We are able, therefore, to pick up automatically only those lethals which are located on the extreme left end of the X-chromosome.

The same method can hold for the study of visible mutations. It is necessary for this purpose to examine both yellow and non-yellow males in the offspring of the cross.

(2) A yellow male is X-rayed and mated to ClB female carrying homozygous yellow and a deletion. F_1 Bar females with a deletion are selected and crossed individually to yellow males. In case a lethal arose in the X-chromosome during treatment in a region not covered by the deletion, no males appear in such a cross; if the lethal arose in the extreme left end of the X-chromosome only males carrying the deletion will survive.

The same principle as that given above will hold true for any region of the X-chromosome; it is only necessary to have a special duplication, producing no lethal or sterile effect on males or females.

The method for the study of the mutation process in a limited region of one of the autosomes is somewhat different. While for the study of mutation in separate regions of the X-chromosome it was necessary to have duplications, in the case of autosomes it is rational to have deficiencies. Let us consider the case of Np deficiency (Bridges, SKoog and Ju-Chi-Li, Genet. 1936). A heterozygous Cy male is X-rayed and mated to Cy/L females. L/+ (or Cy/+) males are selected and mated to females carrying in one of the chromosomes a deficiency Np. The lack of Np flies and the presence of Np/L (or Np/Cy) flies will indicate that a lethal arose in the region Np. All matings ought to be individual. The treated male must have only one normal chromosome which is to be analyzed. About 15 chromosomes are to examined from one X-rayed male, as it is possible that the chromosome under investigation contained originally a lethal in the region of the deficiency. In the latter case all the 15 cultures from one male will show the presence of a lethal.

<u>Spencer, W.P.</u> A new technique for growing Drosophila.

The method of Drosophila culture about to be described is not simply an improvement in current methods but rather a new departure so different that for the present in some laboratories it may be applied gingerly at first and only to certain special problems. The method consists in treating eggs, larvae, pupae and adults as distinct organisms in so far as their culture conditions are concerned. It favors spending as much time on the quantitative and qualitative study of eggs and larvae as has in the past been given to the study of the adult. This method is made possible by the discovery that the Drosophila female will readily deposit her eggs through a fine mesh of silk upon the proper medium. This fact makes it possible to standardize methods of culture all along the line and check each step by the use of adequate controls. With the wealth of material already at hand in the form of genetic tools and the new

technical method of handling Drosophila it now becomes possible to plan and carry out experiments with the precision comparable to that employed in the chemical or physical laboratory.

The Drosophila adult (or adults) are confined in a tiny cage, the design of which will no doubt change with further experiment. The cage in current use consists of a metal gas-hose ferrule 7/8 inch in diameter and 1/2 inch high. Over one end of this ring is stretched tightly a piece of black silk bobbinette trade No. 400 for large Drosophila species and No. 418 for small species such as melanogaster. This silk net is held in place by a small rubber band. Into the smaller end of the ring fits an ordinary cork of such a size that it projects part way into the ring and cut off so that about a quarter of an inch extends out of the ring. The adult flies, either for stocks or experimental cultures, are kept in this tiny chamber. The label goes on a small slip of library card which fits at one side of the cork. This ensures plenty of air entering the chamber. The present system of labeling stock bottles with long symbols will be discarded in favor of the less cumbersome DIS stock list number which may be written on the cardboard slip or on the end of the cork. For experimental cultures generally one female will be used per chamber as the method makes possible new quantitative study of daily and hourly output. For securing eggs the cage is placed directly on the medium on which the eggs are to be laid. This medium will presumably consist for the present of yeast enriched cornmeal agar or banana agar with a little animal charcoal added for contrast. The medium is poured into small water-proofed plates of a size conveniently examined on the stage of the binocular. Yeasting is done by the method described by Müller of spraying with a fine yeast suspension from an atomizer. The yeasting should probably not be done more than an hour before the plate is to be used, if the subsequent exposure of the plate to oviposition lasts for several hours. The best time may be checked for each species. As many experimental cages as will fit conveniently on the agar plate may be placed there. At the time of placing the cages a key to their position is written on the edge of the plate, together with any other pertinent notes on the experiment. An inverted paper plate covers the apparatus and may be held in place by paper clips or two rubber bands at right angles. With this precaution there is even less danger of contamination than in cotton plug bottles. First the wild flies are excluded from the plate and second the investigator is only interested in the spot under each cage which is effectively shielded by the cage itself from contamination. After a given time the cages are lifted off and quickly transferred to a fresh agar plate. Apparatus may easily be designed which will remove agar plates and set fresh ones in place mechanically without disturbing greatly the flies enclosed in the cages.

The agar plate on which oviposition has occurred is then put under the binocular or examined with a hand lens and if the experiment calls for it the egg output of each female is counted. If the experiment involves the raising

of larvae, pupae, or adults a given number of eggs will be cut off with a scalpel or other instrument and these eggs placed in bottles or vials until other better containers are designed. These containers will contain cotton or other porous material soaked in a given amount of yeast suspension known to be more than the minimum amount required for the optimum development of a number of larvae equal to that of the number of eggs used. These vial cultures will, in many experiments, contain eggs all of which have been laid within a few hours. The conditions of development will be standard. The agar plates containing the remainder of the eggs will be placed at the same temperature as the vials and the remaining eggs or portions of them used as an index of hatching. Even though larvae hatch and crawl from spot to spot they do not move unhatched eggs for two days or more, nor is there appreciable shift of egg cases for the first two days. These points may be checked as I have already done repeatedly. With the proper design of larval culture dish the eggs added to the dish may be checked directly for hatching thus eliminating the dangers of fair sampling. In many if not most experiments the investigator will examine the agar plate or larval culture throughout development, observing the larval behavior and genetic differences in cases where visible. Routinely egg and larval mutants will be picked up as are adult mutants today. All larvae (except in very exceptional genetic set-ups) will have pupated in a given dish before the first adults emerge and the handling of adults may be much as in the old technique.

