was no significant effect of increased population density on the interval from first oestrus to first mating and, furthermore, the differences were opposite in direction in the two experiments. However, for reasons concerned with management of C57BL mice, more confidence is placed in the results of experiment B, as indicating the effect of increased population density, than in results of experiment A.

Study of the variance of interval from first oestrus to mating shows this to be greater for grouped mice (except line C57BL, experiment A) than for controls and, also, greater for C57BL controls than 101 controls (though the difference between control means was not significant). These data may indicate greater variation in the performance (libido) of C57BL males when compared to 101 males assuming that in control pairs of mice the delay in mating is mainly due to the male. This suggestion of variability in performance of C57BL males was also supported by observations on the mating behaviour of these animals during the experiments.

Consideration of the manner in which data on C57BL mice were derived for experiments A and B also suggests a reason for the difference in magnitude of the result. Thus, in experiment A, there were four groups of females and the ratio of control to grouped males was 3.5 : 1 (14 : 4). In experiment B there were eight groups of females and the corresponding male ratio was 1.9 : 1 (15 : 8). Owing to the likelihood of variability in C57BL male performance there would seem to be a greater chance of obtaining conclusive results for this line of mice from eight male-groups (experiment B) than four male-groups (experiment A).

The previous history of C57BL males also may suggest a reason for

TABLE 8

Conception rate of C57BL and 101 lines of mice at two levels of population density as shown by the number of first matings resulting in a full-term pregnancy.

Line	Experiment	Density	Number of Full-term Pregnancies	Barren First Matings	% Pregnancies
C57BL	A	Control	10	8	55.6
		Grouped	7	12	36.8
	B	Control	12	2	85.7
		Grouped	22	6	78.6
101	A	Control	10	9	52.6
		Grouped	7	8	46.7
	B	Control	15	2	88.2
		Grouped	7	3	70.0

Comparison of ratio of successful to barren first matings	Line	Experiment	Probability of observed or a more extreme ratio occurring by chance
Control v Grouped	C57BL	A	0.21 [1]
		B	0.46 [2]
	101	A	0.24 [1]
		B	0.25 [2]
		Density	
Experiments B v A	C57BL	Control	0.075 [1]
		Grouped	0.0048 [2]
	101	Control	0.024 [2]
		Grouped	0.23 [1]

[1] Probability by Chi^2.

[2] Probability by Fisher's Exact Test.

differential responses in the two experiments. Thus, in experiment A, two of the C57BL males in groups were litter mates and a third was a male of proven fertility, whereas in experiment B all males were "virgin" and taken at random from a stock cage. Under these circumstances the four C57BL males in experiment A groups may have been of relatively higher and/or more uniform performance than their counterparts in experiment B. Differences in the interval from first oestrus to first mating between individual groups of C57BL mice in both experiments were tested by analysis of variance. A significant difference ($P < 0.05$) was found between groups in experiment B, but in experiment A the differences were well below the level required for statistical significance. If differences between individual groups (of comparable size and line) are mainly due to the males, and the C57BL males of experiment A were of high and/or uniform performance, it would seem unlikely for differences to be shown between individual C57BL groups within experiment A. Therefore, the results for C57BL groups in experiment A might well be expected to differ from those of C57BL groups where the males were randomly selected and of "virgin" reproductive ability (i.e. experiment B).

In contrast to the above arguments, the differential responses of C57BL mice in experiments A and B may have been due to "chance" variation.

Conception Rate following First Mating

The numbers and percentages of full-term pregnancies from first mating (as a measure of conception rate) in the mice are shown in Table 8.

No significant differences were revealed between control and grouped mice for the ratio of females producing litters to those not producing litters as a result of first mating. However, there was a consistent tendency for the percentage of full-term pregnancies to be lower in grouped mice.

A comparison within levels of density of C57BL mice showed that there was a highly significant difference between grouped mice in experiments A and B,

but the difference between controls was not significant. For 101 mice the controls showed a significant difference between experiments but there was no statistical difference between grouped animals. In the latter case, few data were available for comparison from grouped mice in experiment B.

The higher percentage of full-term pregnancies from first matings in experiment B may have been associated with the higher weight of these mice at weaning, as shown in Table 3. At first mating, however, there were no significant differences between experiments in the weights of these mice (Table 9). Nor were there any significant differences in weight at first mating between lines of mice or between levels of population density, except in line 101 in experiment A ($P < 0.01$, grouped mice heavier than controls).

TABLE 9

Weight (gms) at first mating of C57BL and 101 female mice.
(Data for levels of density pooled.)

	C57BL		101	
Experiment	Number of Mice	Weight Mean ± S.E.	Number of Mice	Weight Mean ± S.E.
A	26	18.3 ± 0.22	34	18.4 ± 0.27
B	35	17.7 ± 0.23	17	18.2 ± 0.30

TABLE 10

Rate of pseudopregnancy in C57BL and 101 lines of mice at two levels of population density.

Line	Expt.	Density	Number of Pseudo-pregnancies	Number of Barren Matings not followed by a Pseudopregnancy	% Pseudo-pregnancies
C57BL	A	Control	6	3	66.7
		Grouped	13	3	81.2
	B	Control	1	1	50.0
		Grouped	6	1	85.7
101	A	Control	8	8	50.0
		Grouped	4	11	26.7
	B	Control	3	0	100.0
		Grouped	0	0	-

Comparison of Ratio of Occurrence to Non-occurrence of Pseudopregnancy	Line	Experiment	Probability of Observed or a more extreme Ratio Occurring by Chance	
Grouped v Control	C57BL	A	0.36	2
		B	0.42	2
Control v Grouped	101	A	0.17	1
C57BL v 101		A	0.0059	1

1 Probability by Chi^2.

2 Probability by Fisher's Exact Test.

Incidence of Pseudopregnancy

Following matings of nulliparous mice, observations were made for returns to oestrus. When oestrus failed to occur within seven days of mating, and the animal was not subsequently found to have been pregnant or ill during this period, then pseudopregnancy was considered to have resulted.

The number and mean duration of pseudopregnancies in the two lines of mice were as follows:

Line	Number of Pseudopregnancies	Duration (days) of Pseudopregnancy Mean ± S.E.
C57BL	26	9.5 ± 0.41
101	15	9.7 ± 0.73

The average duration of pseudopregnancy did not differ significantly between females at the two levels of population density or between lines of mice.

The rate of pseudopregnancy in the mice at two levels of population density is shown in Table 10. The relative absence of barren matings in line 101 in experiment B was notable. No adequate reason can be advanced for this result. Pseudopregnancy was not shown to occur more frequently in either control or grouped mice, but the number of animals was few. The frequency of pseudopregnancy in C57BL mice was greater than in 101 mice in experiment A.

TABLE 11

Age (days) at first conception, where pregnancy had been confirmed before completion of the experiment, in C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Mice	Age	
				Mean ± S.E.	Variance
C57BL	A	Control	17	86.0 ± 3.86	253.13
		Grouped	18	86.3 ± 5.28	501.27
	B	Control	19	60.2 ± 2.44	112.73
		Grouped	35	73.9 ± 4.07	579.85
101	A	Control	19	68.2 ± 3.85	281.81
		Grouped	13	110.8 ± 7.74	778.86
	B	Control	20	47.9 ± 2.45	120.13
		Grouped	10	105.6 ± 16.60	2,756.04

Comparison of Density Levels	Line	Experiment	Difference in Ages (days)	Significance of Difference*
Grouped v Control	C57BL	A	0.28	$P > 0.9$
		B	13.70	$0.01 < P < 0.05$
	101	A	42.61	$P < 0.001$
		B	57.75	$P < 0.001$
Comparison of Controls				
C57BL v 101		A	17.84	$0.01 < P < 0.05$
		B	12.36	$0.05 < P < 0.1$
Comparison of Groups				
101 v C57BL		A	24.49	$0.001 < P < 0.01$
		B	31.69	$P < 0.001$

* Probabilities from t-Tests following analysis of variance.

Age and Weight at First Conception*

Results of analysis of data on the ages at first conception in the mice are presented in Table 11. These data refer only to mice which had conceived before completion of each experiment.

The overall age of mice at first conception was greater in experiment A than in experiment B ($P < 0.01$). 101 grouped mice first conceived when much older than their controls, whereas C57BL grouped mice were older than their controls at first conception in experiment B, but not in experiment A. The lack of difference between control and grouped C57BL mice in experiment A is attributed to those factors discussed in Chapter V.

Comparison of control animals shows that C57BL females were older than 101 females at first conception in both experiments, but the difference in ages was significant only in experiment A. However, at the grouped density, 101 mice were older than C57BL mice in both experiments and the differences were highly significant.

A further analysis was carried out on enumerated data because some mice had not conceived at the completion of the respective experiments. The numbers of mice in which full-term pregnancy did not occur were as follows:

Line	Experiment	Control	Grouped
C57BL	A	1	1
	B	0	1
101	A	0	2
	B	0	10

* The term conception here refers only to cases in which the resultant pregnancy was of full-term duration

TABLE 12

Conception rate, as shown by the number of females pregnant (pregnancy being full-term) by 130 days of age, for C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Mice having Conceived	Number of Barren Mice	% Conception
C57BL	A	Control	17	1	94.4
		Grouped	17	2	89.5
	B	Control	19	0	100.0
		Grouped	34	2	94.4
101	A	Control	19	0	100.0
		Grouped	9	6	60.0
	B	Control	20	0	100.0
		Grouped	7	13	35.0

Comparison of Ratio of Parous/Pregnant to Barren Mice	Line	Experiment	Probability of Observed or a more extreme Ratio Occurring by Chance *
Control v Grouped	C57BL	A	0.52
		B	0.42
	101	A	0.0037
		B	0.000 006
	Density		
C57BL v 101	Grouped	A	0.054
		B	0.000 0031
	Line		
Experiments A v B	Grouped	101	0.13 [1]

* Probabilities by Fisher's Exact Test.

[1] Probability by Chi^2.

As these mice were, with one exception, at the grouped density, they also serve to emphasize the delay of first conception associated with increased population density (Table 11). It is not surprising that the single control female which had not conceived was of line C57BL in experiment A.

Examination of the enumerated data was made by comparing the ratios of the numbers of females which had conceived to those that were barren at 130 days of age. These data are presented in Table 12. The age of 130 days was selected as it represented the age of the youngest females at completion of both experiments. It should be noted, however, that some mice first conceived at a greater age than 130 days, the latest record being a grouped 101 female at 222 days of age, and such data were included in the results presented in Table 11.

