GENETIC STUDIES

ON

DROSOPHILA PSEUDO-OBSCURA

Thesis for Degree of Doctor of Philosophy

by

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F O R E W O R D

The thesis consists of the following papers:-

(1) New Mutants and Linkage Data for *Drosophila pseudo-obscura*
(in press, Journal of Genetics)

(2) On the Genetical Constitution of *Drosophila pseudo-obscura*, Race A
(in press, Journal of Genetics)

(3) On the Suppression of Tangled in *Drosophila pseudo-obscura*
(to be read before The Royal Society of Edinburgh)

(4) Fecundity in *Drosophila pseudo-obscura* Race A
(to be extended before publication)

The studies on which these papers have been based were carried out at the Institute of Animal Genetics, Edinburgh, under the direction of Professor F. A. E. Crew, and the author wishes to take this opportunity of acknowledging his indebtedness to Professor Crew for a generous extension of facilities, and for encouragement and assistance.

C O N T E N T S

NEW MUTANTS AND LINKAGE DATA FOR DROSOPHILA PSEUDO-OBSCURA

In the course of experimental work with Drosophila pseudo-obscura a number of naturally occurring mutants have been observed. Such of them as appeared useful for experimental work have been retained and will be described here together with certain linkage data obtained during the process of locating them. No attempt has been made to deal with the question of the homology of these mutants with similar ones in other species; a general discussion of this problem is reserved for a further paper.

All the experiments reported here have been carried out at 23.5 ± .5° C., and the flies cultured on raisin meal food made according to the formula of Offermann and Schmidt in Drosophila Information Service No. 3, 1935.

A. Sex-Linked Mutations.

(1) Dent (de).

The origin of this mutant is rather obscure. It occurred in a progeny which showed several other deviations from normal and was not followed up separately. Since its expression was rather erratic, it was not worked with for some time, but when selection was made, its expression, particularly in the males, improved /

improved rapidly.

Dent shows some similarity to the Deformed of Drosophila melanogaster (3 - 47.5, Bridges and Morgan, 1923). It appears as a depression or flattening of the eye which looks as if dented from the front. In some flies the triangular appearance of the head and the bulging of the posterior edge of the eye can be seen without magnification. The separation of dent males from wild-type is easy, although sometimes the eye is merely flattened, but dent females are less reliable and there is some overlapping with wild-type. Only 20 - 30 per cent. of the expected numbers of dent females were seen in the back-cross progenies of the first outcrosses. A stock of yellow dent flies started from one of these progenies has shown a marked change in the course of four generations of selection. Instead of being almost sex-limited, dent has become merely slightly sex dimorphic in selected stocks.

Although the effect of selection on the dent character appears to show some resemblance to that obtained by Zeleny (1921) on Bar eye in D. melanogaster, it seems that a greater degree of expression has been brought about in both sexes rather than a reduction in sex dimorphism. The sex dimorphic character remains, but at a higher level of expression.

That there is an effective autosomal modifier of dent /

dent is concluded from the quick improvement following selection and from the different results obtained with stock dent males and F_1 dent males from out-crosses. Practically all the expected dent daughters of stock dent males are observed, but only a small proportion of the dent daughters of F_1 males show the character.

Dent is unlike the Deformed of D. melanogaster in being recessive and not lethal. In addition the eyes are usually alike and not reduced in circumference.

The first out-crosses of dent with yellow vermilion flies indicated that dent was sex-linked and located about 30 units from yellow towards beaded. A yellow dent stock was raised from those crosses and selected for dent for four generations. Linkage tests were then made with beaded. On the results of these and other linkage tests (summarised in Table I), dent is located between beaded and yellow at about 30 units from yellow. This can be regarded only as approximate because dent flies, particularly in combination with beaded, are somewhat lower in viability than the non-dent flies of these tests. The variation in the amount of recombination between beaded and dent is a reflection of the effects of low viability and overlapping with wild-type.

Table I. /

Table I.

Summary of linkage tests involving dent.

(Males only)

Cross	Total flies	Cross-overs b - de	Cross-overs de - y	Recombination b - de	Recombination de - y
$\frac{\text{de y}}{\text{b}}$ x de y	519	65	158	12.5	30.4
do.	1143	77	305	6.7	26.7
$\frac{\text{de y}}{\text{b}}$ x b de y	250	21	77	8.4	30.8
$\frac{\text{de y}}{\text{ll}}$ x de y	945	-	263	-	27.8
$\frac{\text{b de y}}{+}$ x b de y	326	31	55	9.5	30.1
Total	3183		858		27.0

Since it is now easily separated from wild-type in both sexes and bridges part of the large gap between beaded and yellow, dent is proving a useful mutant.

(2) Miniature^2 (m^2).

Miniature was first reported for D. pseudo-obscura by Lancefield (1922). Quite recently, it has reappeared twice in genetical experiments with inversions having one end near the miniature locus. It appeared the first time as a single male, and the second time as three males of such a genetical constitution that it is impossible for them to have been due to contamination with stocks of the first-occurring miniature. The conclusion that these two mutations /

tions are both miniatures is based on the exact resemblance of the wings and not on a breeding test, for all three males of the second occurrence were weak and did not produce any offspring. In appearance miniature^2 agrees closely with Lancefield's description and with the miniature of D. melanogaster. The wings are about three-quarters of the length and half the area of wild-type wings and have the characteristic dark colour and prominent veins (Plate I, fig. 8).

When miniature^2 was tested with the miniature of Crew and Lamy (1935) it was found to be non-allelomorphic; the latter shows a slighter reduction in the size of the wing, has a greater variability of expression, and corresponds rather with the dusky of D. melanogaster. It has therefore been renamed dusky (dy), and throughout this paper will be referred to by that name.

The linkage tests so far carried out have been designed merely to give a rough idea of the locus of miniature^2 in the event of its not being the same as Lancefield's, but they show clearly that it is very close to vermilion just as his was. That it is closer still to dusky will be observed from Table II which is based on the progeny of $F_1 \frac{y\ v\ dy\ se}{m^2}$ ♀♀ and which shows that among 400 odd males there was not one which was neither dusky nor miniature^2.

Table II. /

Table II.

Condensed recombination data from the mating

$\frac{y\ v\ dy\ se}{m^2}$ ♀♀ x $v\ m^2$ ♂♂ (7 cultures)

	v dy	v	m^2	$v\ m^2$	dy +	Total
♂♂	132	-	324	5	6 -	467
♀♀	-	354	381	7	- 3	745
Recombination			$v\text{-}m^2$	1.7%		

It is, of course, possible for wild-type flies to have been classified as dusky, but it is unlikely that this happened to any considerable number of them. Since in this and other experiments miniature[2] has shown first-rate viability, and can be classified by naked eye inspection, it serves as a valuable alternative to vermilion.

(3) Sepia (se).

Some data concerning sepia have already been given by Crew and Lamy (1935). They showed that sepia gave almost free recombination with beaded and dusky and was likely to be on the right arm of the X-chromosome (that is, the arm carrying Lancefield's "short" gene. If the suggestion that the arms should be named according to the direction of coiling is adopted, this would be the left arm and the "Pointed" arm, usually called the left arm, would become the right; Koller (1935)). Recent studies of inversions in the X-chromosome /

X-chromosome have shown that the "Pointed" arm is about 90 genetic units long, so that sepia, which is located some 120 units from Pointed, is almost certainly on the other arm.

Because it has good viability and is easily separated from other mutants except purple, sepia is much used as a basis of reference for linkage data. The original basis of reference for this region is the locus of Lancefield's short, but unfortunately no short stock has been available until recently. The results of several linkage experiments involving sepia and dusky are summarised in Table III.

Table III.

Recombination between dusky and sepia

Female parents		Cross-overs	Total	% Recombination
b dy se sp / tt	♂♂	210	511	41.1
ll / b dy se sp	♀♀	211	518	40.7
	♂♂	755	1833	41.2
b dy se sp / + *	♂♂	102	239	42.7
dy se sp / + *	♂♂	301	677	44.5
		1579	3778	41.8

* Crew and Lamy (1935)

As there is no data suitable for the calculation of coincidence in the region between sepia and dusky, the amount of double crossing-over cannot be estimated. However, /

However, if the interference near the spindle-fibre attachment of the V-shaped third chromosome of D. melanogaster is at all comparable with that of the X-chromosome of D. pseudo-obscura there would be about 8 - 10 per cent. of undetected crossing-over between dusky and sepia. The map distance would then be about 50 units.

(4) Lanceolate (ll).

In May 1935 four males with narrowed pointed wing tips were found in a culture of bithorax glass flies. Some of their offspring from females of the same culture showed this lanceolate wing (Plate I, fig. 2) which corresponds to the short description of the lanceolate of Morgan Bridges and Sturtevant (1925). Frequently in males and occasionally in females there is an inward curve of the wing margin about the point where it is joined by the second longitudinal vein. It is easy to separate lanceolate males from the wild-type, but although a tendency to hold the wings slightly spread reduces errors of classification, there is probably a slight overlap among females. A further disadvantage is that present stocks of lanceolate do not show good viability, particularly in combination with certain other mutants such as glass. Apart from its value as a mutant with a useful locus, lanceolate has the added virtue of being an intensifier of the mutant tilt which has hitherto been difficult to deal with on account of overlapping with wild-type. Tilt lies /

lies farthest to the right of our sex-linked mutants, and, as explained later, is of interest in connection with the suppression of tangled. The combination of lanceolate and tilt intensifies both characters (Plate I, fig. 4). As a result, tilt, which normally shows in very few females, can now be followed in both sexes.

A first out-cross to dent yellow flies confirmed the suspicion that lanceolate was a sex-linked recessive, and indicated that it was located well to the right of yellow. Subsequent tests placed it about 25 units to the right of sepia (Table IV.)

Table IV.

Recombination in male progeny of 10 $\frac{dy\ se\ sp}{ll}$ ♀♀

Crossing-over Frequency Region		Class	Number	Crossing-over Frequency Region		Class	Number
None	(0	dysesp	127	Double	(1,2	dysp	29
	(0	ll	310		(1,2	sell	66
Single	(1	sesp	106		(2,3	dysellsp	14
	(1	dyll	224		(2,3	+	129
	(2	sp	51		(1,3	dyllsp	26
	(2	dysell	97		(1,3	se	219
	(3	dyse	306	Triple	(1,2,3	dy	82
	(3	llsp	44		(1,2,3	sellsp	3
Total 1833							

Recombination % se-ll: (a) snapt classes 24.3;
(b) non-snapt classes 26.1

In these tests snapt was quite unreliable, for only about half of the expected numbers were observed. The percentage recombination between sepia and lanceolate was therefore calculated from both snapt and non-snapt /

snapt classes separately. As would be expected if the snapt flies had been present but undetectable, the results of these two calculations are much the same. There appears to be, however, a real absence of lanceolate snapt flies, and this will have to be borne in mind in designing further experiments with these mutants.

If the locus of dusky is taken as 72 units from Pointed, that of sepia becomes approximately 120, and that of lanceolate 145. Previous experiments showed that snapt and tilt are too distant from sepia to allow an accurate determination of their loci, but the presence of lanceolate well beyond sepia should make this possible.

B. Autosomal Characters.

(1) Bithorax (bx).

An autosomal recessive mutation very similar to the bithorax of D. melanogaster (3-58.7, Bridges and Morgan, 1923) was found in April 1934 (Crew and Lamy, unpublished). Eight females and three males with halteres expanded into wing-like structures occurred in a mass culture made for selection of Knobby eyes. This is the second appearance of bithorax in D. pseudo-obscura, as Lancefield has already reported an occurrence of it (Bridges Morgan and Sturtevant, 1925) and has assigned it to the second chromosome. When, later, work on D. pseudo-obscura was begun at this Institute, there /

there were no mutants available by which Lancefield's linkage groups could be identified with any of those known here, and consequently the linkage groups had to be numbered afresh. Thus the orange Scute purple group was allocated to the second chromosome because it was the first described (Crew and Lamy, 1934). However, it is agreed that the reappearance of bithorax, and the cytological demonstration in salivary gland preparations that its linkage group corresponds with the longest autosome (Tan, 1935a,b; Koller, 1936), makes it desirable to revert to the original order decided upon by Lancefield, and this has been done in what follows.

The metathorax of bithorax flies shows the same signs of being a duplication of the mesothorax as that in the bithorax of D. melanogaster. This is doubtless the reason why bithorax flies are unable to fly. The halteres often deviate more conspicuously from the normal than do the bristles, but not so reliably. The amount of wing-like expansion varies considerably. Growths up to half the length of the normal wing as well as normal sized structures are found, but usually there is a small crumpled semblance of a wing with short stout bristles along the anterior margin. Where the halteres are no larger than those of the wild-type they still possess a distinctive row of strong bristles, although the fine surface microchaetae remain the same. Veins cannot often be seen owing to a distension caused /

caused by fluid. This fluid causes much difficulty in breeding from bithorax flies, since, on escaping, it makes the legs sticky and incapacitates the flies.

The third pair of legs are much the same as the second. The preapical and apical bristles are both present and the posterior row of bristles of the proximal tarsal segment are only slightly if at all coarser than those of the second pair of legs. The coxae are rather larger, and their bristles coarser than those of wild-type, and are like those of the second pair of legs. The best diagnostic feature is the duplication of the sternopleural bristles. The extra bristles, although always present, differ from the normal in being frequently bent at a sharp angle, or even somewhat T-shaped. The dark setaceous bodies protruding at the junction of scutellum and abdomen are apparently less constant than in D. melanogaster, since far fewer than half of the flies possess them under usual stock culture conditions. Large coarse bristles, often bent or forked, small coarse bristles, and very fine hairs curled at the tip can be observed on them. The groove which probably corresponds with that between the normal scutellum and mesonotum (Bridges and Morgan, 1923) can sometimes be seen.

When stock flies raised from the new mutants were tested with mutants belonging to the second, third and fourth linkage groups, bithorax was found to belong to the second, i.e., the Stubble-glass group.

The /

The amount of recombination observed between bithorax, Stubble and glass is given in Table V. Bithorax glass flies breed so poorly that it was found necessary to place several males with each F_1 female. In addition, the fact that bithorax flies cannot fly was made use of to avoid etherising where possible, since this seems to delay mating and to render them more liable to stick in the food. Second broods were raised in preference to breeding from a larger number of F_1 females, but no conclusions regarding the effect of age on crossing-over in F_1 females can be drawn. As shown by Bridges (1929) and Plough (1917) considerable changes in the amount of crossing-over may occur within short periods of time, so that comparison of these first and second broods is of little value. However, there is clearly no very large difference between the first and second egg-laying periods under the conditions of this experiment. Bithorax is provisionally located about 25 units from glass on the one side, and 36 units from Stubble on the other.

Table V. /

Table V.

F_1 ♀♀ $\frac{Sb}{bx\ gl}$ x bx gl ♂♂

	First brood		Second brood	
	♂♂	♀♀	♂♂	♀♀
bx gl	164	215	139	153
Sb	219	286	262	289
Sb bx	39	39	28	42
gl	33	42	31	27
Sb gl	75	104	43	52
bx	87	99	102	79
Sb bx gl	100	131	72	77
+	156	184	161	159
Total	873	1100	838	878
Recombination %				
bx-gl	26.8	25.8	24.3	22.8
Sb-bx	37.6	36.0	34.8	34.7

(2) Cross-veinless

Some data on the orange Scute purple linkage group have already been published by Crew and Lamy (1934). Since then they have found another mutant belonging to the group in a culture of $short_4$ flies. It was unrecognised for a time because $short_4$ flies have often a broken or missing posterior cross-vein, but in the F_2 of an out-cross, flies with normal longitudinal veins but missing cross-veins were obtained. (fig.7).

Cross-veinless, morphologically, appears to affect only the cross-veins and wing texture. The posterior and usually the anterior cross-veins are absent. At the same time the wings have a thinner texture and are easily crumpled. Tests with stock flies derived from these indicated that cross-veinless belongs to the third /

third linkage group (Crew and Lamy, unpublished). Further linkage tests have been made with flies from the two matings, orange Scute purple x cross-veinless, and orange purple cross-veinless x Scute, and the results of these are given in Table VI. On account of the marked deficiency of cross-veinless flies in all combinations the linkage values have been calculated from non-cross-veinless classes.

Table VI.

Expt. A. F_1 ♀ $\frac{or\ pr\ cv}{Sc}$ x or pr cv ♂♂ (15 cultures)

Expt. B. F_1 ♀ $\frac{or\ Sc\ pr}{cv}$ x or pr cv ♂♂ (11 cultures)

	A first brood		A second brood		B	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Sc	370	418	299	352	6	19
or pr cv	41	115	27	75	3	3
or Sc	108	102	79	98	6	11
pr cv	13	44	4	22	1	8
or	130	120	124	148	20	20
Sc pr cv	19	43	7	29	33	15
or pr	85	107	64	76	19	21
Sc cv	9	46	6	25	3	7
or Sc pr cv	2	6	-	8	4	32
+	26	32	13	26	35	85
or cv	4	6	1	5	8	25
Sc pr	18	17	12	10	59	66
or Sc cv	3	11	3	10	8	25
pr	33	31	29	36	67	93
or Sc pr	2	3	1	3	140	211
cv	-	-	-	1	27	87
Total	863	1101	669	924	409	728
Non-cv classes	772	830	621	749	352	526

Recombination %

A. all non-cv flies		B.	♂♂	♀♀
or-Sc	20.9	or-Sc	29.5	24.0
Sc-pr	23.0	Sc-pr	27.8	27.4
pr-cv	17.7	pr-cv	19.0	25.7

Since /

Since there was no more than ± 2 per cent. variation with either age or sex in Experiment A, the recombination percentages were based on all non-cross-veinless flies. They are in close agreement with those obtained by Crew and Lamy (1934) for orange-Scute and Scute-purple, and in addition indicate that cross-veinless is located some 20 - 25 units from purple. The generally lower values of Experiment A can probably be traced to the large number of Scute flies which appear to have been much too vigorous in competition with other mutant classes. The shortage of males is largely accounted for by the greater lethal effect of cross-veinless on males than on females.

Table VII. gives the recombination data to date for the orange-Scute-purple linkage group (excluding males of group B above).

Table VII.

Recombination data for or Sc pr linkage group

		or-Sc	Sc-pr	pr-cv	Total
Crew & Lamy (1934)	♂♂	41	30	-	171
	♀♀	37	47	-	159
Experiment A	♂♂	291	326	244	1393
	♀♀	331	359	283	1579
Experiment B	♀♀	126	144	135	526
Total		826	906	662	3828
Recombination %		21.6	23.7	18.9	

A /

A semi-lethal effect of cross-veinless which is rather conspicuous shows itself in large numbers of dead pupae in the last stages of development. Examination of some hundreds of the dead pupae in cultures of the above experiments demonstrated that all but one or two had cross-veinless wings. Further, it was possible to distinguish between purple and wild-type, and this fact indicates, on the basis of work done in this Institute on the development of eye-colours (Cochrane, unpublished), that the pupae died within two days of hatching. In nine out of eleven cultures in which the total number of dead pupae were counted, the number of cross-veinless flies surviving plus the number of dead pupae was approximately equal to the number of surviving non-cross-veinless flies. This may be interpreted to mean that the cross-veinless flies were affected in the later pupal stages by a semi-lethal which their sibs did not possess. Two F_1 females gave distinctly different results in that the total of cross-veinless offspring and dead pupae was less than half that of the number of non-cross-veinless survivors. Since the same results were given in both broods raised from these two females, it seems possible that there was present a genetic modifier of cross-veinless which increased its lethal action by introducing an earlier critical stage in development through which many flies could not pass successfully.

An /

An alternative explanation which seems more likely is suggested by the fact that one of these two females was also characterised by exceptionally high fertility - she produced an average of 122 non-cross-veinless flies in each brood while the average of the nine first mentioned females was 100. This may mean that after the density of larval population passes a certain point an increasing number of cross-veinless larvae die before pupating. Since no attempt was made to ensure that exactly the same quantities of food were put in each vial, and no egg counts were made, it does not seem profitable to attempt to correlate the density of larval population as measured by the numbers of surviving non-cross-veinless flies with the numbers of cross-veinless offspring dying as larvae or as pupae, but it is reasonable to suppose that for some cause or other, cross-veinless flies must pass through two critical periods in development: one during the larval stage when they are susceptible to overcrowding, and one during the last stages of pupation. The latter may be due to overcrowding, for occasionally under optimal food conditions very few dead pupae may be seen. Cross-veinless stocks raised from various types of crossing-over following out-crossing continue to show these effects which are consequently due to the cross-veinless gene or one close to it.

(3) Eyeless^2 (Ey^2).

A /

A stock of a mutant type called Eyeless was kept for some years under selection, but it overlapped with wild-type to such an extent as to be useless and was discarded. Recently, however, another similar mutation turned up which appears to be much more useful. Although there can now be no test of allelomorphism of the new with the old Eyeless, the former has been labelled Eyeless^2. It is sufficiently different to make it certain that it is a new mutation. It appeared first among the progeny of a back-cross involving bithorax, Stubble and glass as a single bithorax female having reduced, flattened eyes with a rough surface like that of a raspberry. This rough surface has turned out to be quite reliable and always enables Eyeless flies to be detected when they occasionally appear without a flattening or a marked reduction in the area of the eyes. Another peculiarity is the frequent appearance near the anterior edge of one or both eyes of a warty structure which may be glassy or black in colour.

The original Eyeless female was mated to stock bithorax glass males and produced both Eyeless and non-Eyeless males and females. Eyeless^2 was therefore dominant. Eyeless^2 flies were then crossed to vermilion-purple-tangled stock flies and the F_1 Eyeless^2 males back-crossed to the multiple recessive. In the F_2 there was no recombination of Eyeless with purple, so /

so that Eyeless^2 belongs to the purple linkage group. From the same F_1 culture two Eyeless females were back-crossed and their progeny gave about 40 per cent. recombination of Eyeless with purple, which places it either near orange or some 20 units beyond cross-veinless (Table VIII.)

Table VIII.

Recombination of Eyeless with purple

	Ey^2	pr	Ey^2pr	+	Total
♂♂	42	36	18	29	125
♀♀	41	40	22	29	132
Recombination % Ey^2-pr			38.1		

So far, all Eyeless flies when bred have produced a proportion of non-Eyeless offspring, and the F_2 of 159 flies raised from the above-mentioned F_1 comprised 107 Eyeless and 52 non-Eyeless, so that it seems likely that Eyeless is lethal when homozygous.

(4) Tangled (tg).

The fourth linkage group includes the mutants tangled (tg), short$_4$ (s_4), and jaunty (j) which have been described by Crew and Lamy (1935). Subsequently a modified type of tangled called shadow-tangled (shtg) appeared which acts as an allelomorph of tangled. The character consists usually of a little extra venation between the distal ends of the second and third longitudinal veins, but it may be reduced to a faint blurring of this region. When linkage experiments

were /

were made by back-crossing $\frac{\text{shtg j } s_4}{+ \; + \; +}$ females to stock shtg j s_4 males, it was found that every shadow-tangled fly was also $short_4$, and that the remaining tangled flies were intermediate between shadow-tangled and the original tangled type. Examples of these three types are given in Plate I, figs. 3,5,6. These results led to the supposition that $short_4$ was suppressing tangled. $Short_4$ flies were then mated to the original tangled and an F_2 raised which gave the expected proportions of tangled and intermediate tangled, but no shadow-tangled. A back-cross to shadow-tangled $short_4$ made at the same time produced none of the original tangled, so that it seems safe to conclude that $short_4$ is a partial suppressor of tangled.

