GENETICS AND CYTOLOGY OF *DROSOPHILA SUBOBSCURA*

IV. AN EXTREME EXAMPLE OF DELAY IN GENE ACTION, CAUSING STERILITY

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(With Nine Text-figures)

Introduction

The genetics of many types of sterility have been investigated. The type here described in *Drosophila subobscura* is unique in being due to a single autosomal recessive mutant gene which causes sterility in both sexes of the progeny of a female homozygous for it.

Its peculiar expression and penetrance, and their resemblance to the manifestations of genes involved in specific barriers in this genus, have made a thorough investigation seem worth while, and the somewhat unusual experimental procedure, analogous to progeny tests in agricultural animals, may be of interest.

In accordance with accepted *Drosophila* terminology we have called this mutant *grandchildless*, symbol *gs*. A female homozygous for *gs* cannot be distinguished from a female heterozygous for it or homozygous for its wild-type allelomorph. Nevertheless, all her children will be abnormal irrespective of what male is their father. The abnormality consists of a deformity of the gonads in both sexes.

We have been unable to recognize this abnormality before the late larval stage, and in practice the phenotype of a female is always scored by examining her sons and daughters in the imaginal stage, i.e. a culture is necessary to score a single female, and by the time her genotype has been identified only her nieces and nephews are alive to be bred from.

Worse than this, the phenotype appears to be female limited, so the genotype of males can only be recognized by the proportions in which *gs/gs* females are reconstituted in the F_2 progeny. The only male recognized as being *gs/gs* in constitution was scored after rearing one F_1 culture, 7 F_2 cultures and 82 F_3 cultures!

Markers used in the experiments

cp (*X*) *copper*, brown eyes, white testes (Spurway, 1945).

c(*X*) (*X*) recessive female-limited crossing over suppressor causing high non-disjunction (*D.I.S.* **20**).

v (*X*) *vermilion*, homologous with *v* in *D. melanogaster* (Spurway, 1945; and Rendel & Suley, 1948).

cv (*X*) *crossveinless*, more extreme than in *D. melanogaster*.

cv^2 (*X*) allelomorph of above; 30% of males have testes abnormalities (*D.I.S.* **19**; and Bird, 1948).

cv^3 (*X*) like cv^2 (*D.I.S.* **20**; and Bird, 1948).

y^6 (*X*) *yellow*^{6}, as in *D. melanogaster* (*D.I.S.* **20**).

y^7 (*X*) *yellow*^{7}, as in *D. melanogaster* (*D.I.S.* **20**).

s (2) *scarlet*, eye colour (Gordon, 1936).

ma (2) *maroon*, eye colour, pale yellow testes (Gordon, Spurway & Street, 1939).

ho (3) *hoary*, white hairs between eye facets, crumpled wing (Gordon *et al.* 1939).

nt (3) *net*, extra veins (*D.I.S.* **17**).

pl (4) *plexus*, extra veins (Gordon *et al.* 1939).

pp (4) *poppy*, scarlet eye colour (Gordon *et al.* 1939), probably homologue of *cinnabar* in *D. melanogaster* (*D.I.S.* **21**; and Rendel & Suley, 1948).

Dl (5) *Delta*, long veins splayed, cut margin as in *D. melanogaster* (*D.I.S.* **17**).

ORIGIN

During the autumn of 1945 Dr Philip and I made an attempt to obtain an *XYYY* male by inbreeding. A female, part of whose constitution was *cp* + *cv*^{2}/+ + *cv*, was mated with unrelated *cp v*+♂♂. Cytological examination of the sons showed that some were *XYY*. An F_2 was made up of the constitution *cp*+*cv*^{2}/*cp v* +♀ × + + *cv* ♂, and only those cultures were examined in which the father had been shown to be *XYY*. An F_3 of the same constitution of the F_1 was made, and again only the cultures sired by *XYY* males were examined.

In one of these F_3 cultures all the *cp*$^+$ males were immediately observed to have abnormally small orange structures visible through the body wall. This was attributed to a genotype having been obtained in which *cv*^{2} had an unexpectedly high penetrance and expression. When Dr Philip dissected them she found no testes or only the minutest rudiments in which no divisions could be found, and suggested that this was a condition independent of *cv*^{2}. The condition was re-obtained in the F_4 from a sister culture of the abnormal one, and also reappeared in another, the F_4 derived from another F_2 mating. Dissection showed in these cultures that even in aged females the ovaries remained vestigial. An extensive F_5 was made up, and selection for the extra *Y*-chromosome was relaxed, though no attempt was made to eradicate it from the line. The mutant *cp* was selected against, as the white testes made it necessary to dissect the males before the condition could be recognized. An attempt was made to keep this line heterozygous for *cv* and *v*, and while this was done several poor cultures showing high non-disjunction were found. As *c* (*X*) had been discovered in a line formed from a third F_2 mating it was assumed to be due to this mutant, though no tests were made for identity. *c* (*X*) was eradicated by making the line homozygous for *v* in the F_9. A non-disjunctional male found in a sterile culture showed the same testis deformity as his regular brothers.