The ratio of parous (or pregnant) to barren females at 130 days of age, shown in Table 12, did not differ significantly between control and grouped C57BL mice in either experiment. However, this ratio was significantly greater for control than for grouped 101 mice in both experiments, and higher for grouped C57BL than for grouped 101 mice (just below statistical significance in experiment A, but highly significant in experiment B). This ratio for 101 grouped mice in experiment A was found not to be significantly higher than that for 101 grouped mice in experiment B.

Since marked differences in age at first conception were apparent, the effect of age on the weight at first conception of these mice was examined by analysis of covariance. The analysis showed that there was a positive regression ($P < 0.001$) of weight on age at first conception. Table 13 shows the corresponding ages and weights at first conception, and the weights adjusted for the effect of age. Having adjusted for this regression, no significant differences in weights at first conception were found either between levels of population density or between experiments. However, differences between the lines were significant: 101 females were heavier

TABLE 13

Weight (gms), adjusted weight (gms) and corresponding age[1] (days) of C57BL and 101 lines of mice at first conception resulting in a full-term pregnancy. (Data for levels of density pooled)

Expt.	Line	Number of Mice	Age	Weight		Adjusted Weight
				Mean ± S.E.	Variance	
A	C57BL	25	92.0 ± 3.89	18.7 ± 0.24	1.38	18.06
	101	31	84.3 ± 5.39	19.1 ± 0.27	2.25	18.78
B	C57BL	41	70.6 ± 3.55	18.4 ± 0.25	2.50	18.60
	101	18	50.3 ± 3.64	18.6 ± 0.40	2.90	19.56

Comparison of Lines	Experiment	Difference in Adjusted Weights (gms)	Significance of Difference*
101 v C57BL	A	0.71	$0.01 < P < 0.05$
	B	0.96	$0.01 < P < 0.05$

[1] Weights not available for all mice whose age at first conception was determined.

* Probabilities from t-Tests following analysis of covariance.

TABLE 14

Number of pups born in first litters of C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Litters	Litter Size	
				Mean ± S.E.	Variance
C57BL	A	Control	17	4.4 ± 0.40	2.76
		Grouped	17	4.5 ± 0.32	1.76
	B	Control	15	5.2 ± 0.48	3.46
		Grouped	28	5.1 ± 0.32	2.81
101	A	Control	22	5.3 ± 0.31	2.11
		Grouped	17	4.4 ± 0.48	3.99
	B	Control	18	6.3 ± 0.39	2.80
		Grouped	9	5.1 ± 0.63	3.61

Comparison of Levels of Density	Line	Experiment	Difference in Litter Size	Significance of Difference*
Control v Grouped	C57BL	A	- 0.06	P > 0.5
		B	0.13	P > 0.5
	101	A	0.92	0.05 < P < 0.1
		B	1.17	0.05 < P < 0.1
Comparison of Controls				
101 v C57BL		A	0.86	0.1 < P < 0.2
		B	1.08	0.05 < P < 0.1

* Probabilities from t-Tests following analysis of variance.

than C57BL females at first conception in both experiments.

Gestation Period

There were no statistically significant differences between the lengths of the first full-term pregnancy of control and grouped mice of either C57BL or 101 lines. However, a significant difference ($P < 0.05$) was found between the lines in both experiments: C57BL animals had a longer gestation period than 101 mice.

The mean length of first full-term pregnancy for mice in each line, with data for levels of population density and experiments pooled, was as follows:-

Line	Number of Mice	Gestation Period (days)	Significance of Difference
C57BL	72	19.8 ± 0.06	$0.01 < P < 0.05$
101	54	19.3 ± 0.06	

Size of First Litter

The numbers of pups born in first litters and results of analysis of these data are shown in Table 14.

There were no statistically significant differences between litter sizes of control and grouped mice of either line. First litter sizes were approximately identical for control and grouped C57BL mice, but for 101 mice in both experiments the control females had larger litters than grouped females and the differences were very close to a significant level.

Although litters tended to be larger in experiment B than in experiment A, and larger in line 101 than in line C57BL, no significant differences were revealed between experiments or between lines. However, comparison of first litter sizes of control mice showed that litters of 101 females were almost significantly larger than those of C57BL females in both experiments. It can be seen that, in each experiment, C57BL control, C57BL grouped and 101 grouped

mice had comparable litter sizes, and that 101 control mice had larger litters than all of these.

A significant reduction in first litter size owing to increased population density has not been shown in these data; nevertheless the results from mice of line 101 may indicate that such an effect exists. However, there is the possibility that the observed decrease in numbers of pups born in first litters of grouped 101 mice was due to increased mortality of pups at parturition followed by devouring of dead pups before the litters were recorded. (Birth of individual litters was not observed.)

Data on the number of live pups per first litter, observed on the day of birth, were examined by analysis of variance which showed a significant difference between levels of population density. Further examination of the data by t-Tests revealed that the number of live pups per litter born to 101 females in experiment A was greater ($P < 0.01$) for control than for grouped mice. The trend towards fewer live pups per litter on day of birth, compared to corresponding controls, was also evident in C57BL grouped mice in both experiments and in 101 grouped mice in experiment B, although the numbers were not significantly less than for control animals.

Observations made on the dead pups suggested that few had been stillborn; most appeared to have died at parturition or within a few hours post partum. As mice readily devour dead (and, sometimes, live) pups, the reduction in first litter size of grouped 101 mice may have taken place immediately post partum and not during pregnancy.

TABLE 15

Mean weight (gms) and adjusted mean weight (gms) of live pups at birth, and corresponding litter size[1], for first litters of C57BL and 101 lines of mice at two levels of population density.

Line	Expt.	Density	Number of Litters	Litter Size	Weight of Live Pups		Adjusted Weight
					Mean ± S.E.	Variance	
C57BL	A	Control	12	4.1 ± 0.48	1.40 ± 0.045	0.024	1.37
		Grouped	8	4.0 ± 0.38	1.35 ± 0.032	0.008	1.32
	B	Control	12	4.8 ± 0.53	1.39 ± 0.032	0.012	1.38
		Grouped	26	5.2 ± 0.30	1.26 ± 0.022	0.012	1.28
101	A	Control	17	5.1 ± 0.41	1.38 ± 0.021	0.007	1.39
		Grouped	14	4.5 ± 0.48	1.29 ± 0.023	0.007	1.27
	B	Control	7	6.0 ± 0.58	1.31 ± 0.021	0.003	1.35
		Grouped	7	5.6 ± 0.65	1.31 ± 0.027	0.005	1.33

Comparison of Levels of Density	Line	Experiment	Difference in Adjusted Weights (gms)	Significance of Difference*
Control v Grouped	C57BL	A	0.054	$0.1 < P < 0.2$
		B	0.107	$P < 0.001$
	101	A	0.115	$P < 0.001$
		B	0.019	$P > 0.5$

* Probabilities from t-Tests following analysis of covariance.

[1] Includes dead pups.

Weight of Live Pups of First Litters at Birth

Mean weights* of live pups at birth, with corresponding litter sizes, and results of an analysis of covariance of these data are shown in Table 15.

There was found to be a negative regression ($P < 0.001$) of mean pup weight on litter size at birth. This regression was verified by graphs which showed the relationship to be linear and uniform for both levels of population density in both lines of mice. The relationship was less distinct in line 101 than in line C57BL due to less variation in the mean weights of 101 pups.

Having adjusted the mean pup weights for litter size, it was found that pups born to control mice were much heavier than those born to grouped mice in line C57BL, experiment B, and in line 101, experiment A. The other two comparisons were affected by scarcity of data. Nevertheless, in line C57BL, experiment A, pups born to control mice were heavier than those born to grouped mice, the difference being almost statistically significant. With line 101, experiment B, even fewer data were available but these showed a similar trend to the other control versus grouped comparisons.

The adjusted mean pup weights did not indicate any statistically significant differences either between the lines or between experiments.

Although pups born to control mothers were heavier than pups born to grouped mothers, it should not be concluded that the differences were due to intra-uterine (pre-natal) factors. Females were examined for occurrence of parturition only once daily and, thus, litters may have been up to 24 hours old when recorded and weighed. This brings immediate post-natal factors into consideration. Some litters would have been suckled before being weighed whereas others would not. Assuming that the diurnal distribution of births was the same for control and grouped mice, then litters of control and grouped mothers should have had an equal chance of being suckled before being weighed,

* Weights of live pups were obtained by dividing the total live litter weight by the number of live pups in the litter.

TABLE 16

Mean weight (gms) of live one-day-old pups, and corresponding number of live pups per litter, for first litters of C57BL and 101 lines of mice at two levels of population density.

Line	Expt.	Density	Number of Litters [1]	Number of Live Pups/Litter	Weight of Live Pups	
					Mean ± S.E.	Variance
C57BL	A	Control	13	4.4 ± 0.46	1.39 ± 0.03	0.015
		Grouped	7	2.3 ± 0.52	1.23 ± 0.08	0.046
	B	Control	8	4.3 ± 0.82	1.41 ± 0.04	0.011
		Grouped	11	3.0 ± 0.50	1.21 ± 0.04	0.017
101	A	Control	20	5.2 ± 0.34	1.52 ± 0.03	0.018
		Grouped	11	4.0 ± 0.47	1.29 ± 0.03	0.010
	B	Control	10	6.0 ± 0.56	1.48 ± 0.04	0.016
		Grouped	4	5.5 ± 0.29	1.28 ± 0.01	0.001

Comparison of Levels of Density	Line	Experiment	Difference in Weight (gms)	Significance of Difference*
Control v Grouped	C57BL	A	0.16	$0.001 < P < 0.01$
		B	0.20	$0.001 < P < 0.01$
	101	A	0.23	$P < 0.001$
		B	0.20	$0.01 < P < 0.05$

* Probabilities from t-Tests following analysis of variance.

[1] Does not include extinct litters.

as daily weighings were completed within one hour. However, it was noted, during the course of experiments, that suckling, before weighing, of pups born to control mice appeared to have been more frequent and of greater magnitude (as evidenced by externally visible milk in the stomach) than suckling of pups born to grouped mice. No data were collected on this aspect but it does suggest that the greater weight, when first weighed, of pups born to control mothers may have been due, partly or entirely, to immediate post-natal factors.

