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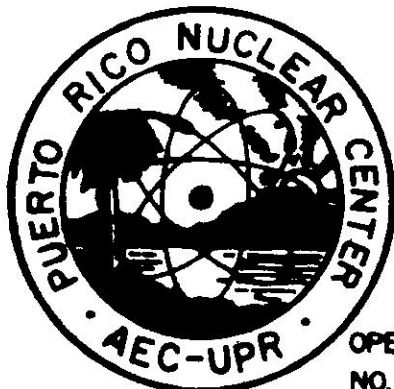
PUERTO RICO NUCLEAR CENTER

INSECT STERILITY PROGRAM

TECHNICAL REPORT 6

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Program Director

February 1972



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Technical Report No. 6: September 1970 - January 1972

Insect Sterility Program

(Formerly Potential for Gamma-Induced Sterility
in Control of the Sugarcane Borer D. saccharalis
(Fab.) in Puerto Rico)

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I. Introduction

This is the sixth technical report to be published for the project (1965, 6, 7, 8, and '70). The Project was begun under the title of Potential for Gamma-induced Sterility in Control of the Sugarcane Borer (Diatraea saccharalis), and the program as now called the Insect Sterility Program. The original motive was to determine if the sugarcane borer could be sterilized by gamma radiation and to develop rearing methods suitable for adapting to a factory rearing method with the objective of releasing fully sterilized borer males into a natural population as a mass sterile release program. Subsequent work has demonstrated that adult males and females can be sterilized by ^{60}Co gamma exposures and field tests showed that it might be practicable as a means of eradication of this species. Several problems prevent a high degree of efficiency for the use of this method for this particular species however. Among these are the short life span of the adults (whether sterilized or normal), necessitating the frequent overflooding to maintain the necessary sterile to normal ratios; the high cost of production of sterile adults for release; the difficulty of adapting to factory rearing methods to produce millions of individuals of this species to sterilize; the long larval developmental period; multiple mating; and the necessity for sterilizing the individuals for release in the adult stage.

To solve the problem of eradicating this species we explored the possibility of giving sub-sterilizing dosages of gamma radiation. This was found to have some important advantages over the release of individuals that are fully sterile-i.e. resulting semi-sterile offspring as contrasted to dominant lethals in embryonic development. Originally we called this effect Delayed Sterility, but later we have coined the phrase Inherited Partial Sterility. In addition to the sugarcane borer we have observed the IPS effect in other lepidoptera Lamprosema indicata, Prodenia ornithogalla, Heliothis zea, and in one hemiptera (Nezara viridula). Some of this work is discussed in this report. In addition North (1968), La Chance (1969), Proshold (1970) and others have observed the same effect in other lepidoptera, in hemiptera and in homoptera. The original observations of this effect were reported by Proverbs (1962) and by Husseiny (1964), but no practical significance was attributed to it. Walker and Pederson (1969), Knipling (1969), North (1970) and others have shown how IPS might be used to suppress or to eradicate a pest population from a theoretical standpoint.

Therefore the original thrust of the program (study of the sugarcane borer intensively with the motive of eradication) has been changed to concentrating upon general means of suppression and eradication of pest species that have holokinetic chromosomes. Our present objectives are in the direction of determining the mechanisms that cause the delay in mortality, and particularly how this mechanism(s) can be used most effectively within the reference of integrated pest management systems. The application of the method to pest population control is still the most important aspect of the program, but in addition the converse concept of how a strain can maintain itself while carrying a heavy gene load is of considerable significance as well. We suspect that the long-term effect over many successive generations is due primarily to point mutations, eliminations and breaks. We believe this to be the case for several reasons. Translocations commonly occur at dosages of one-half to three-fourths of the dose needed to cause complete dominant

lethality (25 kr), however we encounter the IPS effect when there are no observable translocations in meiotic divisions. This of course does not mean that the translocations are not present. Since individual fragments operate as separate and distinct chromosomes we suspect that the code is nearly complete in IPS lines from low doses, but that the code is in a scrambled version. Some indirect evidence can be used to support this view, although the evidence is not sufficient to prove the point.

Subsequent sections of this report discuss the work of the previous year, and evaluate the significance of the work and its relation to other related work particularly in relation to pest management systems, future plans and a narrative report are also included.

At present the program is funded by a grant from the US Atomic Energy Commission Division of Biology and Medicine and a grant from the US Department of Agriculture. In addition to these we are working cooperatively with the Experiment Stations of both the Commonwealth of Puerto Rico and with the USDA Federal Experiment Station, and with the Entomological Laboratory of the Biology Department of the UPR in Mayaguez. These cooperative programs do not entail the interchange of funds.

Among the many individuals deserving acknowledgement for their cooperation and advice I would like to mention Dr. Murray Blum (Univ. of Georgia), Dr. R. C. Von Borstel (Univ. of Manitoba), Drs. Robert Rabson and Charles Edington (DBM of AEC), Drs. Frank Koo, Shreekant Deshpande, Jose Ferrer-Monge and Mr. Jose Cuevas-Ruiz, Mrs. Dolores Ayguabibas de Ruiz and Mr. Samuel de la Rosa, of PRNC, and Drs. Goru Kuno, Chester Moore, and Flavio Padovani and Mr. Ruben Restrepo of the Biology Department, UPR Mayaguez, Dr. Nader Vakili of the USDA Experiment Station and Dr. Niilo Virkki of the UPR Experiment Station, Rio Piedras.

II. Accomplishments

For convenience of presentation results is under general subject headings with sub-divisions by species.

A. Laboratory

Lepidoptera

1. Diatraea saccharalis Fab. (sugarcane borer)

We are maintaining one strain of this species in the laboratory on a modification of the Shorey Bean Diet (Walker, Tech. Rept. 6, 1970) in a ten inch plastic dish with a cover. Larval is good except for the first stage. This method was recommended by Dr. A. N. Sparks, USDA Southern Grain Insects Laboratory at Tifton, Georgia (personal communication) and is a modification of the method used for the European Corn Borer. Theoretically the mature larvae leave the food before pupating, migrate to a wax-covered ring made of corrugated paper and pupated in the food itself. Two generations have been tested with this method and survival was approximately 70 percent in the first generation (700 larvae) and about 60 percent in the second (using

1,200 larvae). Transfer to the container was made by shaking larvae from oviposition cups directly. There was considerable bacterial and mold contamination in both tests. Containers can be washed, sterilized with calcium hypochlorite solution and re-used. Similar tests in smaller rectangular plastic containers have yielded comparable results. Future sugarcane borer rearing will be in these plastic containers. The advantages are fairly high production capacity with minimum handling and attention. The major disadvantage is that larva to larva disease transmission is facilitated.

Since the diet is nutritionally adequate there is a minimum of cannibalism except for accidental feeding of larvae upon pupae. It may be possible to devise an easy method for harvesting adults directly from these containers by using a light source for attracting the adults.

Less than 10 percent of the adults have manifested physical deformities caused by overcrowding or by inadequate diet. Dr. Kuno has isolated material from diseased borer larvae and is testing the inoculum prepared from these into healthy larvae in an attempt to isolate and identify the pathogen(s). Microscopic examinations have not shown fungi nor bacteria so that a virus infection is suspected.

Rearing capacity for this species is apparently limited only by the labor force available. The strain selected and the laboratory regime and diet appear to be adequate for producing several thousands of adults weekly under the proper conditions and with adequate facilities. Production cost is still high, approximately 0.2 cents each, this includes labor costs.

2. Prodenia ornithogalli Gn. (yellow striped armyworm, subfamily Amphipyridae, Phalaenidae)

This species is a large-size larva that attacks vegetable and other succulent crops, it has a wide host range, including (beans, tobacco, weeds, practically all vegetable crops). Mating is nocturnal, and adult lifespan is fairly long in the laboratory. It is rarely encountered during the day on plants since the larvae pass the day in soil and feed at night. The species is particularly damaging to young plants.

This species develops well in the bean diet for sugarcane borer. Two generations were passed in the laboratory and preliminary radiation/sterility tests were made. The strain selected underwent an arrested development after the second generation and the adults produced emerged over such a long period of time that it was not possible to obtain viable offspring for a third generation. This species will be available early next year and we will continue our work with it at that time. Only a single larva can be grown in each cup. Fairly large mating and oviposition chambers are needed to culture this species. Because of the large size of the larvae haemolymph is available in considerable quantity, a decided advantage in the protein electrophoresis work.

3. Lamprosema indicata (Fab.) (pega pega, Pyralidae)

We were able to develop a satisfactory combination of diet and container for rearing larvae of this species (bean diet) however we had great difficulty in providing the proper conditions for mating. Mating frequency was higher when adults were able to fly horizontally over a considerable distance at bean plant height. Mating was better in cages when plants were provided. Even though reproductive rate in nature is very high we were not able to harvest more than 15 to 20 fertile eggs per mated female. Due to these difficulties we have temporarily suspended work with this species.

Hemiptera

4. Nezara viridula (L.) (southern green stink bug, Pentatomidae)

Mr. Ruben Restrepo has performed most of the rearing work on this species. He is working on a volunteer basis. We have cultured this species through 6 generations using a combination of diets. Attractant tests and nutrition tests were mentioned in the previous report (Walker 6, 1970). A meridic (defined) diet has been adequate for supporting 3 generations of this species by Mullet and Rodriguez at UPR Mayaguez. Mr. Restrepo is capable of maintaining it on a combination of guava and acerola nectar (available as a canned juice), bean pods (wild bean) and cabbage leaf. Although the rearing methods tested need considerable refinement it is apparent that we will be able to make evaluations of IPS with the rearing procedure that we have. IPS tests with this species are discussed in a later section of this report. Since the previous report we have changed to one gallon containers for enclosing adults and nymphs of this species: survival and lifespan has been increased as a result. Providing a constant air stream through the net covering over the top has also helped to maintain the laboratory colony. Current production in the laboratory is 20 to 30 adults per week, but under these conditions and with adequate labor this can be increased to 400 to 500 adults per week.

Adult lifespan has exceeded 3 and one-half months in some of the tests. Mating has been observed and egg development has been studied so that fertile eggs can be distinguished from haploid eggs as shown in table 1.

The only serious problems encountered rearing this species is keeping sufficient food available and separating nymphal stages so that predation is prevented. This commonly occurs at the time of molting, mature individuals feeding on younger nymphs while the integument of the younger individual is still soft.

5. Other species cultivated

In addition to the above we have tested artificially rearing the following: Prodenia eridenia (Phalaenidae Lepidoptera); Acrosternum marginatum (Pentatomidae Hemiptera); Leptoglossus gonagra (Coreidae Hemiptera); Diaprepes abbreviata (Curculionidae, Coleoptera); Trichoplusia ni (Noctuidae, Lepidoptera); Phyllophaga vandinei (maybeetle, Scarabaeidae,

Coleoptera); Cosmopolites sordida, Metamasius hemiptera, Cylas formicaria, and an Anthonomus species (Curculionidae, Coleoptera).

We will be capable of rearing P. eridania and T. ni without too much difficulty, however there is no great advantage in rearing the former when we are rearing P. ornithogalli and T. ni is being investigated by USDA scientists.

We had considerable difficulty rearing all of the Coleoptera for a variety of reasons. The food was not acceptable to larvae in some cases, and there was considerable migration from the cups in the root inhabiting species, as well as a very long larval lifespan, for these reasons they were not suitable test insects for laboratory studies.

All of the above species are of some economic importance to commercial crops in Puerto Rico.

B. Inherited Partial Sterility

Lepidoptera

1. Diatraea saccharalis

Tables 2 to 25 and figures 1 to 25 show the mating lineages and reproductive potential of lines at various P generation doseages. Comparable Control lines do not show decrease in fecundity over subsequent generations as the IPS lines do. By itself this data is difficult to interpret with any degree of meaning. It is important to note that there have been no lines that have shown a high degree of chromosome fragmenting (Virkki). This is the case whether the particular line has demonstrated IPS or not. Originally we suspected that the IPS was caused mainly from translocations. This hypothesis appears to have been a valid one, and indeed there may be translocations which we have not observed, however none have been observed upon cytological examination of male meiotic divisions (metaphase). This is in itself an interesting phenomenon, and it raises the point that translocations themselves may not be the sole cause of the late manifestation of lethal factors in subsequent generations.

The fact that we did not observe cytological aberrations while there was still IPS effect indicates that there are other genetic mechanisms that might explain the carrying capacity of these lethal factors.

The preliminary conclusions that can be drawn from these tests include the following:

1. Although the survival rate in the first and second generations of the egg stage is considerably reduced at higher doses the survival rate of ensuing generations is not, i.e. P and F₁ generation produced embryos show a dose-dependent relationship, whereas ensuing generations do not.

2. In non-afflicted lines there is very little embryonic death and larval mortality is highest in the first stage with less in the fifth stage and the pupal stage and virtually no death in the other larval stages. In afflicted lines the proportion of death in embryonic stages is considerably higher and the death rate is uniformly high in all larval stages.
3. Female lines are capable of transmitting, or conversely surviving IPS factors, but to a slightly less extent than male lines. Earlier tests showed that female lines were incapable of transmitting IPS more than two successive generations through the female side only.
4. Development rate in afflicted larvae was considerably longer than in normal larvae, regardless of the generation number.
5. IPS factors are apparently not selected against, even by outbreeding as far as the seventh or eighth generation.
6. No phenotypic abnormalities were observed. Mating frequency was less in afflicted lines, but mating behavior was normal.
7. Preliminary gel electrophoresis work with hemolymph proteins from normal and afflicted lines of larvae are similar in profile, but there is such variation in profiles of larvae of different ages that it would be difficult to make a statement about the similarity between afflicted and normal lines.

One of the most difficult problems encountered in studying the hemolymph proteins has been to develop a method for concentrating the proteins sufficiently to get distinct bands in the gel. Related to this is the labile nature of the proteins, apparently they are of fairly low molecular weight. A graduate student will be working on this problem for her dissertation in the near future.

2. Prodenia ornithogalli

In the preliminary tests at low dosages (4, 6, 8 and 10 Krads) to P generation adults larvae hatched but did not survive to pupate. Further tests are planned in the future. The sterilizing dosage has not been determined.

Hemiptera

3. Nezara viridula

One series of adults was treated with 1.5, 7.5, or 15.0 Krads, mated and observed for egg development as shown in Table at the two higher cases. None of the eggs from treated females survived the nymphal stages to become adults so that it was not possible to observe F₁ egg hatch. At 1.5 Krads we are rearing inbred lines of F₂ nympho from the T₁ generation.

In a previous series F_1 adults had produced offspring that had survived to the second nymphal stage at the 2 K_{rad} treatment. This line was lost at the F_2 egg stage.

Egg development was slower from irradiated parents than from normals, but nymphal development was almost as fast as normals. Lifespan was reduced by irradiation, and in these tests mating frequency was less than in the normals. Dissection after death showed no morphological differences between irradiated and normal females, however egg production was lower in irradiated females. Unfortunately it is difficult to ascertain the number of times that a female has mated since the sperm are not in spermatophores. The courtship behavior is distinctive but not particularly elaborate in this species as is described in Table 1. Development of eggs from irradiated and normal adults is also described in the same table.

C. Fractionated Dose Observations

Sub-sterilizing doses have been given to adults of both the sugarcane borer and the armyworm as fractionated doses in preliminary tests. Exposures were given four hours apart in 2 or 4 K_{rad} increments. The data obtained has not been sufficiently consistent to demonstrate a difference between a total dose of 4 Krads as a fractionated dose or as a single exposure. Likewise there has been no observable difference between 8 Krads as a fractionated or as a single exposure dose.

D. Hormone Treatment

Previous work has shown that some of the juvenile hormones cause chromosome puffing in addition to their main effect on developmental retardation. Because of this observation we have initiated work with the objective of measuring the combined effect of hormone treatment and irradiation.

The effects of Juvenile Hormone B (Cal Biochem) and Ecdysterone (Mann) have been evaluated with larval sugarcane borers. No males survived treatment of 1.0, 10.0, nor 100.0 micrograms of injected juvenile hormone to the fourth stage larvae, however females were able to complete their life cycle and were subsequently irradiated with 4.2 K_{rad}s of gamma irradiation. Offspring have been harvested from the 100.0 microgram treated females after breeding them with normal males.

None of the male fourth or fifth instar larvae treated with 1.0, 2.0 or 5.0 micrograms of moulting hormone, Ecdysterone, survived to become adults. The female larvae that emerged as adults and were subsequently irradiated with 4.2 Krads failed to produce viable offspring when mated with normal males.

These data are shown in Table 26.

III. Narrative Report

The change in the program funding structure resulting in a complete shift of the financial support of the Senior Investigator to the grant funds in December 1968 resulted in a staff reduction from the equivalence of three and one-half employees to one and one-half employees. Prior to this the Senior Investigator was receiving one-half of his salary from the ABS Division of PRNC. This funding structure has reduced program operations considerably, particularly in the capability for performing field tests (of dominant lethality and IPS), host preference tests and pheromone field tests.

In addition the degree of participation by the two cooperating Scientists Dr. Flavio Padovani and Dr. Niilo Virkki (both on ad honorem appointments) has been reduced due to the direct obligations in their own branches of UPR. To correct this problem a proposal was submitted in 1970 to the National Institutes of Health for additional support, and an additional proposal is pending approval by the National Science Foundation. The latter will be presented to the Reviewing Panel of NSF in February of 1972.

Dr. Padovani continues his interest in related aspects of the program mainly through the work of graduate students working with nutritional requirements of hemiptera. Dr. Virkki continues his chromosome observations with preserved material from previous tests as time allows.

The cooperative program with Dr. Nader Vakili (Plant Geneticist) of the USDA Experiment Station has mutually benefitted our program as well as his own bean selection program, because his extensive plots are available to us for collecting a variety of lepidoptera and hemiptera for preliminary rearing tests in the laboratory.

Two species of lepidoptera from bean tests have shown particular promise for sterility tests: Prodenia ornithogalli and Lamprosema indicata. Future tests to evaluate the mechanism of host preference will be possible when time and facilities become available. Dr. Vakili is evaluating several hundreds of species of beans and cowpeas in Puerto Rico and in Central America. In addition to the species tested in the second section of this report (Accomplishments) we have also completed preliminary rearing trials with Acrosternum marginatum and Thyanta perdita (Pentatomidae, Hemiptera), Systema basalis, Ceratoma ruficornis, and Epitrix cucumeris (flea beetles, Chrysomelidae, Coleoptera); Maruca testulalis, Fundella pelluscens, Etiella zinkinella (Lepidoptera); Diaprepes abbreviatus, Metamasius hemipterus, Cosmopolites sordida (Curculionidae, Coleoptera) and Elasmopalpus lignosellus and Protoparce sexta Trichoplusia ni, Prodenia eridenia (Lepidoptera).

Nearly all of these tests were related to the bean resistance program.

Dr. Goru Kuno (Entomological Laboratory, UPR Mayaguez) is collecting and isolating pathogens from our laboratory colony of the sugarcane borer and in addition he has assisted in the maintenance of our insect culture.

Mr. Ruben Restrepo (graduate student in Biology, UPR Mayaguez) has developed rearing methods for Nezara viridula on a voluntary basis during the past year and one half. We are attempting to provide him a short term paid appointment during the spring and early summer of 1972 for Nezara work prior to his return to his appointment at the Universidad Nacional de Columbia (Bogota). Mr. Restrepo has worked diligently in the laboratory and much of the Nezara work in this report has been the result of his efforts. His thesis research is in the taxonomy of the Membracidae (Homoptera).

IPS tests have not been made for all of these species mentioned since a reliable artificial rearing method is a prerequisite for evaluating IPS. Nonetheless from the limited evidence that is available it is apparent that the IPS phenomenon is related to the holokinetic chromosome.

Mrs. Dolores Ayguabibas de Ruiz will terminate her appointment as Research Assistant on the program at an early date at the request of her husband. During the interim period Dr. Lowman has loaned Mr. Sam de la Rosa to the Program until we can find a replacement for Dolores.