Without tangled the $short_4$ gene causes a shortening of the fourth and fifth longitudinal veins and complete or partial elimination of the posterior cross-vein, but its action has not been observed to extend to the second or third longitudinals, nor is there any apparent diminution of its effect in the presence of tangled which affects all four veins more or less equally. In tangled flies which are also homozygous or heterozygous $short_4$ there is always a greater degree of tangling near the end of the second longitudinal vein than there is at the posterior cross-vein, so that $short_4$ exerts varying degrees of suppression in different parts of the wing. The action of the $short_4$ /

$short_4$ gene may therefore be visualised as tending to inhibit the formation of veins but reaching a sufficient intensity to achieve this in the normal wing only in the region of the posterior cross-vein. That it also operates without visible effect in the anterior part of the wing of $short_4$ flies may be concluded from the reduction of tangling in that part of the wing of $short_4$ tangled flies. The failure of tangled to affect the expression of $short_4$ may possibly be due to the latter acting at an earlier stage in the development of the wing.

It has been noted (Crew and Lamy, 1935) that this $short_4$ is very similar to the sex-linked short of Lancefield (1922) located near the end of the right arm of the X-chromosome. In the same region there is a recessive modifier of tangled, known as the tangled inhibitor, which has not yet been separated with certainty from the sex-linked mutant tilt. The effect of this modifier, like that of $short_4$, is to cause almost complete suppression of tangled in the males (and therefore in haploid dose) and a much reduced expression in the females. Further, both modifiers are more effective in the posterior half of the wing than in the anterior half. It became of interest therefore to see if the sex-linked short and tilt would act in this way.

So far only the test with short has been completed and /

and it has justified the supposition that short would have the same effect as short$_4$. It might have been expected that short would have a similar but lesser effect than short$_4$ because it is itself less extensive, but actually it has a greater suppressing effect. Females which are tangled and heterozygous short are not intermediate tangled as they would be with heterozygous short$_4$, but shadow-tangled. It is likely that there is some overlapping of such females with the wild-type, and tangled females homozygous for short will in all probability show no tangling at all, although the short tangled males are, for the most part at least, shadow-tangled. While short has apparently much the same diminishing effect towards the anterior part of the wing, tilt, which causes a shortening or interruption of the third instead of the fourth vein, may have a somewhat different sphere of influence on tangled.

Owing to the difficulty of classifying jaunty in the presence of tangled, there is an element of doubt about the order of the mutants in the fourth linkage group. In Table IX. it has been assumed on the grounds that the jaunty class is consistently smaller than the short$_4$ class, that the order is tangled jaunty short$_4$, but tangled is so far from either of the other two mutants that errors in the classification could easily give a wrong impression of their order. Further, the ten per cent. recombination between short$_4$ and /

and jaunty obtained by Crew and Lamy (1934) is much less than that in the present experiments, where 15 - 16 per cent. occurred; but the difference is not significant, and doubtless it is in part traceable to the same errors of classification.

Table IX.

F_1 $\frac{\text{shtg j } s_4}{+}$ ♀♀ x shtg j s_4 ♂♂

	First brood		Second brood		
Class	♂♂	♀♀	♂♂	♀♀	Recombination %
+	189	235	65	81	First brood:
shtg j s_4	122	153	72	87	s_4 - j, 15.0
s_4	28	37	15	15	tg - j, 43.9
intg j	25	47	19	27	
shtg s_4	17	29	11	12	Second brood:
j	15	26	15	8	s_4 - j, 16.4
s_4 j	128	149	97	93	tg - j, 48.9
intg	128	162	59	70	
Total	652	838	353	393	

shtg = shadow-tangled; intg = intermediate tangled.

Summary.

Descriptions of three new sex-linked and four new autosomal mutations are presented, together with linkage data for the four main linkage groups of Drosophila pseudo-obscura.

Two gene interactions of practical importance have been discovered; namely, an intensification of each other by tilt and lanceolate, and a dominant suppressing effect of the recessives short$_4$ and short on tangled.

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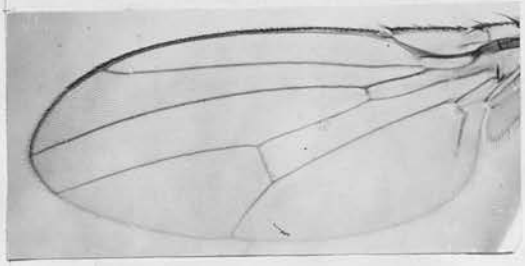
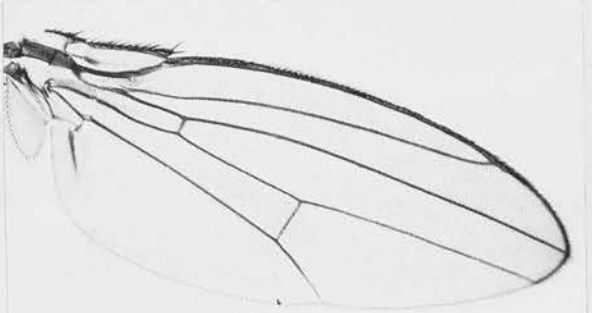
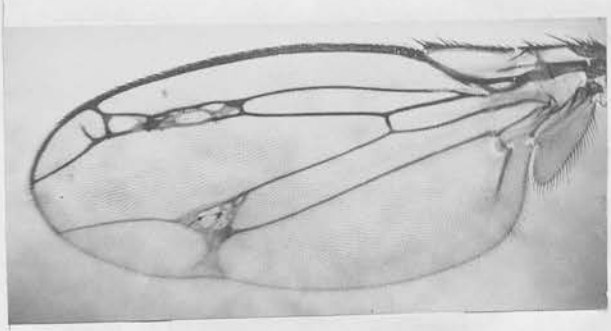
Plate I



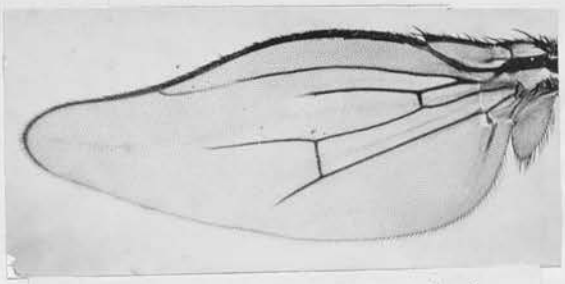
1. Wild-type



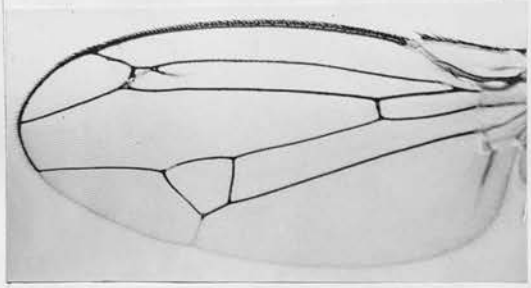
2. lanceolate



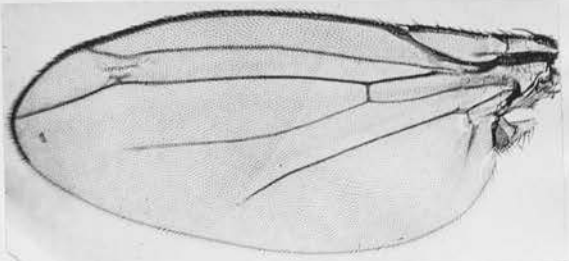
3. tangled



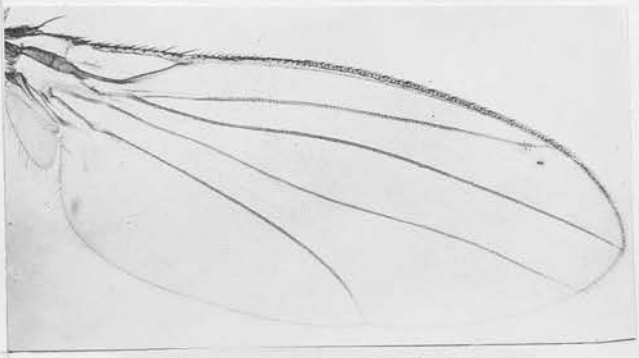
4. lanceolate tilt (♂)



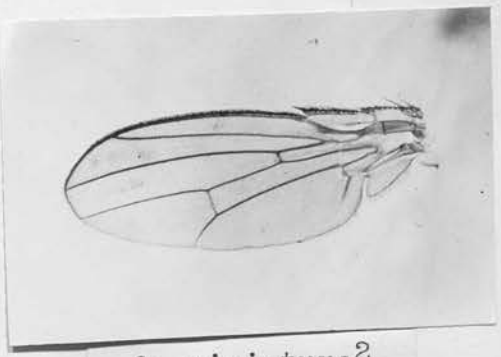
5. intermediate-tangled



6. shadow-tangled short$_4$



7. cross-veinless



8. miniature^2

ON THE GENETICAL CONSTITUTION

OF

DROSOPHILA PSEUDO-OBSCURA, Race A

ON THE GENETICAL CONSTITUTION OF DROSOPHILA PSEUDO OBSCURA, Race A

Some seventeen years ago the researches of Metz on the chromosome groups of the various species of Drosophila induced Lancefield (1922) to undertake the study of the genetic constitution of D. obscura in the hope of securing evidence of the exact relationship of this species to others for which genetic data were available. Since the publication of his extensive work on sex-linked mutants there appears to have been little study of the homology of mutant loci in this species (now D. pseudo-obscura Frol.). More recently the recognition of the value of D. pseudo-obscura as a means for investigating the process of species formation has led to more intensive study and the accumulation of further genetic data. At the same time, the development by Muller of the X-ray technique for inducing an increased rate of mutation and of chromosome rearrangement, and the demonstration by Painter of the possibilities of the salivary gland method of chromosome study, have thrown into prominence the various kinds of change in the constitution of chromosomes which may have played a part in the evolution of species. It has seemed worth while, therefore, to sum up our present knowledge of the mutant forms of D. pseudo-obscura /

pseudo-obscura, Race A, in order to see what light may be shed on the relationship of this to other species and on the processes which led to their differentiation. With this end in view, maps have been prepared which summarize the linkage data at present available. Using these maps as a basis for study, the mutant forms have been compared with possible homologues in other species and the suggestions arising therefrom outlined.

A. The Linkage Maps.

Apart from the linkage map of the sex-linked characters which has been published previously by Lancefield (l.c.), and the summary of the then-known mutants in all linkage groups given by Morgan Bridges and Sturtevant (1925), there have been no published maps in which to incorporate new data. With the exception, therefore, of the sex-chromosome, the map for which has been based on Lancefield's and includes all his mutants, the chromosomes are represented here by completely new maps (Text-fig. 1). Although none of the mutants which Lancefield located in the autosomes have been used, it appears fairly certain that the linkage groups as numbered here correspond with his, partly because there have been reappearances of some of his mutants, and partly because some of them appear to have been available elsewhere.

The maps do not represent a summary of accurate linkage data. At present they serve merely as a ground work /

work for further more detailed investigation of the linkage properties of these chromosomes, and to indicate the kind and position of the landmarks which are available for the study of chromosome constitution. It has not been considered advisable at this stage to adopt the methods available for the construction of accurate linkage maps. The simple procedure of using the cross-over percentages, calculated from the primary data given in Table I, as map distances has been followed. Most of the data for each distance is derived from one linkage experiment, and represents the frequency of single cross-overs between the loci indicated in column 4 of the table. Lancefield's data of 1922 are not included because the revised map given by Morgan Bridges and Sturtevant (1925) is different in some respects and is not accompanied by the figures on which it is based.

Text-fig. 1 /

Text-fig. 1.

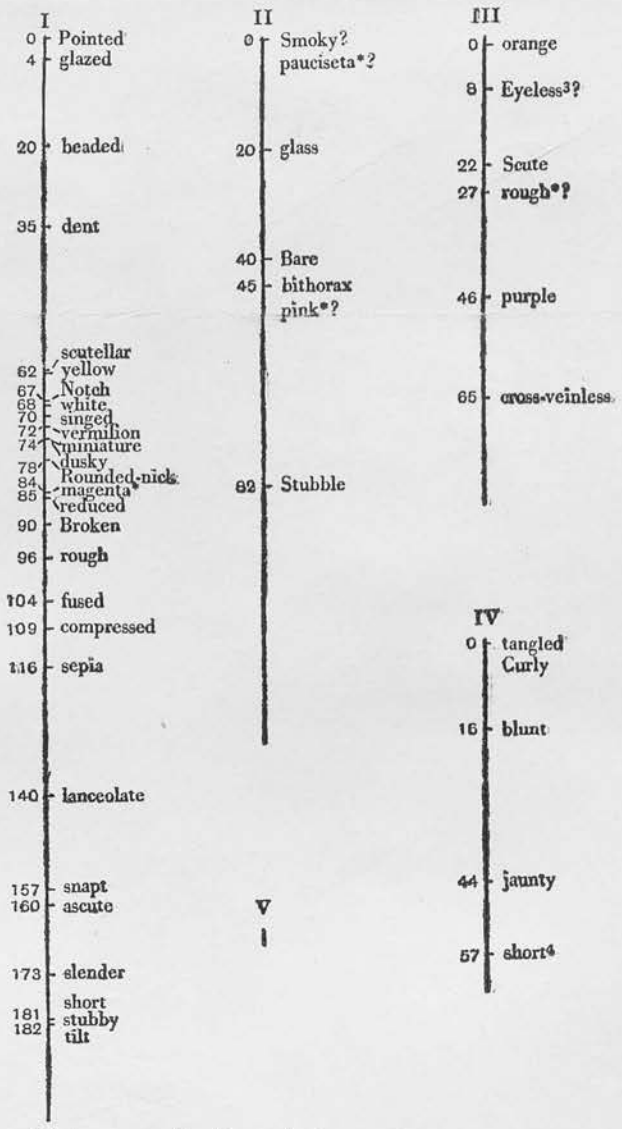


Fig. 1. Current linkage maps for *D. pseudo-obscura*. The position of mutants marked (?) is still doubtful. Mutants received from Dr Dobzhansky and not located with reference to mutants used in constructing these maps are marked with an asterisk.

The map for the X-chromosome from Pointed to compressed is practically the same as Lancefield's except that new loci are shown and that singed is placed to the left instead of to the right of vermilion. Four experiments agreed fairly closely in giving about 42 per cent. of recombination between dusky (74) and sepia (Donald, 1936), so that 116 has been taken as the locus of sepia, and as the basis of reference for mutants to the right of it. The occurrence of three new mutants between Lancefield's compressed and ascute has permitted shorter distances to be measured and has thus had the effect of shifting short and the mutants near it farther to the right than appears in his map.

It has not been possible to find a satisfactory starting point for the second linkage group. Smoky has been placed provisionally at 0.0 on the grounds that Tan's (1935) figure of the inversion found by Sturtevant to reduce crossing-over between Bare and Smoky indicates that one of these two mutants has a locus somewhere near the end of the chromosome. Since Stubble and glass occur at considerable distances upon either side of Bare, it seems likely that Smoky is the one nearest the end.

Another unsatisfactory feature of the map for the second chromosome is that the loci for Stubble, glass and bithorax have been determined with relation to Bare and not to Smoky, and consequently each may lie on /

on the opposite side of Bare to that shown. There are no published accounts of crossing-over between Smoky and Bare, but there are indications that it is about 40 per cent. The amount of information available about this linkage group is shown in Table I. and is clearly inadequate for constructing a map containing all the known mutants. It may be mentioned that the mutant aristipedia has been found to belong here also.

Following Lancefield, the locus of orange on the third chromosome has been placed at 0.0, but, judging from the position of the Scute inversion (Tan, l.c.) in that chromosome and the fact that there is not much crossing-over between orange and the inversion, orange is probably some distance from the end of the chromosome. No three-point experiment has been made with Eyeless, so that its position is doubtful.

In the fourth linkage group the mutant tangled has been given the locus 0.0, not because there is any indication of its whereabouts on the chromosome, but merely because it is at present at one end of the group and is used for fixing the position of other members of the group. On the basis of a small experiment Curly is placed also at the locus 0. Although it is quite possible for two different loci to show no crossing-over especially with small numbers, there is a suspicion that these two mutants may be allelomorphic because $\frac{Cy}{tg}$ flies show Curly and slight tangling at the /

TABLE I

Summary of recent linkage experiments carried out on D. pseudo-obscura. *Flies were cultured at* 23·5 ± 0·5° C. *and counted over a period of about* 10 *days*

Locus	Mutant	Symbol	Loci tested	Cross-overs	Total	% crossing-over	Cross for order
			X-chromosome				
35	dent	*de*	*y–de*	858	3183	27·0	*b* × *y de*
70	singed[2]	*sn*[2]	*v–sn*	7	468	1·5	m^2 × *y v* sn^2
72	vermilion	*v*	*y–v*	639	6551	9·8	m^2 × *y v* sn^2
74	miniature[2]	m^2	*v–*m^2	21	1212	1·7	m^2 × *y v dy se*
74	dusky	*dy*	*v–dy*	21	1212	1·7	m^2 × *y v dy se*
78	Rounded-nick	*Rn*	*v–Rn*	117	2006	5·8	*y* × *v Rn*
116	sepia	*se*	*dy–se*	1579	3778	41·8	+ × *b dy se sp*
140	lanceolate	*ll*	*se–ll*	956	3938	24·3	*ll* × *b dy se sp*
157	snapt	*sp*	*ll–sp*	267	1534	17·4	*sp* × *se ll*
181	short	*s*	*sp–s*	131	541	24·2	*s* × *se ll sp*
182	tilt	*tt*	*ll–tt*	28	67	41·8	*tt* × *se ll*
			2nd chromosome				
20	glass	*gl*	*bx–gl*	672	2678	25·1	*Sb* × *gl bx*
40	Bare	*Ba*	—	—	—	—	—
45	bithorax	*bx*	*Ba–bx*	34	705	4·8	*Ba* × *gl bx*
82	Stubble	*Sb*	*bx–Sb*	724	1973	36·7	*Sb* × *gl bx*
			3rd chromosome				
0	orange	*or*	—	—	—	—	—
8?	Eyeless[3]	*Ey*[3]	*pr–Ey*[3]	98	257	38·1	*pr* × *Ey*[3]
22	Scute	*Sc*	*or–Sc*	826	3828	21·6	*Sc* × *or pr*
46	purple	*pr*	*Sc–pr*	906	3828	23·7	*Sc* × *or pr*
65	cross-veinless	*cv*	*pr–cv*	662	3528	18·9	*Sc* × *or pr cv*
			4th chromosome				
0	tangled	*tg*	—	—	—	—	—
0	Curly	*Cy*	*tg–Cy*	0	144	0·0	*tg* × *Cy*
16?	blunt	*bl*	*tg–bl*	55	337	16·3	*tg* × *bl*
44?	jaunty	*j*	*tg–j*	654	1490	43·9	*tg* × *j* s_4
57?	short$_4$	s_4	*j–*s_4	445	3477	12·8	*tg* × *j* s_4

the end of L2 and L3. Curly is expected to show but not tangled which is a good recessive. As an alternative to this unusual type of allelomorphism, it may be supposed that Curly causes tangled to become partially dominant in somewhat the same way as Abrupt-X causes Scute to become partially dominant in D. melanogaster (Nazarenko, 1930). Although the jaunty-short$_4$ distance is known fairly accurately, they are both so far away from tangled that it has not been possible to determine which is the nearer. Doubtless the use of the intermediate mutant blunt will enable this matter to be settled shortly.

Through the kindness of Dr. Dobzhansky, we have received a number of mutants from the California Institute of Technology, and these have been included in the maps at the provisional loci assigned at Pasadena. With the exception of Bare and Curly their relation to our mutants has not yet been determined, and they have therefore been identified on the maps with an asterisk.

The total number of mutants recorded in these maps is 45, but this number will doubtless be augmented shortly by a considerable number of mutants in hand here and elsewhere. Of these 45 mutants, 10 are dominant, and of these 10, 6 are viable when homozygous, and 4 lethal when homozygous (Notch, Broken, Curly, Eyeless). The mutant Bare is also recorded as lethal /

lethal when homozygous, but in stocks recently obtained all flies are uniformly Bare. This cannot be due to a balanced lethal system as F_1 flies from an out-cross are all Bare. Twenty-seven of these mutants are sex-linked (4 dominant), and 18 autosomal (6 dominant). It will be observed that the sex-chromosome accomodates an unusually long linkage group: even now with several large gaps of uncertain length it appears to be at least 182 units of crossing-over long, and there is little doubt that when all double crossing-over can be detected and possible mutants beyond the farthest now known discovered, it will prove to be nearly 200 units long. As will be indicated later, it seems likely that this is due partly to a high rate of crossing-over per unit of chromosome length as well as to the actual length of the chromosome.

There is still no well-established mutant locus for the small fifth linkage group, but for each of the remaining autosomal groups there are at least four well distributed workable mutants including a dominant, so that the localisation of further mutants should be fairly rapid.

B. Comparison of Mutants in *D. pseudo-obscura* with similar ones in *D. melanogaster*.

1. Sex-linked Mutants.

(a) Pointed (P) and beaded (b). Lancefield (l.c.) and Koller (1932) described in some detail the mutant Pointed /

Pointed of which a second occurrence and two reversions to wild-type have been observed. These authors discussed its possible homology with the Beaded of D. melanogaster, which, under the influence of selection, produced a wing very like that of Pointed. Beaded, however, is unlike Pointed in being lethal when homozygous and in causing a much smaller reduction of the wing when obtained from out-crosses (Bridges and Morgan, 1923) than Pointed. While these differences in themselves do not rule out the possibility of homology, they make it a rather doubtful one, especially as a possibly better case could be made out for the correspondence of Pointed with Beadex of melanogaster (1-59.4). The importance attaching to the homology of Pointed lies in the necessity of explaining the relationships of that portion of the X-chromosome of D. pseudo-obscura which lies to the left of the locus for yellow. This question is narrowed somewhat by a consideration of the mutant beaded which shows a very close resemblance to the cut of D. melanogaster (1-20.0). The wings of some types of beaded are very similar to the figures of cut given by Morgan Bridges and Sturtevant (1925, p.35). Koller (l.c.) figures another type. In both species the mutants are sex-linked recessives with a considerable range of genetic variability and a high rate of mutation. Numerous cut allelomorphs have been recorded, and beaded, /

beaded, which is probably the most frequently recurring mutation in D. pseudo-obscura, has put in at least ten fresh appearances in this laboratory. Unlike cut, beaded causes no observable effect on the eye-shape or on the aristae, and the flattening of the distal antennal joint seen in beaded flies is characteristic of wild-type D. pseudo-obscura. If beaded and cut be now considered to correspond, the speculation might be made that part at least of the Pointed yellow region of the D. pseudo-obscura X-chromosome (with cut (beaded) transferred intrachromosomally) had the same origin as the Beadex region of the X-chromosome of D. melanogaster, and that if bobbed should occur in D. pseudo-obscura there is a possibility of its being found between Pointed and yellow.

(b) Yellow (y) and scutellar (sc). As a rule, the comparison of mutants causing bristle defects is hardly worth while on account of the large number of such mutants, but the proximity of scutellar to yellow as noted by Lancefield (l.c.) creates a high degree of probability that these mutants are both represented in D. melanogaster and other species in which yellow and scute occur together (Morgan Bridges and Sturtevant, l.c., p.207). The appearance of yellow on at least two occasions, and of a yellow allelomorph called cuprous accords well enough with the observed mutability of the yellow locus (1-0.0) in D. melanogaster. /

melanogaster. Both mutants are quite recessive and of good viability. The locus of yellow in the X-chromosome of D. pseudo-obscura is, however, not terminal as in D. melanogaster, but sub-median, and some 60 crossing-over units from Pointed. According to Lancefield's data, scutellar is on the left side of yellow, whereas in D. melanogaster it is on the right.

