# A STUDY OF FILAMENT, THORAX AND OTHER GENES OF PECULIAR INTEREST IN DROSOPHILA.

BY

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Being the thesis presented to the university of Edinburgh for degree of Ph. D.

# I.

## "FILAMENT" A MUTANT IN DROSOPHILA.

C O N T E N T S.



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## INTRODUCTION.

In the course of a study involving egg-production in Drosophila pseudo-obscura a Pointedsnapt (wings narrow and pointed at the tips; second longitudinal vein short) female was observed to lay along with her apparently normal eggs a certain number of peculiarly shaped eggs. These, when separately incubated, yielded twelve females, three of which on testing gave abnormal looking eggs. It was assumed therefore, that a mutation affecting egg-structure had been recognised and since the main effect was on the size, position and disposition of the two filaments, it was decided to call this new mutation "Filament (F)".

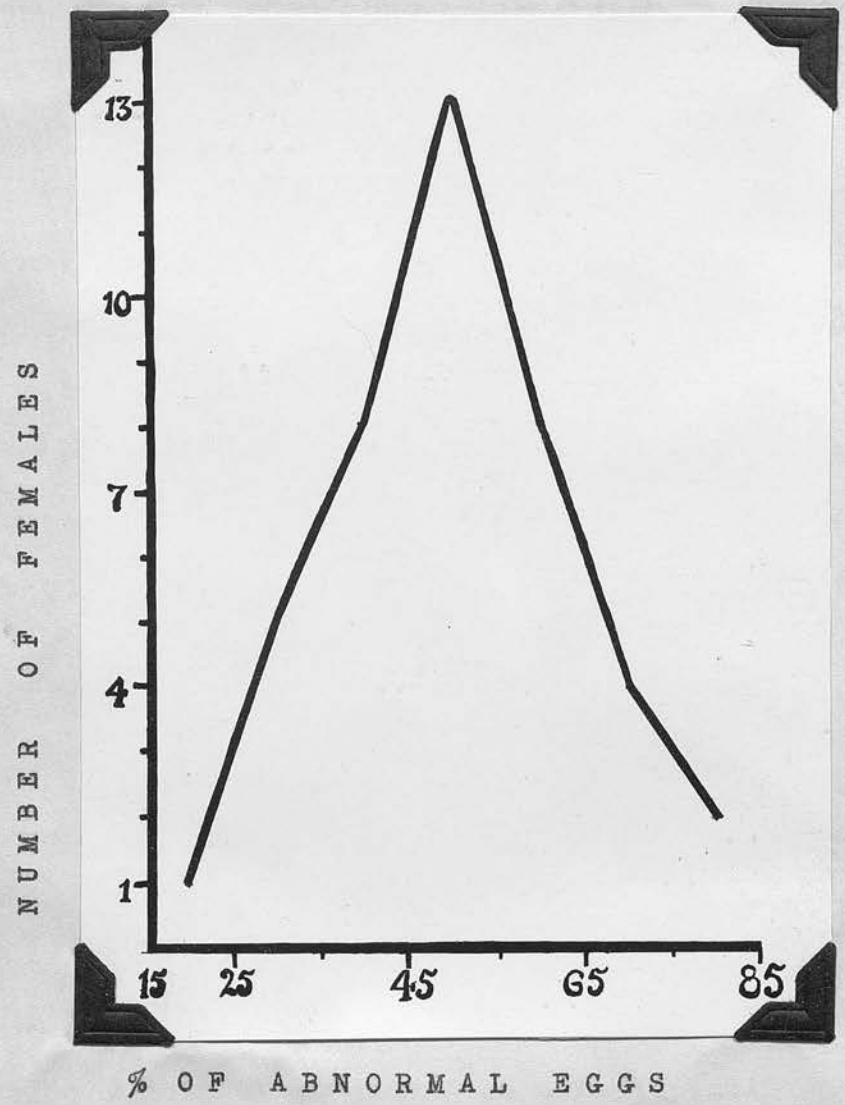
In the following pages a detailed account is given of the various aspects - morphological, genetical, and physiological - of this new mutation together with the possible light thrown on the evolution of the filaments in the Drosophilidae.

Filament (F) forms the second mutation affecting egg-structure to be reported in Drosophila. The other, "spheroidal", was discovered quite recently in Drosophila Funebris (Crew and Auerbach, 1937). It is characterised by extremely low fecundity of the female,

dwarf-shape of the eggs and reduced filaments. The filaments, though in some cases exceedingly rudimentary, are always present. A similar instance of dwarf eggs with deformed filaments was reported by Mohr (1921) in Drosophila melanogaster in homozygous singed (sn) (Bristles and hairs twisted ) females; but he was unable to show that the egg alteration is due to a special gene linked to singed, owing to the complete sterility of the females. Filament has been isolated from the mutants Pointed and snapt. The question therefore, whether the Filament-condition is due to one of several effects of the genes responsible for Pointed and snapt, does not arise.

Filament resembles spheroidal as far as its effect on the general shape of the egg is concerned; but differs markedly from the spheroidal in the apparently normal size of the eggs, and in the comparatively high fecundity of the females. The fecundity of the Filament females in some instances approaches closely that of the wild type females. Moreover, spheroidal females are known to lay, particularly at the end of the laying period, pale yellowish transparent eggs, which are easily overlooked in the food. These eggs seldom, if at all, hatch. Such pale

FREQUENCY DISTRIBUTION OF PERCENTAGE OF ABNORMAL EGGS LAID BY FILAMENT FEMALES.




GRAPH I.

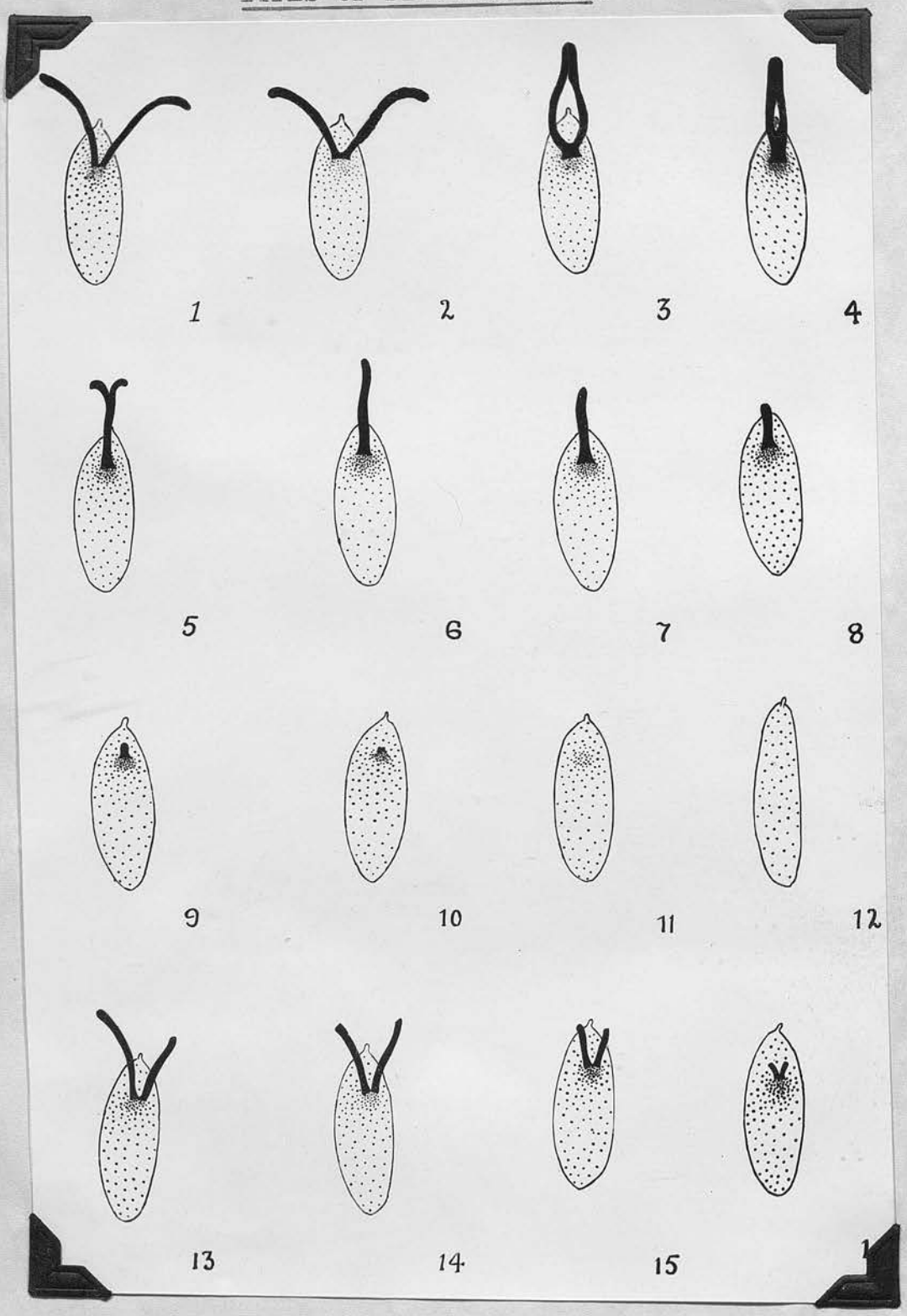
transparent eggs are not uncommon in Filament eggs; they are occasionally as frequent at the beginning of a laying period as at the end of it. One hundred and two tested eggs yielded forty-one flies (20 males, 21 females), showing that they are reasonably viable.

## DESCRIPTION OF "FILAMENT" EGGS.

The eggs laid by genetically "Filament" females are not all alike in appearance; only a few of the eggs laid by the original Filament female were really abnormal looking. The others looked normal to all external appearances. The percentage of abnormal eggs varied from female to female. On an average, about 50 per cent. of the total eggs laid by a Filament female were abnormal. The frequency distribution of the percentages of abnormal eggs laid by 41 Filament females obtained after 7-8 generations of in-breeding is given in Graph I.

The difference in shape between the normal and the abnormal eggs of Filament females is very pronounced; the "normal" eggs of Filament females look exactly like the wild type eggs. The wild type eggs

TYPES OF FILAMENT EGGS.




FIGS. 1 - 16.

of Drosophila pseudo-obscura resemble very closely the eggs of Drosophila melanogaster. The two filaments arise separately at the same level and are spread out side-ways towards the anterior pole of the egg. The abnormal eggs of Filament females showed a good deal of variation in the origin, shape, size and disposition of the two filaments. It was possible, however, to divide them into four different morphological categories according to the characteristics of their filaments. Thus we have (1) normal, (2) fused, (3) 1-filament and (4) no-filament eggs, laid by genetically Filament females.

(1) "Normal" (Fig. 1). These resembled very closely the wild type egg in external appearance.

(2) "Fused". Fig. 3 shows a typical "fused egg". Eggs in this class are marked by a latero-median fusion of the two filaments. The fusion may be slight (Fig. 2), confined to the bases of the filaments only, or nearly "complete" as in Fig. 5. Between the two extremes various degrees of "fusion" of the filaments can be noted. The free ends of the fused filaments usually stick together (Fig. 4).

(3) "1-filament". When the fusion of the two filaments is "complete"- involving the whole length of

the two filaments - 1-filament eggs are obtained (Fig. 6). The single filament so formed occupies a median position and resembles very closely the median filament in Drosophila quinaria, and Drosophila transversa, described by Sturtevant (1921). In thickness sometimes, it shows a greater resemblance to the median filament in Drosophila quinaria, being as thick as or slightly thicker than a normal filament. In some cases it is twice as thick as a normal filament and thus approaches more closely the condition obtained in Drosophila transversa. It may be equal to a normal filament in length (Fig. 6) or may show any degree of reduction in length to a mere vestige (Figs. 7-10).

(4) "No-filament". These eggs, unlike the wild type and the other Filament eggs, have a drawn out anterior end so that the posterior region is much broader than the anterior one (Fig. 12).

Besides the above mentioned types, eggs showing only reduction in size of one or both filaments have been met with. These eggs are rare, (Figs. 13-16).

This classification of the Filament eggs is wholly for descriptive purposes and represents the variation in expression of one and the same gene: 8331

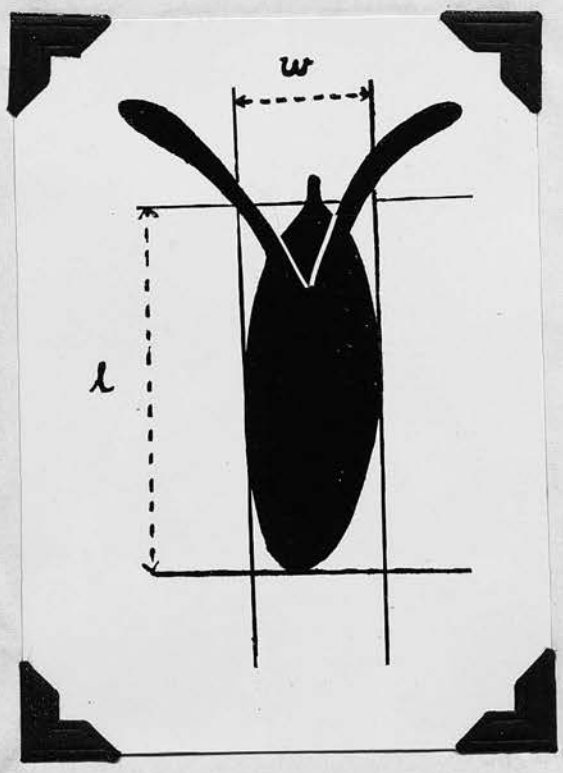
eggs collected from 21 females and graded according to their shape as above described, gave the following percentages of distribution.

| Grade. | No. of eggs. | P/C. of the total. |
|---|---|---|
| 1. "Normal" | 3247 | 38.97 |
| 2. "Fused" | 976 | 11.72 |
| 3. "One-filament" | 2297 | 27.57 |
| 4. "No-filament" | 1811 | 21.74 |

"Normal-looking" forms the highest percentage of the total eggs laid by a filament female. "Fused" forms the lowest percentage. The percentage of the "1-filament" and "no-filament" grade are approximately equal.

## EGG SIZE.

It has been shown by Crew and Auerbach (1937) that the spheroidal eggs are broader and shorter than the normal eggs. To see whether any significant difference in size exists between the Filament and normal eggs, measurements were made of the length and




l ..... length.

w ..... width.

FIG. 17.

width of 451 Filament and 150 normal eggs laid by 20 Filament and 10 normal females of varying ages. The definitions of these dimensions are given in Fig.17. The data obtained are summarised below.

The figures given here are in units of the Zeiss eye-piece micrometer, one unit corresponding to 14$\mu$.

RESULTS.

Length and width of normal and Filament eggs.