In addition to his program activity at PRNC Dr. Walker has given seminars at the Pioneering Entomological Laboratory of UPR Mayaguez, to the Institute of Marine Sciences and to the Sigma Xi Club of the UPR. He served as an advisor to the Environmental Quality Review Board (Office of the Governor of P.R.) for the pending Pesticide Law of the Commonwealth of P.R. The San Juan Star prepared a two page article on the Insect Sterility Program and this was included in the Sunday Supplement, Sept. 19, 1971.

IV. Future Work Planned

Future efforts will concentrate on aspects of Inherited Partial Sterility. We will continue to explore the utility of IPS in population suppression on a laboratory population study basis using the sugarcane borer, the armyworm (Prodenia) and perhaps another moth if rearing methods can be developed with facility, the stinkbug (Nezara) and possibly a scale insect if rearing methods can be developed.

Dr. Kuno has shown an interest in a tissue culture of insect tissues and of this can fit into our chromosome study provided that Dr. Virkki will have sufficient time to give to the program. We are studying haemolymph proteins of Nezara and the sugarcane borer using the disc electrophoresis method and will be combining this with molecular sieve techniques at a later date.

The combination treatment of juvenile hormone and ecdysterone with low radiation dose shows sufficient promise to warrant further consideration. Our early data from fractionated doses is not clear enough to give a forth-right interpretation, however this area should be given further study.

Staff size is too small to consider further field testing because the amount of labor needed to maintain the laboratory colony to the size needed

for field tests is greater than the help that we have available.

V. Publications

Walker, D. W., V. Quintana-Muñiz, and F. Padovani. 1971. Effect of gamma irradiation on immature sugarcane borers. Sterility Principle for Insect Control or Eradication, IAEA, Vienna: pp 513-24.

_____, _____, and Josefa Torres. 1971. Genetic collapse of insect populations. 1. Extinction of inbred and outbred lines in laboratory populations of the sugarcane borer. Journ. Econ. Entom. 64 (3):661.7.

Publications in preparation.

Genetic Collapse. 2. Dose-effect on subsequent generations of sugarcane borers.

Mechanism of Inherited Partial Sterility.

Short distance perception of female sex pherome by male sugarcane borers.

Chromosome abnormalities in IPS lines of the sugarcane borer.

VI. Relation to Other Work and Conclusions

The intense interest in environmental quality in conjunction with the need for high production quotas of agricultural products has stimulated interest in pest management concepts and particularly an integrated systems approach to crop protection. Pesticide pollution has become recognized as a serious threat to the environment yet we do not have satisfactory alternative methods for pest prevention and pest control sufficiently adequate to ensure high yields. This whole problem is further complicated by the mono-cultural practice. The solution to this general problem appears to lie in a combination of practices: more effective and sophisticated pesticide use, particularly the choice of specific insecticides for specific pest species (those available that have short residual half-life and that are least damaging to warm blooded animals); the use of pheromones; judicious use of released parasites and predators of specific pest species such as egg parasites, larval parasites, predatory bugs and orthoptera, etc; use of insect pathogens for creating epizootic among pest populations; crop timing to avoid having the most susceptible growth stage synchronized with the maximum pest population; the use of pest resistant plants; and overflooding the natural population with sterile individuals.

I have mentioned the overflooding technique last since it is most efficiently used when the target pest species is in small numbers. Overflooding can be used in two ways: either for eradication or for suppression. As a consequence our work relates directly with the current concepts of future pest control. This area of technology is developing rapidly as a consequence of the impetus for a clean environment.

The thrust of this new thinking is to develop a number of specific solutions to the specific pest problem and to use these specific methods in such a way that each one will yield a maximum effect. For example a sugarcane borer program in the tropics would probably begin with the use of Endrin^R granules applied to cane that is not developed beyond the stool stage this could be followed by the release of Trichogramma minutum adults (which are egg parasites) and also Lixophaga diatraea (a larval parasite), as well as by setting pheromone traps. If the combination of these methods succeeded in reducing the natural population sufficiently, semi-sterile adults could be released after which time the parasites and pheromone traps would be temporarily removed.

In addition to raising the insects for release it would be necessary to constantly monitor the natural population in order to evaluate the effectiveness of each control measure and to provide the information for timing the sterile release. The overhead for such a program in terms of technological input would be large to begin with, but the overall cost probably would be less than an intensive program if amortized over the period of time that it is effective - for example a two or three year cycle. The obvious dangers of such a method is that an alternate species might take the same ecologic niche from which the pest species is being eliminated. In this example Elasmopalpus lignosellus might occupy this niche. This particular problem could be avoided by including pheromone traps that attract both the sugarcane borer and Elasmopalpus, i.e. traps that have two sex pheromones. The other control measures mentioned are equally effective against both pests. Management techniques are best adapted to permanent crops such as deciduous fruits (apples, pears, peaches, cherries, oranges, etc.), alfalfa, sugarcane, mango, etc.

VII. References

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Table 1. Nezara viridula (L.)

A. Description of Embryonic Development in non-Fertile, Normal, and Eggs from an Irradiated Female Parent

<u>non-Fertile Eggs</u>	<u>from Irradiated Female</u> <u>I. 14 to 16 hours</u>	<u>Normal Eggs</u>
cluster regular but with a small number of eggs in each cluster, often laid singly; bright yellow	cluster irregular, eggs not in neat rows; individual eggs are turgid.	turgid, barrel shaped: apical margin spines directed into margin toward the apex: not elongated; white ring around lmm. or less in diameter, uniform yellow throughout except margin, egg contents milk, and turbid, homogeneous; and egg cluster convex because of swelling of individual eggs; upper cap light reflective, waxy sheen, egg capsule light yellow, eggs tightly packed together in cluster.
bright yellow, spines pointed inward.	II. <u>24 hours</u> eggs with milky turbid contents clear area not distinct.	center of egg with a clear area inside of marginal ring; spines pointing out from argin to outer surface; interior of egg becoming turbid above base.
no clear area, no murky flocculations develop.	III. <u>36 hours</u> clear area does not develop.	clear area in center of egg apex with a ring inside egg margin that is milky white color.
eggs turn dark and become flaccid.	IV. <u>48 hours</u> stylets do not form as discrete	red color at egg apex from developing stylets and forelegs appear like an "eagle" with wings spread.

V. 120 hours (5 days)

dark colored eggs, flaccid.

eggs turn dark yellow and are turbid with clumps of fatty appearing bodies.

body outline distinct, legs visible, embryo surrounded by a clear fluid.

VI. 144 hours or longer (6 days)

same as above.

no further embryo development, eggs become dark brown after 7 to 8 days, and eventually become flaccid.

eggs hatch, lymph leaves from circular hole of egg apex.

B. Description of Courtship Behavior and Egg Laying (Restrepo)

Male moves parallel with female performing a trembling motion, then he moves in front of female and stimulates the female by touching her with his antennae on the frons and on her antennae, they are face to face in this position. Male continues his trembling motion while repeatedly touching his antennae on her frons and her antennae and the anterior dorsum of her thorax, when female stops moving and is quiet he continues palpitating her with his antennae until she raises her abdominal tip. The male moves rapidly to the raised abdominal tip and inserts the aedeagus. There is no spermatophore transferred.

Males will attempt to mate with females at random or with other males. If there is no response from the female or from the other male he will not persist. After coupling the male and female will remain in tandem for as long as an hour or more. This usually takes place on the upper surface of a leaf. Mating takes place at any time of day and has not been observed in the dark.

Females lay fertile eggs in clusters with from 12 to 120 eggs per clusters. Eggs are usually laid during the morning hours, but may be laid as late as 1400 hours.

C. Life Table of Nymphs

Embryonic development 7 to 8 days

First nymphal stage - 5 days

Second - 7 days

Third - 6 days

Fourth - 6 days

Fifth - 8 days

Adult lifespan to and beyond 120 days, average probably 90 days or less.

LEGEND FOR SYMBOLS USED IN COLUMNS ON TABLES 2 THROUGH 25

<u>TITLE</u>	is the code number of a specific mating. It has a letter designation (A,B, etc. to H), followed by a number that is code for the dose, followed by the parent's code number, and terminated by the specific number of that mating.
B	the number of fertile eggs laid
F	the number of fertile eggs that hatched
F/B	percent of the fertile eggs that hatched into larvae
G	is the number of newly emerged <u>larvae</u> that were <u>harvested</u> and placed into artificial diet
M	is the number of <u>adults harvested</u>
M/G	percent survival, <u>first instars to adults</u>

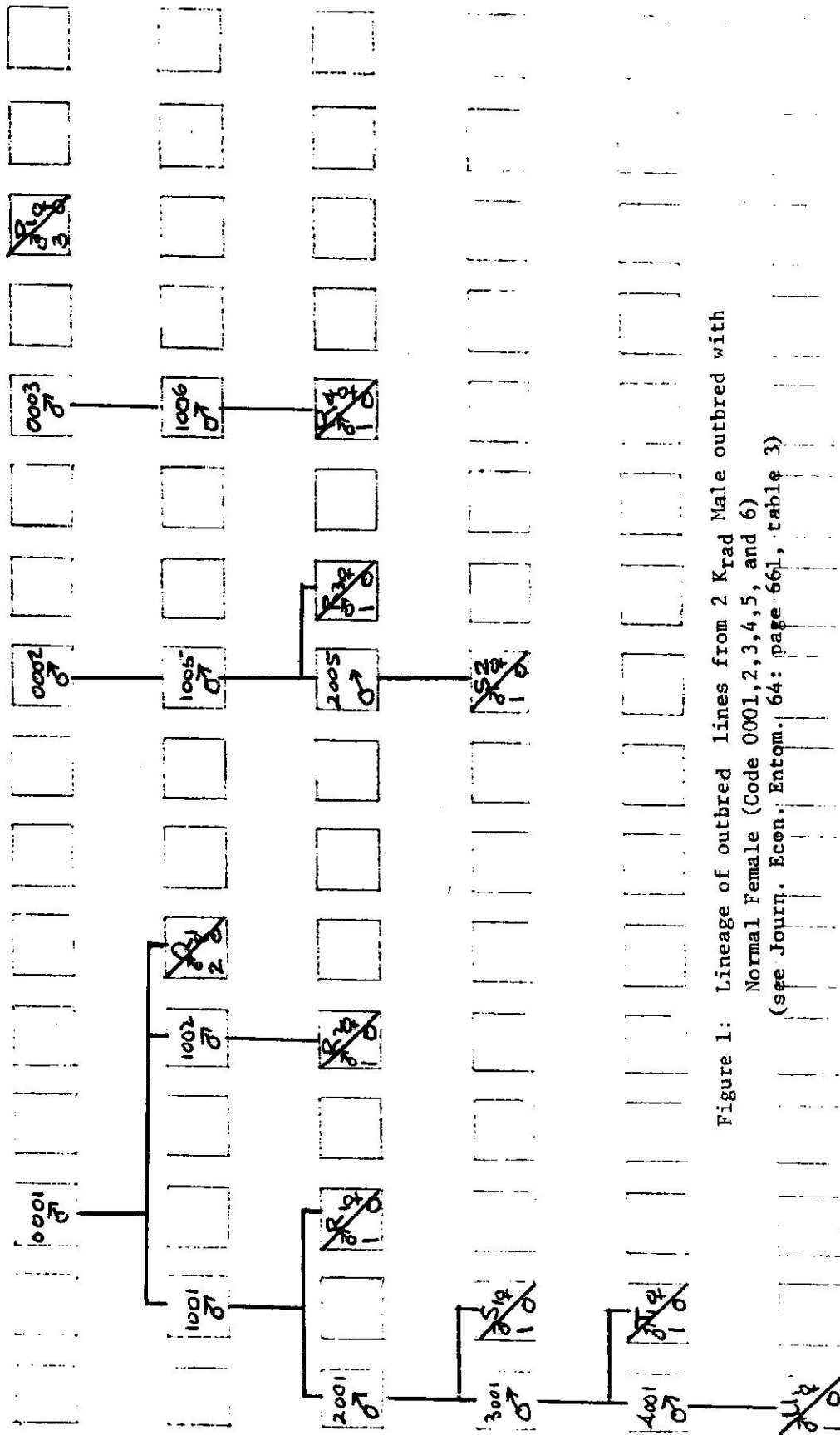


Figure 1: Lineage of outbred lines from 2 Krad Male outbred with Normal Female (Code 0001, 2, 3, 4, 5, and 6) (see Journ. Econ. Entom. 64: page 661, table 3)

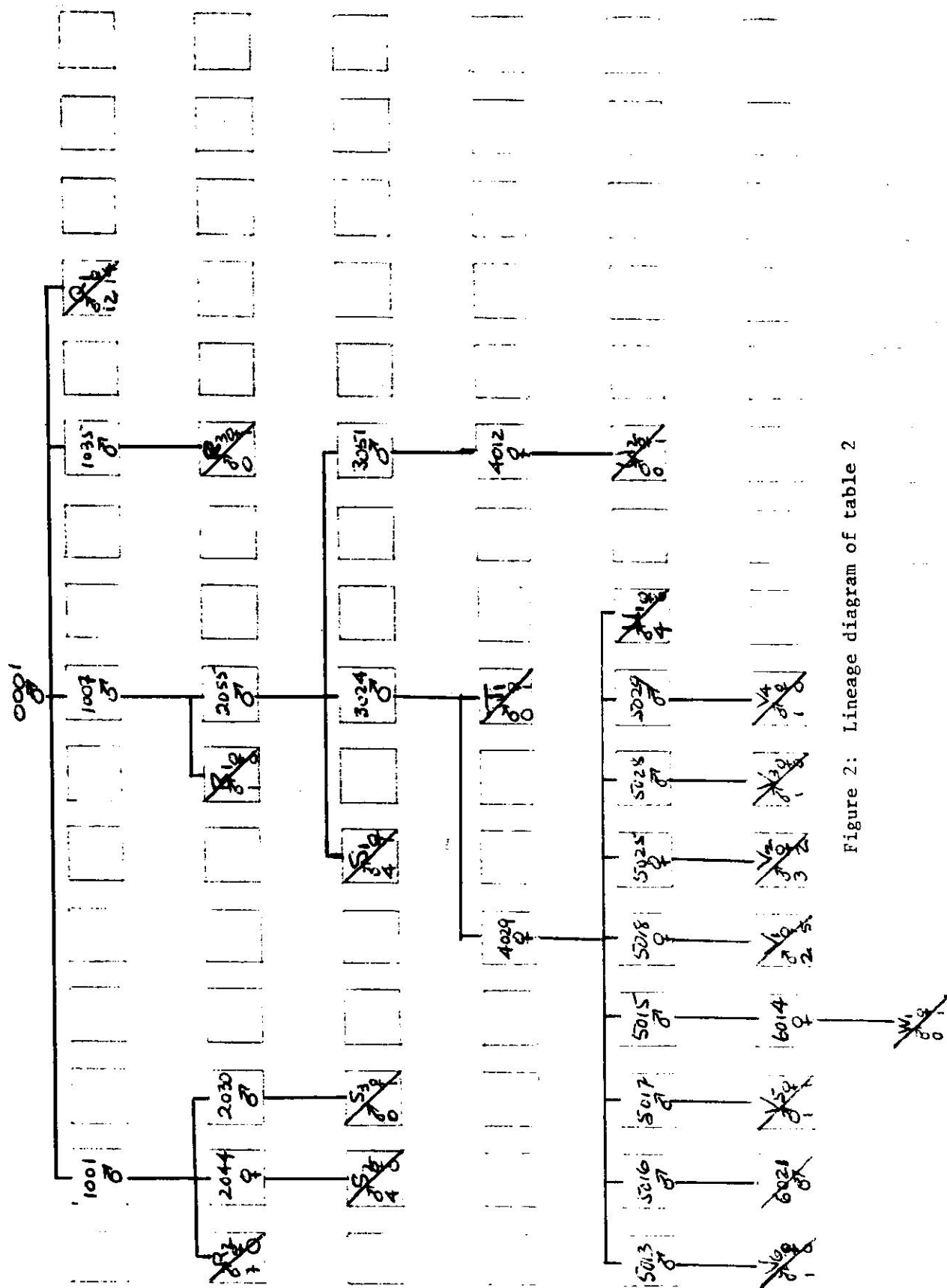


Figure 2: Lineage diagram of table 2

Table 3

Outbred Offspring from 2 Krad Male Mated to Female Normal (Code AI-0003, 4)

TITLE=AI ANCESTOR IS 4													
TITLE	R	F	F/R	G	M	M/G	F/AI	715	105	0.1469	0	0	0.0000
AI 3	351	244	0.6952	210	16	0.0762	AI 41062	715	105	0.1469	0	0	0.0000
AI 31018	685	486	0.7095	365	11	0.0301	AI 41063	330	0	0.0000	0	0	0.0000
AI 31019	592	142	0.2399	171	7	0.0165	AI 41064	410	65	0.1585	0	0	0.0000
AI 31046	64	39	0.6094	30	0	0.0000	AI 41065	500	105	0.2100	0	0	0.0000
AI 31068	295	15	0.0508	0	0	0.0000	AI 41070	86	0	0.0000	0	0	0.0000
AI 31084	468	38	0.0812	0	0	0.0000	AI 41081	210	3	0.0143	0	0	0.0000
AI 31096	535	160	0.2991	130	18	0.1365	AI 41090	383	143	0.3734	110	25	0.2273
AI 10962067	546	265	0.4853	265	10	0.0377	AI 41093	565	265	0.4690	110	7	0.0636
							AI 41101	595	130	0.2185	105	0	0.0000
							AI 41107	311	65	0.2090	0	0	0.0000
							AI 41112	150	45	0.3000	0	0	0.0000
							AI 41116	448	33	0.0737	0	0	0.0000
							AI 41119	618	118	0.1909	60	6	0.1000
							AI 10212009	543	10	0.0184	0	0	0.0000
							AI 10212035	345	293	0.8493	200	47	0.2350
							AI 10212057	269	110	0.4089	80	17	0.2125
							AI 10212058	667	231	0.3463	125	26	0.2090
							AI 10932065	235	95	0.4043	95	17	0.1769
							AI 10932066	141	40	0.2837	40	13	0.3250
							AI 20583022	250	33	0.1320	0	0	0.0000
							AI 20583025	533	75	0.1407	75	47	0.6267
							AI 20583060	406	129	0.3177	0	0	0.0000
							AI 20353044	80	14	0.1750	0	0	0.0000
							AI 20353045	173	90	0.5202	90	17	0.1889
							AI 20353047	737	292	0.3962	0	0	0.0000
							AI 20353048	370	20	0.0541	20	7	0.3509

TITLE=AI ANCESTOR IS 4													
TITLE	B	F	F/B	G	M	M/G	F/AI	715	105	0.1469	0	0	0.0000
AI 4	435	368	0.8460	352	46	0.1307	AI 41116	448	33	0.0737	0	0	0.0000
AI 41003	761	154	0.2024	50	17	0.3400	AI 41119	618	118	0.1909	60	6	0.1000
AI 41012	681	85	0.1248	0	0	0.0000	AI 10212009	543	10	0.0184	0	0	0.0000
AI 41013	961	266	0.2768	200	23	0.1150	AI 10212035	345	293	0.8493	200	47	0.2350
AI 41015	564	282	0.5000	200	24	0.1200	AI 10212057	269	110	0.4089	80	17	0.2125
AI 41020	332	2	0.0060	0	0	0.0000	AI 10212058	667	231	0.3463	125	26	0.2090
AI 41021	861	468	0.5436	85	36	0.4235	AI 10932065	235	95	0.4043	95	17	0.1769
AI 41040	615	395	0.6423	200	10	0.0500	AI 10932066	141	40	0.2837	40	13	0.3250
AI 41045	308	13	0.0422	0	0	0.0000	AI 20583022	250	33	0.1320	0	0	0.0000
AI 41052	74	28	0.3784	0	0	0.0000	AI 20583025	533	75	0.1407	75	47	0.6267
AI 41053	473	323	0.6829	200	2	0.0100	AI 20583060	406	129	0.3177	0	0	0.0000
AI 41058	660	395	0.5985	70	4	0.0571	AI 20353044	80	14	0.1750	0	0	0.0000
AI 41059	645	265	0.4109	0	0	0.0000	AI 20353045	173	90	0.5202	90	17	0.1889
							AI 20353047	737	292	0.3962	0	0	0.0000
							AI 20353048	370	20	0.0541	20	7	0.3509