(c) White (w). Three mutations at the white locus, including an eosin, were discovered by Lancefield (l.c.). Since then, at least three other spontaneously occurring whites have been recorded, and of these w^5 and w^6 are certainly not female-sterile as were w^1 and w^2. In respect of appearance, allelomorphic series, mutability, sexual dimorphism of eosin and inhibiting effect on vermilion, white shows a very good correspondence with the white of D. melanogaster.

(d) Notch (N). A Notch character located close to white, and acting as a recessive lethal has been compared to the Notches of other species by Lancefield (l.c.) Though it has not been cytologically demonstrated that his Notches were caused by deficiencies there can be little doubt that there is a section of chromosome near white which is represented in various species and which has retained its tendency to become deleted.

(e) Singed (sn). Lancefield (l.c.) reported the occurrence of a fairly slight form of singed which was female-sterile, /

female-sterile, recessive, sex-linked and generally similar to the singed of D. melanogaster (1-21.0). Two singed allelomorphs have been found here which differ to some extent from this description. From the point of view of homology, the distinction between singed and forked is one of some importance and has been investigated in detail by Metz Moses and Mason (1923) who could find no reliable criteria for distinguishing them. Short descriptions of these two singed mutants are given, and though it seems that they are closer to the general singed type, it is clear that it is impossible to be sure that they are not really forked.

Singed^2 (sn^2) (Crew and Lamy, unpublished) is a very slight form in which only the long bristles are affected. These are wavy or slightly curled and not much reduced in length. The shorter bristles, hairs and eggs appear to be normal. Low viability and poor separability make it inferior for linkage work.

Singed^3 (sn^3) (Crew and Lamy, unpublished) is an extreme type. All long bristles on the head and thorax are strongly gnarled and depressed, and the short ones slightly wavy. Short acicular branches occur on the aristae, and the hairs on the third antennal segment are slightly wavy. The eggs are normal and the viability and fertility good.

Singed^4 (sn^4) which occurred recently appears to be intermediate between these two, but closer to singed ^{3}.

Since /

Since it has not been possible to test Lancefield's singed with any of these, it is only an assumption that they belong to the same locus. Lancefield's data indicated that singed lay to the right of vermilion, but recently evidence has been obtained (Crew and Lamy, unpublished) that it lies to the left between vermilion and white. This makes the order of the loci for y, w, sn, v, the same for both D. melanogaster and D. pseudo-obscura, although the linkage values involved do not agree.

(f) Vermilion (v). A sex-linked eye colour (Crew and Lamy, 1934) very similar to the mutant vermilion in D. melanogaster (1-33.0) was found by Lancefield (l.c.) and two other spontaneous occurrences have been noted here. Because of its similarity to vermilion, its position in the linkage group, and its interaction with white, there is a presumption in favour of homology. On the other hand, mutations in a variety of sex-linked and autosomal loci produce something like this colour in D. melanogaster, and give the same interaction with white. If, further, the gene producing vermilion does so by preventing the production of brown pigment, then it would have to eliminate more brown pigment in D. pseudo-obscura in order to bring about the same effect. This, of course, is not a real difficulty if the gene for vermilion has no quantitative connection with the absence of brown pigment. /

pigment. The objection to homology raised by Lancefield (l.c.) on account of the yellow colour of the eosin-vermilion compound in D. pseudo-obscura cannot be considered very weighty as there may be just this degree of difference in the production of yellow pigment in the wild-type eyes of the two species.

(g) Miniature (m) and dusky (dy). As with scute and yellow, the appearance of these two characters with closely linked loci in several species provides good evidence of their homology. Lancefield did not intend to signify that his miniature found in D. pseudo-obscura was the same as that in D. melanogaster (1-36.1), but the correspondence of the mutants in appearance, viability, variability and mutability is good. They agree, also, in a tendency to hold the wings extended. As shown previously (Donald, l.c.) dusky also shows a close similarity to the dusky (1-36.2) of D. melanogaster. Unfortunately, the exact relations of vermilion, miniature and dusky to each other in D. pseudo-obscura have not yet been determined, so that it is impossible to say if they lie in the same order as in D. melanogaster. Miniature and dusky are so closely linked in D. pseudo-obscura that so far there has been no crossing-over between them, and it is therefore impossible to decide which is the nearer to vermilion.

(h) Magenta (mg). This mutant has been described fairly /

fairly fully by Sturtevant and Dobzhansky (D.I.S. 1, p.41) and compared with the magenta (1-73.7) of D. virilis which to Metz Moses and Mason (l.c.) suggested the garnet (1-44.4) of melanogaster, and the carmine (garnet?) of simulans. As indicated under vermilion, the probability of error in deciding on the homology of eye colours is high, and consequently the resemblance of these two mutants and the similarity of the position of their loci with reference to the foregoing sex-linked characters, although suggestive, can only be regarded as of doubtful significance.

(i) Sepia (se). This mutant which was named sepia on account of its resemblance to the sepia of melanogaster (Crew and Lamy, l.c.), comes into that category of eye mutations which are characterised by an extra and post-pupal development of brown pigment. Several eye mutants in D. melanogaster, therefore, could possibly be corresponding, but the degree of darkening after emergence points to sepia (3-26.0) as the most likely.

(j) Lanceolate (ll). Since no figures of the mutants called lanceolate or narrow, etc., in other species have been seen, too great significance should not be attached to the use of the name lanceolate here. Nevertheless, it is a fairly distinct type of mutation, and the choice of possibilities is not a very wide one. In Text-fig. 2 it is tentatively made to correspond with /

with the lanceolate (2-106.7) of D. melanogaster.

(k) Short (s), snapt (sp) and tilt (tt). In spite of the inherent difficulties in seeking corresponding mutants for these vein abnormalities, the temptation to trace the affinities of the right arm of the X-chromosome of D. pseudo-obscura has led to the present attempt. Short, which is very similar to $short_4$, may be compared with the abrupt (2-42±) of D. melanogaster. $Short_4$ may equally well be compared with the latter, and whether either, both or neither are homologous is a matter still to be made clear.

As pointed out by Crew and Lamy (l.c.) snapt seems to match the radius incompletus (3-46.0) of Borissenko (1930) fairly well. Both are recessive and of good viability, and appear to affect only the L2 even in the most extreme condition.

Although the tilt of D. pseudo-obscura is more like the tilt (3-40.2) of D. melanogaster than any other mutant described, the agreement is not complete. Gaps in the third longitudinal vein associated with an upward tilt to the end of the wing occur in both mutants, but the tilt flies of D. pseudo-obscura hold the wings in the normal position and have L3 usually short instead of broken. Since some tilt stocks show L2 and L4 short as well as L3, an effect which may also be produced by introducing lanceolate, it is clear that the expression of tilt is easily modified. This may /

may account for its divergence from the tilt of D. melanogaster.

(1) Rough (ro) and ascute (as). On the basis of the descriptions given by Lancefield (l.c.) these two mutants have been compared with the mutants roughoid (3-0.0) and ascute (3-43.5) of D. melanogaster. The probability that the ascutes are the same seems quite high, but the case for the two roughs is weak.

2. Autosomal Mutants.

(a) Bithorax (bx), glass (gl), Stubble (Sb). This group of mutants corresponds very well with those of the same name in D. melanogaster. At present it is immaterial whether bithorax is assumed to be homologous with bithorax (3-58.7) or bithoraxoid (3-59.5) of D. melanogaster since they are so close together, but the fact that there can be some doubt about the homology of such a distinctive character as this illustrates very well the danger of assuming correspondence without a direct mating test.

Glass seems to correspond well with its counterpart in D. melanogaster (3-63). From the descriptions and figures (Bridges and Morgan, 1923) the latter appears to have a colourless rim which is part of the eye and which is caused by limitation of pigment to the central ommatidia. There is also a distinctive colourless rim in the glass of D. pseudoobscura, but here it is due to the reduction in size of /

of the eye which results in the formation of a bright chitinous ring round the eye. The only real difference from the glass of D. melanogaster appears to be that the pigment granules (which are very coarse in both mutants) are distributed in a thin layer just below the facets and extend to the edges of the eye instead of being limited to the central part as in D. melanogaster.

As noted by Crew and Lamy (1935) there is a strong resemblance between the Stubble of D. pseudo-obscura and the Stubble (3-58.2) allelomorphs of D. melanogaster. The measurements made by Dobzhansky (1929) on the legs, wings and bristles of the latter demonstrated the manifold effects of the Stubble genes, and it is clear that the same organs are affected in the same way by the Stubble gene of D. pseudo-obscura.

(b) Bare (Ba). There seems to be some similarity between Bare and the Hairless (3-69.5) of D. melanogaster which also eliminates some of the microchaetae on head and thorax. Homozygous Bare is very like the H/H/+ flies of Gowen (1933) but heterozygous Bare exerts a less selective action on the bristles than heterozygous Hairless and has no associated vein abnormality. The attraction of this comparison, as will be seen later, lies mostly in its corroborative value.

(c) Aristipedia (ar). An example of hereditary homoösis /

homoösis (bithorax) has been known for some time in D. pseudo-obscura, and recently another has been found coming into this category, the significance of which has been shown by Bridges and Dobzhansky (1932). Mutations causing a transformation of the aristae into leg-like organs have already been described (Sturtevant, 1929, Balkaschina, 1929) and appear to be much the same as the one here reported. As it was first seen in large numbers in a stock of pr^4tg which is quite vigorous, it seems unlikely that the low viability investigated by Nikoro (1931) characterises the mutant in D. pseudo-obscura or else it would hardly have established itself in such a stock. Whilst the description by Sturtevant (l.c.) of the aristipedia of D. simulans applies for the most part, it is unsuitable in some respects. There is, for instance, considerable variation in the expression of the character which tends to overlap the wild-type, especially under crowded culture conditions, or at a temperature higher than that of a cool stock room. Further, the legs and bristles are normal, and the third antennal segment retains its usual shape. The extent of sterility in the females is not known. Flies with a good expression of the character show well the reduction of the branches of the aristae, the thickening and segmentation of the stalk of the aristae, and the two tarsal claws. A feature not mentioned by /

by Sturtevant is the occasional expansion of the tip into a spatulate form. The interest of this mutant here lies in the fact that a prediction that its locus would be found to lie in the second linkage group has been borne out by experiment. This is not very remarkable in itself since aristipedia, being probably autosomal from its manner of discovery, must belong to one of four chromosomes. At the same time, the correctness of the prediction strengthens the supposition that the mutants bithorax, Stubble and glass are homologous in D. melanogaster and D. pseudo-obscura because the prediction was based on the fact that the locus for spineless (which is allelomorphic to aristipedia) is close to the loci for these mutants in D. melanogaster. As it has not yet been located within the linkage group, the locus of aristipedia does not appear on the map.

(d) Pink (p). The possibility that this pink is the same as the pink (3-48.0) of D. melanogaster arises as much from the position of their loci in the two species (Text-fig. 2) as from the superficial resemblance of the two characters.

(e) Orange (or), Scute (Sc), purple (pr). Orange was originally obtained from Lancefield under this name. Whether this is the same as the scarlet (Lancefield, 1918) mentioned in the third linkage group (Morgan Bridges and Sturtevant, 1925, p.201)is not known. /

known. It is indistinguishable from the sex-linked vermilion and resembles several autosomal mutant eye colours in D. melanogaster. Comparison with cinnabar shows good agreement, although obviously it is impossible to seek an homologous locus for the orange locus on the grounds of appearance alone. This remark applies almost as well to purple, save that the latter has produced a series of four allelomorphs which give different interaction effects with vermilion (Crew and Lamy, 1934) and so suggests the brown (2-104.5) of D. melanogaster. Brown produces a colourless eye when combined with vermilion or scarlet (Wright, 1932), and has at least five distinguishable allelomorphs. It should be pointed out that purple (2-54.5, mel.) is also very similar, and its interaction with vermilion provided the first example of 'disproportionate modification' (Bridges and Morgan, 1919).

Scute, a dominant mutation causing an elimination of some or all of the large and small bristles on head and thorax, has so far been found of little use in determining the homology of its particular section of chromosome.

(f) Cross-veinless (cv). Phenotypically, this mutant is a fairly exact copy of the cross-veinless (1-13.7) of D. melanogaster, but as shown recently (Donald, l.c.) it differs in having associated with it (at /

(at 23 - 24° C) a semi-lethal effect. Nevertheless, the regularity with which the cross-veins are completely missing rather favours the correspondence of these two; but there are other possibilities such as cross-veinless-c (3-58.3) in D. melanogaster.

(g) Eyeless (Ey). The occurrence of an Eyeless^3 which is rather more extreme than the Eyeless^2 described by Donald (l.c.) strengthens the idea that the Eyeless locus is homologous with the locus of Lobe (2-72.0) of D. melanogaster. Although there has been no approach to the type of Lobe figured by Morgan Bridges and Sturtevant (l.c.) the small flat eyes with nicks in the anterior margin seems to correspond very closely to the description of Lobe^2 (Mohr, 1923) which, however, is viable when homozygous, whereas Eyeless possibly acts as a recessive lethal.

(h) Tangled (tg), short$_4$ (s_4), jaunty (j). In describing short$_4$ and jaunty, Crew and Lamy (1935) drew attention to the possible homology of these loci with the loci for the abrupt (2-42±) and jaunty (2-48.7) of D. melanogaster, so nothing more need be said here about them. It was anticipated that tangled which has shown itself to be very susceptible of genetic modification would be of little use for purposes of homology, but it seems clear that if it is represented at all among the mutants of D. melanogaster, it must correspond to either net (2-0±) or plexus (2-100.5), /

(2-100.5), with the odds in favour of the former on account of its more extreme nature. Like net, tangled is a recessive of good viability.

C. Comparison of the Loci of the foregoing Mutants with the Loci of their Suggested Homologues in D. melanogaster.

For the purposes of the discussion which follows, Text-fig. 2 has been drawn to show the suggested relations between various parts of the chromosome complements of D. pseudo-obscura and D. melanogaster. It shows the mutants mentioned above at their appropriate loci in the various linkage groups with the suggested homologues joined by dotted lines, and incorporates almost completely the previous diagrams of this type (Lancefield, l.c., Crew and Lamy, l.c., Koller, l.c.). If there is any general validity in the conclusions expressed by this figure, there must be a close relation between the left arm of the X-chromosome and the X-chromosome of D. melanogaster, but there can have been little doubt of this since Lancefield published the first evidence on the point. There remains, however, the question of the portion of the chromosome to the left of yellow. Since one of the best cases for homology lies in the similarity of beaded and cut, it seems likely that some of this portion at any rate has the same origin as the rest of this arm. If the suggestion of Koller (1932) concerning Pointed is adopted, /

Text-fig. 2.

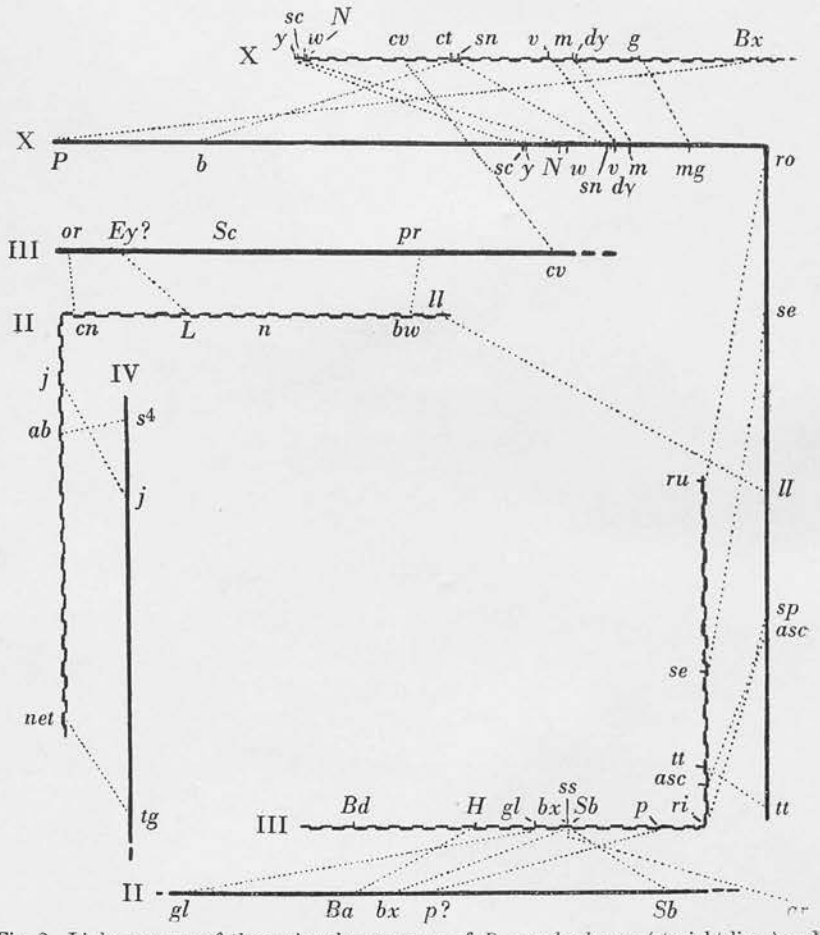


Fig. 2. Linkage maps of the major chromosomes of *D. pseudo-obscura* (straight lines) and *D. melanogaster* (wavy lines) with corresponding loci connected with dotted lines. The mutant *aristipedia* has not yet been located within the second chromosome of *D. pseudo-obscura*.

adopted, then a certain part of the chromosome in the vicinity of this locus would be homologous with the Beaded end of the third chromosome of D. melanogaster. On the other hand, it may have been derived from the source that gave rise to the locus for Beadex which is sex-linked in D. melanogaster. A striking feature of the maps for the two sex-chromosomes is the fact that at least five of the loci (y w sn v m) and the attachment chromomere have the same order. If sections of chromosomes containing the loci for cut and crossveinless be deleted from the region between yellow and singed of D. melanogaster (or the corresponding ones inserted in D. pseudo-obscura) then the two chromosomes would be practically identical for the yellow-garnet-magenta region. Such differences as there are in cross-over values could well enough be due to the influence of the right arm of the X-chromosome on the distribution of chiasmata. Concerning the homology of the right arm, the evidence is not so convincing. Regarded individually, none of the mutant pairs constitute satisfactory evidence, but in the mass they are quite suggestive of a general homology of the right arm with the sepia arm of the third chromosome of D. melanogaster. The figure indicates that the evidence is better than it is, for two of the five lines are based merely on the agreement of the recorded accounts of rough and roughoid, ascute and ascute. However, /

However, these pairs accord very well with the rest, though their value is corroborative rather than intrinsic. According to recent maps (D.I.S. 3) the spindle-fibre attachment lies between the loci for radius incompletus and ascute; if this is so, additional inter-chromosomal exchanges would have to be invoked to account for the positions of the supposed homologues radius incompletus and snapt. Apart from this, however, there is a strong suggestion that the right arm of the X-chromosome of D. pseudo-obscura is equivalent for the most part to the sepia arm of the third melanogaster chromosome, reversed with respect to the attachment chromomeres. Since the former is obviously much longer than the latter, at least one translocation must be postulated to account for the difference in length, and various inversions to account for the difference in relative positions of the loci.

Because it has four mutants (Sb, bx, gl, ar) which may fairly safely be considered to correspond with mutants in the Stubble arm of the third chromosome of D. melanogaster, the second chromosome for the time being may be regarded as fundamentally the same. The fact that none of these four mutants could really be compared with any in D. melanogaster other than the ones indicated must be regarded as supporting this conclusion which was arrived at previously by Crew and Lamy /

Lamy, (1935). For the purpose of establishing this homology, the Hairless - Bare comparison is not of much value; in fact, the possibility that they may correspond is more credible from the positions of their loci than from their actual similarity. Although the order of the loci Sb bx gl is the same for both species, the map distances between them are quite different. This presumably indicates that the process of differentiation has included inversions or deletions affecting this region. If Bare and Hairless were homologous, there would be some evidence that an inversion had actually been involved.

Suggestions concerning the affinities of the third linkage group are somewhat more speculative than those concerning any other group. The credence attaching to them is derived as much from the process of elimination as from direct evidence. Of the five mutants shown in Text-fig. 2, one, Scute, appears to be of no help in this connection, and another, cross-veinless, is best associated with the sex-linked cross-veinless of D. melanogaster. Of the remainder, two are eye colours, and, as such, difficult to compare and open to suspicion when the comparison is made. Nevertheless, the relations indicated in the figure have something to recommend them, and may be used as a working hypothesis until further data justify or condemn them. In discussing the homology of the /

the right arm of the X-chromosome of D. pseudo-obscura above, it was mentioned that if snapt were really homologous with the radius incompletus of D. melanogaster, the existence of the attachment chromomere between the latter and tilt would argue against a simple transference of all the loci mentioned, and would demand a rather special type of exchange to account for the present position of tilt and snapt relative to the attachment chromomere. This situation might therefore be used as evidence that the attachment chromomere of the third chromosome of D. melanogaster is actually between radius incompletus and proboscipedia. A similar type of situation would exist in connection with purple (melanogaster) if it were homologous with the purple of D. pseudo-obscura.

Little can be added to the proposal made by Crew and Lamy (1935) that the fourth linkage group of D. pseudo-obscura is for the most part homologous with the Star-black arm of the second chromosome of D. melanogaster. The comparison of short$_4$ and jaunty with abrupt and jaunty seems very reasonable and receives some support from the resemblance of tangled and net. Since the order of the loci and their relation to the spindle-fibre attachment is not yet satisfactorily established, nothing is to be gained by discussing the tentative order shown with that of the loci shown on the melanogaster chromosome.

D. Discussion.

Many writers have shown that the main contribution of genetics to biology has been the demonstration that it is the material of the chromosomes which is mainly responsible for the phenomena of heredity and variation, and that the causes of evolution must be sought in changes taking place in that material. The methods of genetics have so far revealed the types of changes that can occur, and one of the next steps obviously is to determine the role that each of them has played in the differentiation of species. For the study of this aspect of evolutionary processes, the various species of *Drosophila* offer excellent material, handicapped though they are by the scarcity of inter-fertile forms. Particular interest therefore attaches to those species or sub-species which can be crossed and which offer the opportunity for making direct observations on this point, and the investigations of Sturtevant (1920, 1921) and Kerkis (1936) on *D. melanogaster* by *simulans* hybrids, and of Dobzhansky and Boche (1933), Koller (1936), Tan (1935), and Dobzhansky (1934) on the races of *D. pseudo-obscura* may be cited as examples of the valuable results obtained in this way. These results however can apply in the first instance only to the closely related forms from which they were obtained, and it remains to be seen to what extent the indirect but rapidly accumulating evidence /

evidence from the genetic constitution of inter-sterile and less closely related species supports them. The present paper is an attempt to utilise the information now available for Race A of D. pseudo-obscura for this purpose.

From the point of view of composing a pedigree of the various species of Drosophila, the direct comparison of mutant forms in D. pseudo-obscura with those in D. melanogaster has no more to recommend it than any other procedure, but it has seemed more profitable in other ways to take advantage of the relatively abundant information concerning the latter than to use other less investigated species. It is not therefore to be concluded that there is a gratuitous assumption that either of these species has been derived from the other. A combined genetical and geographical study of the Drosophilinae will be required to elucidate their lineage.

A striking feature of the mutant forms in D. pseudo-obscura is the close resemblance which many of them bear to mutants in D. melanogaster. This resemblance applies not only to phenotypic effects but also to dominance, variability, frequency of mutation and so on. The conclusion to be drawn from this is that during the period of natural selection separating these two species a large number of loci have retained at least some of their major characteristics. This implies /

implies in turn an absence of position effect and a considerable degree of constancy in the biochemical nature of the gene and in the effects of the wild genotype upon the extreme mutant types used in experimental work.