ANATOMY OF THE STERILE FLIES

The culture produced by a *gs*/*gs* female usually, but not invariably, produces fewer flies than the average, frequently less than ten. All, or almost all, the sons contain no testes. These are usually replaced by minute rudiments smaller than the fat globules observed on dissecting a fly. No cell divisions have been observed in these minute bodies. They may be closely attached to the end of the vasa deferentia, or very loosely attached by a tenuous string of connective tissue several times their own length. Sometimes the only substitute for a testis is a frayed region at the end of this connective tissue strand. This tissue, the globular rudiment if present, and the vasa deferentia have the colour appropriate to the genotype of the male that contains them. This is normally bright orange in this species, as is the normal testis. Therefore the absence of the latter can be scored easily in the living fly. If the sons are *cp* or *ma*, dissections are necessary to determine their anatomy.

The vasa deferentia may be fused for about half their length distally. This abnormality only occurs in some cultures.

The remaining ducts are normal. Occasional abnormalities have been found, but it would need extensive series of control dissections of flies with normal parentage to determine whether any of these are rare pleiotropic effects of the *gs/gs* phenotype.

Fig. 3 shows the internal genitalia of a hybrid between *D. pseudo-obscura* ♂ × *D. miranda* ♀. The anatomical similarity of the two conditions is obvious, especially as the complete separation of the paired ducts shown in the drawing of the hybrid is the more usual condition in the abnormal males of *D. subobscura*.

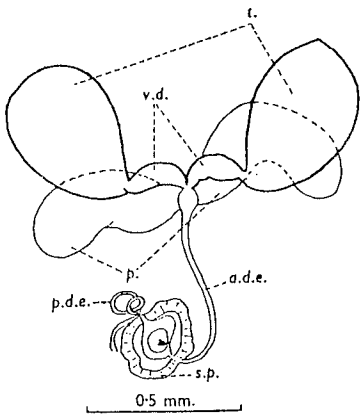

Fig. 1.

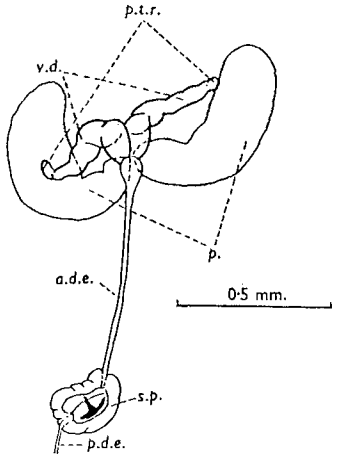

Fig. 2.

Fig. 1. Internal genitalia of a normal male *Drosophila subobscura*, dissected in saline. Explanations of abbreviations: *a.d.e.* anterior ductus ejaculatorius; *p.* paragonium; *p.d.e.* posterior ductus ejaculatorius; *p.t.r.* presumed testis rudiment; *s.p.* sperm pump; *t.* testis; *v.d.* vas deferens.

Fig. 2. Internal genitalia of the son of a homozygous *grandchildless* female dissected in saline. For explanation of abbreviations see Fig. 1.

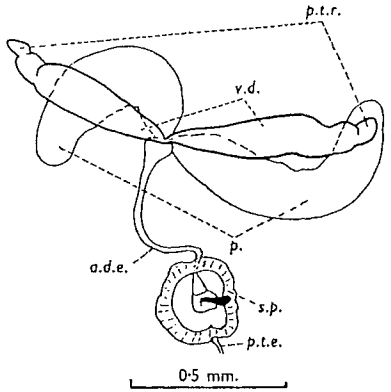

Fig. 3. Internal genitalia of a male hybrid between *Drosophila miranda* ♀ and *D. pseudo-obscura* ♀ dissected in saline. For explanation of abbreviations see Fig. 1.

From these sterile cultures 2242 males can be described as having been examined either by dissection, or, because their testes were the wild-type orange of this species, by examination of the intact fly. Among these eleven were found to have one testis, and twenty-four to have both. These testes appeared normal in size and shape. Three of them were tested and found to be normally fertile. Therefore there is reason to suppose that meiosis is not disturbed in these abnormal lines.

The presence of these exceptional normal flies is significantly commoner in some families than others. It is difficult to group the data on any biological criteria, but Table 1 is an attempt. The χ^2 of these six groups is 26·73 and $n=5$. The main component of the χ^2 is from the experiments using the *pp* stock where the frequency of 'fertile' males rose to 5%.

Table 1

	Testes		
Line	0	1	2
Inbred line F_3–F_9 segregating for *v*, *cv*, *c*(*X*)	570	2	9
Inbred line F_{10}–F_{23} homozygous *v*	402	2	2
F_3 and extraction of outcross to *s*	351	0	2
F_3 and extraction of outcross to *ho ut*	207	2	0
Two F_3 of outcross to *pp*	276	5	9
Two F_3 of outcross to *Dl*	401	0	2
	2207	11	24

The ovaries of the females in these abnormal cultures contain fewer ovarioles than do the normal, and, more striking, these ovarioles remain in the condition usual to the early pupa instead of enlarging during the first week of imaginal life as the eggs develop. Six weeks ageing of the imagines has failed to produce any sign of further development of the ovaries (Figs. 4–7). Of the 186 females from such cultures dissected not one has shown any possibility of being fertile.