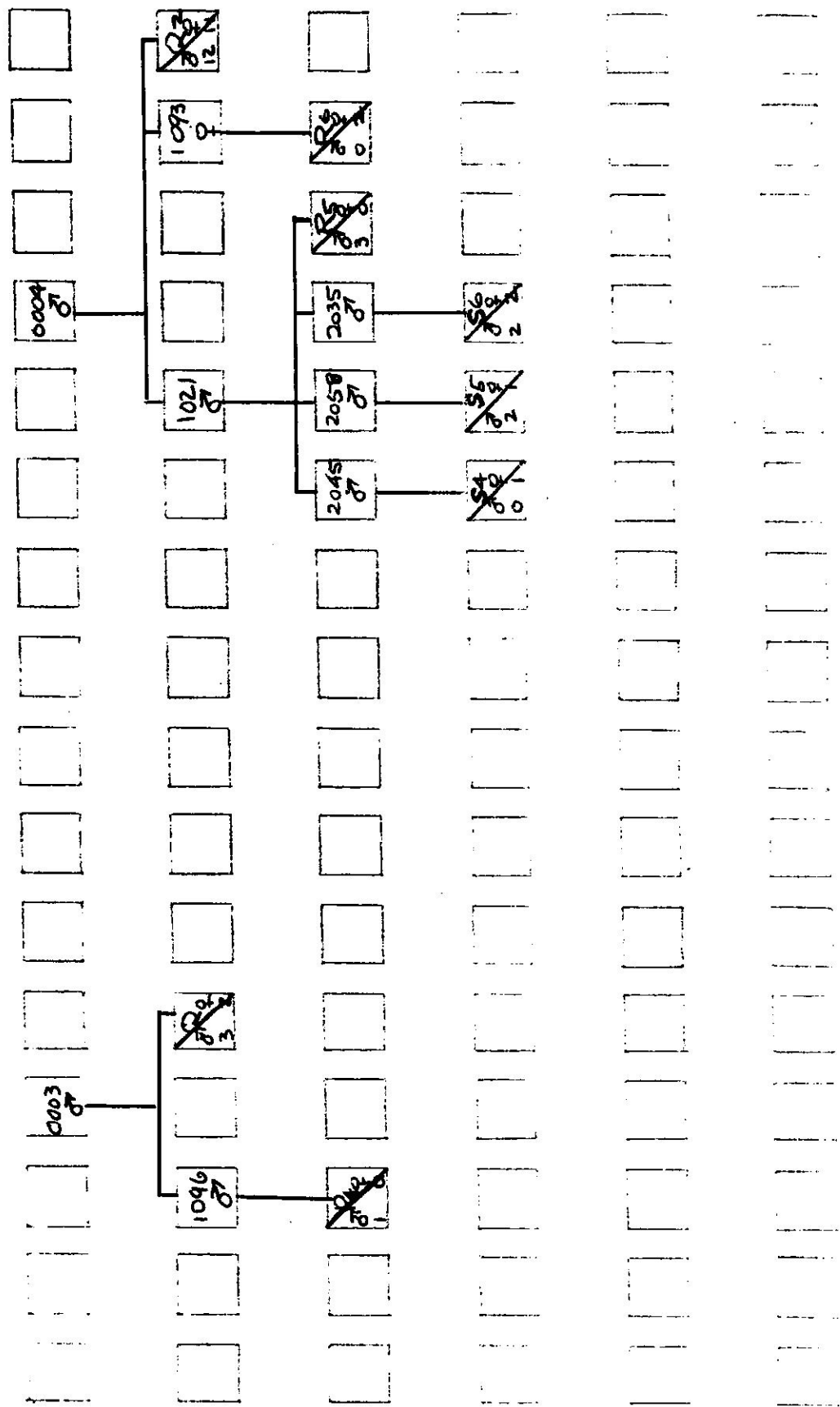


Figure 3: Lineage diagram of table 3

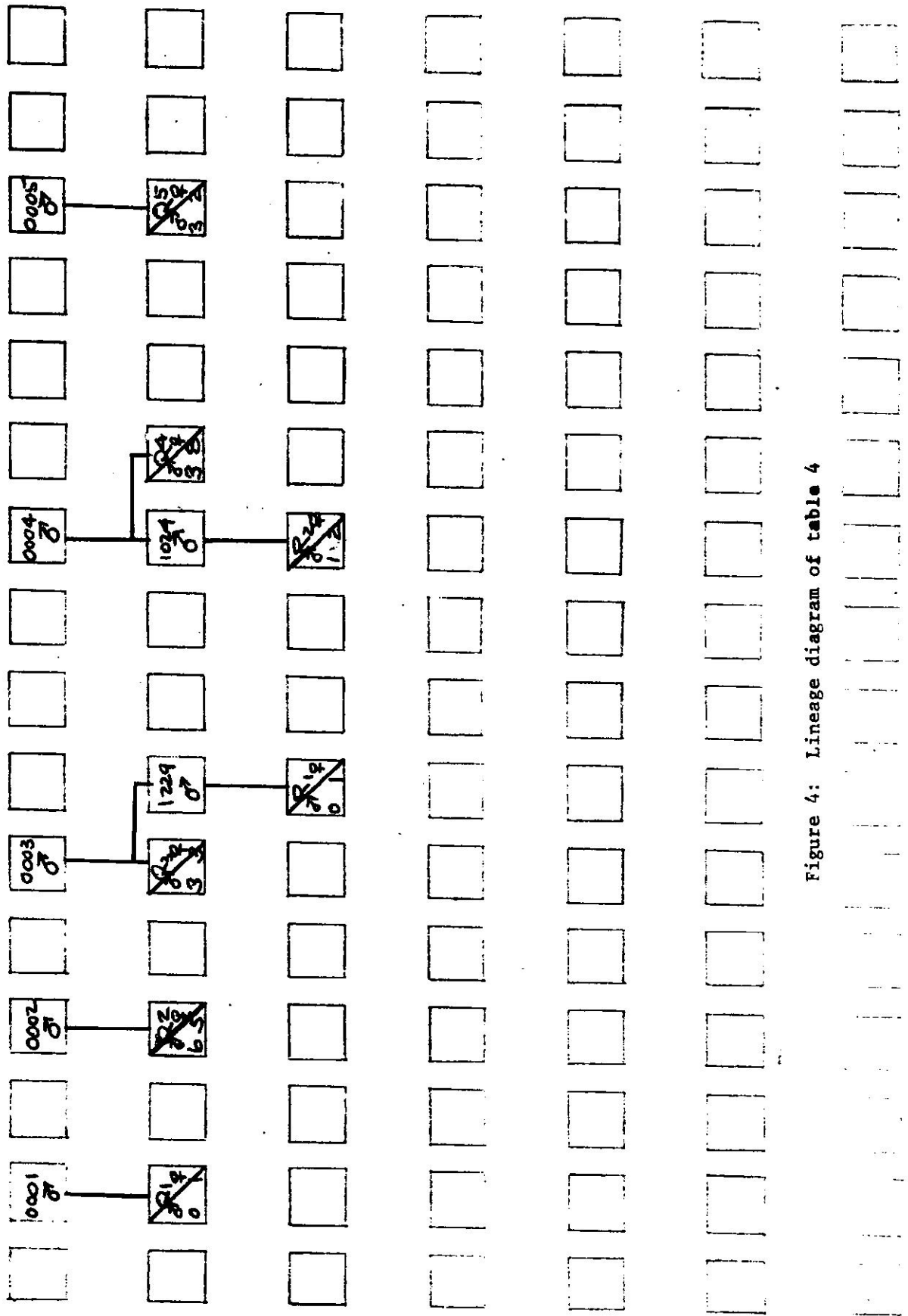


Figure 4: Lineage diagram of table 4

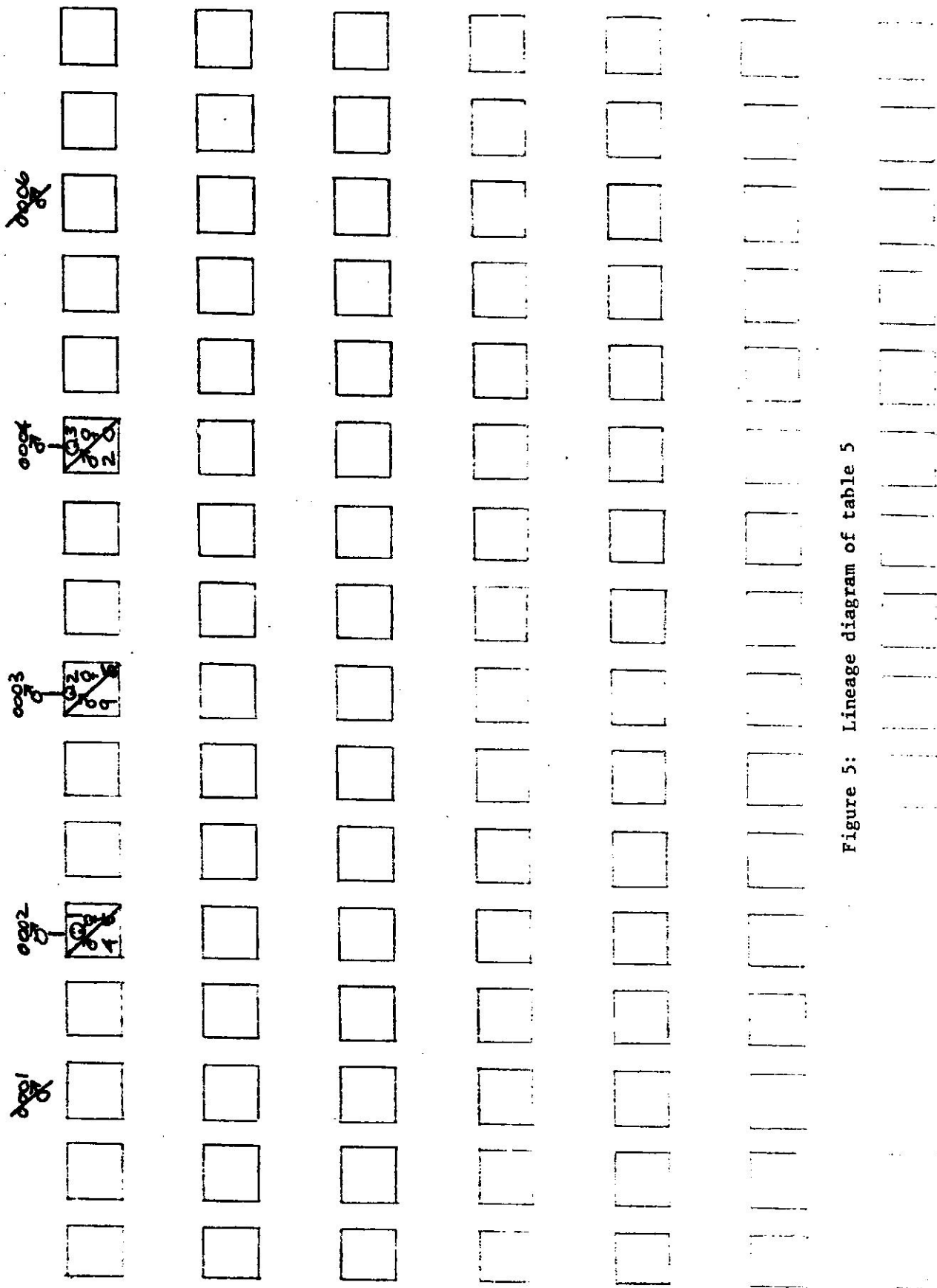


Figure 5: Lineage diagram of table 5

Table 6

Outbred Offspring from 12 Krad Male Mated to Female Normal (Code A3-0005)

TITLE	B	F	TITLE=A3 ANCESTOR IS			5	A330064004	94	0	0.0000	0	0	0.0000
			F/4	G	M	M/G							
A3 51004	1	0	0.0000	0	0	0.0000	A330064003	241	187	0.7743	187	0.0000	
A3 51007	100	45	0.4500	0	0	0.0000	A330064013	674	316	0.4689	240	0.1617	
A3 51015	98	2	0.0204	0	0	0.0000	A330274016	654	155	0.2370	155	0.0238	
A3 51016	668	325	0.4865	290	151	0.5207	A330304010	454	215	0.4715	210	0.0230	
A3 51021	465	5	0.0108	0	0	0.0000	A330404002	293	118	0.4027	118	0.0000	
A3 51029	210	0	0.0000	0	0	0.0000	A330404013	424	314	0.7384	240	0.1617	
A3 51033	315	0	0.0000	0	0	0.0000	A330414005	635	169	0.2661	50	0.0400	
A3 51034	521	8	0.0154	0	0	0.0000	A330444009	723	219	0.3029	139	0.1366	
A3 51035	75	0	0.0000	0	0	0.0000	A330414011	230	177	0.7694	177	0.0000	
A3 51038	370	0	0.0000	0	0	0.0000	A330294014	0	0	0.0000	0	0.0000	
A3 51045	80	0	0.0000	0	0	0.0000	A340085003	969	648	0.6687	375	0.0427	
A3 51048	57	0	0.0000	0	0	0.0000	A340085004	411	143	0.3479	70	0.1371	
A3 51049	270	0	0.0000	0	0	0.0000	A340085010	395	88	0.2228	60	0.0000	
A3 51015	98	2	0.0204	0	0	0.0000	A340085011	750	311	0.4147	240	0.0232	
A3 51016	668	325	0.4865	290	151	0.5207	A340085018	625	360	0.5740	340	0.0334	
A310162001	210	115	0.5500	75	0	0.0000	A340085024	720	430	0.5972	375	0.0533	
A310162002	118	22	0.1864	0	0	0.0000	A340085025	803	425	0.5293	425	0.0165	
A310162003	256	123	0.4805	100	8	0.0000	A340095001	490	164	0.3347	135	0.2815	
A310162004	478	355	0.7427	0	0	0.0000	A340095002	501	245	0.4890	245	0.1142	
A310162005	256	123	0.4805	100	8	0.0000	A340095005	792	394	0.4975	320	0.0949	
A310162006	371	290	0.7817	245	0	0.0000	A340095012	394	370	0.9435	370	0.1895	
A310162007	437	309	0.7071	309	40	0.1294	A340085014	532	0	0.0000	0	0.0000	
A310162008	446	342	0.7668	320	31	0.0949	A340085019	135	0	0.0000	0	0.0000	
A310162014	547	305	0.5575	290	36	0.1261	A340085020	47	0	0.0000	0	0.0000	
A310162015	348	108	0.3103	93	1	0.0108	A340085025	803	425	0.5293	425	0.0165	
A310162024	629	88	0.1399	70	40	0.5714	A340125016	258	127	0.4922	120	0.0647	
A310162025	507	123	0.2426	75	25	0.3333	A340125015	304	0	0.0000	0	0.0000	
A310162028	445	43	0.0966	25	9	0.3600	A340135021	399	302	0.7569	290	0.0144	
A310162031	291	43	0.1478	30	9	0.3000	A340135022	456	330	0.7236	60	0.0000	
A310162032	370	130	0.3514	121	13	0.1074	A340135023	678	541	0.7979	504	0.0417	
A310162035	528	213	0.4034	65	16	0.2462	A340155029	95	0	0.0000	0	0.0000	
A310162036	506	142	0.2806	129	25	0.1953	A340155030	396	130	0.3283	130	0.1539	
A310162039	328	314	0.9573	311	84	0.2701	A340155031	313	165	0.5272	165	0.2806	
A310162040	506	293	0.5791	284	89	0.3134	A340155032	726	704	0.9697	80	0.1250	
A310162043	392	256	0.6531	238	144	0.6050	A340135006	82	0	0.0000	0	0.0000	
A310162044	193	87	0.4508	80	14	0.1875	A340135017	347	30	0.0865	30	0.0333	
A310162049	519	189	0.3642	176	104	0.5909	A340165033	408	0	0.0000	0	0.0000	
A310162050	485	55	0.1134	46	14	0.3043	A350056003	622	209	0.3360	120	0.1167	
A310162054	461	263	0.5705	155	37	0.2387	A350056008	550	90	0.1636	90	0.0556	
A320033048	563	212	0.3766	120	3	0.0250	A350056002	0	0	0.0000	0	0.0000	
A320243038	267	252	0.9438	135	77	0.5704	A350056005	31	0	0.0000	0	0.0000	
A320243049	359	196	0.5462	92	1	0.0109	A350056006	71	0	0.0000	0	0.0000	
A320253006	446	145	0.3251	50	6	0.1200	A350056008	550	90	0.1636	90	0.0556	
A310162007	310	145	0.4677	100	2	0.0200	A350056011	451	30	0.0665	30	0.0333	
A310162009	280	153	0.5294	135	0	0.0000	A350056020	1061	303	0.2854	190	0.0105	
A310162010	133	56	0.4211	0	0	0.0000	A350056030	614	271	0.4414	235	0.0403	
A310162012	354	48	0.1356	22	0	0.0000	A350076004	480	106	0.2228	65	0.0154	
A310162013	239	55	0.2301	20	0	0.0000	A350076019	268	24	0.0896	15	0.0333	
A320393017	397	83	0.2091	60	2	0.0333	A350036012	1021	298	0.2919	180	0.0278	
A320393030	467	178	0.3812	70	19	0.2714	A350036013	920	262	0.2848	230	0.0300	
A320393040	620	226	0.3645	210	72	0.3429	A350036028	688	235	0.3416	235	0.0403	
A320033048	563	212	0.3766	120	3	0.0250	A350056027	376	0	0.0000	0	0.0000	
A310162017	424	95	0.1904	0	0	0.0000	A350125018	287	0	0.0000	0	0.0000	
A310162034	346	0	0.0000	0	0	0.0000	A350126023	274	0	0.0000	0	0.0000	
A320243038	267	252	0.9438	135	77	0.5704	A350126031	79	0	0.0000	0	0.0000	
A320243049	369	196	0.5312	92	1	0.0109	A350126017	853	594	0.6923	355	0.0423	
A310162037	534	30	0.0730	23	0	0.0000	A350126021	543	188	0.3462	15	0.1333	
A310162038	309	156	0.5049	135	0	0.0000	A350126016	774	373	0.4817	90	0.0222	
A320253006	446	145	0.3251	50	6	0.1200	A350126018	652	190	0.2916	115	0.0300	
A320253007	463	194	0.4201	81	8	0.0699	A350046001	717	321	0.4477	185	0.1405	
A310162053	180	0	0.0000	0	0	0.0000	A350116015	271	25	0.0923	10	0.1000	
A320393017	397	83	0.2091	60	2	0.0333	A350126022	885	138	0.1547	55	0.0182	
A320393030	467	178	0.3812	70	19	0.2714	A350126026	840	274	0.3286	155	0.0300	
A320393040	620	226	0.3645	210	72	0.3429	A350126025	702	321	0.4573	75	0.0133	
A320433002	382	118	0.3089	45	17	0.3778	A350126027	777	259	0.3333	194	0.0200	
A320433009	358	251	0.6983	100	17	0.1700	A350126035	517	140	0.2708	35	0.0300	
A320433029	507	267	0.5266	130	20	0.1538	A350126036	165	0	0.0000	0	0.0000	
A320433041	475	113	0.2379	88	9	0.1047	A350216034	201	0	0.0000	0	0.0000	
A320433047	261	91	0.3487	46	3	0.0652	A350126039	424	250	0.5915	200	0.0200	
A320433048	294	150	0.5034	150	0	0.0000	A350186029	476	30	0.0630	30	0.0300	
A320253006	446	95	0.2089	95	2	0.0211	A350216033	473	20	0.0423	20	0.0400	
A320493018	518	221	0.4268	90	4	0.0444	A350216037	819	367	0.4481	221	0.0378	
A320493020	311	177	0.5691	95	33	0.3474	A350216042	259	85	0.3282	85	0.0333	
A320493022	426	98	0.2300	30	1	0.0333	A350216040	130	0	0.0000	0	0.0000	
A320493045	140	1	0.0071	0	0	0.0000	A350216043	332	37	0.0964	37	0.0300	
A320493046	541	75	0.1386	75	8	0.1067	A350236032	940	213	0.2279	150	0.0300	
A320543004	258	64	0.2481	35	7	0.2000	A350236038	398	31	0.0779	18	0.0300	
A320543015	309	89	0.2880	69	0	0.0000	A360017001	512	194	0.3789	130	0.0144	
A320393050	484	2	0.0041	2	0	0.0000	A360017002	983	424	0.4314	155	0.0210	
A320393051	259	0	0.0000	0	0	0.0000	A360017003	785	322	0.4102	60	0.0100	
A320393053	435	80	0.1760	80	0	0.0000	A360017005	410	334	0.8124	270	0.0300	
A320433008	595	148	0.2487	80	3	0.0375	A360017012	550	329	0.5982	95	0.0300	
A320433010	0	0	0.0000	0	0	0.0000	A360037008	751	478	0.6365	390	0.0300	
A320433011	28	22	0.7857	22	3	0.1364	A360037009	671	462	0.6887	330	0.0300	
A320433013	271	7	0.0258	7	0	0.0000	A360037011	423	232	0.5485	190	0.0300	
A320433025	276	68	0.2464	68	0	0.0000	A360017007	137	0	0.0000	0	0.0000	
A330074008	632	390	0.5713	320	53	0.1652	A360037006	63	0	0.0000	0	0.0000	
A330064012	264	100	0.3789	100	2	0.0200	A360037010	747	352	0.4712	313	0.0300	