After the work of Metz and Moses (1923) on the chromosomes of Drosophila, it seemed likely that those species with chromosome groups similar to D. melanogaster and D. virilis would be more closely related to each other than they would be to species like D. pseudo-obscura and D. willistoni which have larger sex-chromosomes. It is very noticeable, however, that there is a much better agreement in the sequence of corresponding loci in the sex-chromosomes of D. melanogaster and D. pseudo-obscura than there is in the sex-chromosomes of D. melanogaster and D. virilis (D.I.S. 2, p.44). If there exists equal opportunity for the incorporation of intra-chromosomal changes in the sex-chromosomes of all species, this fact would seem to show that there may not be such a wide gap between the species with long sex-chromosomes and those with short as previously supposed. As far as D. pseudo-obscura is concerned, there is nothing in the constitution of the autosomes as they are known at present which is at variance with this. Considering how few autosomal mutants are known in it there is a surprising amount of agreement in their grouping in the two species, from /

from which it might be inferred that there had been no extensive intra-chromosomal exchanges since the two species diverged from the parent stock, and that the complete reshuffling of mutants to be expected in distantly related species has not occurred.

The recent work of Kerkis (1936) and Dobzhansky and Tan (1936) on the salivary gland chromosomes of hybrid larvae has shown that the failure of pairing in these chromosomes may be due not so much to inter- and intra-chromosomal rearrangements as to the existence of a sort of physiological incompatability, and it seems reasonable to suppose that this is the underlying cause of the inter-sterility of more widely separated forms than they worked with, because viable induced rearrangements as far-reaching as those indicated here between D. melanogaster and D. pseudo-obscura can be imagined within a species.

Dobzhansky and Tan (l.c.) observed in hybrids between D. miranda and D. pseudo-obscura that most of the chromosome rearrangements were intra-chromosomal; that is, inversions and intra-chromosomal translocations were much more frequent than inter-chromosomal exchanges. That this observation probably has a wider application is shown by Text-fig. 2 of this paper, in which comparatively few inter-chromosomal, but numerous intra-chromosomal, translocations are indicated. As suggested by Koller (1936), this is to be expected /

expected from the sterility induced by the former type of rearrangement.

Summary.

1. A revised linkage map for the sex-chromosomes and original maps for the autosomes of *D. pseudo-obscura* are presented.

2. A comparison of the mutants of this species with similar ones in *D. melanogaster* has been made in order to obtain evidence of the kinds of difference in the genetic constitution of the two species. This evidence, although unreliable when its elements are considered separately, offers, as a whole, support to recent work showing that in the process of differentiation of species, intra-chromosomal rearrangements are more frequent than inter-chromosomal exchanges.

3. It indicates also that there is probably a considerable degree of homology between whole chromosomes or whole arms of chromosomes in the two species.

4. The considerable amount of agreement which was found between the comparatively few mutants of *D. pseudo-obscura* and corresponding ones in *D. melanogaster* suggests that the loci involved have retained to a large extent the same characteristics (including phenotypic effects, variability and mutability) in spite of considerable changes in position.

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ON THE SUPPRESSION OF TANGLED

IN

DROSOPHILA PSEUDO-OBSCURA

ON THE SUPPRESSION OF TANGLED IN DROSOPHILA PSEUDO-OBSCURA

Introduction.

The results of the interaction of any two genes cannot yet be foretold with any degree of accuracy. This is another way of saying that our knowledge concerning the problem of the gene and development is still very meagre even in its grosser details. The work of Schultz (1935) and others has shed much light on the reasons for such phenomena as the disproportionate modification of vermilion by purple (Bridges and Morgan, 1919), and for many other observed effects following the combination of eye-colour mutants, but explanations for similar phenomena in other parts of the body, for instance the non-additive action of bristle modifiers found by Plunkett (1926), and the interaction of the wing and bristle mutants of Lebedeff (1935), are of a somewhat more speculative nature. The accumulating data on these problems do not yet allow the empirical method to be dispensed with in seeking the answer to the question: What will happen when these two mutants are put together. From another point of view, the interaction of two genes, when it can be observed, is a useful method of approach in the investigation of genetic control in development, /

development, and has been used by various workers such as Csik (1934), Goldschmidt (1935), Dunn and Coyne (1935).

Simple summation effects for increasing doses of a given gene or its allelomorphs appear to be the rule (for general discussion of this subject and relevant literature see Mohr, 1932). Plunkett (1926), Stern (1929a), Dobzhansky (1929) and Gowen (1933) have all found that the effects of genes affecting bristle size are more or less proportional to their number. Non-allelomorphic genes have also been found to act in this way. Kikkawa (1934) has reported that in D. virilis the genes Confluent and plexus which, like the gene tangled of this paper, are characterised by extra plexate venation, produce when combined more extra venation than either alone. An additive effect in the opposite direction occurs when the mutants radius incompletus and venae transversae incompletae of D. funebris are combined (Timoféeff-Ressovsky, 1927). A third type of combination of mutants affecting the amount of venation, namely one increasing and the other decreasing venation, has been studied in D. pseudo-obscura, and this has also shown an additive effect. The investigation which is reported here arose from an attempt to discover the nature of two 'inhibitors' of the mutant tangled. These 'inhibitors' proved to be wing-mutants causing gaps in the venation so /

so that the investigation became a study of the interaction of several genes as well as of the nature of 'inhibitors' and 'suppressors'. Up to the present, only the qualitative aspects of the matter have been considered, but there is clearly scope for quantitative work on the suppression of tangled.

Description of Mutants and Stocks used.

1. Tangled.

The mutant tangled (tg), which arose as a spontaneous mutation, is a fourth chromosome recessive located at one end of the known linkage group (Crew and Lamy, 1935). No visible effects can be detected in the heterozygous flies, but in the homozygotes there is considerable disturbance of the venation in the region of the posterior cross-vein and near the ends of the second and third longitudinal veins. The disturbance takes the form of extra development of vein-material which gives rise to large plexate patches and additional cross-veins. As a rule, the wings are also blistered, particularly at the posterior cross-vein, and may occasionally contain bubbles of liquid. The best idea of the phenotypic appearance of this mutant can be obtained by reference to Plate I , figs. 2 and 3, which show typical tangled wings. There is some variation in its manifestation which may be slighter than that shown. The wings are not symmetrical, but they always agree in showing /

showing approximately the same degree of disturbance. The wing shape may be narrower than normal at the tip, and one or both wings curled upward. There is little if any difference between the sexes. The two plexate areas are more or less equal in size in the majority of tangled wings. The one at the posterior cross-vein is always considerable, although sometimes obscured by blistering. The other is more variable in extent. This point is of interest in connection with the effects of introducing the following mutant genes affecting vein-length.

2. Short$_4$

On the fourth autosome, also, the mutant short$_4$ (s_4) (Crew and Lamy, l.c.) has been located 50 or more units from tangled. It appears to be a complete recessive, and when homozygous causes a shortening of the fourth and fifth longitudinal veins, and, nearly always, the elimination of the posterior cross-vein (see Plate I, fig. 4). There is not a great deal of variation in expression in present stocks, but occasionally a fly with the fourth longitudinal complete but weak at the tip will be found.

3. Short.

Another short (s) (Lancefield, 1922), located on the X-chromosome at about 180, also causes a shortening of the fourth and fifth longitudinals, but to a much slighter extent (Plate I, fig. 5). At room temperature /

ture many of the males may show little more than a weakening at the tip of the fifth longitudinal. All the females and most of the males, however, show a short fourth longitudinal and an unaffected posterior cross-vein. The phenotypic expression of this short is therefore much less than that of the autosomal $short_4$.

4. Tilt.

Of greater variability than either of the preceding shorts is the mutant tilt (tt) (Crew and Lamy, l.c.) which is located close to short at about 182 on the X-chromosome. In some tilt stocks only a few of the females may show it at all as a slight gap at the end of the third longitudinal. The rest are wild-type. Nearly all males in such a stock show the mutation and have rather larger gaps in the vein, not necessarily at the end of it (Plate I, fig. 6). The fourth longitudinal may also be slightly shortened. In other stocks the expression is much greater. The shortening of the third vein appears in all females, and frequently gaps in the fourth and fifth, and occasionally in the second longitudinals occur. These gaps are all fairly short. In the males, the third vein is missing almost to the anterior cross-vein, the fourth and fifth to the posterior cross-vein, and the second for a short distance at the tip (Plate I, fig. 7).

5. /

5. Shadow-tangled.

The character shadow-tangled (shtg), which acts as an allelomorph of tangled, appears usually as a little extra venation between the distal ends of the second and third longitudinal veins, but this may sometimes be reduced to a faint blurring, or a dot, or may not show at all (Plate I, fig. 8). It appeared in the F_2 of a mating of one of the original tangled females to miniature $short_4$ jaunty males, as shadow-tangled $short_4$ jaunty flies, and upon a long inbred stock raised from these, part of the present investigation was carried out.

6. Tangled inhibitor.

One of the F_1 females from which the above F_2 was raised had been outcrossed and had produced a son with all the longitudinal veins short. He must have been also heterozygous tangled, for, on outcrossing, he gave rise to an F_2 with tangled flies. These tangled flies were mass cultured for several generations, and on examination they were then found to include many males which were not tangled; some of them were wild-type and some had a gap in the third or fourth longitudinal vein. Several pair matings now showed that certain females were heterozygous for a 'tangled inhibitor' which was sex-linked and recessive. By selection, a stock was obtained in which the females were wild-type or slightly tangled, and in which the /

the males had sometimes gaps in the veins but were usually wild-type. These males, although never tangled, gave only tangled offspring when outcrossed to stock tangled females.

The stock of 'tangled inhibitor' used in these experiments shows a certain amount of variation (Plates I, II, figs. 9 - 13). The males may resemble a weak type of tilt; or may be quite wild-type; or they may have a posterior cross-vein, that is three-quarters to half the normal size, and perhaps slightly askew. This is the only kind of variation on the tangled side of normality which is ever seen. The females, on the other hand, are often wild-type, but rarely have any gap in the venation, and they may show varying degrees of slight tangling in both the usual places. The greatest amount of tangling is at the posterior cross-vein, and as the wild-type is approached the last traces are always seen here.

Experimental Results.

(a) The Suppression of tangled by short$_4$.

It has already been reported (Donald, 1936) that linkage experiments with a stock of shadow-tangled short$_4$ jaunty (shtg s_4 j) flies gave rise to the belief that shadow-tangled was the outcome of the interaction of tangled and short$_4$. This has now been proved by the synthesis of tangled short$_4$ jaunty flies which are shadow-tangled and breed true when mated to stock /

stock shadow-tangled flies. The experimental results are as follows:-

(1) The first crosses made were $\frac{\text{shtg j } s_4}{+ \quad + \quad +}$ ♀♀ x shtg j s_4 ♂♂ and the results published in connection with the linkage data obtained from them. They showed that most of the shadow-tangled flies were at the same time $short_4$ and that the remaining tangled flies were not of the usual stock type as expected, but were intermediate between shadow-tangled and tangled; that is to say, there were clear extra veins in both the usual regions of the wing, but the tangling was 'open' and without the large plexate areas, there were no blisters, and the wings were flat (Plate II, fig. 14). About half the total number of tangled flies (see Table I) was comprised of this type.

Table I. /

Table I.

Offspring of $\frac{shtg\ j\ s_4}{+}$ ♀♀ x shtg j s_4 ♂♂

Class	Genotype	First brood ♂♂	First brood ♀♀	Second brood ♂♂	Second brood ♀♀
+	$\frac{tg\ j\ s_4}{+\ +\ +}$	189	235	65	81
shtg j s_4	$\frac{tg\ j\ s_4}{tg\ j\ s_4}$	122	153	72	87
shtg)	$\frac{tg\ j\ s_4}{tg\ +\ +}$	28	2	11	-
intg)		100	160	48	70
j s_4	$\frac{tg\ j\ s_4}{+\ j\ s_4}$	128	149	97	93
j	$\frac{tg\ j\ s_4}{+\ j\ +}$	15	26	15	8
shtg s_4	$\frac{tg\ j\ s_4}{tg\ +\ s_4}$	17	29	11	12
s_4	$\frac{tg\ j\ s_4}{+\ +\ s_4}$	28	37	15	15
intg j)	$\frac{tg\ j\ s_4}{tg\ j\ +}$	19	43	18	24
shtg j)		6	4	1	3
		652	838	353	393

Total	♂♂	♀♀
(intg) (shtg)	231	278
s_4 shtg	222	281
tg	453	559
Total flies	1005	1231

This obvious reduction in tangling has been interpreted as due to a single dose of the short$_4$ gene in tangled flies arising from crossing-over between the short$_4$ and tangled genes which show free recombination. Subsequent work verified this interpretation. The dominant phenotypic effect thus produced by the heterozygous short$_4$ gene was considerable. The reduced /

duced form of tangling was easily distinguished from the stock type of tangled, but not always from shadow-tangled which it overlapped frequently, especially among the males. Table I. shows that quite a large number of males were classified as shadow-tangled without short$_4$. Since the latter always appears when homozygous even in the presence of homozygous tangled, these males must have been genotypically the same as those classified as intermediate-tangled (intg), namely, $\frac{s_4\ tg}{+\ tg}$.

A corresponding phenotypic effect of tangled in $\frac{s_4\ tg}{s_4\ +}$ flies seems probable, and the impression that the veins were more shortened in such flies than in s_4 tg flies is supported by comparison of stock cultures of short$_4$ with and without tangled, but the effect, if real, is not very marked.

(2) The next step was to produce shadow-tangled short$_4$ flies by starting with the cross tangled by short$_4$. Jaunty was present in this cross but has been disregarded. The F_2's from this cross were not raised to completion after it was observed that the expected classes occurred according to the following scheme:-

Cross	Eggs	Sperm	Phenotype
$\frac{s_4}{tg} \times \frac{s_4}{tg}$	s_4	$\frac{s_4}{tg}$	$\frac{s_4}{+}$
	tg	$\frac{s_4}{tg}$	$\frac{+}{tg}$
	s_4 tg	$\frac{s_4}{tg}$	$\frac{s_4}{intg}$
	+	$\frac{s_4}{tg}$	$\frac{+}{+}$

At /

At the same time as these F_2's were made, two F_1 females were back-crossed to stock shadow-tangled short$_4$ males. The results of these matings are given in Table II.

Table II.

$\frac{s_4}{tg}$ ♀♀ x shtg s_4 ♂♂ (2 cultures)

Class	♂♂	♀♀	Genotype
s_4	30	37	$\frac{tg\ s_4}{+\ s_4}$
intg	28	29	$\frac{tg\ s_4}{tg\ +}$
shtg s_4	12	20	$\frac{tg\ s_4}{tg\ s_4}$
+	24	42	$\frac{tg\ s_4}{+\ +}$

As expected, there were no typical tangled flies. The shadow-tangled short$_4$ cross-over class was exactly the same as stock, and it was considered satisfactorily established that the shadow-tangled character was due to the partial or complete suppression of tangled by short$_4$.

(b) The Suppression of tangled by short.

In order to find out if short would have the same suppressing effect on tangled as short$_4$, short females were mated to tangled males. F_1 females mated to tangled males produced offspring that would be expected if the two shorts had much the same effect (Table III).

Table III. /

Table III.

$\frac{s\ tg}{}$ ♀♀ x tg ♂♂

Class	♂♂	♀♀	Genotype
tg	29	53	tg/tg
+	51	114	tg/+, s/+ tg/+ (♀)
s	42	-	s tg/+, s tg
s shtg	25	-	s tg
shtg	1	20	s/+ tg (♀)
intg	1	9	s/+ tg (♀)

Although not equal in size, the classes of males are otherwise as expected (Plate II, figs. 15 - 17). Apparently a male genetically short tangled may sometimes not show short, and a male genetically tangled may show a rather slight type of tangled. Among the females there are again too few of the reduced tangled type (some were probably classified as wild-type), but it seems clear that the presence of heterozygous short has caused the females to be classified mostly as shadow-tangled and not intermediate-tangled as in the experiments with $short_4$. Short has therefore a greater suppressing effect in the heterozygous condition than $short_4$. In fact, many of the $\frac{s}{+}$ tg ♀♀ showed very slight traces of tangled, traces as slight as those produced by homozygous $short_4$ tangled females.

(c) The Suppression of tangled by tilt.

The inference that the 'tangled inhibitor' might be the mutant tilt was now an easy one, and has been proved correct by the process of making synthetic inhibited-tangled flies and mating them to stock flies.

Tilt /

Tilt females were first crossed to tangled males. All sons should have been tilt, but a few appeared wild-type. This might be attributed to a converse effect, namely, the suppression of tilt by tangled, but the evidence from tilt stocks and from tilt flies heterozygous for tangled is inadequate to show if tilt males which are heterozygous for tangled approach the wild-type more often than tilt males which are not. F_1 females were then chosen to back-cross to tangled males as they should produce inhibited-tangled sons if tilt were the inhibitor, and would at the same time show how heterozygous tilt compared in effect on tangled with the heterozygous shorts.

Table IV.

Offspring of $\frac{tt}{+}\ \frac{tg}{+}$ ♀♀ x tg ♂♂ (5 cultures)

Class	♂♂	♀♀	Genotype
tt	68	-	tt tg/+
tg	44	203	tg, tt/+ tg (♀)
+	122	222	tt tg, tg/+ (♂♂) tt/+ tg/+, tg/+ (♀♀)
intg	52	2	tg, tt tg
tt intg	4	-	tt tg
tt short C_2	5	-	tt tg
short C_2	15	-	tt tg
Total	♂♂ 310	♀♀ 427	

Unfortunately, these results are not very easy to interpret. There can be no doubt that tilt has inhibited tangled to a certain extent, but to what extent /

tent is not clear. One quarter of the total males should have been tilt-tangled, but actually less than one-thirtieth of the total number (9 in 310) appeared. At first sight it seems probable that they are included in the class intermediate-tangled, but there are two objections to this. One is that the intermediate-tangled class differed from that in previous experiments in being difficult to distinguish from tangled rather than from shadow-tangled, so that while it probably contains some tilt-tangled males, the majority will be tangled only. Another objection is that tangled would show a viability much poorer than that of the wild-type, whereas they are consistently about equal in other experiments. The conclusion must be, therefore, that the wild-type class also contains many tilt-tangled males. The classes called 'short C_2' had no other sign of tangled than a shortening of the posterior cross-vein like that occurring in the tangled-inhibitor stock, and on testing proved to be of the constitution tilt-tangled. The four males appearing as'tt intg'are obviously tilt-tangled, and show that tilt can be present in a wing with a good deal of tangling.

It is clear that there is a good deal of variation in the appearance of the tilt-tangled males of this experiment (Plate II, figs. 18, 19). They varied from a fairly good tilt, through wild-type, short posterior cross-vein /

cross-vein with and without tilt, to slight and intermediate tangling, but no typical tangled was found to be at the same time tilt. In stocks of the tangled-inhibitor the tangling in males is further reduced than this, being practically eliminated. The best explanation that can be offered is that selection in the 'tangled inhibitor' stock has accumulated modifiers of tilt which are known to exist, and which increase its expression and at the same time its suppressing effect on tangled. Further evidence on this point is forthcoming from experiments with tilt flies that carried lanceolate (11, 1-140) which is associated with an intensifying effect on tilt (see below). Table IV. shows also that tangled females which are heterozygous for tilt show no appreciable difference from those which are not. In this respect, therefore, tilt differs from the shorts in being practically recessive in its suppressing action.

As anticipated, the various grades of tangling found in this experiment are not exactly the same as those in the previous experiments. For this reason the term shadow-tangled has been dropped as it is properly used only to describe those flies which have a slight degree of tangling at the distal ends of the second and third longitudinals and none at the posterior cross-vein. This is the type produced by the combination of the shorts with tangled. With tilt the /

the tangling disappears last at the posterior cross-vein and is greater there than in the anterior region at all grades of expression. This implies that the shorts have a different 'intensity distribution' from that of tilt.

In order to determine whether or not a more extreme type of tilt would have a greater suppressing effect, lanceolate-tilt females were mated to tangled males, and their daughters back-crossed to tangled males. The offspring of five such F_1 females are classified in Table V. (Plate II, fig. 20).

Table V.

Offspring of $\frac{ll\ tt\ tg}{+\ +\ +}$ ♀♀ x tg ♂♂

Class	♂♂	♀♀	Genotype of males
ll tt	50	-	ll tt tg/+
+	67	183	tg/+, tt tg
ll	33	-	ll tg/+
tt	20	-	tt tg/+, tt tg
tg)	45	177	tg
intg)	20	3	tg, tt tg
shtg	10	4	tt tg
tt shtg	3	-	tt tg
ll shtg	6	-	ll tt tg
ll tt shtg	13	-	ll tt tg
ll tg	10	-	ll tg
Total	♂♂ 277	♀♀ 367	

Among /

Among these flies the intermediate-tangled are again to be regarded as tangled and not tilt-tangled. There may be one or two tilt-tangled flies which appeared as intermediate-tangled, but since tangled and intermediate-tangled were difficult to distinguish, whereas shadow-tangled was of a very slight type and easily separated from intermediate-tangled, it is considered safe to regard the intermediate-tangled flies as tangled. Disregarding lanceolate, the totals become:-

	♂♂	
tt	70	(68)
tg (intg)	75	(96)
+	100	(122)
tt tg	32	(24)
	277	(310)

The figures in brackets refer to the corresponding totals from the previous experiment in which there was no lanceolate. The conclusion drawn from these figures is that many of the flies classified as wild-type in both experiments were actually tilt-tangled, and that some of the intermediate-tangled flies of the previous experiment with tilt were also tilt-tangled. This means, if correct, that the use of the lanceolate-tilt flies has brought about a relative decrease in the amount of tangling; this, in turn, supports the suggestion made above that the absence of even slight tangling in the males of 'tangled inhibitor' stocks is /

is due to the accumulation of genetic intensifiers of tilt.

Females of the constitution $\frac{ll\ tt}{+\ +}$ tg were now mated to stock males carrying tangled and the inhibitor. If tilt is actually the inhibitor, their sons should be half tangled and half tilt, shadow-tangled or wild-type, and their daughters half tangled and half like the females of the inhibitor stock.

Table VI.

$\frac{ll\ tt}{+\ +}$ tg ♀♀ x stock tg inhibitor ♂♂ (3 cultures)

Class	♂♂	♀♀
tg	16	37
intg	-	15
ll tg	1	-
+	6	34
ll	7	-
ll tt	22	-
tt	12	-
Total	♂♂ 64	♀♀ 86

The total number of flies is unexpectedly small owing to the unrealised presence of two mutants of low viability - Knobby eyes and gouty legs - in the parents, but even though the actual proportions of the various classes has little or no significance, the appearance of wild-type sons and daughters proves the identity of the tangled inhibitor with tilt.

As a check, several males classified in Table VI. as wild-type, but showing only a short C_2, were mated to tangled females. These produced only tangled offspring. /

offspring. It is perhaps worth noting that as tilt occurred as a mutation among flies with and without tangled, its role as inhibitor was obscured.

Discussion.

The results obtained may be explained on the following hypothesis. When the mutant tangled is introduced into the wild-type fly the usual process of vein-formation is altered so that excess vein material is formed in definite regions of the wing. Whether this means that the necessary internal environment for the action of tangled exists only near the ends of the wing, or whether the usual processes are less easily disturbed in the proximal parts need not be decided for present purposes. The opposite kind of effect is produced by the vein-shortening mutants which may be supposed to inhibit the process of vein-formation or to remove its products in the distal parts of the wing. If these two opposed influences are at work simultaneously and independently, the result will depend on whichever was the stronger. Underlying the term 'strong' may be actually rate or period of activity, moment of initiation, or combinations of these and other factors.