Weight of Live Pups at One Day post partum

The mean weights and the corresponding number of live pups in first litters at one day post partum are shown in Table 16. The data on mean weights of pups were examined by analysis of variance. An analysis of covariance of these data, with litter size as the concomitant variable, would only have duplicated the results of the analysis of variance since the differences between the adjusted means would have been in the same direction as the unadjusted means but would have been larger and "more significant". However, essentially, the conclusions after either method of analysis would not differ.

To examine the data further, graphs were drawn of mean pup weight plotted against litter size for live pups at one day post partum. In all graphs for control mice a negative regression was evident though there was much variability which was possibly due to the effect of variable amounts of milk in the stomachs of pups at weighing. However, in all graphs for grouped mice there appeared to be no relationship between litter size and mean pup weight. This lack of a relationship was considered to be due to the effect of total litter abandonment in groups (i.e. some litters were probably undergoing total extinction, yet at one day post partum a few pups still survived though they were small and weak).

The graphs were then adjusted by deleting points for litters which subsequently became extinct. This had little effect on the data for control animals except to emphasize the regression of mean pup weight on litter size.

In the graphs for C57BL grouped mice this measure left so few points as to make any indications of a relationship impossible. However, in the graph for 101 groups in experiment A (there were too few data from experiment B) a negative regression became apparent.

Table 16 shows that, at one day post partum, pups born to control mothers were not only in larger litters, but were also considerably heavier than pups born to grouped mothers. Furthermore, the overall mean weight of pups born to 101 females was greater ($P < 0.05$) than that of pups born to C57BL mothers when weighed on the day following parturition.

Mortality of Pups between Birth and Weaning

The numbers of pups surviving per original litter between birth and weaning, as an index of mortality, for all treatments in experiments A and B are shown in Figures 1 and 2, respectively.

The numbers of pups per litter born to 101 control mice appeared to be greater than to mice in the other treatments and the survival rate of these pups to weaning was also greater, so that more pups were weaned per original litter from 101 control mice than from mice in any other treatments. Although in the other treatments litter sizes at birth were comparable (see Table 14), there was a descending order of pups weaned per original litter in these treatments from C57BL control, through 101 grouped, to C57BL grouped mice. The difference between 101 control and 101 grouped mice was less marked in experiment B than in experiment A.

In all treatments pup mortality was greatest in the first three days post partum and during this period differential mortality of pups in the various treatments occurred. Mortality of pups was greater up to three days post partum in litters born to grouped mice than in those born to controls, and greater in line C57BL than in line 101.

The greater pup mortality in grouped mice is consistent with the observed differences in weights of pups born to control and grouped mothers on day of

FIGURE 1

Number of pups surviving per original litter, between birth and weaning (at 28 days of age), in C57BL and 101 lines of mice at two levels of population density - Experiment A.

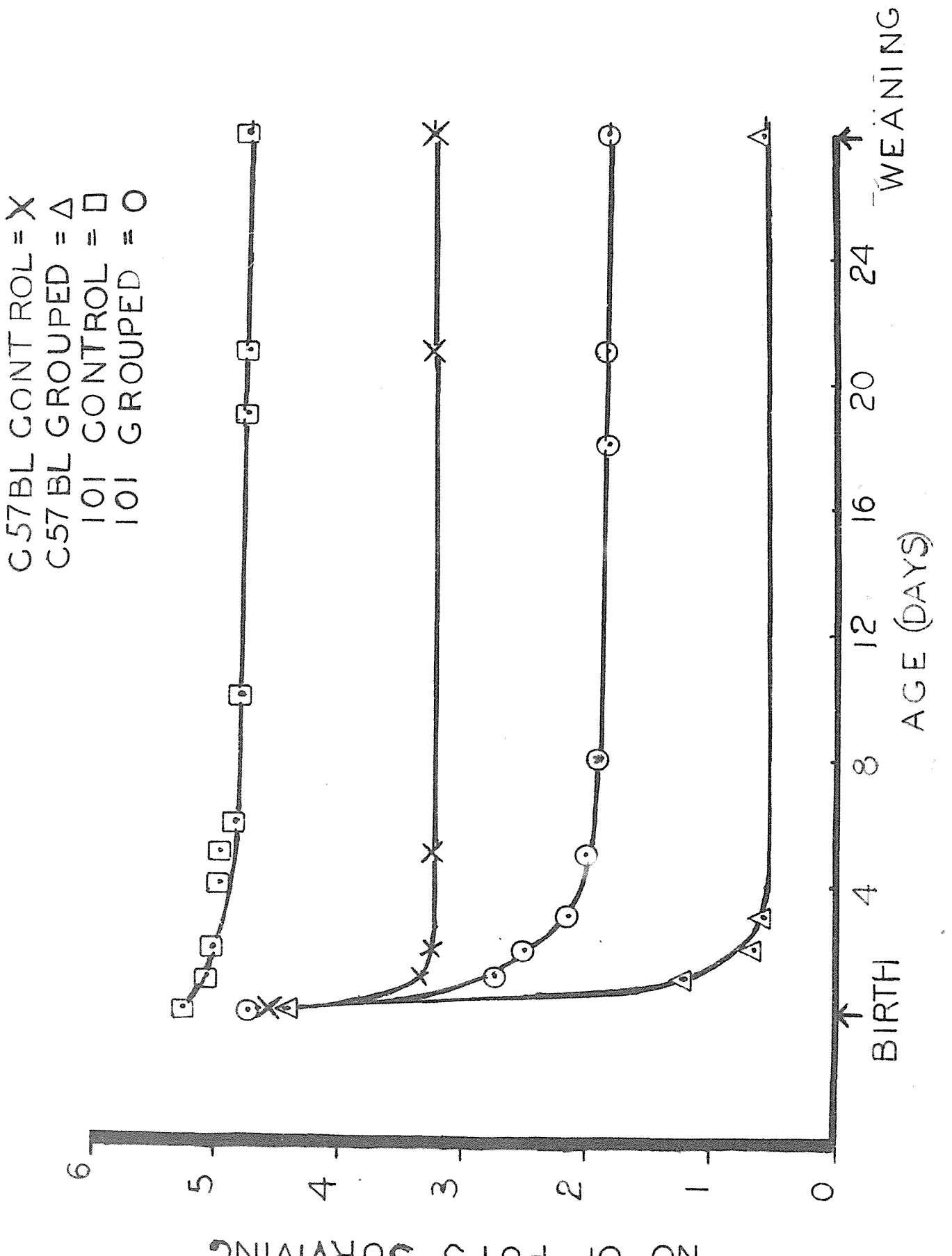

FIGURE 2

Number of pups surviving per original litter, between birth and weaning (at 28 days of age), in C57BL and 101 lines of mice at two levels of population density - Experiment B.

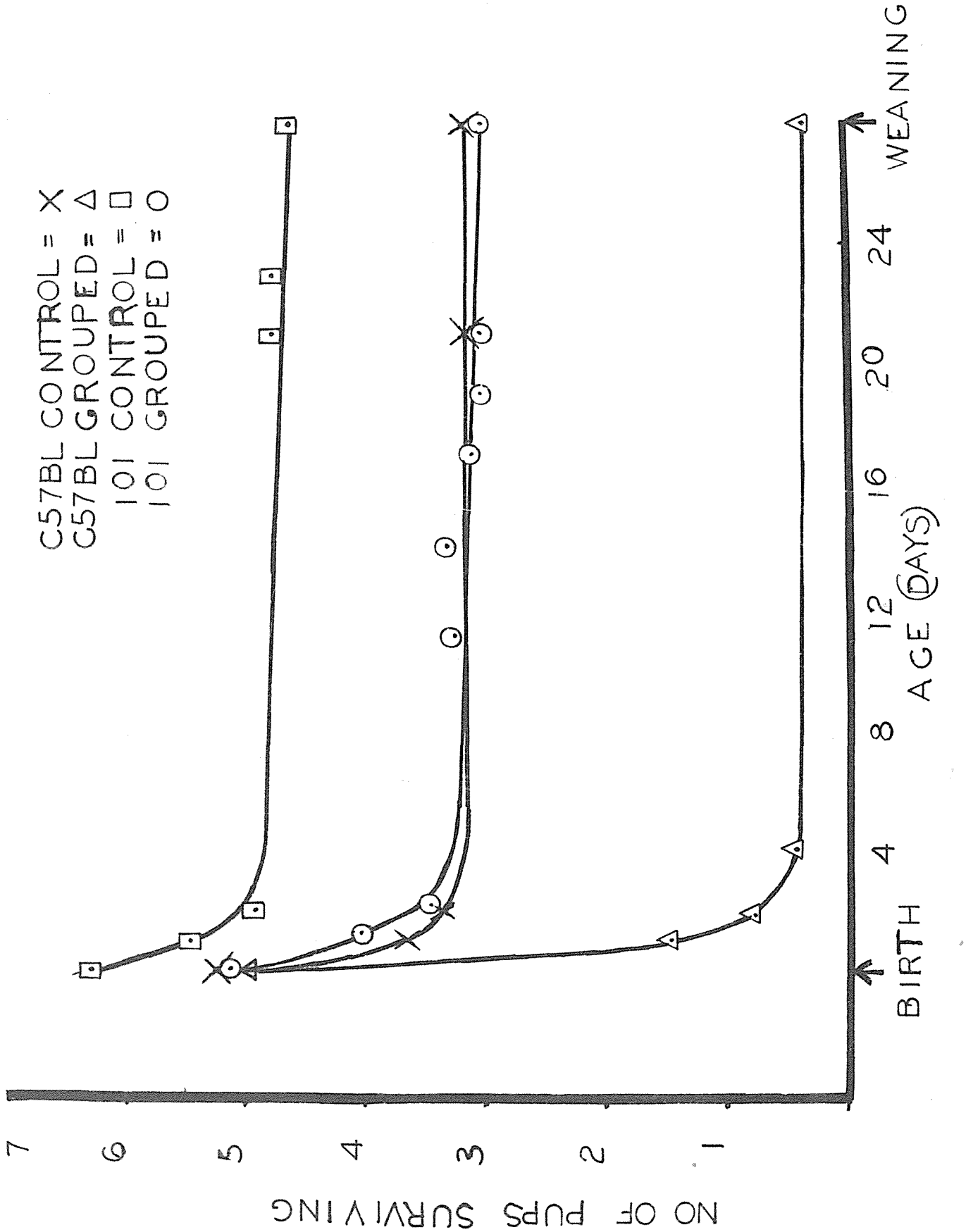

TABLE 17

Survival rate of first litters, as shown by the number of original litters extant at 21 days post partum, in C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Litters Extant	Number of Litters Extinct	% Survival
C57BL	A	Control	13	4	76.5
		Grouped	4	12	25.0
	B	Control	13	6	68.4
		Grouped	3	34	8.1
101	A	Control	21	1	95.5
		Grouped	8	9	47.1
	B	Control	18	3	85.7
		Grouped	7	3	70.0

Comparison of Ratio of Extant to Extinct Litters	Line	Experiment	Probability of Observed or a more Extreme Ratio Occurring by Chance	
Control v Grouped	C57BL	A	0.0045	1
		B	< 0.0001	1
	101	A	0.000 87	2
		B	0.28	2
	Density			
101 v C57BL *	Control		0.033	1
	Grouped		0.0001	1

[1] Probability by Chi^2.