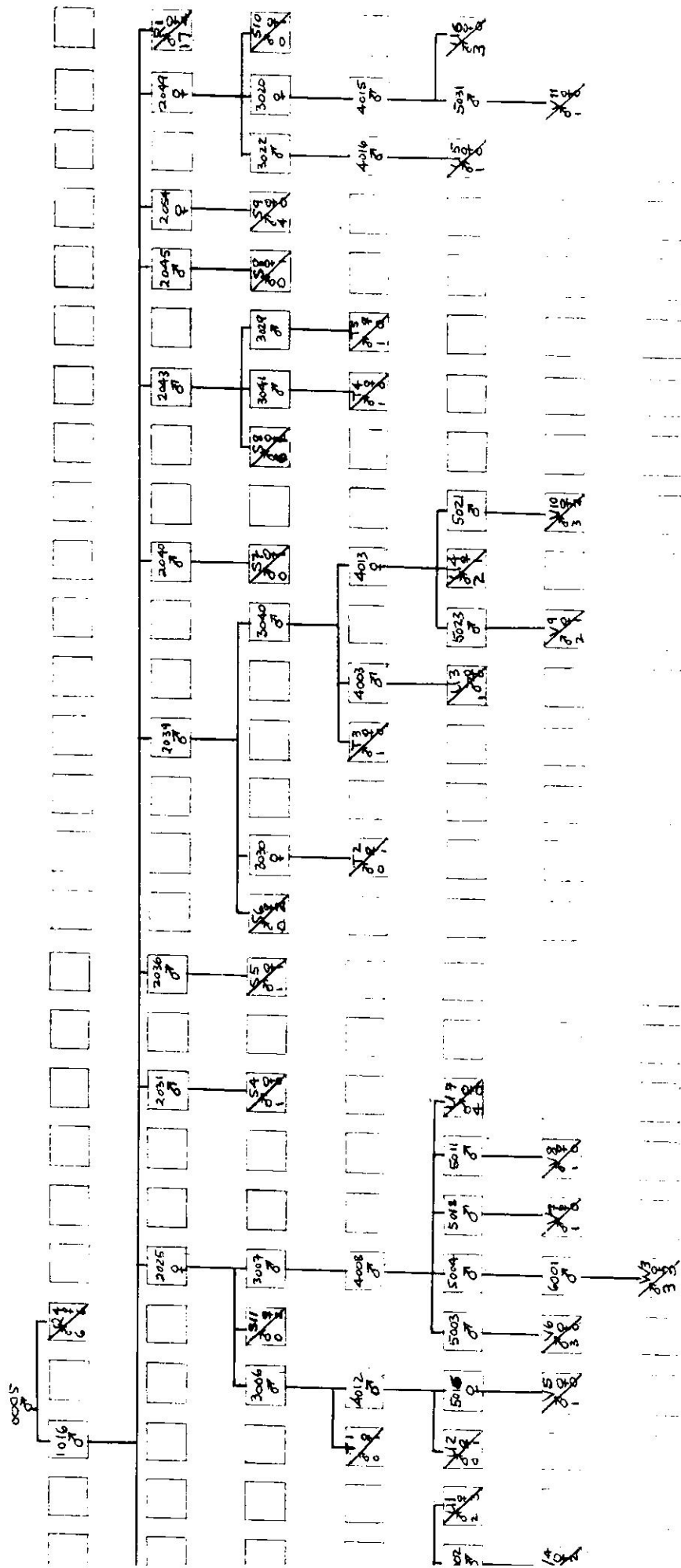


Figure 6: Lineage diagram of table 6

Table 8

Outbred Offspring from 2 K_{rad} Female Mated to Male Normal (Code B1-0001,2)

TITLE		B	F	F/B	TITLE=B1 ANCESTOR IS		2
					G	M	M/G
B1	2 2	593	103	0.1737	89	7	0.0787
B1	21033	115	45	0.3913	10	2	0.2000
B1	21051	750	315	0.4200	70	0	0.0000
B1	21118	859	644	0.7497	514	85	0.1654
B1	21120	39	18	0.4615	0	0	0.0000
B111182071		458	137	0.2991	40	10	0.2500

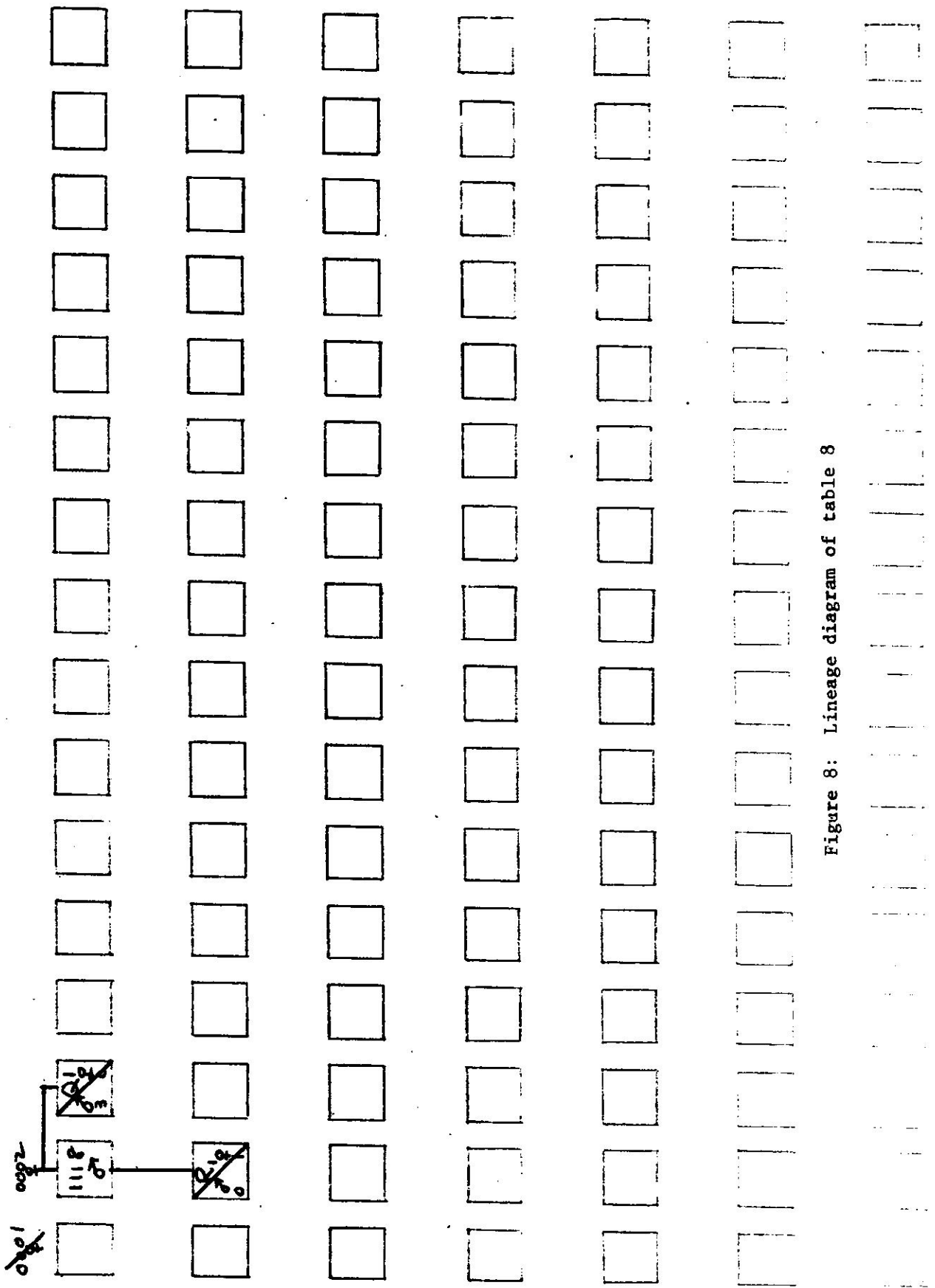


Figure 8: Lineage diagram of table 8

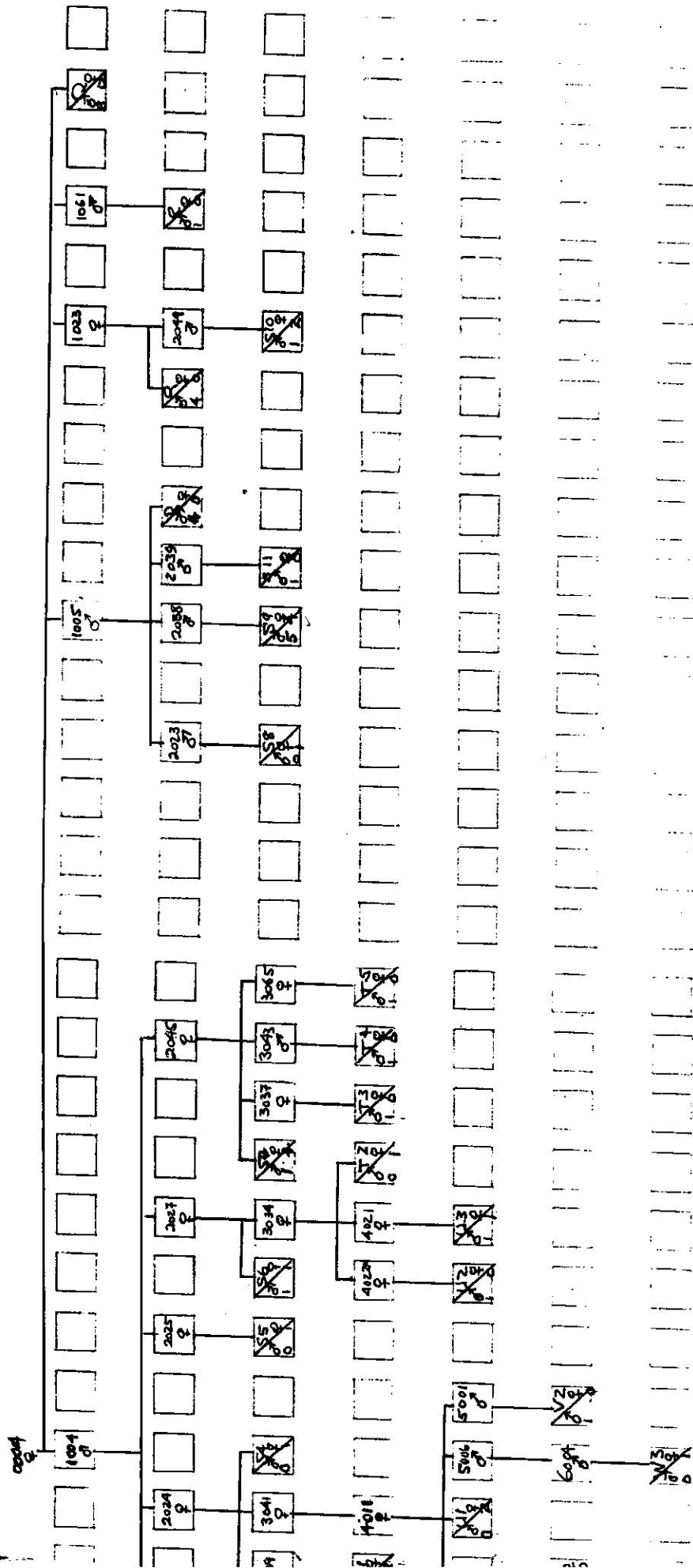


Figure 9: Lineage diagram of figure 9

Table 10

Outbred Offspring from 6 K_{rad} Female Mated to Male Normal (Code B2-0001,2)

TITLE		R	F	F/R	G	M	M/G	TITLE=B2 ANCESTOR IS		1				
R2	1	1	453	0.1317	72	7	0.0972	B220993038	778	134	0.1720	194	4	0.0203
R2	11069	163	23	0.1411	0	0	0.0000	B220543027	963	241	0.2503	50	18	0.3610
R2	11093	335	300	0.8955	160	28	0.1750	B220683024	243	78	0.3210	52	23	0.4423
R2	11110	345	210	0.6087	175	7	0.0400	B220773028	458	321	0.7000	80	13	0.1625
R2	11130	200	0	0.0000	0	0	0.0000	B220773029	415	196	0.4718	160	10	0.1262
R2	11182	320	206	0.6437	190	28	0.1474	B220543070	640	188	0.2927	144	15	0.1042
R2	11183	405	85	0.2099	0	0	0.0000	B220993016	674	519	0.7700	240	44	0.1833
R2	11193	200	0	0.0000	0	0	0.0000	B220993011	552	209	0.3785	185	27	0.2000
R211102166	597	263	0.4405	190	31	0.1532	B220503073	651	280	0.4301	50	7	0.1400	
R210932157	13	7	0.5385	0	0	0.0000	B220473043	513	139	0.2710	120	19	0.1583	
								B210462077	591	280	0.4718	200	84	0.4200
								B210012068	340	52	0.1520	35	12	0.3420
								B220993013	653	252	0.3859	90	27	0.3000
								B230134050	135	0	0.0000	0	0	0.0000
								B230434002	437	183	0.4188	180	17	0.0944
								B230434026	311	100	0.3215	100	4	0.0400
								B230434027	403	192	0.4764	180	24	0.1337
								B230114009	372	113	0.3000	75	0	0.0000
								B230734011	391	183	0.4680	7	0	0.0000
								B230704014	0	0	0.0000	0	0	0.0000
								B230704016	816	465	0.5699	465	25	0.0533
								B230294017	694	122	0.1758	110	5	0.0455
								B230244031	117	0	0.0000	0	0	0.0000
								B230274034	202	12	0.0594	0	0	0.0000
								B230164037	105	40	0.3810	40	0	0.0000
								B230154039	257	94	0.3658	10	1	0.1000
								B230164041	24	7	0.2917	0	0	0.0000
								B230154043	575	137	0.2383	0	0	0.0000
								B240165021	696	526	0.7557	479	89	0.1862
								B240165028	476	298	0.6261	297	5	0.0200
								B240165032	64	0	0.0000	0	0	0.0000
								B230284038	75	0	0.0000	0	0	0.0000
								B240175006	733	0	0.0000	0	0	0.0000
								B240175024	814	875	0.7064	410	50	0.1443
								B240025007	239	34	0.1423	0	0	0.0000
								B240025012	465	116	0.2495	80	2	0.0250
								B240025017	446	189	0.4238	104	29	0.2734
								B240275008	583	245	0.4202	200	11	0.0550
								B240275025	126	0	0.0000	0	0	0.0000
								B240275011	368	0	0.0000	0	0	0.0000
								B240275014	463	200	0.4320	200	14	0.0700
								B240275018	0	0	0.0000	0	0	0.0000
								B240435022	528	105	0.1989	95	3	0.0316
								B240435031	664	163	0.2455	65	9	0.1335
								B250216006	672	256	0.3810	130	0	0.0000
								B250216010	839	339	0.4041	274	3	0.0100
								B250216011	978	237	0.2420	200	9	0.0450
								B250216013	186	0	0.0000	0	0	0.0000
								B250216014	564	327	0.5798	170	0	0.0000
								B250216015	817	464	0.5679	125	1	0.0080
								B250216017	600	66	0.1100	60	10	0.1667
								B250216021	1085	394	0.3631	335	23	0.0687
								B250216024	248	0	0.0000	0	0	0.0000
								B250216025	555	130	0.2342	100	3	0.0300
								B250216026	572	56	0.0979	66	1	0.0179
								B250216028	751	323	0.4301	255	2	0.0078
								B250216030	186	48	0.2581	45	0	0.0000
								B250216032	714	440	0.6162	350	13	0.0371
								B250216034	343	11	0.0321	0	0	0.0000
								B250216035	470	0	0.0000	0	0	0.0000
								B250216036	527	0	0.0000	0	0	0.0000
								B250216037	381	3	0.0079	0	0	0.0000
								B250216038	364	0	0.0000	0	0	0.0000
								B250085007	457	227	0.4957	115	7	0.0600
								B250176008	569	85	0.1494	85	8	0.0941
								B250176012	534	154	0.2884	90	1	0.0111
								B250176023	280	0	0.0000	0	0	0.0000
								B250176027	378	287	0.7593	200	7	0.0350
								B250316039	350	88	0.2514	0	0	0.0000
								B250327017	768	388	0.5055	84	0	0.0000

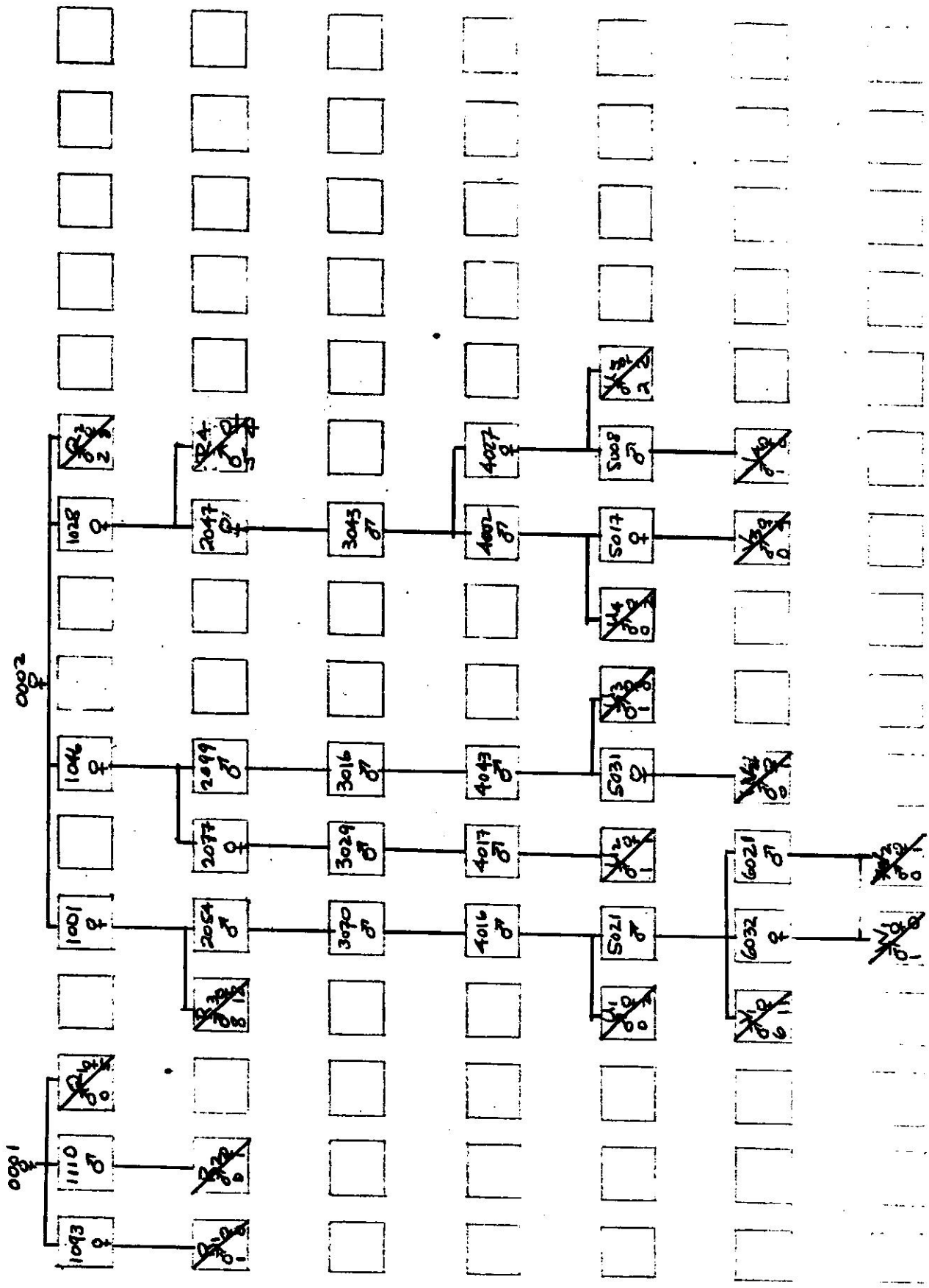


Figure 10: Lineage diagram of table 10

Table 11

Outbred Offspring from 6 Krad Female Mated to Male Normal (Code B2-0003)