It has been found that the suppression of tangled is not confined to those parts of the wing which normally exhibit the interrupted venation. It is therefore necessary to suppose that short, $short_4$ and tilt /

tilt exert a suppressing effect on the tangled venation in regions of the wing where they are never able to suppress normal vein-formation. A more exact study of the variation in the intensity of action of a gene over various parts of its field is possible with bristle modifiers, and the work of Sturtevant and Schultz (1931) and Rokizky (1931) shows that a gene may have a field of action which varies irregularly about a maximum. The actual degree of suppression of tangled achieved by these mutants is therefore regarded as varying in different parts of the wing. Since the shorts, which shorten the fourth and fifth longitudinal veins, have a greater suppressing effect at the posterior cross-vein than they do at the end of the third and second longitudinals, their field of influence must fade away from a maximum which is presumably at or near the point where they produce phenotypically visible effects in the absence of tangled. Tilt causes gaps to appear in the third longitudinal veins and may therefore be considered to have a maximum effect in that region. That its effect may extend farther on both sides is shown both by the appearance of gaps in the other longitudinals in flies with good manifestation of the character, and also in the reduction in tangling on both sides of the third longitudinal. The considerable variation found among flies genetically tangled and short, $short_4$, or tilt, seems to /

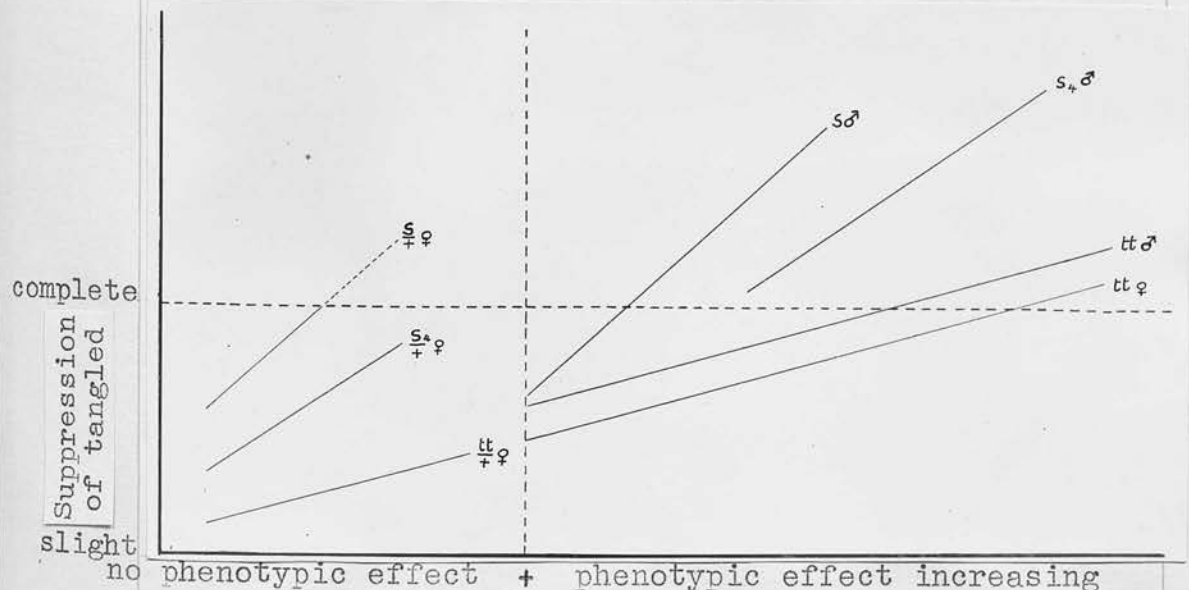
to indicate that the variability of the individual mutants is retained in their combinations. If this is so, then it is necessary to postulate that their mutual suppression may be compatible with a measure of independent activity; that is to say, neither mutant in a given combination actually suppresses the working of the other, but a quantitative adjustment occurs in accordance with the opposed nature of their end-products.

Another source of variability in the degree of suppression is the actual dosage of the mutants. No obvious evidence that tangled when heterozygous can reduce the manifestation of any of its homozygous suppressors has been found, but the reverse is clearly true, at least for the shorts. Heterozygous short or $short_4$ females which are at the same time homozygous tangled show an unmistakable reduction of tangling which may approach complete suppression. Nevertheless, tilt, which may have the most extensive phenotypic effects, does not seem able to produce much suppression in the heterozygous female. These facts provide further evidence (cf. Timoféeff-Ressovsky, 1934) that genes which are completely recessive or dominant are probably very scarce. By introducing tangled into the genotype, short is able to produce a strongly dominant phenotypic effect, $short_4$ a weaker one, and tilt possibly a very weak one.

A /

A certain amount of sexual dimorphism has also been found in the results, particularly in connection with tilt. It is noticeable, for instance, that the single dose of the sex-linked tilt in the male produces a definitely greater suppression than a double dose in the female. This is in keeping with the sex-dimorphism of stock tilt.

On the basis of the foregoing considerations, the following diagram has been constructed to illustrate graphically the preceding hypothesis to explain the phenotypic effects observed in the various combinations of tilt with the vein-shortening mutants.



The diagram represents the hypothetical relation between the 'intensity' of gene action of the suppressors as measured by the degree of phenotypic expression in the absence of tangled, and their suppressing /

suppressing effect on tangling in the region of the posterior cross-vein. Suppression, however, is mutual, and though occasional flies may show a degree of tangling in this region together with a slight shortening of the veins, the suppressors will in general begin to manifest themselves in the presence of tangled at the point where their curves cut the abscissa indicating complete suppression of tangled. On the basis of the comparison of tt tg and ll tt tg flies, of the sex-dimorphism and of the observation that the extreme types of manifestation of the suppressor are not found in the presence of tangled which appears to be incompatible with even a moderate manifestation of the suppressors, it is assumed that the degree of suppression is proportional to the phenotypic expression without tangled. This may be expressed in other words as an additive relation between tangled and its suppressors and is represented by a straight line, the ends of which are determined by the range of variation in phenotypic effect. The appropriate lines have been drawn so that the abscissa for complete suppression of tangled divides them into parts with the same ratio as that of tangled to non-tangled flies in a population carrying both tangled and the suppressor.

In the area to the left of the ordinate marked + the intensity of gene action of the heterozygous suppressors /

pressors is below the threshold for phenotypic manifestation. The end points of the curves here were estimated from the observed variation in suppression and the lines drawn parallel with the corresponding ones in the right-hand section of the diagram.

Since the range of variation of tangled itself is not negligible, the curves refer to one point in that range, and all the observed variation in tangling is ascribed only to the effect of the suppressors.

According to a recent definition which states that "the early term 'inhibitor' may be used in the general sense of a genetic agent of unspecified nature which prevents another genetic factor from showing its effects", and that the term 'specific suppressor' is to be applied when "a given mutant character is inhibited by the action of a second mutant gene, the double mutant type and the second mutant type both appearing like unmutated wild-type" (Bridges, 1932a), the mutants short, $short_4$ and tilt are doubtless to be classed as 'inhibitors' of tangled.

The various specific suppressors which have been recorded affect a fairly wide range of mutants. The original suppressors of vermilion and sable have been succeeded by others affecting such widely different mutants as purple (Stern, 1929b, Bridges, 1932b), black (Plough, 1929), scute, deltex and cut (Bridges, 1932a)./

1932a). The nature of these suppressors is at present uncertain. Recent work by Bridges (1935, 1936) on duplications in salivary gland chromosomes has renewed the possibility that the specific suppressors may be associated with duplications involving the locus of the gene suppressed, but whether they can all be explained in this way is still doubtful. Indeed, the foregoing experimental results might be construed as evidence that some suppressors not of this nature may be found.

It might be argued with some justification that from one point of view, short was an inhibitor of tangled, and from another that it was a suppressor of tangled. For instance, in so far as short produces a shortening of veins, it must be regarded in the light of the above definition as an inhibitor, but by the same token it is a suppressor because it prevents the appearance of tangled at the distal end of the second longitudinal vein where it never appears itself. Short might also be regarded as a suppressor when it is heterozygous in the female, but not when it is homozygous. The same considerations apply with appropriate variations to the other mutants dealt with here.

A comparison of tilt with short shows that a gene with a slight phenotypic effect is not necessarily one with a negligible internal effect. Certainly mutants with slight phenotypic effects have often the least detrimental /

detrimental influence on the viability of the flies which possess them, but this is by no means the rule. Further, Timoféeff-Ressovsky (1934) has drawn attention to the fact that the proportion of dominants among the mutants with poor manifestation is about the same as that among those with good manifestation. It is not surprising, then, to find genes which have no phenotypic manifestation at all suppressing others which have. It would easily be possible to construct a series of mutant genes showing a gradation in visible effect from 0 - 100 per cent., but the position of any gene in this series would be no indication of its ability to prevent the expressions of another gene with an opposed characteristic. Thus specific suppressors could be modifiers without visible effect, but passing gradually into those with visible effects, namely, inhibitors. It seems unlikely that such specific suppressors could be distinguished by the time at which they acted to produce their suppression. It is clear that whether they act at the same time, before, or after the gene of which the phenotypic effects are suppressed, the same latitude must apply to inhibitors as well.

Without prejudice to the actual processes by which somatic effects are produced, the difference between the 'inhibition' of tangled and the 'suppression' of, say, black, might be considered as connected with /

with the fact that the intensity of gene action in the "Wirkungsfeld" of short, $short_4$ or tilt varies so that the point of complete suppression is not attained under all conditions in all parts where tangled appears, whereas the black suppressor passes the threshold of suppression in all parts of the body.

The fact that specific suppressors prevent the appearance of all the characteristics of 'pleiotropic' genes indicates that even if the latter were not really pleiotropic (as suggested by Kamschilow,1935) they are the direct and original cause of the development of all the associated characteristics which could not appear if there were any gene (eg. a suppressor) preventing the primary reactions of the first mutant gene, or removing the products of them. The mutual suppression of $Notch^8$ and Abrupt-X (Nazarenko, 1930) seems to come into a different category. The pleiotropic effects of $Notch^8$ are probably due to a deficiency of a number of genes, and therefore would not arise from one initial deviation from the normal processes of development. A single gene 'suppressing' dissimilar processes involving several genes is less likely to be found that one 'suppressing' a single process, involving one mutant gene.

Schultz and Bridges (1932) have pointed out that suppressors might be used as examples of modifying genes which Fisher (1930, 1932) has postulated for maintaining /

maintaining the dominance of the wild-type. The fact that all the specific suppressors shown to be genes are recessive would indicate on Fisher's theory that they had not originated as neutral or advantageous mutations, but as unfavourable ones which had themselves been modified by selection in the direction of the wild-type. This means that they could have begun as facultative inhibitors. In a population where two mutants are regularly appearing which have opposed end-products there will be as the result of selection the tendency for each to become progressively more effective as a suppressor, and at the same time to have its own phenotypic effects reduced to a minimum. This is more or less the situation now presented by the specific suppressors. They have reached the stage of being phenotypically invisible and at the same time more or less complete inhibitors of another recurring mutation. Put another way, some specific suppressors might be at the last stage in the conversion of a gene complex into a condition in which it no longer reacts to a particular mutation.

Certain of the steps in such a mode of evolution of a specific suppressor might be illustrated as follows:-

(1) Tilt. This mutant has a considerable and obvious disadvantageous phenotypic effect when homozygous, and the genetic environment which enables it to suppress tangled to the greatest extent is the one which increases /

increases its own manifestation.

(2) Short$_4$ suppresses tangled more than tilt, and is still accompanied by a fairly considerable phenotypic effect.

(3) Specific suppressor of Hairy-wing has reached the stage of complete suppression, but its own somatic effects are far from negligible.

(4) Short. A slight phenotypic effect overlapping wild-type and a further increase in ability to suppress tangled.

(5) The recessive modifier of Frizzle in fowls (Hutt, 1932, Landauer, 1933), reduces the expression of Frizzle, and has apparently no somatic effects of its own.

(6) Specific suppressor of vermilion. If there are actually no somatic effects, this mutant would have reached the last stage in which suppression is complete, and somatic effects modified to the point of identity with wild-type.

These stages are obviously not comparable inter se owing to the differences in the nature of the mutants suppressed and of their suppressors, and it is not intended to convey that such a series has any considerable evolutionary significance. But if specific suppressors may have arisen in this way, it is difficult to distinguish them arbitrarily from those which have arisen de novo in the final stages.

The /

The fact that a specific suppressor was recessive in its action would offer no difficulties to the comprehensive theory of dominance proposed by East (1935). On his view, these would be recessive because they caused a restriction in the physiological processes in which they were involved. From the stand-point of Fisher's theory, this fact would be surprising. It would be expected that if a mutant had an advantageous or neutral effect it would show at least partial dominance. This is true of the inhibitors of tangled, which therefore might be considered as examples of mutants with both advantageous and disadvantageous effects of which the former have retained their dominance, and the latter have been modified in the direction of recessivity. Such mutants would unite the two conditions discussed by Fisher (1930, p. 65), namely, the dominance of advantageous mutations and the recessiveness of disadvantageous mutations.

Summary.

1. Two different types of suppression of the character tangled in Drosophila pseudo-obscura have been found to be due to genes causing interrupted venation.

2. /

2. The experimental results suggest that tangled interacts in an additive way with its suppressors, and that modifiers tending to increase phenotypic manifestation act in the same way.

3. Three genes which have been found to suppress tangled have recessive phenotypic effects, but at least two have dominant suppressing effects, and all have suppressing effects in regions of the wing in which they are not themselves manifested.

4. The inverse relation which was found between the phenotypic expression and the degree of suppression effected by these three mutants suggests that there may be no clear line of demarcation between mutants coming into the categories of 'specific suppressors' and 'inhibitors'.

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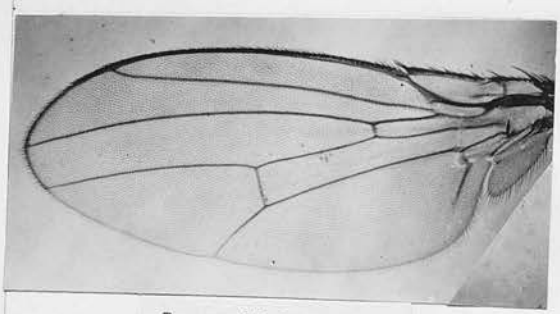
Plate I.



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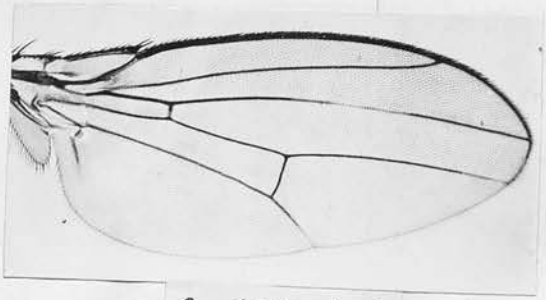
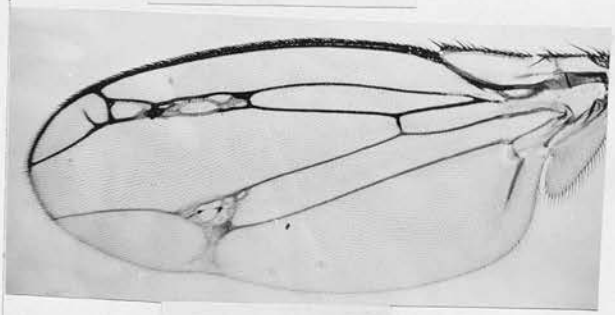
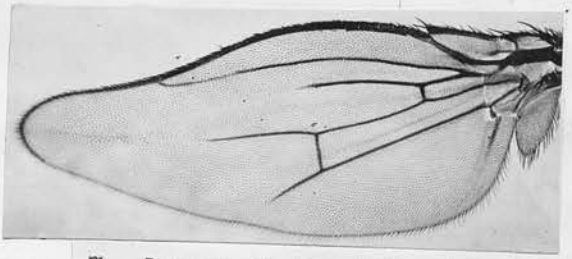


6. tilt (♂)



2. tangled



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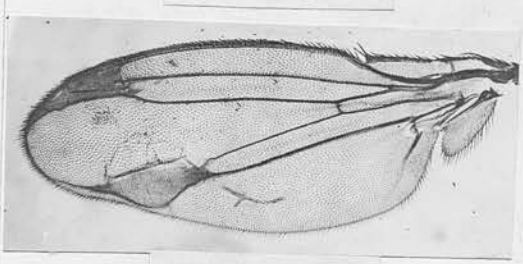
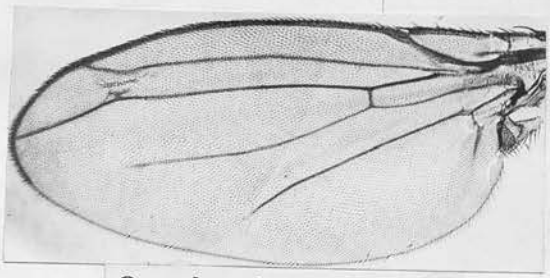


3. tangled



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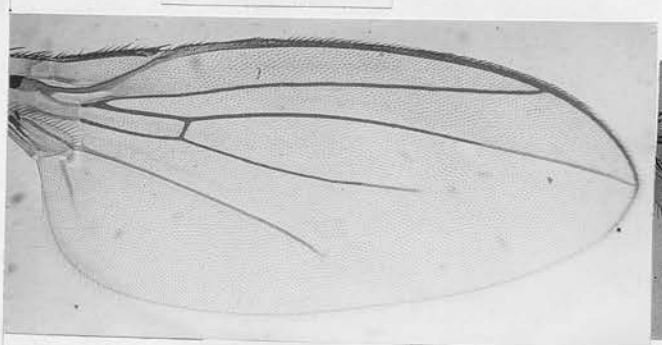
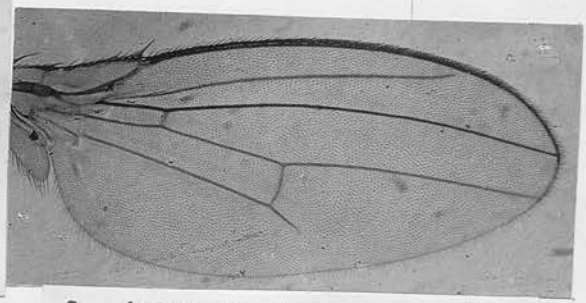
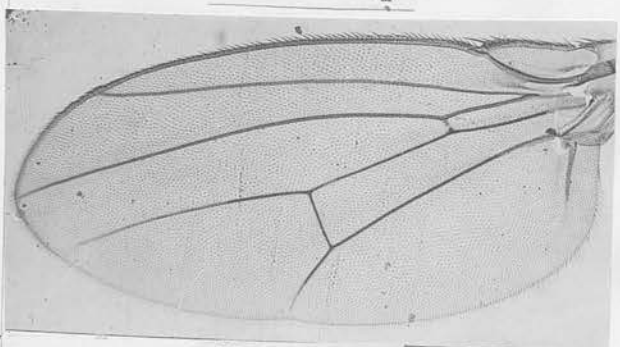


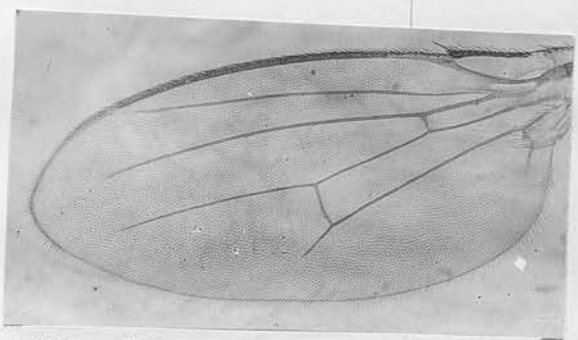
4. short$_4$



9. inhibited tangled (♂)

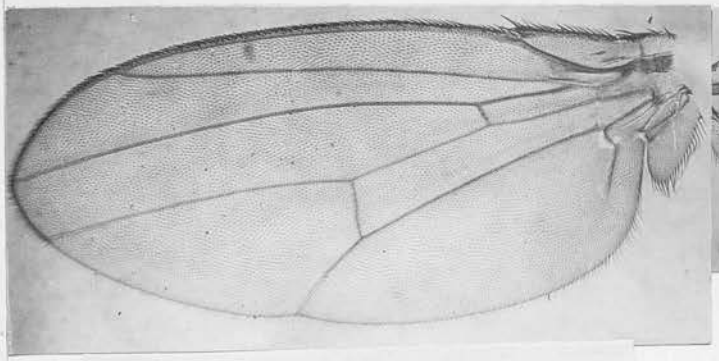


5. short

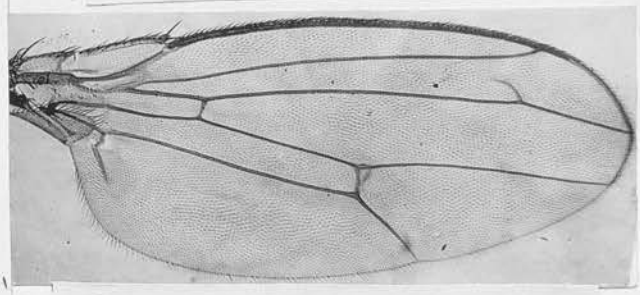


10. inhibited tangled (♂)

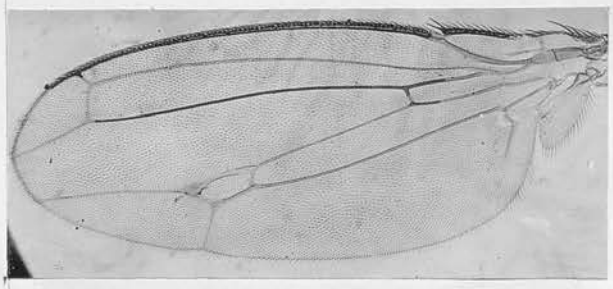
Plate II.



