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The effects of delayed mating on components of fitness, life span, and the geotactic behavior of *Drosophila melanogaster* selected for a postponed senescence

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THE EFFECTS OF DELAYED MATING ON COMPONENTS OF FITNESS, LIFE SPAN, AND THE GEOTACTIC BEHAVIOR OF *DROSOPHILA MELANOGASTER* SELECTED FOR A POSTPONED SENESCENCE.

by

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Abstract

Two strains of Drosophila melanogaster selected for late reproduction and two control strains were studied. All strains were descended from the same ancestral strain.

The following life-history characters or components of fitness were studied in all four strains: fertile matings, mean fecundity, total fecundity, total fertility, and mean fertility proportions. Flies were stored as virgins and tested (sets of 30 females and 30 males of the same age post eclosion, two replicates per strain and age) at ages: 1, 2, 4, 7, 14, 21, 28, and 42 days post-eclosion. The number of eggs, the number of female offspring, and the number of male offspring were counted for three successive days, starting on each of the days indicated above. This experiment provided data on 1,920 females.

The same life-history characters were obtained for the offspring of "old" (42 days post-eclosion) virgin female flies mated to "young" (7 days post-eclosion) virgin male flies and vice versa. These data, combined with the data from the offspring of matings of virgin parents of equal ages, 7 and 7 days post-eclosion, provided data for a comparison of the effect of different virgin female and male parental ages. This experiment provided data on 480 females.

Survivorship distributions of virgins were obtained for populations of virgin flies of both sexes from these strains of D. melanogaster.

Age-dependent behavioral loss of negative geotactic response in virgin female and male virgin flies from the same four strains was also studied. This experiment was done in the dark on an inclined plane raised to 0, 10, 20,30, 40, 50, 60, 70, and 80 degrees in three different orders of presentation. Fly populations of 300 flies per strain and sex were collected. Two groups of 20 flies of each strain and sex, chosen by a randomization procedure, were tested at 0, 3, 7,14, 21, 28, 35, 42, 49, 56, and 63 days post eclosion. All

populations were tested at all of the above ages for negative geotactic response to each angle and order of presentation. Survivorships were recorded for all 8 populations.

The life-history data combined with that for geotactic response changes in these aging populations provide information on how laboratory selection acts on the available genetic variation in D. melanogaster to determine its pattern of senescence. Further these data are used to test several hypotheses that have been used to explain senescence in populations. Data from these experiments were used to test the "rate of living theory", the "mutation accumulation", the "cost of reproduction", and the "antagonistic pleiotropy" hypotheses of aging theory.

The fecundity data from the equal virgin parental ages experiment supports the "antagonistic pleiotropy" hypothesis, but the fertility data does not. Young control females with shorter life spans had significantly higher mean fecundities than longer lived selection strain females. Conversely, at a late age longer lived selection strain females had significantly higher mean fecundities relative to control strain females.

Fertility data was consistent with the hypotheses of "mutation accumulation" and that "age of onset" genes. Survivorship data for long lived strains was consistent with the "rate of living" and the "cost of reproduction" hypotheses.

Analysis of the data from the unequal parental ages experiments supports the hypotheses of "age of onset" genes and the "cost of reproduction."

A comparison of negative geotactic responses for all strains and sexes was consistent with the "mutation accumulation" and "rate of living" hypotheses of aging. No strain or sex differences were detected at the two youngest test ages, but both sexes of the selected strains had greater mean negative geotactic responses at 35 days post eclosion.

Therefore it is concluded that fecundity, fertility, fertile mating in females, and the negative geotactic response of these strains are under the control of different sets of genes with different patterns of action over time in both sexes of the selected and the control strains.

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Chapter 1

Introduction and Background

Aging processes have been defined by Maynard Smith (1962) as "those which render aging individuals more susceptible as they grow older to the various factors, intrinsic or extrinsic, which may cause death." As Rose (1984) points out "the process of ageing is not just one of accumulated damage. An internal biological process of deterioration, known as senescence, is also involved." Medawar and Medawar (1983) define senescence as the "progressive deterioration of bodily faculties and performances that accompanies growing older" and maintain that it is the closing episode of development (ibid. p.5). As these authors point out it is difficult to distinguish between (1) "programmed" changes due to the developmental program and (2) the "cumulative sum of minute and individual trivial accidents or malfunctionings (ibid. p.6)."

In the 19th century Alfred Russell Wallace and August Weissmann (1891) considered senescence to be the consequence of some form of group selection. Individual organisms, in their view, were disposable members of a species and each was discarded by some sort of "death mechanism" to make room for new individuals, but as Rose (1984b) observes, in the wild few organisms survive long enough to die a senescent death.

Williams (1957), also referring to Weismann's concept of a death mechanism, made four strong points against this view: (1) it is a fallacy to equate senescence with mechanical wear and tear, (2) it is rare to find individuals in natural populations old enough to die from the action of such a "death mechanism," (3) several decades of research have not uncovered evidence of such a mechanism, and (4) it is quite difficult to visualize how natural selection could produce such a mechanism. Thus such a mechanism would be superfluous because it would be difficult for natural selection to mold such a mechanism when the majority of the organisms die from accidental causes before such a mechanism could cause the death of an organism.

In the 1940's, theories of aging emerged which incorporated the concepts of modern genetic and evolutionary thought. J.B.S. Haldane (1941) and P.B. Medawar (1942) proposed that special age-of-onset modifier genes somehow repress the action of deleterious genes,

those which reduce the Darwinian fitness of the individual, until organisms reach an advanced post-reproductive age in their life spans. If such modifiers are no longer active later in the life span of an individual organism, there is little or no effect on the fitness of a species, because the individuals have already survived and reproduced when the hypothesized modifiers become active. These authors hypothesized that in the evolution of senescence, natural selection increases or decreases the period of time over which these modifiers repress the deleterious action of other genes. The genes under the control of these modifiers are those with "deleterious" consequences. The consequences for the individual organism would include a senescent decline and a non-accidental death. Survival to later ages is not of great importance for many species since the reproductive period has passed and thus the death of the organism has no effect on the Darwinian fitness of the organism or its ability to survive and reproduce.

According to Medawar and Medawar (1983), there is "a savage exaction of mortality" in nature. When organisms are reared in a laboratory, they live long enough to show the phenotypic consequences of genes which are not expressed in the the natural environment of the species. In nature, natural selection has promoted the fixation of modifiers which prevent expression of the genes they modify at early ages. The question of what causes these modifiers to be turned off remains unanswered under this hypothesis.

As Rose (1984b, p. 17) has noted the genes that affect senescence could be of two kinds: (1) genes whose effects are restricted to the later ages and (2) genes with "effects both early and late, such multiple effects being referred to as 'pleiotropy'." The first type would be neutral in so far as their effect on fitness is concerned, because of the decline in the force of natural selection with increasing age. Such "senescence" genes could accumulate, over time, in a population or species. This hypothetical explanatory mechanism for senescence is now known as the "mutation accumulation" theory (Edney and Gill, 1968; Rose, 1980: Rose,1984b). In an evolving population these "late acting harmful genes can accumulate and intrinsic senescence can develop (Edeny and Gill, p.282)" when the various extrinsic causes of accidental death are removed. Haldane (1941) and later Medawar (1952) had, as was noted above, proposed that

the age of action of such "senescence" genes might be postponed by selection of modifier genes that postpone their effects.

According to the hypothesis of "mutation accumulation" two different populations, with different "senescence genes", will show the onset of changes in a fitness component at different times. The parameter for two populations may have the same mean values early in both of the populations. Late in life, significant differences in the mean values for the measured parameter will be found. The observed differences are assumed to occur when the age of "onset" allele(s) have their effects, resulting in lower mean values late in life (Rose, 1980, p.141).Initial levels for the parameter may be identical, but this is not a necessary condition of the hypothesis. Selection in different environments or niches is expected to "determine a longer or shorter specific longevity (Edney and Gill, 1968, p.282)."

G. C. Williams (1957) modified and extended this line of thinking when he proposed that the genes of the second kind mentioned above exist. The effects of these genes on the process of senescence might be reciprocal pleiotropic effects on the phenotype of an organism at different times in its life span. This hypothesis replaces the modifier concept and replaces it with that of age- of-action genes. The beneficial effects of such genes in the early life of the individual might well be offset by their detrimental consequences in later life. Two possible antithetical consequences of this sort of gene action are : (1) that youthful vigor is accompanied by an early senescence and a short life span, (2) a delayed senescence is possible at the cost of youthful vitality. This formulation of the evolutionary genetic origin of senescence is now known as the "antagonistic pleiotropy" hypothesis (Rose, 1983a, 1984a,b). Antagonistic pleiotropy is currently one of the favored hypotheses to explain the evolution of senescence in Drosophila (Rose, 1984; Luckinbill et al., 1984; Arking and Clare, 1985).

Williams (1957) presented four assumptions as the foundation for his theory of the evolution of senescence: (1) the soma is essential to reproductive success, but no part of it is passed on in either sexual or asexual reproduction, (2) natural selection of alternative alleles is operative in populations, (3) pleiotropic genes have opposing effects on fitness at different

ages and/or in different somatic environments and (4) the probability of reproduction for an individual decreases with increasing age. In this paper, one of the most frequently cited in the literature that incorporates genetic and evolutionary concepts to explain how senescence may have evolved, Williams set forth other ideas and made predictions, some of which have been corroborated by the results of Rose (1984) and Luckinbill et al. (1984), Clare and Luckinbill (1985) and Luckinbill and Clare (1985) in their artificial selection experiments for a postponed senescence in D. melanogaster.

Williams claimed that the entire reproductive probability of an individual will be altered by the effect of any gene after the onset of that effect, and that the action of natural selection will always be to oppose such effects, including ones that cause senescent change, because such effects are unfavorable from the standpoint of Darwinian theory. He also argued that selective forces increase the rate of senescence, because they favor organisms that are vigorous when young even if they are prone to deterioration later. He further stated that senescence "should always be a generalized deterioration, and never due largely to changes in a single system (ibid., p. 403) and thus "natural selection will always be in the greatest opposition to the decline of the most senescence prone system (ibid., p. 406)."

Williams' contribution to the development of the evolutionary theories of aging focuses on the importance of reproduction, and he regarded senescence to be "a consequence of the decline of the reproductive probability with advancing age (ibid., p.,408)." A consequence of Williams' theory is his prediction of a correlation of rapid individual development and a rapid individual senescence, commencing immediately after this rapid development. Thus Williams viewed senescence as one of the events of the developmental sequence.

One of the testable predictions of Williams' theoretical treatment is that a successful selection for increased longevity should be accompanied by an observable decrease in youthful vigor, specifically reproductive vigor. He also maintained that senescence is in some way dependent on "the rate of living" in the sense of Pearl et al. (1928). This is of considerable interest because Leffelaar and Grigliatti's (1984 a, b) data on age-dependent behavioral loss in

adult D. melanogaster support the "rate of living" hypothesis (Pearl,1928) for senescent decline. Their strains of flies were not selected for a postponed senescence, but they did isolate a mutant strain that showed a speeding up of the loss of behavioral responses and significantly shortened life span.

The predictions of the hypothesis of "antagonistic pleiotropy" are that (1) populations with short life spans have significantly higher mean levels of reproductive activity early in life relative to populations with longer life spans and (2) the shorter lived populations show a relatively rapid decline in reproductive activity at later age(s) when the longer lived populations show significantly greater mean levels of reproductive output. Another way of stating this hypothesis is that the reproductive schedule of the longer lived populations is shifted to later ages. This shift results in significantly higher mean values for fitness components at later ages relative to shorter lived populations.

W.D. Hamilton (1966) and Emlen (1970) further developed Williams' concepts and their consequences. Their fundamental concept is that the force of natural selection declines with age. In his mathematical model of the "moulding of senescence by natural selection " (Hamilton, 1966) examined the "consequences to fitness of several types of small age-specific effects on mortality." In this paper Hamilton claimed to show that mortality and senescence are inevitable outcomes of the evolutionary process. This model is an extension of the ideas of Williams (1957) and Medawar (1952,1955), but Hamilton showed mathematically, from a few assumptions, that evolution molds patterns of senescence in populations. Hamilton argued that the model of Medawar is fundamentally correct, because it led to the formulation of a better theory (Medawar, 1952,1955) than had existed, but that the model could be developed in a more rigorous way . A key prediction of the model of Hamilton is that "higher fertility will be a primary factor leading to evolution of higher rates of senescence unless the resulting mortality is confined to the immature period (Hamilton, 1966, p. 12)." Further, most of Hamilton's theoretical and mathematically derived conclusions agree with those of Williams' (1957) qualitative arguments.

Hamilton attempted to show that Fisher's (1958) concept of reproductive value, V_x, is not the most important parameter and instead developed a model based on a mathematical expression for the parameter, w_a, which he maintained "measures more exactly what Williams meant" by the term reproductive probability (Hamilton, 1966,p. 22). Hamilton argued that the problem with Fisher's reproductive value is that unlike w_a it did not take into account the action of natural selection. The model was developed for a population with discrete non-overlapping generations, but many of his results are important to the general discussion of the evolution of senescence. The predictions from Hamilton and Williams' theories have prompted others to design experiments to test them. Both of the larger experiments described in this thesis have been designed to test some of the predictions of this theory.

Hamilton maintained that age-of-onset modifiers are certainly a genetically plausible hypothesis, whereas age-of-action genes could only be subject to "evolutionary improvement" if their phenotypic consequences are manifested during an organism's fertile period (Hamilton,1966,p.29). For non-social species, eg. Drosophila, Williams (1957) and Hamilton (1966) both maintain that natural selection "improves viability for all ages for which there is prospect of any future reproduction (Hamilton, 1966, p.23)." Although Hamilton's paper used human data from Taiwan and India many of his results and observations are relevant to non-human species. For example, he claims that in species which lack a special infant mortality, as in dispersive animals which lay their eggs separately, "it should be found that the highest rates of senescence accompany the highest fertilities (Hamilton, 1966, p. 26)."

In his view attempts to determine how natural selection molds the fertility schedule must consider exactly "what genetic effects selection can be working on (Hamilton, 1966, p.42)." Thus if an artificial selection experiment could produce strains , from the same ancestral strain, with different longevities, rates of senescence etc., some of Hamilton and Williams' predictions could be tested. It is axiomatic in this approach that how selection molds senescence necessarily depends on what sorts of genetical variation are available, whether the selection is natural or artificial.

Charlesworth (1980) elaborated some ways in which the theories of Medawar, Williams, and Hamilton might work in real populations, specifically: "how the strength of selection on age of gene action may contribute to the evolution of senescence (ibid. p.216ff).

Rose (1984b), who has attempted to test these theories and their predictions maintains that , in principle, there are two kinds of senescence genes: (1) senescence genes with phenotypic effects confined to the late stages in the life span and (2) genes with multiple effects i.e. antagonistic pleiotropic action. Rose argues that shifting of the force of natural selection to later stages in the life span of organisms should result in observable phenotypic consequences over a sufficient number of generations of selection. He maintains that shifting the force of natural selection "toward later ages should result in a depression of early reproduction. It is noteworthy that the experiments of Rose (1984), Rose and Charlesworth (1981a,b), Luckinbill et al. (1984), Luckinbill and Clare (1985), and Clare and Luckinbill (1985) with Drosophila melanogaster have provided some data which support this contention.

Charlesworth (1980) in his book Evolution in Age-Structured Populations reviewed the basic theoretical and mathematical arguments for the evolution of senescence. As he noted senescence is defined in demographic terms as " the tendency for the age-specific survival probabilities, P(x) (with discrete age classes) and age specific fecundities, m(x), to decline with increasing age, for individuals of sufficiently advanced age (ibid,p214)." He also noted that "senescent decline at the level of components of fitness reflects the decline in the performance of many different physiological functions with age" emphasized by Comfort (1979).

Drosophila melanogaster has been widely used in gerontological research ,since the work of Pearl and co-workers early in this century (Pearl, 1928; Alpatov and Pearl, 1929). The 1929 paper of Alpatov and Pearl is the 12th in a series of papers Pearl and his co-workers published on aging in Drosophila. Pearl and his coworkers used the results of their studies of Drosophila as the basis for the "rate of living" theory of aging. Under this theory the life span in Drosophila is determined by two parameters: (1) the physiological constitution of the organism, "inherent

vitality", which is genetically determined, and (2) the average metabolic rate of energy expenditure during its life span (Pearl,1932; Lamb,1978).

According to the "rate of living" theory (Pearl, 1928; Alpatov and Pearl, 1929), if the data for some life history character is plotted against time for two populations with different rates of living, the plots of both are predicted to have the same shape. If one plot is shifted relative to the other in time, but has the same shape, then the plausible inference is that the rate of living of one is greater than the other. The same changes occur in both populations, in the same relative order, but in the population that lives faster the entire sequence of events is compressed in time relative to the population with the slower rate of living.

Partridge and Farquhar (1981, 1983) showed that sexual activity reduces the lifespan of male D. melanogaster and have proposed that there is a "cost to reproduction." These "costs of reproduction" (Partridge and Harvey, 1985; Fowler and Partridge, 1989) are hypothesized to be factors such as the intense muscular actiivity of sexual activiity, the energy requiring processes associated with sperm production, and the transfer to females of certain compounds during copulatory activity. These authors maintain that virgin males live longer, have a slower rate of aging, because they do not use up the energy associated with reproduction. This hypothesis is a variant of the "rate of living" theory of Pearl (1982) mentioned above. The hypothesis predicts that virgin males live longer than mated males, because the energy that is saved is available as a stored reserve.

A search of the aging research literature reveals that there is as great a diversity of hypotheses and proposed mechanisms for aging in Drosophila as there is overall in the entire literature of aging in invertebrates and vertebrates (Marx 1974 a, b; Miquel et al., 1979; Rose , 1983). Any theory of aging must account for the linear loss of function and the exponential increase in mortality over time observed in species related as distantly as man and Drosophila melanogaster (Ham and Veomett,1980). The research on aging mechanisms in Drosophila has incorporated many experimental designs and approaches, each reflecting the individual bias of the investigator (Lamb, 1978; Rose, 1984).

Measurements of life span in Drosophila have yielded data that are not uniformly consistent. Different laboratories have used different strains and protocols and thus it is not a simple task to compare data and make valid inferences from the very disparate results. Lamb (1978, Table I, p. 48) summarizes some of the very different results on longevities of various Drosophila species. As Lamb (1978,p.49) correctly observes, a life-table by itself contains no information regarding the age of onset of any change in the physiological state and/or rate of aging, and thus other types of data must necessarily be obtained to better understand the process of aging and senescent changes in aging fly populations.

It is unrealistic to maintain that survival curves for D. melanogaster and other species obtained in laboratories reflect the situation in the wild. Outside the controlled conditions of a laboratory environment, accidental deaths probably eliminate the majority of a population of flies long before senescence causes death. There have been few studies of life span in D. melanogaster in natural conditions. Cannon (1966) found that under more natural conditions, mean life span is roughly nine days and the survival curve tended to be diagonal. Buzzati-Traverso (1955) found the mean life span in his Drosophila melanogaster populations, studied for evolutionary changes in components of fitness, to be 8.82 days. Shapes of the survival curves obtained and the parameters derivable from survivorship data , regardless of the care taken in obtaining the data, depend on the heterogeneity of the population and the specific environmental conditions of the study (Lamb,1978).

Lamb (1978, Table III, p. 53) summarizes life span studies with Drosophila melanogaster mutants. The data show that different mutant strains have different mean life spans for males and females and that there is considerable variation between mutant strains in mean life span.

Gilbert's (1960) analysis of the data of Karp (1940) showed that longevity is a quantitative trait, that different segments of chromosomes affect longevity differently, and that the segments of chromosomes II and III simultaneously affected longevity, fertility, and viability. This is some of the earliest evidence that longevity in D. melanogaster is a polygenic trait and that the species

fitness traits are probably determined by some of the same genes or combinations of genes interacting.

As Ham and Veomett (1980) have noted there has been a "proliferation" of proposed stochastic and programmed mechanisms for aging in recent years possibly because of the entry of physicists (Szilard, 1959; Sacher, 1967; Orgel, 1963; Kirkwood, 1977; Kirkwood and Holliday, 1979) into the field of aging research. These investigators have focused on the fact "that radiation accelerates mortality in a fashion that is additive with natural aging (Ham and Veomett, 1980, p. 743) ." This observation has been coupled with the "superficial similarity between the human survival curve and multiple hit survival curves predicted by target theory (Ham and Veomett,1980;p.743)."

Ham and Veomett maintain that those trained in physics undertook the search for a stochastic theory of aging with an insufficient knowledge of the aging process in somatic cells in relation to the entire life cycle and to the immortality of germ line cells. They also note that the focus has been shifting and that senescence now tends to be viewed as a type of programmed process or "terminal differentiation." If one agrees that this is the proper focus of current aging research then it follows that much more must be known about the programmed processes of gene expression and the details of cellular physiological functions on the cellular level when aging studies are done in any species.

Some of the possible landmarks of age-related changes in animals include (1) biochemical changes (eg. :in the synthesis or catabolism of protein, pigment or other chemicals), (2) anatomical alterations at the cellular or subcellular level (eg.:morphological changes in neurological junctions, the anatomy of muscles, mitochondrial density etc.) (3) alterations in behavior (Leffelaar and Grigliatti, 1984,b). Leffelaar and Grigliatti (1984,b) note that any age-related biomarkers of aging useful for profiling senescence must satisfy three criteria (1) the changes must be easy to detect, (2) quantifiable, and (3) reliable.

Hall (1969) and Burcombe (1972) studied enzyme changes in aging Drosophila, but such changes are subtle, difficult to quantify, and require sequential killings of some members of the

population under study. This is a problem, because it does not provide data on the same organisms over time. Another difficulty with this approach is the choice of enzymes assayed. Criteria for the choice of the enzymes to be assayed as aging markers have not been established and thus any set chosen for study seems somewhat arbitrary.

Drosophila aging studies like those of Miquel (1971), Miquel et al. (1976) , Atlan et al.(1976) , Herman et al. (1971) have detected subtle anatomical changes, but these often occur late in the life span of the species and also require the killing of some of the members of the population under study. An approach (Leffelaar and Grigliatti 1984;a,b) that offers an excellent alternative is the quantitative recording of alterations or age-dependent behavioral losses in responses to specific stimuli without killing any of the individuals of the population being studied. Leffelaar and Grigliatti (1984a,b) examined three simple behaviors that are easy to quantify in Drosophila (1) innate programmed geotactic (2) photactic responses and (3) behavior that is sometimes labeled general motor activity. These behaviors are simply, reliably, and conveniently measured without the killing of any members of a synchronously aging population of flies (Leffelaar and Grigliatti, 1984, b).

Leffelaar and Grigliatti (1983 a; 1983 b) used these behavioral biomarkers to study the process of aging (senescence) in wild-type Oregon R D. melanogaster and a set of X-linked, adult-lethal, temperature-sensitive ethylmethane sulfonate (EMS) induced strains from the same highly inbred laboratory Oregon R base stock. The use of behavioral changes to monitor age-related changes has not been extensive among Drosophila researchers (Miquel et al., 1976; Samis et al., 1981). Leffelaar and Grigliatti studied geotactic, phototactic, and motor activity in synchronously aging populations of flies and are now studying changes in fertility and fecundity in the same wild-type and temperature-sensitive mutant strains. Their data clearly show pattern of behavioral loss in wild- type flies and they argue that one of their temperature-sensitive mutants is probably an aging mutant because the patterns of behavioral losses are the same as those in wild-type flies, but are temporally compressed (Leffelaar and Grigliatti, 1984).

Leffelaar and Grigliatti's (1984a) candidate for an aging mutant in Drosophila exhibited a "temporal compression" of the behavioral losses found in their unselected Oregon R untreated strain. This putative aging mutant strain is a temperature sensitive X-linked EMS mutant (i.e., only a "progeria" type mutant) with a much shorter life span than that of the untreated Oregon R flies. Age-related behavioral loss is very compressed at 29ºC (the non-permissive temperature), but the relative order of the losses of the four measured behaviors in this strain is the same as that in untreated wild-type flies from the Oregon R stock. They do not report any mutant strains that extend the life span of flies (Leffelaar and Grigliatti,1984:a,b).

The behavioral experiments (Leffelaar and Grigliatti,1984 a, b) used flies that developed at 22ºC and that were then divided into two groups: one kept at 22ºC the other shifted to 29ºC after eclosion. Longevity was measured simultaneously.

Introduction to the experiments:

I used two strains of Drosophila melanogaster that Luckinbill et al. (1984) have shown exhibit a delayed or postponed senescence and two strains that they did not subject to selection. The two strains not under selection served as controls. Luckinbill et al. (1984) selected directly for late and early reproduction and therefore indirectly for increased and shortened life span. Exact details of the selection protocols are described in the Materials and Methods section below. The line which they selected for early reproduction and short life span was lost and a new "early line" was later re-established. All four strains used in the experiments to be described descended from the same M4WHC strain (Luckinbill et al., 1984) which was divided into three groups before the selection was initiated in their laboratory. The origin of this stock, the selection protocols, and the techniques of maintaining them are presented in the Materials and Methods section below.

Luckinbill et al. (1984) used strains selected for early and late reproduction. Luckinbill and Clare (1985) and Clare and Luckinbill (1985) also used these so called "late" and "early" strains.

Until recently all attempts to select for increased mean and maximum life spans in eucaryotic organisms have failed. Lints and his coworkers attempted to select for an increased life span in

Drosophila melanogaster (Lints and Stoll, 1978a; Lints and Hoste,1974a; Lints et al., 1979a). The results of their direct and indirect selection experiments led Lints and his coworkers to conclude that it is not really possible to select for an increased life span in Drosophila .

Luckinbill et al. (1984), Rose (1984), Clare and Luckinbill (1985), and Luckinbill and Clare (1985) have all reported successes in their attempts to select for a postponed senescence and increased life span in D. melanogaster. These papers also present similar explanations of the failures experienced by Lints et al. (Lints and Stoll, 1978; Lints et al., 1979). In their experiments Lints and his associates used a highly inbred laboratory strain of D. melanogaster and they controlled the density of the developing larvae. The later successful selection experiments of Rose (1984a,c) and Luckinbill et al. (1984) did not use highly inbred strains and did not control the density of the developing larvae. The results obtained by Luckinbill et al. (1984) and Rose (1984) have been shown to be in part a consequence of uncontrolled density during larval development.

The strains of D. melanogaster used by Luckinbill et al. at Wayne State University (Luckinbill et al., 1984; Arking and Clare, 1985; Luckinbill and Clare 1985) showed a 45% increase in the mean and maximum life span after 21 generations of selection. Lines selected for a short life span laid 22-24% more eggs early in life than did the lines selected for increased life span and there was an approximately a thirty day delay in the onset of senescence in those lines selected for a postponed senescence (Arking and Clare,1985). Clare and Luckinbill (1985) reported that in these strains life span was extended at a considerable cost to early fecundity. They also found that drops in fecundity and fertility could be used as highly accurate biomarkers of senescence and predictors of the death of individuals. Their analysis of the fecundity data compared the strain selected for early reproduction and a short life span and that selected for late reproduction and a postponed or delayed senescence. Luckinbill et al. (1984) compared early fecundity from days 4 to 6 post-eclosion and measured fertility from counts of unhatched eggs. In their experiments fecundity and fertility data were obtained for females that were mated when young and kept continuously with males until they died. It is of interest to note that in

Figure 4 of Luckinbill et al (1984, p.1002) the fecundities, averaged over three day intervals, of the replicate strains selected for late reproduction rise and fall until about 35 days post eclosion, whereas the mean fecundities of the strains selected for early reproduction reach a peak at about 6-7 days post eclosion and then decrease until several days before all the females have died.

Luckinbill et al. (1984, p1001) maintain that their data "supports the broad contention of many genetic and ecological models of life history evolution that age-specific reproductive pattern determines the rate of senescence." They claimed that their selection experiment succeeded because inbreeding had been avoided in the setting up of the base population that was subsequently subjected to 3 different selection protocols, for early, random age, and late reproduction. Luckinbill and Clare (1985) and Clare and Luckinbill (1985) showed that selection for an extended life span only works when the larval density is uncontrolled and that the expression of the genes for life span involves an interaction between these genes and the environment.

Experiments with the short and long-lived selection lines (Clare and Luckinbill,1985) showed an additive genetic variance for longevity in D. melanogaster. Reciprocal crosses of flies from short and long-lived strains yielded flies with life spans intermediate between the parental lines.

Experiments of Rose (1980, 1984a), Rose and Charlesworth (1981) and Luckinbill et al. (1984) support the "antagonistic pleiotropy" model of Williams (1957) described above, whereas those of Giesel (1979 a, b) do not. Williams and others have considered the ways in which natural selection molds the life-history of a species (Hamilton, 1962: Emlen, 1970) as pointed out above. The Williams' model predicts a negative correlation between life-history characters such that selection for a delayed senescence will result in a depression of youthful vigor. Giesel (1979 a,b) found a number of positive correlations in life history characteristics early and late in life span. Le Bourg et al. (1988, p.491) found that "At the individual level, no relation could be detected between early components of fitness and longevity." In an

interspecific comparison of life history characters of 12 Drosophila species (Schnebel and Grossfield ,1988, p.306), , it was found that the predicted relationships of the "antagonistic pleiotropy" hypothesis are "only relevant to the evolution of life-history differences among individuals in the same breeding population confronted by the same environmental constraints." Nesse (1988) reported results of an interspecific comparison of the intensity of selection, using life table data from wild populations. He concluded that "pleiotropic genes may be important causes of senescence in some populations, but not in others (Nesse, p.445).

Leffelaar and Grigliatti (1984a, b) used alterations in simple behaviors as biomarkers of aging in wild type Oregon R D. melanogaster and EMS induced temperature sensitive flies from the same base stock . They studied geotactic, phototactic, and general motor activity in synchronously aging populations descended from differently treated populations. Their data clearly show that there is a pattern of behavioral loss in aging Drosophila melanogaster which was compressed in one of the strains they studied. This population showed the same pattern of behavioral loss as did the original base population, but this pattern was compressed into 10 days instead of the 40 days observed for untreated wild-type flies.

Studies of strains selected for a postponed senescence that have been completed to date have not provided data on the senescent decline of reproductive biomarkers and geotaxis in populations of virgin flies. Experiments described in this thesis have been designed to yield such data.

The work mentioned above on the reproductive activity of D. melanogaster has not been done with flies stored as virgins for up to 6 weeks. The females studied were exposed to males from early in their life spans and could have remated with the same male or with the backup males supplied to them in the course of the experiment (Luckinbill et al, 1984). It has been established that the presence of males is a significant factor in the determination of life span and fertility of females (Bieganska-Pietrzakowa, 1961; Glass,B. 1960; Hoffmann and Harshman, 1985; Malick and Kidwell, 1966).

Studies of strains selected for a postponed senescence that have been completed to date have not provided comparative data on the senescent decline of reproductive function (components of fitness) and geotactic response in virgin flies of various different ages.

Experiments reported here yielded comparative data on fecundity, fertility, and the fertile mating ability in two strains selected for late reproduction and two control strains for 8 different ages where the flies that mated were virgins of the same age. Data on fecundity, fertility, and the fertile mating ability is also presented for matings of "young" females to "old" males and for matings of "old" females to "young" males". Comparative survivorship data for virgin flies, both sexes, for all four strains is presented.

Data from an experiment on the geotactic response of these four strains is presented for 7 different test ages. The survivorship data for the flies tested for geotactic response is also presented.

These experiments were designed to test whether laboratory selection has altered the patterns of variation in life-history characters and behavioral loss (Leffelaar and Grigliatti, 1984b). The life history characters studied here were fecundity, fertility, and fertile mating ability. The behavioral loss studied was geotactic response.

Data from these experiments can be used to test some of the hypotheses mentioned above that have been used to explain senescence: (1) the "mutation accumulation" hypothesis (Medawar, 1952; Edney and Gill, 1968), (2) the "antagonistic pleiotropy" hypothesis (Williams, 1957) and (3) the "rate of living" hypothesis (Pearl, 1928). As Rose (1980, p.141) has remarked either the first or second "could apply in a particular population." Leffelaar and Grigliatti, (1984a, 1984b) have shown that the "rate of living" hypothesis is plausible in the case of geotactic behavioral loss over time in D. melanogaster.

As Rose (1983; p. 20) correctly observes "no one has yet estimated the precise functions giving the interactions between life-history characters, derived quantitative predictions from a 'life-history theory ' model, and then exhaustively tested these predictions statistically over a range of populations." The collection of information on this scale would be an undertaking

requiring the effort of many researchers and agreement on the exact details of the entire project.

Williams and Taylor (1987) note that since the work of Hamilton (1966), who elaborated the logic of the evolutionary basis of senescence, there has been no explicit formula relating mortality rate and age produced by Hamilton or any other theorist. A complete mathematical derivation of the relationship, in their view, may not be possible . As an alternative, computer simulations could be used to explore the consequences of different sets of assumptions about the process of selection, age of action of genes, their interactions, the modifier genes, and environmental factors (Williams and Taylor, 1987).

The objectives of the experiments described in this thesis are to provide data that: (1) show whether significant differences exist between the quantitative values of life history characters, components of fitness, of the selected and control strains when mating is delayed for different time periods, (2) make interstrain and intersex tests of each of the hypotheses of the aforementioned theories of life history possible, (3) permit comparative statistical tests of the survivorships of both sexes within and between selected and control strains when flies are kept as virgins until they die, (4) permit a comparison of the effects of postponed mating when parental ages are both "young", both "old", and when they differ by 5 weeks, and (5) to determine if more than one hypothesis of the theories mentioned above is consistent with the data from each experiment described.

Chapter 2

Materials and Methods

Culture medium

The culture medium (Rockwell and Seiger, 1973) was a Cream of Wheat™, molasses, raisin, dry yeast extract , and agar mix containing Drosophila mold inhibitor (Carolina Biological). The food was stored at 5°C. In the original Michigan selection for longevity (Luckinbill and Clare, 1984) the lines were maintained on a medium consisting of agar, yeast extract powder, sucrose (Dominoe Brand™), propionic acid and distilled water, and was covered with a special yeast solution immediately before use. Exact proportions of the food and yeast solution used in the original selection experiments can be found in Luckinbill et al. (1984). All developing larvae and flies were stored in vials containing the molasses-agar medium in a constant temperature chamber at 25°C, 12:12 LD light-dark cycle, and relative humidity of 50-60%.

In this laboratory the lines have been maintained on the molasses, Cream of Wheat™ medium with sufficient success to warrant its use. No yeast solution was added to the medium at any time during the experiments, but there was yeast extract in the cooked medium. During pilot experiments it was found that addition of yeast made the counting of eggs very difficult. Late-line non density controlled and control flies have done as well on this medium as on that used in Michigan where the selection protocol was initiated several years ago. The original medium used in the selection experiments in Michigan is probably not as rich in nutrients as that used here.

Selection protocols: Since the strains of D. melanogaster arrived here from the laboratory of Dr. Leo Luckinbill at Wayne State University they have been maintained according to the same protocols as in Luckinbill et al. (1984). To be certain that no details of the selection and maintenance protocols were omitted at the time the flies were shipped, I obtained a written copy of the protocols and discussed

them in detail in several phone conversations with Dr. Michael Clare, who had provided me with the protocols and answered various question regarding these strains.

Originally three types of lines were set up in their laboratory : (1) an early line, selected for early reproduction, (2) a so-called random line (RND, control), with no directional selection for age of reproduction, and (3) a late line (NDC), selected for late reproduction. In the experiments outlined below only two random and two late lines were used. The two NDC strains used here arrived from Dr. Luckinbill's laboratory marked NLA and NLB. The two RND strains were received marked RNDA and RNDB. These designations were preserved for the experiments reported here.

Each new generation was set up with 55-60 virgin pairs per bottle. For each line there were two bottles for each replicate (master and a backup bottle). No attempt was made to control the density of the developing larvae. All adults of each replicate were transferred to fresh medium every 48 or 72 hours (50 ml/bottle). When a transfer was done the dead flies in the master bottle were replaced by the appropriate number of live flies, of each sex, from the second bottle. This procedure was followed for both the random (control) and the late (postponed senescence) strain under selection.

When the back up bottle was exhausted in the late lines, selected for late reproduction (NLA and NLB), the bottles were set aside and at eclosion virgins were collected from these dated bottles to establish the next generation. The date for setting up the next generation of the late reproducing line was determined by the exhaustion of the backup flies used at each transfer to replace dead flies . Luckinbill et al. (1984) found that this method of selection resulted in a substantial increase in the time interval between collection times for setting up new generations as their experiments proceeded.

A 45% increase in the mean life span occurred over 17 generations of selection as a consequence of an increase in the maximum life span from about 60 days to 100 days (personal communication to Dr. J. Grossfield from Dr. R. Arking; 8/10/83).

In these experiments the "random" lines (designated: RNDA and RNDB) are the controls. The word random, with regard to lines, denotes the age of the parents of the successive generations in the control strain. The replicates of this strain were maintained without directional selection for early or late reproduction as follows. A long set of random integers between 3 and 30 was generated by a computer program. Integers thus generated were used to determine, the date of the bottles, i.e. age of the adults, the offspring of which were retained for collection to establish the next generation. Control lines (RNDA and RNDB) were therefore reproduced when the parents were between 3 and 30 days of adult life, the adult parental age being determined by which random integer between 3 and 30 was generated by the computer program. The random number program was written in Microsoft Basic™ Version 2.0. for an Apple Macintosh™ microcomputer. Because no directional selection for earlier or later reproduction was applied to the two RND strains it is assumed that these strains have allelic frequencies equal to those in the original base popuiation. If this assumption is correct then the life spans, fecundities, fertilities, and fertile mating abilities of these strains approximate those determined by natural selection in the wild.

In all the experiments on fecundity, fertility, and fertile mating ability, the flies of both sexes were collected within 4 or 5 hours of eclosion and stored until the specified day of a test mating.

In both the experiments on survivorship only virgin flies of both sexes were used.