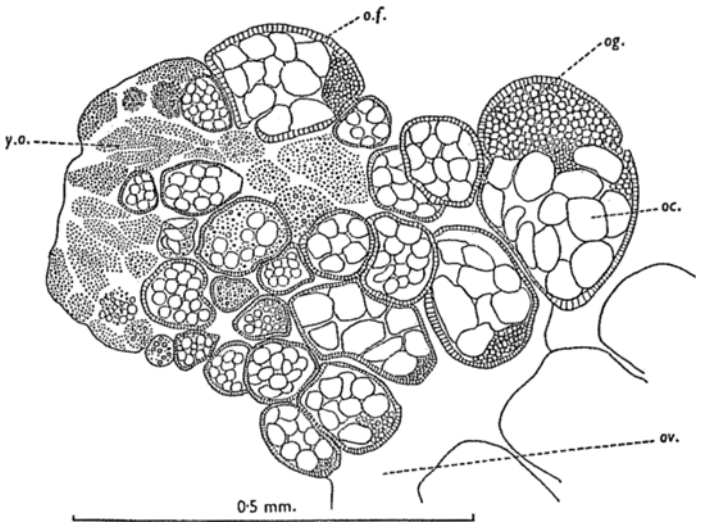

Fig. 4. Ovary of a normal female *Drosophila subobscura* 1 day after eclosion, stained with aceto-orcein. Explanation of abbreviations: *oc.* oocyte; *og.* oogonium; *ov.* oviduct; *o.f.* ovariole follicle; *y.o.* young ovariole.

A very few females have been observed in which a minute area of orange tissue has been included in this vestigial ovary. This tissue resembles the testis sheath. It resembles closely the condition described by Hadorn (1946) in some of his colchicine-treated gonads, and raises the question of whether this condition may not be some form of sex reversal.

In the last instar larvae the ovary and testis rudiments are indistinguishable in these cultures.

The existence of overlaps makes it necessary to make some comment on standards of scoring. In the two prolonged inbred lines in which the condition was segregating,

occasional cultures became commoner in which, though the majority of the flies were normal, a few had only one or even no testes. The proportion of abnormal flies was seldom enough to obscure the determination of a culture, but the following rule was followed. If a female produced only one offspring which had no testes the culture was scored as sterile; if she produced two, one without testes, and one with only one, the culture was

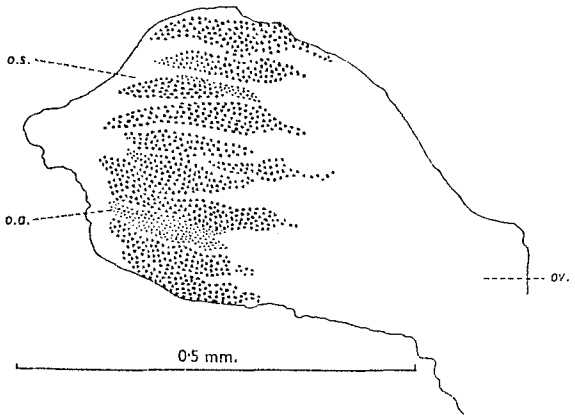

Fig. 5.

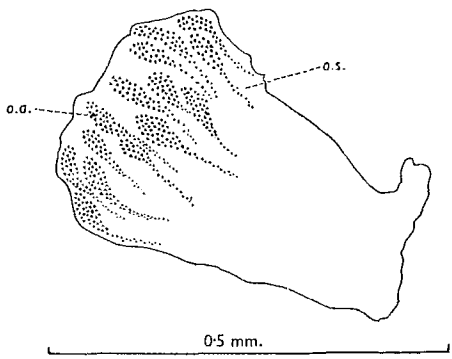

Fig. 6.

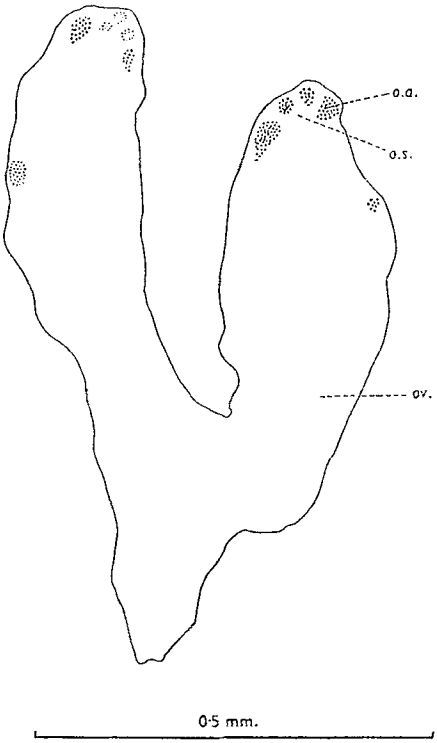

Fig. 7.

Fig. 5. Ovary of a normal female, *Drosophila subobscura* during early pupal life, stained with aceto-orcein. Explanation of abbreviations: *o.a.* ovariole anlage; *o.s.* ovarian stroma; *ov.* oviduct.

Fig. 6. Ovary of the daughter of a homozygous *grandchildless* female 1 day after eclosion, stained with aceto-orcein. For explanation of abbreviations see Fig. 5.