[2] Probability by Fisher's Exact Test.

* Data for experiments A and B pooled.

birth (Table 15) and the succeeding day (Table 16). The higher pup mortality in line C57BL compared to line 101 appeared to be due to maternal factors. Casual observations indicated that either there was delayed onset of lactation, or that there was a delay in onset of nursing in C57BL mice. There was also an observed tendency towards more frequent total litter abandonment in line C57BL. These differences between the lines were evident both in control and in grouped mice.

The aberrant maternal factors observed in C57BL control mice were considerably accentuated in C57BL grouped mice. In addition, interference by other mice and communal nesting were factors contributing to increased pup mortality in groups of mice of both lines. It appeared that increased pup mortality in groups of 101 mice compared to their isolated controls was mainly due to these factors (i.e. interference and communal nesting) which were associated with an increased incidence of total litter abandonment (i.e. deficient maternal behaviour).

Survival of Litters

Table 17 shows the survival rate of first litters up to 21 days post partum, and the results of tests of independence of these data. The survival rate of litters born to grouped mice was significantly lower than that of litters born to control animals. The result for line 101 in experiment B did not reach a statistically significant level, which was probably due to the small number of litters born to the grouped animals.

Furthermore, comparisons of 101 and C57BL lines showed that the survival rate of C57BL litters was lower than that of 101 litters at both levels of population density, and that the difference between the lines was statistically greater for grouped than for control mice.

TABLE 18

Number of pups per first litter at weaning (28 days post partum), for litters extant, in C57BL and 101 lines of mice at two levels of population density.

Line	Experiment	Density	Number of Litters	Litter Size	
				Mean ± S.E.	Variance
C57BL	A	Control	13	4.2 ± 0.43	2.36
		Grouped	4	2.3 ± 0.63	1.58
	B	Control	13	4.6 ± 0.47	2.92
		Grouped	3	3.3 ± 0.33	0.33
101	A	Control	21	5.0 ± 0.27	1.50
		Grouped	8	3.8 ± 0.62	3.07
	B	Control	18	5.3 ± 0.44	3.41
		Grouped	7	4.3 ± 0.64	2.90

Comparison of Levels of Density	Line	Experiment	Difference in Litter Size	Significance of Difference*
Control v Grouped	C57BL	A	1.98	$0.01 < P < 0.05$
		B	1.28	$0.2 < P < 0.3$
	101	A	1.25	$0.05 < P < 0.1$
		B	1.05	$0.1 < P < 0.2$

* Probabilities from t-Tests following analysis of variance.

Numbers of Pups Weaned

The numbers of pups per litter, when weaned at 28 days post partum, and results of analysis of these data, are shown in Table 18. Only litters that were extant have been included.

In all comparisons between control and grouped mice the litter size at weaning was greater for control than for grouped animals. The differences between levels of population density were mainly close to the 5 per cent level of significance, but no significant differences were shown either between the lines or between experiments.

It should be noted that, although the data presented in Table 17 concerns the survival of litters up to 21 days post partum, there was no further total extinction of any litters from 21 to 28 days post partum. Over this latter period deaths of pups totalled three, all being pups of 101 controls, and were, therefore, negligible.

Thus, the results presented in Tables 17 and 18 may be considered together and in conjunction with Figures 1 and 2.

TABLE 19

Mean weight (gms), adjusted for litter size, of first litter pups at 21 days post partum, in line 101 at two levels of population density. (Data refers to litters in which pups survived.)

Experiment	Density	Number of Litters	Adjusted Mean Pup Weight (gms)	Significance of Difference*
A	Control	7	8.62	
				$0.2 < P < 0.3$
	Grouped	5	7.84	
B	Control	8	7.68	
				$0.01 < P < 0.05$
	Grouped	5	6.18	

* Probabilities from t-Tests following analysis of covariance.

Weight of Pups at 21 Days post partum

The numbers of first litters of C57BL grouped mice which survived to 21 days post partum were too small to permit comparison of the mean weights (at 21 days post partum) of pups reared by control and grouped C57BL females.

The available data on mean weights of pups reared by control and grouped 101 females were examined by analysis of covariance with litter size at 21 days post partum as the concomitant variable. There was a negative regression of mean pup weight on litter size but this was not significant ($P < 0.2$). Nevertheless, the regression was sufficient to increase the differences in mean pup weights between levels of population density. There was a significant difference ($P < 0.05$) between experiments, the mean pup weight being greater in experiment A than in experiment B. It was also apparent from the analysis of covariance that differences existed between levels of population density ($P < 0.1$). The mean pup weights, within levels of population density, were then adjusted for litter size and the differences tested for significance. These results are presented in Table 19.

Thus, at 21 days post partum in line 101, pups reared by control mothers were heavier than pups reared by grouped mothers. The difference was significant only in experiment B.

Reproductive Performance of Males

In experiment B, seven individual groups (i.e. of 1 male : 5 females) were set up in line 101. No litters had been born to any of the females in three of these groups at the completion of the experiment. The ages of females in these groups at completion of experiment B were 169, 189 and 192 days, respectively. Moreover, no matings (as evidenced by vaginal plugs) had been recorded in two of these groups. In the third group, two females mated at an early age (both at 49 days old) but neither, if they conceived, had a full-term pregnancy (no abortions were observed). No further matings were detected in this third group.

As the virtual absence of mating was considered to be due mainly or entirely to factors affecting the males, data on females in these three groups have been excluded from previous results subsequent to age and weight at first oestrus (e.g. data presented in Table 12).

Furthermore, in one of the four groups (of line 101 in experiment B) that did produce litters, two females became pregnant and gave birth to two litters each, but no matings were detected after birth of their second litters and the other three females remained barren. In another of these four groups, one female produced litters regularly after first conception at 81 days of age, but the other four females were nulliparous, although one was observed to have been mated once. In the other two groups of 101 mice in experiment B, one and two females, respectively, failed to have full-term pregnancies and, in fact, none of these was observed to have been mated. The single female which failed to become pregnant in one group was isolated with the male at completion of the experiment (the female being 176 days old). She was mated within 24 hours of isolation and became pregnant full-term.

At completion of experiment B the three 101 males, from the groups in which no full-term pregnancies occurred, were immediately paired with isolated, mature, virgin females. Two of these females became pregnant within five days of pairing and each gave birth to a normal litter nineteen days after mating. The third female mated twelve days after pairing and was autopsied at $3\frac{1}{2}$ days post coitum: normally-cleaved ova were recovered from the uterus.

These fifteen barren females were maintained in their respective groups, and other 101 males of proven fertility were introduced singly to the groups over a period of $2\frac{1}{2}$ months. As the length of time during which any particular male co-habited the groups varied, it was not possible to subject the results to statistical analysis. Oestrous cycles in mice are about five days in length and some of these males were present in the groups for only three days. Thus some males may have been present at a time when none of the females in the

group experienced oestrus. The results are presented in Table 20.

TABLE 20

Reproductive performance of 101 males in three groups of 101 females previously barren at completion of experiment B.

Group	Number of Males		Number of Females	Number of
	Introduced	Mated	Mated	First Litters
1	6	3	3	3
2	5	2	3	3
3	3	1	1	0

Two of the males mated twice, the other four only once. It was observed that most males were eager to mate on introduction, but only four were successful: 62.5 per cent of observed matings occurred within 24 hours of introduction of the new male. Thereafter, only a small proportion of the males showed willingness to mate.

The records for 101 males paired with control females in the studies on pre-natal mortality (shortly to be described) were examined for details of reproductive performance. It was found that 101 males were capable of mating with, and causing fertilization of ova in, at least four females per month at the control density. It was notable that these males were from the same stock cage as those used in the groups in experiment B. Moreover, 101 males paired with control females in experiment B mated whenever these females showed oestrus, with few exceptions. Up to three matings per month were recorded.

The results suggest behavioural disturbances including decreased libido in 101 males in groups and also, perhaps, a failure of sperm production in some males.

Similar results might have been obtained with 101 mice in experiment A as, in two of the four groups, no females were pregnant by 93 and 118 days of age, respectively. However, the males were then replaced, as it was thought they

might be sterile (subsequently disproved) and the replacement males mated successfully.

No C57BL males failed to mate with, or to cause pregnancy in, grouped females in either experiment.

Incidence of Disease

An inflammation affecting the external genitalia of some C57BL males has been mentioned previously. This disease may have been contracted from other mice housed contemporarily in the same room, which suffered from the disease.

The clinical symptoms of the disease were that, following inflammation, sepsis developed in the prepuce and penis; the sepsis eventually spread to the adjacent intestines; gangrene and death followed in quick succession. The disease was not observed in any 101 males. None of the C57BL males in control pairs showed symptoms of the disease, but a large proportion of C57BL males in groups were fatally affected.

The C57BL groups were maintained for four months after completion of experiment A. During this time three of the four males died from the disease.

During experiment B, two of the eight C57BL males in groups became infected and died. These were replaced; the replacement males became infected and were killed. One of these groups was then exterminated, but the other was continued. A total of four males had succumbed to the disease in this group when a young male (51 days old) was introduced. This male showed initial symptoms of the disease soon after introduction, but recovered and mated successfully thereafter.

As males, thus diseased, were incapable of mating, data on subsequent mating (by males introduced later) and conception, for females nulliparous when the male became infected, have been excluded from the results.

The incidence of infantile diarrhoea did not appear to be greater in grouped mice than in controls.

No other diseases of any importance were recorded.

TABLE 21

Number[1] of corpora lutea in C57BL and 101 lines of mice at three ages and at two levels of population density.