Components of fitness

Experiment I A - Survivorship of virgin flies:

Toward the end of the mating experiments two sets of thirty virgin flies of each sex and strain were collected on the same day, for a total of 480 flies. At this time the RND flies were at generation 68 and the NL flies at generation 48 of selection in this laboratory. These sets of 30 flies were stored as virgins in 8 dram food vials in the same chamber as the flies for the mass-mating experiment. The environmental conditions were as indicated above. All flies were transferred to fresh food vials every three or four days. At the time of transfer the number of dead flies in each of the 16 vials was recorded. All replicates were scored until all flies had died. No attempt was made to compensate for the effect of lower density on survivorship as flies died in the vials.

From this data LT 10, LT50, and LT90 values for virgin flies of each strain and sex were obtained, survivorship curves plotted and Kolmogorov-Smirnov tests for significant differences in cumulative survivorship distributions of virgin flies of all test strains were made.

Experiment I B - fertile mating, fecundity, and fertility of virgin flies of the same age:

Female and male virgin flies were collected under light CO_2 anesthesia and transferred every seven days to fresh food vials until the flies reached the appropriate age for a mating. Flies were selected from a set of bottles retained after adults had been transferred to new culture bottles as described above. Virgins of each age, sex, and strain were stored in 8 dram food vials. The ages for the mass matings were 1, 2, 4, 7, 14, 21, 28, and 42 days post-eclosion. Until they were mated, the virgin flies were stored in vials containing the molasses-agar medium in a constant temperature chamber at 25°C, 12:12 LD light-dark cycle, and relative humidity of 50-60%. The medium was not yeasted, but contained yeast extract. All storage and mating vials were stored on their sides and stoppered with porous diSPO™ Plugs (American Scientific Products)

Flies for matings were lightly anesthetized with humidified CO_2. Thirty virgin females and thirty virgin males of the same strain were aspirated into a food vial and left for 24 hours in the same chamber that the flies had been reared and stored in previously. After 24 hours, the males and females were lightly anesthetized with humidified CO_2, separated by sex, and the 30 males discarded. Each female was then transferred to a vial numbered from 1 to 30. The females were sequentially transferred again at 24 and 48 hours to fresh food vials numbered from 1 to 30. After the third transfer, the females were discarded. All 90 transfer vials were retained for egg counts and later scoring of eclosed adults for each replicate. Two replicates of each strain, 30 females and 30 males, were tested for all ages post-eclosion indicated above.

Immediately after transfer, each vial was examined under a Wild M5 dissecting microscope with illumination from two high intensity lamps. A thin black line was drawn parallel to the long axis of each numbered vial as a reference marker to prevent duplicate counts as the vial was rotated under the microscope. The number of eggs on the food surface and the glass walls of the vial were counted twice and recorded. A third count was made if there was a disparity between the results of the first two counts. Eggs were quite easy to see because of the dark color of the molasses-agar medium used. For each age and strain two replicates of each line were used. Thus each replicate provided 90 numbers for analysis, since there were 30 females and 3 days of counting.

For the eight ages tested, egg count data was collected for a total of 1,920 females (30 flies per replicate, 2 replicates per strain, 4 strains, 8 test ages post eclosion). In several cases a large number of females died after mating due to failure of the environmental chamber or in some cases for unknown reasons. For any replicate where this happened a new replicate was set up with flies of the appropriate age. This extended the time necessary to do the experiments significantly; however, it

ultimately yielded a complete data set for all ages stated above. A record was kept of the parental age of the collected flies.

After the egg counts the vials were stored in the same constant environmental chamber until the adults began to eclose, in approximately 11 days. From the day that the first adult eclosed until all adults eclosed, the numbers of males and females were recorded daily. Two replicates of flies of two NL lines, NLA and NLB, and of two control lines, RNDA and RNDB, were mated at each of the above 8 ages for a total of 64 replicates.

For each of the 1,920 females there were three vials and thus data was collected from 5,760 vials.

Experiment I C - fertile mating, fecundity, and fertility of virgin flies of unequal ages:

The mating and data collection procedure was identical to that for the virgin flies of equal ages, but the ages of the flies for the matings were as follows. "Young" females (7 days post-eclosion) were mated to "old males" (42 days post-eclosion) and "old" females (42 days post-eclosion) were mated to "young" males (7 days post-eclosion). For each of the four strains and parental age combinations two replicates of 30 virgin females and 30 virgin males were tested. This protocol provided data for an analysis when combined with data from matings of equal aged virgin flies of 7 and 42 days post eclosion to determine if there are interstrain differences in the fecundity, fertility, and the number of fertile matings when there is a 5 week difference (by sex) in the age of mated virgins.

For this experiment there were 240 females for each parental age combination, 2 age combinations, and as above 3 vials per female for a total of 1,440 vials. All data was collected as in the equal parental age experiment.

Experiment II A - Geotaxis experiment with aging virgin flies:

The entire set of experiments was done on virgin females and males from the 4 strains. At the time of the experiment the RND flies were at generation 31 and the NL flies at generation 21 in this laboratory. All flies were collected on the same day and maintained separately in 8 dram food vials under the same conditions as the flies in the mass-mating experiments (see above). Three hundred virgin flies of each strain and sex were collected for each replicate for a total of 2,400 flies.

All flies were maintained in groups of 20 flies/vial and transferred to fresh food vials three times per week until testing. At the time of transfer the number of dead and escaping flies was recorded. This data was used to construct life tables for each replicate and to estimate LT 10, LT50 ,and LT90 values.

Ten numbered vials with 20 virgin females or males were used for storage of experimental flies and the remaining flies maintained for replacement of lost or dead flies in these numbered vials during the experiment. When the flies from the original vials died, backup flies, from the extra 100 flies of each line, replicate, sex, and appropriate age were transferred to the appropriate numbered vial.

A set of random integers ranging from one to ten was used to determine which two numbered vials of each strain and sex were to be tested on a particular day from the sets of vials numbered 1 to 10. These numbers were generated by a program written in Microsoft Basic™ for the random number generator of a Macintosh microcomputer. The entire set of random numbers for all test ages and replicates was prepared before the flies were tested. The four NLA and four RNDA test groups were tested at the same time on the inclined plane and then the four NLB and RNDB sets were tested at same time.

All geotaxis tests were be done between 11 A.M. and 1 P.M. on the predetermined days post eclosion. Positive geotactic response of the populations was tested on an inclined plane that could be rapidly raised to a specified angle. All replicates were tested for their response at: 0, 10, 20, 30, 40, 50, 60, 70 and 80

degrees. Flies from the maintained populations were tested on day 0 (the day of eclosion), 3, 7, 14, 21, 28, 35, 42, 49, 56, and 63 days post-eclosion. In this experimental design it is possible that a fly could be tested at different ages along with sibs that were replacement flies, but the randomization procedure that determined which numbered vials would be tested probably reduced any bias towards the retesting of any individual fly or set of flies.

Flies in all tubes were tested for geotactic response to these angles in three different orders of presentation as follows: (1) 0, 10, 20, 30, 40, 50, 60, 70, 80 degrees ; (2) 0, 50, 10, 70, 20, 80, 30, 60, 40 degrees; and (3) 80, 70, 60, 50, 40, 30, 20, 10, 0 degrees. Two replicates of each line and sex were tested for the three orders of presentation on each day of testing. Each replicate represents 60 "fly decisions" or responses for each test angle.

Before testing, each designated set of 20 flies was removed from its storage vial and transferred to 30-cm-long glass tubes under light CO_2 anesthesia. The flies were set aside in these tubes for 30 minutes before testing to permit recovery from the anesthesia. The eight replicate tubes were aligned parallel to the sides of the inclined plane and held in position by guide nails on the inclined plane.

When testing started, the flies were knocked to the bottom of the tubes, the filter-covered flashlight was turned off and the board was rapidly raised to the appropriate test angle for 1 minute as determined by a pre-set alarm. The filter-covered flashlight was turned on and the number of flies in the top half of each tube was quickly recorded. The next test angle was presented after the flies had been dislodged to the bottoms of the tubes. All testing was done in the chamber where the flies had been stored and reared. A test interval of one minute was selected after a set of pilot experiments showed that longer intervals did not yield very different results and because of the suggestion of Leffelar and Grigliatti (1984 a, b) that time intervals for behavioral tests of aging biomarkers should be kept as short as possible.

All tests were done in a "dark" chamber, where the only light source was a flashlight covered with a uv filter and a red filter previously tested for its absorbance of short wavelength visible light. The light level under these conditions was measured with a 40X Opto-Meter (United Detector Technology, Inc.). The measured light level was between 0.22 and 0.28 footcandles (5-8uW/cm2) at the 15 cm distance from the tubes where the light was held at the time counts were made. The tubes were made of clear glass with a length of 30 cm and inner diameter of 1.5 cm. When testing started, the flies were gently knocked to the bottom of the tubes before the plane was raised to the designated test angle. After testing flies were again lightly anesthetized under light CO_2 and returned to the numbered vials for storage until the next designated test day. At that time, if any flies had died, replacement flies of the appropriate strain and sex were added to the set from the extra storage vials described above.

Each geotaxis replicate tube thus provided 27 numbers for analysis, 3 population responses for each of the 9 test angles for a total of 4,752 data values for the entire experiment for all ages, strains, and sexes tested.

Experiment II B - Survivorship of virgin flies from the geotaxis experiment:

The entire set of experiments was done on virgin females and males from the 4 strains. At the time of the experiment the RND flies were at generation 31 and the NL flies at generation 21 in this laboratory. All flies were collected on the same day and maintained separately in 8 dram food vials under the same conditions as the flies in the mating experiments (see above). Three hundred virgin flies of each strain and sex were collected for each replicate for a total of 2,400 flies.

All flies were maintained in groups of 20 flies/vial and transferred to fresh food vials three times per week until testing. At the time of transfer the number of dead and

escaping flies was recorded. This data was used to construct life tables for each replicate and to estimate LT 10, LT50 ,and LT90 values.

Ten numbered vials with 20 virgin females or males were used for storage of experimental flies and the remaining flies maintained for replacement of lost or dead flies in these numbered vials during the experiment. When the flies from the original vials died, backup flies, from the extra 100 flies of each line, replicate, sex, and appropriate age were transferred to the appropriate numbered vial.

Chapter 3

Statistical Methods

The data for fecundity were analyzed for all parental age combinations as follows: Egg counts for all three days of counting for each test age were summed for each female. The means, variances, standard errors of the means, minimum, maximum and total number of eggs laid by the 60 females of each strain, and replicates pooled within strains, for each of the 4 lines were calculated. These summary statistics for the raw data are shown in Tables 3, 4, 5, and 6.

Examination of Tables 4 and 6 shows that the variances were quite heterogeneous. Several types of square root transformations were applied to the egg count data in an attempt to stabilize the variances across strains within age sets. Before a nested analysis of variance was performed, the egg count data were transformed using a square root transformation (Bartlett, 1947) that was found to produce the most homogeneous sets of variances. In this transformation one adds 0.5 to the original data value and then takes the square root of the result. Bartlett's test for the homogeneity of variances (Snedecor and Cochran, 1967) of the transformed egg count data showed that on this transformed scale, the variances of all egg count data sets, except those for ages 1 and 2 days post eclosion, were homogeneous after transformation.

A nested analysis of variance (replicates grouped within strains) was run using Proc Anova of the SAS statistical package for each parental age combination including the two sets where there were heterogeneous variances even after a transformation was applied to the data. These two age sets and all the egg count data for older ages were also tested by two nonparametric tests; the Wilcoxon 2-sample test was used for all pairwise strain comparisons and the Kruskal-Wallis test for the 4 strains combined (Proc NPAR1WAY). The results of this nonparametric analysis on the untransformed egg count data are shown in Table 9. The reason for using the second type of test on test data from all ages

was to compare the results obtained to those from the parametric nested analysis of variance of the same data.

The equation used for the nested analysis of variance model was : square root(eggs+0.5) = strain rep(strain). The summary statistics from these analyses on the transformed data are presented in Table 12. The SAS program that I wrote for this analysis requested a comparison of the strain means using Tukey's test, alpha=0.05, the lines, and 95% confidence interval options. A detailed description of Tukey's test can be found in Berenson, Levine, and Goldstein (1983, pp. 86-88). This test is appropriate when the sample sizes are equal. The lines option groups those sets of means that are not significantly different into subsets.

Table 7 presents the results of the Tukey's test lines option from this SAS analysis after the data had been backtransformed as follows: each transformed mean was squared, 0.5 was subtracted and the value obtained was corrected by adding the mean square error to the value. The mean square errors used are shown in Table 12. In Table 7 lines have been drawn under sets of means that are not significantly different at the alpha = 0.05 and 95% level of confidence (Tukey's test). The addition of the mean square error to each backtransformed value is a procedure recommended by Snedecor and Cochran (1967: p327) because "the mean of a set of square roots is less than the square root of the original mean. As a rough correction for this discrepancy, add the error mean square in the square root analysis to each reconverted mean." The means thus obtained and reported in Table 7 are in fairly good agreement with the original untransformed means from the raw data reported in Tables 4 and 6. The line segments drawn under strains and means show the sets of means found not to be significantly different.

The Tukey procedure also provides a set of simultaneous family confidence intervals for those pairwise comparisons found to differ significantly by Tukey's test at the level of confidence set in the SAS program. This method permits (1) an estimate of the magnitude of the difference between any two means at a specified level of confidence

and (2) controls for the experimentwise error rate used to control for alpha (here alpha = 0.05) when making a set of simultaneous comparisons. This approach does not pertain to any particular comparison, but "rather to the set of simultaneous statements for all possible pairs of means (Berenson et a., 1983, p . 89)." Tukey's method thus gives a family confidence coefficient of (1-alpha) here 0.95 that is the complement of the experimentwise error rate. Thus one can be confident that if the experiment were to be repeated hundreds of times in 95 % of these replications of the experiment we would expect to find the same significant pair differences . This is a very powerful *a posteriori* method for making comparisons between means when several different pairwise comparisons must be made.

Table 8 presents the results of this analysis after the values were backtransformed. The procedure calculates the difference between the means and a critical range for the number of means in the comparison. Table 8 presents the values for all significantly different pairs (family confidence level =0.95). Further details can be found in Berenson, Levine, and Goldstein (1983, p. 86-88). The values of the upper and lower confidence limits from the SAS output were squared, 0.5 was subtracted from each value, and the mean square error was added to the individual values, as for the means above. Thus each reported comparison in Table 8 gives the 95% confidence interval for the amount by which one of the means exceeds the other mean. For each pair, the strain with the greater mean is listed first in Table 8.

A nested analysis of variance was also performed on the data to compare the mean individual proportion of adults eclosed between strains for each test age and parental age combination using the general linear models procedure (Proc GLM) of SAS. In these comparisons an arcsin(squareroot) transformation was used. I used the transformation recommended by Snedecor and Cochran (1967, p.328 and Bartlett, 1947) in the program to process the data in SAS. I also ran the data through another arcsin(square

root) transformation (Mosteller and Youtz, 1961) and found that the variances were essentially the same.

An arcsin transformation was used, because it is the one that was developed for binomial proportions and because the data to be analyzed had many individual proportions that were either 0 or 1. Snedecor and Cochran (1976, p.327) note that the transformation that they give values near 0 and 1 are "spread out so as to increase their variance."

The transformation of Bartlett (1947), written in the SAS language, set the following conditions for the transformation of the individual egg and adult counts : (1) if (eggs=0 & adult=0) then delete, (2) if (eggs>0 & adult=0) then P= arcsin(square root(1/4*eggs)), (3) if (eggs>0 & adult<eggs) then P=arcsin(square root(adult/eggs)), and (4) if (eggs>0 & adult>0 & adult=eggs) then P=arcsin(square root((eggs-0.25)/eggs)). The values of P were then analyzed with a nested analysis of variance model: P= strain rep (strain). Here the appropriate test for comparison of strain means and the construction of confidence intervals is Scheffe's test, because the sample sizes are not equal. Some females for some ages and strains laid no eggs and thus were excluded from this analysis. Berenson, Levine, and Goldstein (1983, pp. 90-94) note that this *a posteriori* test is appropriate when the sample sizes of the groups are not all equal. The same kinds of inferences for the family confidence intervals and significance of paired means for the Tukey test also apply for the Scheffe test.

All variances for all data sets for all ages and parental age combinations were tested for homogeneity using Bartlett's test for samples with unequal sizes that incorporates a weighted mean of the sample sizes in the test (Snedecor and Cochran, 1967: pp. 296-298). The variances of the transformed data for ages 21 and 42 days post eclosion were found not to be homogeneous even after transformation.

The SAS program that I wrote specified a nested analysis of variance for the transformed variable P and comparison of means by Scheffe's test. It included the options

for the grouping of means and a set of 95% family confidence intervals. Table 13 contains the summary statistics (transformed scale) for this nested analysis of variance for the proportion of adults eclosed. Table 10 presents the backtransformed grouped means with line segments drawn under those sets that are not significantly different (95% family confidence level). I obtained these backtransformed values by taking the sine of each mean strain value and then squaring that value.

Table 11 presents the 95% family confidence intervals for those pairs of proportions found to differ significantly by Scheffe's test. The table shows the amounts by which the mean proportion in one group exceeds the other with 95% confidence. The backtransformations of the upper and lower confidence limit values were performed as for the means comparisons just described.

Table 14 shows the number of females tested and the number of fertile matings for all parental age combinations. A fertile mating was counted as one in which a female had at least one offspring. This data was obtained as output from a SAS program that I wrote. The conditions set in the SAS program that I wrote were (1) if (eggs =0 and adult=0) then delete, (2) if (eggs>0 & adult>0 then MTOT=MTOT+1. The value of MTOT was initialized to zero and the program run for each of the 4 strains and for the NL strains combined and the RND strains combined.

Table 15 shows the results of a chi-square analysis of the results shown in Table 14. The Yates adjusted chi-square values are shown in Table 15 for all age combinations, comparing the NL vs. the RND strains. These tests compared the number of fertile matings of females (replicates combined) between the strains selected for a postponed senescence (NL strains) and the control (RND strains) for each parental age combination test in the mating experiments.

Table 2 shows the LT 10's, LT 50's, LT 90's, and maximum life spans for the virgin flies from the geotaxis experiment and the sets of virgin flies tested for life span toward the completion of the mating experiments for eqaul and unequal parental ages. The

protocols for these survivorship experiments can be found in the Materials and Methods section. The values in Table 2, from Figures 1 and 25, show the data from combined replicates. The data by sex and strain is shown in Figures 2, 3, 26, and 27.

Kolmogorov-Smirnov one- and two-sided tests (Siegel, 1956;Mode et al. 1984) were used to compare the survivorship distributions of the virgin flies tested towards the end of the mass mating experiments. I wrote programs in the S language for both tests, because they are not available as options in SAS or most widely used statistical packages and doing the calculations by hand for several survivorship distributions is very tedious. For a comparison listed in Table 1 the decision to use the one- or two- sided version of the Kolmogorov-Smirnov test was made after the paired differences at all ages were examined. If a one-sided test is indicated in the table then all values of the survivorship distribution for the group listed in column one exceeded those in column two. Thus this test can be used to determine if one survivorship distribution is greater than the other in the predicted direction. This is a one-tailed test.

The two-tailed test was applied whenever the distributions crossed one or more times so that the probabilities indicated in Table 1 for pairs where this test was applied only indicate whether the distributions differ.

Both Kolmogorov-Smirnov tests are nonparametric, in that no assumptions are made about the form or type of the two distributions being compared. The significance values and special chi-square formulas used in the programs that I wrote can be found in Siegel (Siegel, 1956: pp. 128-136, and Table M, p. 279).

A formula from Bean (1977, p.395) was used to quantify the geotactic responses as pure numbers (gt values) where:

$$g_t = \frac{n_b - n_{\frac{1}{2}}}{n_{\frac{1}{2}}}$$

where:

$$n_{\frac{1}{2}} = \frac{n_b + n_t}{2}$$

In this formula n_t = the number of flies in the top half of the testing tube after time t, n_b is the number of flies in the bottom half of the tube and $n_{1/2}$ is the half tube population. In this experiment $n_{1/2}$ =10. As Bean (1977,p.395) notes the g_t value is thus a "dimensionless ratio expressing the proportion of the half tube population that has undergone net redistribution between half-chambers during the period of the assay."

The quantity g_t ranges from -1 to +1, the sign reflecting the net movement, positive values net downward (positive geotaxis) and negative values (negative geotaxis) net upward movement. If all 20 flies went to the top of the tube, the value of g_t = -1 and if all 20 flies went to the bottom of the tube the value of g_t = +1.

For each angle of each age, replicate, and order, the number of flies in the top (n_t) was subtracted from the total of 20 flies in the testing tube to obtain the number in the bottom (n_b). These two numbers were substituted into the half tube formula above to obtain $n_{1/2}$ and then the g_t values were calculated using the first formula above. These derived g_t values were used for the geotaxis plots and analysis throughout.

The analysis of the geotaxis data was done as follows. A SAS procedure (Proc RSQUARE) was used to assist in determining an appropriate model for the maximization of explained variation in the gt values as a function of the strain, angle, age, replicate, order of angle presentation, and sex of the virgin flies. From this test it was determined that a model that included only strain, angle, and replicate as effects was a valid one. For example, the order of presentation of the angles did not change R^2 except in the fourth decimal place.

Table 17 presents the output from a program using the SAS General Linear Models (GLM) procedure to compare the geotactic responses of the two strains selected for a postponed senescence and the two control strains. The analysis of variance results

presented in Table 17 show that the factor sex is not significant. The model used tested the homogeneity of slopes of four regression lines for the linear model in SAS (PROC GLM: model gt= strain angle angle*strain rep(strain)/intercept;).

Table 17 shows the results of this comparison for test ages 0 and 3 days post eclosion for the geotaxis experiment. In these two cases it was determined that there was no strain angle interaction and that sex was not a significant factor. The model tests for homogeneity of slopes (SAS User's Guide: Statistics, Version 5 edition, page 436,1985). This procedure as used is equivalent to a test for an angle*strain interaction (Snedecor and Cochran, 1967, pp. 432-436: Netter and Wasserman,1974, pp.160-167) in covariance analysis. If a significant strain angle interaction existed then the slopes of the four lines would not all be equal. The F-test of the significance of the strain by angle interaction term indicates whether the lines are parallel or not. If the p value is less than 0.05 the slopes are not judged to be significantly different and the slopes are considered to be homogeneous. A significant F-test for angle by strain interaction indicates that the lines do not have homogeneous slopes and makes analysis of covariance inappropriate.

The design of the geotaxis experiment is that of a type I regression (Documentia Geigy Scientific Tables, 1962, p.173), where the values of the independent variable are fixed and the intervals between values are equal. Because of the non-significant interaction values for angle by strain, the lines are parallel, it is legitimate to compare the elevations of these lines for each strain at the two ages separately. Table 17 shows that the mean elevations of the regression lines were tested at 40 degrees, exactly in the center of the set of input angles. This is equivalent to a covariance analysis.

Tables 18 to 22 show significant angle*strain interactions and sex differences in geotactic response for test ages 7, 14, 21, 28, and 35 days. Therefore the means cannot be compared directly for these ages as they were for test ages 0 and 3 days. After having examined the regression plots for these older ages, I determined that there was no clear pattern I could discern for ages 7,14, 21, and 28 days post eclosion.

The question of significant differences in mean geotactic response at older ages for particular angles can be answered by doing a one-way analysis of variance for the strains by sex for age 35 days post eclosion for specified angles. I wrote a SAS program to do a one-way analysis of variance for day 35 post eclosion geotaxis data for input angles 40 and 80 degrees. Table 23 presents the results of this analysis for the strains by sex and angle.

Table 16 shows the statistics for the analyses of variance tests for all regressions for all strains and ages by replicate through test age 35 days. To obtain the statistics for the construction of Table 16, I used the SAS PROC REG and PROC GLM procedures.

Examination of the data for all ages greater than 35 days showed that the geotactic responses obtained could not be used for an analysis of the kind described above, because the data sets consisted mostly of +1's for all four strains and sexes. Thus no analysis was done on the geotaxis data sets for virgin flies more than 35 days post eclosion.

Chapter 4

Results

Experiment I A - Survivorship of virgin flies:

Survivorship of virgin females and males, combined replicates, is shown in Figure 1. Figures 2 and 3 show plots of all replicates for the survivorships of virgin females and males, respectively. NL flies were in the 48th generation of selection and RND flies the 68th generation.

Table 1 shows chi-square values with 2 degrees of freedom for cases in which the one-sided Kolmogorov-Smirnov test has been used to find significant differences in various paired combinations of the cumulative survivorship distributions for the data plotted in Figures 1, 2, and 3. This table also shows the results of the two-sided Kolmogorov-Smirnov test for critical absolute values of D, the maximum difference between the paired values of two cumulative distributions.

In the one-sided test, the null hypothesis is that the values of one distribution are stochastically larger than those of a second distribution (Siegel,1956: p. 128). After determining those paired sets for which all the values of one cumulative survival distribution exceeded those of the other and calculating the maximum difference between paired values over all ages for the two distributions, I used Formula 6.11 on page 134 of Siegel (1956) to determine the chi-square values shown in Table 15.

In those cases where the values of one cumulative survivorship distribution did not uniformly exceed those of the other, the appropriate test is the Kolmogorov-Smirnov two-sided test (Siegel, 1956). Here the null hypothesis is that the two cumulative distributions are different, a two-sided test. For this test I determined the absolute value of the maximum difference between the two paired cumulative survivorship distributions (Siegel, 1956: p.128) and substituted this value into the formula corresponding to the significance level of interest in Table M of Siegel (1956: p.279). This set of formulas permits one to calculate the critical values of D, the absolute value of the maximum difference that must be

exceeded to achieve significance, based on the values of the two sample sizes. Some parts of these calculations used programs written in the S language from Bell Laboratories, because these calculations are "rather extensive" (Mode et al., 1984), particularly when many comparison are being made.

The cumulative survivorship distributions of combined RND females do not differ significantly from the combined RND males, as suggested by Figure 1, and is confirmed by a Kolmogorov-Smirnov 2-sided test ($p>0.05$, $n_1=120$, $n_2=120$). Here the maximum difference is 0.108 and using Table M of Siegel one finds that the minimum significant difference is 0.176 (when $p=0.05$).

A one-sided Kolmogorov-Smirnov test (Table 1) shows that the NL virgin male survivorship is very significantly greater that of NL virgin females (chi-square=43.35, $p<0.0001$, $n_1=120$, $n_2=120$).

NL (combined sexes) survivorship is very significantly greater than RND (combined sexes) survivorship for virgin flies (chi-square=216.01, $p<0.0001$, $n_1=240$, $n_2=240$) as is shown in Table 1.

In Table 1 the dashed lines in the chi-square column indicate that the test does not give a chi-square value (see above). In these cases the calculated absolute values of maximum differences, D values, shown in Table 1 were compared to the critical values obtained when the numbers of individuals in each set are entered into the different formulae in Siegel (1956). The column of Table 1 headed "critical |D| tests" shows the values obtained from substituting the number of individuals in each group into the critical value formulas of Table M in Siegel (1956;p.279). Table 1 shows all the probabilities associated with the pairwise comparisons of interest that were made with this survivorship data.

LT 10, LT 50 and LT 90 and maximum survivorship values for these female and male virgins, replicates combined for each strain, were obtained directly from Figure 1. These values also suggest that the virgin male NL flies survive longer than the virgin NL females,

LT 50 for NL males=57 days vs. LT 50 for NL females=48 days. Maximum survivorship of the NL virgin males is greater than that of NL virgin females by 7 to 10 days (Figure1 and Table 2).

RND virgin females and males differed little in their LT 10, LT 50, and LT 90 values:15 vs. 12 days, 37 vs. 38 days, and 47 vs. 44 days (Table 2). Examination of Table 2, however, indicates that both NL virgin females and males survived longer than either RND females or male. LT 90's of 57 and 68 days vs. LT 90's of 47 and 44 days respectively (Table 2 and Figure1).

Experiment I B - Fertile mating, fecundity, and fertility of virgin flies of the same age:

Table 3 presents the untransformed total numbers of eggs laid, the number of females, the number of males, the total number of adults, and the male to female ratios for all strains (replicates combined) by age for the experiments where the ages of the virgin parents were equal. Table 4 shows the variances, means, standard deviations, standard errors of the means, minimum number of eggs laid, maximum number of eggs laid, and the total number of eggs laid for the untransformed data for all strains and parental age combinations. Tables 5 and 6 present the same statistics as Tables 3 and 4 for the mating experiments where the virgin parents were of unequal ages.

In the analysis of the mass mating experiment data, the two replicates of 30 females were combined for each strain at each test age. All plots and analyses of variance for egg counts, fecundity, therefore, have n=60 for each strain at each test age. Statistics for the untransformed egg count data were used to construct Figures 4 though 19. For each set of 60 females of each strain, age, and replicate the number of eggs contributing to the mean value is the total for the 3 days from the three transfer vials assigned to each female as described in the Materials and Methods section.

Plots of the mean number of eggs laid by each strain at each age for virgin parents of equal ages: 1, 2, 4, 7,14, 21, and 28 days post eclosion are shown in Figures 4, 6, 8,10,

12, 14, and 16. These plots show the mean eggs/female/3 days ± 2 SEM. for all four strains. Three days refers to the 3 consecutive days starting at the specified day for mating. Thus, for example, the means for day 1 females shown in Figure 4 are means for days 2, 3, and 4 post eclosion after the virgin females may or may not have been inseminated.

For these same age groups the total egg counts for each strain are shown in Figures 5, 7, 9, 11, 13, 15, and 17. Each of these bar graphs is labelled with the total number of eggs counted for the 60 females of each of the 4 strains compared. Total egg counts are referred to by the specified age for mating and are counts for the next 72 hours, as in the case of the means above.

Figure 18 shows the the mean eggs ± 2 SEM./female/3 days for all four strains for the parental age combinations (1) female=7 days &male=7 days, (2) female=42 & male=42 days (3) female=7 days & male=42 days, and (4) female=42 days & male=7 days post eclosion. These data have been presented in this way to show more clearly the effects of the parental ages of virgins of different vs. identical ages on egg fecundity for the four strains tested.

In Figure 19 the bar graph shows the total eggs laid for the same 4 parental age combinations as in Figure18 for the same time intervals.

Examination of the plots (Figures 4, 6, 8, 10, 12, 14, 16, and 18) of the fecundity means of the different age groups suggests that for virgin females mated to males of the same age, the means of the two NL strains, selected for a postponed senescence, are significantly less than that for the two RND strains, (Figures 4, 6, 8, and 10) until day 14 post eclosion (Figure 12). By day 14 post eclosion there is a substantial overlap of the intervals for the two standard errors of all four strain means. The overlap of these intervals for the two standard errors of the egg means is also substantial for the 21 day old virgin females (Figure14). These results suggest that for these two ages the selected strains and the control strains do not differ significantly in their mean fecundities at ages 14 and 21

days post eclosion. Figure 16 , day 28 post eclosion means, shows some overlap of all four intervals.

Fig 18. clearly suggests that by day 42 post eclosion the two strains selected for a postponed senescence, NLA and NLB, have greater mean fecundities than the two control strains, RNDA and RNDB, when the mated male and female virgin parents are of the same age.

Examination of total eggs laid by the 60 females of each of the four strains (Figures 5, 7, 9,11,13, 15, 17, and 19) for these same parental age combinations also suggests that for the two strains selected for a postponed senescence, NLA and NLB, the total fecundities are much less (Figures 5, 7, 9, 11) until days 14 and 21 post eclosion (Figures13 and 15), than those of the two control strains. For day 1 flies total RND fecundity is 375% greater than total NL fecundity (579 RND vs. 122 NL eggs), for day 2 flies total RND fecundity is 49 % greater than total NL fecundity (1,681 RND vs. 1,126 NL eggs), for day 4 total RND fecundity is 113% greater than total NL fecundity (1,451 RND vs. 681 NL eggs), and for day 7 total RND fecundity is 143% greater than total NL fecundity (2,604 RND vs. 1,071 NL eggs).

Combined fecundities for age 14 days differ by only 5%, RND fecundity is 95% of NL fecundity (1964 RND vs. 2066 NL eggs) and for day 21 post eclosion total RND fecundity is also 95% of the NLA strains (1,438 RND vs. 1,507 NL eggs).

For the day 28 post eclosion females (Figure 17) the total fecundity for the two selected lines (NLA and NLB) also exceeds that of the combined control strains (RNDA and RNDB). The total for both NL strains is 29% greater than that for the two RND strains (1282 NL vs. 996 RND eggs) for 28 day-old flies. This suggests that by the 4th week post eclosion the strains selected for a postponed senescence have greater fecundities than do the control strains.

For the 42 day-old females the total fecundity of the NL strains exceeds that of the RND strains by 150% (858 NL vs. 342 RND eggs).

Combined total fecundities for days 1, 2, and 4 show that RND female fecundity exceeds that of NL females by 92% (3,711 RND vs. 1929 NL eggs). If all "young ages" are compared (days 1, 2, 4, and 7) then RND fecundity is 111% greater than NL fecundity (6,315 RND eggs vs. 3,000 NL eggs).

For control females , RND strains combined, total fecundity for ages 1 to 42 exceeds that of NL strains combined by 27% (11056 eggs vs. 8713 eggs) for all ages tested when the parents are of the same age post eclosion on the day of mating.

Nested analyses of variance were performed on the fecundity data for equal age virgin parents as described in the Statistical Methods section. It was necessary to transform the fecundity data prior to analysis, because of the heterogeneity of variances of the fecundity means at all ages tested (Table 4). The statistics from these analyses of variance on the transformed egg count data are shown in Table 12 and the backtransformed grouped means are shown in Table 7. For the entire set of ages tested the backtransformed means are grouped with line segments under sets of means that did not differ significantly (Table 7., Tukey's test, alpha=0.05, family confidence level =0.95).

For ages 1, 2, 4, and 7 days post eclosion, the mean fecundities of the RND strains significantly exceeded those of both NL strains (Table 7, Tukey's test, alpha=0.05, 95% family confidence levels). At 7 days post eclosion the two RND strains differ in mean fecundity (alpha =0.05, Tukey's test), but both means are significantly greater than the two NL means (alpha = 0.05, Tukey's test).

Analysis of variance (Table 12) showed that at ages 14 and 21 there are no significant differences between the means of the four strains (F=0.67, df=3,232, p>0.5704 and F=0.07, df=3,232, p>.9758; Tukey's test).

Twenty-eight-day-old virgin female grouped fecundity means are also shown in Table 7. Only the NLB female mean of 11.85 eggs/female/3 days differs significantly from that of the RNDA mean of 7.59 eggs/female/3 days (Tukey's test, alpha = 0.05). The other

pairwise comparisons for this age post eclosion were not found to be significantly different (alpha = 0.05, Tukey's test).

Table 7 further shows that at 42 days post eclosion both NL strains significantly exceeded both RND strains in mean fecundity: NLA=7.35 , NLB=6.66, RNDB=3.15, and RNDA 2.97 eggs/female/3 days (Tukey's test, alpha = 0.05, family confidence level p=0.95). The two RND means do not differ significantly from each other and the two NL means are in the same group. These backtransformed means differ very little from the untransformed means of Table 2. In that table one finds that the means are NLA=7.32, NLB=6.98, RNDB=2.90, and RNDA=2.82.

This relative size order and closeness to the untransformed mean values in Table 4 is of interest, because the analysis of variance was performed after transformation, and the means from this analysis were then backtransformed to construct Table 7 as described in the Statistical Methods section.

In the statistical methods section it was noted that the variances for egg means for ages 1 and 2 were heterogeneous even after transformation. Two nonparametric tests for significant pair differences were used to test differences between the fecundities of strains using the untransformed data for these two ages and for all the other parental age combinations. Although these other age combinations had previously been analyzed by parametric analysis of variance, these tests were used on the data for older flies to determine if the results differed from those obtained by the parametric test. All results for these nonparametric tests of pairwise differences is shown in Table 9.

These nonparametric tests revealed the same sets of significantly different fecundities for paired comparisons as did the parametric test procedure for age 1 day post eclosion. Table 9 shows that the NLA and NLB sets do not differ significantly (Wilcoxon two-sample test, Z=0.1471, p>|Z|=0.8331: Kruskal-Wallis test chi-square=0.02,p>chi-square=0.8808,df=1) and RNDA and RNDB do not differ significantly (Wilcoxon 2-sample test, Z=-0.5624, p>|Z|=0.5738: Kruskal-Wallis test, chi-square=0.32, p>chi-

square=0.5720, df=1). Table 9 shows that for all other pairwise combinations the two nonparametric test give highly significant differences for fecundities at day 1.

These two nonparametric tests applied to the two days post eclosion fecundity data also show a pattern of significant differences identical to that obtained when a parametric analysis of variance was done on the transformed egg data that continued to have heterogeneous variances after transformation (Bartlett's test and see statistical methods section). NLA and NLB fecundities (Table 7) do not differ significantly (Wilcoxon two-sample test, Z=-0.1447 p>|Z|=0.8849: Kruskal-Wallis test, chi-square=0.02, p>chi-square=0.8828, df=1). RNDA and RNDB fecundities do not differ significantly (Wilcoxon two-sample test, Z=0.7833, p>|Z|=0.4334: Kruskal-Wallis test, chi-square=0.62, p>chi-square=0.4319, df=1). All other pairwise combinations for the 4 strains at age 2 days (Table 7) do differ significantly. These results are also in agreement with those from the parametric analysis of variance on the transformed fecundity data with heterogeneous variances.

Results of Tukey's tests, using the GLM procedure of SAS, were also used to construct 95% family confidence intervals for those pairs of means that were found to differ significantly (see Table 8 and Statistical Methods section for details). Table 8 shows the upper and lower limits on the amount by which one mean fecundity exceeds the other mean fecundity of the significantly different pair of means. The pairs for which confidence intervals are shown in Table 8 correspond to the pairs shown to be different in Table 7.

In the statistical methods section the procedures used to analyze the mean individual proportion of adults, by strain, eclosing for all parental age combinations are presented. Further details can be found in Berenson, Levine, and Goldstein (1983, pp. 86-90).

Table 13 contains the statistics from the nested analysis of variance of the transformed fertility data, and Table 10 the grouped mean fertility proportions (alpha=0.05, 95% family confidence level, Scheffe's test), and Table 11 the 95% confidence intervals for those pairs determined to have significantly different mean proportions.