$M_l$ ... mean length

$M_w$ ... mean width

C.V. ... Coefficient of variability

$\sigma$ ... standard deviation.

| | $M_l$ | $\sigma_l$ | C.V.$_l$ | $M_w$ | $\sigma_w$ | C.V.$_w$ |
|---|---|---|---|---|---|---|
| Normal | 32.55 ± ·0952 | 1.73 | 5.31 | 12.15 ± ·0334 | .608 | 5.00 |
| Filament | 32.14 ± ·0802 | 2.54 | 7.89 | 10.80 ± ·014 | .466 | 4.31 |

The differences between the means of normal and Filament are - for length .41 : for width 1.35 . Both differences are statistically significant, showing that the Filament eggs are both narrower and shorter than the normal eggs.

## DIFFERENTIAL HATCHABILITY OF THE FILAMENT EGGS:

Is it conditioned by the shape of the eggs?

The fertility of the Filament females was found to be low, particularly when the percentage of abnormal eggs of extreme types (Figs. 9, 10, 12) was high. The suspicion therefore arose that probably such eggs never hatched. An experiment on a small scale was started to test this point. The result partly confirmed and partly disproved the above suspicions.

About fifteen well fed Filament females of approximately the same age were mated to their brothers, kept isolated from the females for some time (2-3 days) so as to ensure ready mating (D.l.S. No.5). All their eggs in the first few days of laying were collected on spoons, divided into the five different categories as normal, fused, 1-fil., etc., and separately incubated. After 36-48 hrs. the spoons were examined again and the unhatched eggs counted.

The following table gives the total number of eggs examined from each category, with their respective hatchability in percentages.

RESULTS.

| Grade. | No. of eggs kept. | No. of eggs hatching. | % of hatcha-bility. |
|---|---|---|---|
| 1. "Normal" | 605 | 491 | 81.16 |
| 2. "Fused" | 400 | 258 | 68.50 |
| 3. "1-filament" | 745 | 330 | 44.30 |
| 4. "1 fil-short" | 36 | 7 | 18.44 |
| 5. "No filament" | 459 | 0 | 0 |
| 6. Controls | 1153 | 1133 | 98.27 |
| Extreme "1-filament" eggs (Figs. 9 and 10) | | | |

As all the females used were of approximately the same age, well fed before mating, and only the eggs laid within the first few days of laying (4-5) were collected for testing, the possible effect of ageing of females on hatchability may be ignored. The results show a consistent drop in hatchability percentages, as the egg shape shifted further away from the normal. Thus "1-filament" were less viable than the normal looking ones, whereas none of the 459 "no-filament" eggs ever hatched. That the cause of the sterility was in the eggs themselves and not in any

environmental factors, such as total immersion of the eggs in the absence of the filaments, which apparently prevent their sinking, was shown by the non-hatching of the "no-filament" eggs collected on moistened blotting paper and smooth glazed non-yeasted food surfaces.

The results would therefore indicate a positive correlation between egg-shape and hatchability percentages. Such a relation has been observed among the eggs laid by pullets, where various authors (Dunn, 1922; Jull and Hayes, 1925; McClelland, 1931; Warren, 1934; Funk, 1934) have shown an inverse variation of the hatchability percentages with the departure of the egg size from the mean, but has not so far been reported in insects where the egg shape and size show less departure from the mean.

## EFFECT OF AGE ON THE HATCHABILITY OF FILAMENT EGGS.

The age of the females has a considerable effect on the hatchability of the Filament eggs; the eggs laid during the first few days being more fertile than

the eggs laid subsequently.

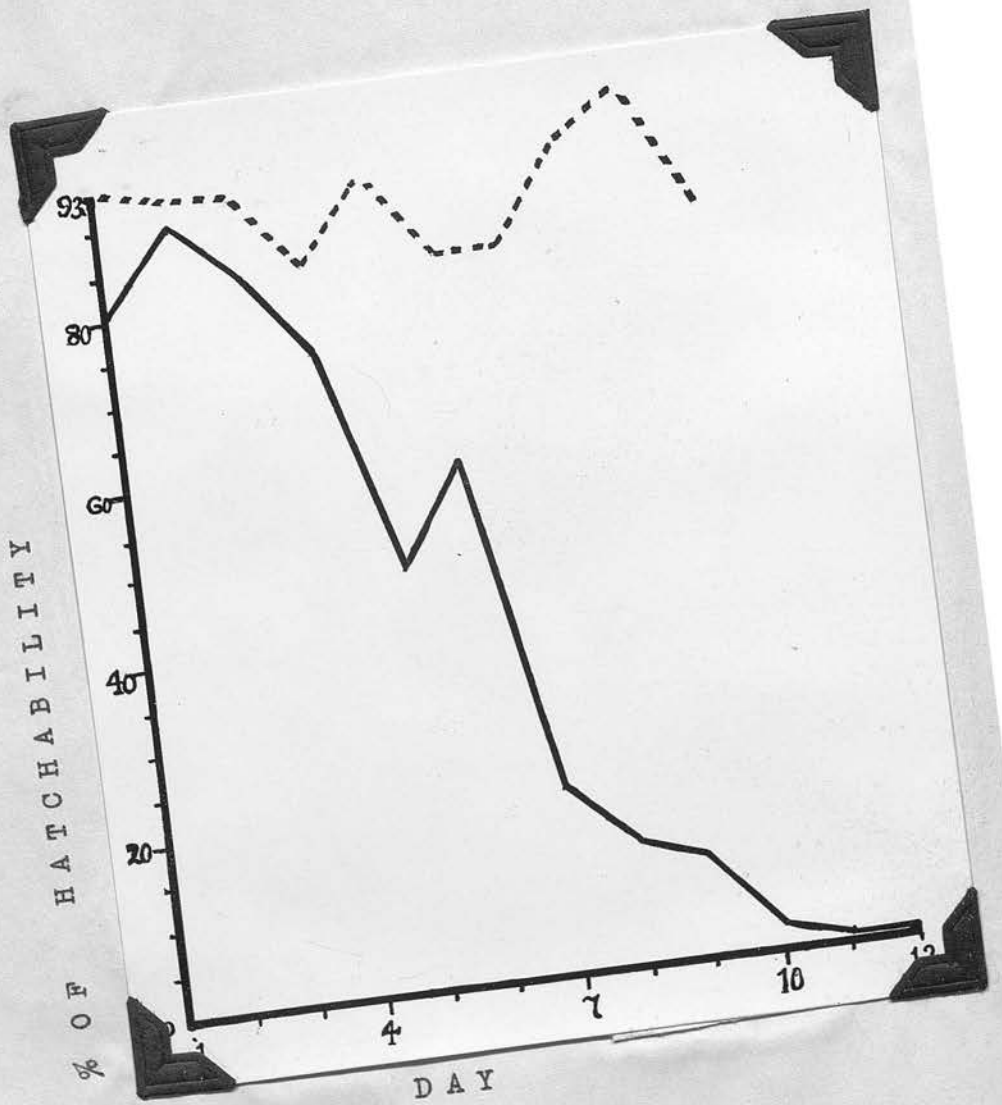
An experiment was devised to test this point. Filament and wild type females of the same age were mated to their brothers. ("Big Bear" wild strain was used as a control.) The technique used will be described later on. Eggs of seven available Filament females and nineteen control females were tested for about fifteen days. The females were kept separately. As their individual records showed no marked difference, only the total results are given in Table 1 and Table 2.

TABLE 2.

CONTROL FEMALES.

| Day | Total eggs kept. | No. of eggs hatched. | % of hatchability. |
|---|---|---|---|
| 1. | 723 | 673 | 93.08 |
| 2. | 399 | 365 | 91.48 |
| 3. | 1128 | 1026 | 90.96 |
| 4. | 610 | 502 | 82.30 |
| 5. | 386 | 353 | 91.50 |
| 6. | 837 | 686 | 81.50 |
| 8. | 790 | 648 | 82.03 |
| 10 | 363 | 336 | 92.56 |
| 12. | 129 | 126 | 97.67 |
| 14. | 360 | 301 | 83.61 |

GRAPH SHOWING THE VARIATION IN THE AVERAGE PERCENTAGE OF HATCHABILITY WITH AGE.




(F)

____ FILAMENT.

----- WILD TYPE.

GRAPH II.

The results are brought out graphically in Graph 11.

Though the number of females used was comparatively small, the results obtained are definitely significant. The Filament eggs started with a fairly high percentage of hatchability (100% is impossible due to the presence of the sterile "no-filament" eggs) but it dropped within the next twelve days to zero. The fall was rapid, the initial high percentage of hatchability never being regained. The controls, on the contrary, started with a high percentage of hatchability and maintained it throughout the period (14 days).

## GENETICAL.

Having established the differential hatchability of the Filament eggs, the problem remains whether the variation in shape observed amongst the Filament eggs is due to somatic or due to genetic causes. In other words, are the normal Filament eggs genetically Filament?

To answer this question, eggs collected from twenty (not virgins) orange Filament females remated to

Big Bear males were divided into the various categories as "normal", "1-filament", "fused", etc., and separately incubated. The emerging flies were expected to be of two kinds.-

1. Flies derived from eggs fertilised by orange sperms.

2. Flies derived from eggs fertilized by Big Bear sperms.

The former can be easily distinguished from the latter by their orange eye colour. The latter would have the wild type eye colour. Both the orange and the wild type daughters were tested for Filament.

If Filament was recessive, only the orange females would be expected to show that character. All the wild type females would be normal layers. If, on the other hand, Filament was dominant, Filament females would be found among both orange and wild type daughters. Secondly, if the normal Filament eggs may be genetically Filament, then some of the flies obtained from the normal eggs would be expected to be Filament (only some of the orange females, if Filament was recessive, or some from both orange and wild type flies, if Filament was dominant). The results obtained are given below.

RESULTS.

| Category | Orange | | Wild type | |
|---|---|---|---|---|
| | No. of ♀♀ tested | No. F. | No. of ♀♀ tested | No. F. |
| 1. "Normal" | 31 | 3 | 22 | 2 |
| 2. "Fused" | 22 | 10 | 3 | 1 |
| 3. "1-filament" | 8 | 6 | 16 | 3 |

Filament females were obtained in both the orange and wild type flies, showing that it was a dominant character; some of the flies obtained from normal eggs proved to be Filament, showing that the normal eggs may be genetically Filament. Filament females were also obtained amongst the flies from "fused" and "1-filament" eggs, showing further that the different types of eggs laid by a Filament female may all be genetically Filament.

## FAILURE TO GET AN HOMOZYGOUS STOCK.

In an attempt to get an homozygous stock, pair matings of tested Filament females with their brothers were made. The resulting daughters from each vial were separately tested. Vials yielding a high percentage of Filament daughters were selected for further breeding. In spite of the fact that this selective breeding was carried on for nearly twenty generations or more, no homozygous stock was obtained. The percentage could never be increased to more than sixty. Following is the result of a recent experiment in which flies from 57 cultures were tested for Filament.

RESULTS.

| No. of cultures. | Total flies examined. | No. of Filament flies. | % of Filament. |
|---|---|---|---|
| 57 | 322 | 177 | 54.97 |

The absence of Filament females amongst the F - 1 flies proves conclusively that the normal layers are genetically normal and not due to an overlapping of Filament with wild type. Filament is therefore proved to be a simple dominant.

## DIFFICULTIES OF LOCATING FILAMENT AND THE NEED OF A SPECIAL TECHNIQUE.

Filament like spheroidal offers no satisfactory means of distinguishing the genotype of the fly from a study of its external morphology. The character becomes visible in the females only from the structure of the eggs they lay, and in the males from the structure of the eggs laid by their daughters. To obtain information on segregation, therefore, it is necessary to test a very large number of individual females. The method now in vogue for collecting eggs in most of the Drosophila laboratories is Beadle's spoon technique. This method no doubt is very satisfactory in dealing with a comparatively small sample of females, but becomes excessively laborious when a large number of females is to be

tested in a reasonably short time. An economical technique which would enable one to handle a large number of females at the same time was therefore found necessary.

Spencer (1937 a, 1937 b) has recently shown that Drosophila females can lay eggs through a silk mesh on a suitable medium. His method consists of keeping the females in a metal hose gas ferrule ($\frac{7}{8}$"d.x$\frac{1}{2}$"h) covered at one end by a silk bobinette, the other end being closed by a cork. The silk net is brought into contact with the medium and the females are allowed to lay eggs through this mesh.

A modification of the above technique was employed which served the purpose admirably. Instead of the metal gas ferrule, two gram pill boxes were used. The bottom of the box was removed, as also the top of the lid, which gave two rings - a broad and a narrow one. The latter will slide over the former ring and acts as a ring to keep the mesh tight and in position. Both the rings were then soaked in paraffin wax to prevent them absorbing the moisture from the medium. The top was closed by an ordinary, non-paraffined pill-box lid, on which appropriate letterings or numbers could be written. Bridge's

food in large petri-dishes containing a fine suspension of yeast served as a medium for laying. The above method was also used in connection with the hatchability experiments, and in all experiments where the females were to be 'tested'.

CHROMOSOME OF FILAMENT.

The following scheme of work was adopted.

1. Pair matings of Filament flies were made.

2. The resulting females from each culture were tested separately for Filament. The culture that yielded the highest percentage of Filament females was retained and the rest discarded.

3. Thirty ♂♂ from this culture were mated individually to thirty v pr tg arr ♀♀ which had been previously tested to see that they contained no Filament.

4. F - 1 ♂♂ from each culture were kept separately from the females. Both the vials along with the original vial were kept held together by means of elastic rubber bands.

5. After four or five days, vials containing the

females were examined for Filament eggs.

6. Only the vials yielding the Filament eggs were retained for further study and the rest discarded. This precaution was undertaken in view of the fact that some of the P-1 males may be normal. Let the names of the retained vials be X-vials.

7. Thirty ♂♂ from each of the X-vials were back-crossed to virgin v pr tg arr ♀♀ known to be normal layers. These thirty vials were kept together by elastic rubber bands, giving as many vial groups as the number of X-vials.

8. F-2 ♀♀ from each group were collected <u>en masse</u> and separated into the various recombinations as v pr tg, v pr, etc., which they showed. At least ten ♀♀ of each recombination, from each group, were tested for Filament. The summary of the data obtained is given below.

If all the F-2 ♀♀ lay normal eggs the obvious conclusion will be that Filament is sex-linked. If, on the contrary, Filament females are obtained, then Filament must be situated on one of the autosomes.

RESULTS.

| Genotype. | Normal Layers. | Filament. |
|---|---|---|
| v | 57 | 36 |
| v arr | 73 | - |
| vpr | 31 | 28 |
| v tg | 48 | 26 |
| v arr pr | 37 | - |
| v arr pr tg | 45 | - |
| v pr tg | 41 | 13 |
| v pr arr | 55 | - |
| | 387 | 103 |

The presence of the Filament females shows that Filament is not sex-linked, and is situated on one of the autosomes. The absence of recombination of Filament with arristopedia shows that Filament belongs to the same group of mutants as arristopedia, i.e. the second group.