Line	Age (months)	Density	Number of Mice	Number of Corpora Lutea	
				Mean ± S.E.	Variance
C57BL	3-4	Control	10	8.8 ± 0.36	1.29
		Grouped	9	7.0 ± 0.67	4.00
	6-9	Control	13	10.0 ± 0.39	2.00
		Grouped	15	9.1 ± 0.25	0.92
	9-12	Control	23	9.3 ± 0.20	0.95
		Grouped	22	8.7 ± 0.31	2.11
101	3-4	Control	13	9.6 ± 0.29	1.09
		Grouped	11	9.5 ± 0.39	1.67
	6-9	Control	18	10.5 ± 0.22	0.85
		Grouped	20	10.1 ± 0.18	0.68
	9-12	Control	27	9.4 ± 0.17	0.78
		Grouped	24	9.7 ± 0.20	1.00

Comparison of Levels of Density	Line	Age	Difference in Numbers of Corpora Lutea	Significance of Difference*
Control v Grouped	C57BL	3-4	1.80	$P < 0.001$
		6-9	0.93	$0.01 < P < 0.05$
		9-12	0.58	$0.05 < P < 0.1$
	101	3-4	0.07	$0.8 < P < 0.9$
		6-9	0.45	$0.2 < P < 0.3$
		9-12	- 0.34	$0.2 < P < 0.3$

[1] Includes data from mice autopsied at $3\frac{1}{2}$ and $18\frac{1}{2}$ days *post coitum*.

* Probabilities from t-Tests following analysis of variance.

Chapter IV

STUDIES ON PRE-NATAL MORTALITY

Introduction

To assess the extent of pre-natal mortality, mice were killed at $3\frac{1}{2}$ days or $18\frac{1}{2}$ days *post coitum* and observations were made on the numbers of ova produced and on the survival of embryos or foetuses.

The females used in these studies were of three ages, the ages being 3 to 4, 6 to 9 and 9 to 12 months, and these females are hereafter referred to as young, intermediate-aged and old, respectively.

It has already been mentioned that mating was often inhibited in groups of 101 mice. This problem was overcome by rotation of males between the groups and the stock cage, as it had been observed that mating usually occurred within 24 hours of introduction of 101 males to groups. 101 males capable of repeated mating in groups, at reasonable intervals of time, were used in groups for longer periods.

Number of Ovulations

Counts of the number of corpora lutea of similar appearance and, presumably, age were used to indicate the number of ova shed at the preceding oestrus. Although corpora lutea were more easily counted at $18\frac{1}{2}$ days *post coitum*, due to their larger size, there was good agreement between the counts of corpora lutea made at $3\frac{1}{2}$ and $18\frac{1}{2}$ days *post coitum*. Thus, for purposes of analysis, these data were pooled. Table 21 shows the number of corpora lutea in all treatments and the results of analysis of these data.

There were no statistical differences in numbers of corpora lutea either between lines or between ages, though there was a tendency towards higher counts in intermediate-aged females.

A relationship between the level of population density and numbers of

TABLE 22

Number and percentage[1] of ova recovered, and number and percentage[2] of viable ova, from C57BL and 101 lines of mice at three ages and at two levels of population density.

Line	Age (months)	Density	Number of Mice	Number of Ova	% Ova Recovered	Number of Viable Ova	% Ova Viable
C57BL	3-4	Control	5	8.4 ± 0.68	97.7	7.4 ± 0.51	88.1
		Grouped	4	6.0 ± 1.35	100.0	5.8 ± 1.60	95.8
	6-9	Control	7	9.6 ± 0.48	94.4	8.1 ± 0.80	85.1
		Grouped	7	8.6 ± 0.53	93.8	7.6 ± 0.69	88.3
	9-12	Control	6	8.2 ± 0.60	92.5	7.3 ± 0.61	89.8
		Grouped	12	8.0 ± 0.37	95.1	7.1 ± 0.42	88.5
101	3-4	Control	4	9.8 ± 0.75	100.0	9.0 ± 0.82	92.3
		Grouped	4	7.8 ± 0.48	81.6	7.3 ± 0.25	93.5
	6-9	Control	9	8.8 ± 0.49	87.8	8.6 ± 0.44	97.5
		Grouped	9	8.4 ± 0.29	82.6	8.1 ± 0.35	96.1
	9-12	Control	10	6.5 ± 0.91	69.2	5.7 ± 0.75	87.7
		Grouped	12	6.5 ± 0.86	65.0	4.8 ± 0.74	73.1

[1] Per cent of corpora lutea.

[2] Per cent of ova recovered.

corpora lutea was evident in C57BL but not in 101 mice. There were fewer corpora lutea in grouped females than in their isolated controls in C57BL mice at all ages. The differences showed a gradation from being close to statistical significance in old females to very high significance in young mice. In line 101, differences between the levels of population density varied in direction between ages and did not approach statistical significance.

Viable Ova

The percentages of ova recovered and the percentages of these ova that were viable, from mice of three ages, are shown in Table 22.

Percentage recovery of ova from C57BL mice was uniformly high but from 101 mice the percentage of ova recovered fell markedly with increasing age. Contraction of the uterus shortly after slaughter, which was apparently responsible for the poorer recovery of ova from line 101, has already been mentioned. It was observed that the uteri were most contractile in old 101 females. Moreover, as the older females were killed first and as the technique for counteracting uterine contractility improved during the course of these studies, recovery of ova from young 101 mice was more successful.

Viable ova were identified as those that had developed to the blastocyst or advanced morula stage at $3\frac{1}{2}$ days *post coitum*. The percentages of the recovered ova that were classified as viable are reasonably uniform for all the mice. There were no statistical differences between mice at the two levels of population density. The lower percentage of viable ova recovered from old 101 females, notably those in groups, was due to a high proportion of non-viable eggs in a few mice.

TABLE 23

Pre-implantation losses of ova in C57BL and 101 lines of mice at three ages and at two levels of population density.

Line	Age (months)	Density	Number of Mice	Number of Corpora Lutea	Number of Implantations	% Ova Not Implanted
C57BL	3-4	Control	5	9.0 ± 0.45	7.6 ± 0.87	15.6
		Grouped	5	7.8 ± 0.37	7.0 ± 0.32	10.3
	6-9	Control	6	9.8 ± 0.75	6.8 ± 0.87	30.5
		Grouped	8	9.0 ± 0.38	7.7 ± 0.84	13.9
	9-12	Control	16	9.5 ± 0.26	6.8 ± 0.58	28.9
		Grouped	10	9.1 ± 0.35	7.7 ± 0.62	15.4
101	3-4	Control	7	9.3 ± 0.29	6.9 ± 0.88	26.2
		Grouped	7	9.3 ± 0.47	7.3 ± 0.64	21.5
	6-9	Control	9	11.0 ± 0.29	7.6 ± 0.56	31.3
		Grouped	11	9.9 ± 0.28	6.9 ± 0.64	30.3
	9-12	Control	14	9.3 ± 0.24	5.9 ± 0.45	36.2
		Grouped	12	9.4 ± 0.34	5.9 ± 0.40	37.2

Pre-implantation Loss

The difference between the number of ova shed and the number of implantation sites at $18\frac{1}{2}$ days *post coitum* represented the pre-natal loss prior to implantation. Table 23 shows the numbers of implantation sites and the corresponding numbers of corpora lutea recorded at $18\frac{1}{2}$ days *post coitum*.

The data were transformed by taking square roots before analysis because of the small size of counts of implantation sites (Snedecor, 1956, p. 315). An analysis of covariance on the transformed data showed that there was a positive regression ($P < 0.1$) of number of implantation sites on number of corpora lutea. There were no statistical differences between levels of population density, between lines or between ages, thus showing that the regression of number of implantation sites on number of corpora lutea had accounted for most of the variation in the data.

However, Table 23 shows that the proportion of ova implanted tended to be higher in line C57BL, in young females, and in C57BL grouped mice compared to their controls.

TABLE 24

Number of intra-uterine resorption sites, at 18½ days *post coitum*, in C57BL and 101 lines of mice at three ages. (Data for levels of density pooled.)

Line	Age (months)	Number of Mice	Number of Resorption Sites		
			Mean ± S.E.	Variance	Transformed Mean[1]
C57BL	3-4	10	1.2 ± 0.20	0.40	1.47
	6-9	14	2.7 ± 0.30	1.30	1.91
	9-12	26	2.9 ± 0.29	2.19	1.94
101	3-4	14	1.1 ± 0.31	1.30	1.39
	6-9	20	1.8 ± 0.45	4.06	1.59
	9-12	26	1.4 ± 0.23	1.37	1.50

Comparison of Lines	Age	Difference in Transformed Means	Significance of Difference*
C57BL v 101	3-4	0.075	$0.6 < P < 0.7$
	6-9	0.311	$0.05 < P < 0.1$
	9-12	0.434	$0.001 < P < 0.01$

[1] Using $\sqrt{x + 1}$ transformation, where x = number of resorption sites.

* Probabilities from t-Tests following analysis of variance.

Post-implantation Loss

The difference between the number of implantation sites and the number of live foetuses found at $18\frac{1}{2}$ days post coitum was taken as a measure of loss occurring after implantation. Observation of the contents of the uterus at $18\frac{1}{2}$ days post coitum showed that post-implantation loss was represented by the presence of implantation sites at which embryos had been, or were being, resorbed (i.e. resorption sites) and also by dead foetuses (the latter representing the most recent pre-natal loss). The numbers of dead foetuses recorded were negligible.

The numbers of resorption sites at all ages of both lines of mice are shown in Table 24. The data were first transformed by using valves for $\sqrt{x + 1}$ (where x = number of resorption sites) because of the small size of counts of resorption sites (Snedecor, 1956, p. 315). Analysis of variance of the transformed data revealed no statistical difference between levels of population density. These data have, therefore, been combined for presentation in Table 24. Differences between ages were not significant, but there was significantly increased resorption of implanted embryos in old C57BL females compared to 101 females of the same age. Comparison of the lines within other ages showed that greater resorption in line C57BL approached significance in intermediate-aged females, but was not significant in young mice.

TABLE 25

Number of live foetuses *in utero*, at $18\frac{1}{2}$ days *post coitum*, in C57BL and 101 lines of mice at three ages. (Data for lines and levels of density pooled.)

Age (months)	Number of Mice	Number of Live Foetuses	Significance of Difference*
3-4	24	6.0 ± 0.40	
			$0.05 < P < 0.1$
6-9	34	5.0 ± 0.39	
			$0.05 < P < 0.1$
9-12	52	4.3 ± 0.23	

* Probabilities from t-Tests following analysis of variance.