The total numbers of females, males, and adults for equal aged parents of all ages is found in Table 3 and for unequal ages in Table 5. It is important to note that the arcsin square root transformation used (see Statistical Methods section) for the analysis of variance gives different proportions than are obtained if one simply divides the total number of adults by the number of eggs for any strain and age in Tables 3 or 5. The conditions of the transformation used excluded from analysis all females without eggs, adjusted the proportion when a female had eggs, but no offspring, and adjusted the value when the number of eggs and adults was equal.

The effect of this transformation is twofold: (1) to spread proportions near 0 or 1 in the angular scale so as to increase their variance (Snedecor and Cochran, 1967, p. 328) before the analysis of variance is performed and (2) results in a different value for n, the number of data values in each data set, compared for many of the data sets. The SAS General Linear models procedure used takes this disparity in n values into account when the analysis is done using Scheffe's test. The procedure uses a harmonic mean of the cell sizes in the computation of the error (Berenson, Levine, and Goldstein, 1983, pp. 90-94). that is used to calculate the minimum significant difference.

Results of the analysis of variance of the mean fertility proportions on the transformed data are shown in Table 10. There are three parental age combinations that did not have significantly different mean proportions, 2 days (equal aged parents), 14 days (equal aged parents), and female age 42 days mated to 7 day old males (Scheffe's test, alpha=0.05). When the parental flies were both 42 days post eclosion, the RND mean proportions were not significantly different from each other (RNDA proportion = 0.1802, RNDB proportion = 0.1945), but differed significantly from the two NL strains (NLA proportion = 0.0835 and NLB proportion = 0.0838) that were not significantly different from each other (Scheffe's test, alpha = 0.05).

For the other ages no particular pattern is evident from Table 10, which shows the grouped mean fertility proportions, when the parental ages are equal. Table 11 shows the

95% family confidence limits as determined by Scheffe's test for the differences between the mean individual fertility proportions. The means compared in this table are those shown in Table 10.

Table 10.1 shows the results of an analysis, using SAS Proc GLM (the general linear models procedure), of the mean fertility proportions from Table 10. The first part of the table shows the results of an analysis of variance comparing the NL and RND fertility proportions, a comparison of all NL vs. all RND proportions, using a model that tested for age by strain interaction. The interaction term was not significant and from this it is legitimate to conclude that the two sets of mean fertility proportions regressed on age have homogeneous slopes. Thus a comparison of the the means of the two groups was made. A significant difference in the mean fertility proportions (RND=0.56331 vs. NL=0.44019, Tukey's test, alpha = 0.05, df =28, mean square error =0.02556) was detected. The second part of Table 10.1 shows the statistics for the two regression lines. Both lines have slopes that differ only in the fourth decimal place (NL=-0.01506 and RND=-0.01524), confirming the non significance of the interaction term, but the intercept of the NL line is less than that of the RND line (NL=0.6643, standard error=0.0684; RND=0.7900, standard error=0.0481). Table 10.1 also shows the results of a covariance analysis of the same data which is appropriate, because the lines have homogeneous slopes as shown in the first part of this table. The covariance analysis also shows that there is a significant difference in the mean fertility proportions (NL=0.44018, RND=0.56331, standard error=0.0393, p=0.0347). Thus the mean fertility proportion of the RND strains is significantly greater than that of the NL strains.

Figures 28a and 28b show the individual plots of the regression line of these two sets of mean fertility proportions on age with their 95% confidence intervals for the slopes and means. The same regression lines for both data sets are shown together in Figure 28c to illustrate the parallelism of the two regression lines that was established by the statistical analysis above.

It is also of interest to compare untransformed counts of adults for the various age combinations: for age 1 day post eclosion the total combined fertility of the RND flies exceeds that of the NL strains combined by 939% (374 RND adults vs. 36 NL adults), day 2 RND fertility exceeds that of NL by 33% (1,400 RND adults vs. 1052 NL adults), day 4 RND fertility exceeds that of NL by 82% (937 RND adults vs. 514 NL adults), day 7 RND exceeds NL by 293% (2,152 RND adults vs. 548 NL adults), day 14 RND exceeds NL fertility by 11% (724 rnd adults vs. 650 NL adults), day 21 RND exceeds NL by 91% (767 RND adults vs. 402 NL adults), day 28 RND exceeds NL by 5% (310 RND adults vs. 296 NL adults), and day 42 RND exceeds NL fertility by 24% (68 RND adults vs. 55 NL adults). Table 3 shows the data used for this summary.

For the ages 1, 2, 4, 7, 14, 21, 28, and 42 days, where the virgin parents mated were of the same age, the total number of adults for the two RND strains is 6,732 and for the NL strain 3,553. Thus there were 89% more RND than NL eclosed adults for these ages. From the same Table 3 that shows egg counts for these matings, the ratio of adults eclosed to eggs counted for NL flies is 3553 adults/8713 eggs = 41% and for RND strains is 6732 adults/11056 eggs = 61%. The 99% confidence interval for the proportion of NL eggs eclosed is 0.41±0.014 and that for RND eggs is 0.61±0.013 (Snedecor and Cochran, 1967, p. 210-211).

The numbers of eggs and eclosed adults for the combined strains from Tables 3 provide data for a comparison between selected (NL) and control (RND) flies at all test ages when the parental ages were equal. This is a comparison of the numbers of adults eclosed vs. the number of eggs laid. I subtracted the numbers of adults from the number of eggs laid and compared the RND and NL strains on the basis of eggs hatched vs. eggs unhatched for combined replicate totals of all the parental age combinations.

Chi-square tests comparing the strains on this basis show that, for all but one of the test ages, the number of eclosed eggs was significantly greater for the RND strains: for the day 1 flies the chi-square is 51.091 (p=0.0001); for day 2 the chi-square is 62.812

(p=0.0001); for day 4 chi-square = 25.335 (p=0.0001); for day 7 chi-square = 315.311 (p=0.0001); for day 14 chi-square = 21.95 (p=0.0001), for day 21 chi-square = 0.013 (p=0.9087, not significant), for day 28 chi-square = 18.536 (p=0.0.0001); for day 42 chi-square= 47.97 (p=0.0001). All these chi-square tests have 1 degree of freedom (Snedecor and Cochran,1967, p.216-217). This confirms the result of the comparative analysis of mean fertility proportions discussed above that compared the slopes of the regression lines and compared mean fertilities using covariance analysis.

I also wrote a simple SAS program to obtain the totals, for all parental age combinations, by strain and for combined replicates of the NL and RND strains, of the numbers of females that produced at least one eclosed offspring, but excluding females with eggs and no adult offspring. These totals are summarized in Table 14 and were used to compare the numbers of fertile matings of the strains at different ages. Chi-square tests of these numbers of females successfully mated from the NL and RND strains were done for all parental age combinations.

Table 15 shows these calculated chi-squares (with Yates correction term) for the entire set of ages where the number of females tested in each group is 120 (NL strains combined and RND strains combined) and the numbers mated are listed in the last column. For ages 1, 2, 4, and 7 the number of RND females mated was significantly greater than the number of NL females (age 1, chi-square = 58.033, p<0.0001; age 2, chi-square =9.12, p<0.0025; age 4, chi-square = 11.504, p<0.0007; age 7, chi-square = 40.587, p<0.0001- all comparisons with df=1).

At 14 days post eclosion (Table 13) there is no significant difference in the number of females mated (chi-square = 0.105 p=0.7462, df=1). For 21 days post eclosion a greater number of RND females mated than NL females (chi-square = 5.504, p<0.0246, df=1). However, non-significant differences (Table 13) in the number of mated females were also found at ages 28 (chi-square = 0.068, p=0.7942) and 42 days (chi-square = 0.628, p=0.4283) post eclosion for equal aged parents.

Experiment I C - Fertile mating, fecundity, and fertility of virgin flies of unequal ages:

Figure 18 includes the plots of the mean eggs/female/3 day±2 SEM for the two combinations of unequal parental ages for all 4 strains. For female flies, age 7 days, mated to males aged 42 days the fecundity means for the NLA and NLB strains do not appear to be very different, RNDA is high ; RNDB is intermediate. The statistical analysis (Table 7) suggests that NLB, NLA, and RNDB form a group with mean fecundities that do not differ significantly (alpha = 0.05, Tukey's test) and that the RNDA mean differs significantly from all three of these. Examination of Figure18 suggests nevertheless that the RND B strain mean is closer to RNDA than to either of the selected strains.

Figure 19 includes the total egg counts for this parental age combination. Combined fecundity for the two RND strains exceeds that of the combined NL strains by 101% (1,695 RND eggs vs. 843 NL eggs). This difference suggests that when the female parent is 7 days old and the male 42 days the RND fecundity is substantially greater than the NL fecundity.

When the female parent is 42 and the male is 7 days post eclosion, the means and confidence intervals appear not to differ except that RNDB appears to be lower than the other three (Figure 18). The analysis of variance and Tukey's test confirms this impression indicating that there are two groups of means (Tables 7 and 12). Combined total fecundities of the two NL strains exceeds that of the combined RND strains by 42% (435 NL eggs vs. 306 RND eggs) for this parental age combination.

As noted, Tables 7 and 12 show the results of nested analyses of variance for these two age combinations. When the female age is 7 days and the male 42 days, the fecundity means fall into two groups (Table 7, alpha = 0.05, Tukey's test). NLB fecundity is the greatest, but does not differ significantly from either NLA or RNDA. For this age combination the only significantly different pair of fecundity means are NLB and RNDB (alpha = 0.05, Tukey's test).

For the matings where the female age is 42 days and the male age is 7 days, the mean for RNDB flies differs significantly from that of the RNDA and NLB lines. The means for NLA, RNDA , and NLB do not differ significantly and there is no significant difference between the means of the RNDB and NLA strains (Table 7, alpha =0.05, Tukey's test).

Table 5 provides data for a comparison of the combined total fertilities of RND and NL strains for the matings where the females were 7 and the males 42 days old: RND fertility exceeds NL fertility by 131% (1259 RND adults vs. 544 NL adults). From the values in this table the ratio of adults to eggs in RND strains is 1,259 adults/1,695 eggs =76% and for the NL strains 544 adults/843 eggs = 65%.

Age 42 day RND females mated to 7 day old post eclosion males had 38 adult offspring and NL flies in the same age combination had 43 (Table 5). From these data and the egg totals in Table 3, the percent of adults from eggs for RND strains is 38 adults/435 eggs= 9% and for NL flies 43 adults/306 eggs= 14%. These data suggest that for strains selected for postponed senescence and for the control strains old females produce small numbers of viable eggs at this advanced age, despite insemination by young vigorous males.

The numbers of eggs and eclosed adults for the combined strains from Tables 5 provide data for a comparison between selected (NL) and control (RND) flies for the two test ages when the parental ages are unequal. This is a comparison of the number of adults eclosed vs. the number of eggs laid, replicates combined within strains. I subtracted the number of adults from the number of eggs laid and compared the RND and NL strains on the basis of eggs hatched vs. eggs unhatched for the combined replicate totals of these two parental age combinations.

The chi-square test comparing the number of eclosed adults for the females 7 days old mated to 42 day old males gives a chi-square = 35.815 (p=0.0001, df=1). This chi-square statistic shows a very significant difference in the numbers of RND and NL adults eclosed for this unequal parental age combination (Snedecor and Cochran,1967, p.216-217).

The chi-square test for the 42 day old females mated to 7 day old males the chi-square = 5.215 (p=0.0224, df=1). This chi-square statistic shows that significantly more RND than NL adults eclosed for this unequal parental age combination (Snedecor and Cochran,1967, p.216-217).

For the two mating combinations where the parents were of unequal ages there were no significant differences in the numbers of fertile matings: the 42 day old females mated to 7 day old males (chi-square = 0.192, p= 0.6610) and 7 day old females mated to 42 day old males (chi-square = 1.662, p = 0.1973; Tables 14 and 15).

Experiment II A -Geotaxis experiment with aging virgin flies:

For the analysis of the geotaxis experiment, only the data sets for ages 0, 3, 7, 14, 21, 28, and 35 days post eclosion, are considered for the reasons given in the statistical methods section. Analysis of variance results for regression of g_t (geotaxis score) on angle for each age, strain, and replicate showed that the linear regressions of geotaxis scores on angle were all significant for all test ages up to 35 days post eclosion (Table 16). The geotactic response of older virgin flies, as measured by the test procedure used, failed to produce data that can discriminate among strains.

Tables 17, 19, 20, 21, 22, and 23 present the results of a nested analysis of variance (replicates within strains) in SAS on the data sets from ages 0 to 35 days post eclosion . The model (see Statistical Methods section) tested for the homogeneity of slopes of the regression of geotaxis scores (g_t values) on angle, and for the significance of sex.

For ages 0 and 3 days post eclosion, sex was not found to be significant (F=2.09, Prob > F=0.1490, df=1,419), and F=2.07,Prob >F=0.1509, df=1,419) as shown in Table 17. From the same Table 17 the interaction between strain and angle is found not to be significant for these two ages (F=0.80, Prob > F=0.4932, (df=3,419), and F=0.74, Prob > F=0.5275, (df=3,419)).

The nonsignificance of the interaction term indicates that the regression lines are parallel. Plots of the regression lines for these two test ages are shown in Figures 20 and

21. Although there is no significant difference in the slopes by sex, the plots are shown by sex, because eight lines on one plot make the graphs difficult to read. It should also be noted that the plots in Figures 20, 21, and 22 do not show the coincidence or overlap of gt values for different strains. For every angle for each strain there were 6 g_t values (replicates combined) and in many cases the g_t values are the same number. These plots are specifically intended to show the parallelism of the lines for ages 0 and 3 (Figures 20, and 21) and the pattern of differences for age 35 days (Figure 22) and their relative positions.

The mean geotactic responses (g_t scores) for the four strains, sexes and replicates combined, with homogeneous slopes were compared at the test angle of 40 degrees for ages 0 and 3 days post eclosion. Table 17 shows the results of these comparisons of the strain means. At age 0 days post eclosion the group of the most negatively geotactic strains contains NLA (0.2287), RNDA (0.2843), and NLB (0.2852) flies, whereas RNDB (0.3713) is a separate group and is the least negatively geotactic strain (Tukey's test, alpha=0.05, df=420, m.s.e.= 0.04984) Thus one of the control strain means (RNDB) is significantly different from the other control and from the two selected strains. The other control strain mean is between the means of the two selected strains .

For the 3 day-old flies none of the means is significantly different from any other: RNDB (-0.2407), NLA (-0.1389), NLB (-0.1352), and RNDA (-0.0713) (Tukey's test, alpha = 0.05, df= 420, m.s.e. = 0.0533). All four strains are more negatively geotactic on day 3 than on day one post eclosion. For one day-old flies, all the means are positive, which indicates that less than half the flies were in the top of the tubes. For three day old flies, all the means are negative, which indicates that more than half the flies were in the top half of the tubes.

RNDB is the least negatively geotactic on day one post eclosion, but is the most negatively geotactic on day 3 post eclosion. RNDA is intermediate in response between NLA and NLB on day one and is the least negatively geotactic on day 3 post eclosion. Both the age 0 day and 3 day results therefore do not indicate a clear pattern of differences in

geotactic response between the RND(control) strains, and the NL strains, selected for a postponed senescence at the two earliest test ages post eclosion.

When the data for ages 7, 14, 21, 28, and 35 days post eclosion are tested for the significance of sex and for homogeneity of slopes (interaction between strain and angle) these factors have significant F values (Tables 19, 20, 21, 22, and 23). The F values and associated probabilities can be found in these tables. When the data for ages 7 through 35 are plotted for all sexes and strains, the significance of the interactions (nonparallelism of the regression lines) is clear, but the plots show no particular pattern of strain and sex differences from age 7 through 28 days post eclosion.

Because the fundamental purpose of the geotaxis experiment was to determine whether NL and RND strains differ in their responses early and late in life, the rest of the analysis is concerned with a comparison of geotactic responses at age 35 days post eclosion. The significant F values for angle by strain interaction ($F=33.96$, $df=3$, $p<0.0001$, Table 22) and that for sex ($F=94.60$, $df=1$, $p<0.0001$) require that the interstrain comparisons of responses be done differently than for the two early ages. At different levels of the independent variable, angle, the mean interstrain responses for that angle can be compared using a one way analysis of variance by sex.

Mean geotactic responses for the two test angles, 40 and 80 degrees, were chosen for comparison. A one-way analysis of variance was done for the responses at each of these angles by sex. Examination of Figure 22, regression plots of geotaxis scores on angle by sex, reveals that for females two strains have regression lines that show a greater mean negative geotactic response across all angles, that these two strains may have different mean responses from each other at each angle, and that they may differ in response from the two strains whose lines are at the top of the graph and are almost superimposed. The relationships between the means are more easily seen in Figures 23 and 24, which show plots of the means $\pm$ 2 standard errors for all strains at 40 and 80 degrees respectively. In Figure 23 there was no variability in the g_t scores for some of the

tested strains and thus no standard errors of the means are shown for those cases. These sets of means for the two test angles were compared by a one-way analysis of variance.

These plots (Figures 23 and 24) suggest that there might be significant differences between the means for at least some pairs of strains. The two graphs indicate that for both test angles the NLA males are much more negatively geotactic and for the greater angle, 80 degrees, the means ± 2 SEM of NL females and NL males do not overlap those of the RND strains.

The results of the one-way analyses of variance by sex and angle for day 35 are presented in Table 18. For the test angle 40 degrees NLB females (0.81667) are the most negatively geotactic and are significantly different from NLA (0.9667), RNDA (1.000), and RNDB (1.000), which constitute a separate group (Tukey's test, alpha = 0.05, mse=0.00417, df=16). For the test angle of 80 degrees, NLA (0.2667) and NLB (0.4500) are the most negatively geotactic, are significantly different from each other, and from the group RNDB (0.9333) and RNDA (0.9167), which are not significantly different from each other (Tukey's test, alpha=0.05, mse=0.00917, df=16). The NLB flies are significantly different from the two RND strains at both of these test angles.

The results of these analyses of variance for females indicate that the postponed senescence females have a significantly greater negative geotactic response than the RND females (controls) at the angle that is the greatest stimulus, 80 degrees (Table 17, Tukey's test, alpha=0.05, 95% family confidence intervals).

Mean geotactic responses for NL and RND males at 35 days post eclosion were compared in the same way as for the females for the test angles of 40 and 80 degrees. Figure 22 shows that males of two strains had very similar responses and that the males of the other two strains had responses for all test angles that were greater for greater angles, but are possibly significantly different at the two test angles chosen for analysis.

Figure 23 shows the mean responses ± 2 SEM for the males at 40 degrees and Figure 24 for 80 degrees. In both plots the greatest mean negative geotactic response is that of

the NLA males. Table 18 shows that the mean NLA response for 40 degrees (-0.1833) is significantly different from that of NLB (0.8667), RNDB (0.9500) and RNDA (1.000) males, all three of which are not significantly different from each other in mean geotactic response (Tukey's test, alpha=0.05, df=16, mse=0.0204).

One-way analysis of variance of the mean male geotactic responses to the test angle of 80 degrees showed that the mean for NLA males (-0.6833) was significantly different from NLB (0.5167) and that both NL male mean geotactic responses are significantly different from the two RND strains (RNDA = 0.7833; RNDB = 0.9167), which were not significantly different in mean geotactic response from each other (Tukey's test, alpha=0.05, df=16, mse=0.0163).

Experiment II B - Survivorship of virgin flies from the geotaxis experiment:

Plots of the survivorship of flies used in the geotaxis experiment are shown in Figures 25, 26, and 27. Examination of these plots shows a sharp initial drop in the percent alive for all strains and sexes at young ages. Due to an error in recording the data, the first and second survivorship counts were made at 7 and 14 days post eclosion. There after the counts were made at 3 or 4 day intervals. These early deaths probably occurred because of changes in the food medium over these longer initial time intervals at the beginning of the experiment.

The plots of survivorship for the geotaxis experiment females (Figure26) show that NLB females did not exceed the RND strains in maximum life span, and that the NLA females from day 48 to day 85 survived in greater numbers than NLB, RNDA, and RNDB females. Table 2 shows that the LT 50 for NL geotaxis females combined is 55 days vs. 45 days for RND females, but that maximum survivorship, for combined postponed-senescence NL females and RND females, was 85 days.

Figure 27 shows plots of the survivorship distributions of males from the geotaxis experiment. These graphs show that both sets of NL males survivorships exceeded those

of both RND male sets. Table 2 shows that the LT 50 for NL males was 60 days vs. 42 days for RND males and the LT 90 for NL males was 76 days vs. 61 days for the RND males. At 35 days the number of NLA males alive was less then that of the RNDB strain and greater than that of the RNDA strain; however, from day 48 until all flies had died, the NLA males consistently outsurvived both RND strains (Figure27). NLB males survived in greater numbers than the other 3 strains from day 28 (Figure27) until all the flies died.

Chapter 5

Discussion

An examination of the theoretical literature on the evolution of senescence might well convince one that as Horn (1978, p. 411) noted " many of the important papers are unintelligible even to the authors of other important papers." The often-cited paper of Hamilton (1962) and Charlesworth's book *Evolution in Age-Structured Populations* (1980) serve as examples of this genre. Despite the mathematical complexities of these theoretical treatments, however, testable hypotheses have been developed. As Rose and Graves (Rose and Graves,1989, p.B28) have noted "to an evolutionary biologist, there is no need for any other general theory of aging. If other biologists could also accept the sufficiency of the evolutionary theory as *the* general theory of aging, then there might be a relaxation of efforts to find general physiological theories of aging." This is a somewhat extreme view . Despite the extensive mathematical formalism of such works, the evolutionary theories of aging derived by the authors of the theoretical papers they do have testable hypotheses. These hypotheses were first set forth in a very clear way by G.C. Williams (1957), and his theory of aging is often called the "antagonistic pleiotropy " theory. His theory is applicable to organisms with a soma that is "essential to reproductive success but no part of which is passed on in either sexual or asexual reproduction (Williams,1957,p.400)."

One of Williams' fundamental predictions was that any successful selection for increased longevity should result in decreased "vigor" in youth. Williams argued that genes favored by natural selection, because they increased fitness early in life, might have opposing detrimental effects later, and that other modifier genes might have evolved that interacted with these major genes. The genes with opposing effects early and late in life could have different alleles, as could their modifiers, according to Williams (1957). These modifier genes would "reduce or delay the unfavorable effects" and therefore would be selected, because senescence is an "unfavorable character" and

the "direct action of selection will always be opposed to it (Williams, 1957, p. 402)." As Williams (1957, p.404) noted, the "death rates of young adults are usually such that very few individuals will live long enough to suffer any gross debilitation through the process of senescence." He also argued that the "critical comparison would be between organisms that have approximately similar life cycles except for adult mortality rates (Williams, 1957,p. 404)."

A key prediction of Williams for the interpretation of the results of the experiments reported here is that "successful selection for increased longevity should result in decreased vigor in youth (Williams,1957,p.410)." Lints et al. (1979) attempted to select for increased longevity in Drosophila melanogaster and failed, but later Luckinbill et al. (1984) and Rose (1984a) succeeded. Furthermore these authors argued that Lints' strains of Drosophila melanogaster were too highly inbred and that he had controlled the density of the developing larvae. Giesel (1979) had also sought to test the predictions of Williams and found all life-history characters to be positively correlated in opposition to Williams' prediction of negative correlations between gene effects on different life-history characters early and late in the life span of a species (Rose, 1983, p.20). Rose (1983, p.21) cogently argued the case for a theory of senescence that does not "preclude any particular set of developmental constraints. All it does is characterize the patterns of allele-action which could predominate among evolving populations using elementary selection theory and that is a theory tantamount to neo-Darwinism, nothing less."

The other major evolutionary theory of aging hypothesizes that "there could be alleles that have strictly age specific effects (Rose and Graves,1989, p. B27) that could have positive effects on fitness at early ages and opposed effects at later ages." Natural selection would not eliminate these age of action alleles, because their negative effects would only be manifested at ages late in or beyond the reproductive time schedule of a population. This theory has come to be known as the "mutation accumulation"

hypothesis. (Hamilton, 1966; Edney and Gill, 1968; Rose and Charlesworth, 1980). As Rose and Graves (1989, p.B27) noted, an important corollary of this theory is "that selection, artificial or natural, should be able to enhance later functions without any early costs or impairment." This hypothesis does not require that there be any significant differences in the mean values of life history characters, fitness traits, early in the life spans of populations with different age of action alleles, but does predict that there will be differences between populations of the same chronological age at later times.

These two genetic evolutionary theories are not mutually exclusive explanations of the aging process and it is thus possible that one or both mechanisms could be operative in an evolving population subject to natural or artificial selection over the same time interval if the population was sufficiently heterogeneous.

As was noted in the Introduction and Background section above another prominent theory is "the rate of living theory" that originated with the work of Pearl (1928). This theory predicts that populations age at different rates, because of intrinsic mechanisms that may interact with the environment. Under this hypothesis two or more populations of the same species may be found to have the same pattern of changes in functional loss, however one population may show a compression of this pattern of changes over time.

Experiment I A - Survivorship of virgin flies:

The results of the survivorship experiment reported here for the four strains reared under conditions very much like those described in Luckinbill et al. (1984), except for a different food medium, show that the populations founded from the offspring of old females outlived the control populations. Artificial laboratory selection has continued to maintain whatever allelic differences contribute to a significantly longer life span in the two strains selected for a postponed senescence (NLA and NLB vs. RNDA and RNDB). All the flies used to set up successive new generations of the two NL strains have been

offspring of females that were at least 55 days post eclosion. The RNDA and RNDB strains were always set up from culture bottles where the parents were never more than 30 days post eclosion. During the maintenance of the NL strains no deliberate attempt was made to select for the maximum possible life span of these strains, nevertheless statistically significant differences in the survivorship distributions of selected and control lines exist..

If time had permitted and the life span had been measured every few generations there might have been some variation in the longevities from generation to generation, as was found by Luckinbill et al. (1984). The number of replicates required in the mating experiments made it logistically impossible to do several survivorship experiments. The results of the survivorship experiment reported here were obtained when approximately two thirds of the mating data had been collected. To date no survivorship data for virgin D. melanogaster selected for late reproduction have been published. The experiments previously reported have used flies mated at young ages only as noted previously.

The statistical analysis of the survivorship experiment data showed that selected strain virgin males live significantly longer than the selected strain virgin females. An explanation for this finding may be found in earlier work on survivorship in D. melanogaster.

It is often believed that the females of most species, including Drosophila, outlive the males, but Lints et al.(1983) have shown that the literature on Drosophila survivorship proves the contrary. Their survey of 18 papers and 228 comparisons published in Experimental Gerontology up to 1981 found that in 128 cases of 228 (56%), male Drosophila outlived females, but their calculations also showed that the potential or maximal life span of females exceeds that of the male. These authors further argued that "when the proportion of senescent deaths is identical for both sexes the mean life span of females will be higher than the males (Lints et al, 1983, p. 346)."

Lamb (1978) in an extensive review of the research on aging in Drosophila noted that methods of rearing and keeping flies cause such variability in the reports of survivorships of flies by different laboratories. Because of environmental differences and handling procedures, mean life spans often differ by a factor of two within the same strain when measured in different laboratories (Lamb, 1978). She thus contends that no standard life table for any strain, mutant, or species is possible. A factor of importance in the survivorships reported here is that the density of the flies in the vials was not uniform throughout the experiment. In this experiment the density decreased as the flies died. As Lamb (1978) points out experiments have repeatedly shown that density is an important factor for survivorship and that the old and young flies may not be exposed to the same stress in an experiment where dead flies are not replaced.

In the original work on life span in these flies (Luckinbill et al, 1974) the flies were kept as male-female pairs in vials and when either sex died a replacement fly of the appropriate sex was added to the vial. In the survivorship experiment reported here, the virgin flies were started off as sets of 30 flies of the same sex and the density was not kept constant as the flies died. Lamb mentions an interesting study by Bilewicz in 1953 "that showed virgin female D. melanogaster lived approximately twice as long as mated females, and suggested that this difference was correlated with the increase in the rate of egg production which followed mating (Lamb, 1978, p. 51)." Thus the different survivorships could be in part explained in part by differences in the densities of the strains and sexes over time. It is possible that the selected line males are the least sensitive to the decreases in density over time and thus live longer than the selected strain females and both sexes of control flies. It could also be true that both sexes of the control strains are equally sensitive to density changes over time which could in part explain the non-significance of the difference in their cumulative survivorship distributions.

Lamb (1978, p.51) also noted that a survey of the literature reveals a complicated relationship between fecundity, environmental conditions, and longevity of female flies and that there is much evidence "of a negative relationship between longevity and egg production." In Table I of Lamb (1978) the mean life span of D. melanogaster is reported as 36.5 days (sexes combined) a value not far from that of the LT 50's of the RND strains used in the survivorship experiment reported here (Table 2: RND females = 37 days and RND males = 38 days). Buzzati-Traverso (Buzzati-Traverso, 1955, Table 8, p. 167), working with 10 groups of 50 Drosophila melanogaster, reported Oregon R adult mean life span to be 30.37 days for 466 flies, the remaining flies escaped. He also determined that longevity was not selected for directly in a competitive environment and that it was not selected as a "correlated response of the improvement in fecundity (ibid., p. 168)."

In a study of heterosexual activity in D. melanogaster males Crowell and Herskowitz (1957) found that (1) mated males had a mean life span of 51.0 days and unmated males of 56.8 days (2) mated males on average lived 90% as long as unmated males, but after two months 23.6% of the mated vs. 20.2% of the unmated males were still alive.

Malick and Kidwell (1966) found that the mating status, breeding type, and the isogenic line used all had significant effects on the longevity of D. melanogaster and that unmated flies generally lived longer than mated ones. They interpreted their results to suggest that strain differences, species, and a spectrum of environmental factors, combined with laboratory techniques, contributed to the conflicting values reported for life span in D. melanogaster .

Partridge and Farquhar (1981) showed that sexual activity reduces the life span of male D. melanogaster. Although in this species the only contribution of the male to reproduction is a gamete, their work demonstrated a "cost to reproduction" for males. Their data showed that virgin males had a median life span of 65 days, single males with

one virgin female a median of 56 days, and single males with 8 virgins a median of 40 days. For the male, Partridge (1981) suggested, the reproductive costs include sperm production, synthesis of seminal fluid, and intense muscular action during mating.

Fowler and Partridge (1989) have recently shown that there is also a cost of reproduction that reduces female life span in D. melanogaster that do not differ in rates of egg-production and egg-fertility. Their results indicated that remating did not increase reproductive output, and no increase in their age-specific progeny production was detected in remated females, but mated female life spans were significantly shortened relative to control females.

The difference in the survivorship distributions reported here may thus be due in part to the virgin status of the populations of the flies. Significantly greater survivorship of NL males relative to NL females and to both sexes of RND, control flies, supports the hypothesis of a "cost to reproduction" for males, of the NL strains used here, of the kind hypothesized by Partridge and Farquhar (1981), Partridge (1986), Partridge and Harvey (1985). As Partridge and Harvey (1985, p.20) note the "general conclusion from properly conducted experiments is that reproduction does have costs; genetic variation has been found in the few cases where it has been looked for, thus providing the raw material for the evolution of reproductive trade-offs." These authors cite the work of Luckinbill et al. (1984) as one of the experimental proofs that there is genetic variation for reproductive fitness characters that can be manipulated in the laboratory or be subject to natural selection in the wild. Another factor, that shortens life span, is the "risk" associated with the copulatory activity for male fruit flies (Partridge and Andrews, 1985). The significantly greater survivorship of the NL male virgins relative to NL females may have also been due to the absence of this "risk". For the selected strain, NL, females there may be costs to mating (Fowler and Partridge, 1989), but the data from the survivorship experiment presented here do not make a quantitative

determination of the costs as in the case of the NL males relative to both sexes of the control strains.

Luckinbill et al. (1984) compared life spans of flies selected for late reproduction to strains selected for early reproduction. The RND strains used in the experiment reported here were also described by Arking (1987) along with further data on the NL strains, but the data were only for females. In Arking's paper the strains were designated as NDC and R respectively. The LT 10 = 57.0, LT 50 = 74.0 and LT 90=104 days values reported by Arking for the 25th generation of the NDC-L (corresponding to the NLA and NLB strains here), are all greater than those reported here (Table 2), but the values for the control R strains were not very different. As was noted above, the selection pressure in this laboratory has not been very strong, but despite the lower LT values, the NL strains nevertheless had survivorship distributions for both sexes that differed very significantly from the those of the control flies here.

The significant differences in survivorships between the NL and RND strains reported here suggest that these strains from the same ancestral base population (Luckinbill et al., 1984) differ in the frequencies of whatever alleles determine longevity, despite the different laboratory conditions and the fact that virgin, not mated survivorship, was measured in this laboratory. The experiments on stored virgin D. melanogaster reported here show that in more than one replicate the virgin males of the NL strains outlived the males of the RND strains (Fig. 3, Table 1) and the females of the NL strains outlive the RND females (Fig. 2, Table 1).

A recent report on the patterns of amino acid incorporation in these strains (Pretzlaff and Arking, 1989) included data on the life span parameters of adult flies. In this paper the measured LT 10, LT 50, LT 90, LT 100, and mean life spans of flies reared under high density conditions, 10 pairs per vial and 5 vials per strain, showed that male flies outlived female flies (Pretzlaff and Arking, 1989, Table 1, p. 71) in both the NL and RND strains. These were not virgin flies and when flies died, the density was kept constant

by adding ebony replacement flies. The LT values in Table 1 of their paper are in very good agreement with the values found in the experiment here (Table 2) with virgin flies and decreasing densities over time, with the exception of the control males. Control males lived longer than control females according to Pretzlaff and Arking (1989), but in this laboratory a Kolmogorov Smirnov two-sided test (Table 1) established that the cumulative survivorship distributions did not differ for RND males and females. Pretzlaff and Arking used t-tests to determine significant differences of mean survivorship.

The significantly greater survivorship of NL males relative to NL females was not anticipated. There was no significant difference for RND females and males. This result with virgin RND flies is not in agreement with those of Pretzlaff and Arking (1989). Selection strain virgin males may live significantly longer than selection strain virgin females, because in the absence of sexual activity the males may save more of the energy that goes into reproductive activity as suggested by Partridge (1981) than females. This suggests that the "cost of reproduction" for the selected strain males is greater than for the control strain males, because the control strain males did not live significantly longer than control females, however the selected strain males lived significantly longer than selected strain females and both sexes of control flies.The relative virgin survivorship results reported here are in agreement with the "rate of living" hypothesis (Pearl, 1928) . The form of the survivorship plots (Figure 1) of the RND flies, both sexes, and the non-significant difference of these cumulative survivorship distributions suggest that both sexes of the control flies aged at the same rate. The plot of selected strain virgin male cumulative survivorship appears to have the same form as that of the selected strain females after day 33 post eclosion, but to be shifted to the right for greater ages (Figure 1). Further, the plots for both sexes of the selected strains appear to be shifted to right, and to have a very similar appearance to the plots of both sexes of the control flies after approximately 70% of the flies had died in all four groups.

A combination of factors could thus contribute to the differences in the cumulative survivorships reported here: differential density sensitivities, mating status, and the conservation of resources that would ordinarily be allocated to reproductive activity.

Further experiments of the type reported by Kosuda (1985), Fowler and Partridge (1989), Partridge (1980), Fowler and Partridge (1989) would help to answer the questions raised by the differences in female and male virgin survivorships. Experiments of this type could use selection and control strains to extract information on the costs of reproduction in these strains and their relationships to longevity.

Experiment I B - Fertile mating, fecundity, and fertility of virgin flies of the same age:

The interstrain mean and total fecundity differences for all test ages where the parents were the same age lend support to the antagonistic pleiotropy theory for the evolution of senescence. Both control strains had significantly higher mean fecundities at early age: 1, 2, 4, and 7 days post eclosion than the two selection strains. At a late age, 42 days post eclosion, both control strains had significantly lower mean fecundities.

Young flies from the two selected strains , ages 1, 2, 4, and 7 days, had significantly lower means than the two control strains of these ages. No significant difference in mean individual 3-day fecundity between strains was found at ages 14 and 21 days post eclosion. When the flies were 28 days post eclosion, one only of the means, NL strain, differed significantly from one of the control means, NLB vs. RNDA, but the relative magnitude of the means shows that this age post eclosion, the selection lines both have greater mean 3 day mean fecundities than either of the control strains (Table 7).

In the case of the "old" flies, 42 days post eclosion, the mean fecundities of both of the NL strains were significantly greater than the means both of the RND (control) strains (Table 7). The two NL and the two RND strain means were found to belong to significantly different sets of means.

Thus the strains selected for postponed senescence have lower mean fecundities early in their life spans, their mean fecundities by days 14 and 21 post eclosion are the same as the controls, and at a late age, 42 days post eclosion, both of the selected strains have significantly greater mean fecundities than either of the control strains. These differences in mean fecundities support the hypothesis of Williams (1957), who predicted lower reproductive output, at early ages, in a population subjected to either natural or artificial selection for postponed senescence relative to a population with a shorter life span and a higher relative reproductive output at later ages.

In the earlier work Luckinbill et al., (1984) comparing the fecundities of strains from the same base population as the strains used here, the comparison was made between lines selected for early and late reproduction. In the experiments reported here the comparison is between two control strains and two strains selected for late reproduction. In their 1984 paper, Luckinbill et al., used female flies mated when young and kept with males or replacement males until reproduction had ceased and the female flies died. In the experiment here, each of the age comparisons is based on data for different sets of females stored as virgins and that mated, if at all, only within a 24 hour interval. Furthermore, the large number of replicates were collected and processed over a period of 16 months. It is of considerable interest that despite these several differences in experimental design and approach, the predictions of the hypothesis are supported by the pattern, over time, of significant differences between selected and control strain means reported here. The protocol for establishing new RND generations, in theory at least, is intended to maintain the original populational genetic variation of the flies caught in the wild. The comparison here may be thought of as one between the wild population and another derived from it by selection carrying alleles for life-history characters in different proportions.

The mean individual fecundity values for strains do not reveal the totality of the significance of the differences, because an individual whose fecundity is at the mean of

a population does not necessarily have the highest Darwinian fitness relative to all the other individuals in that population. Because the comparison is between populations, it is quite likely that there are flies from each of the selected lines that have the same alleles that influence fecundity that have a higher frequency in the control lines and vice versa Thus it is useful to examine the percentage differences in total fecundities which are additional evidence in support of the correctness of the antagonistic pleiotropy theory as an explanation of the results reported here.