## FECUNDITY AND THE OVARIAN RHYTHM OF FILAMENT FEMALES.

The Fecundity of Filament females is lower than that of the controls (Big Bear). While a control female produced on an average a total of 906 eggs (average of ten females showing nearly equal "performances") in the course of the first fifteen days of laying, the total average output of the Filament female for the same period approximated to 767 eggs (average of twenty Filament females showing nearly equal "performances". The former took on an average of 22.4 actual laying periods (one period = 12 hrs.) to deposit 906 eggs, while the latter took a longer time (25.8 actual laying periods) to lay the lesser number (767) of eggs. This result is to be expected, if egg production is due (as suggested by Donald and Lamy, 1936) to the interaction of two independent factors, one determining the number of egg primordia formed at the apices of the egg strings, the other influencing the rate at which these primordia develop into eggs ready for fertilisation and laying. Thus a disturbance in the normal functioning of the ovary, as no doubt shown by the abnormal eggs produced by the Filament females,

may cause a corresponding disturbance in the "normal" functioning of the two factors which influence egg production. If the factors were inter-dependent we should obtain, when the total output of a female is reduced (as in Filament), a proportional reduction in the actual laying time.

The results obtained support this assumption. It would therefore appear that the two factors for egg production are independent variables.

"RHYTHM".

The characteristic phenomena of egg production known as the rhythm, which consists of periods of rapid or high laying followed by periods of little or no laying, recognised for the first time by Shapiro (1932) in Drosophila pseudo-obscura females and later on established by Dobzhansky (1935), was exhibited by all the Filament females under observation. As a quantitative method of determining this rhythm has recently been described by Donald and Lamy (1936), who confirmed the results of the above authors, it was thought that a study of the rhythm in Filament females might possibly throw further light on the processes of egg-formation and laying.

Accordingly, the following experiment was undertaken.-

METHOD.

Pair matings of the controls (Big Bear) and Filament were made. Flies were removed daily to fresh vials to prevent overcrowding. Sixteen pairs of control and sixteen pairs of Filament flies so obtained were allowed to lay eggs on paper spoons; these spoons were changed daily at 9.30 a.m. Eggs collected on a particular day (third) were divided into lots of 35 and distributed in vials (3¼ x 1") containing approximately 1" of standing Schmidt and Offermann Drosophila food (D.1.S. No. 5) prepared at the same time. The eggs were incubated at 23.5±.5°C. Of the hatching females, 19 controls and 27 filament of the same age were selected and without etherisation placed in 4" x 1" vials with one male in each vial. Subsequently dead males were replaced by fresh ones.

Spoons were prepared at least six hours before actual use, the spoons for the morning being prepared the previous night. All operations were carried out in the constant temperature room (23.5±.5°C). Great care was taken to give the least possible disturbance to the females when changing the spoons.

Spoons were changed twice a day (9.30 a.m. and 9.30 p.m.). Separate records of eggs laid by individual females were kept.

In measuring the rhythm, the method suggested by Donald and Lamy (1936) was adopted, as it would also afford an opportunity of verifying their results which were based on a smaller number of flies. The rhythm of individual females was sub-divided into its most obvious parts, namely the individual waves of which it was made up. The interval between the central points of the successive hollows of the rhythm - expressed in 12 hour units - gave the "length" of the wave for which the total number of eggs was known. With this method it is possible to follow the changes in the number of eggs elaborated by a female in a particular wave and the length of time taken to deposit them as wave followed wave and the females got older. It is to be remarked, however, as already pointed out by Donald and Lamy (1936), that this method is an arbitrary one (the real limits of a wave not being accurately determined), so that the result obtained could give only a rough idea as to the actual changes occurring in egg production. This however, should not reduce the significance of the results

obtained when we are comparing the egg production and laying of two sets of separate females. Thus in this particular experiment it is proposed to compare the rhythm in Filament females with the rhythm in the wild type females. The rhythm is found to exist in both the sets of females considered. An identical method is used to measure it in both cases. If a difference in rhythms does exist between a wild type female and a Filament female, it will be brought out even if the method used to detect such a difference is not absolutely accurate.

The results given below are the average performance of 41 r-h-filament and thirty-three control females. In the original experiment, only nineteen control and twenty-seven Filament females had been used. The egg records of the additional females included were obtained in a subsequent experiment and were in agreement with the earlier results. The records varied from 10-30 days, the majority of the females being kept for 15 days.

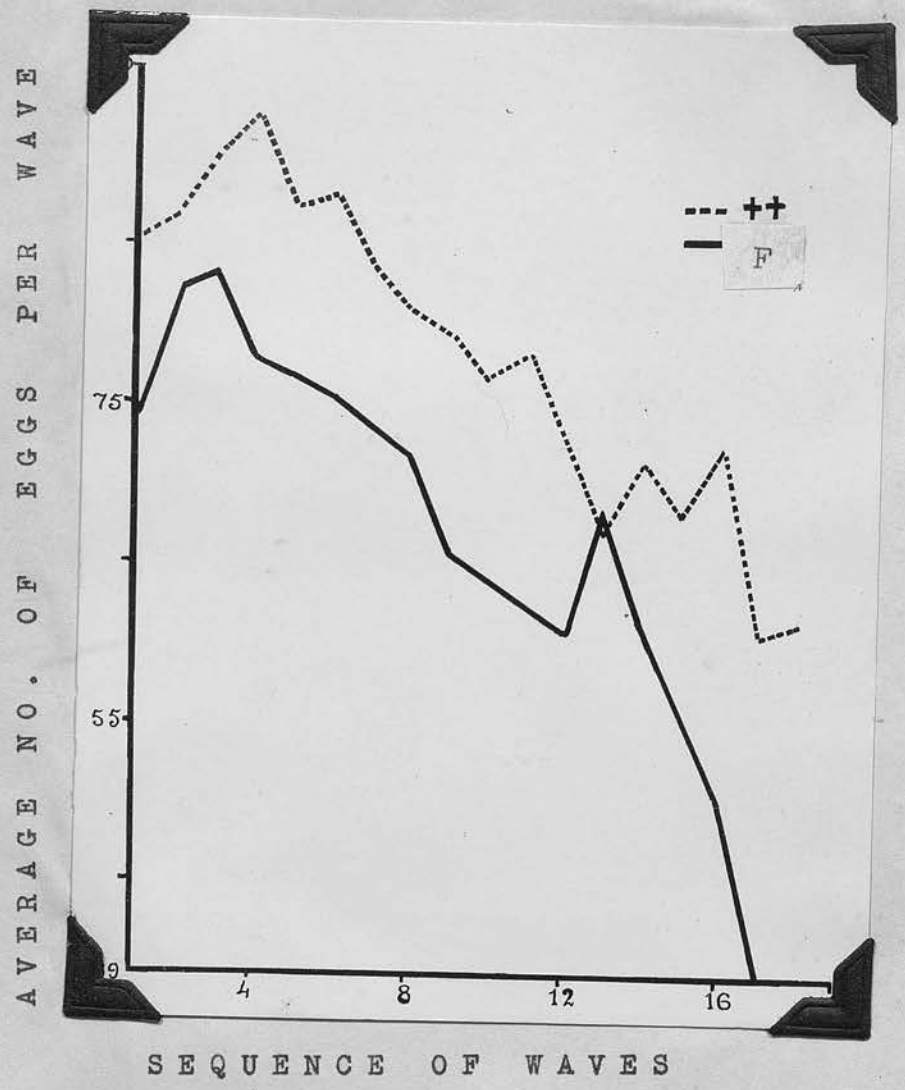
RESULTS.

TABLE 3.

CONTROL FEMALES.

| Wave No. | Total No. of eggs laid. | No. of females. | Aver. No. of eggs per wave. |
|---|---|---|---|
| 1. | 2611 | 33 | 79.12 |
| 2. | 2874 | 33 | 87.09 |
| 3. | 3309 | 33 | 91.18 |
| 4. | 3089 | 33 | 93.61 |
| 5. | 2818 | 32 | 88.06 |
| 6. | 2839 | 32 | 88.72 |
| 7. | 2696 | 32 | 84.25 |
| 8. | 2525 | 31 | 81.45 |
| 9. | 2403 | 30 | 80.10 |
| 10. | 2005 | 26 | 77.12 |
| 11. | 1225 | 16 | 78.44 |
| 12. | 583 | 8 | 72.63 |
| 13. | 268 | 4 | 67.00 |
| 14. | 287 | 4 | 71.75 |
| 15. | 273 | 4 | 68.25 |
| 16. | 291 | 4 | 72.75 |
| 17. | 243 | 4 | 60.75 |
| 18. | 185 | 3 | 61.67 |

CHANGE IN THE AVERAGE NUMBER OF EGGS PER WAVE WITH WAVE.




GRAPH III.

TABLE 4.

FILAMENT FEMALES.

| Wave No. | Total No. of eggs laid. | No. of females. | Aver. No. of eggs per wave. |
|---|---|---|---|
| 1. | 3081 | 41 | 75.15 |
| 2. | 3403 | 41 | 83.00 |
| 3. | 3432 | 41 | 83.71 |
| 4. | 3206 | 41 | 78.20 |
| 5. | 3168 | 41 | 77.27 |
| 6. | 3047 | 40 | 76.18 |
| 7. | 2667 | 36 | 74.08 |
| 8. | 2387 | 33 | 72.33 |
| 9. | 1919 | 29 | 66.17 |
| 10. | 1544 | 24 | 64.33 |
| 11. | 816 | 13 | 62.77 |
| 12. | 366 | 6 | 61.00 |
| 13. | 138 | 2 | 69.00 |
| 14. | 123 | 2 | 61.50 |
| 15. | 56 | 1 | 56.00 |
| 16. | 50 | 1 | 50.00 |
| 17. | 39 | 1 | 39.00 |

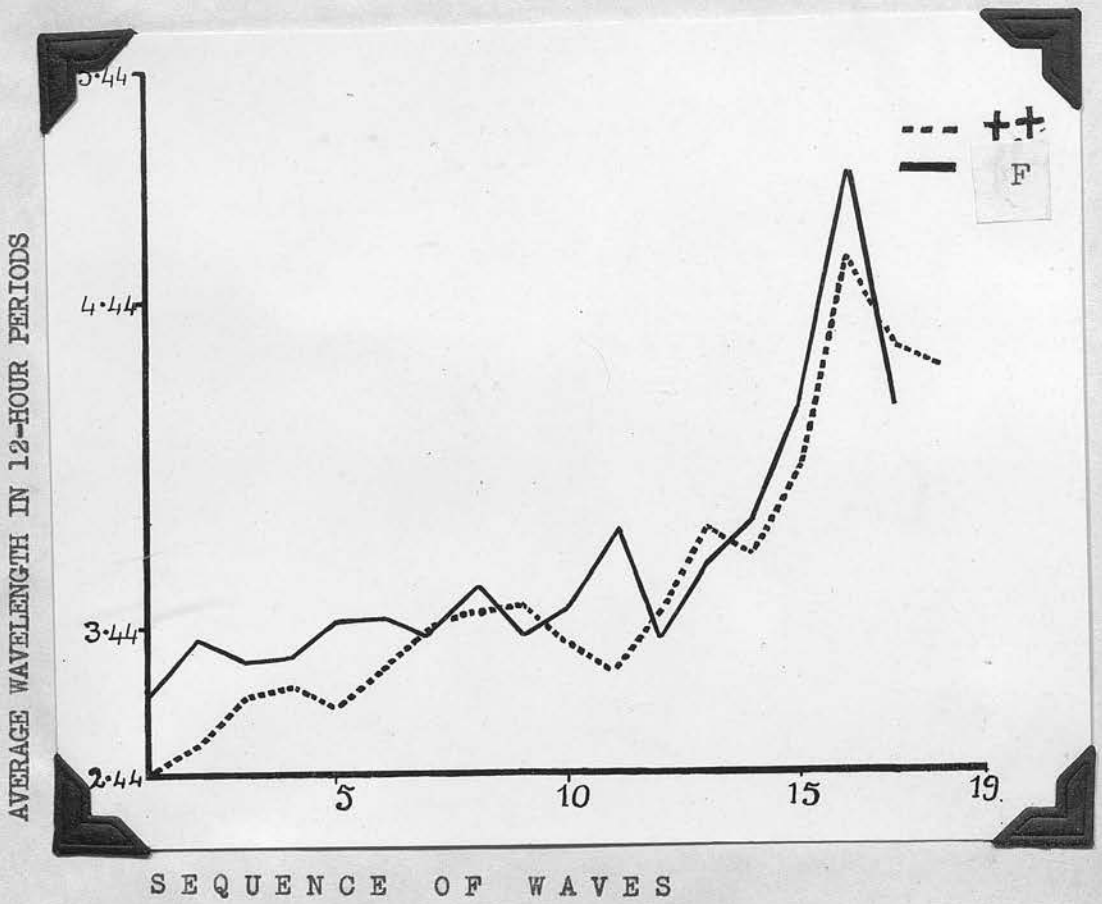
TABLE 5.

CONTROL FEMALES.

| Wave No. | Total wave length. | No. of females. | Aver. wave length. |
|---|---|---|---|
| 1. | 80.5 | 33 | 2.44 |
| 2. | 84.0 | 33 | 2.55 |
| 3. | 91.0 | 33 | 2.76 |
| 4. | 92.0 | 33 | 2.79 |
| 5. | 87.0 | 32 | 2.72 |
| 6. | 92.50 | 32 | 2.89 |
| 7. | 98.0 | 32 | 3.06 |
| 8. | 97.0 | 31 | 3.13 |
| 9. | 91.5 | 29 | 3.16 |
| 10. | 78.0 | 26 | 3.00 |
| 11. | 46.0 | 16 | 2.88 |
| 12. | 25.0 | 8 | 3.13 |
| 13. | 14.0 | 4 | 3.50 |
| 14. | 13.5 | 4 | 3.38 |
| 15. | 15.0 | 4 | 3.75 |
| 16. | 18.5 | 4 | 4.63 |
| 17. | 17.0 | 4 | 4.25 |
| 18. | 12.5 | 3 | 4.17 |

CHANGE IN THE AVERAGE LENGTH OF THE WAVE

WITH WAVE.




GRAPH IV.

TABLE 6.

FILAMENT.

