Research Notes

Auerbach, C. The unsolved statistical problem of calculating mutation rate when eggs are treated. In order to test a possible effect of non-volatile chemicals on mutation rate it is usual to expose eggs to a fluid in which the chemical has been dissolved. Other methods that have been used are: injection of the fluid into larvae or imagines, and feeding of larvae on a medium which contains the chemical. Treatment is thus applied exclusively or mainly to immature germ cells. These subsequently develop into the much larger population of mature germ cells, out of which a random sample is used for the F_1 matings and represented by an identical number of F_2 cultures.

There is thus no longer the 1:1 correspondence between treated cells and examined F_2 cultures which obtains when treatment is applied to mature spermatozoa. In some experiments of this kind, notice is taken of this fact by grouping the F_1 according to the P_1 parent, and scoring as one any cluster of identical mutations which occurs in any of these groups. In the writer's opinion, this is misleading, since similar clusters without mutation escape notice. In the average of an adequately large sample the mutation rate should come out correctly when each mutation--whether belonging to a cluster or not--is counted as one. On the other hand, there is a real difficulty involved in the estimate of the error. Apparently in most of these experiments the standard error of the difference between treated and control series is calculated by the usual formula substituting for $n_{tr.}$ and $n_{contr.}$ the numbers of F_2 cultures in the two series. Obviously the correct values of n would be the numbers of treated control cells which gave rise to the F_2. These numbers are unknown and the writer does not see a way of estimating them from the observed data. Since the increase in mutation rate to be expected even from an effective chemical is of the order of size of a few per cent only, a valid error estimate is indispensable for evaluation of the results in all but really spectacular cases. The writer would be very grateful for suggestions how to arrive at one.

U. Fano. Remarks on the preceding note by Miss Auerbach. Two different problems seem to be involved in the issue raised by Miss Auerbach: (a) What is the standard error of the rate of mutations induced by treatment in immature germ cells? As Miss Auerbach points out, this standard error is substantially dependent on the number of treated germ cllls involved in the experiment, and not merely on the number of F_2 cultures tested. (b) What is the standard error of the rate of "spontaneous" mutations detected by standard methods in control material? It has generally been assumed that this error is given by the formula

$$\sqrt{\frac{\text{number of mutants (number of tests-number of mutants)}}{(\text{number of tests})^3}} .$$

Serious doubts arise regarding the validity of this formula when one considers that mutations in control material may also occur in immature germ cells. (See, for instance, the discussion of an analogous problem concerning mutations in bacteria, by Luria and Delbrück in GENETICS, 28: 491-511, November 1943.)

No theoretical solution to either of these problems is available, so far as I know. (I hope, however, to be able to report soon on some progress which I have been making toward this goal.) Miss Auerbach's main interest at present seems to be the preliminary question of how to determine whether the mutational effect of a treatment of germ cells is at all significant; and I should like to emphasize the fact that problem (a) is not involved in this question. In fact,

the test of significance of the effect of a treatment is carried out by assuming first that the treatment was not effective. Thus the difference observed between the frequencies of lethal-bearing F_2 cultures from treated and from control P1 males must be compared with its standard error calculated on the basis of the "no effect" hypothesis; i.e., from the solution to problem (b). If one rejects the usual theoretical solution of this problem, and if no more accurate theoretical solution is available, it should still be possible to solve it experimentally; that is, by carrying out a series of determinations of the frequency of spontaneous lethals and scoring the departures from their means. It could then be seen, even without much help from statistical formulae, whether the results of one or more determinations of the frequency of lethals in treated material depart so much from those of the controls that they cannot be considered as belonging to the same population.

Cooper, K. W. Meiotic pairing of the sex chromosomes of male Diptera.