For the test days (1, 2, 4, and 7) the total fecundities of the RND strains were consistently higher than were those of the NL strains, but the percentage differences varied over these different test ages. The greatest difference found, RND total fecundity 375% greater than NL, was for the day 1 post eclosion flies. The next three ages post eclosion showed RND total fecundity to be 49%, 113%, and 143% greater than NL total fecundity. Thus there was a substantial drop in the relative magnitude of the percentage difference at age 2 days.

The mean fecundities reported here for the NL strains for these four early test ages showed an increase from day 1 to the day 2 values, a decrease from day 2 to the day 4 values, and an increase in the mean values from days 4 to 7 post eclosion (Table 5). Luckinbill et al. (1984) also found oscillations in the mean and total fecundities of young flies from the strains selected for a postponed senescence although their flies were mated and monitored from the time of mating. More recently Pretzlaff and Arking (1989, Fig. 4, p. 76) measured fecundities for selected and control strains of flies. Their figures show oscillations in the mean number of eggs laid per 3 day period for both strains until approximately day 22 for the controls and day 31 for the selected strain. Mean 3-day fecundities then decline exponentially for both strains until approximately day 53 post eclosion. After this their R strain control means fall quickly toward zero, but their selection strains have two more peaks, one at about 58 days and another at about 73

days, after which the means for these strains fall exponentially toward zero at around 90 days.

In pilot experiments here on mating of virgin flies aged 55-60 days post eclosion, the number of virgin females that could mate was very small in the selection and control strains and the number of control flies of a stored population of virgins very small. Because of these preliminary observations, the maximum test age of 42 days post eclosion was chosen for this experiment. Some workers (Kosuda, 1985) consider a 28-day-old fly to be an "old" fly. As has been pointed out above, measured strain longevities vary considerably from laboratory to laboratory and within the same laboratory at different times. Butz and Hayden (1962) found that 40 day old male-female pairs of D. melanogaster did not produce offspring.

At this point the results of some other studies on aging and fecundity may provide hints as to the biological processes in D. melanogaster that control and regulate fecundity as the females of this species age. These results from other laboratories provide some clues as to why the fecundities of the virgin females of various ages in the experiment here were somewhat low when compared to those from flies of the same base population as Luckinbill et al. (1984) and Pretzlaff and Arking (1989).

Courtright, Sonstein, and Kumaran (1985) studied the age specific regulation of vitellogenic activity in Drosophila melanogaster. They found lower rates of incorporation of labeled amino acids into three yolk proteins (YP1, YP2, and YP3) in older female flies, but that aging does not affect the amount of YP1 transcript present in normal flies. Further, their data showed that senescence, defined as a decline in fecundity, is correlated with a decline in vitellogenesis. Data from other experiments reported in their paper suggest that there may be changes in the activity of regulatory genes as flies age.

More recently Pretzlaff and Arking (1989) showed that females of longer, selected strains, and shorter lived control strains, from the same base population as the flies used here, have a similar pattern of amino acid incorporation *in vivo*, but that the onset of the decline is delayed by "10 to 20 days in the long-lived animals." Examination of the plots for the age-related incorporation of amino acids for females and males shows that control females have higher rates of incorporation until 25 days post eclosion and the males until about 27 days post eclosion (Pretzlaff and Arking,1989, Figs. 2 and 3). The differences found for females were, however, significant only for day 1 and day 50. For the males the only significant difference found was that for 10-day-old flies when the rate for the control strain mean value was significantly greater than for the selected strain males. These authors concluded that the strains " differ form each other mainly in the pattern and the timing of their decrease of protein synthesis (Pretzlaff and Arking, 1989, p.78)."

Malick and Kidwell (1966) found that mating status, breeding type and the isogenic line used had significant effects on longevity. Hoffmann and Harshman (1985) determined that the presence of males had a stimulatory effect on oviposition and that vials preconditioned by the presence of males had a residual effect on female fecundity resulting in 22% more eggs in conditioned than control vials. It is also known that females kept with males have higher fecundities than females kept in isolation (Hoffmann and Harshman, 1985).

Kosuda (Kosuda, 1985) has shown that male D. melanogaster virility, measured as the mean number of females mated per male, shows a more than twofold increase in genetic variability between 3 and 28 days post eclosion. His data also showed that some young males mated with as many as 12 females in 24 hours. 'The far greater coefficients of variation for old males, 28 days post eclosion, were interpreted by Kosuda as an indication of the expression of deleterious genes at an older age. Kosuda's result is in agreement with the "mutation accumulation" hypothesis in that it

suggests that different males have different alleles which determine the differences in virility at older ages.

Narain (Narain et al., 1962) found very wide fluctuations in mean fecundities from generation to generation even in the total absence of selection. In another experiment using virgin flies (Narain, 1962) the critical time for comparison of fecundities was found to be from days 6 to 8 post eclosion. A mean lifetime fecundity mean of 52.5 eggs per day was calculated (Narain, 1962).

In a study on viability and longevity in D. melanogaster (Cannon, 1966) it was found that Oregon R flies lived approximately 9 days in population cages that were a modified version of those used by Buzzati-Traverso (1955) designed to simulate the process of natural selection.

The experiments of Long, Markow, and Yaeger (1980) showed that male mating success increased as males aged. Virgin males, Canton S strain, were found to have 50% mating success at 2 days, 64% at 4 days and 66% at 8 days post eclosion. Virgin males were found in this study to have a statistically significant advantage over males that had mated previously.

Obin, Vander Meer and Ehrman (1988, p.140) comment that mate finding, courtship, and oviposition, components of sexual behavior, all provide "reliable indicators of particular age-associated changes at the cellular, organ, and organ system levels."

The work of Wilson, King and Lowry (1955) showed that virgin females were not as fecund as mated females in D. melanogaster tu^{W} mutants. This result was confirmed by McMillan et al. (1970b) who suggested that mating acts "possibly as a trigger to stimulate oviposition" and that it is possible that in "in the absence of the mating stimulus, oviposition does not commence until the ovary contains all the mature eggs that it can hold (ibid, p.364)."

Delayed mating has not been an extensively explored area of Drosophila biology, but Patterson et al. showed in 1932 that "retention of mature eggs in the ovary greatly retards development of the younger eggs in D. melanogaster (Patterson, Brewster, and Winchester, 1932)."

Examination of the spindle fiber regions of eggs from virgins stored and then mated at 6 days shows increased levels of recombination events in the first eggs laid after mating, but the recovery to normal levels occurs soon after matings (Redfield, 1966). Redfield also found that delayed mating is associated with (1) changes in the normal rates of egg production, egg deposition, and egg mortality, all of which are affected by the vitality of each sex, the age of the male, and the number of males present in the mating chamber. This report is of interest here, because during the process of counting eggs from the many replicates and ages, the number of eggs generally appeared to increase on the second and third days. The pooling of the data over the 3 days, for each individual, for the present analysis prevents a formal statistical test of this impression.

Jallon (1984) presented some of the finest biochemical research on the sexual activity of D. melanogaster. He showed that cuticular hydrocarbons in both sexes act as pheromones and aphrodisiacs and are produced in different quantities while the gonads and the neuroendocrine systems of the flies mature. His results showed that male peptides modify female behavior, that they stimulate virgin female egg production, and that if these compounds are injected into females the rate of oviposition is increased. In D. melanogaster sexual activity, Jallon discovered peptide compounds that are transferred from male to female which control female receptivity and egg laying.

The somewhat low values for the observed differences in mean fecundities and total fecundities reported here for delayed mating of virgin females may have been due to absence of these compounds at some critical time in the life history of the virgin females that were prevented from mating for up to 42 days post eclosion. Results of

Redfield (1966) suggest that to some extent the effects of sexual isolation can be overcome, in that females begin to lay normal eggs after mating. Such a pattern of recovery may be different in the NL and RND strains used in the mating experiment. If females are kept isolated from males for long periods of time relative to the time that they mature sexually, it is quite possible that they miss important chemical signals and behavioral stimuli necessary for fecundities quantitatively equivalent to those observed in flies mated early in their life spans and kept with males until they die.

The design of the mating experiment made it impossible to determine the number of males that mated, the number of multiple matings by individual males, or if some females were inseminated by more than one male. Veiulle and Marzeau (1988, p.400) concluded that "vigor acts on sexual success in neither sex, or at most in one. In no way does it play a simultaneous role in males and in females." Levels of male vigor by age and strain, or mating success, had not been determined in this experiment.

Hedrick and Murray (1983) in a major review of the research on selection and fitness in Drosophila, noted that selection occurs at many different stages in the life cycle. In Table II of their paper, they list many of the "intrinsic and extrinsic factors that potentially affect different components of selection (Hedrick and Murray, 1983)." Several of the "intrinsic" factors may have had different effects in the control and selected strains in this experiment: (1) sexual factors such as age, mating speed, mating experience, female receptivity, male vigor, and pheromonal differences in males; (2) factors effecting fecundity such as age, adult nutritional status, egg production ability, and sperm production ability. Their Table II also lists extrinsic factors that could influence the fecundity and fertility of the strains differently: temperature, humidity, light, population density, frequency of different genotypes, mating substrate, and the sex ratio. Most of the extrinsic factors listed were controlled in this experiment, but very little is known about the sexual factors in these strains.

Control strain females from single pair matings of flies placed together soon after "eclosion was complete" (Luckinbill et al., 1984, p.998) lay 22-24% more eggs during the third to fifth days of their lives (Clare and Luckinbill, 1985) than do the strains selected for late reproduction. In the mating experiment here, control strains combined laid 33% more eggs in the interval from day 2 to day 5. Such direct comparisons are probably misleading, because the flies here were not from single pair matings and did not have the various stimuli provided by males discussed above that were present in their experimental design.

Comparative analysis of the mean fertility proportions presented here suggests that the RND strain females are more fertile at all ages than NL strain females. The homogeneity of the slopes of the two regression lines suggests that the rate of decline over time is the same for selected and control females, however covariance analysis indicates that the control flies have a significantly higher mean fertility at all ages.

This result is not in agreement with the antagonistic pleiotropy hypothesis, because the control, shorter lived flies, have higher mean fertilities at both early and late ages than the selected strain females. It is also not in agreement with the "mutation accumulation" hypothesis, because under that hypothesis no differences would exist at the younger ages and differences would only be detected at later ages as a consequence of the action of "age of onset" genes, influencing fertility, of the kinds previously discussed.

This conclusion is supported by the results of the chi-square analysis of the differences in the number of control adults that eclosed which showed that, for all ages except day 21 post eclosion, the number of adults that eclosed from control females was significantly greater than from the selection strain. These data thus also suggest that the control females in this experiment had higher mean and total fertilities than did the selection strains, despite their shorter life spans.

These data on fertility suggest that, for delayed mating, the productivity of the selection strains is much less than that of the control strains. As has been noted by Cannon (1966, p. 130) " A population with greater productivity in relation to longevity is considered to have a higher evolutionary potential than one with lower productivity and longer longevity." Cannon regarded the total number of a population as a measure of its fitness (1966, p. 128). Delayed mating of the NL strains results in a significantly lower productivity than in the RND strains. Total RND strain eggs (11,056) exceeded total NL eggs (8,713) by 27% and RND adults (6,732) exceeded NL adults (3,553) by 89%. The significantly greater numbers of eclosed adults from the RND strains at all ages, except 21 days post eclosion, strongly suggests that the fitness of the control strains relative to the selected strains is greater when mating is postponed.

Luckinbill et al. (1984, p. 998) indicate that fertility was "estimated" from counts of eggs that remained unhatched, but they do not present this data in their paper and thus it is difficult to know if it also supported Williams (1957) theory.

Luckinbill et al (1984, p. 1001) measured longevity and fecundity concluding that "the effects of this selection may be manifested in a wide variety of life history features." These authors assumed that early fecundity "should accurately reflect what Williams termed 'early vigor'." The data reported here, for fecundity from the mating experiment on virgins of equal age, lends further support to Williams (1957) theory for the evolution of senescence.. The chi-square tests comparing the numbers fertile matings, in the selected and control strains, over all test ages when the parental ages are equal showed (see Results Section above) that more RND females mated at all early ages (1, 2, 4, and 7 days post eclosion) and that for older flies nonsignificant differences existed on for test ages 14, 28, and 42 days post eclosion. These results also suggest that the control females may have a greater ability to recover from the absence of the stimuli provided by males, at younger ages, or that they may react differently when they can exercise "mate choice". In single pair matings, the number of flies mated might be quite

different if (1) the flies were with males from the time of eclosion or (2) they were older virgins with only one male to "choose" from.

When the selected and control lines are mated early in their life spans, the mean daily egg production of the controls is greater than that of the selected strains, however the lifetime mean egg output of the selected strains exceeds that of the controls, because of their substantially greater mean life span (Arking, 1987, Fig.7, p.213). Data presented here suggest that the lifetime egg output of virgin control females greatly exceeds that of selected line virgins. As has been noted above, the fecundity and fertility measurements reported here are not directly comparable to those obtained for flies mated early and kept with males until they die.

Recent results have been obtained (Arking et al. 1988) on the metabolic rates for strains of Drosophila melanogaster selected for a postponed senescence and control strains, all derived from the same base population of flies used in the experiments reported here. It was found that the mean daily metabolic rates of the different strains are not very different. This would suggest that the rate of living theory (Pearl, 1928) as originally stated does not fully explain the observed significant differences between strains found here. As Arking et al. (1988) noted this theory has recently been revised to include aspects of the free radical theory of aging. His laboratory has recently been investigating whether there are interstrain differences in superoxide dismutase activity that support this theory. Arking (personal communication) has indicated that a preliminary analysis of the data suggests that there are interstrain differences in the activity levels of this enzyme at older ages such that longer lived flies have higher activity levels of this enzyme at later ages than control flies.

Recent work has succeeded in determining the chromosome on which the genes are located that are responsible for the greater longevities of the selected strains used by Luckinbill et al. (1984) and in the experiments here. The localization of the

"longevity genes" to chromosome III has been accomplished (Wells et al, 1987), but the gene products and their modes of action are not yet known.

Fleming et a. (1986), using 2-D gel electrophoresis, showed that there are quantitative, but not qualitative changes in the polypeptides produced by older D. melanogaster. They found a significant increase in the heterogeneity of protein gene expression over time. From these results they concluded that there may be some form of activation or repression of some genes in fruit flies as they age.

The lower mean fertilities at all ages, lower total fecundities for most ages, the lower lifetime fecundity, and the differences in the numbers of fertile matings of virgin flies, at younger ages, may all be due to absence of certain stimuli that are present when females are mated early in their life spans. The kinds of stimuli that influence fecundity, fertility, and mating ability have been described above.

It would be of considerable interest to know what gene products are produced in quantitatively different amounts over time in flies that are not mated for different time intervals as in the case of the mass mating experiment reported here. It may be that certain genes that have an important role in determining the reproductive output of females all require the presence of males. The significant difference in the cumulative survivorships of NL males and females suggests that this may be the case for the selection strains. It was hypothesized above (Discussion of Experiment I A) that there is a greater cost to reproduction for the NL males. Whatever resources they did not put into reproductive effort may have contributed to their longer life spans as virgins. Virgin males, of the NL strains, may be able to use the saved energy and resources in some ways that extend their life spans significantly relative to females.

Le Bourg et al. (1988, p.491) recorded the daily egg production of 322 Drosophila melanogaster (Oregon R strain) females and found that at "the individual level, no relation could be detected between early components of fitness and longevity." It is important to note that the Oregon R strain of flies has been maintained in laboratories

for many years and it is quite possible that populations of this strain have become highly inbred and/or evolved in different laboratories in different ways. The strain may have adapted to differences in laboratory environments in such different ways that significantly different values for life history characters exist between many of these lab adapted strains. These workers (Le Bourg et al., 1988) selected three lines for spontaneous locomotor activity: a high, a low, and a control line. They reported "absolutely no response to selection and no variation among lines in either fecundity parameters, or life span (ibid, p. 493". This is in sharp contrast to the significant differences in life spans and fecundities of the strains used in the experiments reported here and those of Luckinbill et al. (1984). As was noted in the Materials and Methods section they were derived from flies recently caught in the wild and the allelic frequencies and thus the responses to selection may well be very different from those reported by Le Bourg et al. (1988) for a possibly much more homogeneous strain of flies.

Le Bourg et al. (1988) used life history data of flies from the three lines to test the antagonistic pleiotropy theory by looking for negative correlations among "individuals" and not at the level of a population as in the experiments described here. They found a small, but positive and significant, correlation between early fecundity and life span. The meaning of correlations for individuals is somewhat difficult to understand as a statistically meaningful parameter.

Further, they used the method of principal components analysis to determine the relationship between the variables measured. Principal components analysis is a method that is used to partition the total variation in terms of the factors chosen by the experimenter to be possible sources of the variation. This type of analysis, as done by LeBourg et al. (1988), showed that (1) the females surveyed had substantial diversity in egg laying potential and (2) there is "no relationship between the variations in laying potential, on the one hand, and in longevity, on the other hand (ibid. p.496)." Their

analysis also showed that some flies are quite fecund for a long portion of their life span whereas others are not and that in general the observed variations in individual fecundity patterns are not linked to the variations observed in life spans. These authors concluded that the principal components analysis revealed no clear antagonistic relationships of the sort predicted by Williams (1957). These results do not agree with those for fecundity differences found here within and between strains at early and late ages.

Schnebel and Grossfield (1988) examined 12 species and semispecies of Drosophila, including D. melanogaster (Oregon R strain) , in an interspecific comparison of early- and late-life fitness characters and life spans. Their results showed a wide variety of life history patterns: in some cases short life span and high early life fertility and low late life fertility, in others, longer life spans and a low early life and higher late life fertility, and others such as D. melanogaster , one of the longest lived of the 12 species studied, did not show a significant decrease in mating ability or fecundity late in life. These authors thus concluded that the antagonistic pleiotropy hypothesis does not hold for this species.

Environmental factors and details of the ecological niches occupied by these species that might have been important in the molding of the pattern of life history characters by natural selection for each were not discussed by Schnebel and Grossfield (1988). Their comparative approach, although of interest, has not shown whether the differences detected in their analysis were the consequence of long term laboratory adaptations or are patterns that have been fixed by natural selection in each species studied prior to their introduction to the laboratory environment.

It is important to note that the flies used in the experiments of Schnebel and Grossfield (1988) came from populations where young and old flies were both used to set up the new stock bottles from which flies for test matings were obtained. Population densities varied in these stock bottles and there was no selection for early or delayed

fertility in the species studied. Williams (1957), Hamilton (1966), and Charlesworth (1980) all developed the antagonistic pleiotropy model for the evolution of senescence based, in part, on the assumption of distinct non-overlapping generations. Thus the use of overlapping generations of a possibly inbred line of the Oregon R strain of D. melanogaster that has evolved in the laboratory for hundreds of generations and the fact that the flies were not selected (Schnebel and Grossfield,1988) makes a direct comparison of their results and those reported here tenuous at best. The life history parameters and fitness components of this population of the Oregon R strain and the 11 other species and semispecies studied by Schnebel and Grossfield (1988) may have evolved in the laboratory so as maximize the intrastrain fitnesses in the laboratory and thus the observed life history characters may not, as in the work of Le Bourg et al (1988), reflect the patterns observed when a heterogeneous population of a species is subjected to selection early or late in life. Thus their experiments do not constitute a direct test of the predictions of the theory. Furthermore the frequencies of the loci that control fecundity, fertility, and longevity in the RND strains used in the experiments reported here may be closer to the actual frequencies of this species in the wild than are the frequencies of these alleles in the Oregon R strain used in their experiments.

Schnebel and Grossfield (1988, p.310) pointed out that the "inconsistency of the data with interspecific patterns predicted by antagonistic pleiotropy implies that genetic differences in longevity among taxa need not primarily involve genes with pleiotropic effects on the fecundity schedule." They noted that different loci may affect fecundity schedules or life spans or both simultaneously and that the proponents of the antagonistic pleiotropy theory have focused attention on loci only of the latter kind. These authors suggest that fecundity patterns, fertility patterns, and longevities may be subject to independent "adjustment (ibid. p. 310)" and that the patterns predicted by the antagonistic pleiotropy model might only be observed in flies that are allowed to reproduce early in life.

The higher mean fertilities of the control strain female flies, their higher fertile mating abilities, and greater total fecundities may have resulted from independent control of fertility patterns by genetic loci with a different kind of adjustment as suggested by Schnebel and Grossfield,1988).

It would be of considerable interest to have data from such a comparative analysis of populations of species recently caught in the wild and subjected to laboratory selection for late and early reproduction and a control strain for each. D. melanogaster is a cosmopolitan species with a world-wide distribution. It may be that its genome is so constituted that this species responds to selection very differently than some of the more ecologically limited species studied by Schnebel and Grossfield (1988).

Nesse (1988) developed a method to calculate the intensity of selection acting against senescence using life table data from the literature for wild populations of animals. His analysis , based on coefficients expressing the intensity of selection of hypothetical vs. actual populations for each species, showed that for bird species senescence has little effect on fitness. These small coefficients he interpreted as evidence in support of the mutation accumulation theory for the evolution of senescence (Edney and Gill, 1968; Comfort, 1979). According to this theory "senescence persists because the genes that cause it have not been exposed to natural selection (Nesse, 1988, p.446)."

Many of the species Nesse (1988) examined, however, have much larger selection coefficients that he interpreted as proof of strong selection against senescence. For these species Nesse (1988) concluded that selection for genes with antagonistic pleiotropic effects at different times in the life of a species was the better explanatory hypothesis (Williams, 1957; Charlesworth, 1980). He concluded that there is considerable variation in the "extent of pleiotropic contributions to senescence " with the greatest effects to be found in large mammals (Nesse, 1988, p.450). As Nesse

(1988) noted the two theories are not mutually exclusive. Rose (1980, p.141) has also noted that "Either or both of these theories could apply in a particular population."

In the work of Le Bourg et al. (1988) and that of Schnebel and Grossfield (1988) (with Oregon R strains of Drosophila melanogaster) the following details must be kept in mind: (1) the strains may have been highly inbred and/or adapted to the particular environments of the laboratories, (2) in the work of Schnebel and Grossfield (1988) a fundamental assumption of the antagonistic pleiotropy theory was not incorporated into the design of their experiments, and (3) no selected and control strains were compared in either paper. This suggests that the results reported here for the NL and RND strains cannot be directly compared to the results of their work. The experiments of these two groups do not show the consequences of selection.

Experiment I C - Fertile mating, fecundity, fertility of virgin flies of unequal ages:

In the analysis of the fecundity data for 7 day old females test mated to 42 day old males it was shown that one of the control strain mean fecundities, RNDA, differed significantly from the other control, RNDB, and from the two selected strains (Table 7). If we look at the results for the same data (Table 9) from two nonparametric tests we see that the difference between NLA and RNDB approaches significance ($p>|Z|=0.0781$, Wilcoxon two-sample test; p>chi-square = 0.0776, Kruskal-Wallis test). And for the difference between NLB and RNDB ($p>|Z|=0.0.0671$, Wilcoxon two-sample test; p>chi-square = 0.0667, Kruskal-Wallis test). There were very significant differences for the fecundity between NLA and RNDA ($p>|Z|=0.00001$, Wilcoxon 2-sample test; p>chi-square = 0.0001, Kruskal-Wallis test) and for NLB vs. RNDA ($p>|Z|=0.00001$, Wilcoxon 2-sample test; p>chi-square = 0.0001, Kruskal-Wallis test). These highly significant differences and the two that border on significance suggest that young virgins mated to old males in the RND strains have relatively higher fecundities than either of the NL strains with the same combination of parental ages.

An analysis of variance comparison, on the transformed values, of the mean individual fecundities for this same combination of parental ages showed that the mean individual fecundities fell into two groups (Table 8). One of the controls, RNDA, had the highest mean and one of the selected strains the lowest. Further the second highest was the other selected strain, NLA. The four means fell into two groups according to Scheffe's test, which grouped the RNDB strain mean with those of the two NL strains.

These results suggest that the mean fecundities of the control strains exceed those of the selected strains for this parental age combination. The total fecundities (Table 3), combined replicates, for this age combination also support this contention (NL = 843 eggs, vs. RND = 1695 eggs). Control strain total fecundity exceeds that of the selected strains by 101%. A chi-square test comparing the total number of eclosed adults shows that the control strains had a very significantly greater total fertility (chi-square = 35.815, p=0.0001, d.f. =1) than the selected strains for this parental age combination.

There was no significant difference in the number of fertile matings of selected vs. control females for this age combination.

These results suggest that the young RNDA females are more fecund and fertile than the NL females of either strain when they are mated to 42 day old virgin males. Examination of Figure 18 shows that the mean ±2 S.E.M. fecundity of the RNDA strain for this age combination overlaps that of RNDA when both parental ages are 7 days. This same figure shows that RNDB, for females aged 7 days and males aged 42 days, overlaps the two NL strains where both sexes were 7 days old. This result suggests that on day 7 post eclosion the RNDA females, mated to old males, are little affected by the advanced age of virgin males. Further the overlap (Figure 18) of the intervals for the two NL strains when the female is 7 days old and the male 42 is very substantial with that when the NL parents are both 7 days old. This result suggests, combined with that for

the RNDA flies, of the same age combinations, that the age of the female is more important than that of the male in determining mean individual fecundity. Further it does not support the antagonistic pleiotropy hypothesis because the RND females are more fecund and fertile when mated to old RND males than one might expect under the hypothesis. If the data supported the hypothesis then the RNDA mean fecundity, for example would not be nearly what it is when both RND parents are 7 days post eclosion. The two NL mean fecundities are much lower and are close to the values when both RND parents are 7 days old, suggesting that the 42 day old males are more senescent than the RND males of this age. It was suggested above that the longer life spans of NL male virgins might be the consequence a different pattern of energy resource utilization. Old NL virgin males may not be able to reallocate their resources as quickly as RND virgin males when presented with young virgin females.

These findings are not in agreement with the earlier work of Butz and Hayden (1962), who showed that female age in D. melanogaster, strain not indicated, is more important than male age in determining fecundity, fertility, and mating success. These authors (Butz and Hayden,1962) mated flies of equal and unequal ages ranging from 0.5 to 40 days old. The oldest matings producing progeny were from matings where either parent was 35 days post eclosion. They found that 5 day old females mated to 35 day old males were nearly as fecund and fertile as females 5 days old mated to males 5 days old. As in the experiment reported here the age of the female is more important than the age of the male.

For all the parental age combinations used Butz and Hayden (1962), unfortunately, reported only the adult longevities for the numbers of females and males eclosing, and the mean numbers of flies emerging without any standard deviations or errors. No statistical tests were applied to the data and their conclusions were based on an examination of the tabulated averaged data for six females for each age combination (Butz and Hayden ,1962; Table 1, p.618).

A comparison of the total fecundities (Figure 19), the total fertility by a chi-square test, number of fertile matings by a chi-square test, mean fecundities by parametric and nonparametric tests, and mean fertilities by a parametric test does not yield a pattern consistent with the hypothesis of antagonistic pleiotropy. Data consistent with this hypothesis would have shown the longer lived, NL, strains to have significantly higher values for the fitness characters examined than for the control, NL strains. The mutation accumulation hypothesis could be invoked to explain the lower mean fecundity of the RNDB strain relative to that of the RNDA strain. An allele or alleles of "age of onset" genes could exist in different frequencies in the RNDA and RNDB females. That this is possible is suggested by Figure 2 which shows that the survivorship of both replicates of the RNDA strain females are greater than both of the RNDB female replicates. Table 1 confirms this visual impression. RNDA female survivorship does significantly exceed RND female survivorship ($p<0.0001$, Kolmogorov-Smirnov test). Furthermore Table 1 shows that RNDB males and females have significantly different cumulative survivorships ($0.01<p<0.025$, Kolmogorov-Smirnov test).

Thus although both the sexes of the RND strains live less long than both sexes of the NL strains, both sexes do not have equivalent survivorships in both RND strains (Table1). From this one may tentatively infer that the frequencies of the "longevity genes" are not equal in both sexes of the control strains.

If different sets of 7 day old females, of all 4 strains used here, were all mated to old males of the control strain and separate sets all mated to old males of the selected strains the interstrain differences in the fecundities of the females might be more completely understood.

When 42 day post eclosion females were mated to 7 day old males, the intervals of the mean fecundities ±2 S.E.M. showed substantial overlaps for the two NL and the RNDA strains. Further RNDB showed overlap with NLA. Analysis of variance on the transformed egg count data showed that the means fell into the two overlapping groups

(Table 5) suggested by examination of Figure 18. Nonparametric tests (Table 9) of fecundity showed the same significant differences as the parametric test. This result does not support the hypothesis of antagonistic pleiotropy, because the NL virgin females do not have significantly greater mean fecundities at a later age when mated to young virgin males.

Examination of Table 4 shows that, for untransformed data; the variances, standard deviations, standard errors of the mean,and maximum number of eggs laid for the two NL and the RNDA strains are quite similar for the untransformed data, whereas these statistics for the RNDB strain are all much lower values. This result suggests that the RNDB strain females may interact with RNDB males differently than do the RNDA females with RNDA males. That this is possible is suggested by Figure 18. A visual examination of the Figure shows that RNDA mean fecundity± 2 S.E.M., when both parents are 7 days old, is nearly the same as when the RNDA females are 7 and the males 42 days old. The same comparison for the RNDB strain shows no such overlap. For RNDB, if both parents are 7 days old, the mean almost exceeds that for RNDA, it is the greatest of all the means of the 16 shown in the figure, however when the female is 7 and the male 42 days old the mean is significantly less than that of the RNDA strain. This suggests that the frequencies of the genes determining fecundity may be different in the RNDB strain. Because the mean fecundities of the RND strains did not differ significantly for young equal aged parents but did for both combinations of older parents and since the variances are quite different for the RND strains, when the females are 42 and the males 7 days old, it is possible that this strain has a different allele(s) for some "age of onset" gene(s) that is important for fecundity. If this is correct then the "mutation accumulation" hypothesis would be a plausible explanation for the detected differences between RND strains for the 42 day old virgin females combined with 7 day old virgin males..

Table 10, however ,shows that there are no significant differences across all four strains in their mean fertilities. RNDB that was the lowest in mean fecundity has the highest mean. This result, showing mean fertilities to be differently related than mean fecundities, suggests as did Experiment I B that different alleles determine the values these two fitness characters. This is in agreement with the result above for Experiment I B where the results of the analysis suggested that fecundity and fertility may be independently regulated by different genes.

The total combined fecundities (Figure 19) of the NL exceeded that of the RND strains by 42% (NL = 435 eggs, RND = 306 eggs). A chi-square test of the number of NL vs. RND adults eclosed (Table 5) showed a significant difference (chi-square = 5.215, p = 0.0224, d.f. = 1) in total fertilities (NL= 38 adults, RND = 43 adults). Thus the total fertility of the RND females significantly exceeded that of the NL females when the female parents were 42 and the males 7 days old.

There was no significant difference in the number of fertile matings of selected vs. control females for this age combination (Tables 14 and 15).

These results suggest that the 42 day old females of the selected strains are more fecund, less fertile, and do not differ from the control strain females in the number of fertile matings when mated with young, 7 day old males. The higher total fecundity of the old NL females suggests that the hypothesis of antagonistic pleiotropy can be used to explain the fecundity difference, because the NL females have been shown to have significantly longer cumulative survivorships than the RND females and were selected for reproduction at late age. This result for total fecundity is in agreement with that for Experiment I B. The mean fecundity comparison, however does not support the hypothesis (Table 7 and Figure 18), because the means fall into two groups. The RNDA strain mean is lower than that of NLB and higher than that of NLB. Also the RNDB mean is not significantly different from that of the NLA strain. This result for old virgin females and young virgin males does not support the the hypothesis of antagonistic pleiotropy,

because from the hypothesis one would predict that the females NL strains would, at this "old" age, have significantly higher fecundities than the RND strains.

Examination of Figure 18 and Table 7 suggests that the fecundity means for both NL and the RNDA strain (and possibly RNDB), when the females are 42 and the males 7 days old, are very comparable to those of the two RND strains when both parents are 42 days old. The low mean fecundity values for these RNDB females could possibly be due to the significant differences in the cumulative survivorships (Table 1) for the females of the two control strains. RNDB females have a significantly lower cumulative survivorship distribution than the RNDA females. Thus the low mean fecundity of this strain for this age combination is in agreement with the antagonistic pleiotropy hypothesis, but that for RNDA is not. The old, shorter lived, RNDB females are in fact less fecund when mated to young RNDB males. That the difference can be attributed to the survivorship differences of the females is confirmed by the non- significant difference of the survivorships of the RNDA and RNDB males. This result for females is in agreement with the "mutation accumulation" hypothesis because it suggests that the for this life history character the females of the two strains have different "age of onset" changes in mean fecundity. Laboratory selection may have resulted in different frequencies of the genes for longevity and/or fecundity in the two control strains.

The relative contributions of the males and females to these observed differences could be better understood if young males of the control strain were mated to old females of selected and control lines and if young selected strain males were mated to these two lines of females.

Table 10 shows no significant differences for all four of the the fertility means for 42 day old virgin females and 7 day old virgin males. This also suggest that the genes important to the regulation of fertility may be different from those regulating fecundity. RNDB has the lowest fecundity mean (Table 7), but the highest fertility mean (Table 10). The relative order and the pattern of significant differences of the fecundity and fertility

means (Tables 7 and 10) changes as follows: (1) for mean fecundity : RNDB<NLA<RNDA<NLB ; RNDB does not differ from NLA ; NLA, RNDA, and NLB do not differ. (2) for mean fertility NLA<RNDA<NLB<RNDB and there is no significant difference. This inconsistency in pattern, relative magnitude and pattern of significant differences, for these two life history characters does not support the antagonistic pleiotropy theory. To agree with the hypothesis, the means for both characters for both NL strains would have been greater than those for the RND strains. That the difference cannot be attributed only to the males is suggested by the results from the matings of 7 day old flies of all four strains. Although it is true that the RND females were more fecund than the NL females (Figure 18 and Table 7) for that age combination, the mating of old RND females to young males results in no clear pattern of differences. The RNDB is the lowest and RNDA mean±2 S.E.M. overlaps substantially with both intervals of the selected strains.

Experiment II A - Geotaxis experiment with aging virgin flies:

Geotaxis has been defined by Grossfield (1978, p. 53) as " a directed movement mediated by gravity" in his extensive review of the nonsexual behavior of Drosophila. Furthermore as noted by Grossfield the minimum angle required to elicit a geotactic response in Drosophila has not been determined experimentally in a manner analogous to the minimum threshold for phototactic responses (Grossfield, 1978). Most of the researchers on the geotactic response in Drosophila species have used either mazes (Ricker and Hirsch, 1988) or vertical cylinders (Miquel, Lundgren and Binnard, 1972; Miquel et al.,1976; Leffelaar and Grigliatti, 1984b). Hirsch and his co-workers have done many selection experiments on geotaxis using vertically oriented mazes, although this does detect "discrete alterations in response" it does not have very high predictive power for the analysis of this behavior. Grossfield suggested that modified techniques might be able to "detect mutations or discrete alterations in response" and

would "seem to offer an approach with higher predictive value for studies of behavioral mechanisms (1978, p.57).

In this laboratory, Edgar Schnebel has shown that an inclined plane of the type described in the Materials and Methods section could be used to discriminate between the geotactic behaviors of different Drosophila species (personal communication) that were either positively or negatively geotactic. Schnebel's use of the inclined plane set at various test angles suggested to me that this apparatus, if modified, might be used to test for a decline in geotactic response in aging populations of D. melanogaster selected for postponed senescence relative to the control populations. A comparison of the responses of selected and control might show differences by strain and/or sex at different ages.

Further, because it was known that the interactions between the stimuli of light and gravity were known to be complex (Grossfield, 1978), I modified the protocol used by Schnebel to eliminate the effect of light in the determination of geotactic responses in these strains as they aged. Leffelaar and Grigliatti (1984b) had shown that a diminution in negative geotactic behavior is a reliable behavioral biomarker of aging in Drosophila populations that age at different rates, but they did not remove light as a factor in their experiments. They had also tested aging populations for loss of phototactic response and motor activity in separate experiments (Leffelaar and Grigliatti, 1984b).

The time interval of 1 minute, used in the geotactic experiment reported here, was chosen " to amplify the apparent age-related loss of behavior (Leffelaar and Grigliatti, 1984b, p. 214)." An analysis of preliminary results showed that a shorter time interval, 30 seconds, was not sufficient to discriminate between population results and a longer interval did not yield statistically significant results. A longer time interval might also have yielded data that measured the motor activity unintentionally, because the flies might tend to be distributed differently over longer times after the initial geotactic stimulus.

Since these geotaxis experiments, using NL and RND strain flies were completed here, strains derived from the same base population have been tested in the laboratory of Dr. Robert Arking (personal communication 1988; Arking, 1988, p. 130). These additional experiments used a test protocol very much like that of Leffelaar and Grigliatti (1984b) and thus the sensitivity of the test procedures is different, because of the substantial differences in designs. The procedure used by Arking (1988, p.130) was used to determine the time in the life span of these strains when half the test population "cannot do a geotaxis run of 11 cm in 10 seconds." In the case of the control strains this age was determined to be about 22 days post eclosion and for the selected strains about 37 days post eclosion.

Tests of geotactic behavior here showed that at the two earliest ages, zero and 3 days post eclosion, there is no difference in the strain responses, by sex, to gravity in the dark. The interactions of strain and angle for these ages were not significant and it was shown above that sex was not a significant effect for the first two test ages. A comparison of the mean responses of the strains, sexes combined, showed that on the day of eclosion one of the control strains is the least negatively geotactic, significantly different from the other control and from the two selected strains. These three strains are not significantly different from each other and one of the selected strains is the most negatively geotactic on the day of eclosion. This finding suggests that if the response to gravity is to be used as one of the biomarkers of aging and as a measure of "vigor" in the comparison of these strains then the vigor of the longevity strains is not less, relative to the control strains, within 24 hours of eclosion.