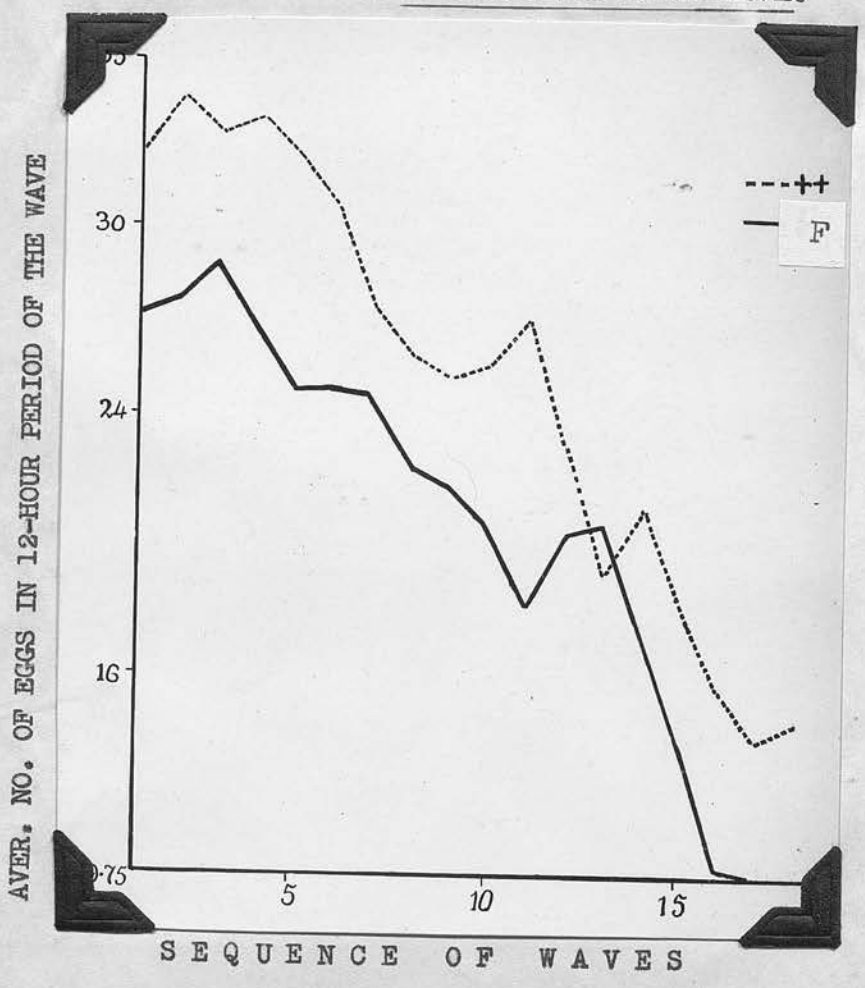
| Wave No. | Total wave length. | No. of females. | Aver. wave length. |
|---|---|---|---|
| 1. | 113.5 | 41 | 2.77 |
| 2. | 122.5 | 41 | 2.98 |
| 3. | 119.0 | 41 | 2.90 |
| 4. | 119.5 | 41 | 2.91 |
| 5. | 126.5 | 41 | 3.09 |
| 6. | 122.0 | 40 | 3.05 |
| 7. | 109.0 | 36 | 3.03 |
| 8. | 106.0 | 33 | 3.21 |
| 9. | 87.5 | 29 | 3.02 |
| 10. | 75.0 | 24 | 3.13 |
| 11. | 45.0 | 13 | 3.46 |
| 12. | 18 | 6 | 3.00 |
| 13. | 10 | 3 | 3.33 |
| 14. | 7 | 2 | 3.50 |
| 15. | 4 | 1 | 4.00 |
| 16. | 5 | 1 | 5.00 |
| 17. | 4 | 1 | 4.00 |

TABLE 7.

CONTROL.

| Wave No. | Aver. No. of eggs in a wave. | Aver. leng. of a wave. | Aver. No. of eggs per 12 hr. period within a wave. |
|---|---|---|---|
| 1. | 79.12 | 2.44 | 32.43 |
| 2. | 87.09 | 2.55 | 34.15 |
| 3. | 91.18 | 2.76 | 33.04 |
| 4. | 93.61 | 2.79 | 33.55 |
| 5. | 88.06 | 2.72 | 32.38 |
| 6. | 88.72 | 2.89 | 30.70 |
| 7. | 84.25 | 3.06 | 27.53 |
| 8. | 81.45 | 3.13 | 26.02 |
| 9. | 80.10 | 3.16 | 25.35 |
| 10. | 77.12 | 3.00 | 25.71 |
| 11. | 78.44 | 2.88 | 27.24 |
| 12. | 72.63 | 3.13 | 23.20 |
| 13. | 67.00 | 3.50 | 19.14 |
| 14. | 71.75 | 3.38 | 21.23 |
| 15. | 68.25 | 3.75 | 18.20 |
| 16. | 72.75 | 4.63 | 15.71 |
| 17. | 60.75 | 4.25 | 14.28 |
| 18. | 61.65 | 4.17 | 14.78 |

GRAPH SHOWING THE CHANGE IN THE AVERAGE NO. OF EGGS IN UNIT PERIOD OF THE WAVE WITH WAVE.




GRAPH V.

TABLE 8.

FILAMENT.

| Wave No. | Aver. No. of eggs in a wave. | Aver. leng. of a wave. | Aver. No. of eggs per 12 hr. period within a wave. |
|---|---|---|---|
| 1. | 75.15 | 2.77 | 27.13 |
| 2. | 83.00 | 2.98 | 27.85 |
| 3. | 83.71 | 2.90 | 28.87 |
| 4. | 78.20 | 2.91 | 26.77 |
| 5. | 77.27 | 3.09 | 25.01 |
| 6. | 76.18 | 3.05 | 24.98 |
| 7. | 74.08 | 3.03 | 24.45 |
| 8. | 72.33 | 3.21 | 22.46 |
| 9. | 66.17 | 3.02 | 21.91 |
| 10. | 64.33 | 3.13 | 20.55 |
| 11. | 62.77 | 3.46 | 18.14 |
| 12. | 61.00 | 3.00 | 20.33 |
| 13. | 69.00 | 3.33 | 20.72 |
| 14. | 61.50 | 3.50 | 17.57 |
| 15. | 56.00 | 4.00 | 14.00 |
| 16. | 50.00 | 5.00 | 10.00 |
| 17. | 39.00 | 4.00 | 9.75 |

Filament females started laying at approximately the same time as the wild type females (i.e. on the third day of adult life).

Change in the number of eggs per wave with successive waves is represented graphically in Graph III. The Filament and the wild type females show a very close similarity in the general trend of their respective curves. Both start with a fairly high initial production per wave, which increases in the subsequent waves to reach a peak production in the third (as in Filament) or fourth wave (as in the wild type). The number of eggs elaborated per wave afterwards shows a gradual decrease. "Wave-performance" of the Filament females, however, is lower than the corresponding "wave-performance" of the wild type females throughout the rhythm. If we take the wave performance of the wild type as "normal" then it would follow that the ovaries of the Filament females are working at a lowered activity.

Graph 4 depicts the change in the length of the wave with successive waves. Unlike the previous graph, wave length shows a continuous increase in length from the beginning. A close similarity is again observed between the curves of the Filament

females and control females. It can be seen that longer lengths are observed among the waves of Filament females.

Changes taking place in individual waves are brought out in Graph 5. A striking resemblance could be observed between these curves and the curves showing the change in the number of eggs per wave with successive waves (Graph 3). Average egg production per 12-hour unit within the wave, in both the controls and Filament females, is fairly high for the first wave; it increases in the subsequent waves to attain the peak in the second (as in controls) or third (as in Filament) waves. The average number of eggs per unit period in the wave afterwards drops down gradually in the succeeding waves. The drop does not occur earlier than the corresponding drop in Graph 2 as reported by Donald and Lamy (1936), but at the same time. Again Filament females gave throughout a lesser number of eggs per unit period within the wave, than the wild type females. The changes occurring per unit period within a wave, therefore, are exactly the same as the changes occurring in the number of eggs in the waves as a whole. A concrete example from data will make this point clear.

A look at the initial, highest and lowest production in Filament and wild type females, shows that.-

FILAMENT:

| Wave | Aver. No. of eggs per wave. | Aver. wave length. |
|---|---|---|
| 1 (initial) | 75.15 | 2.77 |
| 3 (highest or peak) | 83.71 | 2.90 |
| 17 (lowest) | 39.00 | 4.00 |

If we divide the initial wave production (75.15) by the time taken to lay (2.77), we get the number of eggs laid in unit period:

$$\frac{75.15}{2.77} = 27.13$$

(This does not mean that 27.13 eggs are laid per period (12 hours) and is done only for comparative study.)

In the peak wave production 83.71 eggs are laid, showing that the ovaries are more active during that particular wave, if so we should get a higher number

of eggs per unit-period:

$$\frac{83.71}{2.90} = 28.87,$$ which is exactly the case.

In the minimum wave production, it appears that the ovaries during that particular wave are at their minimum activity and a minimum number of eggs per unit period are expected:

$$\frac{39}{4} = 9.75,$$ which is exactly the case.

Similar results are obtained in the controls:

CONTROLS:

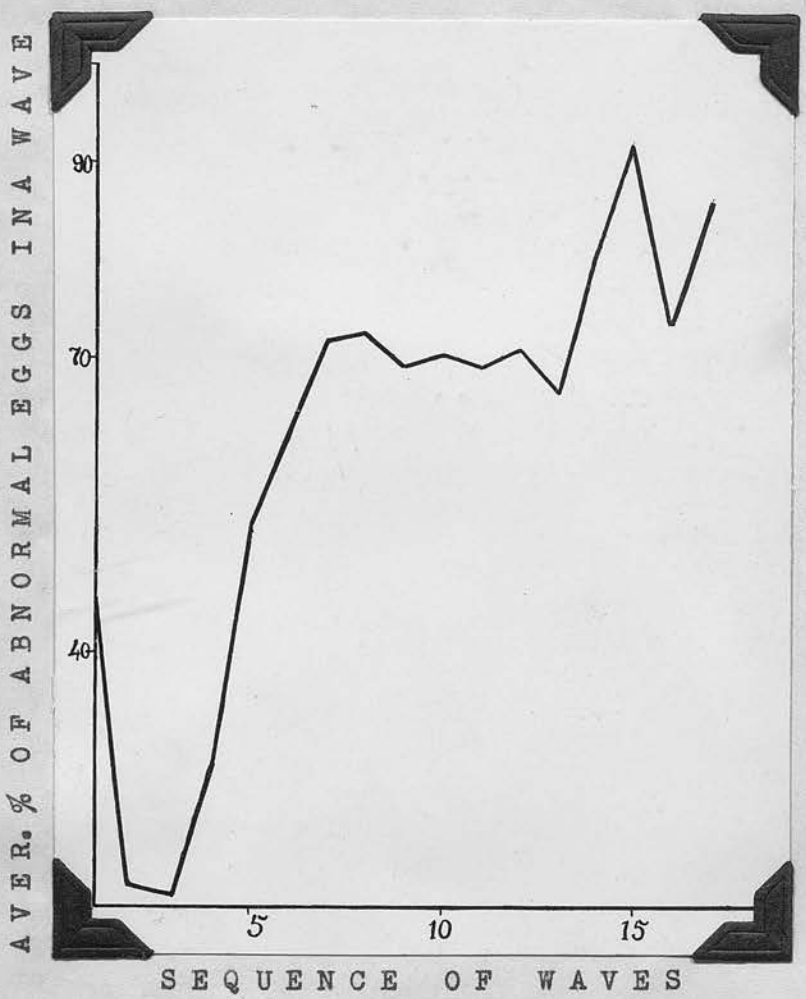
| Wave | Aver. No. of eggs in a wave. | Aver. wave length. | Aver. No. of eggs per unit period in a wave. |
|---|---|---|---|
| 1 (initial) | 79.12 | 2.44 | 32.43 |
| 4 (peak) | 93.61 | 2.79 | 33.55 |
| 17 (minimum) | 60.75 | 4.25 | 14.28 |

The changes taking place in the number of eggs laid per 12 hrs. within each wave therefore are exactly the same as the changes occurring in the number of eggs produced in a wave as a whole.

## CHANGE IN THE PRODUCTION OF % OF ABNORMAL LOOKING EGGS IN A WAVE WITH SUCCESSIVE WAVES.

As a count of abnormals had been noted as well as the total egg count it was possible to investigate the changes that occur in the percentage of abnormal eggs produced as the fly aged. The results are given below.

GRAPH SHOWING THE VARIATION OF THE PERCENTAGE OF ABNORMAL EGGS WITH WAVE.




GRAPH VI

RESULTS.

| Wave No. | Aver. No. of eggs in a wave. | Aver. No. of abnor. males in a wave. | % of abnors. in a wave. |
|---|---|---|---|
| 1. | 75.15 | 34.29 | 45.63 |
| 2. | 83.00 | 13.41 | 16.16 |
| 3. | 83.71 | 12.51 | 14.94 |
| 4. | 78.20 | 21.59 | 27.61 |
| 5. | 77.27 | 40.10 | 51.90 |
| 6. | 76.18 | 53.88 | 70.73 |
| 7. | 74.08 | 53.50 | 72.22 |
| 8. | 72.33 | 52.63 | 72.73 |
| 9. | 66.17 | 46.07 | 69.62 |
| 10. | 64.33 | 45.45 | 70.65 |
| 11. | 62.77 | 43.62 | 69.49 |
| 12. | 61.00 | 43.50 | 71.31 |
| 13. | 69.00 | 46.00 | 66.67 |
| 14. | 61.50 | 51.50 | 83.74 |
| 15. | 56. | 52 | 92.86 |
| 16. | 50 | 37 | 74.00 |
| 17. | 39 | 34 | 87.18 |

The percentage of abnormals is fairly high in the first wave. It drops to a minimum in the succeeding waves, thereafter increasing consistently with age, so that as the fly gets aged, a greater percentage of abnormal looking eggs are produced.

## F E R T I L I T Y.

### 1. Fertility of Filament females mated to their brothers.

Eggs of twelve Filament and fifteen control females from the original experiment were tested for 'fertility' (the number of adult flies obtained). Eggs of individual females were separately kept. Not more than 50 eggs were placed in a vial. As care had been taken to keep the eggs belonging to a particular batch as far as possible in separate vials, it was found possible to follow the fertility of the females with reference to the successive batches of eggs they laid. The results are given below.

RESULTS.