Stocks will consist of adult stocks and larval stocks. The adults of the two sexes will be kept separately in cages identical with or larger than the one described above, sitting on enriched media which have been treated to prevent mold and bacterial growth and to inhibit oviposition. They will be kept at temperatures so low that the same set of adult flies may be held for a long period of time (possibly three months). The length of life is prolonged both by the very low temperature (12°) and rich feeding accompanied by low metabolism and lack of expenditure through egg laying. Always moreover, a parallel set of larval stocks will be kept in vials on yeast. Possibly ingredients may be added to these larval cultures to inhibit growth without being harmful. Certainly low temperature may be employed here. The details as to the exact form in which larval and adult stocks are kept will probably vary from laboratory to laboratory.

Mite infections can never be serious since the life cycle of the mite is broken up by the use of two sets of stocks. Suppose a mite infection to occur among adult stocks. The life cycle of the mite would be broken up by several mass transfers of these stocks to fresh media. This would be the work of a few minutes as the cages may be fixed to holders, allowing a slight movement of the cage on the holder (so that no cage would ever accidentally be held off the medium) and a hundred cages lifted from the old agar plate to a new one at once. If a mite infection broke out in the larval stocks, first new larval stocks would be established from the adult breeders and then old ones discarded en masse. However, the real reason that

mites have flourished in Drosophila laboratories is because the stock keeper could not see what was going on in a half-pint milk bottle and generally did not trouble to see what occurred in a culture vial. Egg and larval stages will be under the microscope as frequently as adults and mites will have no chance. They might, however, prove interesting objects for physiological or genetic research when we no longer fear their depredations.

No doubt improvements will be made in the technique here suggested, and suggestions will be welcomed. The method offers too many advantages to be held out of use while all details are perfected. I have only recently developed the method above described, using such suggestions as have already appeared in DIS and the literature in regard to yeasting, charcoal, growing larvae in yeast. It works for studies in life histories, egg counts, egg hatch counts, examination and comparison of eggs and larvae of different species en masse, discovery of egg laying rhythms in different species, time at which hatching per cent of eggs begins to lower (probably due to sperm supply running out) when females mated to males are then put alone into oviposition cages. The value in the method lies in the opportunity to check every factor at every point in the life history of the fly. Exact quantitative checks and controls may be employed and it is bound to lead to a toning up of our technical methods at all points. For instance if one questions the size of the breeding cage either as regards volume or area exposed to food, the comparison is immediately open to exact quantitative experiment. I have checked food exposure areas and two types of silk nets and done the two things in the same set of experiments. The introduction of this method makes relatively easy the investigation of many problems in the behavior, physiology, and genetics of Drosophila hitherto unapproachable because of the time involved and the impossibility of standard conditions in the experiment. It is to be hoped that many laboratories will give it a trial.

During the course of developing the apparatus I have kept in mind the matter of expense of equipment and labor involved in the procedures. As soon as certain problems on size of cage, etc. have been more thoroughly tested it should be possible to have breeding cages of perhaps several sizes and designs made up to sell at low cost. This is more likely to happen if those interested go directly to the manufacturer rather than putting the matter in the hands of supply houses. In the past I have corresponded with the Aluminum Company of America relative to aluminum shipping vials which could be used to send stocks by airmail letter with no additional postage and know that vials could be secured and made over for a little more than two cents each. Cellophane will probably play a part in some cage designs. Whatever material is used, cages must be light in weight for best results. Otherwise an exudate is pressed from the agar plate.

Cost of material used in the above tests

1 yd. 72 inch width, of black silk bobbinette No. 400		$3.00
1 yd. 72 inch width, of black silk bobbinette No. 418		$4.00
(A yard will make up 1000 or more cages)		
Metal gas-hose ferrule, 7/8 inch by 1/2 inch per	100	$2.50
Water-proofed paper plates, 5 inch diameter	18	.05
Atomizer for spraying yeast		.85

There is no doubt but that in terms of research production the cost both in money and in time will be far below that now expended. (See also Science 85:298. 1937).

Stalker, H.D. Mounting and handling dried specimens pf Drosophilinae. For cementing Drosophilinae and other small insects to cardboard points we have found the following much superior to mucilage, shellac, etc.

10 cc. glacial acetic acid, equal volume of powdered gelatin, (Knox).

The gelatin is dissolved in the cold acid and the cement is ready for use.

For immediate relaxation of dried specimens, household ammonia, applied a drop at a time until the insect is thoroughly wet, will cause complete relaxation in two or three minutes. If, in the case of insects on points, the excess ammonia dissolves the cement, the specimen is limp enough to be remounted with safety. When the specimen dries out it will be found to be unharmed by the treatment.

Williams, C.R. Collecting eggs and larvae. Size number 47 ramekins, made by American Lace Paper Co., Milwaukee, Wis., which have been previously dipped in paraffin are used. About six mm. of banana-agar-yeast food is placed in the ramekin. About eight pairs of mated flies are placed in a 3x9 1/2 cm. empty shell vial. The ramekin containing the food is then placed over the shell vial; the whole is inverted so that the food is ventral to the flies. They are then put away in a dark container for the desired egg-laying period, after which the ramekin is removed and capped with an ordinary milk bottle cap. With this method the ramekin can be observed under the binocular with ease. The larvae should not be left in ramekins more than fifty hours as the food dries up after this length of time. The flies in the shell vials may be immediately used in another egg-laying period or else returned to vials containing food.