Live Foetuses

The numbers of live foetuses at 18½ days *post coitum* are presented in Table 25. Analysis of variance of the data revealed that variation due to lines and to levels of population density was not statistically significant. Thus these data have been pooled for presentation in Table 25.

The major part of variation in the data was associated with ages of the mice. There were more live foetuses per pregnancy in young than in intermediate-aged females, and likewise in intermediate-aged than in old females. Those differences approached statistical significance, whereas the difference between young and old females was significant ($P < 0.02$).

Examination of data already presented on pre-natal loss shows that the differences in numbers of live foetuses associated with increasing age in both lines of mice have arisen due to pre-implantation losses (Table 23) and post-implantation losses (Table 24).

Chapter V

DISCUSSION

These studies were undertaken to determine some effects of increased population density on reproduction in two inbred lines of mice in laboratory populations of fixed size. The two lines selected for study showed certain differences in behaviour that are of interest in view of the quantitatively different responses of mice of these lines to increased population density.

Animals of the C57BL line were much more aggressive than those of line 101. Observations on mice kept in cages showed that fighting was more prevalent between C57BL males than between 101 males. In cages of 101 males fighting essentially ceased within 24 hours of introduction of strange males; none of the animals were scarred; and the death rate was higher than that of C57BL males in cages, but was not apparently due to fighting. In cages containing C57BL males fighting was frequent, more so where "non-virgin" males were present; many, and sometimes all, of the mice were scarred; and deaths were few, although those which did occur were often a direct result of fighting. In the case of females in cages and in groups, fighting was apparently very rare, but C57BL females in cages were frequently scarred at the base of the tail due to "shepherding" by one or more dominant animals.

It is suggested that dominance and subordinance were more clearly defined in line 101. Thus fighting was not important as a means to maintain any particular animal in a position of relative dominance. On the other hand, mice of the C57BL line may have been of more even social rank; consequently, fighting, to maintain or to secure a position in the social hierarchy, was more frequent. With such a distinct social hierarchy, variability of reproductive criteria might be greater within line 101 than within line C57BL when the animals were placed in groups, and the effects of grouping might be more severe, as has been shown.

Therefore, 101 mice appear to have been more stressed than C57BL mice when subjected to increased population density.

Fighting in laboratory populations of fixed size, in relation to adrenal weights and stress, has been discussed previously and it was concluded that no constant relationship existed between fighting and stress. Christian (1963) has stated that the apparently contradictory results with relation to fighting and dominance in no way invalidate the conclusions that purely psychological social pressures are responsible for stimulating increased adrenocortical and decreased reproductive activity in groups of mice and rats, and that fighting *per se* has little or no effect on the adrenal hypertrophy observed in groups of animals.

However, Bronson and Eleftheriou (1963) have compared adrenal responses to crowding in deermice (*Peromyscus maniculatus bairdii*) and the C57BL/10J strain of house mice. The latter mice have been found to be far more aggressive than deermice (King, 1957), and Bronson and Eleftheriou showed that the adrenal response to crowding was evident in C57BL mice but that there was no significant change in the indices of adrenal function relative to density in deermice.

These findings appear to contradict the suggestion that 101 mice were more stressed than C57BL mice when grouped, as the aggressiveness of 101 animals is perhaps comparable to that of deermice in relation to the aggressiveness of mice of the C57BL strain. However, the comparison made by Bronson and Eleftheriou refers to mice of two different species which may respond differently to crowding. Moreover, all mice in their experiments were males, and responses may differ in populations of mixed sex: for instance, these authors observed that female deermice with young were as aggressive as trained "fighter" mice of either species.

The statements of Bronson and Eleftheriou throw some doubt on the suggestion that 101 mice were more stressed than C57BL mice when crowded,

where the populations were unisexual, but they do not necessarily invalidate such a suggestion where the populations were of mixed sex. In view of this, comparisons of adrenal responses to crowding in 101 and C57BL mice from populations of mixed sex would be worthy of investigation.

Variability of Results

Greater variation in some reproductive characteristics occurred with grouped mice than with their controls, e.g. age at first oestrus, interval between first oestrus and first mating, and age at first conception. Moreover, the variances of these data were proportional to the corresponding means. This necessitated transformation of the data for their valid interpretation. With results for age at first oestrus, transformation of the data did not alter the significance of differences as determined by analysis of variance, but care should be taken in accepting marginal significances of difference where no transformation has been made.

The observed variability of reproductive responses to increased population density is of interest in view of the findings of Welch and Klopfer (1961). These authors studied adrenal responses to crowding of male white Swiss mice. The variance, as well as the mean, of adrenal weight increased markedly with increase in population size. The nonparametric rank correlation of variance with population density was significant. Moreover, there was an increasingly skewed distribution in the direction of larger adrenals as population size increased, due mainly to extremely deviant individuals. Even after censoring the deviant individuals, however, the mean adrenal weight of the remaining animals still showed a significant increase with population size, although virtually all of the increase in variance was accounted for by such censoring.

Relationships between adrenal weight, adrenocortical activity and reproductive function have been discussed earlier. Assuming the existence of such relationships, the results of the present study are in agreement

with the findings of Welch and Klopfer. Thus, for example, consideration of the data for age at first conception shows that mean and variance were greater in grouped mice than in their controls. Furthermore, the greater variance in grouped mice was mainly due to a higher proportion of extremely deviant individuals in the groups than in the controls.

The importance of relative position of dominance or subordinance in the social hierarchy of grouped animals should be noted in connection with endocrine variability. Welch and Klopfer (1961) and others, as mentioned previously, have observed an inverse relationship between adrenal weight and social rank, and a direct relationship between reproductive function and social rank. Christian (1963) has comprehensively discussed these relationships. From the statements of these authors, it would appear reasonably certain that social pressures related to social rank are the major factors causing greater variability of adrenal weight and of reproductive function in grouped mice.

Weaning

Females at weaning in experiment B were heavier than in experiment A. This may have been because of improved husbandry methods in rearing pups to weaning for use in experiment B. For example, a supplementary loose feed mixture (buttermilk powder and barley meal) was fed to litters that appeared unthrifty, and stunted pups were culled. Such methods were not used in rearing pups to weaning for experiment A.

Moreover, in line C57BL, the males used in experiment B were older and more aggressive than those used in experiment A. When paired or grouped with females, fighting occurred almost invariably in experiment B and some females were killed or were fatally injured, but comparatively few such deaths occurred in experiment A. Animals killed thus, which have been excluded as regards data on weight at weaning, were found to be lighter than average in weight. This factor was also responsible for raising the mean weight of C57BL females

weaned for experiment B.

It is important to stress that, although females were heavier at weaning in experiment B than in experiment A, and although 101 mice were heavier than C57BL mice, there were no differences between the weights (at weaning) of females subjected to the control and the grouped density in either line or in either experiment. Thus comparisons of control and grouped mice are not biased by initial advantages conferred on females at either level of population density.

It has been shown that data on reproductive performance, at two levels of population density, of 101 mice in experiment B were in good agreement with those data for this line in experiment A. However, there were differences between the two experiments in the responses of C57BL mice to increased population density. The data on line C57BL indicated a greater response to increased population density in experiment B than in experiment A, and it has been suggested that this may have been due to the lower weight at weaning of C57BL females in experiment A. These weanlings tended to have thin coats due to poor thrift. When placed in groups they were able to conserve body heat by huddling together, whereas control females could not do this.

As a result of failure to conserve warmth, the control females possibly reacted by increased thyroid activity. Exposure to low temperatures results in increased release of thyroid hormone (Brown-Grant, Von Euler, Harris and Reichlin, 1954; Woods and Carlson, 1956). Furthermore, there are relationships between the thyroid gland and the reproductive system (Kraatz, 1939; Maqsood and Reineke, 1950; Hess, 1953; Denison and Zarrow, 1955; Bruce and Sloviter, 1957). Christian (1963) observes that results reported in the literature seem to indicate an optimal level of thyroid activity for proper functioning of the reproductive system. Any appreciable deviation from this level in either direction apparently leads to diminished reproductive function.

It seems likely, therefore, that maturation of control C57BL females in

experiment A may have been delayed partly because of their inability to conserve body heat. This may have resulted in increased thyroid activity and, hence, suppression of reproductive function.

On the other hand, two of the four groups of C57BL mice in experiment A were given supplementary feed immediately after the females were weaned, because of the low weight of some of the mice. This may have caused earlier maturation of females in these groups.

On the basis of these considerations, it is suggested that environmental conditions were biased in favour of grouped C57BL females, compared to their controls, in experiment A, and that the results from experiment B give a more accurate indication of the response of C57BL mice to increased population density.

Puberty

These studies did not reveal an effect of increased population density on age at puberty, as indicated by age at vaginal opening, in either line of mice. Vaginal opening is used as an index of puberty in mice because it is generally followed immediately by oestrous cycles, which are accompanied by ovulation - the ultimate criterion of puberty in females. However, it was observed during the course of experiments A and B that, after vaginal opening, complete closure of the vaginal orifice occurred in some females, notably in line C57BL. Moreover, no further changes (such as would indicate oestrous cycles) occurred for some time in the condition of the vaginal orifice of certain other females. Thus some time elapsed between vaginal opening and first oestrus: the interval was least for 101 control females; greater for C57BL controls and for C57BL grouped mice in experiment A; and greatest for 101 groups and for C57BL groups in experiment B. If it is considered that first oestrus indicates attainment of puberty, then data on age at vaginal opening should be ignored.

Laurie (1946) states that the condition of the vaginal orifice alone,

whether perforate or imperforate, is not a reliable criterion of ovulatory activity. Parkes (1925) found an interval of seven days between vaginal perforation and the first ovulation. However, Southwick (1955a) and Crowcroft and Rowe (1957) found a high correlation between the condition of the vaginal orifice and ovulation as determined at autopsy. Crowcroft and Rowe state that at post-mortem examination of anoestrous mice, in which the vulva was usually partly or completely sealed, most females were found to have thread-like uteri and to lack corpora lutea.

Therefore, age at first oestrus appears to be a more suitable criterion for determining puberty than age at vaginal opening. Vaginal opening may occur by physical means, may not be accompanied by ovulation and, as shown in the present studies, may be followed by a period of anoestrus.

Hence, as indicated by first oestrus, females in groups reached puberty later than their controls in all comparisons except within line C57BL in experiment A.