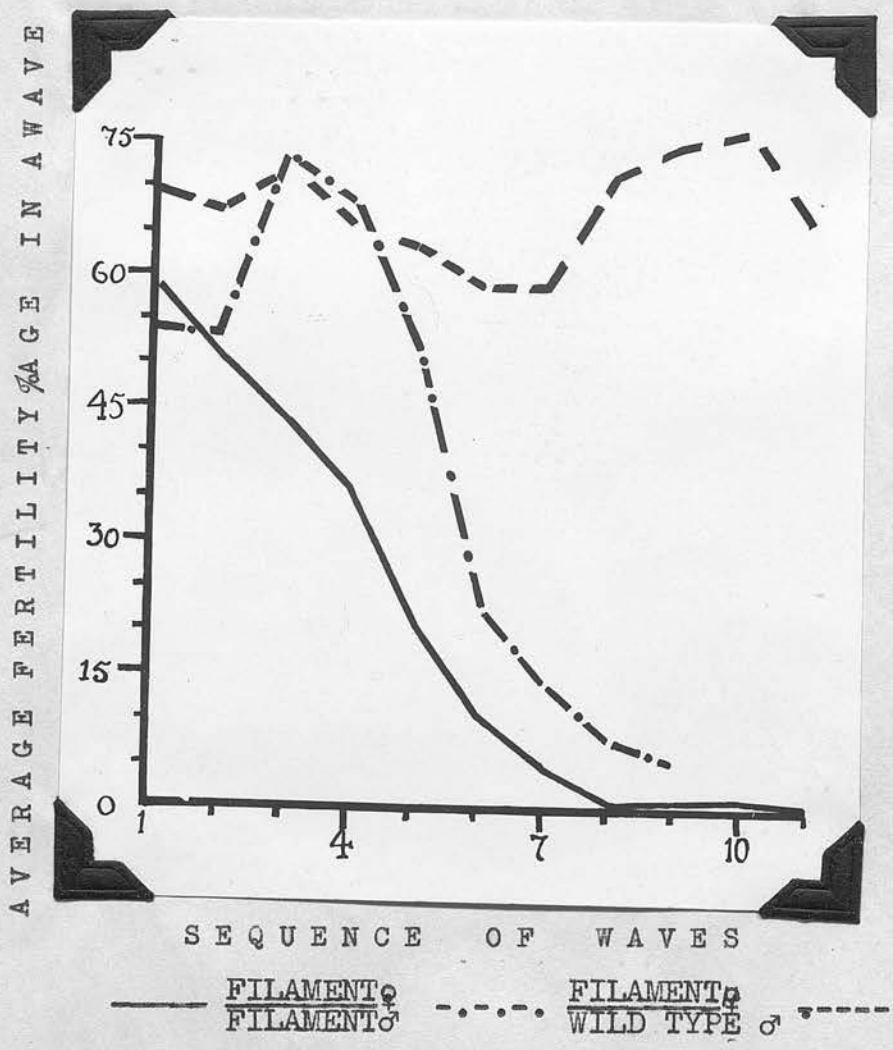
CONTROLS.

| Wave No. | Aver. No. of eggs kept. | Aver. No. of adult flies obtained. | % of fertility. |
|---|---|---|---|
| 1. | 78.26 | 54.14 | 69.18 |
| 2. | 91.26 | 60.93 | 66.76 |
| 3. | 91.00 | 64.86 | 71.27 |
| 4. | 100.33 | 65.79 | 65.57 |
| 5. | 91.90 | 57.70 | 62.79 |
| 6. | 92.00 | 54.00 | 58.70 |
| 7. | 85.00 | 49.93 | 58.74 |
| 8. | 83.60 | 59.14 | 70.74 |
| 9. | 81.14 | 60.29 | 74.30 |
| 10. | 74.50 | 56.92 | 76.06 |
| 11. | 73.30 | 49.00 | 66.81 |
| 12. | 53 | 48.00 | 81.61 |

FILAMENT.

| Wave No. | Aver. No. of eggs kept. | Aver. No. of adult flies obtained. | % of fertility. |
|---|---|---|---|
| 1. | 83.90 | 48.83 | 58.20 |
| 2. | 89.00 | 45.17 | 50.75 |
| 3. | 88.33 | 38.00 | 43.05 |
| 4. | 87.83 | 31.33 | 35.67 |
| 5. | 79.33 | 16.00 | 20.17 |
| 6. | 79.50 | 7.91 | 9.95 |
| 7. | 66.92 | 2.91 | 4.35 |
| 8. | 66.00 | 0.09 | 0.14 |
| 9. | 60.60 | 0.22 | 0.36 |
| 10. | 66.00 | 0.29 | 0.44 |
| 11. | 68.17 | - | - |
| 12. | 55.67 | - | - |

GRAPH SHOWING THE VARIATION OF FERTILITY WITH THE WAVE.




GRAPH VII.

The fertility of the Filament eggs laid in the first batch is fairly high; but falls rapidly with the successive batches so that at the end of the ~~end of the~~ tenth wave the fertility is zero. The controls, on the contrary, maintain a high percentage of fertility throughout the period for which they are kept.

The results are shown graphically in Graph Vll.

The graphs show a close resemblance to the graphs obtained for 'hatchability' though the hatchability percentages are greater than the corresponding percentages of fertility, which show that some of the larvae that hatch do not live up to the adult stage.

2. Fertility of the Filament females mated to wild type males.

When the above experiment was repeated with ten Filament females, using the wild type males for mating, the following results were obtained.

RESULTS.

| Wave No. | Total No. of eggs kept. | Total No. of flies obtained. | % of fertility. |
|---|---|---|---|
| 1. | 438 | 234 | 53.42 |
| 2. | 659 | 346 | 52.42 |
| 3. | 606 | 442 | 72.94 |
| 4. | 485 | 322 | 68.45 |
| 5. | 457 | 235 | 51.42 |
| 6. | 553 | 125 | 22.60 |
| 7. | 507 | 72 | 14.20 |
| 8. | 407 | 31 | 7.62 |
| 9. | 298 | 16 | 5.37 |
| 10. | 44 | 7 | 1.59 |

As before, the fertility was fairly high to start with; but instead of dropping with successive batches of eggs laid as in the previous experiment, it increased rapidly in the next two batches, maintained a high level of fertility for a further period of two waves and then started to drop rapidly, so that at the end of the ninth wave the fertility was practically nil.

The rapid drop in fertility after the 5th wave , may at first be supposed to be due to the effect of age; especially, since it has been shown elsewhere, that the percentage of abnormal eggs in a wave increases with age. This interpretation seems improbable, as a look at the data given below will show, though it cannot be totally denied that such an effect is absent.

TABLE COMPARING THE % OF ABNORMAL EGGS IN A WAVE AND THE % OF FERTILITY OF THAT WAVE.

| Wave No. | % of ab. eggs in a wave | % of fertility of the eggs in a awave. |
|---|---|---|
| 5. | 51.90 | 51.42 |
| 6. | 70.73 | 22.60 |
| 7. | 72.22 | 14.20 |
| 8. | 72.73 | 7.62 |
| 9. | 69.62 | 5.37 |
| 10. | 70.65 | 1.59 |

Whereas the % of abnormal eggs remain more or less the same from the 6th to the 10th wave, the corresponding fertility decreases sharply. The effect of age therefore, is inadequate to explain the rapid drop in fertility completely. A "progressive abnormality of the ovary" of the Filament females seems to me to be

the only assumption that fits in with the results obtained. The presence in the older batches of eggs, greater % of abnormal eggs and a larger number of extreme types of abnormal eggs, lend support to this idea. Filament therefore, may be described as a "progressive abnormality" of the ovary.

The higher % of fertility obtained when the Filament females were fertilised by wild type males than when they were fertilised by their brothers, suggest that possibly the homozygous Filament flies die, either during the larval or pupal stages. Filament female by Filament male, on the above assumption is expected to give about3/4 of the offspring obtained when Filament is fertilised by wild type male. The total fertility obtained in the two cases is given below. As no flies are obtained after the 10th wave the eggs after the 10th wave are not considered in calculating total fertility.

| Cross | Total fertility |
|---|---|
| Fil.♀. ♂ | 35.0 % |
| Fil.♀.Fil.♂ | 22.3 % |
| Difference .. | 12.7 % |

The result conformsto the expection.

GENERAL CONSIDERATIONS.

Sturtevant (1921) has noted the remarkable diversity and constancy in the number and size of the filaments in the different species of Drosophila.

The number varies from 0 to 10. He found as many as 10 filaments in the eggs of Chymomyza amoena, whereas Chymomyza procnemis showed usually 8 filaments. The eggs of the other species showed either 2 filaments (D. affinis, D.caibbea, D. earlei, D.pseudo-obscura, D.simulans), 3 filaments (D.quinaria, D.transversa), or 4 filaments (D.busckii, D.cardini, D.funebris, D. hydei, D. immigrans, D. putrida, D. repleta, D. robusta, D. similis, D. torrei, D. tripunctata, D. virilis). Eggs of Scaptomyza exhibited a reduction in size of their 2 (S. graminum) or 4 (S.adusta) filaments. All the above characteristics, though apparently different were constant and characteristic of the species described.

A study of Filament would seem to indicate one possible process in the evolution of these filaments in geological times. Thus the 10 filament type (Chymomyza amoena) may be considered as a "fundamental", from which all the varying number of filaments could be derived, through a process of "total'fusion" or "reduction" or both. Complete fusion of one or more pairs of the 10 filament type would

give rise to the 8 and then to the 4 filament types. The 2 filament type must have arisen from the 4 filament type by the complete fusion of the 2 filaments of the same side. It is to be emphasised however that the sequence given here is purely imaginary; the only important fact established as aresult of the study of Filament is the role of the processes of "total fusion" and "reduction" in the evolution of the number of filaments.

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## SUMMARY

1. Filament forms the first dominant eggs mutation to be reported in Drosophila. As yet, no satisfactory means of determinigg its genotype from the study of the external morphology of the fly has been discovered. Like many known dominant mutations, it is lethal when homozygous. When present in the heterozygous condition, it lowers the total fecundity of the females and the size of the eggs they produce. Its main visible effect however, is on the two filaments - causing a variable amount of their fusion and reduction in size. Hatchability tests show that aging of the females lessens the viability of the eggs produced - the older the females the less viable are their eggs. When the effect of age is practically nil, namely, during the first 3 or 4 days of laying, the viability of the Filament eggs is conditioned by the structure of the eggs. The more extreme the departure of the egg structure from the normal, the less viable the eggs become. Viability of the eggs with no filaments is zero.

2. A comparison of the hatchability percentages with the fertility (no. of adult flies obtained) percentages as also the larger number of adult flies obtained when Filament female was fertilised by wild type male than when it is fertilised by Filament male, suggest that possibly Filament is lethal when homozygous

and that the lethal effect acts probably during the larval or pupal stages.

3. The shape of the egg is not necessarily determined by the genetic possibilities of the embryo. Filament flies have been obtained from apparently anormal Filament eggs. The gene for Filament is on the second chromosome.

4. A study of the fecundity and ovarian rhythm in the normal and Filament indicates the presence of a rhythm (periods of high laying with periods of little or no laying) in both; but fewer number of eggs per wave are developed in Filament. The curve for the average number of eggs produced per batch with successive waves is one of first rapid increase followed by a gradual decrease, the usual curve for egg-production. The curve for the average time taken to lay these eggs in terms of 12-hr units with successive waves, follows on the contrary, a linear trend. These results substantiate s the assumption made by Donald and Lamy (1937) that egg-production is due to the inter-action of two factors - one determining the number of egg-primordia formed at the apices of the egg-strings, the other, influencing the rate at which these primordia develop into eggs ready for fertilisation and laying. The different nature of the two curves show these factors to be independent.

5. The presence of the ovarian rhythm, lower number of eggs produced per wave and the longer time taken to lay them, point to the conclusion that the effect of Filament on egg-production, is one of lowering the normal activity of the ovary; in other words, reducing the efficiency of the mechanism of egg-production and laying. As this mechanism, as already shown, is dependent on two factors, say factor A (determing the number of eggs produced per wave) and factor B (determining the rate of development into ripe eggs), a corresponding repercussion in their normal rate of activity is also expected. Thus there will be a reduction in the rate of activity of the two factors. Reduction in factor A activity will lead to the production of lower number of eggs per wave; reduction in factor B activity will lead to the lowering of the rate of laying- lengthening the total period taken for laying. This has been shown to be exactly the case in Filament.

6. The study of Filament has established at least 2 processes that went to evolve the number of filaments in insects, esp., Drosophiladae. One is "total fusion" and the other is "reduction" of the filaments.

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II.

# THORAX - A MUTANT IN DROSOPHILA PSEUDO)-OBSCURA.

CONTENTS.

## INTRODUCTION

Amongst the progeny of pair matings of Minute flies, several males and females with unequal wings were observed by Miss R.Lamy, in Feb. 1937. These when bred together gave rise to a stock with a high percentage of abnormal flies. Careful study of the abnormals, showed that the irregularities were not confined to the wings alone, but a small percentage of flies also appeared showing defects of the thorax and legs. It was decided therefore, to call this new mutation "thorax" (th).

## DESCRIPTION

The visible effects of thorax are on the general structure of the thorax and its appendages, and may thus be classified under 2 heads.

1. Effects on the thorax.
2. Effects on the appendages.

The latter may be further considered under 2 sub-heads.

a. Effects on the wings and halters.

b. Effects on the legs.

1. The effect on the thorax is less constant than on the wings; only a small percentage of the thorax flies show defective thorax. In less

extreme cases of defective thorax, a meadian groove may be seen on the dorsal surface of the thorax dividing it into 2 halves. The groove may be deep with the 2 halves quite separate. In extreme case s, one of these halves may be reduced in size, or may be completely absent, with the result that the head droops over the side with the missing half. Deformities in structure can also be noticed amongst the micro- and macro-setae of the thorax. The former may show stunted growth, whereas, the latter are frequently bent at an angle - not unlike thoracic bristles in "blunt" (bl) of D. pseudo-obscura.

Defective thorax is quite common among st the offspring of X-Rayed males of Drosophila melanogaster and has been described by Morgan, Bridges and Sturtevant (1925) under "abnormalities that are not inherited".

2.

a. Wings

Perhaps the most remarkable peculiarities occur in the wing - the size, shape and structure of which may be appreciably altered. Ordinarily only one of the wings is affected. Instances of both the wings being affected are not quite as numerous. In the simplest instances, shape and structure remain normal and only the size

MICROPHOTOGRAPH OF A DUPLICATED WING.

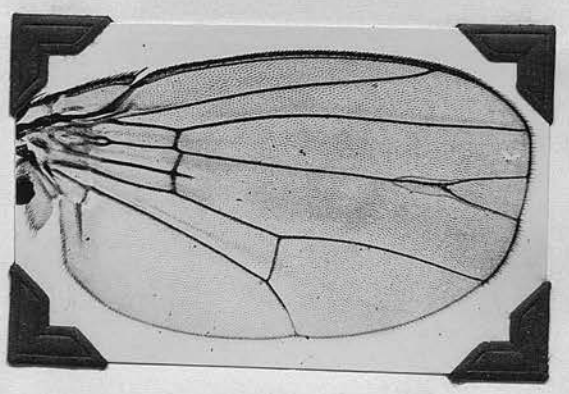

FIG. 1

MICROPHOTOGRAPH OF A "MIRROR IMAGE" DUPLICATED WING.