Fig. 7. Ovaries and oviducts of the daughter of a homozygous grandchildless female 26 days after eclosion, stained with aceto-orcein. For explanation of abbreviations see Fig. 5.

scored as fertile. The largest numbers of normal overlaps in 'sterile' cultures are given below:

2 testes	1 testis	No testes
1	—	10 (twice)
2	2	19
2	1	18
2	—	14
2	2	56
1	—	5
4	—	33

The largest numbers of sterile flies found in "fertile" cultures are as follows:

Generation of flies observed	2 testes	1 testis	No testes
F_7	2	3	3
F_8	—	1	1
F_8	2	1	—
F_9	17	1	5
F_{17}	6	6	—
F_{17}	3	3	—
F_{20}	4	3	—
F_{22}	1	1	—
F_{23}	1	1	—
$F_3(s)$	7	6	5
$F_3(s)$	8	3	3
$F_3(s)$	10	4	1
$F_4(s)$	2	2	—
$F_{x+3}(y's)$	2	4	1
$F_{x+4}(y's)$	11	7	3

Flies with one testis may have two or one vasa deferentia. Those found among the cultures scored as sterile always have two ducts, but a high proportion of the deformed animals found among predominantly fertile cultures may have only one vas deferens.

The above cultures were found during the time the experiments were being made and suggest that the rules made for scoring were adequate.

Unfortunately, at the very end of the work, during a final experiment made chiefly to save the abnormality during the excessive heat of the summer of 1947, when the bulk of the material after wide outcrossing had been given to colleagues and the original lines had been lost, I found the following culture from a single paired mating in an F_3 involving *pp*:

2 testes	1 testis	No testes	2 ovaries	1 ovary	No ovary	♀♀ not dissected
5	10	16	6	6	19 (1 with 3 rudiments)	2

This culture has been scored as 'fertile', and the question is raised whether the genetic basis for it is the same as that described in this paper. Though the question is open I do not think that it undermines the basis of classification used in the following experiments.

FIRST GENERATION OF OUTCROSSING FLIES FROM ABNORMAL LINE

Table 2. *Results of outcrossing close relatives of gs/gs females*

Relationship to *gs/gs* ♀	Sterile cultures	Normal cultures
Sisters	9	30
Nieces	0	4
Nephews	0	5
Sons	0	3
Brothers	0	14

The result of crossing flies from the abnormal line to unrelated flies is shown in Table 2. It is seen that females from the abnormal line when crossed to unrelated males produce sterile cultures indistinguishable from those produced by brother and sister matings within the line, and also that the proportion in which they appear is approximately the same, i.e. 27 % (see p. 138). When males from the abnormal line are outcrossed to unrelated females no abnormal cultures were obtained, though two of these males produced abnormal cultures when mated with their own sisters.

Second generation from outcrossed males

That the mother but not the father is influential in determining whether a culture shall be sterile suggests that the character is determined by the maternal genetic constitution and not by the constitution of the abnormal flies.

Since all, or almost all, the ancestors of a sterile culture have been fertile, the sterility can hardly be due to a plasmagene or virus transmitted in the female line. It seems more likely to be due to a gene with a maternal (Dobzhansky, 1935) or delayed zygotic (Haldane, 1932) effect. Moreover, if the effect has a cytoplasmic cause it should rarely be transmitted by a male, if a genic cause it should be transmitted regularly. Therefore F_2 generations were reared from four of the outcrossed males.

The number of F_2 cultures examined in these four F_2 experiments are shown in the fourth column of Table 3. In all these cultures at least twelve males and twelve females

Table 3

Relationship of fly outcrossed to *gs/gs* ♀	Markers carried by its mate	Chromosome carrying these markers	No. of F_2 cultures examined by inspection	F_2 data obtained by rearing an F_3, i.e. by test mating F_2 females: Total no. of F_2 cultures	No. of F_2 cultures segregating for *gs*	Segregation in those F_2 cultures where at least one *gs/gs* ♀ recorded: + +	+*gs*	Mutant *gs*+	Mutant *gs*
Brother	*s*	2	10	7	2	14	6	2	2
Nephew	*ho nt*	3	8	8	4	17	8	17	0
Son	*pl pp*	4	13	10	5	22	8	19	3
Son	*Dl*	5	8	7	7	39	11	24	8
Sister	*Dl*	5	8	2	1	2	0	2	1
Great niece	—	—	12	8	2	26	2	—	—

were examined, and in most very many more. They were all vigorous cultures. No sterile cultures were found, nor were any animals showing the abnormal testes found segregating among the males. This result in the F_2 would be expected whether the character was cytoplasmic or chromosomal.

Third generation from outcrossed males

If the character was due to genes, however, some of the F_2 females should have the genetic constitution to produce abnormal offspring. The segregation in the original line was leading us to suspect that it might be due to a mutation at a single autosomal locus. If we represent the recessive mutant at this locus as *gs grandchildless*, the original outcrossed brothers or nephews of abnormal females could have one of three constitutions gs^+/gs^+, gs^+/gs or gs/gs, and the sons of abnormal females could have the constitution $gs/+$ or gs/gs. If the female that produces the sterile cultures is gs/gs it can be seen that if the outcrossed male was $gs/+$ a quarter of the females in a quarter of the F_2 cultures would, if bred from, give sterile offspring. If the outcrossed male was gs/gs a quarter of the females in all the F_2 cultures would give abnormal cultures.

If the male was gs^+/gs^+ no abnormal cultures would be obtained and the experiment would be inconclusive. A further complication might be expected, i.e. the gs/gs male might be sterile or he might be inviable.