TITLE	H	F	F/R	G	M	M/G	TITLE=B2 ANCESTOR IS	3						
R2 3 3	207	130	0.5280	128	45	0.3516	R221073031	882	306	0.3449	130	37	0.2945	
R2 31002	407	94	0.2310	91	54	0.5934	R220873021	550	336	0.6109	200	4	0.0200	
R2 31045	420	183	0.4357	150	51	0.3400	R220883033	734	247	0.3365	140	14	0.1070	
R2 31070	378	348	0.9206	300	62	0.2067	R220883034	315	156	0.4952	0	0	0.0000	
R2 31073	1029	498	0.4840	345	110	0.3188	R220883052	459	188	0.4096	51	1	0.0196	
R2 31077	815	595	0.7301	295	174	0.5898	R221203035	120	85	0.7083	85	1	0.0118	
R2 31080	496	0	0.0000	0	0	0.0000	R221203036	369	317	0.8455	0	0	0.0000	
R2 31081	60	0	0.0000	0	0	0.0000	R221213001	411	111	0.2701	105	1	0.0095	
R2 31101	65	0	0.0000	0	0	0.0000	R221213056	459	80	0.1743	80	20	0.2500	
R2 31102	627	92	0.1467	65	0	0.0000	R221213057	656	251	0.3826	95	14	0.1474	
R2 31119	315	195	0.6190	85	5	0.0706	R221213076	548	141	0.2573	0	0	0.0000	
R2 31122	619	590	0.9532	445	83	0.1865	R220303006	417	146	0.3501	35	7	0.2000	
R2 31123	180	105	0.5833	80	5	0.0625	R220303007	209	44	0.2105	18	9	0.4444	
R2 31134	446	46	0.1031	0	0	0.0000	R220303072	591	300	0.5076	170	46	0.2706	
R2 31161	468	18	0.0385	0	0	0.0000	R220230010	216	106	0.4907	95	17	0.1787	
R2 31162	635	110	0.1732	50	0	0.0000	R220423023	718	420	0.5850	220	75	0.3409	
R2 31175	58	0	0.0000	0	0	0.0000	R220423046	300	153	0.5100	0	0	0.0000	
R2 31189	566	66	0.1166	0	0	0.0000	R220423047	536	226	0.4216	125	2	0.0160	
R2 31213	651	301	0.4654	215	3	0.0140	R220873070	622	192	0.3087	55	21	0.3818	
R2 31217	90	5	0.0556	0	0	0.0000	R210022087	581	261	0.4492	60	12	0.2000	
R2 31234	243	105	0.4321	0	0	0.0000	R230104040	814	320	0.3938	520	11	0.0212	
R210022087	581	261	0.4492	60	13	0.2167	R230404004	48	3	0.0625	0	0	0.0000	
R21022088	477	377	0.7904	160	44	0.2750	R230404005	174	90	0.5172	90	0	0.0000	
R21022141	341	6	0.0176	0	0	0.0000	R230404006	521	206	0.3954	140	1	0.0071	
R210452073	394	111	0.2817	15	8	0.5333	R230404007	519	196	0.3776	110	24	0.2182	
R210452105	516	80	0.1550	0	0	0.0000	R230404013	161	28	0.1739	0	0	0.0000	
R21045 274	9	0	0.0000	0	0	0.0000	R230404018	423	137	0.3239	137	3	0.0219	
R210452106	518	40	0.0772	0	0	0.0000	R230404029	541	148	0.2734	148	7	0.0473	
R210452107	614	271	0.4414	95	11	0.1158	R230514019	307	0	0.0000	0	0	0.0000	
R210452108	461	50	0.1085	0	0	0.0000	R230514020	0	0	0.0000	0	0	0.0000	
R210452111	444	24	0.0541	0	0	0.0000	R230274024	297	150	0.5051	150	4	0.0267	
R210452120	513	270	0.5263	105	76	0.7238	R230724028	694	320	0.4611	320	16	0.0500	
R210452143	580	163	0.2810	0	0	0.0000	R230404008	695	387	0.5568	240	0	0.0000	
R210732014	386	180	0.4663	70	9	0.1286	R230204015	70	20	0.2857	15	0	0.0000	
R210772069	104	3	0.0288	0	0	0.0000	R230204046	0	0	0.0000	0	0	0.0000	
R210772075	376	222	0.5904	150	6	0.0400	R240405028	646	500	0.7740	500	19	0.3380	
R210772080	476	278	0.5870	20	11	0.5500	R240405030	295	0	0.0000	0	0	0.0000	
R210772082	214	31	0.1440	0	0	0.0000	R240075003	43	33	0.7674	0	0	0.0000	
R210772118	261	31	0.1188	0	0	0.0000	R240075010	220	99	0.4500	85	16	0.1882	
R210772119	284	40	0.1408	30	1	0.0333	R240285004	725	141	0.1945	135	2	0.0148	
R210772135	179	130	0.7263	0	0	0.0000	R240285005	672	107	0.1592	80	1	0.0125	
R210022006	295	130	0.4407	125	48	0.3840	R240285013	456	16	0.0351	0	0	0.0000	
R210022007	274	16	0.0584	0	0	0.0000	R240285016	868	480	0.5530	325	67	0.2062	
R210022012	535	134	0.2505	115	0	0.0000	R240285019	536	41	0.0765	25	1	0.0400	
R210022013	206	64	0.3107	45	0	0.0000	R240065009	646	348	0.5387	200	6	0.0300	
R210022015	160	7	0.0437	0	0	0.0000	R240185015	132	2	0.0152	0	0	0.0000	
R210022024	524	386	0.7366	319	4	0.0125	R240245027	996	731	0.7339	520	5	0.0095	
R210022025	155	67	0.4323	35	13	0.3714	R240245029	602	462	0.7674	390	8	0.2005	
R210022026	176	71	0.4034	71	4	0.0563	R230046022	433	0	0.0000	0	0	0.0000	
R210022028	64	0	0.0000	0	0	0.0000	R250046016	807	25	0.0310	25	1	0.0400	
R210022029	393	261	0.6641	180	31	0.1722	R250106001	851	455	0.5347	360	54	0.1500	
R210022030	636	502	0.7893	220	31	0.1409	R250106002	837	601	0.7180	270	18	0.0667	
R210022031	370	100	0.2703	50	4	0.0800	R250106003	733	460	0.6276	460	3	0.0065	
R210022038	12	0	0.0000	0	0	0.0000	R250106005	78	0	0.0000	0	0	0.0000	
R210022040	482	337	0.6992	214	17	0.0794	R250166004	765	250	0.3268	95	6	0.0632	
R210022041	193	0	0.0000	0	0	0.0000	R250166009	854	349	0.4087	230	37	0.1609	
R210022042	699	432	0.6170	303	2	0.0065	R250166018	735	388	0.5279	300	11	0.0367	
R210022043	153	84	0.5490	0	0	0.0000	R250166019	916	519	0.5666	365	8	0.0219	
R210022044	263	65	0.2471	0	0	0.0000	R250166020	516	115	0.2229	115	10	0.0870	
R210022045	484	325	0.6715	155	5	0.0323	R250046022	433	0	0.0000	0	0	0.0000	
R210022047	553	310	0.5606	110	29	0.2635	R260017001	328	110	0.3354	75	0	0.0000	
R210022049	301	6	0.0199	0	0	0.0000	R260017005	222	0	0.0000	0	0	0.0000	
R210022102	319	131	0.4107	0	0	0.0000	R260017010	948	441	0.4652	390	0	0.0000	
R210022130	257	29	0.1128	0	0	0.0000	R260187002	813	319	0.3924	130	2	0.0154	
R210022131	257	149	0.5798	25	5	0.2000	R260187004	615	165	0.2683	114	0	0.0000	
R210732002	466	45	0.0966	0	0	0.0000	R260187013	766	102	0.1332	102	0	0.0000	
R210452121	441	176	0.3991	176	150	0.8573	R260047003	721	85	0.1179	85	0	0.0000	
R220063077	0	0	0.0000	0	0	0.0000	R260027006	79	0	0.0000	0	0	0.0000	
R220063078	355	24	0.0674	0	0	0.0000	R260027008	117	31	0.2650	0	0	0.0000	
R220063079	457	230	0.5033	230	64	0.2783	R260027009	581	342	0.5875	340	0	0.0000	
R220293049	67	9	0.1343	0	0	0.0000	R260027011	529	255	0.4820	255	0	0.0000	
R220293051	303	148	0.4884	148	0	0.0000	R260027012	44	0	0.0000	0	0	0.0000	
R220073065	651	178	0.2735	128	5	0.0391	R260007007	938	96	0.1023	70	0	0.0000	
R220243066	239	131	0.5504	0	0	0.0000	R260007015	125	5	0.0400	0	0	0.0000	
R220243069	136	22	0.1618	0	0	0.0000	R260007016	190	0	0.0000	0	0	0.0000	
R220243081	0	0	0.0000	0	0	0.0000								

Table 12

Outbred Offspring from 6 K_{rad} Female Mated to Male Normal (Code B2-0004)

TITLE	B	F	F/B	G	M	M/G
R2 4 4	569	159	0.2794	147	32	0.2177
R2 41003	604	1	0.0017	0	0	0.0000
R2 41014	18	6	0.3333	0	0	0.0000
R2 41017	298	2	0.0067	0	0	0.0000
R2 41038	389	143	0.3676	105	2	0.0190
R2 41043	790	465	0.5886	300	121	0.4033
R2 41044	776	122	0.1572	20	11	0.5500
R2 41062	950	414	0.4358	245	68	0.2776
R2 41072	410	275	0.6707	190	75	0.3947
R2 41083	526	206	0.3916	61	33	0.5410
R2 41116	495	290	0.5859	40	17	0.4250
R2 41117	660	265	0.4015	235	44	0.1872
R2 41142	76	0	0.0000	0	0	0.0000
R2 41158	370	250	0.6757	75	1	0.0133
R2 41163	490	440	0.8980	310	21	0.0677
R2 41185	382	50	0.1309	0	0	0.0000
R2 41199	170	0	0.0000	0	0	0.0000
B211632161	648	355	0.5478	130	27	0.2077
R210432085	72	0	0.0000	0	0	0.0000
R210432140	306	155	0.5065	0	0	0.0000
R210432145	618	35	0.0566	0	0	0.0000
B210442094	529	292	0.5520	110	18	0.1636
R210442095	301	40	0.1329	0	0	0.0000
R210622059	216	52	0.2407	45	1	0.0222
R210622064	385	73	0.1896	0	0	0.0000
R210622084	180	86	0.4778	0	0	0.0000
R210622103	295	59	0.2000	0	0	0.0000
R210622112	402	125	0.3109	0	0	0.0000
R210622114	248	41	0.1653	0	0	0.0000
B210622136	314	98	0.3121	0	0	0.0000
R210622139	186	13	0.0699	0	0	0.0000
R210622141	341	6	0.0176	0	0	0.0000
R210622146	146	2	0.0137	0	0	0.0000
R220943022	761	551	0.7240	103	24	0.2330
R220943039	686	287	0.4184	220	60	0.2727
R230224003	188	61	0.3245	0	0	0.0000

Table 13

Outbred Offspring from 12 K_{rad} Female Mated to Male Normal (Code B3-0001,2,3)

		TITLE=B3 ANCESTOR IS 1					
TITLE	R	F	F/R	G	M	M/G	
B3 1 1	401	46	0.1147	43	3	0.0698	
B3 11006	781	66	0.0845	0	0	0.0000	
B3 11019	183	9	0.0492	0	0	0.0000	
B3 11071	330	80	0.2424	0	0	0.0000	

		TITLE=B3 ANCESTOR IS 2					
TITLE	R	F	F/R	G	M	M/G	
B3 2 2	537	27	0.0503	15	4	0.2667	
B3 21027	678	227	0.3348	160	26	0.1625	
B3 21043	328	103	0.3140	0	0	0.0000	

		TITLE=B3 ANCESTOR IS 3					
TITLE	R	F	F/R	G	M	M/G	
B3 3 3	500	149	0.2980	104	5	0.0577	
B3 31028	314	224	0.7134	190	44	0.2316	
B3 31031	121	58	0.4793	25	1	0.0400	
B3 31039	65	0	0.0000	0	0	0.0000	
B3 31042	60	0	0.0000	0	0	0.0000	
B3 31044	585	250	0.4274	0	0	0.0000	
B3 310282055	167	50	0.2994	0	0	0.0000	

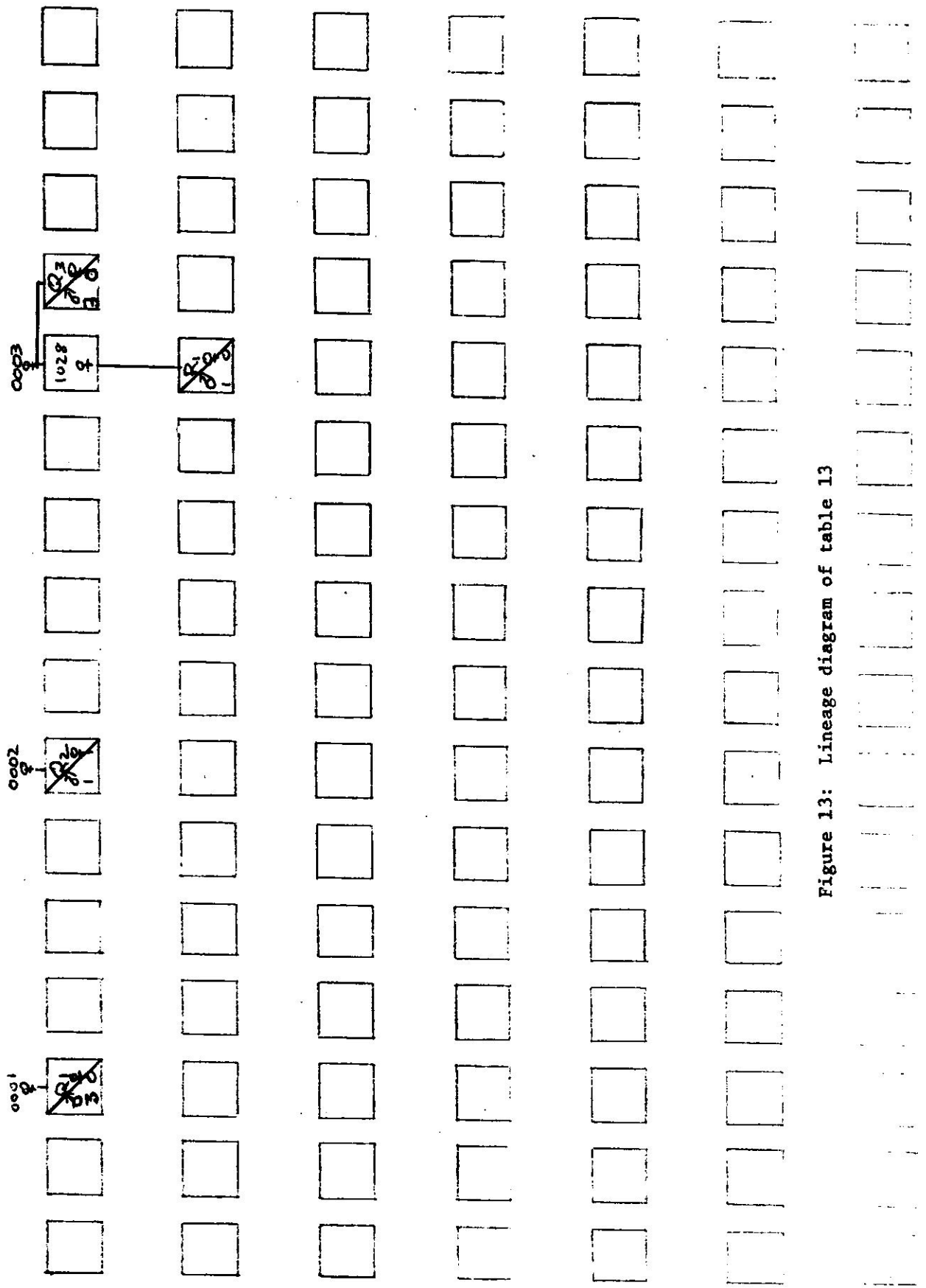


Figure 13: Lineage diagram of table 13

Table 14

Outbred Offspring from 14 K_{rad} Female Mated to Male Normal (Code B4-0001,2,3)