11. inhibited tangled (♀)



12. inhibited tangled (♀)



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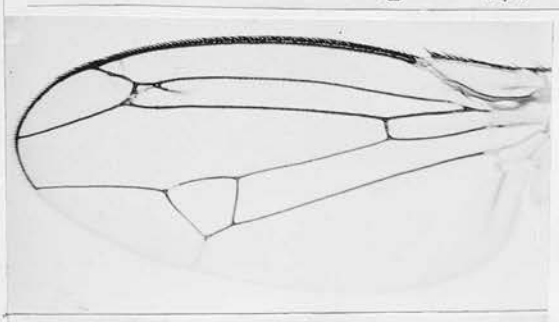
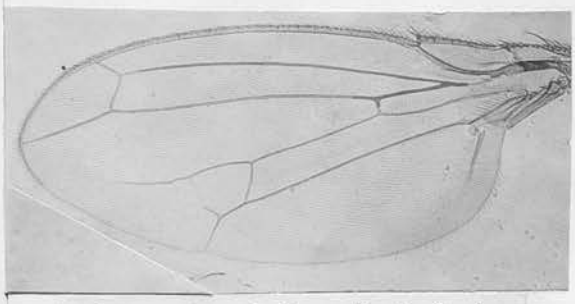
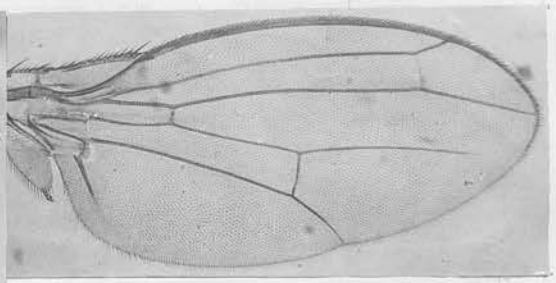


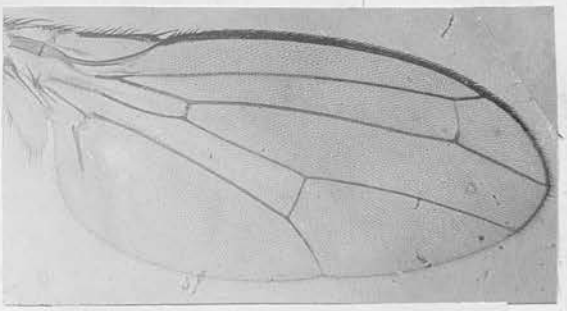
14. intermediate-tangled



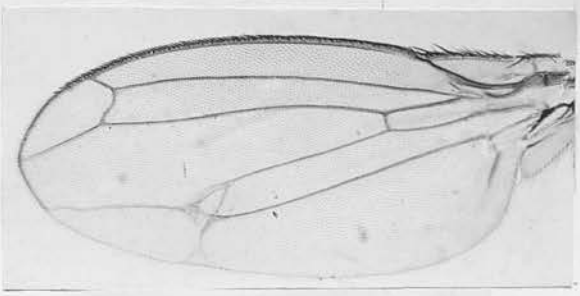
15. short tangled (♂)



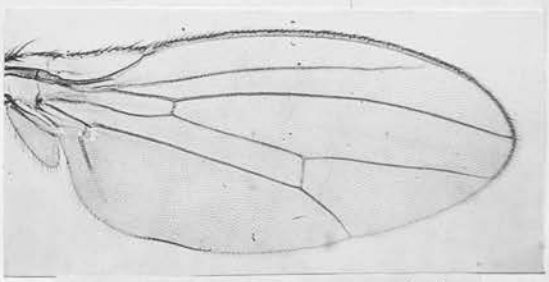
16. short tangled (♂)



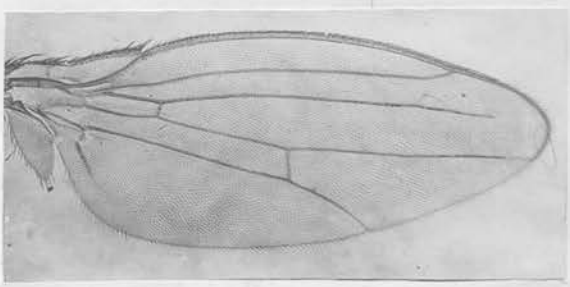
17. short tangled (♂)



18.tilt tangled (♂)



19. tilt tangled (♂)

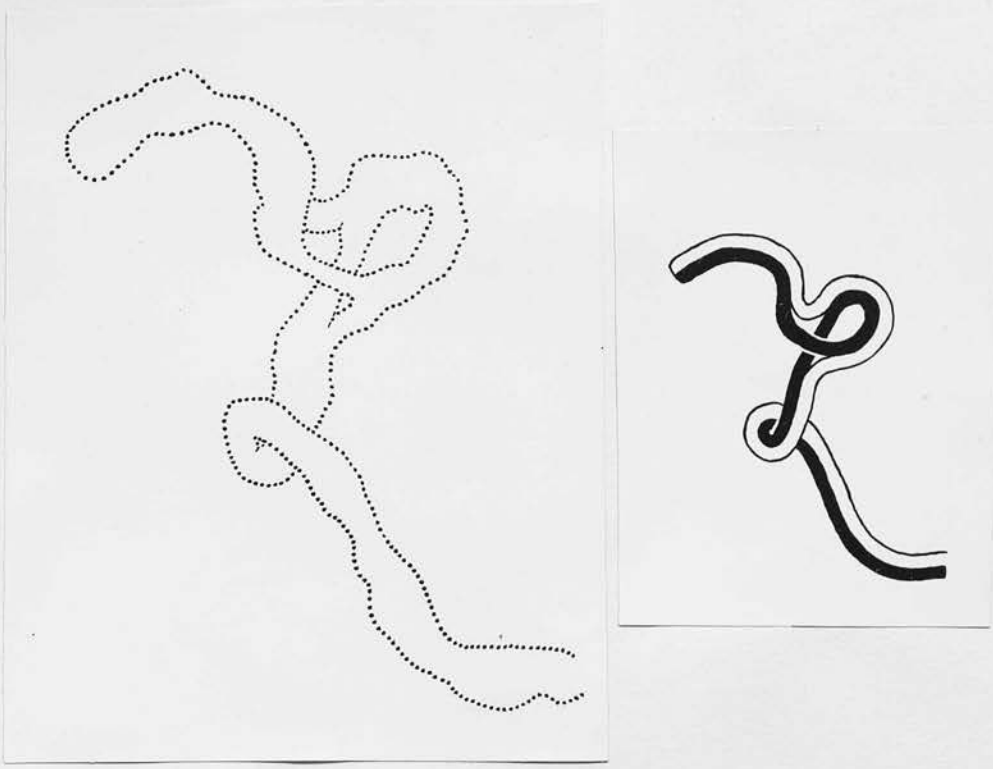


20.lanceolate tilt tangled (♂)

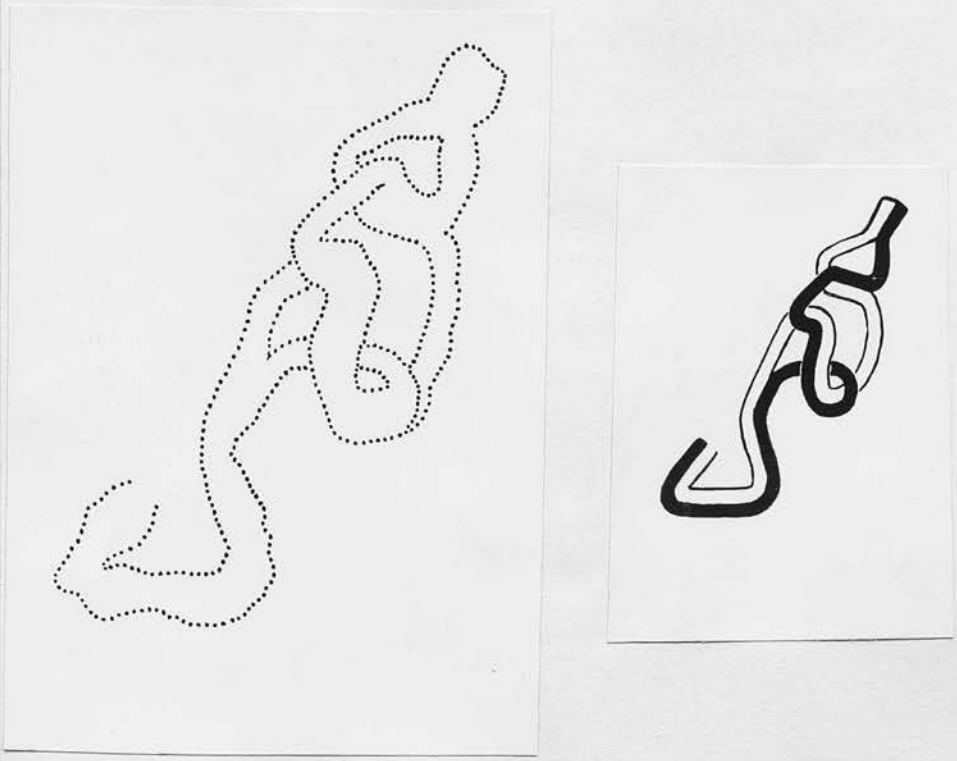
FECUNDITY

IN

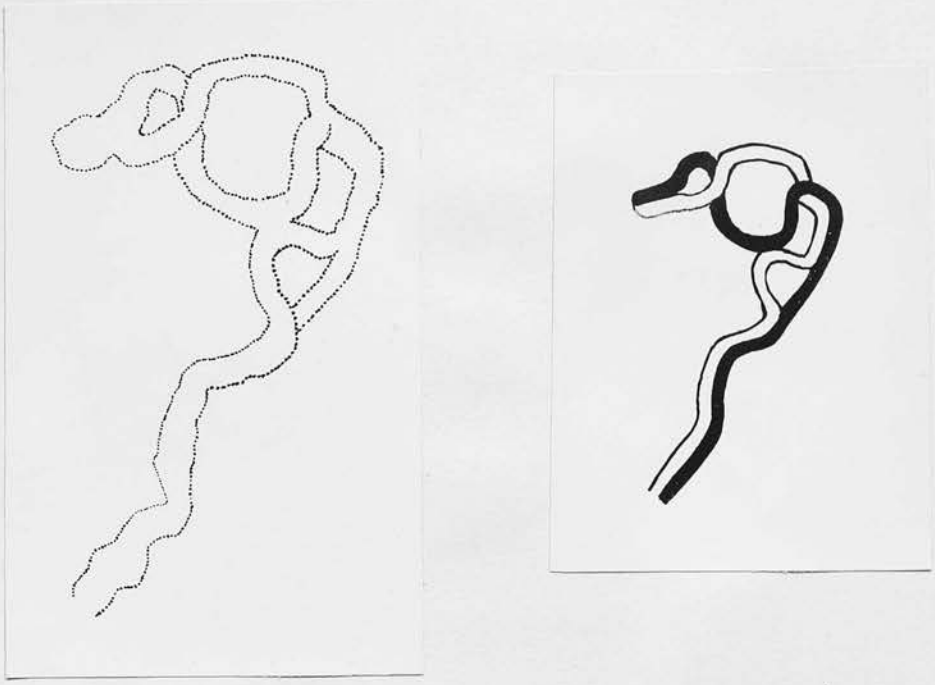
DROSOPHILA PSEUDO-OBSCURA, Race A



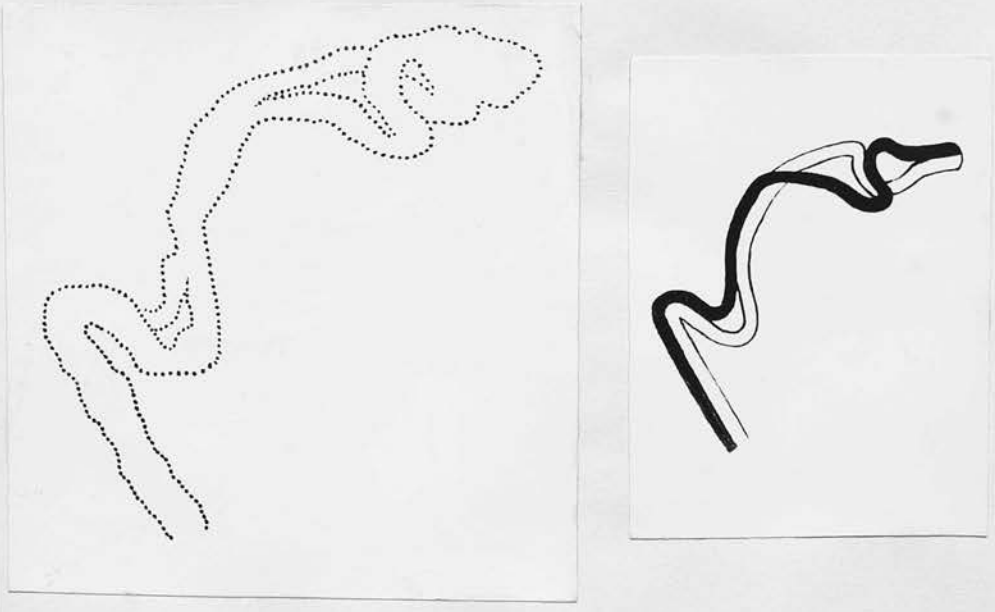
Text-fig. 1. Outline camera-lucida drawing and diagram from salivary gland preparation of inversion in the left arm of the X-chromosome associated with the Rounded-nick (Rn) character.



Text-fig. 2. Outline camera-lucida drawing and diagram of inversions in the left arm of the X-chromosome associated with the Plexus (Px) character.



Text-fig. 3. Outline camera-lucida drawing and diagram of left arm of X-chromosome heterozygous for inversions associated with Px and Rn characters.



Text-fig. 4. Similar to Text-fig. 3, except that the proximal loop has not paired.

appeared. The fecundity records obtained could not be compared with others of the same species for normality because the only published material is partly inadequate and partly unsuitable. As a basis for future work on these lines it has been considered desirable to write an account of the breeding behaviour to be expected from females of this species.

2. Literature

General reviews of the literature dealing with this subject are to be found in papers by Adolf (1920), Sturtevant (1921), Hanson and Ferris (1929), Alpatov (1932), and in the 'Genetics of Drosophila' (Morgan Bridges and Sturtevant, 1925). A further review will not be given here, but relevant literature will be referred to in the text.

There are two papers dealing with fecundity in D. pseudo-obscura. Shapiro (1932) who used practically the same methods as those of the present experiments, but who gives no details of his results with D. pseudo-obscura, found that Race A females began to lay about two days after emergence, and Race B females about three days after. He noted also that in comparison with D. melanogaster females, D.pseudo-obscura females laid their eggs very irregularly,but, nevertheless, their egg-laying curves could be represented by the same equation as could those of D. melanogaster females. Dobzhansky (1935), using a different technique which involved the counting of eggs /

eggs from groups of five females, also found that Race A females began to lay sooner than Race B females and reached the peak of their productivity earlier. In addition, the relative productivity of the two races changed with temperature. Dobzhansky drew attention to the mode of laying which he found irregular as compared with the even day-to-day production of the female D. melanogaster.

3. Methods

The susceptibility of Drosophila to changes in the internal and external conditions has been well established by various authors. Guyénot (1912) demonstrated that the fecundity depends on the nutrition during the larval stages, on the medium on which the flies have to lay, on the temperature, and on the presence of males. Adolph (1920) gives an account of the effects of various stimuli on fecundity and specifies several internal conditions which have an influence on it. Numerous experiments, of which those by Pearl Allen and Penniman (1926) with an early synthetic medium, and those of Bridges and Darby (1933) may be mentioned, have shown that the composition, texture and micro-organisms of the food play an important rôle in determining fecundity. Another factor which has been shown by Pearl (1932) and Alpatov(1932) to be most important is the density of the population per unit area of the food surface. According to Pearl this is because slight disturbances either by companions or by external agents interrupt laying. It

is clear, therefore, that experiments connected with fecundity have to be very carefully controlled to ensure uniform conditions in all cultures. It might be mentioned here, however, that if the analogy which Pearl draws between the collision of molecules of a gas and the disturbance by one another of flies in a culture has any validity, the influence of density of population on fecundity will be of a lower order in D. pseudo-obscura than it is in D. melanogaster as a result of the much slower motion of the 'particles'.

The females to be tested were raised at 23.5±5°C and subsequently kept at the same temperature except during counting and replacement of food. All females would be exposed to room temperatures during these processes for about 1½ - 2 hours each day. A few hours after emergence the females were separated from the males and placed in masses on food for 2 - 3 days on the last of which a number of them were subjected to X-rays.* These were enclosed in gelatine capsules 1 cm. long by ½ cm. wide at a distance of 30 cm., and given a dosage of 3,700r units (Tungsten anti-cathode water-cooled Muller tube, 80 K.V., 5 m. amps., .1 mm. Al. filter, 74r units per minute for 50 minutes). After the treatment the females were again left in masses until the next day when they were paired off singly with males (P sp) from stock. Most of the females were mated to two males, but some were given only one until more hatched a day or two later. They were kept in /

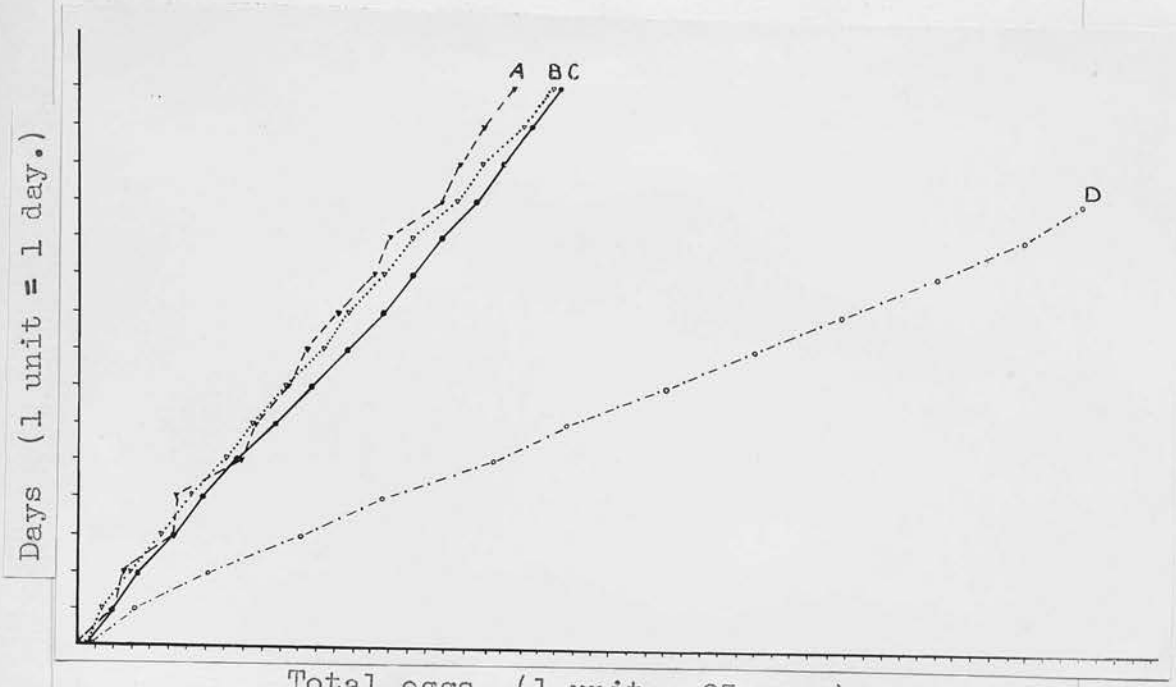
* The author is indebted to Dr. C.M.Scott of the Pharmacology Department, Edinburgh University, for carrying out the X-raying.

in vials 100 cm. long and 2.5 cm. in diameter, with a paper spoon carrying a slice of food prepared according to the formula of Bridges (1932, p. 268). Counting took place daily between 11 a.m. and 3 p.m. and always in the same order so that each culture was counted at approximately the same time each day. For the production of a maximum number of large flies, the agar-cornmeal-raisin-molasses medium of Offermann and Schmidt (D.I.S. 3, p.52) is generally used, but the raisins in it make it difficult to slice evenly and it was discarded for this purpose in favour of the older type of food but retained for the cultures into which the eggs were transferred after counting. It was found unsatisfactory to pour drops of food into the spoons because the operation is awkward and because thin edges and cracks develop which impede counting and make it difficult to remove the eggs without damage. Food was therefore set in a narrow cylindrical mould, and sections cut from this were later placed on the spoons with a drop of live yeast. Such a section has narrow and smooth edges, and provided it is not left in the vial for more than a day it does not dry out appreciably in a constant temperature room with a humidity of approximately 65 per cent. The smooth edges are important for it has been found that the flies do not lay freely on such a surface, and counting is thus restricted to the rough upper surface. The influence of the texture of the food has been noted before by Adolf (1920) and Schweitzer (D.I.S. /

(D.I.S. 4, p.66) for D. melanogaster, but under the conditions of the present experiments, females of this species seem to prefer the smooth edges. For both accuracy and speed in counting eggs under a binocular microscope, it has been found expedient to mark off the slice of food with a needle into several parallel strips.

The published records of fecundity in D. pseudo-obscura do not permit the comparison of actual numbers of eggs laid which might provide some idea of the effectiveness of the various techniques employed. Dobzhansky (1935) appears to have obtained about 250 - 380 eggs in sixteen days from his masses of five Race A females at 25°C. From Shapiro's graphs, a single D. melanogaster female heterozygous for vestigial laid about 1600 eggs in that time. In the experiments of Hanson and Ferris (1929) with white-eyed melano-gaster females about 660 eggs were obtained, and in present experiments about 600. These relations are brought out in Text-fig. 5.

Text-fig. 5. /



Text-fig. 5. Total egg production during first 16 days of laying in Drosophila.

A - - - - data from 10 $\frac{y\ Px}{v\ Rn}$ D. pseudo-obscura ♀♀ (mean)

B • • • • data from 10 $\frac{y}{v}$ D. pseudo-obscura ♀♀ (mean)

C ———— data of Hanson and Ferris (1929) on D. melanogaster ♀

D -·-·-·-·-data of Shapiro (1932) on D. melanogaster ♀

4. Accuracy of Counting

During the period of egg-counting a subsidiary experiment was carried out to determine the accuracy of counting with the spoon technique. Five spoons were chosen which gave a range of numbers of eggs from about 60 - 110, and these were submitted to three counters two of whom were experienced and one inexperienced. Each spoon was counted ten times by each. In order to guard against the results being influenced by any knowledge of previous counts, the spoons /

spoons were presented in varying order. This factor, however, had little if any importance. Counting becomes a mechanical process in which no attempt is made to memorise any particular situation. The range of variation in the results is shown in the following table and may be compared there with the first count which is the one recorded in practice.

Table I.

Counter \ Spoon	1	2	3	4	5
	Range of variation				
A. Inexperienced	72-77	62-66	109-118	94-101	71-74
B. Experienced	74-78	63-65	117-123	96-101	76-79
C. Experienced	75-78	62-65	112-119	95-102	75-78
	First count				
A. Inexperienced	77	65	109	96	74
B. Experienced	78	63	118	99	76
C. Experienced	78	64	116	95	77

The degree of accuracy attained in the counts may be observed from the following table of standard deviations and co-efficients of variation.

Table II.

Counter \ Spoon	1	2	3	4	5
	Standard deviation				
A	1.4	1.2	2.6	2.3	.9
B	1.2	.7	1.8	1.7	.9
C	1.0	.9	2.0	2.3	1.1
	Co-efficient of variation %				
A	1.9	1.9	2.3	2.4	1.3
B	1.6	1.1	1.5	1.7	1.2
C	1.4	1.5	1.8	2.3	1.5

It may be concluded from this that experience need not count for very much as far as accuracy goes, but /

but it does make a difference to the speed of counting. In this experiment, Counter A required an average of 1.8 minutes for each count, and Counter B, 1.4 minutes, so that for a given speed the experienced counter would probably be the more accurate. First counts appear to be high as often as they are low, and consequently there is no reason to suppose that they are any less reliable than any subsequent count.

Observation shows that there are several factors affecting the difficulty and reliability of counting:

(a) The number of eggs. The number of eggs, when considered in relation to their distribution on the slice, has probably some importance. When the eggs are particularly numerous they are often laid closely together, and even on top of one another, and at these times a second count may vary more than usual from the first. The co-efficients of variation for the higher egg numbers are consistently greater than for the smaller egg numbers (see Table II.)

(b) Condition of the food. The presence of lumps, cracks, etc., sometimes hides the eggs, raises doubts as to whether an egg has been counted or not, and inhibits an even distribution. Eggs may also be completely buried in soft food.

(c) Appearance of the eggs. Most eggs are a bright white but certain females have been observed to lay numbers of pale eggs which are difficult to see.

5. Experimental Results

A. /

A. General Breeding Behaviour.

The general breeding behaviour of all females in the experiment is given in Table III. It shows in the seventh column the number of days from mating to start of laying required by the females within the various groups. The variation here is not certainly greater than variation in age for the $\frac{y}{v}$ group except for the first and third X-rayed lots. In the $\frac{y\ Px}{v\ Rn}$ group the variation is certainly greater than the variation in age. Sterility accounts for much of the delay in starting to lay, but when this is allowed for there is still considerable irregularity.

In the eighth and ninth columns appears the distribution of eggs from sterile and fertile females respectively. The whole period of laying, namely 16 days, has been divided into four periods of four days each, and, for the sake of brevity, these have been used in describing the distribution of eggs instead of the actual days. It will be observed that the sterile females behaved in much the same way as the fertile females, and that among the $\frac{y\ Px}{v\ Rn}$ flies both the fertile and sterile females were often very tardy in beginning to lay. In this respect the first two groups were exceptional, but at present no explanation can be given either for their regularity or for the irregularity of apparently similar groups. From this data there arises no suggestion that X-raying has delayed the onset of laying.

Table III. /

TABLE III.

Egg laying behaviour of all females for first 18 days after mating.