Therefore about a dozen F_3 paired matings were examined from each of the number of F_2 cultures shown in column 5. These matings are best considered as test matings of F_2

females. The original outcrosses had been made to stocks carrying markers for the four long autosomes, and the F_2 cultures containing *Delta* were the offspring of matings *Dl* ♂ × +♀. Therefore if the locus for *gs* and the marker were on the same chromosome no F_2 female showing either of the markers on that chromosome should give a sterile culture. The females carrying the markers in the F_2 could be recognized, and more than a quarter of them were test mated.

The last five columns in Table 3 give the data derived from these test matings. The following conclusions can be drawn concerning the inheritance of the character:

(1) Sterile cultures reappear in all four experiments in every way similar to those observed in the original line. Therefore the genetic basis for the abnormality is transmitted through the male, and therefore most probably by the chromosomes.

(2) Since females producing sterile cultures are produced in the F_2 from a male, the character cannot be due to a sex-linked gene.

(3) Assuming one locus to be involved, three males behaved as heterozygotes. We should expect a quarter of their F_2 cultures to segregate for *grandchildless*. In the crude data given in the table, eleven out of twenty-five did so. This exceeds the expected value by only 1·1 times the standard error. However, the cultures recorded as not segregating were not adequately tested.

This inadequacy of testing was even more marked in the experiment with *ho* and *nt*, where *gs* females were not selected at random because of their linkage with the markers.

(4) In the experiment with *Dl* females producing sterile cultures segregated in all the F_2 families, i.e. their grandfather behaved as a homozygote. Therefore the homozygous male is viable and fertile. As he had produced a normal F_1 this confirms that the father is irrelevant to the production of the abnormality and that the gene is female-limited in its effect.

(5) Among the segregating F_2 from crosses involving the chromosomes 2, 4 and 5, 38 out of 158 females gave sterile cultures, the Mendelian expectation being 39·5. Thirteen of these females also carried the marker, the expectation being 13·9. Thus the agreement with non-linkage is almost as good as possible.

(6) In the cross to chromosome 3 the total cannot be considered, as mutants were tested preferentially. On a 2 × 2 table the probability that the recessives should by chance have produced no sterile cultures is 1265/137,047 or 0·00923. There is thus strong evidence that the character is due to a recessive autosomal mutant which is female limited and delayed zygotic in its effect and which we call *grandchildless*.

OUTCROSS OF NORMAL FEMALES FROM THE ORIGINAL LINE

Some of the females of the original line that produce normal cultures should be gs/gs^+ in constitution. Only two of the outcrossed females have been tested further. The data are given in Table 3. Eight F_2 cultures from the matings *Dl* ♀ × + ♂ were examined and were normal, but only two of these were examined by F_3 test matings. One tested by four females failed to show *grandchildless*, the other tested by five females produced one sterile culture.

At the end of the original line the last surviving female was outcrossed to *ma pp* ♂♂. She herself was *v/v*. As *ma* has pale yellow testes, necessitating dissection to score, and as *v* and *pp* are indistinguishable, no linkage data could be obtained from this culture.

At least eleven females were test mated from eight F_2 cultures, and one *grandchildless*

culture was found in two of these, though the anomalous culture described on p. 131 is from a sister of one of these *grandchildless* females.

Thus the abnormality, as expected, is transmitted by females that produce fertile cultures. An essential part of the hypothesis here put forward is that no female producing a fertile culture should prove by the test mating of her F_2 granddaughters to have been *gs/gs*.

I have not tested large numbers of females to discover whether *grandchildless* females ever segregate in all their F_2 cultures, because of the amount of work and food required in obtaining a convincing negative.

Segregation of *gs* in later generations

On three occasions an F_4 and later generations were bred. The first was a continuation of the F_3 in which the gene first segregated. The second was a continuation of the outcross to *s*, and the third was the continuation of the outcross to *ho nt* on chromosome 3. This last experiment may be described in a few words. As previously described, no F_2 female that was either *ho* or *nt* was shown by her F_3 progeny to be *gs*, but of the eight F_2 *gs* females no less than four were shown by their F_3 progeny to be heterozygous for *ho* or *nt* or both, and more might have been shown to have been if the F_2 males to which they were mated had been chosen so that the F_3 was a test cross. This result means that there is considerable recombination between the three genes on chromosome 3, and led me to hope that by selection of parents a few generations of the line could be made to yield test cross data from which the recombination could be calculated. Unfortunately, though, an F_9 was raised, the extremely large mortality encountered made the experiment unsuccessful, and after no *gs/gs* females had been observed for two generations the line was discontinued. The selection of flies in each generation for mutants believed to be linked to *gs* prevented these later generations providing evidence for the segregation of the gene.

The pedigrees of the other two lines are shown in Figs. 8 and 9.

The conventions are as follows. Each square represents a single female who has been tested by growing a culture from her. She was usually mated to her brother, and in all cases where another generation was raised from her children this is assumed in the pedigrees, except at the beginning of the lines where the parentage is stated. A white square means that she produced a normal fertile culture, a shaded square means a culture in which all or almost all the flies showed abnormal gonads showing that she was of the constitution *gs/gs*. The numbers in the squares are the number of females producing cultures of that type in the segregations. For example, the F_{21} of the line shown in Fig. 9, thirty-four females, all from one culture in the F_{20}, produced progeny; eleven of these gave sterile progeny and twenty-three gave fertile progeny. From two cultures out of these twenty-three an F_{22} generation was raised, one consisting of thirteen single female cultures and one of twenty-six.