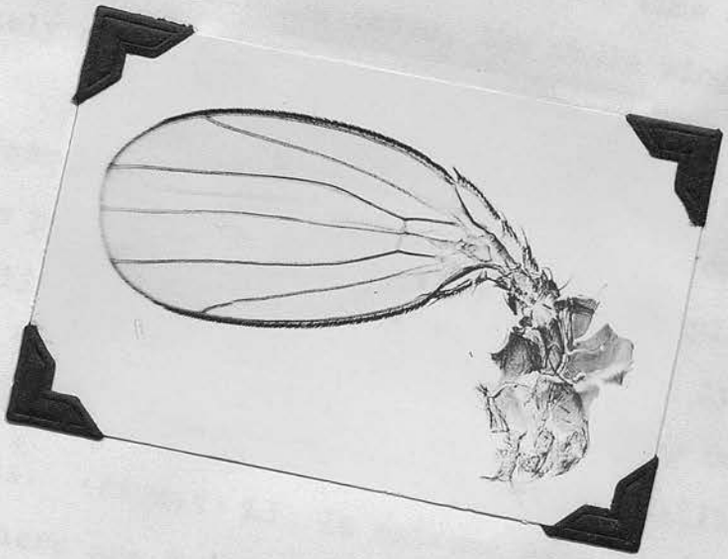

FIG. 2

of the wing is modified. The modified wing is held slightly raised up at an angle, and can be easily distinguished from the normal flies. The wing may be distended with fluid only, presenting an inflated cushion-like appearance or with fluid and air bubbles giving a blistered appearance. The general shape and size of the wing may at the same time change profoundly. In extreme cases, the whole wing is completely absent.

Modification in wing - structure may take the form of presence of extra veins, or of duplication in parts or of the whole wing. The duplication when slight is confined to a vein or two. It may extend to the main roots of the vein and may cause duplication both in the radius and in the axillary sclerites. (Figs.1 & 2.) In extreme cases of duplication there are 2 wings, a. growing separately side by side, facing the same direction and joined only at the proximal portion. (Fig.1), or b. 2 wings not growing separately, but facing each other, one half being the mirror image of the other half and having a comman outer margin. (Fig.2) This type of duplication may be termed "mirror image duplication" as distinct from the other ordinary "duplication". I have come across in this stock of an extraordinary instance of "triple" wing. The wing belongs to th e

MICROPHOTOGRAPH OF A "TRIPLE" WING DUPLICATION.

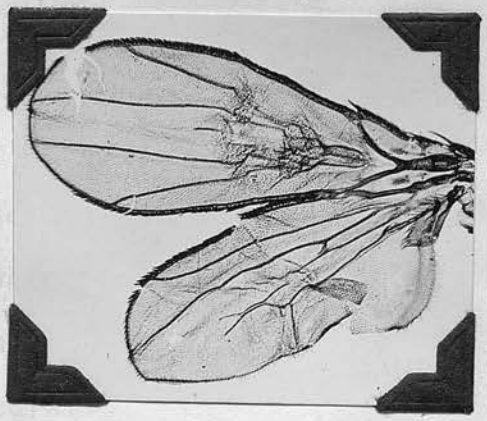

FIG.3

to the first order of extreme duplications, with 2 distinct wings, the posterior being an ordinary wing and the anterior a "mirror image" duplicated wing. (Fig.3)

Duplications may occur in one or both the wings. Duplicated wings are larger than the normal wings and are held in an out-stretched position.

Halteres.

One or both the halters may be absent. In some instances they are enlarged - resembling the character "enlarged balancer" or held drooping down resembling the character "balancer down" of D. pseudo-obscura.

b. Legs.

Very rarely flies are obtained with one of the third pair of legs missing. Apart from this , no other visible leg abnormality has been observed.

All the above characters are inherited together. All attempts at getting pure stocks of one or other of the many abnormalities free from the rest of the abnormalities have, so far, failed. It is possible therefore, that all the characters observed are due to one and the same gene.

## GENETICAL

Thorax ♀♀ when mated to wild type ♂♂ yielded all normal individuals. When an F $_{-2}$ was raised, thorax again appeared in the offspring, proving that it is a simple autosomal recessive.

## FAILURE TO GET A "PURE" BREEDING STOCK.

Though selective inbreeding has been going on for nearly a year and a half, a pure breeding thorax stock is not yet available. A certain percentage of normal flies invariably appears amongst the progeny. The percentage no doubt varies from culture to culture, but on an average, about 30% of the flies obtained from the pair matings of thorax are normal. The record of flies obtained from 55 pair matings of thorax, is given below.

## RESULTS.

| No. of cultures. | Total number of flies examined. | Total number of th. flies obtained. | % of th. flies. |
|---|---|---|---|
| 55 | 3425 | 2445 | 71.39 |

ARE THE NORMAL FLIES REALLY NORMAL OR ARE THEY THE RESULT OF OVERLAPPING OF THORAX WITH WILD TYPE ?

To test this point, virgin normal and thorax females from stock were isolated and mated to brothers having the same appearance and their off - spring separately recorded. The results are tabulated below :-

1. Result of pair matings of normal flies from thorax stock.

| No. of cultures. | Total number of flies examined. | Total number of th. flies obtained. | % of th flies |
|---|---|---|---|
| 19 | 1346 | 956 | 71.03 |

2. Result of pair matings of thorax flies.

| No. of cultures. | Total number of flies examined. | Total number of th flies obtained. | % of th flies. |
|---|---|---|---|
| 36 | 2079 | 1489 | 71.62 |

The percentage of thorax flies obtained in both the cases is more or less the same, showing that the normal flies found in thorax stock are genetically thorax.

LOCATION OF THORAX (th).

In a prelimnary experiment carried out to find the chromosome of thorax, thorax ♂♂ were out crossed to v pr tg arr ♀♀ and an $F_2$ raised. Out of the 1789 $F_2$ offspring (817 ♂♂; 972 ♀♀) examined, no recombination of thorax with pr was obtained. It was assumed therefore that the gene for thorax be - longs to the same group of mutants as pr - namely the third group.

Location of thorax was carried out by first making up a stock - orange plexus thorax (or px th); or px th ♂♂ were then crossed to wild type virgin ♀♀, and the normal $F_1$ ♀♀ backcrossed to or px th ♂♂ from stock.

The recombination data obtained is given below: -

Cross.

$$\text{or px th } ♀ \times \frac{\text{or px th}}{\text{or px th}} ♂$$

(26 pair matings).

| | Genotype | ♂♂ | ♀♀ |
|---|---|---|---|
| Non cross overs. | or px th | 398 | 443 |
| | Wild type | 450 | 527 |
| Single cross overs. | or | 102 | 99 |
| | px th | 79 | 99 |
| | or px | 25 | 27 |
| | th | 3 | 8 |
| Double cross overs. | or th | 1 | - |
| | px | 1 | 5 |

As only 71.39% of the thorax flies look phenotypically thorax, correction had to be made in considering the percentage of recombinations of thorax with or and px. The corrected recombination of thorax with or is 22.4% and with px 3.1%. px gives with or 17.5% recombination, which means that th is to the right of px and the order of the genes is or px th

Thorax (th) is therefore, located at 22.4 units to the right of or, i.e. if or is at zero. But recent work of Bhattacharya (1938) had placed or at 1.0, which would mean that th is at 23.4 on the lll chromosome.

## DISCUSSION

Many genes are now known in Drosophila that produce changes in more than one character. Mullar's classical analysis of the "truncate" wing series in Drosophila melanogaster, has shown that different mutant genes at the same locus, may cause either a shortening of the wing, an eruption on the thorax, a lethal effect, or any combination of two or more characters. Dobzhansky (1927) noted that ten out of the twelve different mutants differing in characters as eye-colour, wing-size, etc., of D. melanogaster examined for shape of spermathecae, showed distinct differences. Similar manifold effects of a single gene were observed by Savelieu (1928) in vestigial flies of D. melanogaster; the vestigial flies had reduced halteres, modified location of post-scutellar bristles, decreased productivity, another variability of the number of egg-tubes and delayed development. Recent work of Prabhu (1938) on the egg-mutant "Filament" in D. pseudo-obscura, has shown that an alteration in the morphological character, egg-structure, is attended by such physiological changes as lowered productivity, poor hatchability and fertility as compared with the normal. The foregoing data on "thorax" support the idea of manifold effects of genes. Further analysis of the

known genes in Drosophila may establish the universal presence of the manifold effects, and indeed, as already pointed out by Dobzhansky (1937) all genes have manifold effects, - the effects being more pronounced in some and easily overlooked in other cases.

It would therefore appear, thatthe process of development of the various characters are closely dependent on each other; and that a change or changes in any one or more of the genes determining these processes, is likely to cause corresponding disturbances in the whole train of developmental processes that go to form an organism. The fact that the disturbances are pronounced in some and negligible in others, is probably determined by the direct dependance of these processes on the altered process or processes. A direct analysis of these processes is outside the scope of genetics and belongs to "developmental mechanics". These effects are interpreted by Mullar as possibly "due to either changes of different types occurring in the same material or with changes (possibly quantitative changes, similar in type) occurring in different component parts of one gene". Further work on the nature of the gene may settle this point.

SUMMARY.

A full morphological description of a new autosomal gene in Drosophila pseudo-obscura - thorax (th) - that partly overlaps wild type and affects the thorax and its appendages, along with its linkage data is given. It is found to be situated on the third chromosome, 22.4 units to the right of orange (or).

ACKNOWLEDGMENTS.

The author acknowledges with deep gratitude the generous hospitality and assistance that he has received from Professor F.A.E. Crew during the course of this work. He is also greatly indebted to Miss R.Lamy for her help and advice.

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DIAGRAM SHOWING THE BASAL PORTION

OF A NORMAL WING.

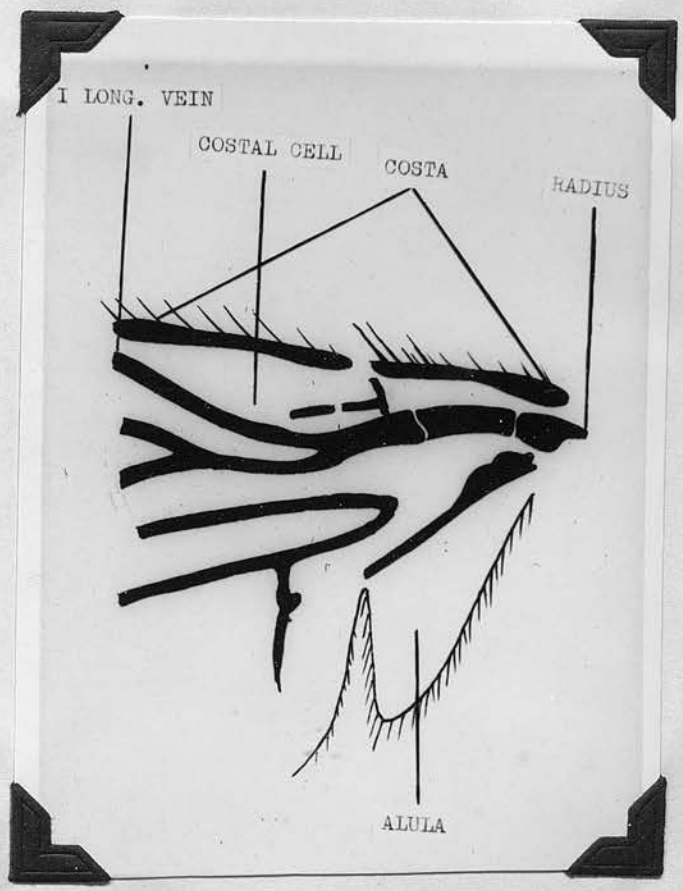


DIAGRAM SHOWING THE BASAL PORTION OF AN EXTREME TYPE OF DUPLICATED WING. THERE ARE TWO DISTINCT RADII AND THEIR BRANCHES.

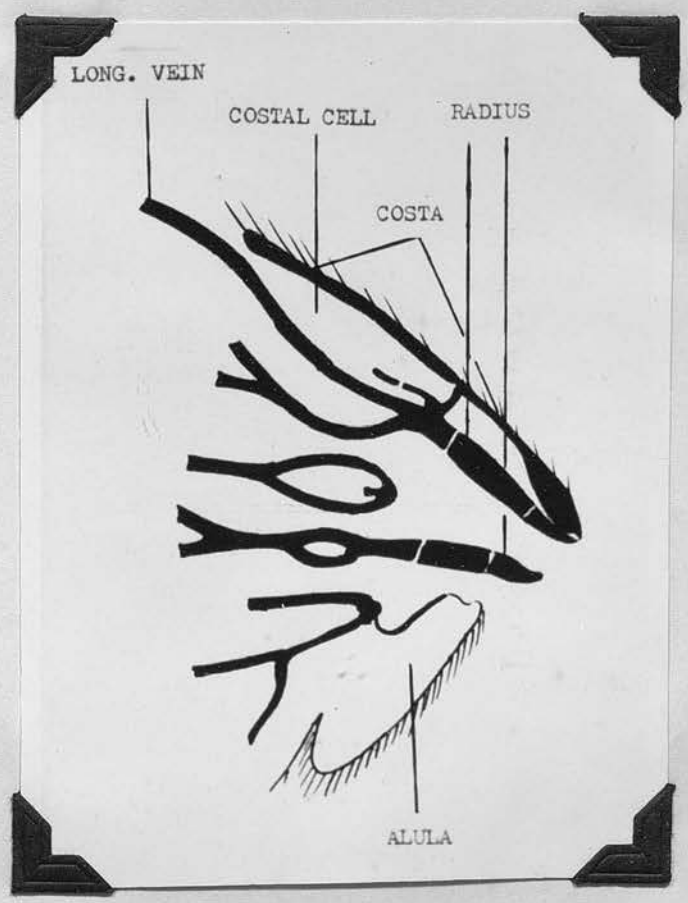



FIG.1 a

DIAGRAM REPRESENTING THE BASAL PORTION OF AN EXTREME TYPE OF DUPLICATED WING WITH A THREE BRANCHED RADIUS.

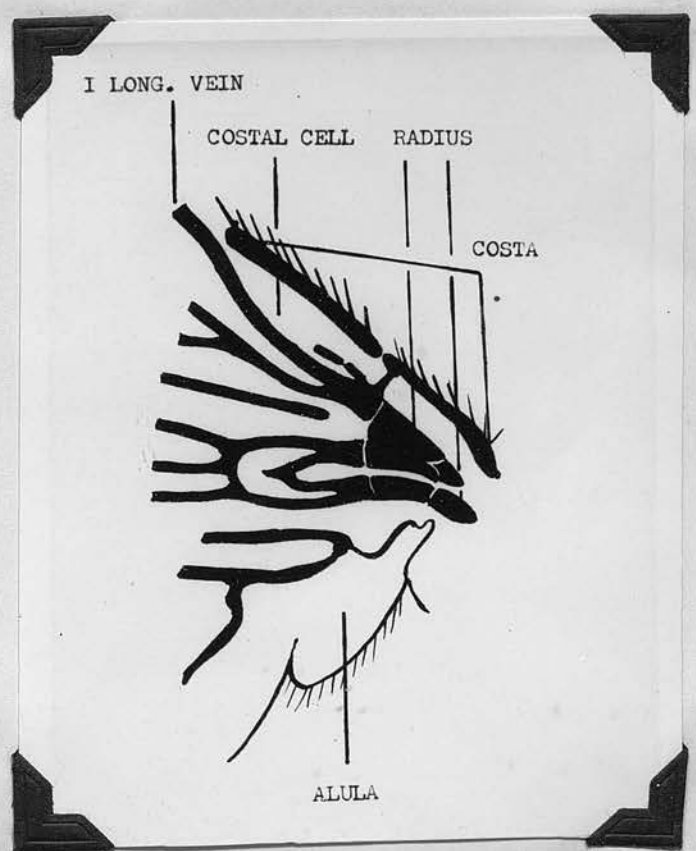



FIG. 1 b

DIAGRAMATIC REPRESENTATION OF THE BASAL PORTION OF A "MIRROR IMAGE" DUPLICATED WING SHOWING INDISTINCT RADII.