N.B. All females laid eggs.

Genotype	♂♂	X-ray	Origin of females.	Date of mating.	No. of ♀♀	Distribution of ♀ ♀ according to days between mating and start of laying.						Distribution of eggs from sterile ♀♀ by periods			Distribution of eggs from fertile ♀♀ by periods			
						2	3	4	5	6	7 - 10	1234	234	34	1234	234	34	4
	3 P sp.	U	v♀a x y ♂♂	9/1	11	3	7	-	-	-	1	-	1	-	10	-	-	-
	"	T	"	"	10	1	3	1	1	3	1	4	-	-	5	-	1	-
y / v	"	U	v♀b x y ♂♂	"	10	3	7	-	-	-	-	-	-	-	10	-	-	-
	"	T	"	"	10	4	6	-	-	-	-	-	-	-	10	-	-	-
	"	U	y ♀ x v ♂♂	"	6	3	2	-	-	1	-	-	-	-	5	1	-	-
	"	T	"	"	8	1	2	4	1	-	-	-	-	-	5	1	-	-
	2 P sp.	U	vRn♀ x y Px (X1) ♂♂	27/11	10	1	7	2	-	-	-	-	-	-	10	-	-	-
	"	T	"	"	10	-	10	-	-	-	-	-	-	-	10	-	-	-
	"	U	"	28/11	11	2	2	3	-	2	2	1	1	-	5	4	-	-
	"	T	"(X5)	"	12	4	5	-	-	-	2	1	-	-	9	-	-	2
	"	U	"	27/11	8	1	3	2	2	-	-	3	-	-	1	2	2	-
y Px / v Rn	"	T	"	"	8	-	6	2	-	-	-	3	-	-	2	2	-	1
	"	U	"	29/11	4	-	1	1	2	-	-	-	-	-	-	-	3	1
	"	T	"	"	5	-	-	-	1	2	2	-	2	1	-	1	1	-
	2 sc y w	U	"(X1)	"	5	-	1	2	-	-	2	1	-	-	2	-	2	-
	"	T	"	"	7	-	-	-	-	3	4	-	1	-	-	4	2	-
	2 sc y sw	U	"(X5)	"	5	1	-	2	1	1	-	2	-	-	1	1	1	-
	"	T	"	"	6	-	-	-	-	-	6	-	3	-	-	1	2	-

T = Treated: U = Untreated.

B. Fecundity of Fertile Females.

As it was impracticable to incubate separately the daily egg-output of each female, the eggs from four days' laying were incubated together. The sixteen days during which the counts were made were divided into four periods of four days each for the reason that in four days a female would lay about 150 - 200 eggs at the most, and the usual vial cultures are considered to contain sufficient food to enable this number of eggs to develop without undue crowding. The four-day period had the further advantage that it would include no more and no less than two of the peaks of the rhythmical production which is characteristic of most females. In order to compare fecundity with fertility, the four-day averages have been calculated and will be used in presenting results although it has to be realised that they conceal a great amount of variation.

The average numbers of eggs produced by the groups of females which laid fertile eggs in the first period are given in Table IV. That these females were fertilised soon after mating is shown by the production of fertile eggs in the first period and by the fact that sterile or unfertilised females lay definitely fewer eggs in this period. No group in which less than five females were promptly fertilised is included in this table.

Table IV. /

females. Their curves of production were, however, more or less of the same shape.

The total production of a $\frac{y}{v}$ female in sixteen days was about 750-800 eggs, that of a $\frac{y\ Px}{v\ Rn}$ female about 600-630. Genetic effects on fecundity have also been found by Dobzhansky (1935) and Saveliev (1928) and many others. Treatment of the females with X-rays has in every group reduced the fecundity. The reduction is greatest in the first period, and becomes progressively less as time goes on. In the fourth period one of the six pairs of results shows that treated and untreated females laid the same number of eggs in the fourth period but the data are insufficient to show if the treated females would ever attain the fecundity normal for a given age. Among treated flies the onset of laying is not appreciably delayed, and the maximum production occurs as usual during the fifth to twelfth days, so that it would be expected that after the sixteenth day egg production would be dropping among both groups of flies.

C. Fecundity of Sterile Females.

A large number of females among both treated and control groups, although mated to two or more males, produced no offspring. The majority of these occurred among the $\frac{y\ Px}{v\ Rn}$ groups in which about 24 per cent. were sterile. The distribution of their eggs is shown in Table III, and their average production during these periods is given in Table VI.

Table VI. /

Table IV.

Average number of eggs produced by fertile females in 4-day periods

Genotype	X-rays	No.	No. of eggs in periods 1	2	3	4	Total eggs
$\frac{y}{v}$	U	10	163.1	206.0	197.7	186.8	736.9
	T	5	66.8	86.8	121.4	128.8	403.8
	U	10	212.8	213.9	194.5	187.3	808.5
	T	10	82.8	108.7	183.1	150.5	525.1
	U	5	220.2	230.o	189.2	189.4	828.8
	T	8	72.6	62.1	163.5	163.4	461.6
$\frac{y\ Px}{v\ Rn}$	U	10	133.3	175.3	176.2	114.5	599.3
	T	10	57.0	133.0	158.5	119.8	468.3
	U	5	137.8	180.6	161.4	152.0	631.8
	T	8	61.9	111.3	132.4	119.4	423.1

There can be little doubt from inspection of these figures that both treatment with X-rays and period of laying have a large influence on the number of eggs laid. The individual variation among females was so large, however, that these data were submitted to an analysis of variance which is summarised in Table V.

Table V.

Analysis of variance in data providing results given in Table IV. (after Fisher, 1930)

Variance due to	D.F.	Variance	Mean sq.	$\frac{1}{2}\log_e$	z	P
Genotype	1	65,357	65,357	5,544	1.705	<.01
Treatment	1	333,818	333,818	6.359	2.520	<.01
Periods	3	108,062	36,027	5.246	1.407	<.01
Remainder	314	677,933		3.839		
Total	319	1185,170				

This analysis bears out the conclusions regarding treatment with X-rays and period of laying, and in addition shows that at all times during the first sixteen days the $\frac{y}{v}$ females laid more than the $\frac{y\ Px}{v\ Rn}$ females. /

Table VI.

Average number of eggs produced by sterile $\frac{y\ Px}{v\ Rn}$ ♀♀

X-rays	No.of ♀♀	Eggs 1	2	3	4	Total
Untreated	8	37.5	139.9	158.1	147.5	483.0
Treated	11	9.3	44.6	103.6	115.7	273.3

As with the fertile females, treatment with X-rays has resulted in a lowering of the fecundity, although the controls are themselves much less productive than the fertile control females. In comparison with the 600 eggs produced in sixteen days by the fertile control females, these latter produced 480. This difference is less than that shown by the corresponding treated flies which produced about 450 and 270 eggs respectively. It will be noticed that the peak of production in the control females occurs during the third period, that is, at the same time as that of the fertile females. The amount of sterility found was about equal in X-rayed and control groups, but the data are scarcely adequate for conclusions on the point. The egg-laying behaviour of the sterile females appears to be more irregular than that of fertile females as judged by the fact that the variance of the individual totals for each period is consistently greater among the former than it is among the latter although the number of eggs is lower (Table VII.)

Table VII. /

Table VII.

Comparison of variance in totals of eggs from fertile and sterile $\frac{y\ Px}{v\ Rn}$ ♀♀

X-rays	♀♀	D.F.	Mean sq.	$\frac{1}{2}\log_e$	z	P
Untreated	sterile	28	2383	3.888	.36	<.05>.01
	fertile	36	1160	3.528		
Treated	sterile	40	1328	3.596	.51	<.01
	fertile	36	476	3.083		

D. Variation in Daily Egg Production.

All available published data on D. melanogaster and records from fifteen females obtained by the author go to show that there is a fairly regular production of eggs from day to day. Hanson and Ferris (1929) quote Guyénot (1912) as recording an initial periodicity in laying followed by regularity, but they do not mention that this applied to unmated females. Guyénot repeatedly mentions that control flies lay "à raison de 20 à 25 oeufs par jour". It may be concluded that in this species there is not much day to day variation, although several observers have reported that the females tend to lay in spurts during the day.

Shapiro (1932) and Dobzhansky (1935) both found a marked irregularity in daily egg production in D. pseudo-obscura, but do not commit themselves as to its probable nature. Dobzhansky started his cultures with a mass of five females and ten males, and a rhythm in the laying was still observable. If the ovaries functioned rhythmically there was no apparent reason why the peaks and hollows of production should coincide /

coincide in a given bottle as they must have done to some extent for the rhythm to have been observed in mass cultures. If the food conditions were responsible the peaks of production would be expected to coincide in the different bottles on any given day, but this was not so.

There is abundant evidence in the entomological literature of periodical laying by various insects, but, as a rule, it is difficult or impossible to dissociate the egg-laying habits from the mode of nutrition. No satisfactory evidence has been found that adult feeders such as Drosophila lay irregularly, but this may well be due to the methods of feeding and egg collection employed. Back and Pemberton (1914) found that the melon fly laid up to 30 eggs in batches at intervals of 3 - 30 days, but it is uncertain whether this periodicity or that noted by the above workers with D. pseudo-obscura was the result of the conditions under which laying took place or whether it was a specific habit of the flies.

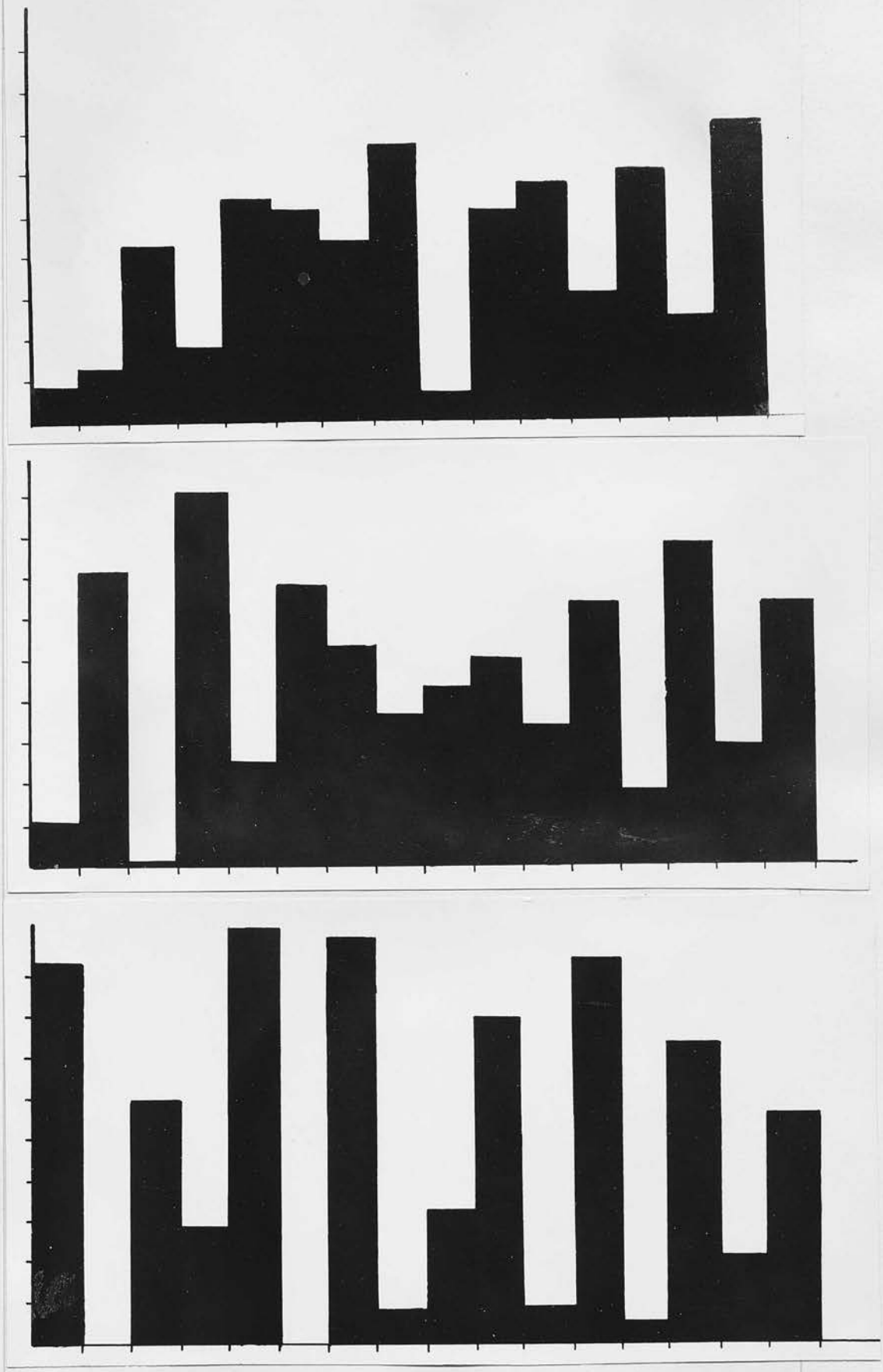
The present experiments have provided adequate substantiation of the fact emerging from previous experiments that females which are kept singly tend to lay many eggs one day and few the next, and that under culture conditions controlled as closely as those of experiments with D. melanogaster, D. pseudo-obscura females show a periodicity in laying which does not appear in the daily egg records of the other species.

From a study of all the individual records, it seems /

seems that although deviations from the daily rhythm are fairly frequent, the rhythm itself is fundamental, either to the method or to the flies, for there is always a recovery of the rhythm after a deviation from it. Most females show it from the beginning, but very few maintain it without interruption for the first sixteen days. The time of interruption varies; sometimes it occurs right at the beginning, most frequently during the eighth to twelfth days, and sometimes towards the end of the sixteen days. From this it might be argued that the rhythm is characteristic of the flies and that deviations from it are produced by unfavourable variations in the technique which are imperceptible to the observer but important to the flies.

Samples of the daily records obtained from control $\frac{Y}{v}$ females are shown graphically in Text-fig. 6. They have been chosen to illustrate the different types of record and the recovery of the rhythm after a break, and do not indicate the frequency with which these types occur. This remark applies equally to similar figures which follow. The actual records of twenty females are given in Tables VIII and IX.

Table VIII. /



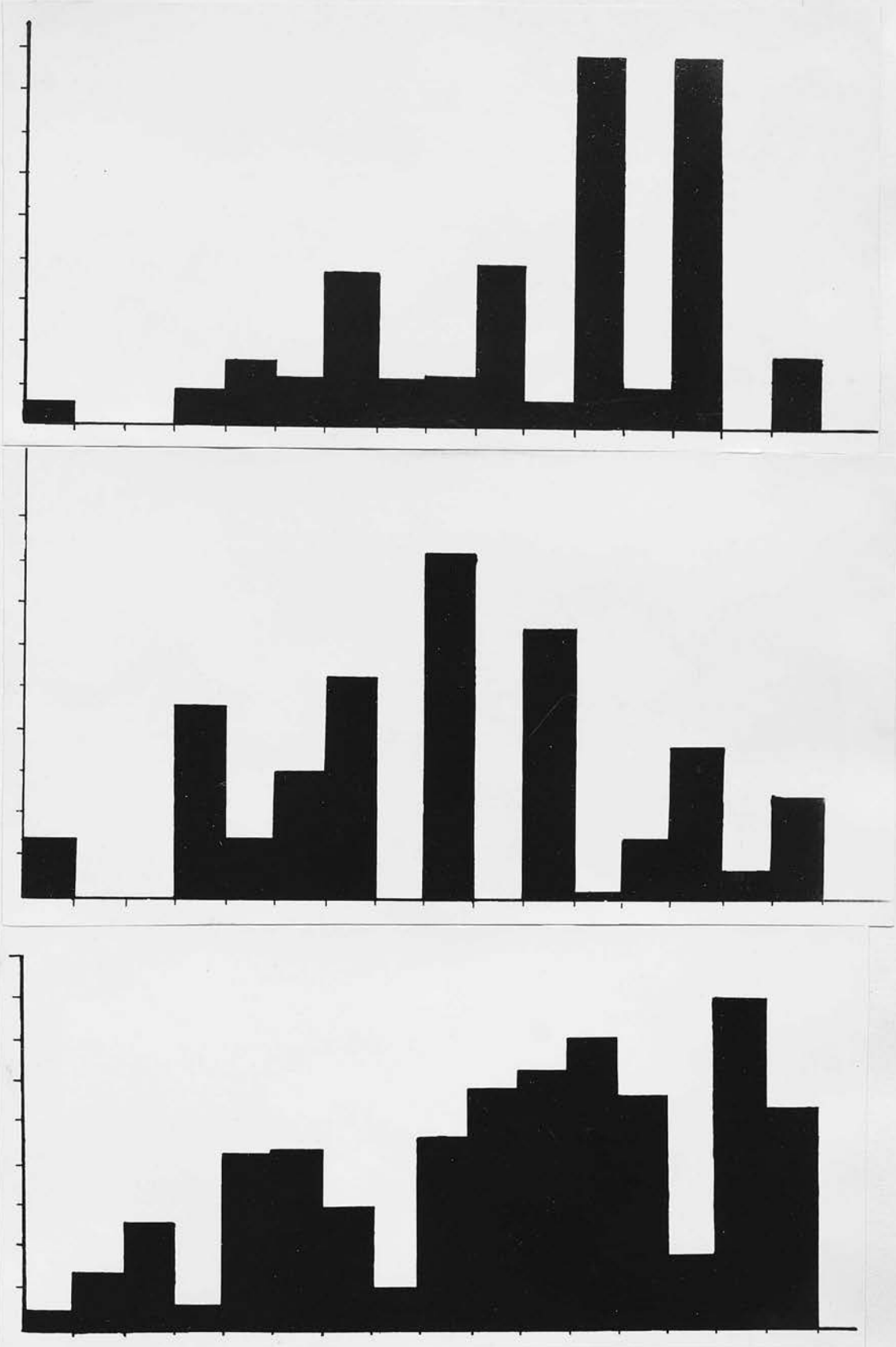
Text-fig. 6. Samples of daily egg records from $\frac{V}{V_1}$ ♀♀
(ordinate, 1 unit = 10 eggs; abscissa, 1 unit = 1 day)

Table VIII.

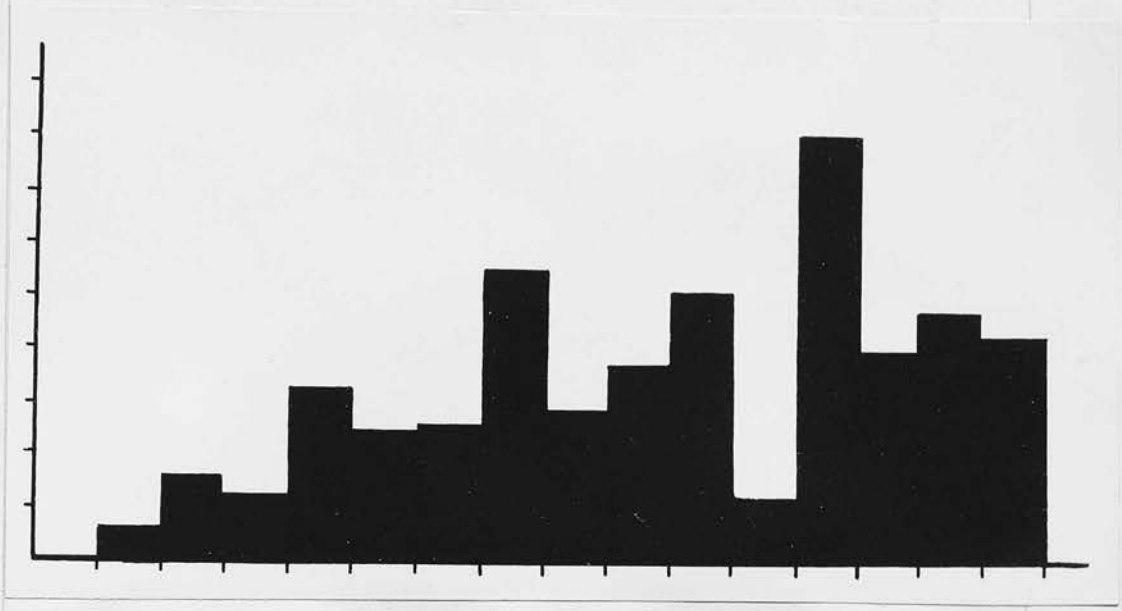
Sample egg records from control flies producing fertile eggs in all periods

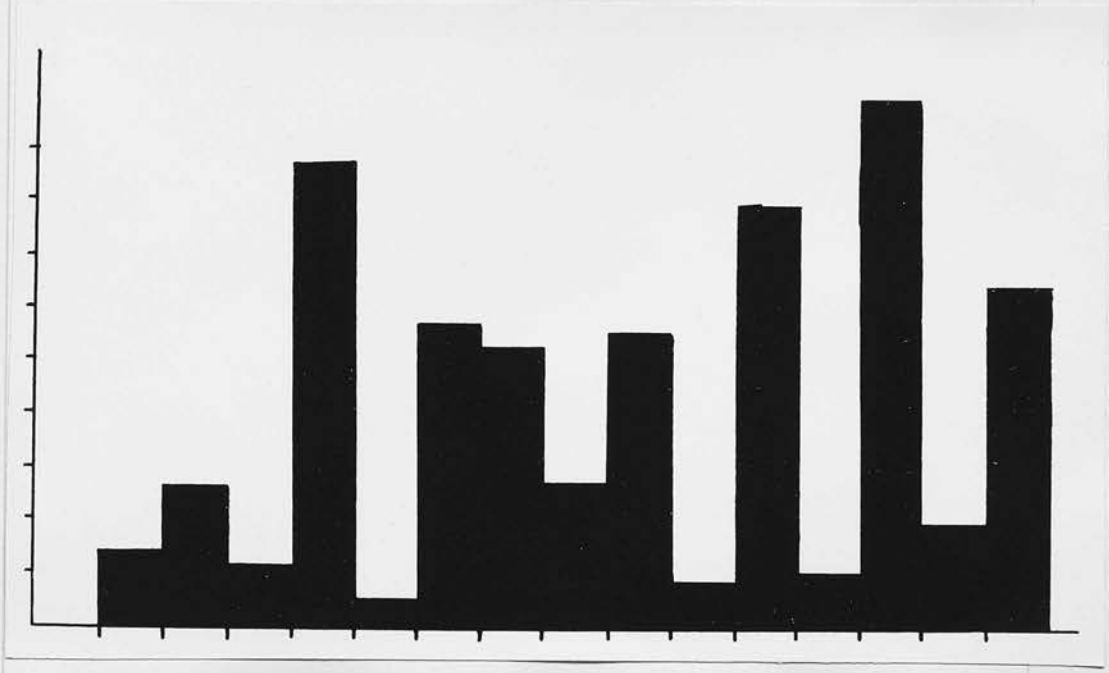
Count	y v ♀♀					y Px v Rn ♀♀				
1	28	53	9	30	11	0	21	0	25	0
2	99	25	14	35	73	0	61	0	21	50
3	29	72	43	90	1	23	49	3	10	8
4	45	99	19	13	91	33	58	63	26	78
5	86	8	53	97	26	84	103	4	34	21
6	17	102	51	12	66	23	1	104	9	68
7	73	47	44	85	53	59	80	o	31	0
8	36	62	67	38	36	34	24	100	0	69
9	78	80	7	58	43	55	118	0	55	10
10	49	23	51	3	50	75	22	0	61	49
11	52	97	58	94	35	9	82	103	25	35
12	99	0	32	2	64	80	35	0	66	30
13	0	106	60	97	20	46	72	36	1	70
14	102	22	26	2	77	41	22	69	84	0
15	25	86	72	105	29	59	85	0	0	75
16	41	59	1	1	64	28	0	30	87	0
Total	859	941	607	762	739	649	833	512	535	563