The finding that the four mean geotactic responses do not differ at 3 days post eclosion further supports this conclusion. It is noteworthy that the mean responses for all four strains at 3 days post eclosion were greater than the mean responses on the day of eclosion. This finding seems to agree with that of Leffelaar and Grigliatti (1984b,

Figs.5 and 7) which show the fitted curves for the percents of wild-type flies, by sex, that climbed to a height of 15 cm. and 5 cm. in 20 seconds.

It was noted above that there were significant strain by angle interactions beginning at day 7 post eclosion and from that test age until the last usable data set, 35 days post eclosion, that no particular pattern was evident when the plots of the data were examined. It was noted above that the day 35 data analysis had to be done differently, because of significant strain by angle and sex by angle interactions.

The analysis, by sex, showed that significant differences in mean response were detected at the two test angles chosen. For the lesser angle, 40 degrees, one of the selected strains had a significantly greater mean response. For the greatest angle, 80 degrees, both selected strains had significantly greater mean responses than the control strains, and were significantly different from each other in mean response. Comparison of the survivorship distributions showed that the longest living strain of females, NLA, had the most negative geotactic mean response.

These results suggest that NL females do have a greater mean negative geotactic response at 35 days relative to the RND females, especially for greater angles.

The analysis also showed that the NLA males had the greatest mean geotactic response at both 40 and 80 degrees and that NLB males were also more negatively geotactic than either RND strain male population at 80 degrees. Despite the fact that fewer NLA males, the most negatively geotactic strain at 35 days post eclosion, were alive at 35 days they outsurvived both of the control strains over time.

These results suggest that males selected for a postponed senescence are more negatively geotactic at a late age than are control males.

Significant differences were found for both males and females between the control strains and the strains selected for late reproduction . There was no direct selection for increased or decreased geotactic response of the strains used here. It may be that different alleles in different frequencies are present in these strains that are directly or

indirectly responsible for the significant differences in behavior between strains. The differences could be due to different frequencies of alleles that determine geotactic behavior or to different allelic frequencies of other genes that only indirectly influence this behavior. Differences were not found by strain or sex at the two earliest test ages. The data thus support the hypothesis of mutation accumulation hypothesis for the evolution of postponed senescence (Rose,1989). The results might, alternatively be explained as consequences of some overall level of "vigor" that is characteristic of the selected strains at a later age. This "vigor" might be due to genotypic differences that indirectly affect geotactic response. Alleles that have different ages of action could explain the observed differences in behavior.

Hirsch and Ricker (1988) have used chromosomal inversions, in D. melanogaster selected for high and low response over 500 generations, to show that long term changes in geotactic response are controlled by genes on all three major chromosomes.

Arking and his coworkers (personal communication,1989) have concluded that the geotactic response differences between populations are "under the primary influence of genes on chromosomes one and two" and that longevity is "controlled only by genes on chromosome three." These results were also obtained using chromosome substitutions (Arking, personal communication, 1989; Wells et al., 1987).

It is thus not surprising that the inter-strain differences found in the experiments reported here for, longevity, fecundity and geotaxis do not follow the same pattern. Different genes on different chromosomes determine the observed responses. This does not exclude the possibility that there are interactions between the products of these genes in these strains. The exact details of such interactions, if they exist, are not known at this time. Detected differences may reasonably be attributed to differences in allelic frequencies on chromosome III and possibly chromosomes I and II in the selected and control strains. In the strains used here, selection for reproduction at late ages

might have resulted in different allelic frequencies of those genes that are responsible for the geotactic response on the third, but not of those on the first and second chromosomes. Such possible differences could exist because as Hirsch and Ricker (1988) have shown there are genes on all three major chromosomes that are important in determining the geotactic response.

The data from geotaxis experiment does not support the antagonistic pleiotropy theory. This theory (Williams, 1957) predicts that populations that are shorter lived (here the RND strains) are more "vigorous" when young and that longer lived populations (here the NL strains) are less "vigorous" when young. It would also be true, according to the antagonistic pleiotropy hypothesis that when the shorter lived flies were "old" they would be less active. Here it has been shown that the longer lived flies do not differ in geotactic response from the control flies at young ages, but have, in the case of one strain for each sex , a significantly lower mean response at a late age.

Arking (1988, p.131) has suggested that "the involvement of the neuroendocrine" is implied by the differences observed between the longer and shorter lived strains. The results of the geotaxis experiment reported here could be the consequence of changes in this system that are not manifested until later ages in the geotactic response, but these changes could be entirely and exclusively in the reproductive system. Thus the differences "vigor" that were quantified by the geotaxis experiment could be in no way whatsoever associated with genes that ordinarily influence geotactic behavior. Thus the differences between strains and sexes are also consistent with the "rate of living" hypothesis, because after the long lived strains reach a certain age they may age more slowly due to a more gradual loss of "vigor". A relatively slower loss of function in the neuroendocrine system of longer lived flies could be responsible for the differences in response detected in 35 day old virgin flies.

Geotactic behavior could be considered to be a component of fitness. A fly that is more responsive to a geotactic stimulus may reasonably be considered to be more

"vigorous". It is likely that such an individual is more active and thus more capable of mating and thus of having offspring than is a less active fly. In this sense, geotactic behavior can be thought of as a component of Darwinian fitness. This greater relative "vigor", measured as a negative geotactic response, might be due to differences in the state of the neuroendocrine systems of individual flies of the same age.

The finding of no significant difference in geotactic response by sex or strain at young, but not at an older age is consistent with the "mutation accumulation hypothesis", because there could be different "age of onset" alleles that have different levels of activity when the flies are 35 days post eclosion.

Whatever the differences in geotactic response between strains reported here, this new method of testing has been shown to be effective in discriminating between responses over a range of angles. The method can be used to test strains that are positively or negatively geotactic. Further, the method has been shown to yield geotactic responses that can be regressed on the stimulus angle to yield highly significant slopes and intercepts to discriminate responses by sex. This method of testing could also be semi-automated. The distributions of the flies could be videotaped and the distances, from the bottom of the test apparatus, could be measured for each individual fly for each set tested. This data could be used to calculate the mean response even more accurately than was done in the experiment reported above.

The data for the control strains provide information about the geotactic response of a population of D. melanogaster that has been shown to be heterogeneous and that is descended from flies that were recently caught in the wild (Luckinbill et al., 1984).

Experiment II B - Survivorship of virgin flies from the geotaxis experiment:

The data of this survivorship experiment suggest that the NL males lived somewhat longer than the NL females. Although the LT10 and LT90 values did not differ for

selected line males and females, the LT50 of the males was 9% greater than that for the females. Figure 25 suggests that from day 46 to day 74 post eclosion, more males of the selected strains were alive. Both NL sexes had greater LT50 values than both sexes of the RND flies. The NL females LT50 was 22% greater than that of the RND females and 31% greater than that of the RND males. For the NL males their LT50 was 33% greater than that of the RND females and 43% greater than that of the RND males. At day 45 when only 300, 50 %, of the RND females were alive 420 NL females were still alive. When 300 NL females were alive, 50%, only 240 RND females were alive. In the case of the males, when 300 RND males, 50%, were still alive 420 NL males were still alive. At 60 days post eclosion 300 NL males, 50%, were alive, but only 90 RND males survived to that age post eclosion. All of these comparisons are based on the data as plotted in Figure 25 for combined cumulative survivorships of NL and RND flies by sex. Thus each group had 600 flies on the day of eclosion.

The results of these calculations and Figure 25 strongly suggest that substantially greater numbers of the NL flies of both sexes had longer life spans, but that the maximum life span of the RND females was not less than that of either sex of the flies selected for a postponed senescence.

In Experiment IA, the survivorship of the NL males was found to be very significantly greater than that of the NL females. Although there was a difference detected here in LT50's, the distributions do not seem to be so very different. The NL flies in Experiment IA were in the 48^{th} generation of selection, whereas those in this experiment were in the 21^{st}. In Experiment I A the control flies were in the 68^{th} generation of selection, whereas in this experiment these strains were in the 31^{st} generation.

Examination of the pattern for all LT values (Table 2) suggests that over time laboratory selection changed the pattern of relative differences between strains and sexes so as to increase the life spans of the NL flies relative to the RND flies by increasing the relative magnitude of the LT90 values..

The error in data recording of survivorships of the geotaxis flies, described in the Results section, may have had a substantial effect on the life spans of the flies of all four strains. Because the flies were not transferred to fresh food as frequently as in Experiment IA until day 14, the early adult environment could have significantly altered survivorships.

Chapter 6

CONCLUDING DISCUSSION

Fisher, in his classic *The Genetical Theory of Natural Selection*, (1958, p.47) noted that: " It would be instructive to know not only by what physiological mechanism a just apportionment is made between the nutriment devoted to the gonads and that devoted to the rest of the parental organism, but also what circumstances in the life-history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction." Analyses of the data from the experiments reported here, comparing the fecundities, fertilities, and the fertile matings of virgin D. melanogaster of various ages, suggest that the "apportionment" of resources is not equivalent in the four strains studied. Statistical tests of data from mating experiments, using virgin flies of various parental age combinations from the strains selected for late reproduction and control strains, have shown that these strains differ significantly in their life history characters at different times in their life spans.

It has been shown above (Experiment IA), for strains selected for late reproduction, that the cumulative survivorship distribution of virgin males is greater than that of females from the same strains. This difference is very highly significant. For the control strains, no significant difference was found between males and females. Both sexes of the strains selected for a postponed senescence had very significantly greater cumulative survivorship distributions than either sex of the control strains.

These results are consistent with the hypothesis of "costs of reproduction for males" (Partridge and Farquhar, 1981; Partridge and Farquhar, 1983; Partridge and Andrews, 1985; Partridge and Harvey, 1985; Partridge, 1986) in D. melanogaster. The very significantly greater survivorship of the selected strain, NL virgin males relative to the NL virgin females vs. the nonsignificant difference between RND virgin males and RND virgin females, strongly suggests that the NL males use their resources differently from RND males , as they age. This inference

would not have been plausible if: (1) the survivorship distributions of the NL virgin males and females did not differ significantly from each, (2) the survivorship distributions of the RND virgin males and females did not differ significantly from each, and (3) if the survivorships of both of the virgin NL sexes significantly exceeded the survivorships of both virgin RND sexes, then this inference might not be plausible. If these three relationships between the survivorships had been found, then one might conclude that the NL strains had different alleles for longevity from the RND strains, but that the males of the selected and control strains did not apportion their resources for reproduction differently over time.

Here virgins of both sexes of all strains did not have an opportunity to mate, however the NL male survivorship differed very significantly from the other three. This finding and the nonsignificant difference between the sexes of the RND strains, suggest that when the NL males do not mate their life spans are extended. The work on reproductive activity in D. melanogaster, cited earlier, suggests that the energy or resources not used for reproductive activity may thus have been available for utilization in other ways that extend the life spans of these NL males. Mated RND and virgin males could possibly allocate resources differently, but this cannot be known from the data of the experiment reported here. The data do suggest that, when not mated, the selected and control strain males do allocate resources quite differently. The differences detected between the survivorships of these virgin flies can reasonably be attributed to genetic differences between the sexes and strains and not to environmental factors, because all of the flies descended from the same base population (Luckinbill et al., 1984) and there was no difference in the environments of the strains or sexes .

The mean fecundity comparisons for 8 different ages from mating experiments where the parental ages were the same, have shown a pattern over time that is consistent with the "antagonistic pleiotropy" hypothesis. Females from strains selected for late reproduction had significantly lower mean fecundities at young ages relative to controls. Conversely, these strains had significantly higher mean fecundities at a late age, 42 days post eclosion, relative to control strain females at this late age.

An analysis of covariance of mean fertilities on age showed that the control females had consistently higher mean fertilities relative to the selected strain females for all ages post eclosion. Further, it was shown that the rate of decline in mean fertility is the same in selected and control strain females over all test ages where the parental ages are equal.

The number of fertile matings of control females relative to selected strain females was shown above to be: (1) very significantly greater for young ages: one, two, four , and seven days post eclosion, (2) not to differ significantly for ages 14, 28, and 42 days post eclosion, and (3) to be significantly different for 21 days post eclosion. An interstrain comparison of the number of adults eclosed showed that for all test ages, except 21 days post eclosion, the number of RND adult offspring was very significantly greater than the number of NL adult offspring.

These results suggest that different genes control and/or regulate the fecundity and fertility of females and that there may be different alleles of these genes in different frequencies in the selected and control strains. The data also suggest that postponed mating may have different effects on the activity of these genes in the control and selected strains. It was indicated above that the NL males may apportion their resources differently when they are not mated. The lower relative fertilities of NL females in postponed matings could plausibly be attributed to a reapportionment that cannot be reversed in the NL males such that they are not capable of reversing the effects of postponed mating. For these males longer life span may been gained if matings do not occur, but if the NL males are presented with NL females at later ages the reproductive output of the NL females may be irreversibly lowered relative to the RND females . In an aerlier section, possible important factors supplied by the presence of males that affect female fecundity and fertility have been cited.

Hamilton (1966) and Charlesworth (1980) have both shown that an allele, with an action at a specific time in the life history of a population, can have very important consequences for reproductive output or relative fitness in an environment where selection is operative. Thus the differences in fertility detected here are consistent with the hypothesis of "age of onset" or "age of action" genes and/or modifiers of such genes and with the hypothesis of a "cost of mating" in

both female flies (Fowler and Partridge, 1989) and in male flies (Partridge and Farquhar, 1981). The very significantly greater cumulative survivorship of NL females relative to RND females, could be a consequence of a reallocation of resources by these long-lived virgins which is not reversible and also be in part a consequence of the absence of important male stimuli of the kinds cited earlier.

The nonsignificant differences, between NL and RND strains, in the numbers of females mated at older ages could be a consequence of the more rapid aging, or earlier senescence, of RND males. It is possible that fewer control males could have had motile sperm at later ages such that the numbers of fertile matings no longer differed significantly. However, with the exception of 21 days post eclosion, the control females had total fertilities that were very significantly higher than the selected females. This finding suggests that even if there are fewer control virgin males that can inseminate virgin control females these females may be inseminated by a few virile control males or that virgin control females may allocate their resources for reproduction differently and thus are more fertile when presented with their virgin male sibs at later ages. These results thus suggest that postponed mating has very different effects for control strain virgin female fertility from that for virgin females of long-lived strains.

The results of the interstrain data analysis for the offspring of unequal virgin parental ages, also showed significant differences between the NL and RND strains. When the female parent is 7 and the male 42 days post eclosion, the total fecundity and total fertility of the control, RND females, is much higher than that of the selected strain, NL, females. The number of fertile matings did not differ, however. These differences in fecundity and fertility may be due to a shift in the reproductive schedule to later ages in the NL females as is suggested by the comparison of mean fecundities where the parental ages were equal. The data of that experiment showed that, at 7 days post eclosion, the mean egg output of both strains of RND females is significantly greater than that of both strains of NL females. These interstrain differences, for 7 day-old virgin females test mated to 42 day-old virgin males, are consistent with the hypothesis of "age of onset"

or "age of action" genes with different alleles and modes of action in the selected and control strains.

When the virgin female parental age was 42 and the virgin male 7 days post eclosion, the total fecundity of the selected strain females was significantly greater than that of the control strain females, however the total fertility of the control females was significantly greater than that of the selected strain females. Thus the total fecundity result for this parental age combination is consistent with the "antagonistic pleiotropy" hypothesis of greater reproductive output at a late age in a longer lived population. The total fertility result for the same age combination is consistent with the hypothesis of "age of onset" or "age of action" genes and/or modifiers, because the total fertility of females selected for late reproduction is significantly lower than that of control females.

It is therefore concluded that fertility and fecundity in these strains of D. melanogaster are controlled by different genes with different patterns of action over time.

Analyses of the data for geotactic responses of virgin flies, showed a pattern of no strain or sex differences at the first two test ages post eclosion. Significant differences between strains within sexes were found to exist at 35 days post eclosion. NL males and females were more negatively geotactic at this age. This pattern of differences is consistent with both the "rate of living" hypothesis and the "age of onset" genes and/or modifiers hypothesis. It is consistent with "the rate of living" theory, because the significantly lower negative mean geotactic responses of control flies at 40 and 80 degrees in both sexes of the control flies could be a consequence of lowered vigor due to a more accelerated aging process in these strains relative to the selected strain flies. Alternatively the detected differences are consistent with "age of action" genes and/or modifiers that have some important consequences for a greater overall responsiveness of the selected strain flies at late ages. Although the selected strain females were less fertile at 42 days post eclosion they were more fecund when the parental ages were the same. As Arking (1988, p.131) noted the observed differences between the selected and control line flies imply the involvement of the neuroendocrine system. It is thus a plausible inference that different alleles of

genes and/or modifiers of these genes that control and regulate this system are present in different frequencies in selected strain and control populations of these flies.

It is thus further concluded that fecundity, fertility, fertile mating in females, and the negative geotactic response of these strains are under the control of different sets of genes with different patterns of action over time in both sexes of the selected and the control strains.

set 1	set2	n1	n2	max. diff	test	chi-square (df=2)	critical |D| tests	prob
NL	RND	240	240	0.671	1-sided	216.01		p<0.0001
NLm	RNDm	120	120	0.758	1-sided	138.02		p<0.0001
NLm	NLf	120	120	0.425	1-sided	43.35		p<0.0001
NLm	RNDf	120	120	0.675	1-sided	109.35		p<0.0001
NLf	RNDm	120	120	0.692	1-sided	114.82		p<0.0001
RNDm	RNDf	120	120	0.108	2-sided	--------	|D|<0.176	p>0.05
NLf	RNDf	120	120	0.583	1-sided	81.67		p<0.0001
RNDAm	RNDAf	60	60	0.217	2-sided	-------	|D|<0.248	p>0.05
RNDBm	RNDBf	60	60	0.283	2-sided	-------	0.270<|D|<0.298	0.01<p<0.025
NLAm	NLAf	60	60	0.500	1-sided	30.00		p<0.001
NLBm	NLBf	60	60	0.350	2-sided	--------	0.314<|D|<0.356	0.001<p<0.005
RNDAm	RNDBm	60	60	0.183	2-sided	-------	|D|<0.248	p>0.05
RNDAf	RNDBf	60	60	0.433	1-sided	22.53		p<0.001
NLAm	NLBn	60	60	0.167	2-sided	------	|D|<0.248	p>0.05
NLAf	NLBf	60	60	0.183	2-sided	------	|D|<0.248	p>0.05
NLAf	NLBf	60	60	0.700	1-sided	58.80		p<0.001
NLAf	RNAf	60	60	0.367	1-sided	11.00		p<0.001

note 1: critical values for D calculated from Table M (Siegel,1956;p279).
note 2: chi-square values calculated using Formula 6.11 (Siegel,1956;p.134)

Table 1: Kolmogorov-Smirnov tests for survivorship of virgin flies.

Mass mating combined replicates and strains

strain	sex	LT10	LT50	LT90	max. lifespan
NL	f	33	48	57	75-78 days
NL	m	36	57	68	64-68 days
RND	f	15	37	47	61-64 days
RND	m	12	38	44	57-61 days

note: LT values for days post-eclosion; 120 virgin flies per strain.
NL generation 48, RND generation 68.

Geotaxis combined replicates and strains

strain	sex	LT10	LT50	LT90	max. lifespan
NL	f	5	55	76	85
NL	m	5	60	76	85
RND	f	5	45	76	85
RND	m	5	42	61	62

note: LT values for days post-eclosion; 600 virgin flies per sex and strain.
NL generation 21, RND generation 31.

Table 2: LT values for survivorship of virgin flies.

strain	female age	male age	eggs	females	males	adults	(m/f) ratio
NLA	1	1	72	10	9	19	0.90
NLB	1	1	50	10	7	17	0.70
NL	1	1	122	20	16	36	0.80
RNDA	1	1	290	78	67	145	0.86
RNDB	1	1	289	109	117	229	1.07
RND	1	1	579	187	184	374	0.98
NLA	2	2	561	268	244	522	0.91
NLB	2	2	565	260	269	530	1.03
NL	2	2	1126	528	513	1052	0.97
RNDA	2	2	866	348	336	684	0.97
RNDB	2	2	815	353	363	716	1.03
RND	2	2	1681	701	699	1400	1.00
NLA	4	4	257	100	75	175	0.75
NLB	4	4	424	188	151	339	0.80
NL	4	4	681	288	226	514	0.78
RNDA	4	4	841	338	249	587	0.74
RNDB	4	4	610	313	216	529	0.69
RND	4	4	1451	551	386	937	0.70
NLA	7	7	522	170	183	353	1.08
NLB	7	7	549	121	110	231	0.91
NL	7	7	1071	291	293	584	1.01
RNDA	7	7	1142	503	417	920	0.83
RNDB	7	7	1462	662	570	1232	0.86
RND	7	7	2604	1165	987	2152	0.85

Table 3: Egg totals, total females, total males, total adults, and male to female sex ratios for equal virgin parental mating experiment.

strain	female age	male age	eggs	females	males	adults	(m/f) ratio
NLA	14	14	1113	331	338	669	1.02
NLB	14	14	953	329	321	650	0.98
NL	14	14	2066	660	659	1319	1.00
RNDA	14	14	952	322	402	724	1.25
RNDB	14	14	1012	292	376	666	1.29
RND	14	14	1964	614	778	1390	1.27
NLA	21	21	783	114	96	210	0.84
NLB	21	21	724	91	101	192	1.11
NL	21	21	1507	205	197	402	0.96
RNDA	21	21	731	207	181	388	0.87
RNDB	21	21	707	183	196	379	1.07
RND	21	21	1438	390	377	767	0.97
NLA	28	38	573	35	53	88	1.51
NLB	28	38	709	97	111	208	1.14
NL	28	38	1282	132	164	296	1.24
RNDA	28	28	469	60	58	118	0.97
RNDB	28	28	527	90	103	192	1.14
RND	28	28	996	150	161	310	1.07
NLA	42	42	439	15	10	25	0.67
NLB	42	42	419	18	12	30	0.67
NL	42	42	858	33	22	55	0.67
RNDA	42	42	169	12	14	26	1.17
RNDB	42	42	174	27	15	42	0.56
RND	42	42	343	39	29	68	0.74

Table 3 (continued): Egg totals, total females, total males, total adults, and male to female sex ratios for equal virgin parental mating experiment.

strain	f. age	m. ag	N	var	mean	stdev.	s.e.m.	min	max	sum
NLA	1	1	60	3.96	1.20	1.99	0.26	0	11	72
NLB	1	1	60	0.89	0.83	0.94	0.12	0	4	50
RNDA	1	1	60	28.04	4.83	5.30	0.68	0	22	290
RNDB	1	1	60	17.81	4.82	4.22	0.54	0	17	289
NLA	2	2	60	55.52	9.35	7.45	0.96	0	28	561
NLB	2	2	60	46.86	9.42	6.85	0.88	0	23	565
RNDA	2	2	60	32.79	14.43	5.73	0.74	1	27	866
RNDB	2	2	60	43.67	13.58	6.61	0.85	1	37	815
NLA	4	4	60	28.07	4.28	5.30	0.68	0	23	257
NLB	4	4	60	72.81	7.07	8.53	1.10	0	34	424
RNDA	4	4	60	66.36	14.02	8.15	1.05	0	43	841
RNDB	4	4	60	61.94	10.17	7.87	1.02	0	30	610
NLA	7	7	60	81.20	8.70	9.01	1.16	0	28	522
NLB	7	7	60	75.21	9.15	8.67	1.12	0	34	549
RNDA	7	7	60	153.29	19.03	12.38	1.60	2	59	1142
RNDB	7	7	60	145.63	24.37	12.07	1.56	0	51	1462
NLA	14	14	60	143.74	18.55	11.99	1.55	1	51	1113
NLB	14	14	60	143.94	15.88	12.00	1.55	1	48	953
RNDA	14	14	60	110.93	15.87	10.53	1.36	0	47	952
RNDB	14	14	60	135.24	16.87	11.63	1.50	0	57	1012
NLA	21	21	60	161.30	13.05	12.70	1.64	0	46	783
NLB	21	21	60	119.15	12.07	10.92	1.41	0	46	724
RNDA	21	21	60	108.69	12.18	10.43	1.35	1	35	731
RNDB	21	21	60	103.09	11.78	10.15	1.31	0	56	707
NLA	28	28	60	79.74	9.55	8.93	1.15	0	37	573
NLB	28	28	60	82.16	11.82	9.06	1.17	1	35	709
RNDA	28	28	60	75.00	7.82	8.87	1.12	0	36	469
RNDB	28	28	60	55.09	8.78	7.42	0.96	0	37	527
NLA	42	42	60	34.15	7.32	5.84	0.75	0	25	439
NLB	42	42	60	43.68	6.98	6.61	0.85	0	29	419
RNDA	42	42	60	20.49	2.82	4.53	0.58	0	22	169
RNDB	42	42	60	20.43	2.90	4.52	0.58	0	27	174
NLA	7	42	60	46.64	6.80	6.83	0.88	0	28	408
NLB	7	42	60	58.90	7.25	7.67	0.99	0	27	435
RNDA	7	42	60	144.53	17.52	12.02	1.55	0	54	1051
RNDB	7	42	60	109.59	10.73	10.47	1.35	0	38	644
NLA	42	7	60	22.22	2.98	4.71	0.61	0	24	179
NLB	42	7	60	28.20	4.27	5.31	0.69	0	23	256
RNDA	42	7	60	25.74	3.58	5.07	0.66	0	22	215
RNDB	42	7	60	6.56	1.52	2.56	0.33	0	15	91

Table 4: Statistics for untransformed mating data from all virgin parental age combinations.

strain	replicate	fem. age	male age	eggs	females	males	adults	m/f (ratio)	% adults
NLA	rep1	7	42	251	96	90	186	0.94	74.10%
NLA	rep2	7	42	157	57	50	107	0.88	68.15%
NLB	rep1	7	42	225	63	51	114	0.81	50.67%
NLB	rep2	7	42	210	78	59	137	0.76	65.24%
RNDA	rep1	7	42	544	234	228	462	0.97	84.93%
RNDA	rep2	7	42	507	206	181	387	0.88	76.33%
RNDB	rep1	7	42	330	157	121	278	0.77	84.24%
RNDB	rep2	7	42	314	65	67	132	1.03	42.04%
NLA	rep1	42	7	115	4	5	9	1.25	7.83%
NLA	rep2	42	7	64	3	4	7	1.33	10.94%
NLB	rep1	42	7	157	9	11	20	1.22	12.74%
NLB	rep2	42	7	99	1	1	2	1.00	2.02%
RNDA	rep1	42	7	73	4	0	4	0.00	5.48%
RNDA	rep2	42	7	142	11	17	28	1.55	19.72%
RNDB	rep1	42	7	38	2	2	4	1.00	10.53%
RNDB	rep2	42	7	53	4	3	7	0.75	13.21%

Table 5: Egg totals, total females, total males, total adults, sex ratios, and percent adults eclosed for unequal virgin parental age mating experiment.

Strain	rep	f.age	m.age	N	variance	mean	stdev.	s.e.m.	eggs
NLA	1	7	42	30	67.274	8.367	8.202	1.490	251
NLA	2	7	42	30	22.530	5.233	4.747	0.866	157
NLB	1	7	42	30	58.466	7.500	7.646	1.397	225
NLB	2	7	42	30	61.241	7.000	7.825	1.429	210
RNDA	1	7	42	30	155.223	18.133	12.459	2.275	544
RNDA	2	7	42	30	138.024	16.900	11.748	2.145	507
RNDB	1	7	42	30	149.379	11.000	12.222	2.231	330
RNDB	2	7	42	30	73.430	10.467	8.569	1.565	314
all	1	7	42	120	122.048	11.250	11.067	1.010	1350
all	2	7	42	120	91.990	9.900	9.591	0.876	1188
NLA	1	42	7	30	24.488	3.833	4.949	0.903	115
NLA	2	42	7	30	19.230	2.133	4.384	0.800	64
NLB	1	42	7	30	27.289	5.230	5.224	0.954	157
NLB	2	42	7	30	28.148	3.300	5.305	0.969	99
RNDA	1	42	7	30	12.116	2.433	3.481	0.635	73
RNDA	2	42	7	30	37.513	4.730	6.125	1.118	142
RNDB	1	42	7	30	3.168	1.267	1.780	0.325	38
RNDB	2	42	7	30	10.047	1.767	3.170	0.578	53
all	1	42	7	120	18.576	3.191	4.310	0.393	383
all	2	42	7	120	24.487	2.983	4.948	0.452	358
all	1	mix	mix	480	78.257	6.831	8.846	0.404	3279

Table 6: Egg totals , means, variances, standard deviations, and total eggs from the unequal virgin parental age experiment.

fem. age	male age				
1	1	NLB 1.28	NLA 1.44	RNDA 4.34	RNDB 4.52
2	2	NLA 8.80	NLB 9.08	RNDB 13.88	RNDA 14.96
4	4	NLA 4.70	NLB 6.48	RNDB 10.06	RNDA 14.33
7	7	NLA 8.56	NLB 9.32	RNDA 19.01	RNDB 24.50
14	14	NLB 15.75	RNDA 16.17	RNDB 16.66	NLA 18.61
21	21	NLB 11.74	RNDB 12.02	RNDA 12.03	NLA 12.53
28	28	RNDA 7.59	RNDB 9.21	NLA 9.27	NLB 11.85
42	42	RNDA 2.97	RNDB 3.15	NLB 6.66	NLA 7.35

Table 7: Grouped egg means for all virgin parental age mating combinations (Tukey's test).

fem. age	male age				
		RNDB	NLA	RNDA	NLB
42	7	1.95	2.97	3.37	4.04
7	42	NLB	NLA	RNDB	RNDA
		7.46	7.47	10.35	17.25

Table 7 (continued): Grouped egg means for all virgin parental age mating combinations (Tukey's test).

f. age	m .age	comparison	lower limit	upper limit
1	1	RNDB-NLA	0.43	1.80
		RNDB-NLB	0.52	2.00
		RNDA-NLA	0.38	1.69
		RNDA-NLB	0.46	1.88
2	2	RNDA-NLB	0.82	2.62
		RNDA-NLA	0.85	2.76
		RNDB-NLB	0.73	2.24
		RNDB-NLA	0.75	2.36
4	4	RNDA-RNDB	1.13	2.27
		RNDA-NLB	1.64	4.82
		RNDA-NLA	2.43	6.62
		RNDB-NLB	1.13	2.76
		RNDB-NLA	1.37	4.02
7	7	RNDB-NLB	3.39	8.98
		RNB-NLA	3.78	9.75
		RNDA-NLB	2.15	6.03
		RNDA-NLA	2.37	6.64
14	14	NONE	NONE	NONE
21	21	NONE	NONE	NONE
28	28	NLB-RNDA	1.38	3.29
42	42	NLA-RNDB	0.82	2.76
		NLA-RNDA	0.88	2.93
		NLB-RNDB	0.70	2.37
		NLB-RNDA	0.75	2.53
42	7	NLB-RNDB	0.43	1.61
		RNDA-RNDB	0.38	1.23
7	42	RNDA-RNDB	1.74	4.51
		RNDA-NLA	2.36	6.62
		RNDA-NLB	2.36	6.63

Table 8: 95% family confidence limits for egg mean comparisons for all virgin parental age combinations.

Table 9 (3 pages): Nonparametric test comparisons for fecundity data from all virgin parental age combinations.

	Wilcoxon 2-sample test				Kruskal-Wallis test		
comparison	f. age	m. age	Z	p>|Z|	chi-square	df	p>chi-square
NLA vs. NLB	1	1	0.1471	0.8831	0.02	1	0.8808
NLA vs. RNDA	1	1	-5.3494	0.0000	28.64	1	0.0001
NLA vs. RNDB	1	1	-5.6037	0.0000	31.43	1	0.0001
NLB vs. RNDA	1	1	-5.8999	0.0000	34.84	1	0.0001
NLB vs. RNDB	1	1	-6.0086	0.0000	36.14	1	0.0001
RNDA vs. RNDB	1	1	-0.5624	0.5738	0.32	1	0.5720
4 lines	1	1	-	-	65.71	3	0.0001
NLA vs. NLB	2	2	-0.1447	0.8849	0.02	1	0.8828
NLA vs. RNDA	2	2	-3.7227	0.0002	13.88	1	0.0002
NLA vs. RNDB	2	2	3.0836	0.0020	9.53	1	0.0020
NLB vs. RNDA	2	2	-3.8574	0.0001	14.90	1	0.0001
NLB vs. RNDB	2	2	-3.1323	0.0017	9.83	1	0.0017
RNDA vs. RNDB	2	2	0.7833	0.4334	0.62	1	0.4319
4 lines	2	2	-	-	24.46	3	0.0010
NLA vs. NLB	4	4	-1.2108	0.2260	1.47	1	0.2250
NLA vs. RNDA	4	4	-6.7617	0.0000	45.76	1	0.0001
NLA vs. RNDB	4	4	-4.5192	0.0000	20.45	1	0.0001
NLB vs. RNDA	4	4	-4.7909	0.0000	22.98	1	0.0001
NLB vs. RNDB	4	4	-2.6763	0.0074	7.18	1	0.0074
RNDA vs. RNDB	4	4	2.5590	0.0105	6.56	1	0.0104
4 lines	4	4	-	-	52.50	3	0.0001
NLA vs. NLB	7	7	-0.8937	0.3715	0.80	1	0.3701
NLA vs. RNDA	7	7	-5.2248	0.0000	27.33	1	0.0001
NLA vs. RNDB	7	7	-6.7033	0.0000	44.97	1	0.0001
NLB vs. RNDA	7	7	-4.9910	0.0000	24.94	1	0.0001
NLB vs. RNDB	7	7	-6.4569	0.0000	41.72	1	0.0001

Table 9 (3 pages): Nonparametric test comparisons for fecundity data from all virgin parental age combinations.

	Wilcoxon 2-sample test				Kruskal-Wallis test		
comparison	f. age	m. age	Z	p>|Z|	chi-square	df	p>chi-square
RNDA vs. RNDB	7	7	-2.8576	0.0043	8.18	1	0.0042
4 lines	7	7	-	-	73.64	3	0.0001
NLA vs. NLB	14	14	0.9641	0.3350	0.93	1	0.3337
NLA vs. RNDA	14	14	1.4997	0.1337	2.26	1	0.1330
NLA vs. RNDB	14	14	0.7717	0.4766	0.51	1	0.4750
NLB vs. RNDA	14	14	0.1707	0.8644	0.03	1	0.8624
NLB vs. RNDB	14	14	-0.3204	0.7487	0.10	1	0.7467
RNDA vs. RNDB	14	14	-0.5437	0.5867	0.30	1	0.5849
4 lines	14	14	-	-	2.05	3	0.5620
NLA vs. NLB	21	21	0.2763	0.7823	0.08	1	0.7803
NLA vs. RNDA	21	21	0.1552	0.8767	0.02	1	0.8746
NLA vs. RNDB	21	21	-0.0210	0.9832	0.00	1	0.9811
NLB vs. RNDA	21	21	-0.2130	0.8313	0.05	1	0.8293
NLB vs. RNDB	21	21	-0.0868	0.9308	0.01	1	0.9288
RNDA vs. RNDB	21	21	0.0684	0.9455	0.01	1	0.9434
4 lines	21	21	-	-	0.07	3	0.9955
NLA vs. NLB	28	28	-1.9229	0.0545	3.71	1	0.0542
NLA vs. RNDA	28	28	1.3192	0.1862	1.75	1	0.1862
NLA vs. RNDB	28	28	-0.0158	0.9874	0.00	1	0.9853
NLB vs. RNDA	28	28	2.8776	0.0040	8.30	1	0.0040
NLB vs. RNDB	28	28	1.6537	0.0982	2.74	1	0.0976
RNDA vs. RNDB	28	28	-1.4420	0.1493	2.09	1	0.1486
4 lines	28	28	-	-	9.29	3	0.0257

Table 9 (3 pages): Nonparametric test comparisons for fecundity data from all virgin parental age combinations.

comparison	Wilcoxon 2-sample test f. age	m. age	Z	p>|Z|	Kruskal-Wallis test chi-square	df	p>chi-square
NLA vs. NLB	42	42	0.7895	0.4298	0.63	1	0.4283
NLA vs. RNDA	42	42	5.7780	0.0000	33.42	1	0.0001
NLA vs. RNDB	42	42	5.7097	0.0000	32.63	1	0.0001
NLB vs. RNDA	42	42	4.6913	0.0000	22.03	1	0.0001
NLB vs. RNDB	42	42	4.4928	0.0000	20.21	1	0.0001
RNDA vs. RNDB	42	42	-0.6481	0.5169	0.42	1	0.5152
4 lines	42	42	-	-	54.73	3	0.0001
NLA vs. NLB	42	7	-1.6565	0.0976	2.75	1	0.0971
NLA vs. RNDA	42	7	-0.4683	0.6396	0.22	1	0.6377
NLA vs. RNDB	42	7	2.2987	0.0215	5.30	1	0.0214
NLB vs. RNDA	42	7	1.1822	0.2371	1.40	1	0.2361
NLB vs. RNDB	42	7	3.9336	0.0001	15.49	1	0.0001
RNDA vs. RNDB	42	7	2.6042	0.0092	6.80	1	0.0091
4 lines	42	7	-	-	15.88	3	0.0012
NLA vs. NLB	7	42	0.2982	0.7656	0.09	1	0.7636
NLA vs. RNDA	7	42	-5.1387	0.0000	26.43	1	0.0001
NLA vs. RNDB	7	42	-1.7619	0.0781	3.11	1	0.0776
NLB vs. RNDA	7	42	-4.9643	0.0000	24.67	1	0.0001
NLB vs. RNDB	7	42	-1.8308	0.0671	3.36	1	0.0667
RNDA vs. RNDB	7	42	3.2521	0.0011	10.59	1	0.0011
4 lines	7	42	-	-	34.26	3	0.0001

Table 9 (continued)

Table 10: grouped fertility means for all virgin parental age combinations (Scheffe's test).

female age	male age				
1	1	0.3378 NLA	0.4112 NLB	0.5701 RNDA	0.7486 RNDB
2	2	0.8103 RNDA	0.8784 NLA	0.8839 RNDB	0.9017 NLB
4	4	0.49006 RNDB	0.6896 NLA	0.7305 RNDA	0.7629 NLB
7	7	0.3203 NLB	0.5574 NLA	0.7672 RNDA	0.8388 RNDB
14	14	0.5977 NLB	0.6164 NLA	0.6647 RNDB	0.7571 RNDA
21	21	0.1905 NLA	0.1986 NLB	0.4141 RNDA	0.4761 RNDB

Table 10: grouped fertility means for all virgin parental age combinations (Scheffe's test).

female age	male age				
28	28	0.1633 RNDA	0.1756 NLA	0.2381 NLB	0.3252 RNDB
42	42	0.0835 NLA	0.0838 NLB	0.1802 RNDA	0.1945 RNDB
42	7	0.1511 NLA	0.1519 RNDA	0.1526 NLB	0.1967 RNDB
7	42	0.4835 NLB	0.5021 RNDB	0.6637 NLA	0.77 RNDA

Table 10 (continued)

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	2	NL RND

NUMBER OF OBSERVATIONS IN DATA SET = 32

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FERT

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FERT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	4	9.54126653	2.38531663	93.31
ERROR	28	0.71577347	0.02556334	PR > F
UNCORRECTED TOTAL	32	10.25704000		0.0001

R-SQUARE	C.V.	ROOT MSE	FERT MEAN
0.674788	31.8655	0.15988539	0.50175000

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

Table 10.1 (continued)

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	8.05609800	315.14	0.0001
STRAIN	1	0.12127812	4.74	0.0380
AGE	1	1.36384208	53.35	0.0001
AGE*STRAIN	1	0.00004833	0.00	0.9656

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	7.71868286	301.94	0.0001
STRAIN	1	0.05776287	2.26	0.1440
AGE	1	1.36384208	53.35	0.0001
AGE*STRAIN	1	0.00004833	0.00	0.9656

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: FERT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 CONFIDENCE=0.95 DF=28 MSE=.0255633
CRITICAL VALUE OF STUDENTIZED RANGE=2.897
MINIMUM SIGNIFICANT DIFFERENCE=.11579

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

STRAIN COMPARISON	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT	
RND - NL	0.00733	0.12313	0.23892	***
NL - RND	-0.23892	-0.12313	-0.00733	***

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

Table 10.1 (continued)

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: FERT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=28 MSE=.0255633
CRITICAL VALUE OF STUDENTIZED RANGE=2.897
MINIMUM SIGNIFICANT DIFFERENCE=.11579

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	STRAIN
A	0.56331	16	RND
B	0.44019	16	NL

GENERAL LINEAR MODELS PROCEDURE

MEANS

STRAIN	N	AGE
NL	16	14.8750000
RND	16	14.8750000

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

Table 10.1 (continued)

FERTILITY PROPORTIONS REGRESSIONS
STRAIN=NL

DEP VARIABLE: FERT

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	1	0.67382682	0.67382682	19.701	0.0006
ERROR	14	0.47882962	0.03420212		
C TOTAL	15	1.15265644			

ROOT MSE	0.1849381	R-SQUARE	0.5846
DEP MEAN	0.4401875	ADJ R-SQ	0.5549
C.V.	42.01349		

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR H0: PARAMETER=0	PROB > |T|
INTERCEP	1	0.66425103	0.06845372	9.704	0.0001
AGE	1	-0.01506309	0.003393646	-4.439	0.0006

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

Table 10.1 (continued)

FERTILITY PROPORTIONS REGRESSIONS

STRAIN=RND

DEP VARIABLE: FERT

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	1	0.69006358	0.69006358	40.773	0.0001
ERROR	14	0.23694386	0.01692456		
C TOTAL	15	0.92700744			

ROOT MSE	0.1300944	R-SQUARE	0.7444
DEP MEAN	0.5633125	ADJ R-SQ	0.7261
C.V.	23.09454		

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR H0: PARAMETER=0	PROB > |T|
INTERCEP	1	0.79005952	0.04815366	16.407	0.0001
AGE	1	-0.01524350	0.002387255	-6.385	0.0001

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

Table 10.1 (continued)

FERTILITY PROPORTIONS ANALYSIS OF COVARIANCE MODEL

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	2	NL RND

NUMBER OF OBSERVATIONS IN DATA SET = 32

FERTILITY PROPORTIONS ANALYSIS OF COVARIANCE MODEL
GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FERT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	1.48512020	0.74256010	30.08
ERROR	29	0.71582180	0.02468351	PR > F
CORRECTED TOTAL	31	2.20094200		0.0001

R-SQUARE	C.V.	ROOT MSE	FERT MEAN
0.674766	31.3124	0.15710987	0.50175000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
STRAIN	1	0.12127812	4.91	0.0347
AGE	1	1.36384208	55.25	0.0001

Table 10.1
Parametric tests comparing NL and RND strain fertility means vs. age.