	D	F	F/B	G	M	M/G
B400010001	369	0	0	0	0	0
B400020002	176	0	0	0	0	0
B400030003	0	0	0	0	0	0

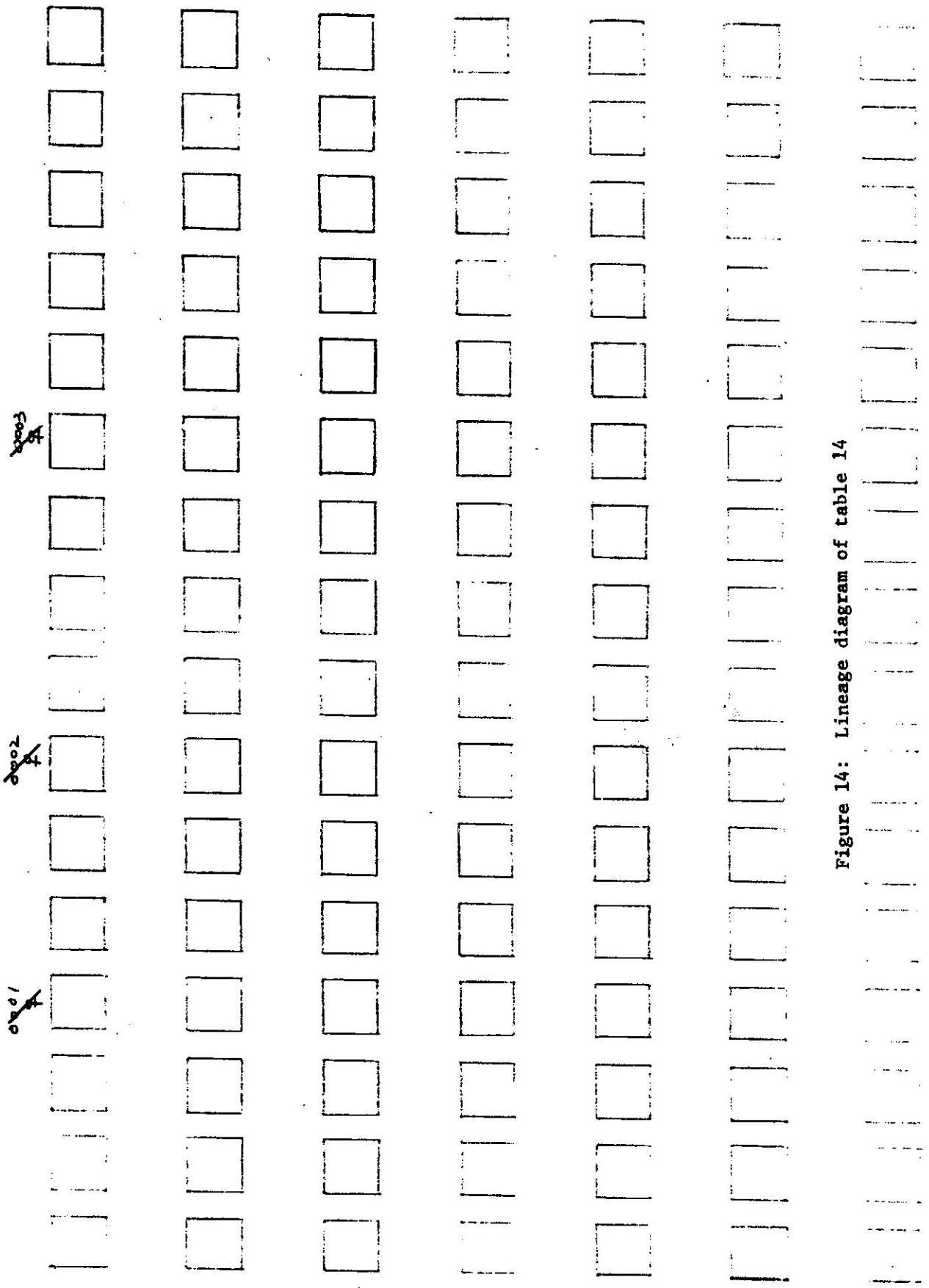


Figure 14: Lineage diagram of table 14

Table 15

Outbred Offspring from 1 K_{rad} Female Mated to Male Normal (Code D1-0002,3,4)

TITLE		B	F	F/B	G	M	M/G
D1	2 2	262	185	0.7061	0	0	0.0000

TITLE		B	F	F/B	G	M	M/G
D1	3 3	391	195	0.4987	0	0	0.0000

TITLE		B	F	F/B	G	M	M/G
D1	4 4	202	0	0.0000	0	0	0.0000

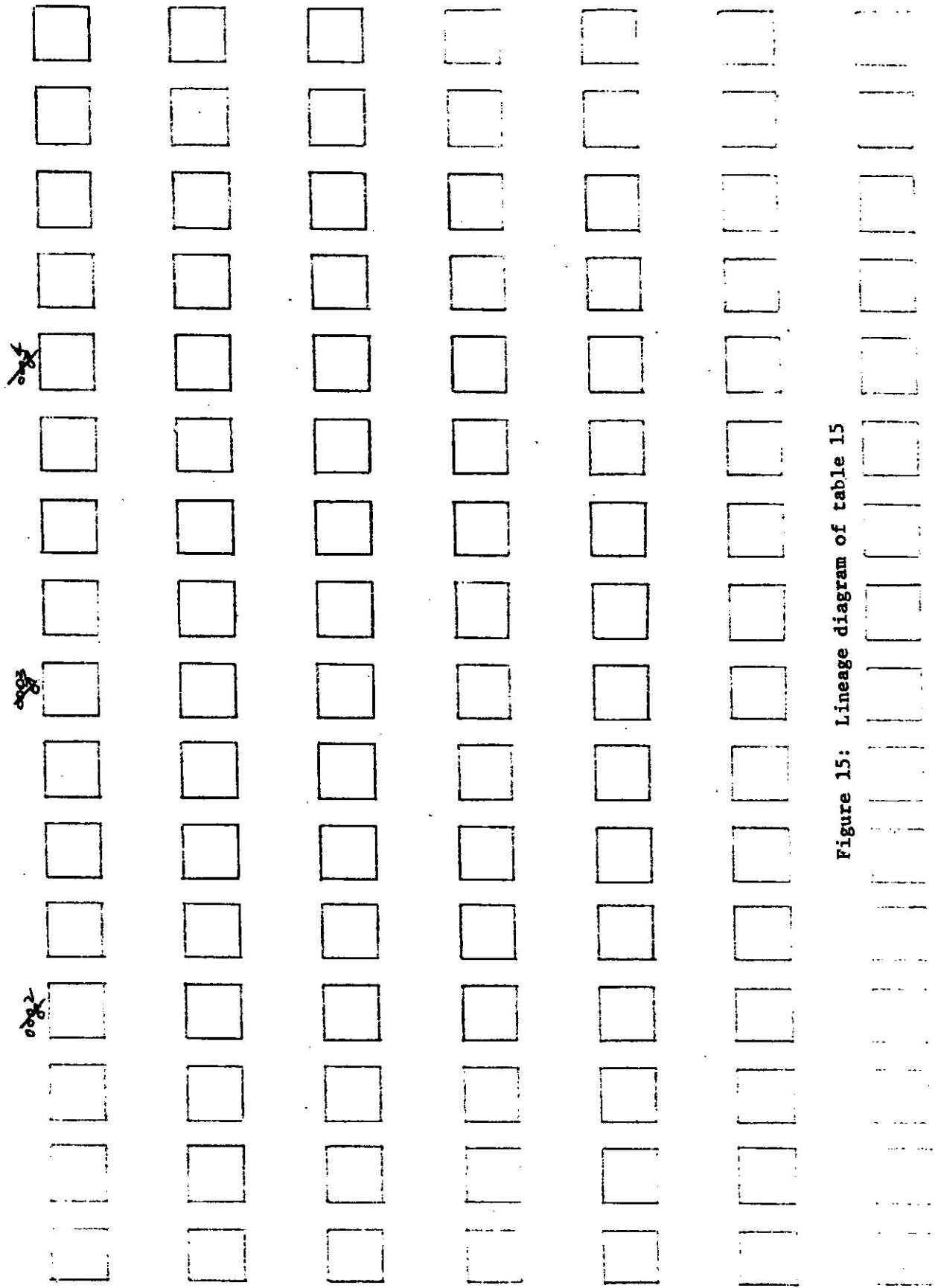


Figure 15: Lineage diagram of table 15

Table 16

Outbred Offspring from 2 K_{rad} Male Mated to Female Normal (Code D2-0002)

	TITLE	B	F	F/R	G	M	M/G
D2	2	266	147	0.5526	147	19	0.1293
D2	21003	175	0	0.0000	0	0	0.0000
D2	21034	672	252	0.3750	252	1	0.0040
D2	21052	344	246	0.7151	235	0	0.0000
D2	21057	60	40	0.6667	40	0	0.0000
D2	21062	282	175	0.6206	130	0	0.0000
D2	21067	418	276	0.6603	264	0	0.0000
D2	21079	123	101	0.8211	95	0	0.0000
D2	210032044	355	8	0.0225	8	0	0.0000
D2	210342023	411	158	0.3844	158	0	0.0000
D2	210622039	322	185	0.5745	0	0	0.0000

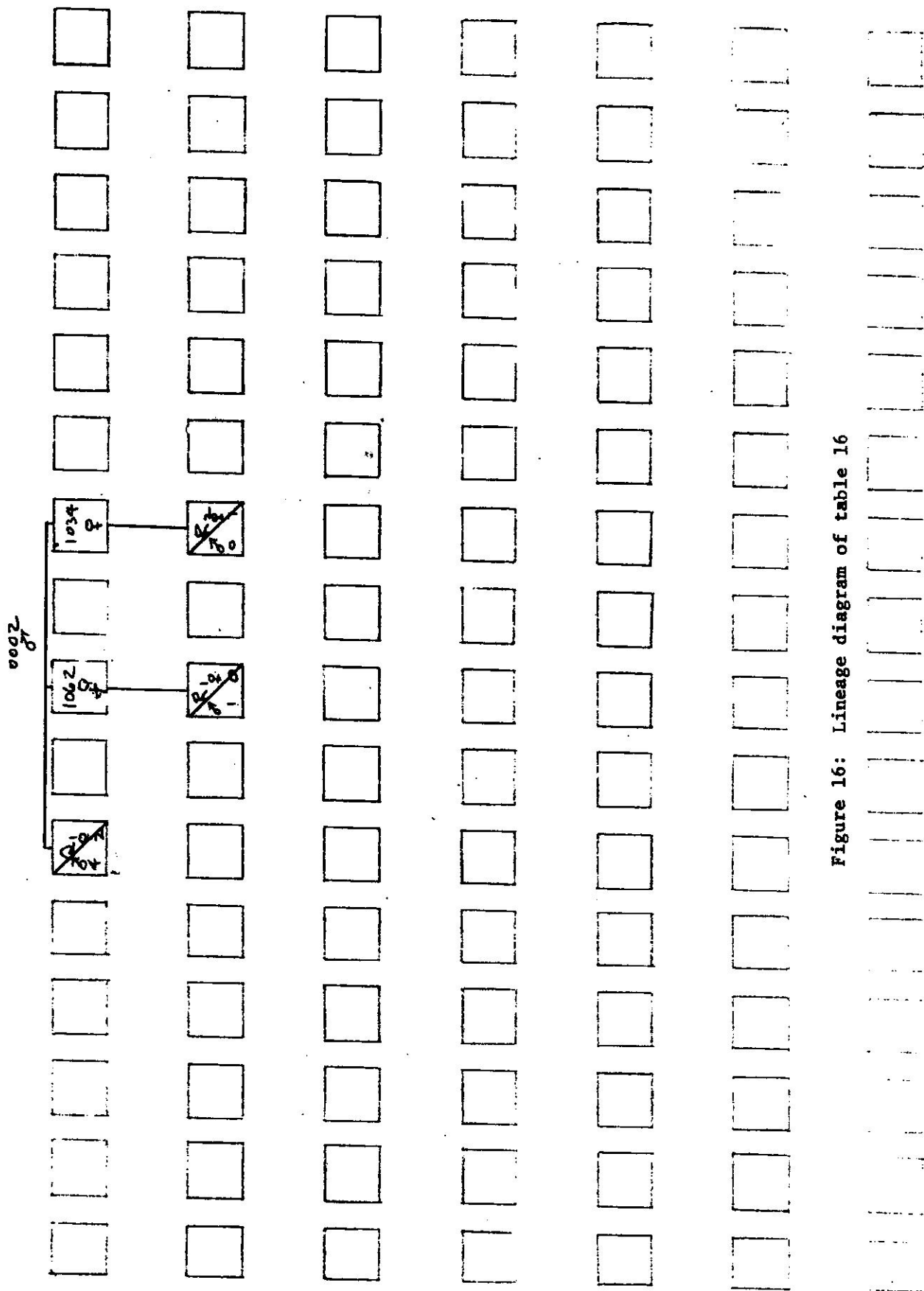


Figure 16: Lineage diagram of table 16

Table 17

Outbred Offspring from 4 Krad Male Mated to Female Normal (Code D3-0001,2)

TITLE	TITLE=03 ANCESTOR IS 1				TITLE=03 ANCESTOR IS 2			
	R	F	F/R	M/G	R	F	F/R	M/G
03 11178	525	395	0.5810	0.1574	90	92	0.9111	0.0000
03 11179	750	0	0.0000	0.0000	465	317	0.6817	0.0000
03 11180	191	29	0.1518	0.0000	227	1	0.0044	0.0000
03 11181	281	201	0.7153	0.0721	139	0	0.0000	0.0000
03 11182	347	117	0.3372	0.0400	206	168	0.8155	0.0000
03 11183	48	0	0.0000	0.0000	283	209	0.7385	0.0000
03 11184	548	520	0.9489	0.0201	395	210	0.5316	0.0000
03 11185	678	271	0.3997	0.0443	362	176	0.4862	0.0000
03 11186	392	92	0.2347	0.0278	216	88	0.4074	0.0000
03 11187	447	291	0.6510	0.0069	593	186	0.3137	0.0000
03 11188	316	81	0.2563	0.0000	413	46	0.1114	0.0000
03 11189	352	55	0.1563	0.0000	346	0	0.0000	0.0000
03 11190	418	262	0.6268	0.0000	341	77	0.2258	0.0000
03 11191	365	199	0.5452	0.0052	309	111	0.3592	0.0000
03 11192	339	250	0.7375	0.0040	64	0	0.0000	0.0000
03 11193	418	289	0.6914	0.0000	399	124	0.3108	0.0000
03 11194	355	84	0.2366	0.0000	476	128	0.2689	0.0000
03 11195	366	211	0.5765	0.0071	301	101	0.3355	0.0000
03 11196	180	25	0.1389	0.0000	670	119	0.1776	0.0000
03 11197	421	106	0.2518	0.0000	427	100	0.2342	0.0000
03 11198	303	57	0.1450	0.0000	435	120	0.2759	0.0000
03 11199	90	82	0.9111	0.0000	188	97	0.5160	0.0000
03 11200	465	317	0.6817	0.0000	80	80	1.0000	0.0000
03 11201	281	201	0.7153	0.0721	0	0	0.0000	0.0000
03 11202	347	117	0.3372	0.0400	0	0	0.0000	0.0000
03 11203	548	520	0.9489	0.0201	0	0	0.0000	0.0000
03 11204	678	271	0.3997	0.0443	0	0	0.0000	0.0000
03 11205	447	291	0.6510	0.0069	0	0	0.0000	0.0000
03 11206	316	81	0.2563	0.0000	0	0	0.0000	0.0000
03 11207	352	55	0.1563	0.0000	0	0	0.0000	0.0000
03 11208	418	262	0.6268	0.0000	0	0	0.0000	0.0000
03 11209	365	199	0.5452	0.0052	0	0	0.0000	0.0000
03 11210	339	250	0.7375	0.0040	0	0	0.0000	0.0000
03 11211	418	289	0.6914	0.0000	0	0	0.0000	0.0000
03 11212	355	84	0.2366	0.0000	0	0	0.0000	0.0000
03 11213	366	211	0.5765	0.0071	0	0	0.0000	0.0000
03 11214	180	25	0.1389	0.0000	0	0	0.0000	0.0000
03 11215	421	106	0.2518	0.0000	0	0	0.0000	0.0000
03 11216	303	57	0.1450	0.0000	0	0	0.0000	0.0000
03 11217	90	82	0.9111	0.0000	0	0	0.0000	0.0000
03 11218	465	317	0.6817	0.0000	0	0	0.0000	0.0000
03 11219	281	201	0.7153	0.0721	0	0	0.0000	0.0000
03 11220	347	117	0.3372	0.0400	0	0	0.0000	0.0000
03 11221	548	520	0.9489	0.0201	0	0	0.0000	0.0000
03 11222	678	271	0.3997	0.0443	0	0	0.0000	0.0000
03 11223	447	291	0.6510	0.0069	0	0	0.0000	0.0000
03 11224	316	81	0.2563	0.0000	0	0	0.0000	0.0000
03 11225	352	55	0.1563	0.0000	0	0	0.0000	0.0000
03 11226	418	262	0.6268	0.0000	0	0	0.0000	0.0000
03 11227	365	199	0.5452	0.0052	0	0	0.0000	0.0000
03 11228	339	250	0.7375	0.0040	0	0	0.0000	0.0000
03 11229	418	289	0.6914	0.0000	0	0	0.0000	0.0000
03 11230	355	84	0.2366	0.0000	0	0	0.0000	0.0000
03 11231	366	211	0.5765	0.0071	0	0	0.0000	0.0000
03 11232	180	25	0.1389	0.0000	0	0	0.0000	0.0000
03 11233	421	106	0.2518	0.0000	0	0	0.0000	0.0000
03 11234	303	57	0.1450	0.0000	0	0	0.0000	0.0000
03 11235	90	82	0.9111	0.0000	0	0	0.0000	0.0000
03 11236	465	317	0.6817	0.0000	0	0	0.0000	0.0000
03 11237	281	201	0.7153	0.0721	0	0	0.0000	0.0000
03 11238	347	117	0.3372	0.0400	0	0	0.0000	0.0000
03 11239	548	520	0.9489	0.0201	0	0	0.0000	0.0000
03 11240	678	271	0.3997	0.0443	0	0	0.0000	0.0000
03 11241	447	291	0.6510	0.0069	0	0	0.0000	0.0000
03 11242	316	81	0.2563	0.0000	0	0	0.0000	0.0000
03 11243	352	55	0.1563	0.0000	0	0	0.0000	0.0000
03 11244	418	262	0.6268	0.0000	0	0	0.0000	0.0000
03 11245	365	199	0.5452	0.0052	0	0	0.0000	0.0000
03 11246	339	250	0.7375	0.0040	0	0	0.0000	0.0000
03 11247	418	289	0.6914	0.0000	0	0	0.0000	0.0000
03 11248	355	84	0.2366	0.0000	0	0	0.0000	0.0000
03 11249	366	211	0.5765	0.0071	0	0	0.0000	0.0000
03 11250	180	25	0.1389	0.0000	0	0	0.0000	0.0000
03 11251	421	106	0.2518	0.0000	0	0	0.0000	0.0000
03 11252	303	57	0.1450	0.0000	0	0	0.0000	0.0000

Table 18

Outbred Offspring from 5 K_{rad} Male Mated to Female Normal (Code D4-0001)

TITLE			TITLE=D4 ANCESTOR IS				I	
		B	F	F/R	G	M	M/G	
D4	1	1	540	28	0.0519	28	2	0.0714
D4	11174		472	387	0.8199	50	1	0.0200
D4	11175		124	68	0.3505	0	0	0.0000
D4	11742549		506	436	0.8617	436	0	0.0000

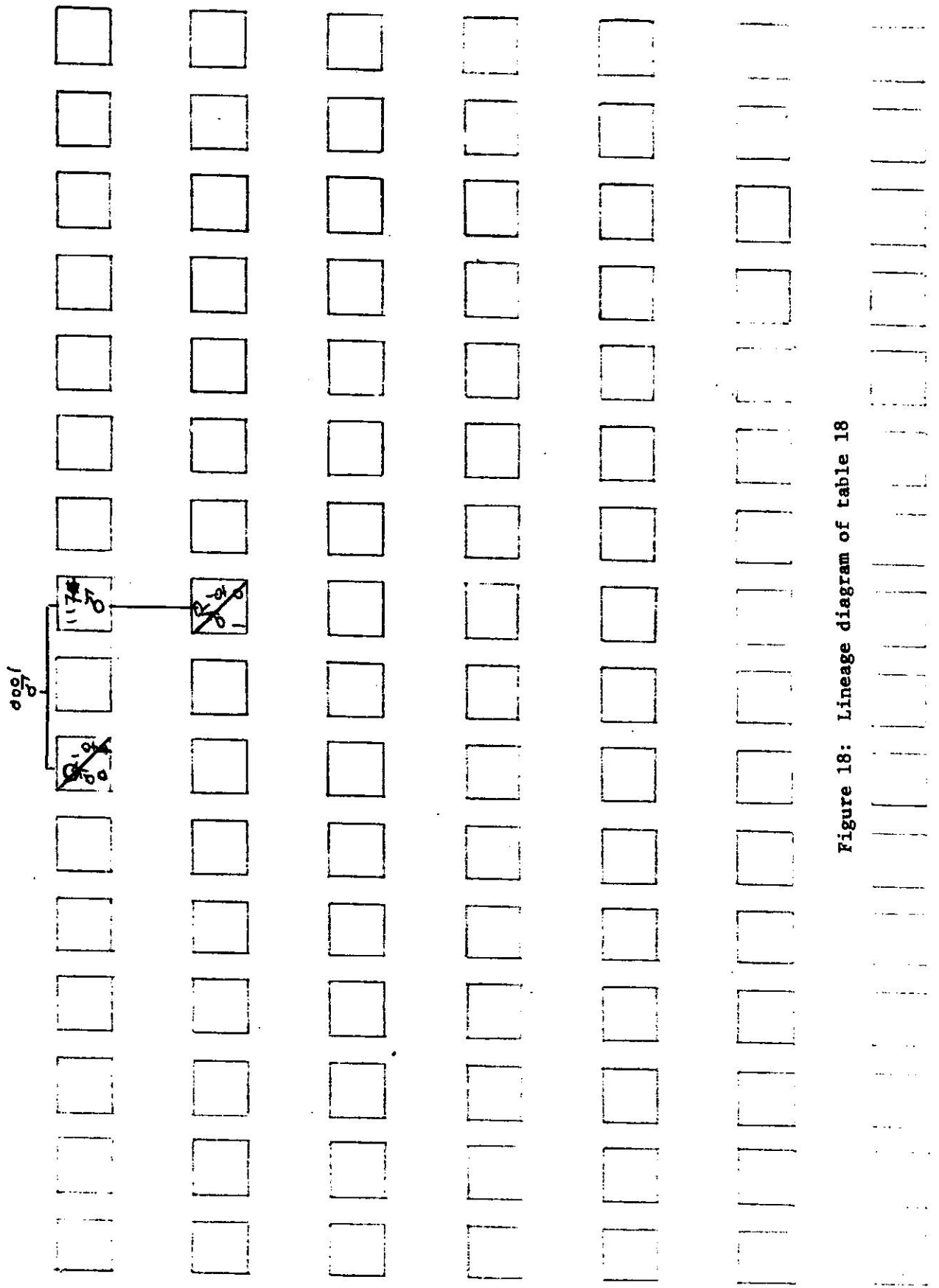


Figure 18: Lineage diagram of table 18

Table 19

Outbred Offspring from 1 K_{rad} Female Mated to Male Normal (Code E1-0002,4)