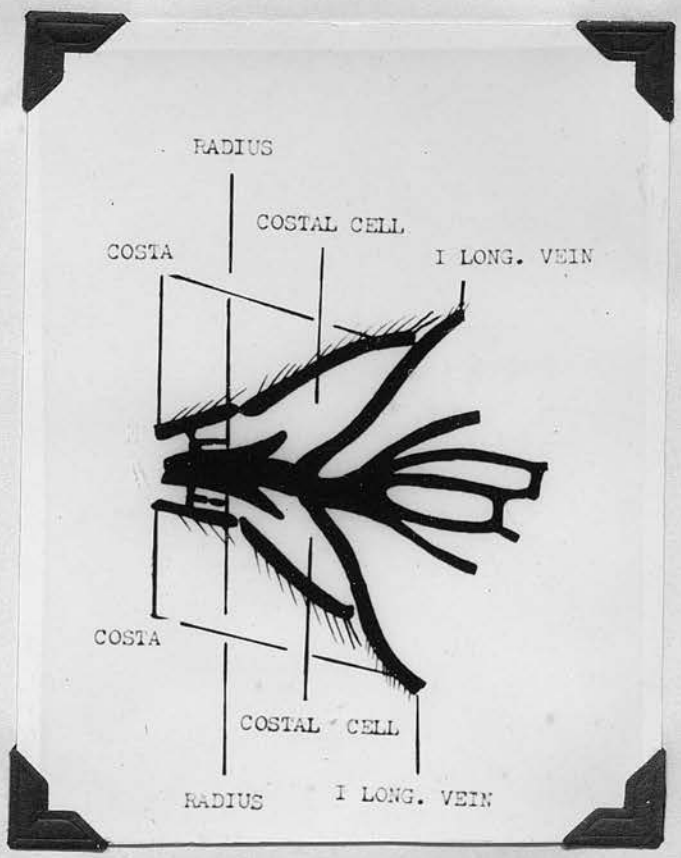



FIG. 2 a

DIAGRAMATIC REPRESENTAION OF THE BASAL PORTION OF A "MIRROR IMAGE" DUPLICATED WING SHOWING DISTINCT RADII .

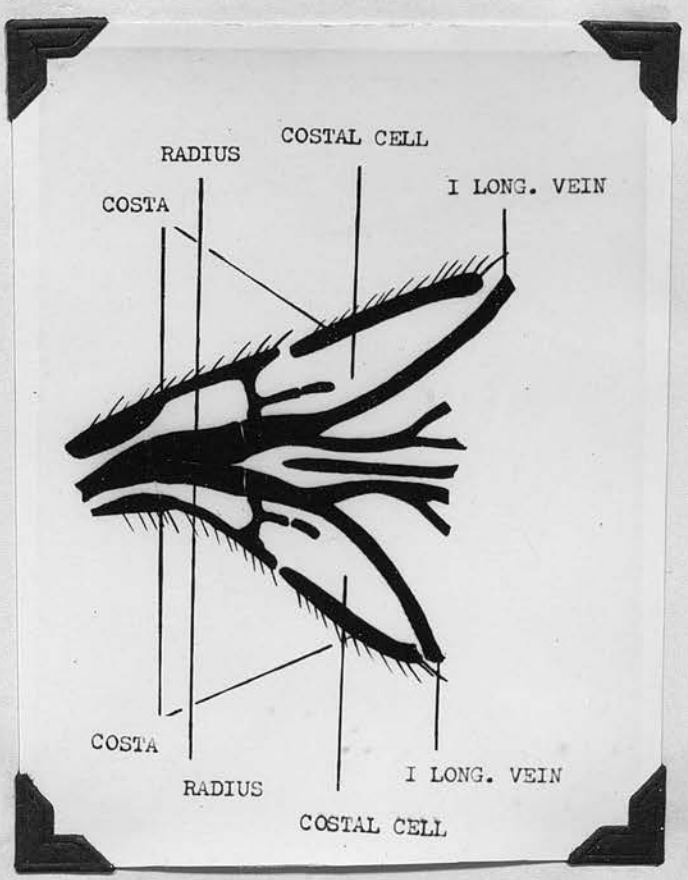



FIG. 2 b

DIAGRAMATIC REPRESENTATION OF THE BASAL PORTION OF A "MIRROR IMAGE" DUPLICATED WING SHOWING DISTINCT RADII.

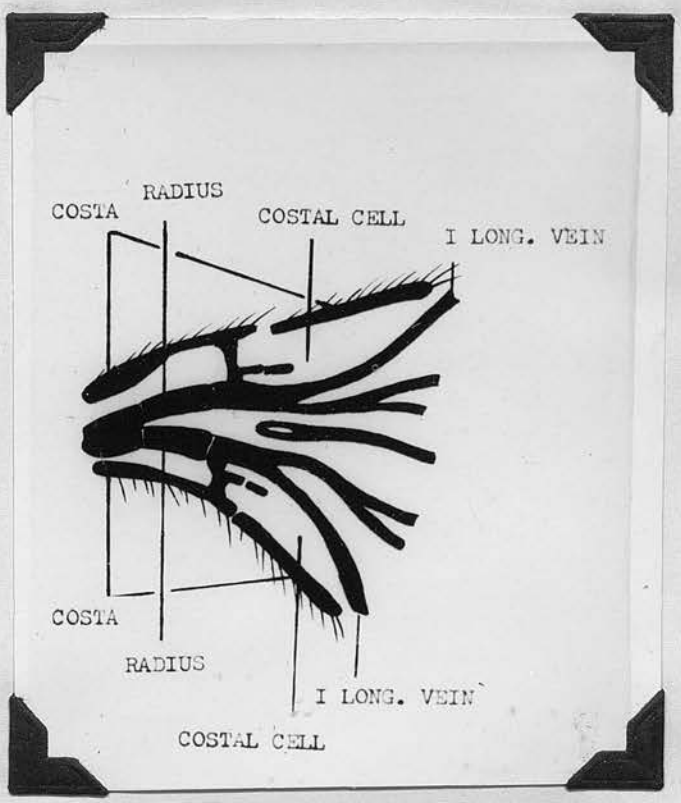



FIG.2 c

DIAGRAMATIC REPRESENTATION OF THE BASAL PORTION OF A "MIRROR IMAGE" DUPLICATED WING SHOWING DISTINCT AND SE PARATE RADII.

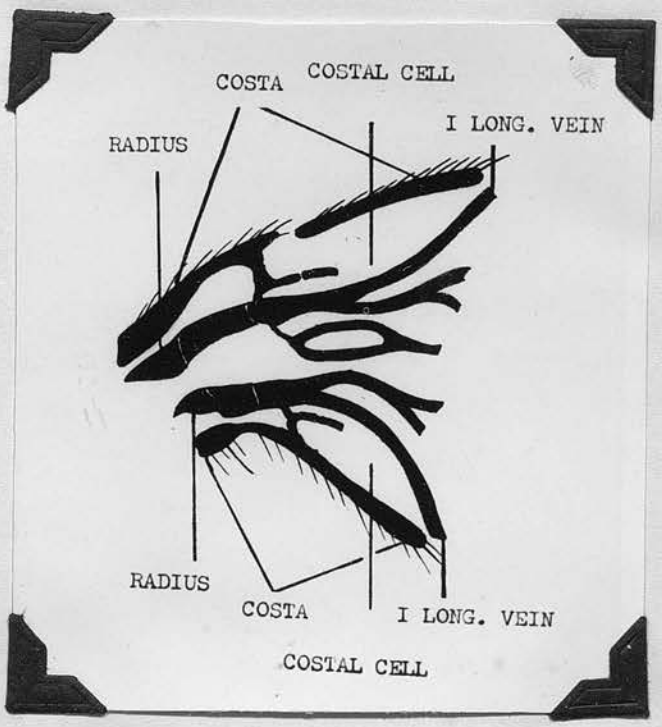



FIG. 2 d

III.

# STERILITY MUTATIONS IN DROSOPHILA MELANOGASTER.

CONTENTS.

## INTRODUCTION

As a result of the exhaustive studies of Goldschmidt (1911-1920, 1934) on intersexuality in Lymantria and that of Bridges (1922,1925,1932) on triploids, intersexes and supersexes of Drosophila melanogaster, it has been established that sex is a quantitatively variable character. The sex of an individual is determined by the quantitative relation or balance between female and male determining genes. These genes, according to Goldschmidt are either single or completely linked; the male determining gene is in the X-chromosome and the female determining one in the cytoplasm. However, the more recent Drosophila work of Dobzhansky and Schultz (1931, 1934), of Patterson, Stone and Bedichek (1935) show that in this form the sex genes are numerous and distributed throughout the X-chromosome and the autosomes; there being a greater preponderance of the female determining genes over the male determining genes in the X-chromosome, whereas, in the autosomes, the male determining genes are either more numerous or stronger than the female determining ones.

In recent years, chiefly, 2 avenues of research have been followed up in the study of the genetic basis of sex.

1. Using Painter and Mullar's (1929) technique of obtaining small pieces of the X-chromosome, Dobzhansky and Schultz (1934) studied the effect of adding or subtracting small sections of the X-chromosome of Drosophila melanogaster, on the phenotypic expression of the sexual types of both male and female intersexes. Their results showed that the duplications for any section of the X-chromosome studied, except the inert region, produced a shift towards femaleness in the average type of intersexes. The extent of the shift, on the whole, was proportional to the cytological lengths of the duplicated segments. They concluded therefore, that the sex determining role of the X-chromosome of Drosophila, is due to a co-operative effect of numerous female modifiers located in all regions of the X-chromosome, except the inert region.

Goldschmidt (1935) however, has challanged this conclusion on the grounds that the types of intersexes may be easily changed by temperature or by modifying genes (Dobzhansky, 1930) and therefore, the decisiveness of the intersex-test for sex genes is doubtful. The other abnormalities observed, he considers have nothing to do with the hyperfeminine (a term, which as applied to Drosophila, he claims, is a misconception) sex-constitution ; they are the

affects of hyperploidy. This view has been substantiated by the more accurate studies of Patterson, Stone and Bedichek (1937), on the effects of aneuploidy of relatively short segments of the X-chromosome on viability, fertility and sex in diploid individuals od Drosophila melanogaster. Their method consisted in the judicious combination of two known translocations, so as to produce a short overlap of a known section of the X-chromosome - the section overlapped will thus be in a duplicated condition. By this method it is possible to produce duplications in very small sections of the X-chromosome and therefore, this method may be considered as more accurate than the one used by Dobzhansky and Schultz - as the latter consisted of relatively large segments.

Two points of special interest are established by Patterson, Stone and Bedichek (1937) and they are:-

1. The hyperploid females are more viable than the hyperploid males.

2. The viability and fertility of the hyperploid males differ with the segment of the X-chromosome. Thus 89% of the males containing the w 13 R + 17R duplication were fertile, whereas, the fertility of the males containing 4 L + 12 R dupli - cation was only 2%, though the relative lengths of the 2 segments are more or less the same (.06;.062 of

the X-chromosome). These facts will be considered later on, in the discussion.

2. Through studies on sterility and the distribution of the sterility factors.

Inter-specific sterility has been noted both in plants and animals and has been the subject of many investigations. In Drosophila, it has been used in proving the existence of factors causing sterility, when combined with other factors from other race or species. Kollar and Lancefield (1932 ) showed the presence of such factors in the X-chromosome of Drosophila pseudo-obscura. Recent work of Dobzhansky (1937), not only confirmed his previous work, showing their existence in the autosomes as well, but, using testis-size as a measure of the degree of sterility, he was able to show that, in the interracial crosses of Drosophila pseudo-obscura (where the male hybrid is sterile), the X-chromosome produces the strongest effect, the second chromosome follows next, and the third and the fourth last. According to him, the effectiveness of each of the chromosomes is,on the whole, proportional to its cytological length.

Intra-specific sterility is well known in Drosophila. Certain mutations as, fused, deltax, rudimentary, cleft, dwarf, etc., in Droso-

phila melanogaster, have been known to be accompanied by a partial or complete sterility of one or the other sex. Clara Lynch (1919), who investigated 7 such cases, was unable to isolate a factor for sterility, in any of these instances, independent of the mutant factors for the visible character noted. She therefore concluded that in these cases the sterility is due to one of the manifold effects of the gene responsible for the visible mutation.

We are concerned however, not with indirect evidence for the existence of sterility factors in the X-chromosome and the autosomes, but with direct proof for their existence, and their frequencies in the X-chromosome and the autosomes, should be at least approximately known, before we attempt to solve the problem of sex. Such a proof is forthcoming.

Muller (1927) has already shown the possibility of producing sterility mutations by X-Rays. Neuhaus (1934) later obtained more definate evidence of their high frequency. Berg (1937) was the first to follow this up and find their frequencies in the different chromosomes. According to her researchees there are "much more numerous sterility mutations in the X-chromosome than in the second chromosome and possibly more than in all the autosomes taken together". The relative frequencies of the lethals and the semi-

lethals occurring in the same experiment in one of the large autosomes (Chromosome II), was 3 times as numerous as that occurring in the X. These results are interpreted by her as due to an evolutionary process of "differentiation". The genes in the X-chromosome according to this theory have differentiated towards sex-determination. This postulate cannot yet be considered as proved for the following reason.

The hemizygous nature of the Drosophila males makes it impossible to distinguish between the purely recessive sterility mutations and dominant sterility mutations affecting only the males, occurring in the X-chromosome, as both these types will show in the males, irrespective of the fact of whether they are recessive or dominant. It can, therefore, be argued on theoretical grounds that the number of sterility mutations observed by Berg in the X-chromosome, represent the sum total of the purely recessive and dominant specifically male sterility mutations. The sterility mutations in the II chromosome considered by her, on the other hand, belonged to one of these types only - namely, the recessives. Before comparing therefore, the total number of sterility mutations produced in the X-chromosome with that produced in the autosomes and drawing conclusions regarding their relative roles in sex-determination, it is desirable to know the frequency of the second type steriles -

namely, the dominant male sterility mutations occurring in the autosomes, as well as that of the first type.

In the following pages, an attempt has been made to find out whether such dominant autosomal steriles occur or not and if they do, with what frequencies.