The estimation of the variance due to daily variations has not been worked out as the data are not considered as sufficiently homogeneous to make it worth while. It is therefore not possible to say definitely if the variables involved in this experiment have had any effect on the daily rhythm. Inspection of the records, however, gives the impression that the rhythm is not prevented from appearing by any of them. Females treated with X-rays do not lay so many eggs as the untreated during the first five or six days, and the rhythm is often not very conspicuous. Also there is often a period of two days in which no eggs are laid. As the egg production becomes higher the rhythm becomes manifest again (see Text-fig. 7). Sterile females also show the rhythm whether they have been X-rayed or not (see Text-figs.8 and 9), but especially if the egg production is low it is rather obscure. /

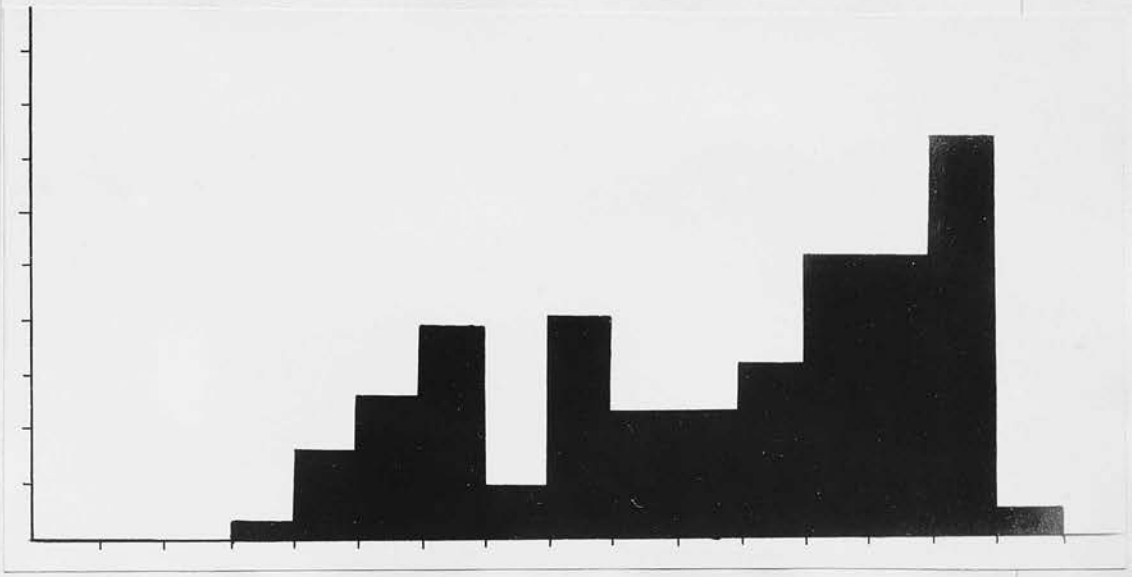


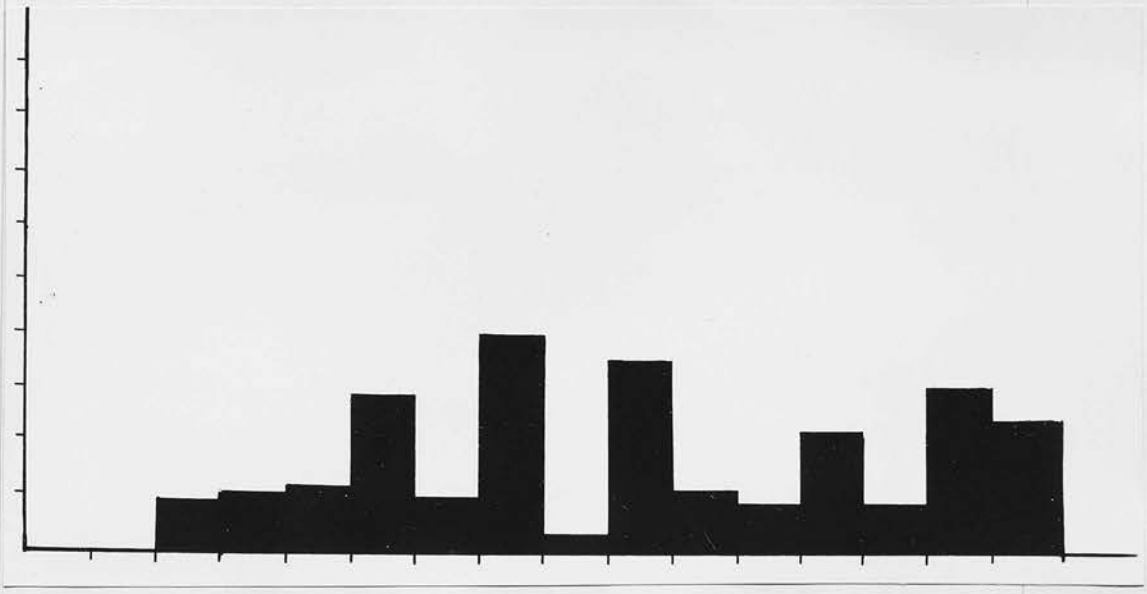
Text-fig. 7. Samples of daily egg records of X-rayed $\frac{Y}{Y}$ ♀♀ (ordinate, 1 unit - 10 eggs; abscissa, 1 unit - 1 day).





Text-fig. 8. Samples of daily egg records of sterile $\frac{y\ Px}{v\ Rn}$ ♀♀.





Text-fig. 9. Samples of daily egg records from X-rayed sterile $\frac{y}{v}$ ♀♀.

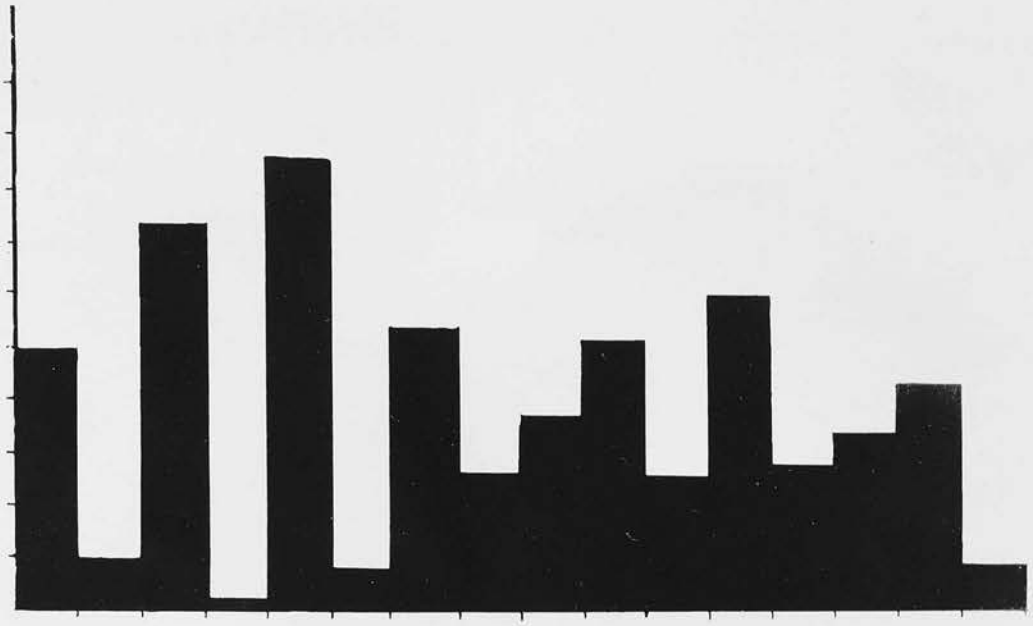
obscure. When the egg production is high the rhythm is often quite as marked as it is in fertile females. The records of females which were not fertilised until the second or third period also show the rhythm.

The total daily egg production for the ten females which is shown in Table IX explains the rhythm found by Dobzhansky in his mass cultures. Although these totals represent the number of eggs laid in a day by ten females, there is still a clear indication of a rhythm which may be compared with that obtained by Dobzhansky from five females by comparing Text-figs. 10 and 11, the latter of which has been constructed from his data. Both figures are based on samples, but if they may be taken as typical, it may be inferred that a group of females of the same age and mated at the same time under the same conditions will begin to lay at the same time and with coincident rhythms. If one or two flies in the mass get out of step, the rhythm will still be present, but not so evident. When half are out of step the rhythm is lost, but may be recovered later. The rhythm is apparently not easily suppressed either by internal or external variables. It has been found in three experiments by different investigators who have not used a standard technique, and it has occurred among several races and strains of flies subjected to various treatments. There is therefore a strong presumption that the rhythm is a specific property of D. pseudo-obscura that is not shared by D. melanogaster.

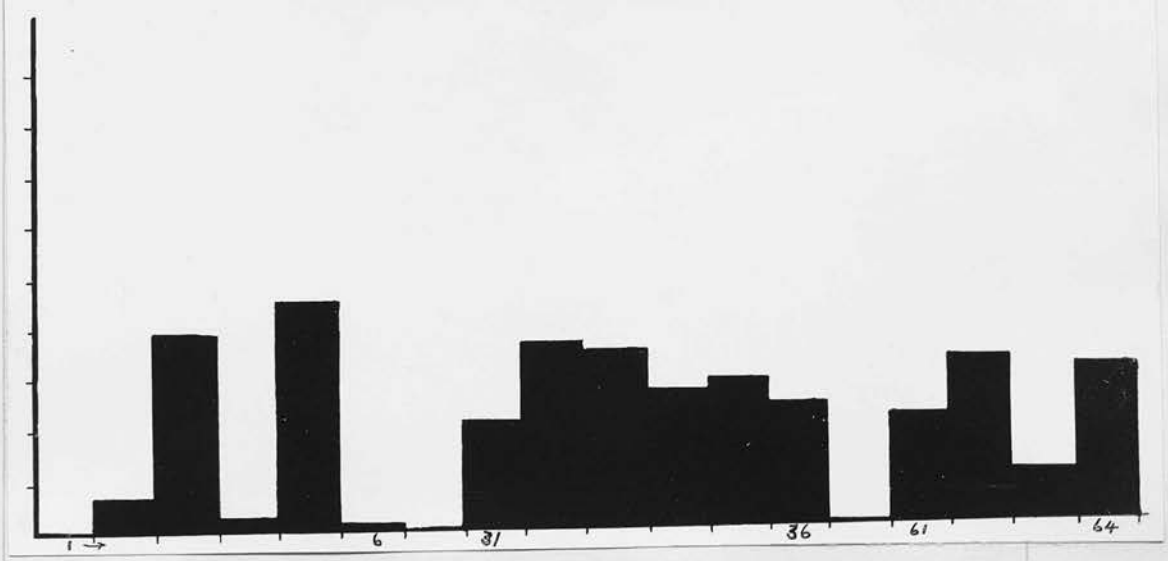
Table IX. /

TABLE IX. Variation in daily egg production of ten $\frac{\text{♂ Px}}{\text{♀ Rn}}$ ♀♀ comparable in all controllable respects.

♀ No. \ Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total.
19	62	0	89	5	94	3	44	35	93	27	71	22	84	3	66	2	700
20	55	0	48	0	91	0	91	0	89	8	83	17	71	13	75	4	645
21	74	13	94	0	110	0	100	12	66	21	70	23	71	32	52	43	781
22	65	1	101	0	106	0	89	0	37	39	0	98	0	71	27	20	654
23	0	41	46	0	106	0	31	43	4	74	0	69	25	36	55	0	530
24	0	8	40	4	42	38	0	55	0	80	1	73	18	39	37	2	437
25	60	26	71	0	88	0	38	43	2	75	0	90	0	39	51	1	584
26	55	0	87	0	72	32	17	38	19	78	0	98	0	36	42	11	585
27	57	10	77	0	89	0	87	0	32	59	15	62	2	26	16	8	540
28	58	0	86	0	56	20	39	44	38	53	24	52	11	38	16	2	537
Total	486	99	739	9	854	93	536	270	380	514	264	604	282	333	437	93	5,993



Text-fig. 10. Mean daily egg production of 10 comparable y Px/v Rn ♀♀ for first sixteen days of laying.



Text-fig. 11. Mean daily egg production of 5-4 D. pseudo-obscura ♀♀ in mass (from Dobzhansky, C 23, p. 453, 1935). Three selected periods.

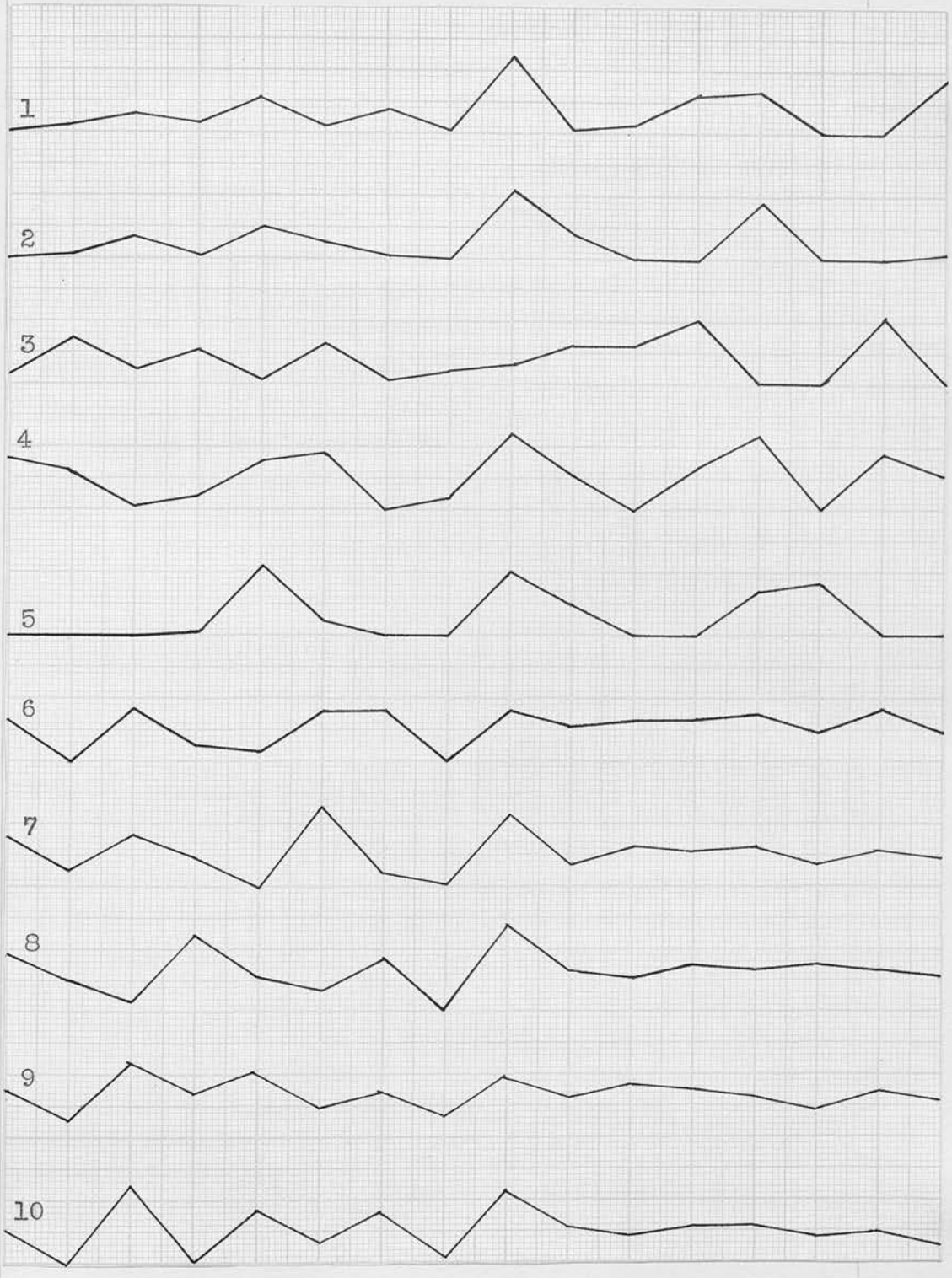
6. Further Experiments connected with Periodicity of Laying

Two possible explanations of the rhythm were (1) that the ovaries produced eggs continuously which were retained until the pressure induced rapid laying, and (2) that there was an ovarian rhythm as suggested by Dobzhansky (1935). As a preliminary to the experiments necessary to determine which of these alternatives is correct, two small pilot experiments to act as a guide in designing more conclusive ones were carried out. The results from these, however, leave very little doubt that the second alternative is the right one, although, as with the previous experiments, there are clear indications that unrecognised factors exist which at present prevent any detailed rules of behaviour being formulated.

The first experiment was concerned with providing more information about alternative (1). If a 'pressure of eggs' hypothesis were correct, D. melanogaster females might also have a rhythm which, as a result of their smaller size, was completed within one day instead of two. D. melanogaster females lay as many eggs as do the females of D. pseudo-obscura, and these eggs are approximately .53 x .18 mm. in size as compared with eggs .45 x .16 mm. in size laid by the latter females, so that laying would necessarily have to take place oftener in D. melanogaster. A test of this was made by counting the eggs from females of both /

both species treated in exactly the same way, twice a day, at 9 a.m. and 9 p.m. The results from five females of each species are shown graphically in Text-fig. 12. In general they are not conclusive, but the expected shape of curve for D. pseudo-obscura females appears during the later counts from all females, and very well during the whole period for numbers 4 and 5. In the first half of the counting period, the results from the melanogaster females seemed to show clear signs of a daily rhythm, but in the latter half the curves of all have flattened out so that the possibility of a rhythm must be left open. The similarity in shape of the curves, however, and the results which follow strongly suggest that fluctuating food conditions have been responsible for any semblance of a rhythm here. It may be observed from the graphs that there is no obvious night and day effect.

The second experiment involved the dissection of females of both species after their laying had been watched for some days. The number and condition of the eggs in the ovaries could then be correlated with the immediately preceding numbers of eggs laid. The results which are given in Tables X and XI are vitiated somewhat by the fact that there was a lapse in the food supply of certain of the melanogaster females which has upset the normal regularity of their laying. This, however, does not affect greatly the general conclusion that there is a distinct difference /



Text-fig. 12. Comparison of egg laying curves of single females drawn from counts made twice daily. Numbers 1 - 5 D.pseudo-obscura ♀♀; 6 - 10 D.melanogaster ♀♀. (Abscissa, 1 cm. = 1 day; ordinate, 1 cm. = 50 eggs.)

difference in the functioning of the ovaries of the two species. The ovary of a D. melanogaster female is composed of egg-strings which contain eggs gradually diminishing in size towards the tip of the egg-strings just as figured by Nonidez (1920). This leads to continuous maturation and deposition of eggs. In D. pseudo-obscura, the ovaries ripen the eggs in batches; the immature eggs are divided into groups at distinctly different stages of development, so that a large number of eggs become ready for laying at once. Table X. shows that females such as E6, 9, 12, 22, which have paused for a day after laying many eggs, have large numbers of mature eggs in their abdomens, while those such as E10, 17, 19, 24, which have just laid a large number, have no mature eggs in the abdomen. Females such as E13 appear to have retained some eggs.

Table X. /

Table X.

Number of eggs found on dissection of laying *D.pseudo-obscura* females; ovaries separately where possible

No.	Eggs laid on last four days of life	Eggs in abdomen on dissection	Remarks
E 6	48, 3, 56, 0,	32, 31	Eggs mature
7	0, 0, 0, 0,	116	Eggs mature
8	0, 7, 9, 3,	25, 21	
9	12, 20, 58, 0,	41, 38	
10	25, 27, 0, 62,	0, 0	Very young eggs
11	0, 0, 75, 1,	38, 38	
12	74, 7,111, 0,	45, 44	Some not quite mature
13	0, 19, 19, 74,	34, 29	Mature; also many half-formed clearly two stages
14	0, 23, 35, 78,	42, 34	About half-formed
15	40, 18, 31, 51,	14, 10	Approx.; many immature
16	0, 0, 62, 0,	50, 56	Not quite mature
17	66, 62, 27, 63,	0, 0	Very young eggs
18	0, 0, 0, 0,	22, 32	Various stages of maturity
19	0, 7, 13, 95,	0, 0	Very young eggs
20	0, 0, 0, 33,	36,	Not quite mature
21	0, 0, 20, 17,	4, 0	Ovaries small; eggs very young
22	57, 50, 37, 4,	32, 29	
23	0, 0, 0, 0,	30, 18	
24	28, 15, 0, 79,	0, 0	Very young eggs

Table XI. /

Table XI.

Number of eggs found on dissection of laying D.melanogaster females; ovaries separately where possible

No.	Eggs laid on last four days of life	Eggs in abdomen on dissection	Remarks
31	39, 28, 35, 60,	8, 20	Also many immature
32	50, 31, 48, 4,	72	Various stages of maturity
33	80, 47, 48, 0,	40, 48	Retained eggs
34	45, 69, 1, 0,	42, 41	do.
35	46, 39, 0, 67,	6, 14	Also many half-formed
36	33, 39, 0, 56,	20, 21	do.
37	34, 0, 13, 60,	2, 22	do.
39	48, 44, 14,102,	6, 8	do.
40	42, 0, 1, 61,	31, 19	do.
41	86, 46, 12,104,	12, 13	do.
42	39, 0, 7, 87,	18, 16	do.
43	41, 23, 19, 35,	35, 26	do.
44	28, 0, 7, 70,	12, 11	do.

7. Discussion

The value of the foregoing results lies mainly in the light which they shed on the difficulties underlying the use of fecundity as an index of the biological activity of Drosophila pseudo-obscura. It is apparent that even under the relatively uniform conditions of the laboratory the processes of egg-formation and laying are modified considerably by the slightest deviations from a standard technique. An explanation is forthcoming for the previous failure of experiments designed to test fertility on various food media, and it is clear that the efficiency and usefulness /

fulness of experiments concerned with fecundity will be seriously impaired unless precautions are taken to standardise every possible variable and to reduce the enormous variance exemplified in Table V. p. 117.

The reduction in fecundity following treatment with X-rays is in keeping with present knowledge of the biological effects of X-rays. A further discussion of this matter is projected in connection with the effects of the X-rays on the fertility of the eggs, but it may be mentioned here that studies by others of the effects of X-rays on the biological processes of the cell indicate that a slowing up of the rate of division of the germ-cells rather than metabolic changes will be responsible for the drop in fecundity observed above. Whether or not the same explanation would apply to the sterile control females would largely depend on the cause of the sterility. Genetic sterility of the type met with here may have an effect similar to that of X-rays.

The discovery of an ovarian rhythm in Drosophila creates an interesting problem. It is well known that such rhythms in mammals are conditioned by hormones, but at present the inference that hormones exist in insects is supported by rather scanty evidence (Fraenkel, 1935). Whilst the immediate reason for the difference between D. melanogaster and D. pseudo-obscura may be found in the conditions imposed by the natural habitats of the two species, there must /

must be some internal difference responsible for the dissimilar behaviour of the ovaries under the same conditions, and the question remains as to whether the functioning of the ovaries is autonomously controlled, or whether it is subject to the influence of other internal agents.

8. Summary

1. The random variance of any group of comparable females was found to be very large, and suggests that the females were very susceptible to slight variations in the conditions and that only gross differences in the fecundity of this species will be detectable until more stringent control can be devised.

2. The counting of eggs on spoons was subject to an error of about 1.5 - 2.0 per cent.

3. The fecundity of Drosophila pseudo-obscura females has been found to be strongly affected by the genotype and by treatment with X-rays.

4. Sterile females have a lower fecundity than fertile females and treatment with X-rays lowers it still further. As with fertile females, the peak of egg laying usually occurs during the eighth to twelfth days of laying.

5. There appears to exist an ovarian rhythm in Drosophila pseudo-obscura females which requires approximately two days for its completion.

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