TITLE			B	F	TITLE=E1 ANCESTOR IS			2
				F/R	G	M	M/G	
E1	2	2	234	99	0.4231	57	0	0.0000

TITLE			B	F	TITLE=E1 ANCESTOR IS			4
				F/R	G	M	M/G	
E1	4	4	209	0	0.0000	0	0	0.0000

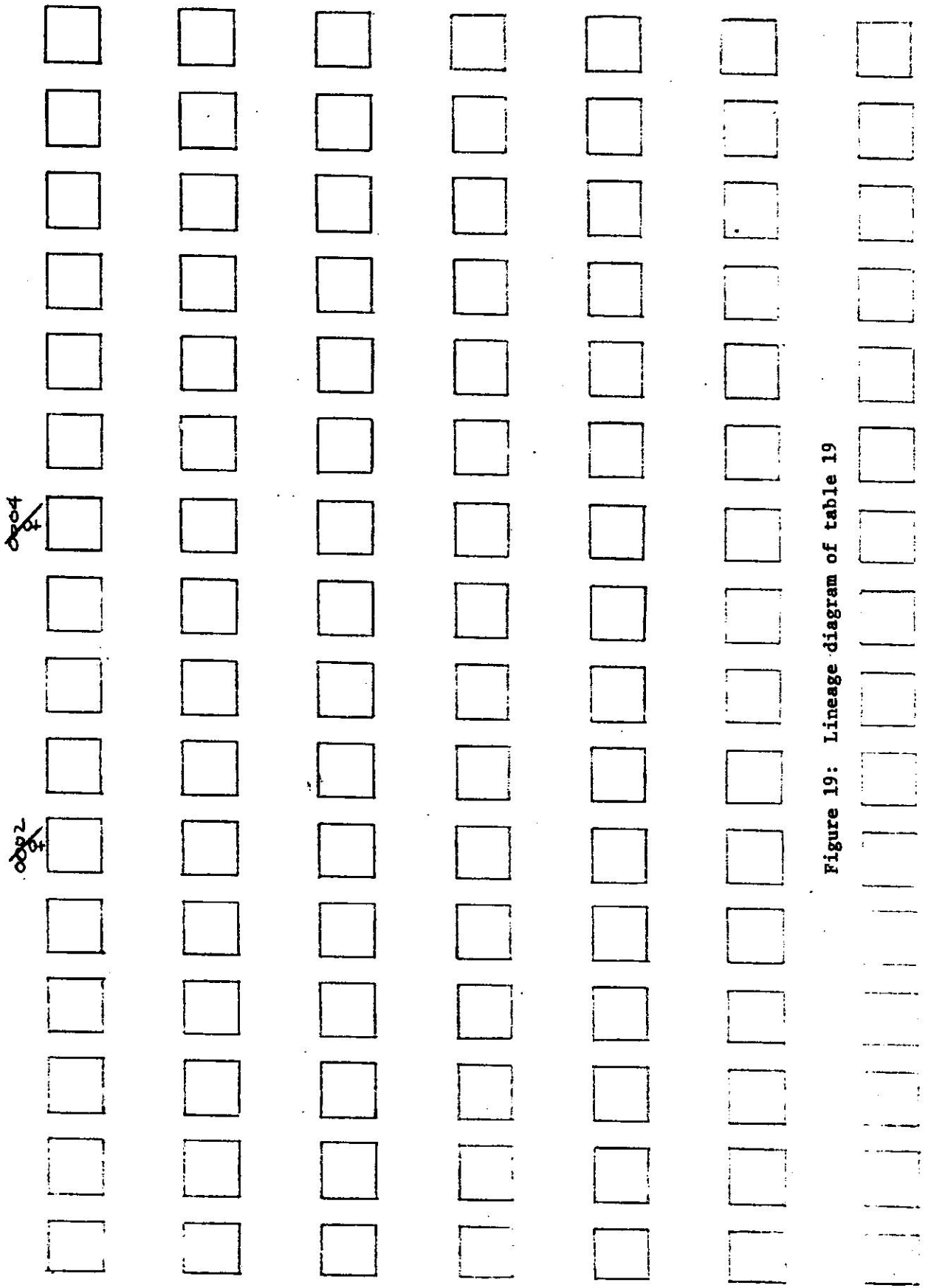


Figure 19: Lineage diagram of table 19

Table 20

Outbred Offspring from 2 K_{rad} Female Mated to Male Normal (Code E2-0002)

TITLE	R	F	TITLE=F2 ANCESTOR IS 2			
			F/R	G	M	M/G
E2 2 2	350	208	0.5943	208	16	0.0759
F2 21100	94	84	0.8936	30	1	0.0333
E2 21114	540	428	0.7926	174	31	0.1782
F2 21129	80	0	0.0000	0	0	0.0000
F2 21130	345	60	0.1739	50	0	0.0000
E2 21151	332	127	0.3825	123	0	0.0000
F2 21163	268	222	0.8284	208	0	0.0000
F2 21164	140	0	0.0000	0	0	0.0000
E2 21171	186	97	0.5215	93	0	0.0000
F2 21172	429	321	0.7483	321	0	0.0000
E211632561	235	102	0.4340	0	0	0.0000

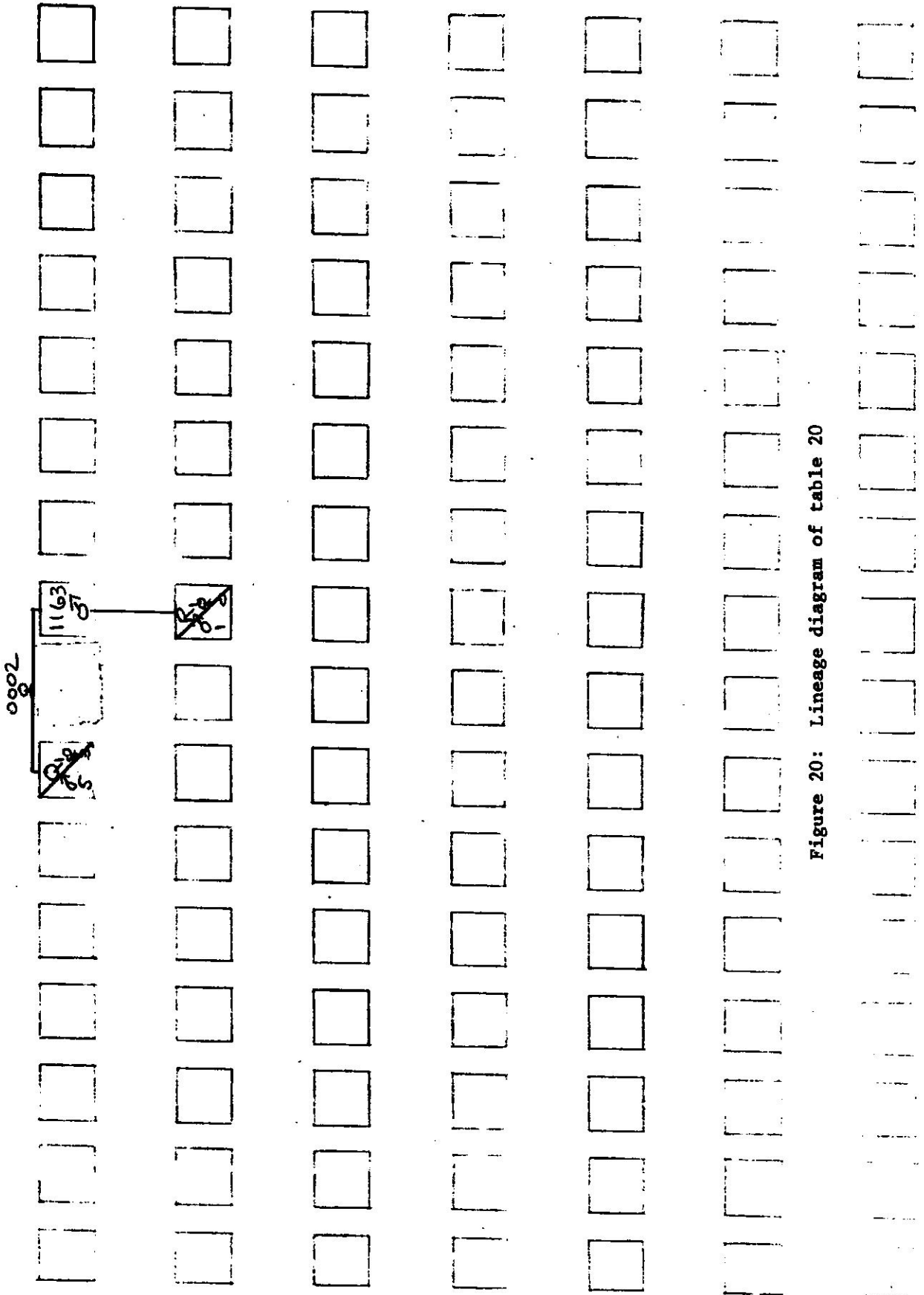


Figure 20: Lineage diagram of table 20

Table 21

Outbred Offspring from 4 K_{rad} Female Mated to Male Normal (Code E3-0002)

TITLE	B	F	TITLE=F3 ANCESTOR IS			
			F/R	G	M	M/G
E3 7 2	535	33	0.0617	33	3	0.0909
E3 21119	620	450	0.7258	421	9	0.0214
F3 21127	413	266	0.6441	231	2	0.0087
E311192515	106	66	0.6226	55	0	0.0000
E311192517	297	116	0.3906	0	0	0.0000
E311192519	281	0	0.0000	0	0	0.0000
E311192534	358	252	0.7039	120	0	0.0000
E311192537	129	103	0.7984	71	0	0.0000
E311192544	210	106	0.5048	34	0	0.0000
E311192548	471	302	0.6412	125	0	0.0000
E311192571	49	49	1.0000	0	0	0.0000

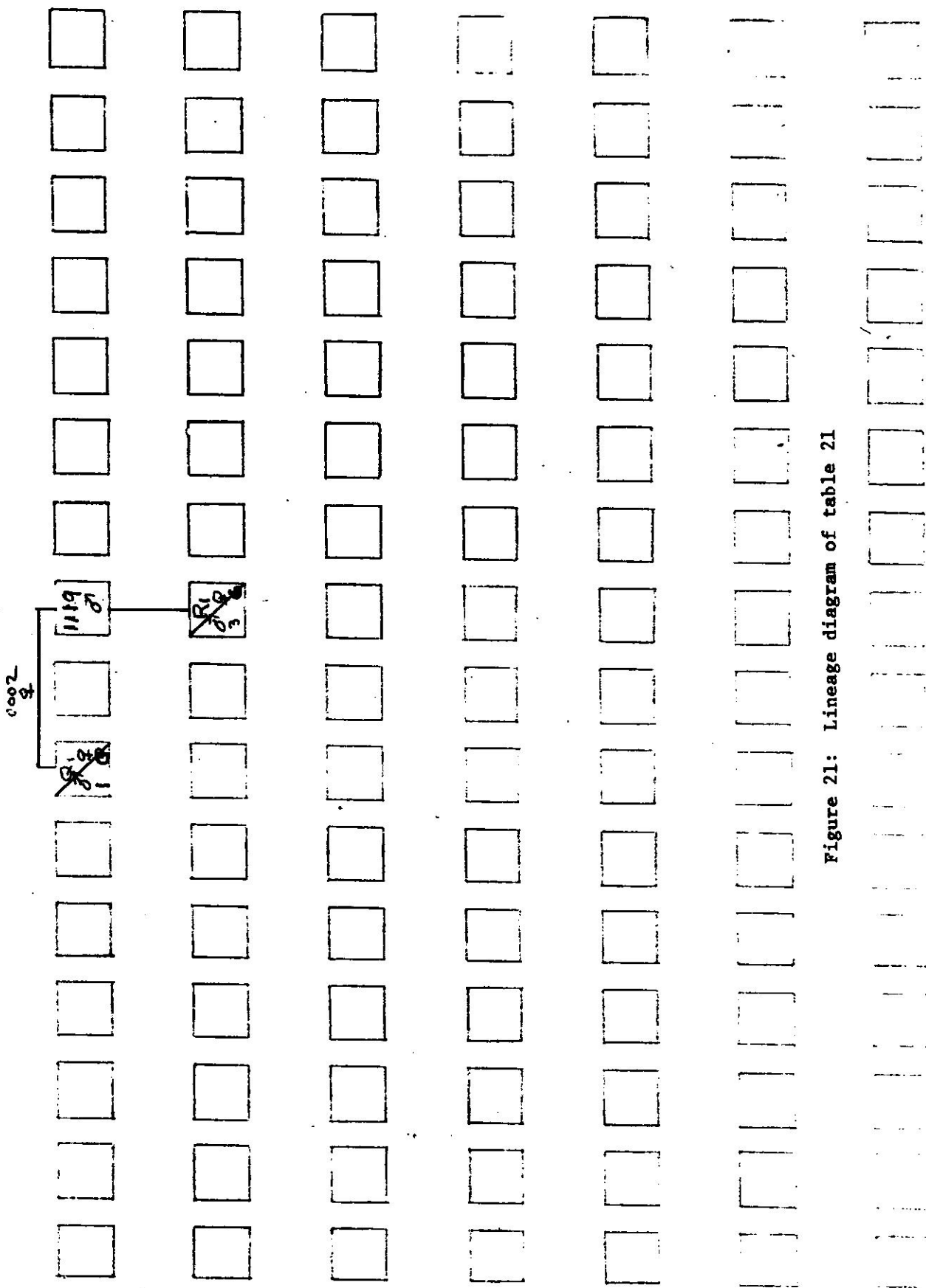


Figure 21: Lineage diagram of table 21

Table 22

Outbred Offspring from 5 K_{rad} Female Mated to Male Normal (Code E4-0001,2)

TITLE			R	F	TITLE=E4 ANCESTOR IS		1	
					F/R	G		M
F4	1	1	442	40	0.0905	40	3	0.0750
E4	11125		486	342	0.7058	203	11	0.0542
F411252505			390	142	0.3641	0	0	0.0000
F411252507			338	79	0.2337	20	0	0.0000
F411252509			91	54	0.5934	50	0	0.0000

TITLE			R	F	TITLE=F4 ANCESTOR IS		2	
					F/R	G		M
E4	2	2	234	27	0.1154	27	2	0.0741

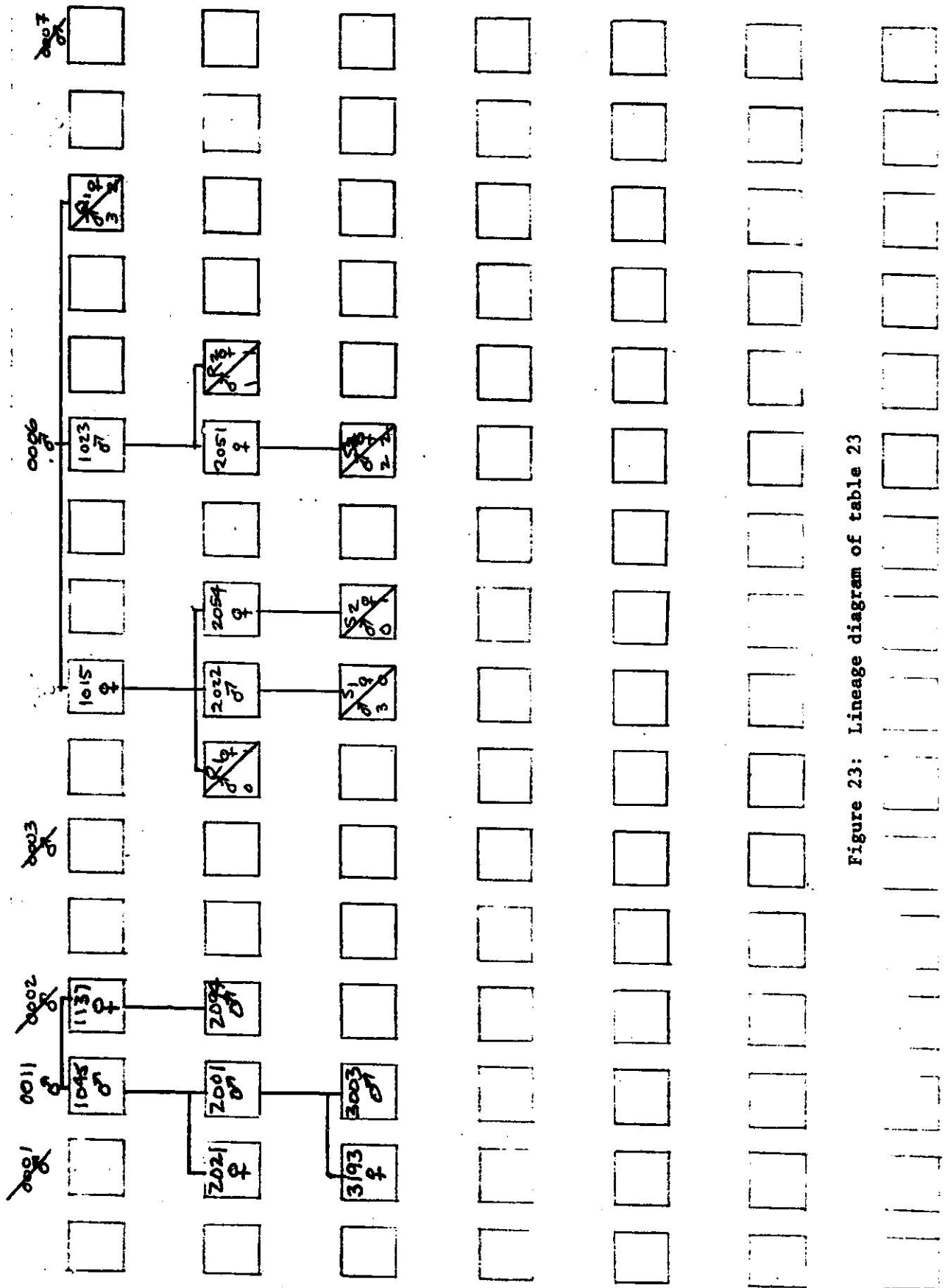


Figure 23: Lineage diagram of table 23

Table 24

Outbred Offspring from 2 K_{rad} Female Mated to Male Normal

(Code G1-0001,4,5,7,9,11,13,15,16,17)