Inasmuch as Drosophila has in the past proved rather refractory material, a comparative cytological study is being made of meiosis in the Drosophila male and male pupiparous flies. In the hippoboscid fly Olfersia the male forms a sex chromosome bivalent identical with that of Drosophila pseudoobscura, showing all those "criteria" on which Darlington has based his reciprocal chiasmata hypothesis. Nevertheless in Olfersia the conjunction of the sex chromosomes is brought about not by chiasmata, but by a region of interstitial cohesion or pairing. This follows from the fact that the sex chromosomes separately condense in the nucleus prior to diakinesis and then come together to form the definitive bivalent at diakinesis. The mechanical properties of such "cohesive" segments and their role in Drosophila are being further investigated.

Harrison, B. J. An effect of culture conditions on mating.

Five females x five males from the B ♀ were placed in each of four bottles, A_1.....D_1. The flies were removed from A_1 after 1 day's laying, from B_1 after 2 days, from C_1 after 3 days, and from D_1 after 4 days.

The offspring emerging on the first day from each bottle were transferred to fresh bottles, A_2.....D_2. The second day's emergence were left in the old bottles, which were now transferred to room temperature to minimize further emergence. After 4 days, females were dissected to determine the proportion still virgin, with the following results:

Bottle	No. dissected	% virgin	Bottle	No. dissected	% virgin
A_1	8	25	A_2	11	27
B_1	17	29	B_2	14	7
C_1	18	67	C_2	16	25
D_1	21	62	D_2	11	27

Mating frequency is constant in the fresh bottles, A_2.....D_2, but varies in the old bottles, A_1.....D_1, being least where the bottles had been most crowded with larvae.

Kalmus, Hans. The optomotor responses of some eye mutants of Drosophila.

Drosophila flies of many species are circling inside a striped cylinder, when the latter is turned. The response can be measured by applying a series of striped patterns of different widths. Female melanogaster flies react slightly better than males. The reaction of flies carrying Bar genes is reduced, parallel with the reduction in facet number. The same applies to

eyeless flies. D. subobscura eyeless flies with less than 12 facets do not show any optomotor reactions. Various facet abnormalities in the two mentioned species do not noticeably decrease the reaction. White mutants of D. melanogaster and D. pseudoobscura do not react optomotorically, nor do white-eyed melanogaster flies homozygous for brown and cinnabar. Homozygous apricot flies and homozygous brown and vermilion flies showing a similar eye color react markedly worse than wild-type flies. The eye-color mutants brown, cinnabar and vermilion in D. melanogaster and scarlet, maroon and vermilion in D. subobscura do not singly diminish optomotor reactions, but the mutant prune in the latter species abolishes it. The contour-blind flies mentioned for the most part show ordinary phototropism.

Lamy, R. Hidden divergence in laboratory strains of D. pseudoobscura. In experiments involving Race A/Race B pseudoobscura hybrids, it was found that the majority of old strains of Race A carrying sex-linked markers contained genes which acted as inter-racial lethals in the backcross generation. Suitable tests showed that genes on the autosomes as well as on the X were responsible for the lethal effects. Similar effects were not observed when equally old wild-type strains were used. It is thought, therefore, that by means of inter-racial crosses a type of genetic divergence is revealed, which is not readily observable in the pure race, and which is apparently connected with the artificial selection and maintenance of major mutations as "marker genes" in stocks. It is suggested that such divergence may be due to a relaxation of natural selection in respect of whole gene-systems controlling developmental processes which are in some way interfered with as a result of the changes produced by the markers. In consequence of this relaxation, mutations of a "sub-functional" type would tend to accumulate in these genes by a process which may be described as "directional drift." The resulting deterioration in the genotype would be scarcely observable intra-racially, because although in some degree impaired or undermined, the gene systems would still hold together and retain their coordinated effects, which are more important than the effects of the genes individually. In the two races, however, the gene systems are not built up on identical patterns either in respect of linkage or of concentration of function, even though the genetic content may be largely similar. In the backcross generation, where both racial systems are mixed up, single genes may have an importance in development which they do not have in the pure race, due to lack of appropriate coordination. Hence a sub-functional mutation, which is practically harmless when the gene-system functions as a whole, may be lethal or semi-lethal if some part of that system is absent or inadequately represented, as in the unbalanced genotype of the partial hybrids of the backcross generation. Full details in press.