This result agrees with the findings of Kalela (1957) in his study of subarctic populations of voles (Clethrionomys rufocanus). Southwick (1955a) and Crowcroft and Rowe (1957) reported a high proportion of non-ovulating females at higher densities in their freely-growing, confined populations of wild house mice. This may have been partly due to delayed puberty in younger females in their populations.

Andervont (1944) has reported that oestrous cycles occurred at an earlier age in segregated mice than in their littermates placed in groups of eight each. However, these females were grouped without males. Whitten (1959) and Lamond (1959) observed that female mice entered into anoestrus when caged in groups, but that oestrous cycles promptly resumed when a male was introduced into each cage. Thus the delay in onset of oestrous cycles observed by Andervont may not have occurred had each group of mice contained a male.

It was of particular interest that logarithmic transformation of the

data on age at first oestrus did not alter the statistical significance of the differences between control and grouped mice. Evidently, even when the relative variability of this criterion at the higher level of population density had been considerably reduced, grouped females were still significantly older than their controls at first oestrus (with the exception of line C57BL in experiment A).

Mating and Conception

Age at first mating and at first conception are determined by factors affecting both males and females. Before either can occur, the essential requirement is that the female be oestrous. It is of equal importance that the male be capable of copulation. Hence, the following discussion refers to the results for first mating and first conception in relation to male reproductive performance.

It has already been shown that puberty was delayed in most grouped females. However, the delay in onset of first oestrous cycles did not account entirely for the much greater age of most grouped females, compared to their controls, at first conception. The first full-term pregnancy of control mice of both lines commenced, on average, within two weeks of puberty, whereas that of grouped females commenced, on average, more than two weeks after puberty in line C57BL and more than six weeks after puberty in line 101.

The longer interval from first oestrus to first conception in most grouped females, compared to their controls, was due partly to a delay of first mating, and partly to a decreased proportion of full-term pregnancies from first matings.

The lower proportion of full-term pregnancies from first matings in grouped mice was not due to abortions. Only one case of abortion was observed; that being in a grouped 101 female. The cause may, however, have been an increased incidence of failure of implantation of ova, or of

resorption of complete litters in early pregnancy, in grouped females, as suggested by Christian and LeMunyan (1958).

The delay of first mating appears to have been due to inhibition of mating in grouped mice. It has been suggested previously that there may have been behavioural disturbances, including decreased libido, in males in the groups. There is also the possibility that females may have suffered behavioural disturbances. For example, subordinate females may have been unwilling to mate or may have been prevented from mating by dominant females. As the interval from first oestrus to first mating was not significantly different between control and grouped C57BL mice, these considerations are mainly applicable to line 101.

A further consideration with 101 mice is that some males may have become infertile when placed with grouped females. Southwick (1955a) found a high correlation between fertility as determined by scrotal testes and fertility as determined by macroscopic visibility of tubules of the cauda epididymis (Laurie, 1946). Using scrotal testes as the criterion of fertility, Southwick observed that fewer males were fertile at higher levels of population density in his freely-growing, confined populations of house mice. No data were collected on presence of scrotal testes in males in the present study, but it seems possible that 101 males which did not mate, when placed with groups of females, may have become temporarily infertile. This would explain the consistent observation that many 101 males were eager to mate upon introduction to groups of females, but later apparently became impotent. It would also appear that, if these males were in fact temporarily infertile, fertility quickly returned when the males were paired with isolated females.

Christian and LeMunyan (1958) observed that male mice were fertile within a few days of being removed from crowded cages containing mice of both sexes. No females became grossly pregnant in the crowded cages. The authors attribute this to failure of reproductive processes in the females,

but they do not present evidence that mating actually occurred. It is possible that matings did not occur or were infrequent, and that there was a failure of reproductive processes in males as well as in females in the crowded cages.

Evidently all C57BL males in groups remained fertile as all were capable of successful mating with grouped females, even after being present in the groups for some time.

However, such suggestions should be considered with reservations. Christian (1963) has observed that the reproductive competence of males has not been examined in detail for any species from freely-growing populations other than house mice; and no studies have adequately explored the problem. The criteria of fecundity (fertility) used are poor indicators of relative fertility and do not provide information on subtle changes in fertility. He concludes that the ability of males to fertilize females with respect to population density has not been investigated.

As a result of delayed puberty in females, inhibition of mating and decreased conception rate to first mating, the onset of first full-term pregnancy was considerably delayed in most grouped mice. This was also observed by Christian and LeMunyan (1958). The delay was greater in 101 grouped mice than in those of line C57BL. C57BL females in experiment A responded to grouping by a decreased conception rate to first mating, but there was no response in terms of age at first conception.

Gestation Period

Dickson (1964) has reported a delay in implantation of fertilized ova, following superovulation, in mice subjected to mild crowding. This suggested that there might be a lengthening of the gestation period at the higher level of population density in the present study, although in Dickson's experiments the superovulation procedure itself may have been the cause of the observed effects. However, there was no indication of the first gestation period

being prolonged in grouped females, compared to controls, in the present study.

The duration of first gestation within the C57BL line agrees with that noted by Snell (1941).

Size of First Litter

Evidence for decreased first litter size in grouped mice in these experiments was not conclusive. While there may have been such an effect in line 101, it should also be noted that there was a good (negative) correlation between size of first litter, as first recorded, and the degree of mortality of pups immediately post partum. Thus 101 controls in both experiments had both greatest first litter size and least post-natal mortality compared to 101 grouped mice and to both control and grouped C57BL mice. With greater post-natal mortality more pups may have been devoured before the litters were recorded. Thus the probability that pups would die and be eaten before recording of the litter was greater for grouped 101 mice and all C57BL animals, and least for 101 controls.

The literature records decreased litter size in house mice and in deermice subjected to crowded conditions in the laboratory, but not in natural populations of rats and voles (Clethrionomys rufocanus) at high levels of density. There are several further reports of diminished birth rate in confined laboratory populations of house mice and voles (Clarke, 1955; Crowcroft and Rowe, 1957; Christian, 1959c, 1961).

A further consideration, with regard to first litter size in line 101, is that grouped females were older than control females at onset of first full-term pregnancy. The difference in ages is the equivalent of two to three gestation periods. First litter size increased with age of the dam, where dams were young, in rats (Asdell, Bogart and Sperling, 1941); and litter size increased initially with successive pregnancies in mice (Biggers, Finn and McLaren, 1962), though this may have been due to the effects of

pregnancy rather than of age.

Therefore, it seems possible that grouped 101 mice in the present study might have had smaller litters had they become parous at the same age as their controls. This would have increased the difference between first litter sizes at the two levels of population density. To obtain more accurate information on the difference in first litter size, control 101 females should have been paired with males at a later age, so that their first litters would have been born at the same average age as that at which first litters were born to grouped 101 females.

The difference in ages of C57BL grouped and control females, at onset of first full-term pregnancy, was not great and is unlikely to have affected the relative size of first litters.

Weights of Pups

It has been found that pups born to grouped females were lighter than pups born to control females both on the day of birth and on the following day. The difference in weights was greater at one day post partum, which indicates that mothering of pups born to grouped mice was deficient.

The importance of being cautious in attributing the lower weight, on the day of birth, of pups born to grouped mothers to pre-natal factors has been stressed. It would have been necessary to weigh all pups immediately after birth in order to obtain conclusive evidence that the intra-uterine environment was responsible for any differences in weights of pups.

Christian and LeMunyan (1958) found that there was no significant difference between the mean weights, at birth, of pups born to crowded and isolated mothers. Moreover, in these experiments the crowded mice were segregated into isolated pairs before the litters were born. Thus the post-natal environment was similar for all pups, irrespective of whether they were born to isolated or to (previously) crowded mothers.

There is a good (negative) correlation between the weights of live pups

at one day post partum and the degree of post-natal mortality. Hence there was greater post-natal mortality in litters born to grouped females than in those born to control mothers; and 101 pups had a higher rate of survival than C57BL pups.

An indication of lactational performance of the dam is given by the weights of pups at 21 days post partum. Most of the variation in pup weight at this age is due to maternal performance, but a small proportion may be due to other factors, as pups begin eating solid food before 21 days of age. In line 101, pups reared by grouped mothers were lighter than pups reared by control females at 21 days post partum. Christian and LeMunyan (1958) also reported similar findings. Thus diminished lactation is indicated in 101 mice subjected to increased population density.

Mortality of Pups between Birth and Weaning

Mortality in pups born to grouped mice was considerably greater than in pups of control females. This effect was due to an increased incidence of total litter extinction, and to greater mortality of pups within surviving litters, in grouped mice.

Although there is evidence for diminished lactation in 101 females in response to grouping, it is most unlikely that this was the main cause of the high incidence of total litter extinction in litters born to grouped mice. The primary causes of complete loss of litters appear to be interference by other mice, communal nesting and behavioural disturbances. The severity of pup mortality in grouped mice suggests that this cannot be due to interference and communal nesting alone, and that deficient maternal behaviour must also be involved.

There are several reports of high litter mortality in rodent populations of high density (Strecker and Emlen, 1953; Brown, 1953; Clarke, 1955; Southwick, 1955a, b; Christian, 1956a; Louch, 1956; and Rowe, Taylor and Chudley, 1964). Decreased litter survival has been attributed to diminished

lactation (Christian, 1956a; Christian and LeMunyan, 1958) and to behavioural factors (Brown, 1953; Strecker and Emlen, 1953; Southwick, 1955b) in dense populations of house mice and voles. However, the problem of determining whether post-natal litter mortality is due either to inhibition of lactation, changes in maternal behaviour, or interference, or to combinations of these factors, requires investigation in greater detail (Christian, 1963).

Social rank may be involved in pup mortality in groups. It seems probable that total litter extinction applies mainly to litters born to subordinate females, whereas litters born to dominant females may suffer only partial mortality. This suggestion is supported by Calhoun (1949, 1950, 1962) who has proposed that socially subordinate rats, in freely growing confined populations, were incapable of raising their young because of physiological and psychological disturbances.

Aberrant maternal factors have been noted in C57BL mice at the control level of population density. This may partly explain the greater mortality of pups born to C57BL grouped mice compared to the mortality of pups born to 101 grouped females; and may also explain the low weight at weaning, associated with a slow initial growth rate, of C57BL females used in experiments A and B.

Unlike most of the other reproductive criteria examined in this study, the data on litter mortality showed that C57BL mice were affected by increased population density to a greater degree than were 101 mice.