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 1	43	279	0.1541	43	1	0.0233	50	229	0.2193	30	0	0.0000
G1 11017	64	211	0.3033	40	1	0.0250						
G1 11047	64	211	0.3033	0	0	0.0000						
G110472029	235	413	0.5690	235	12	0.0511						
G120293008	247	247	0.0000	0	0	0.0000						
G120293010	278	394	0.7056	0	0	0.0000						
G120293023	190	190	0.1053	12	1	0.0833						
G120293024	227	227	0.3216	73	2	0.0274						
G120293025	407	407	0.3391	0	0	0.0000						
G120293026	303	303	0.0000	0	0	0.0000						
G120293028	80	350	0.2286	0	0	0.0000						
G120293029	266	266	0.2669	0	0	0.0000						
G120293098	256	256	0.0625	0	0	0.0000						
G120293099	242	242	0.1116	0	0	0.0000						
G120293087	220	220	0.2955	65	0	0.0000						
G130234025	179	179	0.5754	102	3	0.0294						
G140255023	216	216	0.0556	12	0	0.0000						
G140255025	84	325	0.1600	84	0	0.0000						
G140255029	363	363	0.1102	40	0	0.0000						

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 4	76	365	0.2082	76	1	0.0132						
G1 41005	157	292	0.5567	157	0	0.0000						

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 5	39	482	0.0809	39	4	0.1025						
G1 51002	110	277	0.3971	110	10	0.0909						
G1 51006	0	134	0.0000	0	0	0.0000						
G1 51011	0	0	0.0000	0	0	0.0000						
G1 51036	63	220	0.2864	60	0	0.0000						
G110022000	309	407	0.7592	0	0	0.0000						
G110022004	24	226	0.1062	24	1	0.0417						
G110022012	255	394	0.6641	0	0	0.0000						
G110022013	25	405	0.0617	0	0	0.0000						
G110022015	0	126	0.0000	0	0	0.0000						
G110022019	48	211	0.2275	0	0	0.0000						
G110022024	93	392	0.2435	93	2	0.0215						
G110022035	185	462	0.4004	185	2	0.0198						
G110022038	24	152	0.1342	0	0	0.0000						
G120022063	182	437	0.4145	0	0	0.0000						
G120042022	96	359	0.2692	96	0	0.0000						
G120240054	25	246	0.0977	25	0	0.0000						
G120243126	0	121	0.0000	0	0	0.0000						
G120250055	24	224	0.1171	24	0	0.0000						
G120353073	101	301	0.3355	101	0	0.0000						

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 13	58	237	0.2447	58	1	0.0172						
G1 131021	150	373	0.4021	150	2	0.0133						
G110212011	74	91	0.8132	74	0	0.0000						
G110212042	0	372	0.0000	0	0	0.0000						

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 15	31	124	0.2500	31	0	0.0000						

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 16	25	284	0.0890	25	0	0.0000						

TITLE	TITLE=G1			ANCESTOR IS			TITLE=G1			ANCESTOR IS		
	F	R	M/G	F	R	M/G	F	R	M/G	F	R	M/G
G1 17	0	1	0.0000	0	0	0.0000						

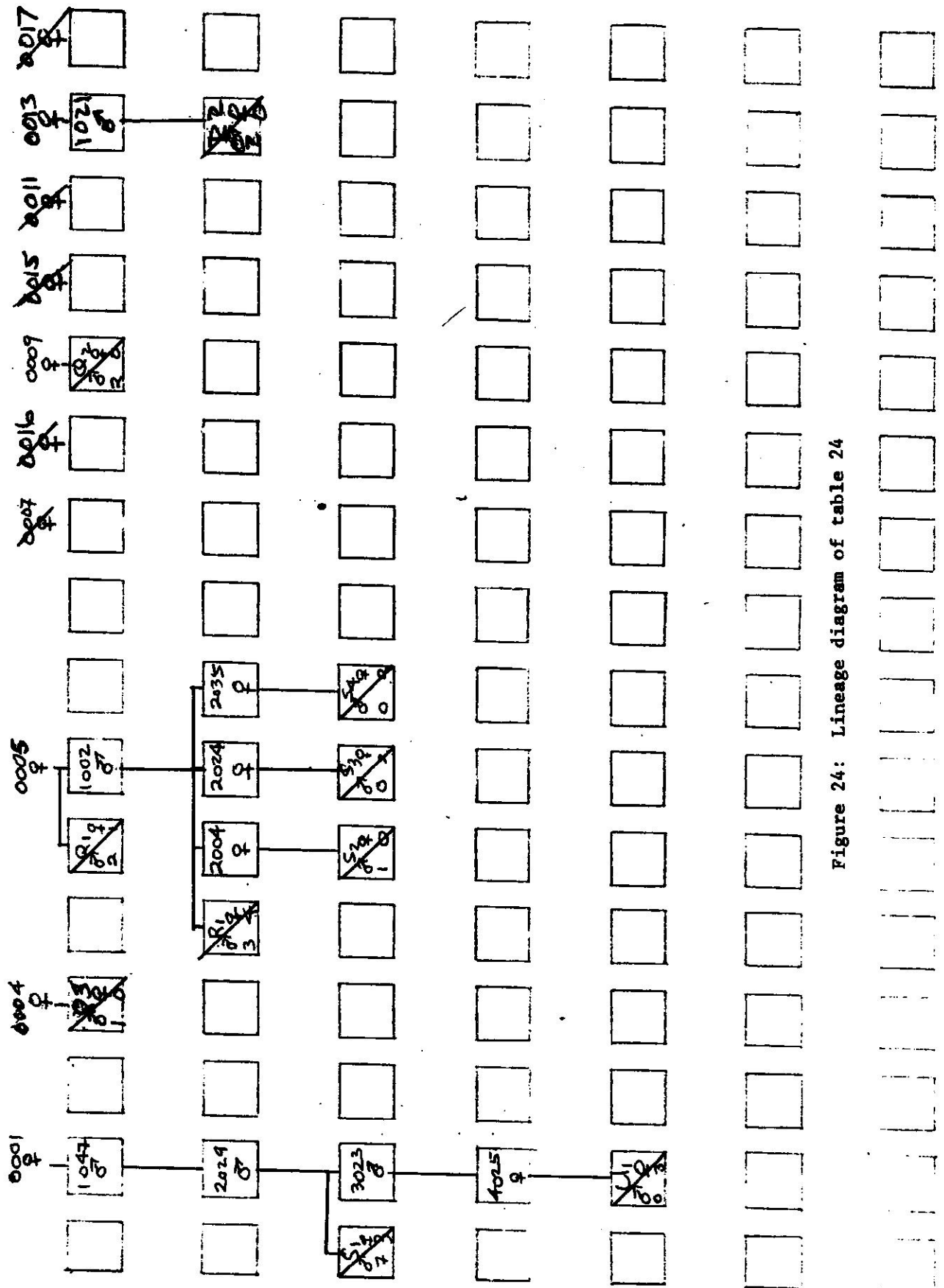


Figure 24: Lineage diagram of table 24

Table 26 IPS in Nezara

<u>dose</u> <u>Krads</u>	<u>No. inds.</u> <u>and sex</u> <u>irradiated</u>	<u>No. Fertile</u> <u>Eggs Laid</u>	<u>No. Fertile</u> <u>Eggs Hatched</u>	<u>% Egg</u> <u>Hatch</u>	<u>No. F₁</u> <u>Adults</u> <u>Harvested</u>
<u>P Generation Reproduction</u>					
ck	9	659	276	72.5	200
1.5	9 F	121	20	50.0	10
7.5	6 F	298	62	20.8	0
15.0	9 F	563	8	1.4	0
<u>F₁ Generation Reproduction</u>					
from 1.5 Krad female	3 F ¹	163	44		5 F ₂ adults reproducing currently: 2 M x 3 F siblings

1 siblings, 2 M and 3 F

2 still reproducing, all eggs not yet hatched, most appear to be fertile.

APPENDIX A

Appendix A
Diet Evaluations Report

Crianza de Nezara viridula (L.) en el Laboratorio
Rubén Restrepo-Mejía

Nezara viridula (L.) es un insecto de la familia Pentatomidae, cosmopolita y con amplio rango de plantas hospedantes, muchas de las cuales son cultivadas en gran escala. En algunos casos, este hemiptero se ha presentado como plaga importante, por lo cual ha sido objeto de la realización de trabajos en medidas de control. Una de estas ha sido la erradicación utilizando diferentes técnicas ya sean por químicos o por radiación. La utilización de estas medidas conlleva la disposición de gran cantidad de individuos.

Este trabajo en el Centro Nuclear de Puerto Rico bajo la dirección del Dr. David Walker tuvo por objeto desarrollar un método de crianza para N. viridula en condiciones de laboratorio, con miras a obtener una colonia suficientemente estable para proveer individuos en cantidades suficientes como para permitir estudios en relación con radiación y esterilización.

Se consideraron dos aspectos dentro del método de crianza: a) manutención y alimentación de los individuos, es decir cómo tratar y modo de suministrar los requerimientos nutritivos y b) evaluación de dietas artificiales líquidas. Se realizaron observaciones de actividad, preferencia y mortalidad.

Materiales y Métodos:

Se dispuso para este estudio de ninfas y adultos colectados en cultivos de habichuela y "cowpeas" de Mayaguez e Isabela. En condiciones de laboratorio a $23 \pm 4^{\circ}\text{C}$ y a $50 \pm 20\%$ de humedad relativa se procedió a separar las ninfas de los adultos, colocando en vasos de plástico de 4 onzas, 5 adultos o 10 ninfas. Cada vaso se cerró con una tapa plástica la cual tenía un agujero tapado con algodón para permitir el paso de aire y humedad. Como alimento se les suministró vainas de habichuelas tiernas.

Los anteriores recipientes tuvieron buen resultado con ninfas de 1er, 2do. y 3er. instares, no así con adultos, por cuanto las excreciones de los insectos dieron fuerte olor y humedad al interior del vaso, afectando posiblemente la supervivencia. Por esta razón se ensayaron también recipientes de cartón parafinado ("ice cream cartons") de 1 galón, lo que permitió colocar de 10 a 30 individuos en cada uno. Se obtuvo mejor resultado, con menos mortalidad e interior del recipiente más limpio. Estos envases se usaron en todas las pruebas con dietas artificiales. Se taparon con tela de nylon o con "saran" sujetos con bandas de caucho.

Las dietas artificiales se pusieron en vasos plásticos de 1 onza, cerrados con tapa de cartón perforadas con el objeto de pasar un mecha de algodón absorbente ligeramente prensado y amarrado con hilo para evitar el esponjamiento de las mismas con el líquido. En lugar de las mechas de algodón se usaron también trozos alargados de esponja plástica ya colocados a través del agujero de las tapas, o impregnados con las dietas.

Las dietas se prepararon al momento de usarlas, utilizando los ingredientes preparados con anterioridad.

El extracto de vainas de habichuelas tiernas se preparó semanalmente, licuando las vainas de habichuelas, agregando un poco de agua destilada y filtrando de modo que se obtuviera un líquido sin partículas vegetales gruesas.

Cada 2 o 3 días a los insectos se les cambió el alimento ya fueran las vainas o las dietas líquidas; esto con el objeto de que dispusieran siempre de alimento fresco, evitando también la fermentación de las mismas.

Hay que anotar que las diferentes dietas a evaluar en las diferentes pruebas se prepararon siempre con el mismo extracto. La preparación de los extractos fué similar en todos los casos, utilizando la misma variedad de habichuelas, de tal modo que no hubo diferencias: "siempre el extracto tuvo igual preferencia por parte de las "chinchas".

Durante las pruebas se mantuvieron continuamente los que completaron su ciclo de vida en el laboratorio, usando vainas de "cowpeas" (Vigna sinensis). Se usaron las chinchas de esta colonia para las diferentes pruebas con dietas artificiales, además de ninfas y adultos del campo.

Antes de iniciar cada prueba se pusieron los insectos que se iban a utilizar, a dieta de hambre, es decir sin alimento por 12 horas más o menos.

Los datos de ciclo de vida en el laboratorio se presentan en la tabla I.

Resultados:

Para la cría de N. viridula (L.) se descartaron los vasos plásticos de 4 onzas, considerando los factores mencionados anteriormente y el reducido espacio que se presentó especialmente para los adultos.

Los cartones de 1 galón cubiertos con tela de nylon fueron los más efectivos, siempre y cuando el número de individuos no excediera de 20. La temperatura parece que fué un factor importante en la cría de los insectos.

Las "mechas" de esponja plástica fueron muy adecuadas, siempre y cuando se mantuvieran un poco saturadas con las soluciones de dietas.

A. Pruebas Preliminares. Cuatro Dietas

1. Dieta de vainas de habichuelas tiernas.

Los insectos se alimentaron bien y completaron su ciclo hasta adultos; a pesar de la alta mortalidad en éstos hubo apareamiento y oviposición. No se obtuvieron nuevos individuos. En conclusión las vainas son adecuadas para alimentar los "chinchas". Las causas de la alta mortalidad en adultos no se pudieron confirmar, sospechándose fué en parte la temperatura y humedad del laboratorio.

Las "chinchas" aparentemente rechazaron el olor y el sabor.

B. Pruebas de Preferencia por: diferentes niveles de azúcar (Sucrosa)

1. Dietas Artificiales IV y V

Composición:	IV	V
Agua	20 cc	20 cc
Gerber "Baby Food Strained Beans"	10 gr	10 gr
Solución de Vitamina (Vanderzant)	2 cc	2 cc
Sucrosa	2 cc	5 cc

Estas dos dietas se evaluaron durante una semana en dos grupos de ninfas y adultos. Se utilizaron recipientes de cartón parafinado dentro de los cuales y en cada uno se colocaron los dos vasos con las dietas IV y V.

No hubo diferencias en esta prueba de preferencia. Los insectos se alimentaron bien y en ambas dietas.

C. Pruebas de Preferencia por Amino Acidos. Dieta Compuesta VII

Composición:

Extracto de vainas de habichuelas tiernas	60 cc
Solución de Vitaminas (Vanderzant)	3 cc
Sucrosa	6 gr
Acido Ascórbico	1 gr

5. Amino Acidos:

a) Glycina	6 gotas
b) Asparagina	" "
c) Isoleucina	" "
d) Alanina	" "
e) Arginina	" "
f) Fenilalanina	" "
g) Tyrosina	" "

Se probaron ocho dietas diferentes para preferencias a partir de la Dieta Compuesta VII:

Dieta No.

1. Todos los ingredientes, 1 a 5g inclusive.
2. Ingredientes 1 a 4 más 5a.
3. Ingredientes 1 a 4 más 5b.
4. Ingredientes 1 a 4 más 5c.
5. Ingredientes 1 a 4 más 5d.
6. Ingredientes 1 a 4 más 5e.
7. Ingredientes 1 a 4 más 5f.
8. Ingredientes 1 a 4 más 5g.

Para esta prueba se usaron ocho "cartones" de 1 galón. Dentro de cada recipiente se colocaron las ocho diferentes dietas. Inicialmente se introdujeron de 10 a 15 individuos por cartón. Posteriormente para facilitar las lecturas se redujo el número a 5 individuos por cartón y se aumentó el número de cartones.

Se hicieron lecturas durante 2 a 2 y media semanas, anotando cuántos individuos estaban alimentándose sobre cada una de las dietas. Las observaciones se realizaron por un mínimo de 2 diarias.

Los individuos adultos utilizados en estas pruebas, vinieron de ninfas criadas en el laboratorio con vainas de habichuelas tiernas y antes de iniciar la prueba se les suprimió todo alimento por un tiempo de 12 horas aproximadamente.

Las diferentes dietas alimentaron satisfactoriamente a los insectos. Estos produjeron gran cantidad de excreciones líquidas. No hubo aumento significativo en la producción de huevos a pesar de que se apareaban.

En conclusión podemos decir que todos los amino ácidos presentaron igual preferencia. Un amino ácido específico en las concentraciones usadas no fué significativamente diferente individualmente o en combinación.

D. Pruebas para preferencia por diferentes dietas artificiales

Se probó preferencia por varias de las dietas usadas anteriormente, tratando de comparar con la dieta VII.

Se utilizaron siete "cartones" de a galón. En cada uno se colocaron las siete diferentes dietas.

Las dietas probadas fueron: I, II, III, V, VI, VII y una dieta con la siguiente composición:

Extracto de vainas de habichuelas tiernas	30 cc
Sucrosa	5 cc
Solución de Vitamina (Venderzant)	3 cc

Se utilizaron adultos provenientes de ninfas colectadas en el campo y criadas en el laboratorio y ninfas de tercer instar provenientes de huevos ovipositados en el laboratorio por adultos provenientes de ninfas del campo. Esta prueba se hizo por un periodo de dos semanas al cabo del cual las conclusiones fueron:

- a) El ingrediente más atractivo fué el extracto;
- b) La dieta VII fué más satisfactoria que la I, II, III, V y VI y la de composición dada en esta parte;
- c) La producción de huevos fué bastante reducida y fueron o infértiles o no se desarrolló el embrión.

Los nuevos fueron depositados tanto en el cartón como en la tela de nylon; nunca en el "sarán". No se obtuvieron nuevos individuos: se presentó ovofagia por parte de los adultos.

2. Dieta Artificial I

Composición:

Agua destilada	30 cc
Azúcar (Sucrosa)	1.5 gr
Solución de Vitamina (Venderzant)	2 cc

Con esta dieta las ninfas no sobrevivieron para completar el ciclo biológico. Los adultos presentaron alta mortalidad; las hembras fertilizadas no ovipositaron.

Característico de esta dieta y todas las líquidas fué la abundante excreción líquida de los insectos.

Esta dieta no fué aceptable para proveer los requerimientos nutritivos de los insectos.

3. Dieta Artificial II

Composición:

Agua destilada	20 cc
Gerber "Baby Food Strained Beans"	10 gr
Sucrosa	5 gr

Los adultos y ninfas sobrevivieron por una semana más que con la dieta anterior (o sea 3 semanas a partir de la iniciación de la prueba). Los adultos se aparearon; no hubo oviposición.

Al cabo de 3 semanas los adultos perecieron.

4. Dieta Artificial III

Composición:

"Corn Hidrolizate" (NBC)	2 cc
Sucrosa	5 grs
Solución de Vitamina (Vanderzant)	3 cc
Acido Ascórbico	1 gr
Extracto de vaina de habichuelas tiernas	30 cc
Agua	20 cc

Esta dieta se acidificó con HCl a un pH de 5, similar al del jugo de las vainas de habichuela.

Esta dieta fué poco atractiva; los insectos demoraban en gustarla y no permanecían mucho tiempo alimentándose sobre ella. El "corn hidrolizate" dió a la dieta un fuerte olor a maíz y se fermentó con frecuencia.

Conclusiones Generales:

1. Esta especie completa su ciclo de vida alimentandose sólo de vainas de habichuelas tiernas, pero la producción de huevos es baja;
2. las dietas a base de extracto de vainas de habichuelas fueron más atractivas que cualquier otra sin extracto;
3. la sucrosa actuó como estimulante y atrayente;
4. los amino ácidos en conjunto o individualmente no obraron ni como estimulantes ni como atrayentes de acuerdo a las concentraciones usadas (0.0015%/150 mgrs/10 grs de H₂O).

Tabla 1 - Ciclo de vida de Nezara viridula (L.) en el laboratorio a $70 \pm 10^\circ\text{F}$ y a $50 \pm 20\%$ de humedad relativa, alimentados con vainas de habichuelas tiernas

Periodo	Tiempo promedio en días de cada periodo	Tiempo promedio en días desde la postura	Tiempo promedio en días desde la eclosión
Incubación	6	6	
Ier Instar	5	11	5
IIer Instar	7	18	12
IIIer Instar	6	24	18
IVto Instar	6	30	24
Vto Instar	8	38	32
Adulto			