Table 10.1 (continued)

SOURCE	DF	TYPE III SS	F VALUE	PR > F
STRAIN	1	0.12127812	4.91	0.0347
AGE	1	1.36384208	55.25	0.0001

FERTILITY PROPORTIONS ANALYSIS OF COVARIANCE MODEL
GENERAL LINEAR MODELS PROCEDURE

LEAST SQUARES MEANS

STRAIN	FERT LSMEAN	STD ERR LSMEAN	PROB > |T| H0:LSMEAN=0	PROB > |T| H0: LSMEAN1=LSMEAN2
NL	0.44018750	0.03927747	0.0001	0.0347
RND	0.56331250	0.03927747	0.0001	

female age	male age	comparison	lower limit	upper limit
1	1	RNDB-RNDA	0.0003	0.1253
		RNDB-NLB	0.0236	0.2683
		RNDB-NLA	0.0477	0.3478
		RNDA-NLA	0.001	0.1816
2	2	NONE	NONE	NONE
4	4	NLB-RNDB	0.0042	0.2328
		RNDA-RNDB	0.002	0.1919
7	7	RNDB-NLA	0.0207	0.2175
		RNDB-NLB	0.1421	0.4405
		RNDA-NLA	0.003	0.6196
		RNDA-NLB	0.0855	0.3515
		NLA-NLB	0.0046	0.1623
14	14	NONE	NONE	NONE
21	21	RNDB-NLB	0.0177	0.2016
		RNDB-NLA	0.0211	0.2081
		RNDA-NLB	0.005	0.1528
		RNDA-NLA	0.0069	0.1586
28	28	RNDB-NLA	3.4225E-06	0.1157
		RNDB-RNDA	0.0002	0.1293
42	42	RNDB-NLB	0.0038	0.0682
		RNDB-NLA	0.0042	0.0673
		RNDA-NLB	0.0016	0.061
		RNDA-NLA	0.0018	0.0602
42	7	NONE	NONE	NONE
7	42	RNDA-RNDB	0.009	0.2061
		RNDA-NLB	0.012	0.2247

Table 11: 95% family confidence intervals for fertility means for all virgin parental age combinations (Scheffe's test).

fem. age	male age	ss model df=7	ss error df=232	ms model	ms error	F	Pr>F
1	1	66.3245	138.268	9.4749	0.596	15.9	0.0001
2	2	54.8085	273.889	7.8298	1.1806	6.63	0.0001
4	4	116.1922	376.742	16.599	1.6238	10.22	0.0001
7	7	209.5172	500.104	29.931	2.1556	13.89	0.0001
14	14	21.3422	537.943	3.0489	2.3187	1.31	0.2439
21	21	63.8788	500.561	9.1255	2.1576	4.23	0.0002
28	28	34.5862	4330.75	4.9409	1.8667	2.65	0.0119
42	42	56.6499	245.718	8.0928	1.0591	7.64	0.0001
42	7	22.8053	204.865	3.2579	0.883	3.69	0.0008
7	42	98.1107	498.265	14.0158	2.1477	6.53	0.0001

femal age	male age	ss strain df=3	F	pr>f	rep(strain) df=4	F	Pr>F
1	1	55.133	30.84	0.0001	11.1914	4.69	0.0012
2	2	42.2257	11.92	0.0001	12.5828	2.66	0.0333
4	4	105.5746	21.67	0.0001	10.6176	1.63	0.1663
7	7	200.9062	31.08	0.0001	8.557	0.99	0.4124
14	14	4.67045	0.67	0.5704	16.6717	1.8	0.1301
21	21	0.4549	0.07	0.9758	63.4239	7.35	0.0001
28	28	16.7353	2.99	0.0319	17.8509	2.39	0.0516
42	42	55.6574	17.52	0.0001	0.9925	0.23	0.9189
42	7	14.0769	5.31	0.0015	8.7284	2.47	0.0454
7	42	94.6543	14.69	0.0001	3.4564	0.4	0.8069

Table 12: Analysis of variance statistics for fecundity for all virgin parental age combinations.

fem. age	male age	ss model df=7	ss error	df error	ms model	ms error	F	Pr>F
1	1	8.2711	14.8836	156	1.1815	0.0954	12.38	0.0001
2	2	1.2682	16.699	221	0.1812	0.0756	2.4	0.022
4	4	3.0572	28.0791	193	0.4367	0.1454	3	0.0052
7	7	12.5459	22.7015	220	1.7923	0.1031	17.37	0.0001
14	14	6.2206	26.4398	227	0.8887	0.1165	7.63	0.0001
21	21	5.5685	22.5859	226	0.7955	0.0999	7.96	0.0001
28	28	2.014	22.1676	213	0.2877	0.1041	2.76	0.009
42	42	1.3018	6.2945	194	0.186	0.0324	5.73	0.0001
42	7	0.1942	6.2912	162	0.0277	0.0388	0.71	0.6598
7	42	6.1183	24.7743	203	0.874	0.122	7.16	0.0001

fem. age	male age	ss strain df=3	F	pr>f	rep(strain) df=4	F	Pr>F
1	1	4.2031	14.68	0.0001	4.068	10.66	0.0001
2	2	0.5714	2.52	0.0588	0.6967	2.31	0.0593
4	4	2.4962	5.72	0.0009	0.5609	0.96	0.4284
7	7	10.5245	34	0.0001	2.0214	4.9	0.0008
14	14	1.0528	3.01	0.0309	5.1679	11.09	0.0001
21	21	4.4759	14.93	0.0001	1.0926	2.73	0.0298
28	28	1.2412	3.98	0.0088	0.7727	1.86	0.1193
42	42	1.203	12.36	0.0001	0.0988	0.76	0.5516
42	7	0.0932	0.8	0.4957	0.101	0.65	0.6274
7	42	3.3146	9.05	0.0001	2.8037	5.74	0.0002

Table 13: Analysis of variance statistics for fertility for all virgin parental age combinations.

Table 14: Total numbers of fertile matings for virgin females of all parental age combinations.

strain	female age	male age	n tested	n mated
NLA	1	1	60	12
NLB	1	1	60	17
RNDA	1	1	60	44
RNDB	1	1	60	45
NL	1	1	120	29
RND	1	1	120	89
NLA	2	2	60	53
NLB	2	2	60	52
RNDA	2	2	60	59
RNDB	2	2	60	59
NL	2	2	120	105
RND	2	2	120	118
NLA	4	4	60	40
NLB	4	4	60	34
RNDA	4	4	60	57
RNDB	4	4	60	44
NL	4	4	120	77
RND	4	4	120	101
NLA	7	7	60	39
NLB	7	7	60	31
RNDA	7	7	60	57
RNDB	7	7	60	56
NL	7	7	120	70
RND	7	7	120	113
NLA	14	14	60	55
NLB	14	14	60	50
RNDA	14	14	60	56
RNDB	14	14	60	49
NL	14	14	120	105
RND	14	14	120	105
NLA	21	21	60	34
NLB	21	21	60	30
RNDA	21	21	60	38
RNDB	21	21	60	44
NL	21	21	120	64
RND	21	21	120	82

Table 14: Total numbers of fertile matings for virgin females of all parental age combinations.

strain	female age	male age	n tested	n mated
NLA	28	28	60	23
NLB	28	28	60	30
RNDA	28	28	60	15
RNDB	28	28	60	35
NL	28	28	120	53
RND	28	28	120	50
NLA	42	42	60	9
NLB	42	42	60	8
RNDA	42	42	60	5
RNDB	42	42	60	7
NL	42	42	120	17
RND	42	42	120	12
NLA	42	7	60	4
NLB	42	7	60	9
RNDA	42	7	60	6
RNDB	42	7	60	4
NL	42	7	120	13
RND	42	7	120	10
NLA	7	42	60	47
NLB	7	42	60	34
RNDA	7	42	60	53
RNDB	7	42	60	38
NL	7	42	120	81
RND	7	42	120	91

Table 14 (continued)

comparison	fem. age	male age	chi-square (Yates)	p> chi-square
NL vs. RND	1	1	58.033	0.0001***
NL vs. RND	2	2	9.116	0.0025***
NL vs. RND	4	4	11.504	0.0007***
NL vs. RND	7	7	40.587	0.0001***
NL vs. RND	14	14	0.105	0.7462 n.s.
NL vs. RND	21	21	5.054	0.0246*
NL vs. RND	28	28	0.068	0.7942 n.s.
NL vs. RND	42	42	0.628	0.4283 n.s.
NL vs. RND	42	7	0.192	0.6610 n.s.
NL vs. RND	7	42	1.662	0.1973 n.s.

Table 15: Chi-square statistics for comparisons of the number of fertile matings of NL vs. RND females for all parental age combinations.

Note:
* = significant
*** = very highly significant
n.s. = not significant

Table 16 (4 pages): Statistics for analysis of variance of geotaxis experiment regressions.

Strain	sex	code	Age	Rep	ss model	ss error	ms	ms error	F	prob>F
NLA	m	-1	0	1	5.4427	1.3069	5.4427	0.0523	104.12	0.0001
NLA	f	1	0	1	4.8020	0.8451	4.8020	0.0338	142.00	0.0001
NLA	m	-1	0	2	4.0201	1.3518	4.0201	0.0541	74.35	0.0001
NLA	f	1	0	2	5.0000	1.0067	5.0000	0.0420	124.17	0.0001
NLB	m	-1	0	1	5.6180	1.1687	5.6180	0.0467	120.18	0.0001
NLB	f	1	0	1	5.9769	1.1231	5.9769	0.0449	133.04	0.0001
NLB	m	-1	0	2	6.5742	1.1376	6.5742	0.0455	144.47	0.0001
NLB	f	1	0	2	4.7694	1.3869	4.7694	0.0555	85.97	0.0001
RNDA	m	-1	0	1	5.9405	0.8536	5.9405	0.0341	173.99	0.0001
RNDA	f	1	0	1	4.9336	0.6864	4.9336	0.0275	179.68	0.0001
RNDA	m	-1	0	2	4.4180	1.4020	4.4180	0.0561	78.78	0.0001
RNDA	f	1	0	2	4.5125	1.2149	4.5125	0.0486	92.86	0.0001
RNDB	m	-1	0	1	3.8136	2.4064	3.8136	0.0963	39.68	0.0001
RNDB	f	1	0	1	3.3620	1.3847	3.3620	0.0554	60.70	0.0001
RNDB	m	-1	0	2	7.0805	0.9802	7.0805	0.0392	180.58	0.0001
RNDB	f	1	0	2	5.0333	1.3585	5.0333	0.0543	92.63	0.0001
NLA	m	-1	3	1	5.7961	1.8625	5.7961	0.0745	77.80	0.0001
NLA	f	1	3	1	5.0334	1.4385	5.0334	0.0575	87.48	0.0001
NLA	m	-1	3	2	7.8542	1.1776	7.8542	0.0471	166.74	0.0001
NLA	f	1	3	2	6.6509	0.6091	6.6509	0.2436	272.98	0.0001
NLB	m	-1	3	1	5.6180	1.1887	5.6180	0.0475	118.16	0.0001
NLB	f	1	3	1	5.1342	0.8021	5.1342	0.0321	160.03	0.0001
NLB	m	-1	3	2	10.7067	1.2140	10.7067	0.0486	220.48	0.0001
NLB	f	1	3	2	9.2934	1.5629	9.2934	0.0625	148.66	0.0001
RNDA	m	-1	3	1	4.9667	0.9896	4.9667	0.0396	125.48	0.0001
RNDA	f	1	3	1	6.3845	0.5185	6.3845	0.2074	307.86	0.0001
RNDA	m	-1	3	2	7.4827	0.7202	7.4827	0.0288	259.73	0.0001
RNDA	f	1	3	2	9.2934	1.4007	9.2934	0.0560	165.87	0.0001
RNDB	m	-1	3	1	3.3347	0.7171	3.3347	0.0287	116.25	0.0001
RNDB	f	1	3	1	6.0500	1.7367	6.0500	0.0695	87.09	0.0001
RNDB	m	-1	3	2	8.2347	1.0460	8.2347	0.0418	196.81	0.0001

Table 16 (4 pages): Statistics for analysis of variance of geotaxis experiment regressions.

Strain	sex	code	Age	Rep	ss model	ss error	ms	ms error	F	prob>F
RNDB	f	1	3	2	9.6605	1.0958	9.6605	0.0438	220.40	0.0001
NLA	m	-1	7	1	4.3245	0.9762	4.3245	0.0390	110.74	0.0001
NLA	f	1	7	1	3.6694	1.5380	3.6694	0.0615	59.65	0.0001
NLA	m	-1	7	2	8.6681	0.6038	8.6681	0.0242	358.90	0.0001
NLA	f	1	7	2	8.8002	1.1205	8.8002	0.0448	196.34	0.0001
NLB	m	-1	7	1	5.1005	1.3462	5.1005	0.0538	94.72	0.0001
NLB	f	1	7	1	4.9667	0.8999	4.9667	0.0360	137.97	0.0001
NLB	m	-1	7	2	11.4005	1.0062	11.4005	0.0403	283.27	0.0001
NLB	f	1	7	2	10.8536	10.8536	10.8536	0.0158	688.94	0.0001
RNDA	m	-1	7	1	5.5476	0.9376	5.5476	0.0375	147.91	0.0001
RNDA	f	1	7	1	7.2401	0.8199	7.2401	0.0328	220.75	0.0001
RNDA	m	-1	7	2	9.1576	1.1276	9.1576	0.0451	203.03	0.0001
RNDA	f	1	7	2	11.9609	1.4021	11.9609	0.0561	213.27	0.0001
RNDB	m	-1	7	1	3.9014	0.9394	3.9014	0.0376	103.83	0.0001
RNDB	f	1	7	1	5.6181	0.9816	5.6181	0.0393	131.63	0.0001
RNDB	m	-1	7	2	8.1494	0.8314	8.1494	0.0333	245.06	0.0001
RNDB	f	1	7	2	8.8002	0.7472	8.8002	0.0299	294.45	0.0001
NLA	m	-1	14	1	5.0000	1.5919	5.0000	0.0637	78.53	0.0001
NLA	f	1	14	1	4.8347	0.6119	4.8347	0.0245	197.52	0.0001
NLA	m	-1	14	2	5.1342	1.7110	5.1342	0.0684	75.02	0.0001
NLA	f	1	14	2	3.0681	1.3349	3.0681	0.0534	57.46	0.0001
NLB	m	-1	14	1	5.2361	0.9625	5.2361	0.0385	136.01	0.0001
NLB	f	1	14	1	6.4601	0.8118	6.4601	0.0325	198.94	0.0001
NLB	m	-1	14	2	7.0409	0.6710	7.0409	0.0268	262.34	0.0001
NLB	f	1	14	2	8.6242	0.5743	8.6242	0.0230	375.43	0.0001
RNDA	m	-1	14	1	5.2020	1.2432	5.2020	0.0497	104.61	0.0001
RNDA	f	1	14	1	9.4761	0.5469	9.4761	0.0219	433.17	0.0001
RNDA	m	-1	14	2	4.2936	1.1872	4.2936	0.0475	90.42	0.0001
RNDA	f	1	14	2	8.4934	0.3207	8.4934	0.0128	662.13	0.0001
RNDB	m	-1	14	1	8.0645	0.8622	8.0645	0.0345	233.84	0.0001
RNDB	f	1	14	1	10.3201	0.2385	10.3201	0.0095	1081.94	0.0001

Table 16 (4 pages): Statistics for analysis of variance of geotaxis experiment regressions.

Strain	sex	code	Age	Rep	ss model	ss error	ms	ms error	F	prob>F
RNDB	m	-1	14	2	7.8542	0.7065	7.8542	0.0283	277.92	0.0001
RNDB	f	1	14	2	9.4761	0.6506	9.4761	0.0260	364.12	0.0001
NLA	m	-1	21	1	3.0942	0.5399	3.0942	0.0216	143.29	0.0001
NLA	f	1	21	1	5.9769	0.5216	5.9769	0.0201	286.45	0.0001
NLA	m	-1	21	2	3.5842	0.4810	3.5842	0.0192	186.30	0.0001
NLA	f	1	21	2	7.1601	0.6340	7.1601	0.0254	282.33	0.0001
NLB	m	-1	21	1	2.2445	0.2207	2.2445	0.0088	254.27	0.0001
NLB	f	1	21	1	10.1769	0.4794	10.1769	0.0192	530.70	0.0001
NLB	m	-1	21	2	2.0056	0.7152	2.0056	0.0286	70.11	0.0001
NLB	f	1	21	2	10.0820	1.1787	10.0820	0.0471	213.83	0.0001
RNDA	m	-1	21	1	4.0201	0.3140	4.0201	0.0126	320.05	0.0001
RNDA	f	1	21	1	7.8542	0.3287	7.8542	0.0131	597.30	0.0001
RNDA	m	-1	21	2	4.5442	0.4521	4.5442	0.0181	251.30	0.0001
RNDA	f	1	21	2	9.0676	0.4510	9.0676	0.0180	502.68	0.0001
RNDB	m	-1	21	1	7.6880	0.5587	7.6880	0.0223	344.03	0.0001
RNDB	f	1	21	1	9.8000	0.4496	9.8000	0.0179	544.89	0.0001
RNDB	m	-1	21	2	6.6125	0.9060	6.6125	0.0362	182.46	0.0001
RNDB	f	1	21	2	11.3001	0.3666	11.3001	0.0147	770.58	0.0001
NLA	m	-1	28	1	0.0347	0.1905	0.0347	0.0076	4.56	0.0428
NLA	f	1	28	1	0.5014	0.4253	0.5014	0.0170	29.47	0.0001
NLA	m	-1	28	2	0.0347	0.1594	0.0347	0.0064	5.45	0.0279
NLA	f	1	28	2	1.9014	0.5482	1.9014	0.0219	86.70	0.0001
NLB	m	-1	28	1	0.0027	0.0691	0.0027	0.0028	0.98	0.3306
NLB	f	1	28	1	1.9101	0.5527	1.9101	0.0221	86.01	0.0001
NLB	m	-1	28	2	0.0005	0.0758	0.0005	0.0030	0.17	0.6881
NLB	f	1	28	2	1.4045	0.3407	1.4045	0.0136	103.06	0.0001
RNDA	m	-1	28	1	0.0161	0.0936	0.0161	0.0037	4.29	0.0488
RNDA	f	1	28	1	1.0276	0.1687	1.0276	0.0067	152.24	0.0001
RNDA	m	-1	28	2	0.0720	0.0487	0.0720	0.0019	36.93	0.0001
RNDA	f	1	28	2	2.3805	0.1514	2.3805	0.0061	393.21	0.0001

Table 16 (4 pages): Statistics for analysis of variance of geotaxis experiment regressions.

Strain	sex	code	Age	Rep	ss model	ss error	ms	ms error	F	prob>F
RNDB	m	-1	28	1	0.1027	0.4039	0.1027	0.0162	6.36	0.0184
RNDB	f	1	28	1	6.0500	1.1130	6.0500	0.0445	135.90	0.0001
RNDB	m	-1	28	2	1.2667	0.2733	1.2667	0.0109	115.88	0.0001
RNDB	f	1	28	2	4.9005	1.7847	4.9005	0.0714	68.65	0.0001
NLA	m	-1	35	1	4.0500	1.2167	4.0500	0.0487	83.22	0.0001
NLA	f	1	35	1	1.4580	0.7316	1.4580	0.0293	49.82	0.0001
NLA	m	-1	35	2	4.1102	1.1772	4.1102	0.0471	87.29	0.0001
NLA	f	1	35	2	0.7867	0.4585	0.7867	0.0183	42.00	0.0001
NLB	m	-1	35	1	0.7476	0.2243	0.7476	0.0090	83.32	0.0001
NLB	f	1	35	1	1.0889	0.1711	1.0889	0.0068	159.09	0.0001
NLB	m	-1	35	2	0.6125	0.2927	0.6125	0.0117	52.32	0.0001
NLB	f	1	35	2	0.4302	0.3083	0.4302	0.0123	34.89	0.0001
RNDA	m	-1	35	1	0.0681	0.0986	0.0681	0.0039	17.25	0.0003
RNDA	f	1	35	1	0.0180	0.0583	0.0180	0.0023	7.72	0.0102
RNDA	m	-1	35	2	0.0436	0.0964	0.0436	0.0039	11.29	0.0025
RNDA	f	1	35	2	0.0125	0.0416	0.0125	0.0017	7.52	0.0111
RNDB	m	-1	35	1	0.0201	0.1518	0.0201	0.0061	3.30	0.0812
RNDB	f	1	35	1	0.0036	0.0150	0.0036	0.0006	5.94	0.0222
RNDB	m	-1	35	2	0.0889	0.1452	0.0889	0.0058	15.31	0.0006
RNDB	f	1	35	2	0.0036	0.0150	0.0036	0.0006	5.94	0.0222

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

HOMOGENEITY-OF-SLOPES MODEL

AGE=0

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	0
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432

HOMOGENEITY-OF-SLOPES MODEL

AGE=0

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	122.77953704	9.44457977	189.98
ERROR	419	20.83046296	0.04971471	PR > F
UNCORRECTED TOTAL	432	143.61000000		0.0

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.804748	76.2646	0.22296795	0.29236111

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	36.92520833	742.74	0.0
STRAIN	3	1.12321759	7.53	0.0001
ANGLE	1	80.56767014	1620.60	0.0
SEX	1	0.10391204	2.09	0.1490
ANGLE*STRAIN	3	0.11962153	0.80	0.4932
REP(STRAIN)	4	3.93990741	19.81	0.0001

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	117.43648039	2362.21	0.0
STRAIN	3	0.35526253	2.38	0.0690
ANGLE	1	80.56767014	1620.60	0.0
SEX	1	0.10391204	2.09	0.1490
ANGLE*STRAIN	3	0.11962153	0.80	0.4932
REP(STRAIN)	4	3.93990741	19.81	0.0001

HOMOGENEITY-OF-SLOPES MODEL

AGE=0

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

AGE	1	0
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432
HOMOGENEITY-OF-SLOPES MODEL

AGE=0

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	12	122.67562500	10.22296875	205.10
ERROR	420	20.93437500	0.04984375	PR > F
UNCORRECTED TOTAL	432	143.61000000		0.0

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.803774	76.3635	0.22325714	0.29236111

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	36.92520833	740.82	0.0
STRAIN	3	1.12321759	7.51	0.0001

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

ANGLE	1	80.56767014	1616.40	0.0
ANGLE*STRAIN	3	0.11962153	0.80	0.4944
REP(STRAIN)	4	3.93990741	19.76	0.0001

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	117.43648039	2356.09	0.0
STRAIN	3	0.35526253	2.38	0.0695
ANGLE	1	80.56767014	1616.40	0.0
ANGLE*STRAIN	3	0.11962153	0.80	0.4944
REP(STRAIN)	4	3.93990741	19.76	0.0001

HOMOGENEITY-OF-SLOPES MODEL

AGE=0

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 CONFIDENCE=0.95 DF=420 MSE=.0498438
CRITICAL VALUE OF STUDENTIZED RANGE=3.648
MINIMUM SIGNIFICANT DIFFERENCE=.07836

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

STRAIN COMPARISON	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT	
RNDB - NLB	0.00775	0.08611	0.16448	***
RNDB - RNDA	0.00867	0.08704	0.16540	***
RNDB - NLA	0.06423	0.14259	0.22096	***
NLB - RNDB	-0.16448	-0.08611	-0.00775	***

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

NLB	- RNDA	-0.07744	0.00093	0.07929	
NLB	- NLA	-0.02188	0.05648	0.13485	
RNDA	- RNDB	-0.16540	-0.08704	-0.00867	***
RNDA	- NLB	-0.07929	-0.00093	0.07744	
RNDA	- NLA	-0.02281	0.05556	0.13392	
NLA	- RNDB	-0.22096	-0.14259	-0.06423	***
NLA	- NLB	-0.13485	-0.05648	0.02188	
NLA	- RNDA	-0.13392	-0.05556	0.02281	

HOMOGENEITY-OF-SLOPES MODEL

AGE=0

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=420 MSE=.0498438
CRITICAL VALUE OF STUDENTIZED RANGE=3.648
MINIMUM SIGNIFICANT DIFFERENCE=.07836

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	STRAIN
A	0.37130	108	RNDB
B	0.28519	108	NLB
B			
B	0.28426	108	RNDA
B			
B	0.22870	108	NLA

HOMOGENEITY-OF-SLOPES MODEL

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

AGE=0

GENERAL LINEAR MODELS PROCEDURE

MEANS

STRAIN	N	ANGLE
NLA	108	40.0000000
NLB	108	40.0000000
RNDA	108	40.0000000
RNDB	108	40.0000000

HOMOGENEITY-OF-SLOPES MODEL

AGE=3

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	3
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432
HOMOGENEITY-OF-SLOPES MODEL

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

AGE=3

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	116.63235185	8.97171937	168.59
ERROR	419	22.29764815	0.05321634	PR > F
UNCORRECTED TOTAL	432	138.93000000		0.0

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.831779	189.8222	0.23068668	-0.12152778

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	6.38020833	119.89	0.0001
STRAIN	3	0.36506944	2.29	0.0781
ANGLE	1	109.16128125	2051.27	0.0
SEX	1	0.11020833	2.07	0.1509
ANGLE*STRAIN	3	0.11845486	0.74	0.5275
REP(STRAIN)	4	0.49712963	2.34	0.0549

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	54.88192157	1031.30	0.0
STRAIN	3	0.23075163	1.45	0.2290
ANGLE	1	109.16128125	2051.27	0.0
SEX	1	0.11020833	2.07	0.1509

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

ANGLE*STRAIN	3	0.11845486	0.74	0.5275
REP(STRAIN)	4	0.49712963	2.34	0.0549

HOMOGENEITY-OF-SLOPES MODEL

AGE=3

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	3
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432

HOMOGENEITY-OF-SLOPES MODEL

AGE=3

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	12	116.52214352	9.71017863	182.00
ERROR	420	22.40785648	0.05335204	PR > F

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

UNCORRECTED TOTAL	432	138.93000000		0.0

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.830948	190.0640	0.23098060	-0.12152778

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	6.38020833	119.59	0.0001
STRAIN	3	0.36506944	2.28	0.0787
ANGLE	1	109.16128125	2046.06	0.0
ANGLE*STRAIN	3	0.11845486	0.74	0.5286
REP(STRAIN)	4	0.49712963	2.33	0.0554

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	54.88192157	1028.68	0.0
STRAIN	3	0.23075163	1.44	0.2301
ANGLE	1	109.16128125	2046.06	0.0
ANGLE*STRAIN	3	0.11845486	0.74	0.5286
REP(STRAIN)	4	0.49712963	2.33	0.0554

HOMOGENEITY-OF-SLOPES MODEL

AGE=3

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 CONFIDENCE=0.95 DF=420 MSE=0.053352
CRITICAL VALUE OF STUDENTIZED RANGE=3.648
MINIMUM SIGNIFICANT DIFFERENCE=.08108

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

STRAIN COMPARISON	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT
RNDA - NLB	-0.01719	0.06389	0.14496
RNDA - NLA	-0.01348	0.06759	0.14867
RNDA - RNDB	-0.01163	0.06944	0.15052
NLB - RNDA	-0.14496	-0.06389	0.01719
NLB - NLA	-0.07737	0.00370	0.08478
NLB - RNDB	-0.07552	0.00556	0.08663
NLA - RNDA	-0.14867	-0.06759	0.01348
NLA - NLB	-0.08478	-0.00370	0.07737
NLA - RNDB	-0.07922	0.00185	0.08293
RNDB - RNDA	-0.15052	-0.06944	0.01163
RNDB - NLB	-0.08663	-0.00556	0.07552
RNDB - NLA	-0.08293	-0.00185	0.07922

HOMOGENEITY-OF-SLOPES MODEL

AGE=3

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=420 MSE=0.053352
CRITICAL VALUE OF STUDENTIZED RANGE=3.648
MINIMUM SIGNIFICANT DIFFERENCE=.08108

Table 17 (continued): General linear model analysis of geotaxis data for zero and 3 day-old virgin, all strains.

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY	GROUPING	MEAN	N	STRAIN
	A	-0.07130	108	RNDA
	A			
	A	-0.13519	108	NLB
	A			
	A	-0.13889	108	NLA
	A			
	A	-0.14074	108	RNDB

HOMOGENEITY-OF-SLOPES MODEL

AGE=3

GENERAL LINEAR MODELS PROCEDURE

MEANS

STRAIN	N	ANGLE
NLA	108	40.0000000
NLB	108	40.0000000
RNDA	108	40.0000000
RNDB	108	40.0000000

Table 18 : General linear models analysis of geotaxis data for 7 day-old virgins.

HOMOGENEITY-OF-SLOPES MODEL
AGE=7

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	7
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432
HOMOGENEITY-OF-SLOPES MODEL
AGE=7

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	143.54918981	11.04224537	230.17
ERROR	419	20.10081019	0.04797329	PR > F
UNCORRECTED TOTAL	432	163.65000000		0.0

Table 18 : General linear models analysis of geotaxis data for 7 day-old virgins.

Table 18 (continued)

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.859499	100.3395	0.21902807	-0.21828704

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	20.58446759	429.08	0.0001
STRAIN	3	2.39247685	16.62	0.0001
ANGLE	1	113.76450000	2371.41	0.0
SEX	1	0.24557870	5.12	0.0242
ANGLE*STRAIN	3	0.48077778	3.34	0.0193
REP(STRAIN)	4	6.08138889	31.69	0.0001

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	42.25949047	880.90	0.0
STRAIN	3	1.61193110	11.20	0.0001
ANGLE	1	113.76450000	2371.41	0.0
SEX	1	0.24557870	5.12	0.0242
ANGLE*STRAIN	3	0.48077778	3.34	0.0193
REP(STRAIN)	4	6.08138889	31.69	0.0001

Table 19 : General linear models analysis of geotaxis data for 14 day-old virgins, all strains..

HOMOGENEITY-OF-SLOPES MODEL
AGE=14

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	14
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432
HOMOGENEITY-OF-SLOPES MODEL
AGE=14

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	139.81219907	10.75478454	275.65
ERROR	419	16.34780093	0.03901623	PR > F
UNCORRECTED TOTAL	432	156.16000000		0.0

Table 19 : General linear models analysis of geotaxis data for 14 day-old virgins, all strains..

Table 19 (continued)

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.878346	87.9700	0.19752527	-0.22453704

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	21.78009259	558.23	0.0
STRAIN	3	6.86527778	58.65	0.0001
ANGLE	1	105.83833681	2712.67	0.0
SEX	1	2.19592593	56.28	0.0001
ANGLE*STRAIN	3	1.52423264	13.02	0.0001
REP(STRAIN)	4	1.60833333	10.31	0.0001

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	37.36230093	957.61	0.0
STRAIN	3	5.71040605	48.79	0.0001
ANGLE	1	105.83833681	2712.67	0.0
SEX	1	2.19592593	56.28	0.0001
ANGLE*STRAIN	3	1.52423264	13.02	0.0001
REP(STRAIN)	4	1.60833333	10.31	0.0001

Table 20: General linear models analysis of geotaxis data for 21 day-old virgins, all strains.

HOMOGENEITY-OF-SLOPES MODEL

AGE=21

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	21
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432

HOMOGENEITY-OF-SLOPES MODEL

AGE=21

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	134.55117593	10.35009046	276.95
ERROR	419	15.65882407	0.03737190	PR > F
UNCORRECTED TOTAL	432	150.21000000		0.0

Table 20: General linear models analysis of geotaxis data for 21 day-old virgins, all strains.

Table 20 (continued)

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.889434	137.1321	0.19331812	0.14097222

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	8.58520833	229.72	0.0001
STRAIN	3	2.79377315	24.92	0.0001
ANGLE	1	99.19800347	2654.35	0.0
SEX	1	22.45891204	600.96	0.0
ANGLE*STRAIN	3	1.34814931	12.02	0.0001
REP(STRAIN)	4	0.16712963	1.12	0.3474

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	99.14117647	2652.83	0.0
STRAIN	3	0.58943573	5.26	0.0014
ANGLE	1	99.19800347	2654.35	0.0
SEX	1	22.45891204	600.96	0.0
ANGLE*STRAIN	3	1.34814931	12.02	0.0001
REP(STRAIN)	4	0.16712963	1.12	0.3474

Table 21 : General linear models analysis of geotaxis data for 28 day-old virgins, all strains.

HOMOGENEITY-OF-SLOPES MODEL
AGE=28

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	28
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432
HOMOGENEITY-OF-SLOPES MODEL
AGE=28

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	235.08166204	18.08320477	487.00
ERROR	419	15.55833796	0.03713207	PR > F
UNCORRECTED TOTAL	432	250.64000000		0.0

Table 21 : General linear models analysis of geotaxis data for 28 day-old virgins, all strains.

Table 21 (continued)

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.734680	28.9045	0.19269684	0.66666667

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	192.00000000	5170.73	0.0
STRAIN	3	16.61111111	149.12	0.0001
ANGLE	1	12.54792014	337.93	0.0001
SEX	1	11.40750000	307.21	0.0001
ANGLE*STRAIN	3	2.24476042	20.15	0.0001
REP(STRAIN)	4	0.27037037	1.82	0.1239

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	110.05735539	2963.94	0.0
STRAIN	3	0.90636683	8.14	0.0001
ANGLE	1	12.54792014	337.93	0.0001
SEX	1	11.40750000	307.21	0.0001
ANGLE*STRAIN	3	2.24476042	20.15	0.0001
REP(STRAIN)	4	0.27037037	1.82	0.1239

Table 22: General linear models analysis of geotaxis data for 35 day-old virgins.