Pre-natal Mortality

The mice used in the studies on pre-natal mortality had been kept in cages (sexes segregated) for up to 10 months before experimental pairs and groups were set up. The numbers of mice in these cages were sufficient to constitute crowding at a density similar to that which obtained for the mice studied by Christian and LeMunyan (1958). As previously mentioned, these

authors observed that effects of crowding persisted for some time after segregation of the mice into isolated pairs. The magnitude of the response to increased population density is apparently greater in populations of mixed sex than in unisexual populations (Christian, 1960). Although the mice studied by Christian and LeMunyan, which were in populations of mixed sex when crowded, may have been affected more by the period of crowding that the mice used in the present study, it seems likely that these latter mice must also have been adversely affected. The subsequent effects of crowding may have served primarily to reduce differences in response between control and grouped mice. Thus it seems unlikely that results of the studies on pre-natal mortality give an accurate indication of responses of 101 and C57BL mice to increased population density.

The ability to show statistically significant differences between mice at the two levels of population density was further limited by the small numbers of animals in these studies. This limitation was accentuated by the variation due to ages of the mice, which necessitated classification of the data according to age as well as to level of population density.

There were fewer corpora lutea per pregnancy in grouped females than in controls in line C57BL but not in line 101. Other studies give indirect evidence that numbers of corpora lutea are reduced in response to increased population density: crowding of mice may result in increased secretion of adrenal androgens (Christian, 1959c, 1960), and adrenal androgens can cause inhibition of formation of corpora lutea (Varon and Christian, 1963). This evidence is not entirely satisfactory, however, as certain doses of some adrenal androgens caused increased formation of corpora lutea in mice (Varon and Christian, 1963), and grouping of female mice resulted in an increase in the number of corpora lutea (Mody and Christian, 1962).

It should be noted that corpora lutea were counted with the naked eye in the present study, and not by microscopic examination of stained sections, as

done by the authors mentioned above. Thus it is possible that fewer corpora lutea were counted in grouped C57BL females than in their controls because of poorer development of corpora lutea in the grouped mice. If so, this would mean that the number of ovulations was not greater in control females than in those in groups, and this would account for the higher proportion of viable ova and of implanted ova in grouped C57BL females than in the C57BL controls.

These studies did not reveal any effects of increased population density on the proportion of ova that were viable, on pre-implantation loss of ova or on the number of resorbing embryos per pregnancy. An increase in the number of resorbing embryos per pregnancy with increased population density has been reported by several investigators, as mentioned previously.

Data on the number of live foetuses per pregnancy did not show a significant difference between levels of population density, but there was a significant decrease between the young and the old females. Decline of litter size with ageing of mice has been reported in the literature (Sugiyama, 1961; Roman and Strong, 1962; Finn, 1963), and has been observed to be greater where the aged females had been kept virgin (Nishimura and Shikata, 1960).

Although pre-implantation losses of ova tended to be greater as the mice aged, the number of implantation sites was more or less constant for all ages of mice (except in old 101 females which had slightly fewer). This result agrees with the findings of Finn (1962). The decline of litter size with increasing age of the females was due almost entirely to increased embryonic resorption, as also observed by Nishimura and Shikata (1960), Roman and Strong (1962), and Finn (1962, 1963).

Incidence of Disease

The occurrence of an apparently infectious disease affecting the external genitalia of male mice has been noted. The disease was not observed in 101 males which may have been due to inherent resistance to this disease in

particular, or to disease in general, in line 101. The greater susceptibility of grouped C57BL males to the disease may have been the result of their environment, as boxes containing groups of mice became soiled more quickly than boxes containing pairs. There is also the possibility that grouped C57BL mice had decreased resistance to the disease compared to C57BL controls. Such an explanation is in agreement with reports of depressed inflammatory response in grouped mice, as noted previously.

The infectiousness of the disease is indicated by the occurrence of inflammation and sepsis in the vagina of a female following mating by a male which then showed initial symptoms of the disease. Resistance to the disease appears to have decreased with increasing age of males.

Chapter VI

SUMMARY and CONCLUSIONS

The reproductive performance of two inbred lines of mice (strains 101 and C57BL), maintained in boxes at population densities of 1 male with 1 female (control) or 1 male with 5 females (grouped), was studied.

In two experiments, females were paired or grouped at weaning with males of greater age. Data on age and weight of females were collected at puberty, mating and conception. Further observations were made on the incidence of pseudopregnancies, success of first mating, length of gestation period, size of first litter, survival of pups and weights of pups from birth to weaning. Only data which refer to first full-term pregnancy and first litter have been presented. In a third experiment, the extent of pre-natal mortality in control and grouped mice was assessed. Nulliparous females of various ages were autopsied at $3\frac{1}{2}$ and $18\frac{1}{2}$ days *post coitum*. Data were collected on numbers of corpora lutea, viable ova, implantation sites, resorption sites and live foetuses.

Vaginal opening, as an indication of puberty, was considered to be of doubtful validity in these mice because oestrous cycles did not always commence at this stage. First oestrus has, therefore, been the indicator of puberty. A significant delay of puberty was found in females of both lines when subjected to grouping. The delay was greater in 101 than in C57BL mice. Following puberty, grouped mice of line 101 were less inclined to mate than control mice of this line. Further investigation of inhibition of mating in line 101, in response to increased population density, indicated that deficient reproductive ability of the males was the main cause. Complete suppression of effective reproductive activity apparently occurred in some 101 males at the grouped density. Results on readiness to mate following puberty in mice of line C57BL at the two levels of population density were

inconclusive.

Fewer, but not significantly fewer, full-term pregnancies resulted from first matings of grouped females than of their controls in both lines. Pseudopregnancies were found to be neither of greater frequency nor of longer duration in grouped mice than in controls of either line.

First conception was regarded as that which resulted in the first full-term pregnancy. Grouped mice of both lines were older than their controls at first conception, notably in line 101. The overall difference in ages of control and grouped 101 females at first conception cannot be assessed as, at completion of the studies, a significantly lower proportion of grouped than of control 101 females had conceived. Such a difference did not occur in line C57BL. Delay of first conception in grouped 101 mice was largely due to inhibition of mating.

There was no effect of population density on weights of mice at first oestrus, first mating and first conception.

No differences were revealed between control and grouped females of either line in terms of the length of the first gestation period.

Although the sizes of first litters of control 101 mice were larger than those of grouped 101 mice, this difference should be regarded with caution as there is a good reciprocal correlation between size of first litters and the degree of post-natal mortality of pups. Dead pups may have been eaten before first litters were recorded. The sizes of first litters of C57BL mice at two levels of population density did not differ.

Pups born to control females were heavier than pups born to grouped mothers in both lines, both on the day of birth and the following day. It has been emphasized that differences in weights of pups on the day of birth may have been due to greater suckling of pups by control mothers, rather than to differences in the intra-uterine environment. The greater disparity in pup weights on the second day was probably due to effects of litter abandonment.

Differences in weights of 101 pups at 21 days post partum reflected the initial differences in weights. Data were limited because of high pup mortality.

Pup mortality was a major factor limiting the reproductive rate of grouped mice of both lines. Increased mortality in response to grouping occurred both in terms of increased total litter extinction and of increased mortality within surviving litters. Interference by other mice, diminished lactation and deficient maternal behaviour were considered contributing factors to increased mortality of pups born to grouped mice. Maternal performance of C57BL females was poorer than that of 101 females at both levels of population density.

Consideration of variation in the data on certain reproductive criteria suggested that social hierarchies were formed in groups of females, with reproductive performance being related to rank. It was postulated that social hierarchies were more distinct and stable in line 101 than in line C57BL. This could account for the greater variance of certain reproductive responses to increased population density in 101 mice than in C57BL animals. A further suggestion was made that 101 mice were more stressed than C57BL mice when subjected to crowding in populations of mixed sex. This would account for the greater response to grouping of 101 mice in terms of certain reproductive criteria.

No marked differences due to population density were found in the numbers of viable ova, implantation sites, resorption sites or live foetuses at autopsy. The validity of results obtained on pre-natal mortality, in the mice which were available for this investigation, is questioned owing to possible carry-over effects of the pre-experimental environment. These effects may have precluded differences in responses of the mice when subjected to the two levels of population density. The only statistically significant difference revealed between control and grouped mice was in the number of corpora lutea

in line C57BL, the number being greater in control females.

A disease, apparently infectious, affecting the external genitalia of males of line C57BL, was found to occur only at the higher level of population density. Susceptibility to the disease appeared to increase with age.

Studies on some problems which have arisen from the present investigation include:

1. Examination of adrenal responses to crowding of mice of these two lines would give an indication of the relative degree of stress generated by increased population density. Comparison of the two lines of mice could be done both for unisexual and bisexual populations, but preferably the latter.
2. Determination of onset of ovulation in relation to vaginal opening and first oestrus at both levels of population density. This may reveal a suitable externally-detectable criterion for determination of age at puberty.
3. Investigation of the duration and frequency of oestrous cycles in these mice at the two levels of population density may reveal that delays of first mating and first conception in mice at the higher level of density are partly due to disturbances of oestrous cycles. Vaginal smears should be taken to determine accurately the phases of oestrous cycles.
4. Fertility of males in relation to population density has not been sufficiently investigated. The suggestion that some 101 males became infertile when placed with groups of females could be examined.
5. The effect of increased population density on litter size could be further elucidated, particularly in line 101. Sizes of first litters of 101 females in groups should be compared with those of control 101 females mated at such a time that first litters are born at the same mean age as those of the grouped females. This would reduce any effects due to age of the dam.

Actual observation of births would be of great value in assessing the true sizes of litters and the proportion of stillborn pups.

6. Pre-natal mortality should be investigated using females that are paired or grouped with males at weaning, and not using females that are accumulated in cages beforehand. Larger numbers than those used in the present study may be necessary to reveal statistically significant differences.

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APPENDIX

Incidence of Tapeworms (*Hymenolepis nana*)

Further to examination of the reproductive tract at autopsy, the intestines were examined for tapeworms. The parasites were found almost exclusively in the posterior part of the small intestine. Of 34 C57BL females examined, only one was found to have tapeworms, whereas 19 per cent of the 47 females of line 101 examined were parasitised.

Tapeworms were found most frequently in young females. As a large proportion of 101 females examined was young, the percentage occurrence of tapeworms quoted for this line, as indicating a general sample of the population, may be exaggerated. There was no evidence of differences between levels of population density of the mice in the incidence of tapeworms.