Fig. 8 shows the complete data on the outcross of a brother of a *gs/gs* female to a *s* female. The F_2 data derived from the F_3 generation has been given in Table 3. Three further generations were bred as shown in the pedigree.

In the F_3 one culture produced three *yellow* males and another one *yellow* male. These F_3 cultures had been derived from different F_2 cultures which had been adequately examined. Therefore there must have been two independent mutations at the *yellow* locus. These were called y^6 and y^7 and were indistinguishable from the original *y* and each other and

allelomorphic with them. Stocks were derived from both, and kept from mass cultures which contained some males with minute testes. This would, of course, select for any mimic phenotypes that might arise in the stock.

After about seven months nine paired matings were obtained from a single mass stock bottle of y^7 which had arisen among the children of the brother and sister of a *gs*/*gs* female. Three produced the typical sterile cultures showing that their mothers were *gs*/*gs*. From two others paired matings were made, and from the one shown to be segregating for the mutant another line was established, four generations of which are shown in the table.

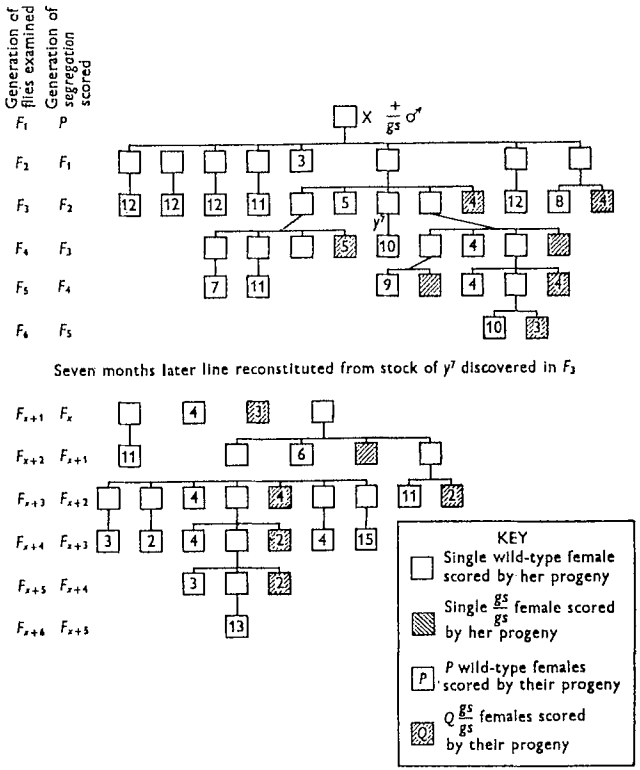

Fig. 8. Pedigree of line extracted from outcross of male from balanced line.

The mutant reappeared in generation F_{x+7}, and the line homozygous for y^7 was given to an embryologist.

In Fig. 9 is shown the complete pedigree of the line in which the mutant was originally observed, including the immediate ancestry of its first appearance, and ending with the single female which was outcrossed.

Before discussing these two lines a technical difficulty must be emphasized. As a female is scored solely by her offspring, a number of females remain unscored because they are sterile for various reasons. For example, in the original line (Fig. 9) between the F_7 and F_{22} inclusive, the number of females in the three classes were

Normal ♀♀	*gs* ♀♀	Unscored ♀♀
309	90	112

In the F_{23} and F_{24} the numbers were

Normal ♀♀	*gs* ♀♀	Unscored ♀♀
30	1	69

This increased sterility of flies with normal gonads may have been an effect of inbreeding (Philip, Rendel, Spurway & Haldane, 1944) but seems more likely to have been due to the extremely hot summer of 1947, as a comparable sterility affected the whole species in our laboratory.