HOMOGENEITY-OF-SLOPES MODEL
AGE=35

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	35
REP	2	1 2
ORDER	3	1 2 3
SEX	2	-1 1

NUMBER OF OBSERVATIONS IN BY GROUP = 432
HOMOGENEITY-OF-SLOPES MODEL
AGE=35

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	13	303.46163426	23.34320264	458.80
ERROR	419	21.31836574	0.05087915	PR > F
UNCORRECTED TOTAL	432	324.78000000		0.0

Table 22: General linear models analysis of geotaxis data for 35 day-old virgins.

Table 22 (continued)

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.650243	28.8637	0.22556408	0.78148148

SOURCE	DF	TYPE I SS	F VALUE	PR > F
INTERCEPT	1	263.82814815	5185.39	0.0
STRAIN	3	22.06722222	144.57	0.0001
ANGLE	1	7.23003125	142.10	0.0001
SEX	1	4.81333333	94.60	0.0001
ANGLE*STRAIN	3	5.18345486	33.96	0.0001
REP(STRAIN)	4	0.33944444	1.67	0.1565

SOURCE	DF	TYPE III SS	F VALUE	PR > F
INTERCEPT	1	122.50045779	2407.67	0.0
STRAIN	3	0.60652369	3.97	0.0082
ANGLE	1	7.23003125	142.10	0.0001
SEX	1	4.81333333	94.60	0.0001
ANGLE*STRAIN	3	5.18345486	33.96	0.0001
REP(STRAIN)	4	0.33944444	1.67	0.1565

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=-1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	35
REP	2	1 2
ORDER	3	1 2 3
SEX	1	-1

NUMBER OF OBSERVATIONS IN BY GROUP = 24

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=-1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	8	16.17333333	2.02166667	99.02
ERROR	16	0.32666667	0.02041667	PR > F

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

UNCORRECTED TOTAL	24	16.50000000		0.0001

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.980202	21.7043	0.14288690	0.65833333

SOURCE	DF	TYPE I SS	F VALUE	PR > F
STRAIN	4	16.12333333	197.43	0.0001
REP(STRAIN)	4	0.05000000	0.61	0.6599

SOURCE	DF	TYPE III SS	F VALUE	PR > F
STRAIN	4	16.12333333	197.43	0.0001
REP(STRAIN)	4	0.05000000	0.61	0.6599

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=-1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 CONFIDENCE=0.95 DF=16 MSE=.0204167
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.23602

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

STRAIN	SIMULTANEOUS LOWER CONFIDENCE	DIFFERENCE BETWEEN	SIMULTANEOUS UPPER CONFIDENCE

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

COMPARISON	LIMIT	MEANS	LIMIT	
RNDA - RNDB	-0.18602	0.05000	0.28602	
RNDA - NLB	-0.10269	0.13333	0.36936	
RNDA - NLA	0.94731	1.18333	1.41936	***
RNDB - RNDA	-0.28602	-0.05000	0.18602	
RNDB - NLB	-0.15269	0.08333	0.31936	
RNDB - NLA	0.89731	1.13333	1.36936	***
NLB - RNDA	-0.36936	-0.13333	0.10269	
NLB - RNDB	-0.31936	-0.08333	0.15269	
NLB - NLA	0.81398	1.05000	1.28602	***
NLA - RNDA	-1.41936	-1.18333	-0.94731	***
NLA - RNDB	-1.36936	-1.13333	-0.89731	***
NLA - NLB	-1.28602	-1.05000	-0.81398	***

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=-1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=16 MSE=.0204167
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.23602

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	STRAIN
A	1.00000	6	RNDA

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

```
                A
                A            0.95000       6  RNDB
                A
                A            0.86667       6  NLB

                B           -0.18333       6  NLA
ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE
                                                        AGE=35 SEX=-1 ANGLE=80

        GENERAL LINEAR MODELS PROCEDURE

            CLASS LEVEL INFORMATION

    CLASS      LEVELS    VALUES

    STRAIN          4    NLA NLB RNDA RNDB

    AGE             1    35

    REP             2    1 2

    ORDER           3    1 2 3

    SEX             1    -1

   NUMBER OF OBSERVATIONS IN BY GROUP = 24
ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

            AGE=35 SEX=-1 ANGLE=80

        GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE                      DF       SUM OF SQUARES          MEAN SQUARE       F VALUE
```

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

MODEL	8	13.16000000	1.64500000	101.23
ERROR	16	0.26000000	0.01625000	PR > F
UNCORRECTED TOTAL	24	13.42000000		0.0001

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.980626	33.2545	0.12747549	0.38333333

SOURCE	DF	TYPE I SS	F VALUE	PR > F
STRAIN	4	13.12666667	201.95	0.0001
REP(STRAIN)	4	0.03333333	0.51	0.7273

SOURCE	DF	TYPE III SS	F VALUE	PR > F
STRAIN	4	13.12666667	201.95	0.0001

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=-1 ANGLE=80

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 CONFIDENCE=0.95 DF=16 MSE=0.01625
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.21057

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

STRAIN COMPARISON	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT	
RNDB - RNDA	-0.07723	0.13333	0.34390	
RNDB - NLB	0.18943	0.40000	0.61057	***
RNDB - NLA	1.38943	1.60000	1.81057	***
RNDA - RNDB	-0.34390	-0.13333	0.07723	
RNDA - NLB	0.05610	0.26667	0.47723	***
RNDA - NLA	1.25610	1.46667	1.67723	***
NLB - RNDB	-0.61057	-0.40000	-0.18943	***
NLB - RNDA	-0.47723	-0.26667	-0.05610	***
NLB - NLA	0.98943	1.20000	1.41057	***
NLA - RNDB	-1.81057	-1.60000	-1.38943	***
NLA - RNDA	-1.67723	-1.46667	-1.25610	***
NLA - NLB	-1.41057	-1.20000	-0.98943	***

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=-1 ANGLE=80

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=16 MSE=0.01625
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.21057

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

TUKEY GROUPING	MEAN	N	STRAIN
A	0.91667	6	RNDB
A			
A	0.78333	6	RNDA
B	0.51667	6	NLB
C	-0.68333	6	NLA

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
STRAIN	4	NLA NLB RNDA RNDB
AGE	1	35
REP	2	1 2
ORDER	3	1 2 3
SEX	1	1

NUMBER OF OBSERVATIONS IN BY GROUP = 24

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=1 ANGLE=40

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	8	21.62333333	2.70291667	648.70
ERROR	16	0.06666667	0.00416667	PR > F
UNCORRECTED TOTAL	24	21.69000000		0.0001

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.996926	6.8246	0.06454972	0.94583333

SOURCE	DF	TYPE I SS	F VALUE	PR > F
STRAIN	4	21.60833333	1296.50	0.0001
REP(STRAIN)	4	0.01500000	0.90	0.4870

SOURCE	DF	TYPE III SS	F VALUE	PR > F
STRAIN	4	21.60833333	1296.50	0.0001
REP(STRAIN)	4	0.01500000	0.90	0.4870

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

ALPHA=0.05 CONFIDENCE=0.95 DF=16 MSE=.0041667
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.10662

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

STRAIN COMPARISON	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT	
RNDB - RNDA	-0.10662	0.00000	0.10662	
RNDB - NLA	-0.07329	0.03333	0.13996	
RNDB - NLB	0.07671	0.18333	0.28996	***
RNDA - RNDB	-0.10662	0.00000	0.10662	
RNDA - NLA	-0.07329	0.03333	0.13996	
RNDA - NLB	0.07671	0.18333	0.28996	***
NLA - RNDB	-0.13996	-0.03333	0.07329	
NLA - RNDA	-0.13996	-0.03333	0.07329	
NLA - NLB	0.04338	0.15000	0.25662	***
NLB - RNDB	-0.28996	-0.18333	-0.07671	***
NLB - RNDA	-0.28996	-0.18333	-0.07671	***
NLB - NLA	-0.25662	-0.15000	-0.04338	***

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=1 ANGLE=40

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

```
     BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

        ALPHA=0.05  DF=16  MSE=.0041667
        CRITICAL VALUE OF STUDENTIZED RANGE=4.046
        MINIMUM SIGNIFICANT DIFFERENCE=.10662

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

     TUKEY     GROUPING              MEAN       N  STRAIN

                  A              1.00000       6  RNDB
                  A
                  A              1.00000       6  RNDA
                  A
                  A              0.96667       6  NLA

                  B              0.81667       6  NLB
       ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

                AGE=35 SEX=1 ANGLE=80

              GENERAL LINEAR MODELS PROCEDURE

                  CLASS LEVEL INFORMATION

           CLASS      LEVELS    VALUES

           STRAIN          4    NLA NLB RNDA RNDB

           AGE             1    35

           REP             2    1 2

           ORDER           3    1 2 3

           SEX             1    1
```

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

NUMBER OF OBSERVATIONS IN BY GROUP = 24

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=1 ANGLE=80

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: GT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	8	12.01333333	1.50166667	163.82
ERROR	16	0.14666667	0.00916667	PR > F
UNCORRECTED TOTAL	24	12.16000000		0.0001

R-SQUARE	C.V.	ROOT MSE	GT MEAN
0.987939	14.9209	0.09574271	0.64166667

SOURCE	DF	TYPE I SS	F VALUE	PR > F
STRAIN	4	11.91000000	324.82	0.0001
REP(STRAIN)	4	0.10333333	2.82	0.0604

SOURCE	DF	TYPE III SS	F VALUE	PR > F
STRAIN	4	11.91000000	324.82	0.0001
REP(STRAIN)	4	0.10333333	2.82	0.0604

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

AGE=35 SEX=1 ANGLE=80

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 CONFIDENCE=0.95 DF=16 MSE=.0091667
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.15815

COMPARISONS SIGNIFICANT AT THE 0.05 LEVEL ARE INDICATED BY '***'

STRAIN COMPARISON	SIMULTANEOUS LOWER CONFIDENCE LIMIT	DIFFERENCE BETWEEN MEANS	SIMULTANEOUS UPPER CONFIDENCE LIMIT	
RNDB - RNDA	-0.14148	0.01667	0.17482	
RNDB - NLB	0.32518	0.48333	0.64148	***
RNDB - NLA	0.50852	0.66667	0.82482	***
RNDA - RNDB	-0.17482	-0.01667	0.14148	
RNDA - NLB	0.30852	0.46667	0.62482	***
RNDA - NLA	0.49185	0.65000	0.80815	***
NLB - RNDB	-0.64148	-0.48333	-0.32518	***
NLB - RNDA	-0.62482	-0.46667	-0.30852	***
NLB - NLA	0.02518	0.18333	0.34148	***
NLA - RNDB	-0.82482	-0.66667	-0.50852	***
NLA - RNDA	-0.80815	-0.65000	-0.49185	***
NLA - NLB	-0.34148	-0.18333	-0.02518	***

ANOVAS FOR GEOTACTIC RESPONSE AT EACH ANGLE

Table 23 (continued) :Analysis of variance for day 35 geotaxis data for angles 40 and 80 degrees.

AGE=35 SEX=1 ANGLE=80

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: GT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=16 MSE=.0091667
CRITICAL VALUE OF STUDENTIZED RANGE=4.046
MINIMUM SIGNIFICANT DIFFERENCE=.15815

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	STRAIN
A	0.93333	6	RNDB
A			
A	0.91667	6	RNDA
B	0.45000	6	NLB
C	0.26667	6	NLA

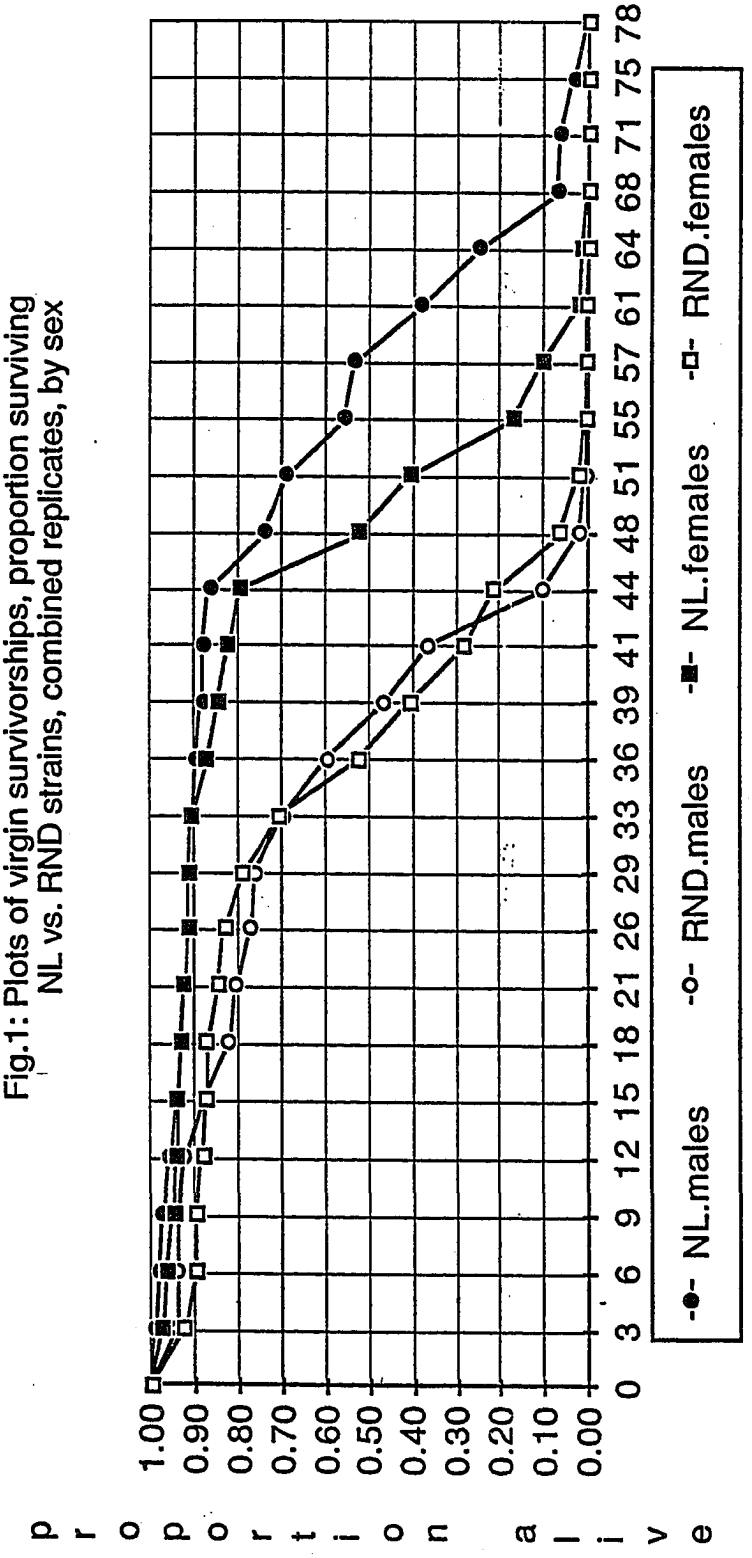


Fig.1: Plots of virgin survivorships, proportion surviving
NL vs. RND strains, combined replicates, by sex

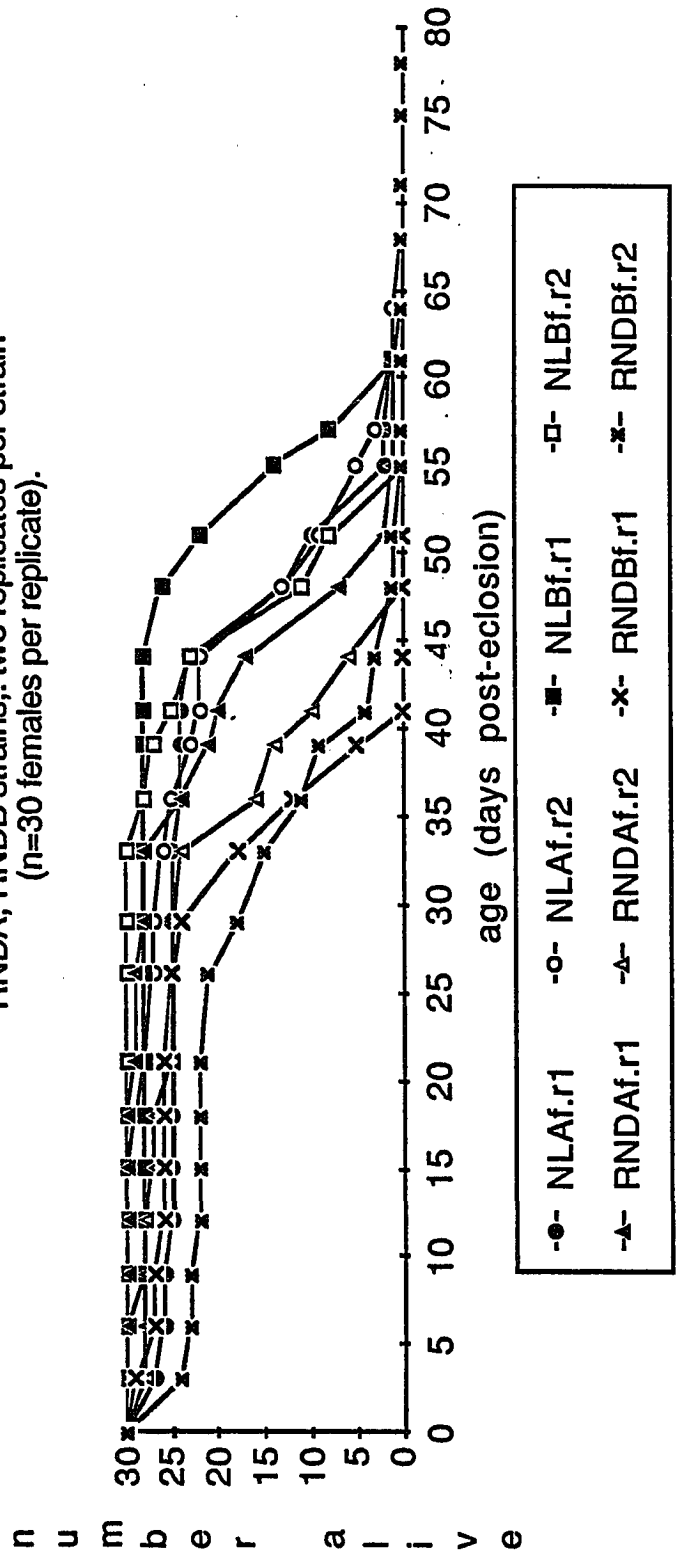


Fig. 2: Plots of virgin survivorships, females NLA, NLB, RNDA, RNDB strains,: two replicates per strain (n=30 females per replicate).

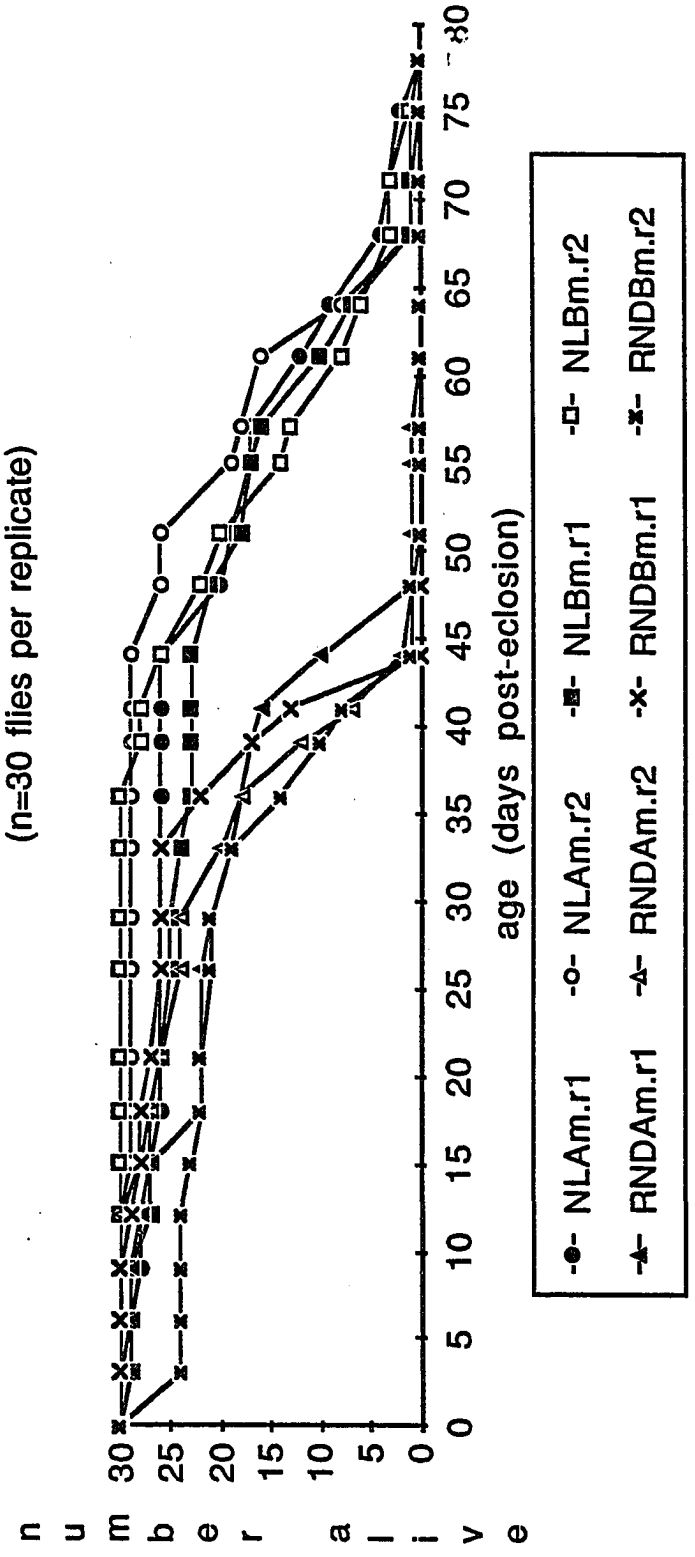


Fig. 3: Plots of virgin survivorships, males NLA, NLB RNDA, RNDB strains; two replicates per strain (n=30 flies per replicate)

Fig. 4: One day-old virgin egg means.

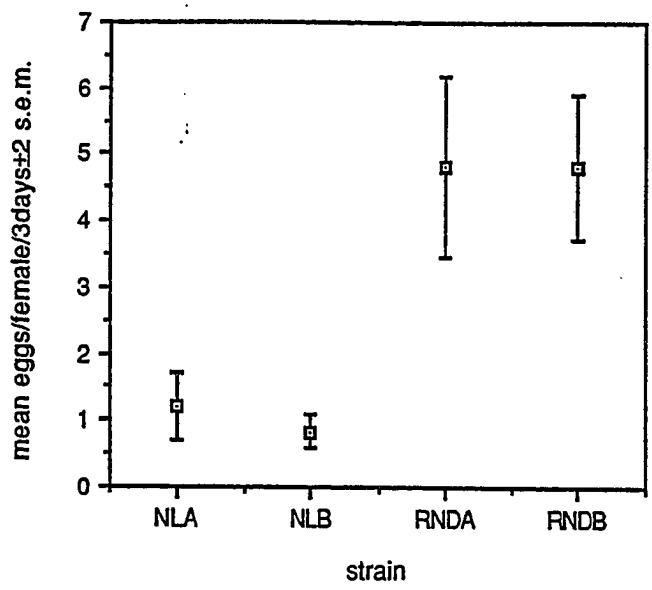


Fig.5: One day-old virgin egg totals.

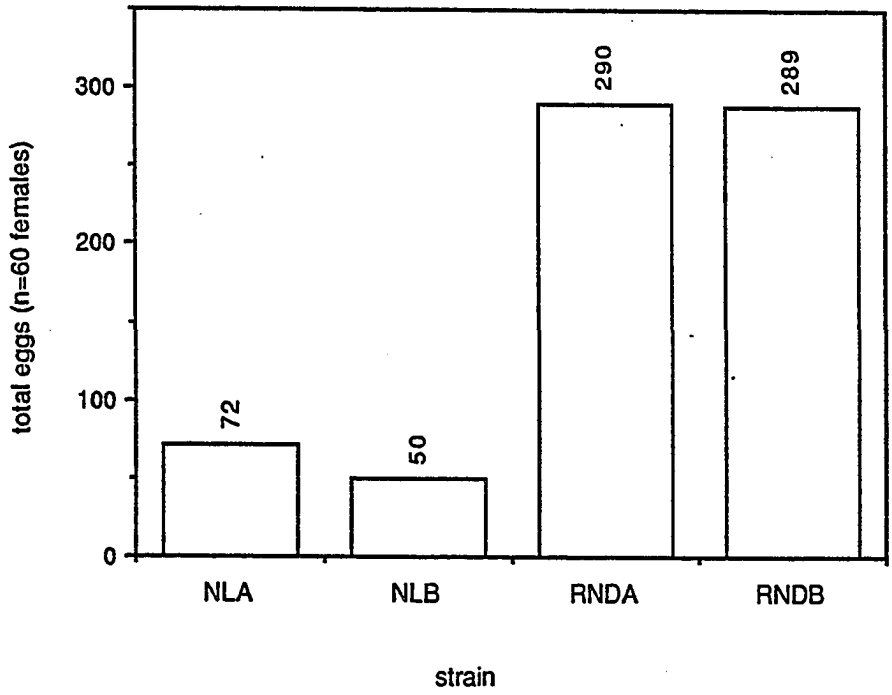


Fig.6 Two day-old virgin egg means.

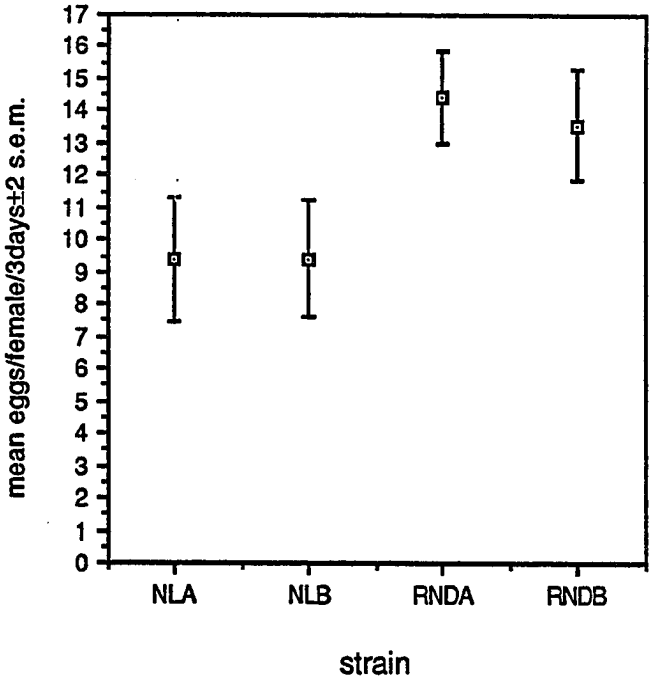


Fig.7 :Two day-old virgin egg totals.

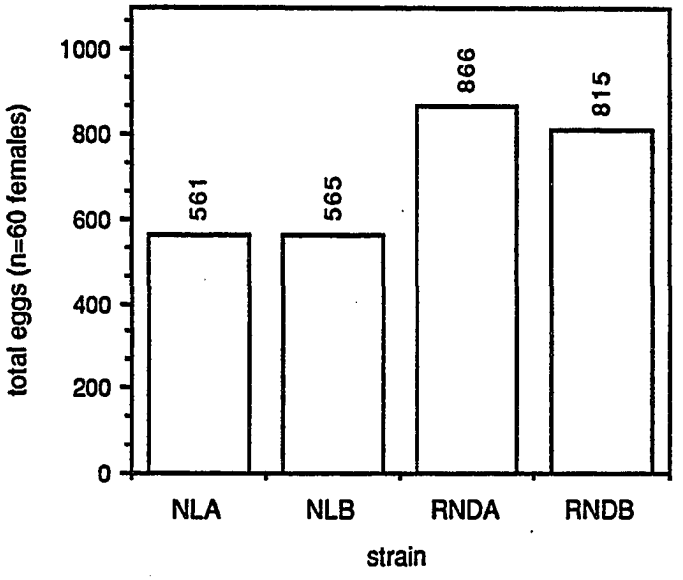


Fig. 8 : Four day-old virgin egg means.

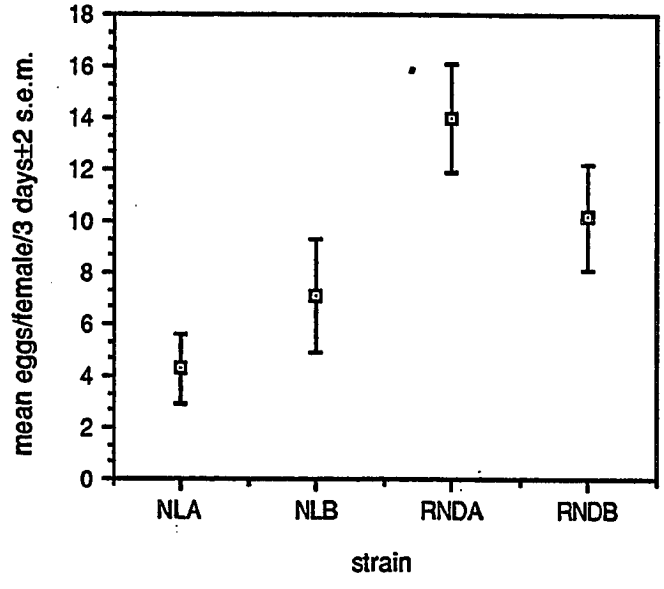


Fig.9 : Four day-old virgin egg totals.

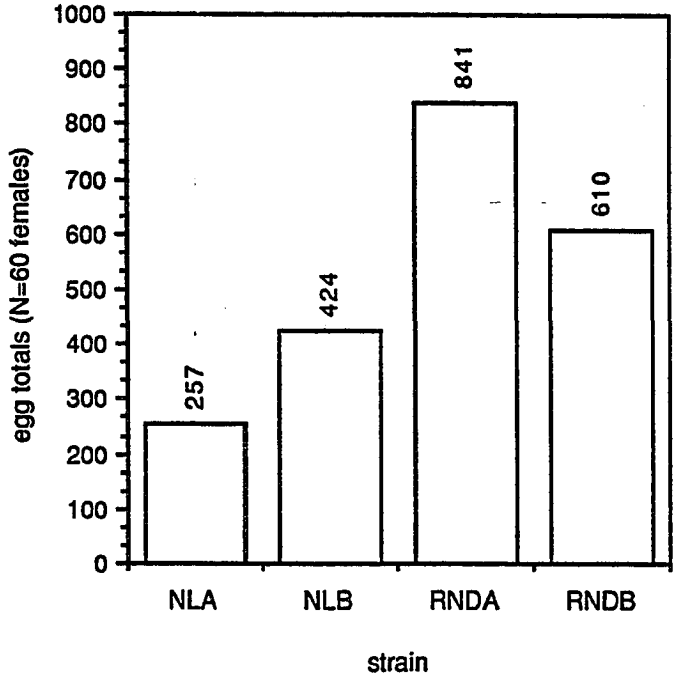


Fig. 10: Seven day-old virgin egg means.

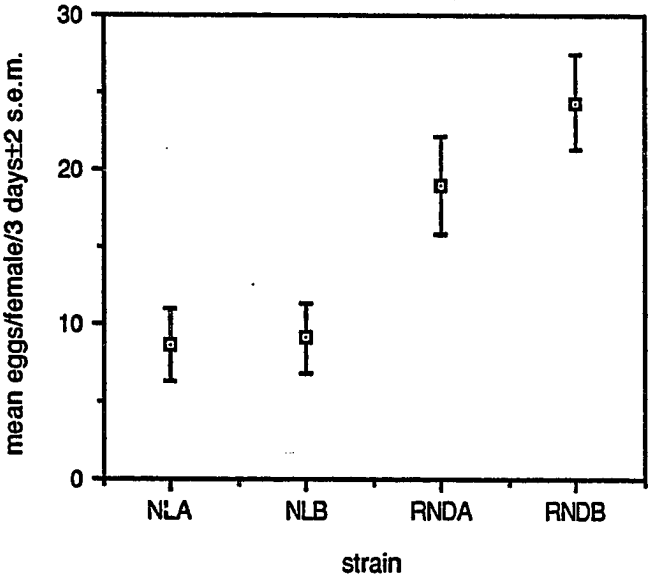


Fig. 11: Seven day-old virgin egg totals.

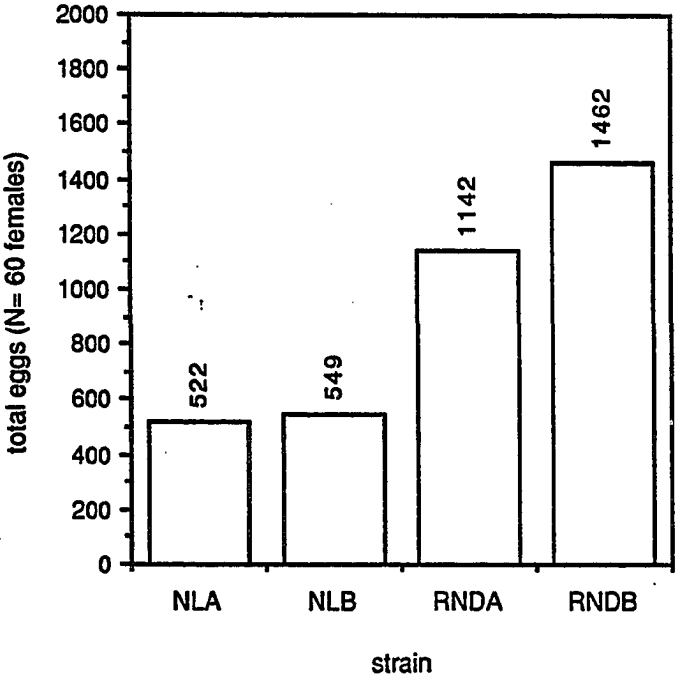


Fig.12: Fourteen day-old virgin egg means.

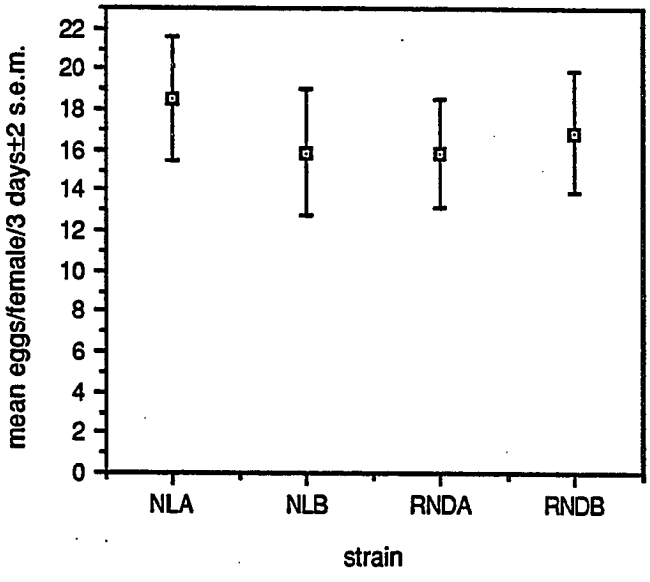


Fig. 13: Fourteen day-old virgin egg totals

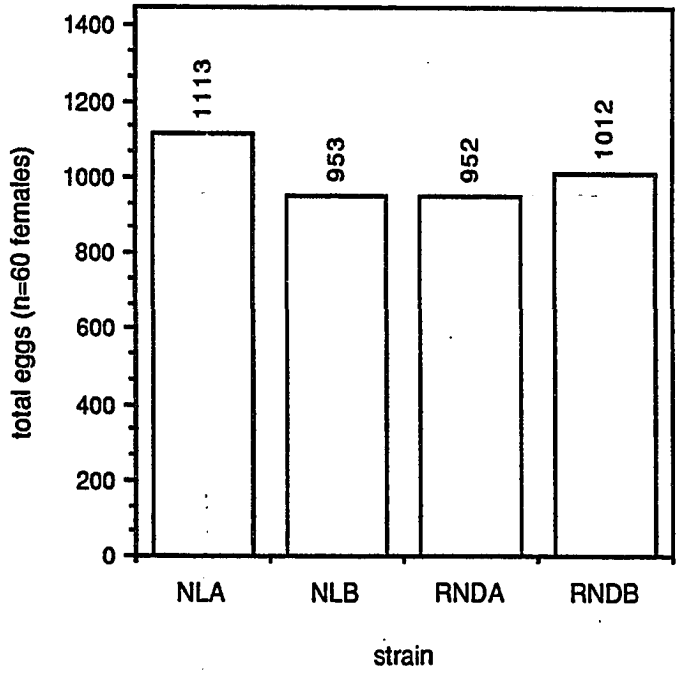


Fig.14: Twenty one day-old virgin egg means.

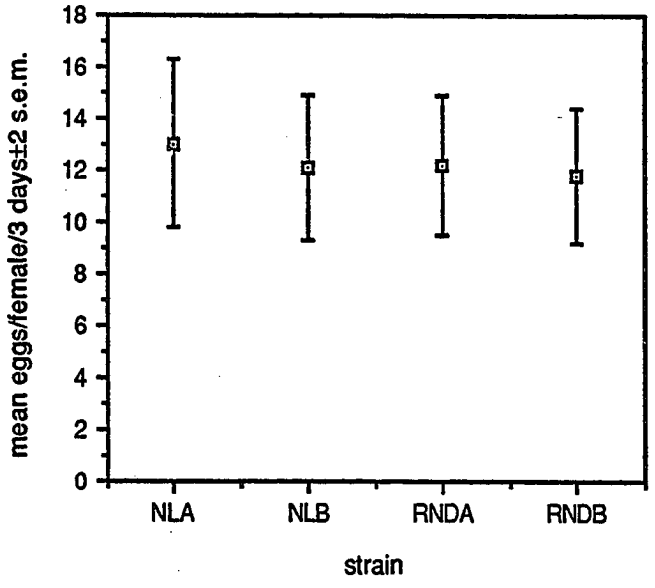


Fig.15: Twenty one day-old virgin egg totals.