Muller, H. J. A stable double X chromosome. Stock of a double X chromosome has been obtained which is superior to the ordinary attached-X chromosomes for most stock-keeping and crossing operations, by virtue of its stability in breeding, since it does not break up, by crossing over with the Y, to form viable single X's. Hence stock containing any desired X chromosome in the male and this double X in the female does not have to be watched, as ordinary attached-X stocks do, to avoid the breakdown into single X's whereby the desired single X may be lost.

The new double X, denoted by the symbol : together with symbols of any contained genes which it may be desired to represent (as explained below), arose by X-ray irradiation of early germ cells (oogonia) of a female containing one X chromosome having the formula y w dl-d9 f and one having the formula sc^8 B y^{3P} (this having the left part of a scute 8 chromosome, bearing y+ and Bar, and the right part of a $yellow^{3P}$ chromosome, bearing the usual dark-bristled yellow allele present in the latter). Deletion of the sc^8 y^{3P}

chromosome occurred. A deleted X, doubtless derived from this breakage, having the left and right ends of this chromosome, and therefore giving the non-yellow phenotype, was in fact recovered in one of the offspring of the irradiated female. The long middle portion of the X, that was deleted out from the rest, became inserted or otherwise attached, presumably by both its broken ends, to the other X, that having the formula y w dl-49 f, and this (nearly) double X was recovered in a sister of the individual having the deleted X, in an egg laid at about the same time (some 12 days after irradiation). The doubleness of the attachment is indicated by the two dots of the colon (:) used in designating the double X thereby produced. The resulting double X, not having either the y+ of the left end or the y^{3P} of the right end of the sc^{8} y^{3P} chromosome, and having y in the part derived from the y w dl f chromosome, gives the phenotype ordinary yellow (y). The deleted piece most probably became inserted at some point into the y w f X, to form one double-length X having almost terminal attachment, since this process would require only one break of the y w f X-chromosome. Alternatively, but far less probably, the deleted piece might have become attached at (nearly) the two ends of the y w f X, if the latter had become broken within the dispensable heterochromatic region at the free left end and at the same time in that at the right end, beyond the centromere (as happened, for example, when the ring chromosomes Xc1 and Xc2 were formed). In this latter case the double X would have the form of a large (double-sized) ring. Which of these alternatives is correct is now being investigated.

Owing to the double attachment, and the presence heterozygously, of the dl-49 inversion in addition, the two X's cannot form viable recombinants with one another except by double crossing over, and this is very rare. The original double X in this case gives not only the mutant phenotype ordinary yellow but also heterozygous Bar, owing to the B of the original sc^{8} y^{3P} chromosome. By the very rare double crossing over between the members of this double X, however, Bar is occasionally lost, and then forked nearly always appears so that a yellow forked stock of this chromosome has also been obtained. The latter chromosome is represented by the abridged formula yf:= (where = represents another chromosome bearing, in effect, the same genes as those already shown; although in this case the complete structural formulae of the two X's are of course different from one another). This representation should be contrasted with that of ordinary attached X's bearing yellow forked, which are designated as y f.= in the present author's notation or, in the notation of some other authors, as y f. The yellow heterozygous Bar double-X cannot be accurately represented in so simple a fashion, but may best be shown, in our notation, as y B : y (omitting the w and f which it contains heterozygously and which do not manifest themselves).

It is planned to substitute the y f: = chromosome as rapidly as possible in place of the ordinary attached X's (y.=, y v f.=, etc., sometimes represented as y, y v f, etc.) in all our stocks that exist primarily for the maintenance of the X chromosome of the male. It is also planned to use it in place of ordinary attached X's in stocks in which special Y's are being maintained, since crossing over between the X's and the Y in the female, of such a kind as to lead to breakdown of a special Y, should not occur as readily in this case as in the case of ordinary attached X's. Cultures of either the y f:= or the y B;y chromosome may be obtained on application.