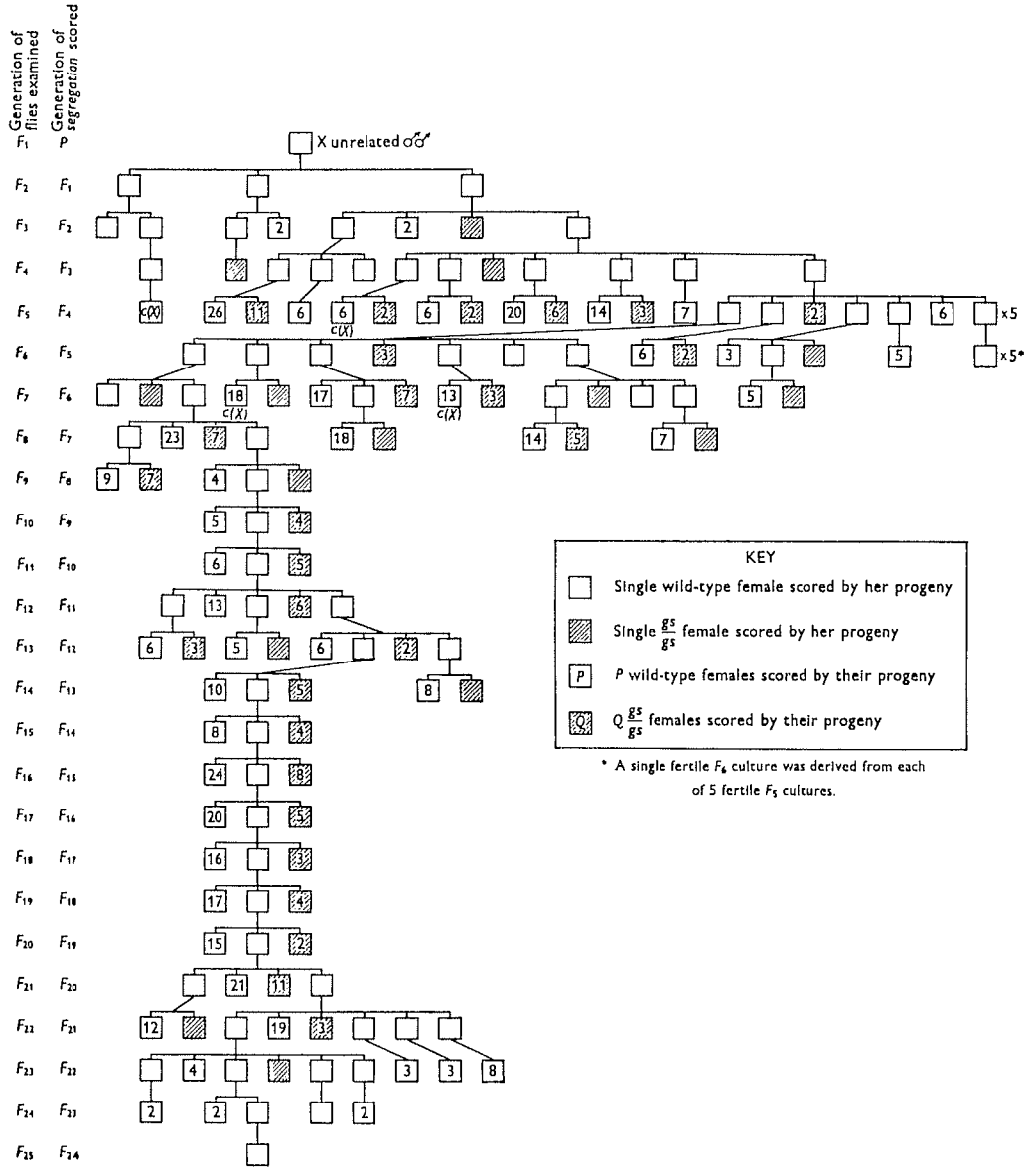

Fig. 9. Pedigree of line balanced for *gs* including original appearance of the mutant. Outcrosses of females are included but line is always continued through a sib pair.

Cultures producing flies without gonads seem to contain less flies than normal, and frequently only one or two. This strongly suggests that *gs/gs* females form a larger proportion of the group of unscored females than they do of scored females. Confirmation of this is provided by the data given in the last paragraph which are not unique among our families.

This consideration makes very doubtful the comparison of the segregation ratios observed with any Mendelian hypothesis, but does not prevent the two lines from being compared with one another.

The most striking difference between the two lines is the almost inevitable segregation of *gs/gs* females in all families of what is called the original line, the highest number examined in a family that did not segregate being eight. Before the homogeneity of the two lines can be considered it is necessary to consider which cultures can properly be included.

In considering the homogeneity of segregation in the F_3 and subsequent generations in the two lines I have included in the two samples (1) all cultures grandchildren of paired matings, i.e. the *segregation scored* occurred in the offspring of a paired mating; (2) all cultures descended, however remotely, from a mating of brothers and sisters, all of which were sibs of a *gs/gs* female.

In discussing these data it is convenient to use the word family for a set of cultures whose mothers and usually both parents were derived from a brother-sister mating. We can then investigate a set of families for homogeneity in respect of the frequency of sterile cultures derived from *gs/gs* mothers among them. Using χ^2 as a test of homogeneity we find that in the line containing *s*, $\chi^2 = 41{\cdot}41$ with $n-1=18$, so $P=0{\cdot}0013$, and the line is clearly heterogeneous. On the other hand, in the original line $\chi^2 = 48{\cdot}28$ for $n-1=56$; so $P=0{\cdot}76$, and there is no sign of heterogeneity.

This last result is clearly unexpected, for three types of families are to be expected from the normal sibs of *gs/gs* females if there is no selective mortality or sterility except that due to *gs*:

Type *A* from mating $+/gs$ ♀ × gs/gs ♂, giving 1 $+/gs$: 1 gs/gs.

Type *B* from mating $+/gs$ ♀ × $+/gs$ ♂, giving 1 $+/+$: 2 $+/gs$: 1 gs/gs.

Type *C* from matings where at least one parent is $+/+$, giving no *gs/gs* progeny.

All later generations are derived from families of type *A* or type *B*, that is to say, segregating for *gs*. Now type *A* matings giving fertile progeny give rise to families in the frequencies $\frac{1}{2}A$ and $\frac{1}{2}B$. Type *B* matings similarly give rise to families in the frequencies $\frac{1}{6}A$, $\frac{1}{3}B$, $\frac{1}{2}C$. But since type *C* families were not bred from, their progeny should actually have been $\frac{1}{3}$ type *A*, $\frac{2}{3}$ type *B*.

It follows that, whatever bias may have existed, between $\frac{1}{2}$ and $\frac{1}{3}$ of the families segregating for *gs* should have been type *A*, the remainder of type *B*.