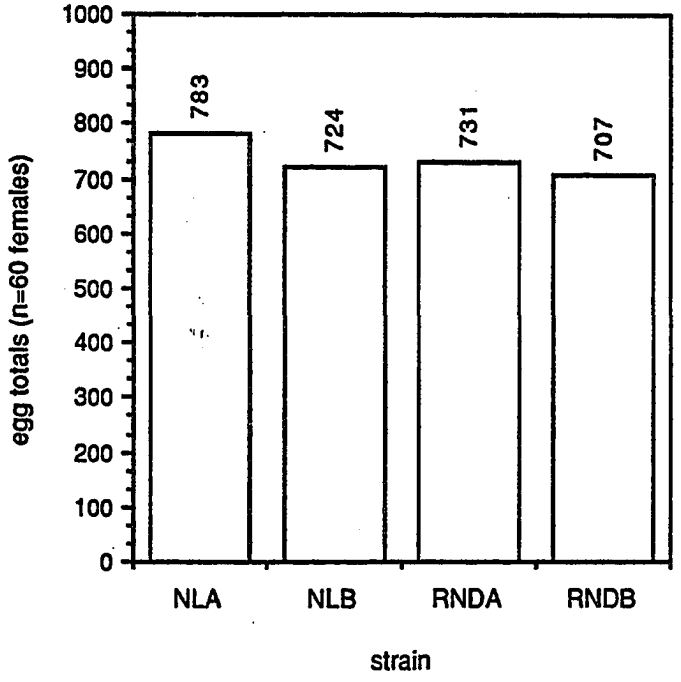


Fig.16: Twenty eight day-old virgin egg means.

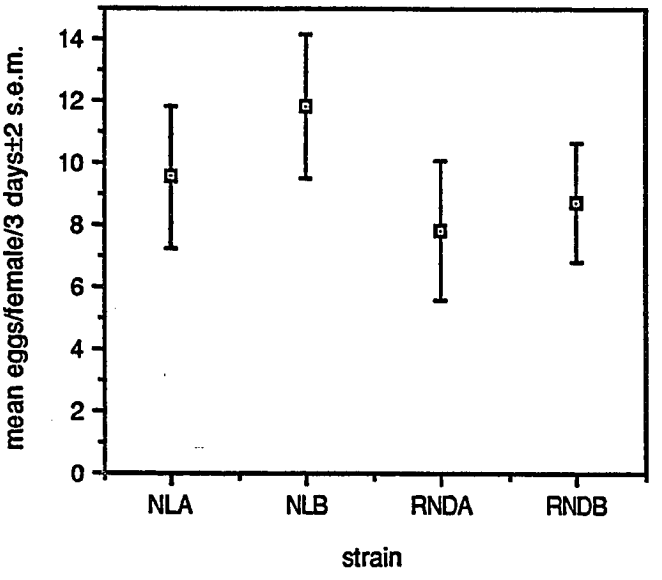


Fig. 17: Twenty eight day-old virgin egg totals.

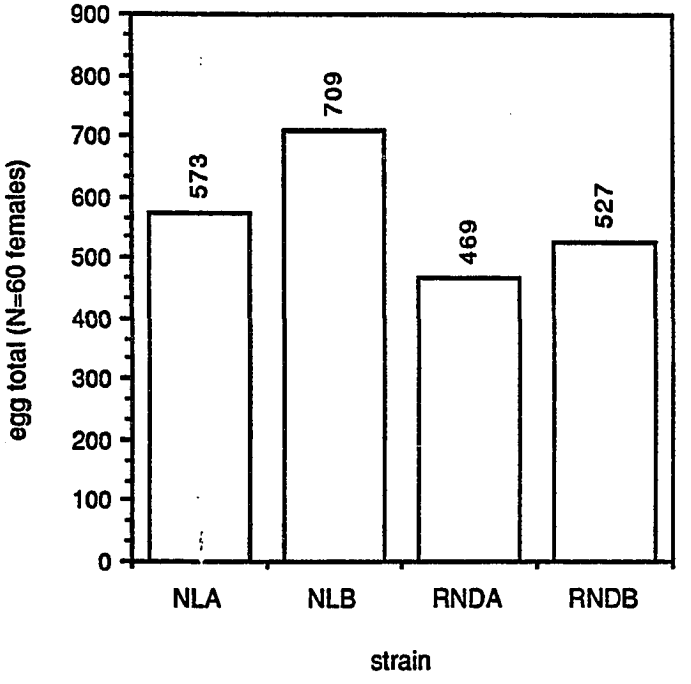


Fig.18 : Egg means for all combinations of female and male virgin parental ages of 7 and 42 days.

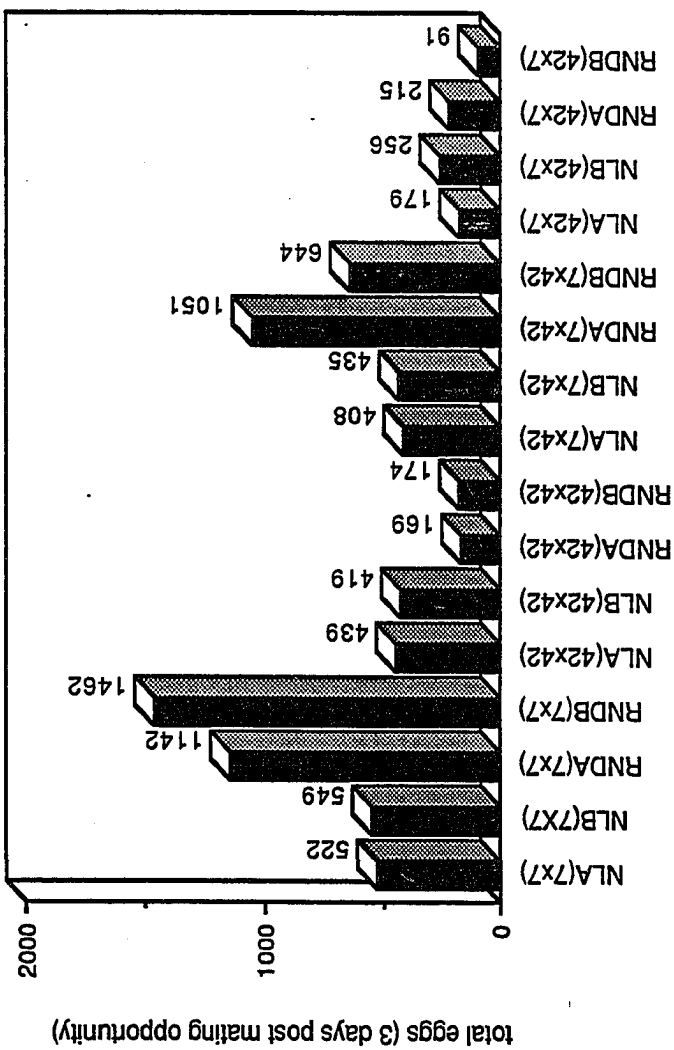


Fig. 19: Total egg counts (combined replicates) for all combinations of virgin parental ages of 7 and 42 days post eclosion by strain. Each total is for 60 females (2 replicates of 30 for each strain and parental age combination) for 3 days following the 24 hour mating opportunity on the day of the specified ages.

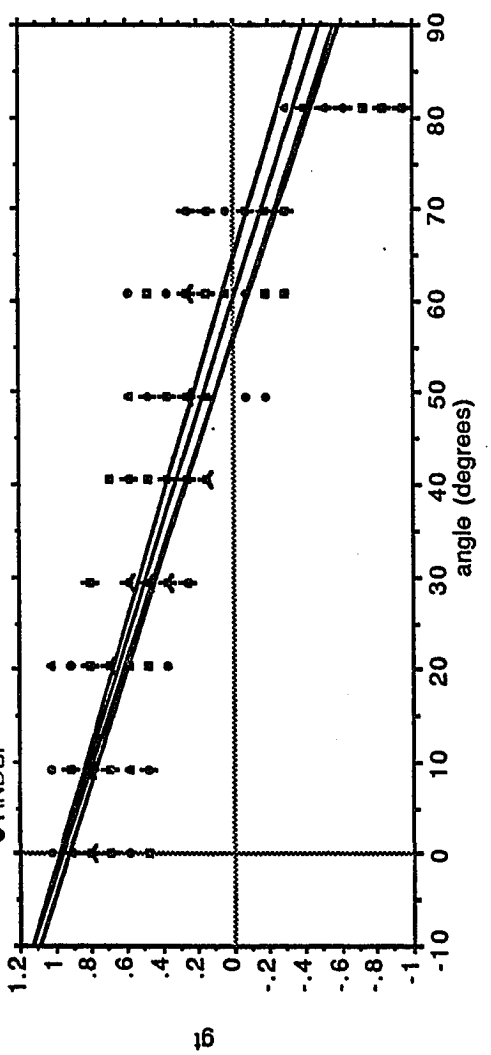


Fig 20a: Day 0 post eclosion ovelaid regression plots of gt values for virgin females. Replicates combined for each strain.

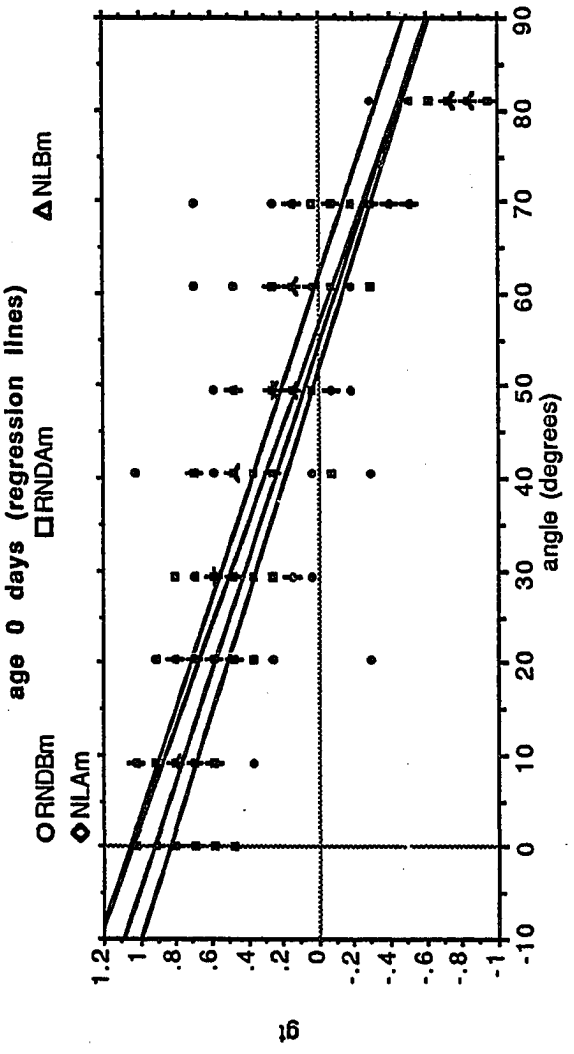


Fig 20b: Day 0 post eclosion ovelaid regression plots of gt values for virgin males. Replicates combined for each strain.

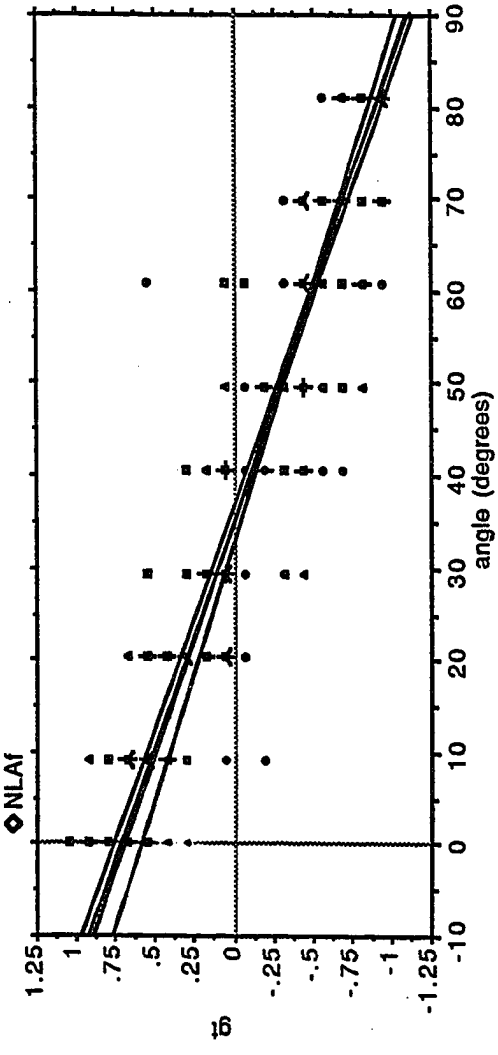


Fig. 21a: Day 3 post eclosion overlaid regression plots of gt values for virgin females. Replicates combined for each strain.

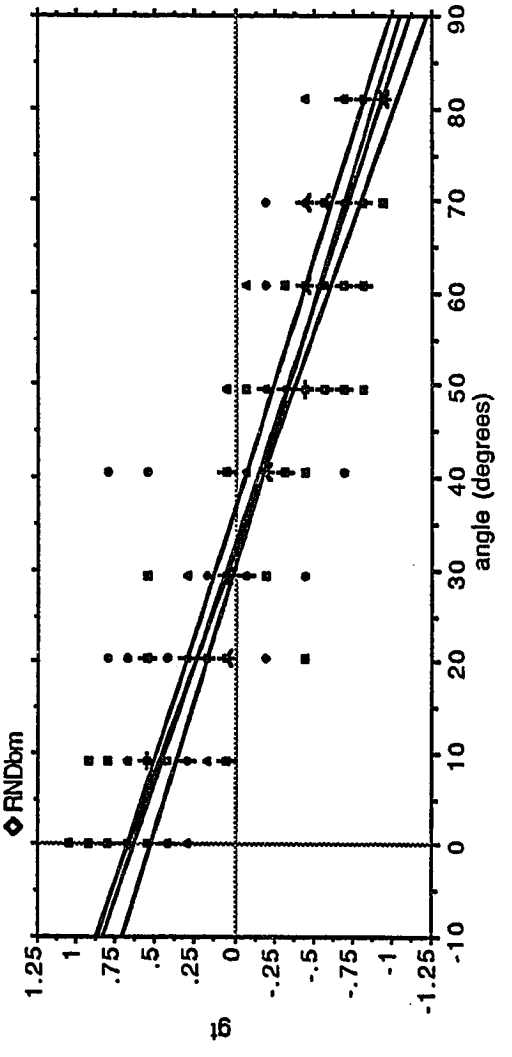


Fig. 21b: Day 3 post eclosion overlaid regression plots of gt values for virgin males.
Replicates combined for each strain.

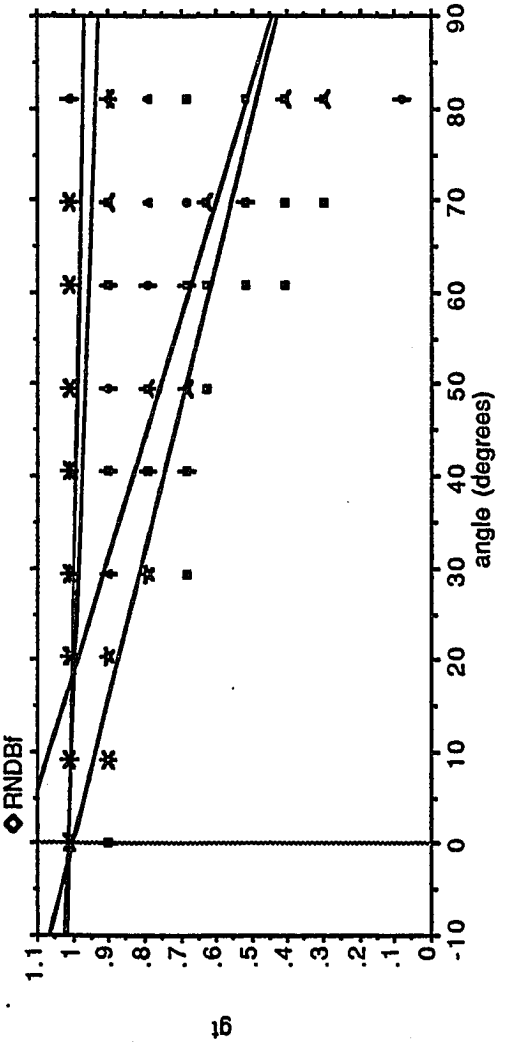


Fig. 22a: Thirty five days post eclosion overlaid regression plots of gt values for females. Replicates combined for each strain.

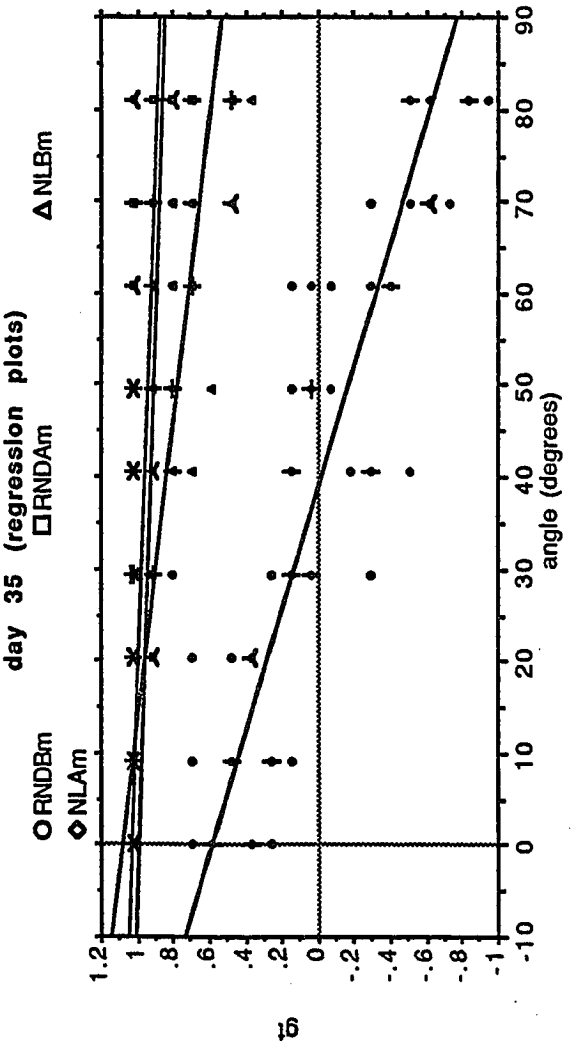
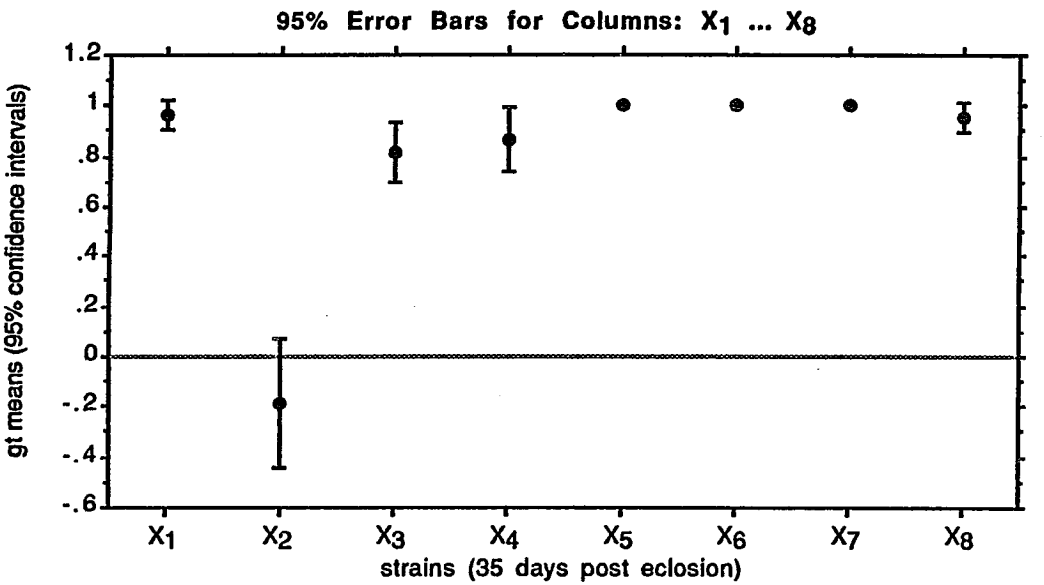


Fig. 22b: Thirty five days post eclosion overlaid regression plots of gt values for males. Replicates combined for each strain.

Fig.23: Mean gt scores with 95% confidence intervals, both sexes and all strains, for test angle of 40 degrees.



Legend:
X1= NLA females, X2= NLA males
X3= NLB females, X4= NLB males
X5= RNDA females, X6= RNDA males
X7= RNDB females, X8= RNDB males

Note: For the strains for which no error bars are shown all gt values were the same.

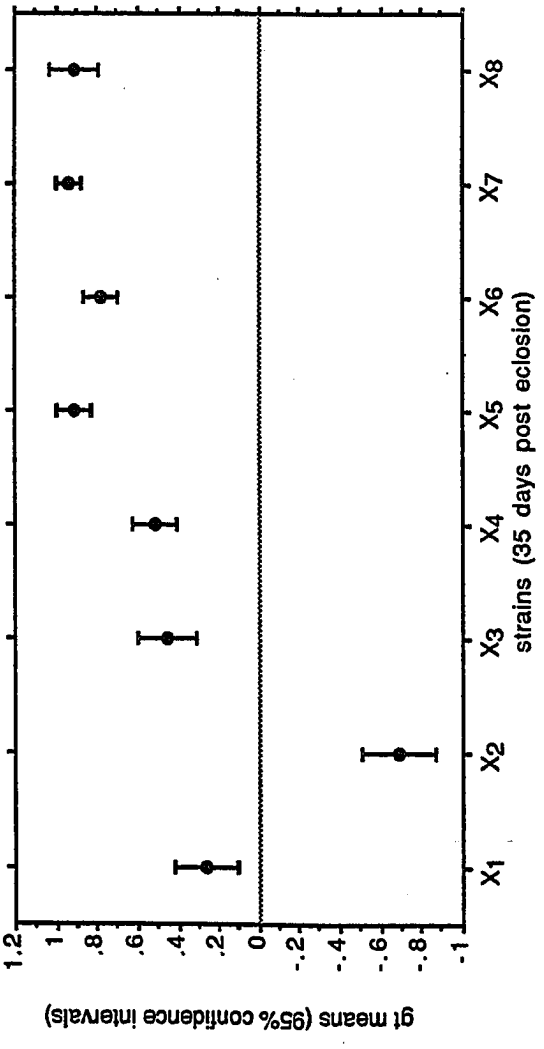


Fig.24: Mean gt scores with 95% confidence intervals, both sexes and all strains, for test angle of 80 degrees.

Legend:
X1= NLA females, X2= NLA males
X3= NLB females, X4= NLB males
X5= RNDA females, X6= RNDA males
X7= RNDB females, X8= RNDB males

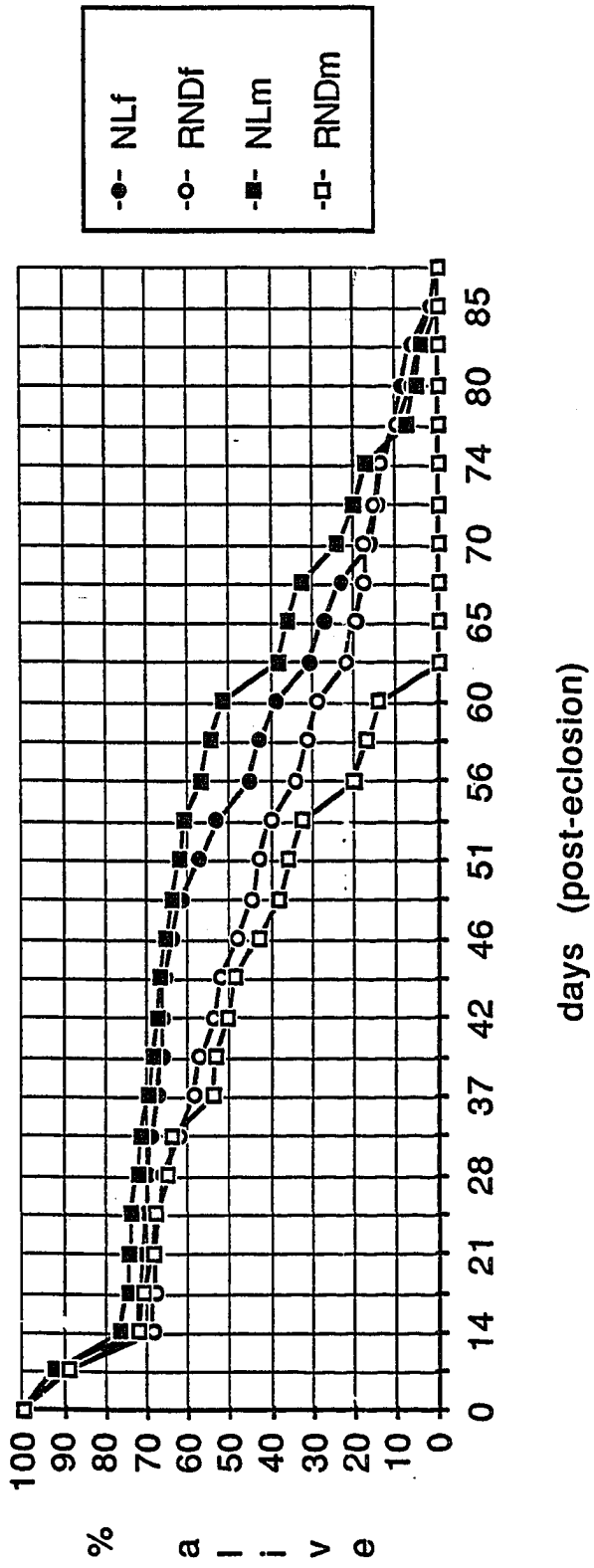


Fig. 25: Survivorship plots for virgin flies from the geotaxis experiment, by sex (n=600 for NL females, NL males, RND females, and RND males).

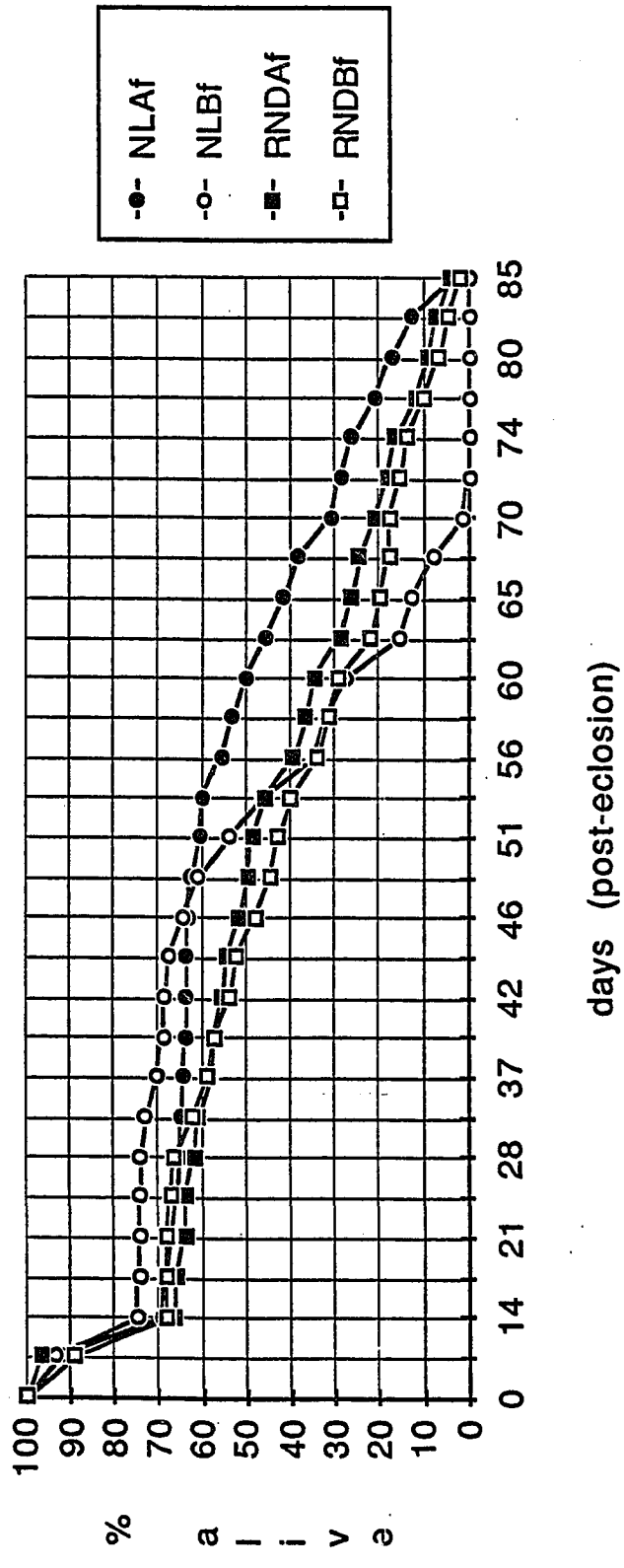


Fig. 26: Survivorship plots for virgin females from the geotaxis experiment (n=300 per strain).

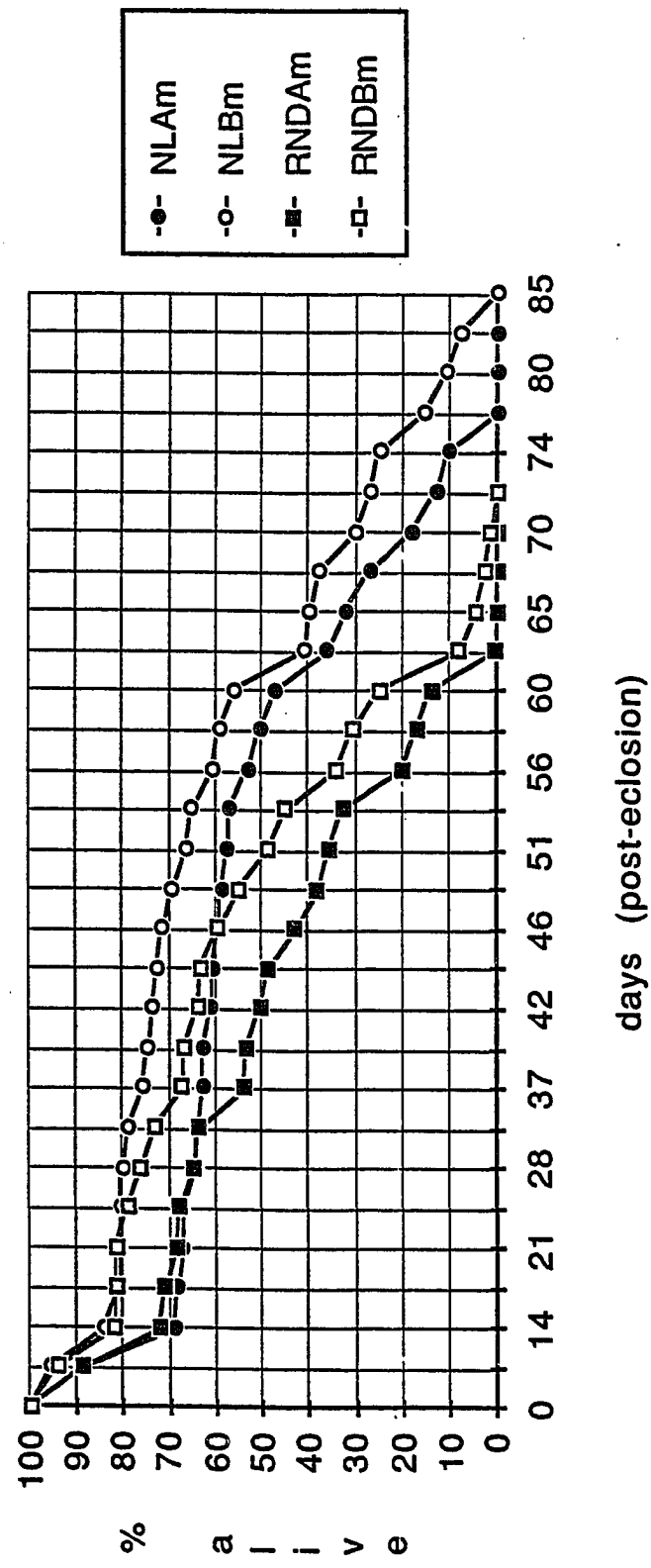


Fig. 27: Survivorship plots for virgin males from the geotaxis experiment (n=300 per strain).

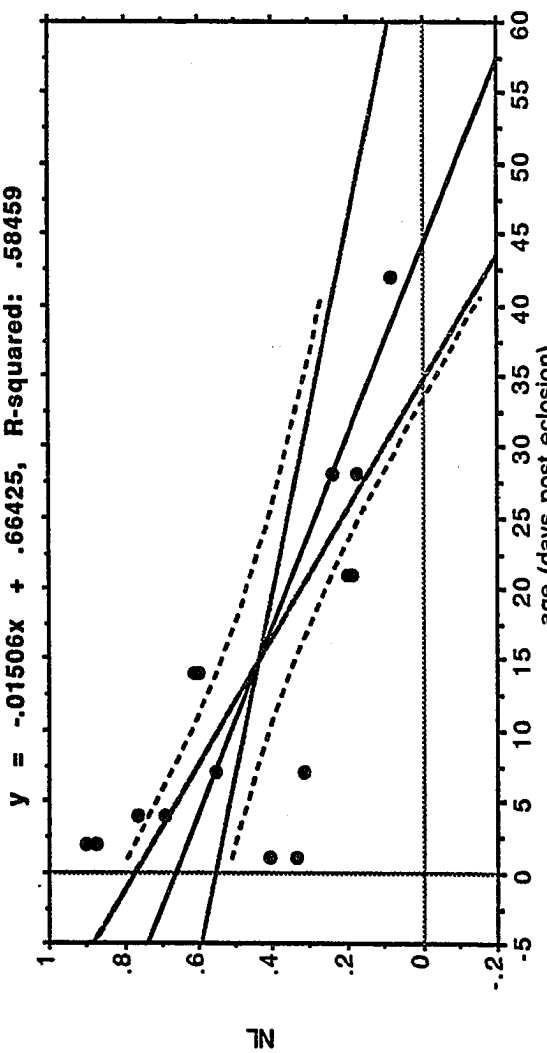


Figure 28a: Regression plots of mean fertility proportions on age post eclosion for NL virgin females. Replicates combined.

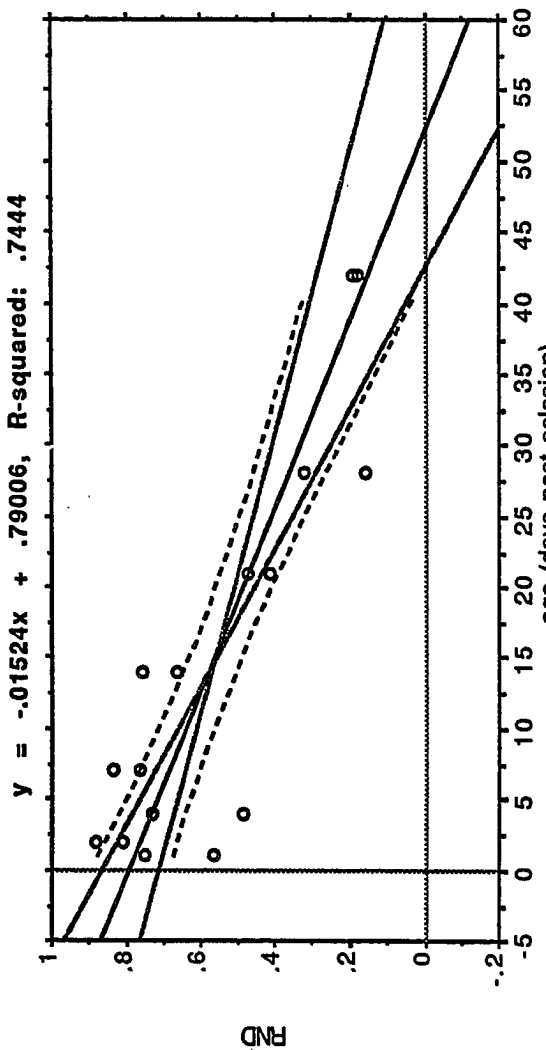


Figure 28b: Regression plots of mean fertility proportions on age post eclosion for RND female virgins. Replicates combined.

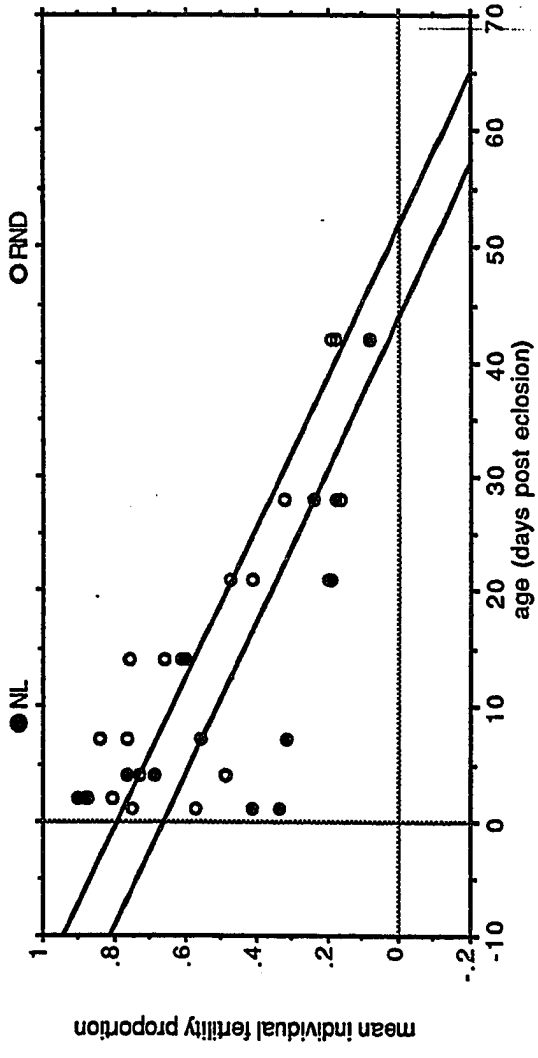


Figure 28c: Regression plots of mean fertility proportions on age post eclosion for NL and RND virgin females. Replicates combined.

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