It should be noted that flies having this double X, unlike those with ordinary attached X's, should have increased crossing over in their autosomes, because of the considerable reduction of crossing over in their X's.

Nolte, D. J. Appearance of unexpected eye colors. Certain unexpected and as yet unidentified eye colors appeared among the F_2 of crosses with y w stock, being the sex-differentiated eye colors of cadmium orange in the ♂ and nasturtium red in the ♀. The following numbers were obtained: first, 24 ♀ and 8 ♂ among 3063 individuals of the F_2 of a cross y w x wild; second, 12 ♂ among 2062 individuals of the F_2 of a cross se x y w; and third, 126 ♀ and 74 ♂ among 12,230 individuals of the F_2 of a cross y w x se.

This strain breeds true and has been proved to be an allele of w. Out of its progeny, however, have appeared some brown-eyed individuals called "Maroon," which have yielded a true-breeding strain and which, though proving allelomorphic to w, have in the few trials completed not proved allelomorphic to its parent strain.

Phillip, Ursula. Cytologically homozygous stock of D. subobscura. During the last year 11 wild females of D. subobscura were inbred. They yielded a number of mutants listed elsewhere. Ten females gave progeny which were heterozygous for the three pairs of autosomal inversions that are present in all our stocks. The progeny of one female, however, was homozygous with regard to chromosome order. The offspring of this fly crossed freely with all laboratory stocks against which they were tested. The female yielded two mutants, dried wing (dw) and short (st). The short stock, extracted after outcrossing to delta, was found to be homozygous. This is most remarkable, as so far all attempts to breed a cytologically homozygous stock have failed.

Philip, Ursula and Spurway, Helen. In(1)1. Salivary-gland chromosomes show two abnormal chromosome orders B and A. Order B carries a single inversion occupying 2/9 of the chromosome, 1/3 from spindle attachment and 4/9 from the other end. B/standard (s) chromosomes pair with a normal loop. A carries a reinversion of one-third of the region inside B. Pairing takes place in the two ways described by Sturtevant, although the two chromosomes often stay unpaired.

Genetically B/S and A/S cannot be distinguished. Recombination between ct and cd (see map in this issue) markedly reduced. Actual figures: non-crossovers, 17,192; single crossovers between ct and left break of B, 142; single crossovers between right break of B and cd, 6; crossovers in both these regions, 2; double crossovers within inverted segment, 1; N.B. Coincidence of 31.

The mother of the double crossover within the inversion was S/B. The linkage map of the B chromosome, based on 1416 flies, is ct - cp 37.6; cp-sin 3.7; sin - cd 26.8. Crossing over in A/B not yet counted.

Poulson, D. F. Cytological extent of $Df(1)N^B$. Acetic orcein smears of salivary glands of females carrying $Df(1)N^B/y$ Hw m^2 g^4, d1-49 indicate that the deficiency involves bands 3C 5 - 10. It could not be determined with certainty whether bands 4 and 11.12 are involved.

Redfield, Helen. Mosaic eyes in melanogaster intersexes. Mosaic eyes appeared in the intersex progeny of free-X eosin triploids--a definite proportion of the light eyes (like the 2N male eosin eyes) of these intersexes, 25%, show patches of a darker color (like that of the 2N eosin females). A dark patch is apt to include about a quarter of the eye area; it may be either smaller or larger;

in extreme cases it covers an entire eye. All eosin triploid mothers of the strain, mated to eosin males, gave mosaic intersexes.

An unrelated strain of triploids with two attached-X chromosomes was crossed to males of the eosin stock. The first eosin triploids obtained did not give mosaic intersexes, but further backcrossing gave triploids whose intersex offspring had the mosaic eyes.

Free-X eosin triploids were crossed to apricot, an allele in which the color relations of the sex types are normally the reverse of those shown by eosin. As expected, the mosaicism on appearing in apricot intersexes after some generations showed light patches (female-like) on a darker background (male-like). Thus the mosaic patches always take on the color of the female eye.