## MATERIAL AND METHOD

The material used here was obtained from Mr. Makhijani's experiment dealing with the effects of X-Raying under different conditions of temperature, on the frequency of translocations and mutations.

Males possessing special marker genes on their chromosomes were used. On one of the II chromosome was the dominant gene Curly wings, associated with a crossing over suppressor that prevents crossing over in the greater length of the chromosome, whereas, on the other, there were a string of recessive genes, popularly called "apl". The III chromosome had the dominant gene Dicheate wings on one of its chromosome with a crossing over suppressor, similar in affect to the one in the II chromosome, and a string of recessive genes called popularly "rucuca", on the other.

These males ($\frac{Cy.C}{"apl"}$ $\frac{D.C}{"rucuca"}$) were sub - jected to a fairly high dose of X-Rays and crossed to $\frac{C\ 1\ B}{"scar"}$ (one of the X-chromosome contained a crossing over suppressor C, a lethal 1 and a dominant gene affecting the shape of the eye called Bar - B ; the other chromosome contained recessive genes scute, ver milion, forked, carnation - popularly called "scar") virgins.

Out of the $F_1$ flies, males showing Curly and Dicheate wings were crossed individually, to virgin normal females (2 or 3 per male) and the female s with Bar, Curly and Dicheate characters were cross - ed individually to "scar" males.

From the $F_2$ of the first $F_1$ cross, fe - males from each culture possessing Curly, Dicheate, Curly Dicheate, and wild type were subjected separate- ly, to fertility tests. Not more than 3 females were kept in a vial.

In a similar way, Curly, Dicheate, Cur- ly Dicheate and wild type $F_2$ males from each culture of the second $F_1$ cross, were subjected "en masse" se- parately, to fertility tests. Not more than 3 males were kept in a vial and only cultures having no sex- linked lethals and no translocations were used.

DETAILS OF THE CROSS

$P_1$ $\frac{Cy\ C}{}$ "apl" $\frac{D\ C}{}$ "rucuca" ♂♂ X $\frac{C\ 1\ B}{}$ "scar" Vir.♀♀

$F_1$ (1). (2).

$\frac{Cy\ C}{}$ $\frac{D\ C}{}$ ♂♂ X Vir.♀♀ $\frac{C\ 1\ B}{}$ $\frac{Cy\ C}{}$ $\frac{D\ C}{}$ ♀♀

X ♂♂.

$F_2$

C y, D, Cy D and wild type ♀♀ TESTED.

Cy, D, CyD and wild type ♂♂ TESTED.

A record of all the sterile $F_1$ males and females was kept which gave the number of sterility mutations occurring in all the chromosomes.

The markers used enabled one to detect the dominant steriles occurring in the different chromosomes and the method employed to detect the "male specific" or "female specific" dominant sterility mutations.

It may be observed that the $F_1$ males have received their Y-chromosome from the irradiated father and so part of the sterility obtained in them may be due to the breakage of the Y-chromosome, as a result of X-Ray treatment (Stern's sterility). Such an objection however, doesnot arise in the case of the

F2 males tested, as their Y-chromosome comes from a normal father. Sterility observed in the $F_2$ males therefore, must be due to mutations produced in the X-chromosome or the autosomes.

Testing of all the different (4) types of $F_2$ males and females was done purposely, as it afforded an opportunity of checking up the results obtained. After 4 days the vials were examined and the flies from apparently sterile cultures (cultures containing no grubs) were transfered to fresh vials. These cultures were re-examined after the lapse of a further period of 4 days, after which period, it was fairly clear that a sterility mutation had occurred in that culture. Further testing, however, was carried out before arriving at a final conclusion. This test will be described in the paragraph dealing with the "balancing of the sterility mutations". Only those steriles that successfully stood the tests were considered as "sterile". The experiment was carried through twice. The results obtained are given below:-

DOMINANT STERILES OBSERVED IN THE F1 FLIES.

| Individual tested. | Total number of cultures | | | % ofsterility. | % of lethals (sex-linked). |
|---|---|---|---|---|---|
| | Started | Bad | Sterile | | |
| Male | I. 449 | 150 | 5 | 1.7 | 6.5 |
| | II. 502 | 294 | 6 | 2.5 | 6.4 |
| Female | 1.2035 | 433 | 2 | 1.13 | 6.5 |
| | II.1544 | 232 | 2 | 1.19 | 6.4 |

The flies in the first experiment were examined after 10 days and in the second, after 5 days. Only those flies that survived up to this period and produced no grubs were considered as sterile.

Dominant sterility mutations were found in both the experiments, affecting both the sexes. The frequency of the dominant steriles as compared with the frequency of the sex-linked lethals in these experiments, is much lower than that obtained by Hanson (1933). This may be due to the poor viability of the individuals containing the sterility gene and the ease with which the Curly Dicheate wings get stuck to the food. In the absence of the grubs, the chances of the Curly Dicheate flies getting stuck to the food are even greater. The large percentage of bad cultures (cultures in which the tested individual

have died.

male/or females as the case may be) point to the same conclusion.

As the F1 males have received their Y-chromosome from the treated father, part of the sterility observed in them must be due to the breakage of the Y-chromosome, following X-ray treatment. The rest of the sterility is all due to dominant sterility mutations produced in the X-chromosome and all the autosomes put together. Though it cannot be definately asserted, it is quite conceivable that a small fraction of this total sterility mutations mayt have occurred in the autosomes.

In the same way it can be argued that a small number of dominant sterility mutations may have occurred in the autosomes, in the case of the $F_1$ females.

## DOMINANT STERILITY MUTATIONS OBSERVED IN $F_2$ FLIES.

A.

### FEMALE SPECIFIC

| Experiment No. | Total No. of chromosomes tested | Total No. of steriles obtained | No.of X-steriles | No.of Cy steriles | No.of D steriles | %of sterility. |
|---|---|---|---|---|---|---|
| I. | 294 | - | - | - | - | - |
| II. | 232 | - | - | - | - | - |

B.

### MALE SPECIFIC

| Experiment No. | Total No. of chromosomes tested | Total No. of steriles obtained | X-steriles | Cy steriles | D steriles | % of sterility |
|---|---|---|---|---|---|---|
| I. | 476 | 12 | 12 | - | - | 2.7 |
| II. | 311 | 7 | 7 | - | - | 2.3 |

In the I.experiment 6.5% lethal mutations were obtained. The II. experiment the lethal mutation rate was 6.4%.

Out of a total of 526 cultures tested, no dominant female specific sterility mutations were noticed.

Out of a total of 787 cultures tested

to detect male specific sterility, 19 sterility mut - ations were found. They were all sex-linked, show - ing that most probably they are recessive; for if they had been dominant, we should have expected more evidence of thei: existence of such dominant sterility mutations in the autosomes as well.

It would therefore appear that dominant sterility mutations producing sterility in only one sex while having no affect in the other sex, are either very rare or do not occur.

## "BALANCING THE X)-STERILES"

Though the males from X-sterile cultures were completely sterile, their like-looking sisters were all, without exception, fertile. Hence, no difficulty was encountered in continuing the stocks. Balanced stocks of the X-steriles were obtained as follows:-

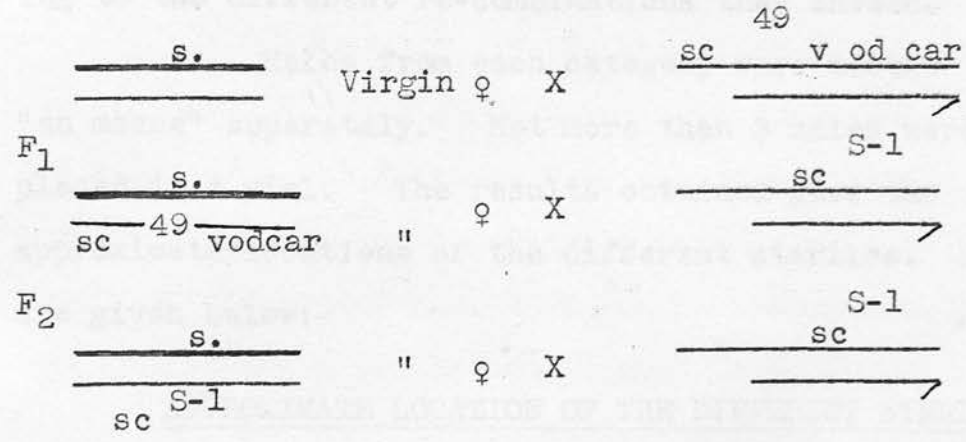
Normal looking virgin females from each sterile (these will be heterzygous for the sterility mutation) were crossed individually to males contain - ing rearranged X-chromosome termed "delta 49" that undergoes practically no crossing over except near the right end, with a normal X-chromosome and containing the recessive genes scute, vermilion , out-stretched and carnation.

The normal looking $F_1$ virgin females

that would have the sterility gene on one of the X-chromosome and the crossing over preventing "delta 49" inversion on the other, were collected and crossed individually, to males possessing a rearranged X-chromosome called scute$^{\text{Stone 1.}}$ that prevents crossing over with a normal X-chromosome.

In the $F_2$, the non-scute virgin females were again selected and crossed individually to scute Stone 1 males.

PLAN OF THE MATINGS.




BALANCED STOCK.

In each generation the normal looking males were subjected to fertility tests. Only those steriles which gave through out sterile normal looking males were considered as definately sterile. It is to be remarked, however, that though the majority of the normal looking males were sterile, a few exceptional fertile males were also found in some cases.

## LOCATION OF THE X-STERILITY GENES.

A prelimnary test for locating the X-steriles was carried out as follows:-

Females heterozygous for the sterility gene were crossed to males containing a series of recessive genes on the X-chromosome called X ple and the $F_1$ non-scute virgin females backcrossed individually to X ple males.

In the $F_2$ all the cross-over males were selected and divided into different categories according to the different re-combinations they showed.

Males from each category were tested "en masse" separately. Not more than 3 males were placed in a vial. The results obtained gave the approximate locations of the different steriles. They are given below:-

### APPROXIMATE LOCATION OF THE DIFFERENT STERILES

| Between sc & ec | Between ec & ct | Between ct & v | Between v & g | Between g & f | Between f & - |
|---|---|---|---|---|---|
| 0.0-5.5 | 5.5-20. | 20.-33. | 33.-44.4 | 44.4-56.7 | |
| ) - | - | - | - | 5 | - |

| Between ct & f<br>20. -56.7 | Between ct & g<br>20.-44.4 | Between v & f<br>33.-56.7 |
|---|---|---|
| 1 | 1 | 1 |

Out of the 8 steriles located, 5 proved to be between garnet and forked. The location of the other 3 was not precisely determined, because of the reduced crossing over, though it was observed that all of them lay to the right of cut.

Crowding of the sterility genes in a certain part of the X-chromosome is in agreement with the suggestion put forth by Berg that there are a greater number of genes affecting sex per unit of length of the active region of the X-chromosome and is in agreement with her unpublished data. (1938). Out of the 17 X-steriles located, she found 9 to be situated between vermilion and forked (33.0 - 56.7). The results obtained here narrows the length of the active sex influencing region of the X-chromosome from vermilion to forked (33.0 - 56.7) to garnet to forked (44.4 - 56.7).

## DISCUSSION

The peculiar role of the X-chromosome in the determination of sex was emphasied by Berg (1937) as a result of her work on the relative fre - quencies of lethals and semi-lethals occurring in the X-chromosome and one of the larger autosomes - the II chromosome, of Drosophila melanogaster on the one hand and the relative frequencies of the sterility mutations occurring in the X-chromosome and the auto - somes on the other. She found that, whereas the num- ber of lethals and semi-lethals in the II chromosome was 3 times as high as that occurring in the X-chro- mosome, the number of the sterility mutations found in the X-chromosome in the same experiment was much more numerous than those in the II chromosome and probably more than in all the autosomes taken together. She therefore concluded that the genes in the X-chro- mosomes must have been modified, during the process of evolution, towards a special function, namely that of sex-determination. By comparison of the numbers of semi-lethals and full lethals she also showed the existence of another factor, namely that of "stabi- lisation" of these genes with respect to injurious effects both internal and environmental, for the semi-lethals were relatively to the full lethals more numerous in the X-chromosome. As already pointed

out, the results concerning steriles in the autosomes are only based on the detection of the recessive sterility mutations occurring in them, while those in the X-chromosome included both purely recessive and dominant male specific sterility mutations. It may be argued that though the recessive steriles in the autosomes are few in number the number of dominant male specific sterility mutations occurring in the autosomes may be far greater in number ( thus upset - ting the ratio between the number of steriles in the X-chromosome to the number of steriles found in the II chromosome found by Berg) unless the contrary is proved. Our results show that such dominant steri- lity mutations are rare and negligible in number. Berg 's original conclusion therefore holds good.

It was also suggested by Berg that probably the sterility genes in the X-chromosome are more crowded in certain regions. Her own unpublish- ed data, dealing with the location of 17 X-steriles show that 9 are situated between vermilion and forked. Our results on the location of 8 steriles show them to be crowded in the same region. 5 out of the 8 steriles lay between garnet and forked. The effect ive X-chromosomal region in producing sterility is therefore reduced from between vermilion and forked (33.0 - 56.7) to between garnet and forked (44.4 - 56 .7).

The peculiar property shown by the garnet - forked region is in agreement with the results obtained by Patterson, Stone and Bedichek (1937) on the effects of hyperploidy on viability, fertility and sex in diploid individuals of Drosophila melano - gaster. They found males containing the 4 L 12 R duplication (i.e. comprising a part of the region between garnet and forked, namely, that half of it lying just to the right of garnet) were highly sterile. (98.0%), The corresponding females were not so affected.(only 11.0%sterile). Larger duplications of nearly all other regions had not so much sterilising affect. It can also be shown from the results of Dobzhansky and Schultz(1934), though the duplications in this experiment were much larger, that the same results are produced when fragments of different lengths but containing this region, were added to 3 A, 2 X. Such results had in fact made Goldschmidt (1935) in his 'critique' on their work suggest that most probably the sex gene is situated in this region- a suspicion that had still earlier been voiced by Patterson, on the his and his co-workers' results.

SUMMARY.

In an attempt to test Berg's hypothesis namely that there are "much more numerous sterility mutations in the X-chromosome than in the second chromosome and possibly more than in all the autosomes taken together", experiments were deviced to find out the frequency of the male "specific" (producing sterility of the males only, leaving females fertile) and female "specific" (producing sterility in the females only while leaving the males fertile) dominant sterility mutations produced in the autosomes, as these were not considered by Berg.

Our results show that such dominant sterility mutations are either very rare or do not occur. Berg's original conclusion therefore, holds good.

Localisation of 18 of the X-steriles show 5 to be located between the garnet and forked region, which lends support to Berg's suggestion that there are found in the X-chromosome, greater number of genes per unit of length of the active region, affecting sex and is in agreement with the unpublished data of Berg dealing with the location of 17 X-steriles.

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