The above crosses would lead one to suspect that the effect is due to something originally present in the eosin stock, not the eosin gene itself, which can manifest its presence in the presence of the sex-limited alleles of white. To test a possible relation of the presence of a Y chromosome and the appearance of the mosaicism, it was desirable to distinguish between 2X3A intersexes and 2XY3A intersexes. A stock of yellow, Hairy wing, eosin was derived and was crossed to the eosin triploids. The Hairy wing intersex offspring contain no Y (unless the triploid mother contained an extra Y), but the non-Hairy wing intersexes have received their father's Y. The classification of Hairy wing was for some reason not completely satisfactory in these intersexes, but it was definite enough to show that both Hairy wing and non-Hairy wing intersexes may be mosaics. The mosaicism, then, is not a direct Y effect unless Y chromosomes are derived from the triploid mothers. It is possible that an effect of another type is involved--somatic crossing over, for example, or autosomal chromosome elimination giving 2X2A patches.

Rendel, J. M. D. pseudoobscura A x D. subobscura.

Matings have been successfully made between pseudoobscura and subobscura in both directions. The ♀'s always resist the ♂'s and struggle to kick them off once they are in copula. The ♂'s succeed in about 30% of cases in inseminating the ♀'s. The matings are sterile. Sperm are injected into the ♀'s and eggs are laid, but none hatch. Eggs have not yet been sectioned to determine whether they are fertilized. The sperm plug formed by the pseudoobscura ♂ ejaculate in the subobscura ♀ is much more substantial than that of the subobscura ♂, and sometimes causes eggs to be caught up and prevents laying. This is not always the case, and given time, the subobscura ♀'s inseminated by pseudoobscura ♂'s do lay. The sperm bundle of pseudoobscura, when injected into subobscura ♀'s, is sometimes slow to break down, and remains a sticky, coherent bundle in the sperm receptacle.

Schultz, Jack. A derivative of $In(1)w^{m\,4}$ showing a different reaction to heterochromatin.

In $(1)w^{m4}$ was found by Muller to give derivatives showing a change in the degree of mottling. A number of new ones have been found, and in the case to be described tests have been made (1) of the locus of the difference between the original and the derived chromosome and (2) of the reaction of the derivative to modifiers of variegation. In contrast to w^{m4}, which has a ground color in the ♂ resembling the white allele coral, the derivative has an almost white eye. This difference, by appropriate tests with crossovers between w^{m4} and other not quite identical inversions, turns out to be located at the junction between the white locus and the heterochromatin of the X. Salivary-gland analysis shows no gross difference in the rearrangement. It might be suspected that a mutant at the white locus, tp some slighter allele,

is involved. By the addition of extra Y chromosomes, and experiments also with suppressors of the variegation effect, it was shown that the wild phenotype can be attained by the derivative as well as by the original w^{m4}. The difference is that two additional Y's are required to give the effect in the derivative that is reached with one in the original type. Conversely, experiments with enhancers of variegation show that the derivative gives a more extreme reaction. Thus the change concerns the reaction of the white locus to the heterochromatin balance of the nucleus.

Slizynska, H. and Slizynski, B. M. The distribution of sex-linked lethals.

As a preliminary step in the study of the nature and type of the chemically induced sex-linked lethals, the position of 89 out of more than a hundred obtained by Dr. C. Auerbach was determined genetically. The distribution of these lethals in the genetical map is as follows: (0-5) : 17; (5-10) : 2; (10-15) : 8; (15-20) : 4; (20-25) : 9; (25-30) : 5; (30-35) : 8; (35-40) : 4; (40-45) : 3; (45-50: : 3; (50-55) : 5; (55-6C) : 4; (60-65) : 9; (65-70) : 4; The lowest frequency was found between 5 and 10 due to sudden drops around locus 10. Five lethals out of 10 so far as investigated were found to be deficiencies.

Steinberg, Arthur G. Is a chromosomal aberration necessary for a position effect?

Existing evidence has shown that in general a position effect is obtained (a) when certain mutants which are normally situated close to heterochromatin are translocated, by means of a chromosomal aberration, to a region far removed from heterochromatin (e.g., ci), and (b) when certain mutants which are normally situated in a region far removed from heterochromatin are translocated to a heterochromatic region (e.g., w). It has been shown that the presence of an extra Y chromosome enhances the position effect of the former group of mutants and inhibits or suppresses the position effect of the latter group of mutants. This contradictory action of the Y chromosome may be only apparent. If we reason that the important factor in the position-effect phenomenon is the percentage of the total heterochromatin contained in the cell which lies adjacent to the affected locus, and not the absolute amount of heterochromatin near the affected locus, it follows (a) that a position effect of ci arises when the relative amount of heterochromatin adjacent to it is reduced and (b) that a position effect of w arises when the relative amount of heterochromatin adjacent to it is increased. Since an additional Y chromosome increases the total heterochromatin content of the cell without affecting the absolute quantity of heterochromatin adjacent to the locus under observation, it follows that the relative amount of heterochromatin adjacent to the locus is reduced; hence the position effect of ci is enhanced and that of w suppressed. Finally, this reasoning leads to the conclusion that it should be possible to obtain a position effect of ci in the absence of a chromosomal aberration, by increasing the total quantity of heterochromatin in the cell so as to reduce the amount of heterochromatin adjacent to the ci locus. At the time of writing, ♀♀ containing three Y chromosomes and heterozygous for ci have been obtained. They show no position effect for ci. Experiments designed to obtain ♀♀ containing four and possibly five Y chromosomes are in progress.

Technical Notes

Auerbach, C. Treatment of dechorionated eggs. When dechorionated eggs are exposed to solutions, the main difficulty experienced is the tendency of the eggs to float on the surface. In order to obtain complete immersion, the writer used the following method. Batches of from 20 to 30 eggs are dechorionated in the usual way on a slide. A small piece of filter paper is soaked with the required fluid and stuck into the bottom of a block dish or watchglass. The eggs are transferred as a cluster into the center of the paper. With fine needles the paper immediately surrounding the cluster is roughened up and the loosened fibers bent over the eggs so that a kind of closed pocket is formed in the center of the paper. Only then the fluid is poured into the dish. For washing eggs, the piece of paper can easily be transferred with a forceps into other dishes without disturbing the enclosed eggs. For removing the eggs, the paper is lifted out of the fluid and put on several layers of filter paper. The pocket is then opened, and the eggs can be transferred to food.

Gowen, J. W. Mold A green mold, of unusually vigorous and resistant strain, has been bothering us lately. To overcome it, we have tried Moldex and more recent product, Spergon, in the concentrations indicated below. The Moldex was not too successful. Seven rather difficult stocks were chosen, two pairs from each. Egg productions are for 7-day periods.

Mold preventive	Amount added to medium	Pairs counted	Total eggs	% cultures moldy
Spergon	1/1500	14	1284	0
	1/3000	14	1598	14
	1/6000	14	1484	36
	2/12000	14	1772	64
Moldex	1/250	14	760	21
	1/333	14	1140	50
	1/500	14	974	71

The mold preventive was added to the medium just before it was poured. The bottles were seeded with Fleischmann's Yeast in tap water and paper toweling added. The toweling was soaked in the same concentration as that added to medium, then dried before use.

We interpret the results as follows. Moldex reduces the flies more than Spergon in concentrations used. For this mold, 1/1500-1/3000 of Spergon is about the right concentration. Spergon has another effect, however. It encourages acid fermentation so that cultures become quite acid. This acid may sometimes liquify some of the agar and leave the culture very wet. To discourage the organism which causes this fermentation, we are now adding 0.1% Moldex as well as 1/5000 Spergon.

Schultz, Jack. An improvement in technique for the agarless cornmeal-molasses-rolled oats medium. The difficulties encountered in the pouring of the agarless cornmeal medium depend upon ascertaining the correct stage at which to stop cooking. There is unfortunately a very slight differential between the stage at which a degree of consistency is reached sufficient for a proper culture medium, and one at which the food is too thick to be poured easily. A procedure which obviates this difficulty follows:

(1) Ingredients in the proportions suggested by Lewis (DIS-16: 71) are mixed. Some experimentation, dependent on the type of cornmeal, will be necessary, since in the present technique, which permits more thorough cooking, less cornmeal may be used. Salt is not necessary.

(2) Cooking proceeds until the cornmeal no longer settles to the bottom.

(3) Medium is poured into the unsterilized bottles. At this stage the medium is quite fluid, and can be poured easily, by any of the techniques used for the previous agar-containing media.

(4) The bottles with the medium in them are now sterilized (either in a large autoclave or, where such is not available, in a large garbage can). This provides the additional cooking necessary to make the medium sufficiently stiff for Drosophila culture. Care is necessary to prevent the accumulation of water of condensation inside the bottles; either paper milk-bottle tops can be used, or a mass cover for each layer of bottles--a simple procedure in either case.

(5) Yeasting and papering are carried out as desired after cooling. If the bottles have been covered individually, these final steps can be left until the appropriate time before use, an advantage when food is not prepared daily.

Spassky, B. Cream of wheat-molasses fly medium.

Due to the shortage of agar, several non-agar mediums have been tried out in Professor Dobzhansky's laboratory. For the last year, cream of wheat-molasses medium, introduced by the writer, has been used widely. It was found to be satisfactory with D. pseudoobscura, D. persimilis, D. melanogaster, D. miranda, D. azteca, and others.

Formula for 100 half-pint bottles is as follows:

Water	4500 cc.	
Cream of wheat	625 gr.	
Molasses	670 cc.	
Salt	7 gr.	
Moldex	40 cc.	(10% solution in 90% alcohol)

Regular cream of wheat of "The Cream of Wheat Corp., Manf., Minneapolis, Minn." is used. The other kinds, as well as the "5-minute" variety of the same manufacturers, have not been tried. Molasses of the same specification as for agar food is used.

The procedure is the same as for agar food, but agar is omitted and cream of wheat is used instead of cornmeal. About 3/4 of the indicated amount of water is put on the fire, while the balance of the water is left aside cold. Salt, Moldex and molasses are added to the water on the fire. When the mixture is boiling, the needed amount of cream of wheat is put into the cold water that was left aside, stirred and dumped into the boiling mixture at once. From this point on the food has to be stirred constantly to prevent burning. After the whole mixture starts boiling again, it has to be boiled for 3-7 minutes, depending on the intensity of the fire and the quantity of food.

Food is poured into the bottles through the tin funnel with its nasal cut off. A fast worker can pour as many as 200 bottles. For a larger number, the food is poured as long as it is thin enough for pouring, and then the balance of it is packed into a pastry bag and pushed into bottles. The idea of using a pastry bag was introduced by one of the men who prepared the fly food at

Columbia University (see DIS-16). Vials too are filled with a pastry bag, although for a small number of vials the food can be poured.

A larger quantity of food per bottle or vial is used. The ratio between the quantity of agar and of cream of wheat food per bottle is 1 to 1 1/2(1:1½). One-third of the vial is filled with food; only a slight slant is made. A thin strip of paper folded twice is pushed through the food to the bottom of each vial to allow gases to escape. A drop of fresh yeast suspension is put in each bottle or vial, as usual. Dry brewers' yeast was tried and is not recommended for this kind of food. The addition of dry yeast causes too strong immediate fermentation, which is harmful to flies and makes the food watery.

This food is a jello-like substance. It does not come off at the "shaking-out" operation, which allows systematic counting of flies and collecting of virgins. The other non-agar food tried did not have this desirable property.

The favorable condition of the food cake persists indefinitely, provided the temperature and humidity are normal. In our particular case when the temperature in the cold room is 15-17 degrees C. and the humidity is abnormally high this food gets watery in about 35 days (the other non-agar food in less than 2 weeks under the same conditions). By using a 1-month rotation for stocks, transferring them in a month's time, we were able to overcome this difficulty.