In all generations families were bred from several brother-sister matings, and several biases must certainly have operated in various directions determining in which of these families was the segregation examined. The most serious, and also the most likely of these, is that as type *B* families contain less *grandchildless* females they contain fewer females that are sterile or relatively sterile, and are thus more likely to be chosen for the continuation of the line. This should increase the probability of obtaining type *C* families. On the other hand, where several families were examined there would be a tendency to continue breeding from type *A* families as they would contain more *gs/gs* females.

If we consider all the twenty-seven families of over eight cultures, the probability that no family should have been of type *C* is about 3×10^{-5}, though it cannot be exactly estimated. However, there is no doubt that type *C* families are much below expectation, if they occur at all. Hence +/+ zygotes are either handicapped by inviability or sterility. If +/+ zygotes are quite sterile or inviable, we should expect between $\frac{1}{2} \times \frac{1}{2} + \frac{1}{2} \times \frac{1}{4}$, or $\frac{3}{8}$, and $\frac{1}{3} \times \frac{1}{2} + \frac{2}{3} \times \frac{1}{4}$, or $\frac{1}{3}$, that is to say, between 37·5 and 33·8 % of sterile cultures.

Considering the whole line from the F_4 onwards, the total number of *gs/gs* females recorded was 137 out of a total of 667 or 20·5 %. This may mean that there is a selection against these homozygotes even before breeding, but more likely that *gs/gs* females often give poor cultures and form an abnormally high proportion of the females unscored.

This line may therefore be said to be balanced, as some agent has preserved an unexpectedly high proportion of +/*gs* flies in it. As this line no longer exists we can only guess at the mechanism at work.

Philip *et al.* (1944) have described the balanced heterozygosis involving three of the long autosomes of *D. subobscura*. Apparently in our stocks no fly homozygous for more than one of these autosomes is normally fertile, and homozygotes are always deficient in segregating cultures at the age group at which salivary gland preparations are made.

I suggest that this balanced line has become homozygous for one of these autosomes, but not for the autosome on which *gs* is situated. As the *gs* autosome is known to belong to the system, larvae in which this chromosome becomes homozygous then have two chromosomes homozygous and are at a disadvantage.

Discussion

If sufficient data have been presented to sustain that *gs* is a sex-limited autosomal recessive mutant, then the production of these sterile offspring is one of the most extreme examples of delay in gene action yet discovered. A pair of genes in the mother produces no detectable effect upon her. It only acts on her progeny. It can be detected on the last instar male larva where the gonad rudiment remains small as in the female.

In the female larva it cannot be recognized until mid-pupal life, and then only with high magnifications. In practice one waits for the imaginal stage. Most examples of delayed gene action can be scored much earlier, though Professor C. Jucci permits me to quote his unpublished observation of quantitative characters in the silk of *Bombyx mori* determined by the mother's genotype and not by the genotype of the larva that secretes the silk.

The maternal effects recorded in *Drosophila melanogaster* are mainly lethals, i.e. they produce inviable effects some time before eclosion. *l*(2) mat. of Redfield (1926) kills before hatching over 80 % of the daughters of females homozygous for it, though the proportion varies from culture to culture.

A similar effect is ascribed by Bridges & Brehme (1944) to the interaction of *l*(3) *a* and *M*(3) *w*, but full details are not given.

The closest parallel occurs in the hybrids of the North American members of the *obscura* group of the genus *Drosophila*. The deformity observed in the male hybrids between *D. pseudo-obscura* and *D. miranda* is almost identical, at least in gross structure, with that found in the sons of *gs* females (Dobzhansky, 1937). As this species hybrid is completely sterile no further genetic analysis has been possible. In the hybrid *D. pseudo-obscura* ♂ × *D. persimilis* ♀, the females are fertile but the males are sterile (Dobzhansky, 1935). These males are comparable with the sons of *gs* females, but their testes, though

variable in size, are always larger, and the meiosis is also considerably disturbed. Dobzhansky (1935*a*), by the use of autosomal markers, showed that this effect was due to genes in all the autosomes of *D. persimilis*, which acted on the cytoplasm of the egg before fertilization in the manner in which we suppose our single mutant *gs* must act. Other abnormalities in later generations of the species cross can also be attributed to similar genes (Dobzhansky, 1935*a*).

Sturtevant (1946) discovered in *D. neorepleta* a gene unlike ours in the anatomical differences that it produces, but like it in that it segregates in a clear-cut Mendelian manner. This gene, like those in *D. persimilis*, has only been demonstrated by its action in specific hybrids with *D. repleta*. *D. neorepleta* carries an autosomal gene which, when present in single dose in a hybrid female, makes her eggs male in potentiality. This predisposition to maleness is only partially overcome by two *repleta X*'s, and male-like intersexes result. One *repleta X* and one *neorepleta X* are sufficient to produce normal females. *D. neorepleta* eggs are homozygous for this gene which is probably part of the normal sex-determining mechanism, and confirms Bridges's classical conclusions that maleness is determined by the autosomes. Sturtevant's gene produced gross abnormalities not only in the gonads but also in the internal and external genitalia. But for the rare occurrence of orange tissue in the ovaries, *gs* produces no intersexual characters, and it is difficult to see how it could be assumed to do so, as the female gonad develops approximately normally until early pupal life, i.e. for considerably longer than the male gonad. This development of a fairly normal gonad rudiment apparently makes it unlikely that *gs/gs* acts by altering the germinal pole of the insect egg, though this is known to be determined before fertilization. The occurrence of genes of the kind postulated by Dobzhansky and Sturtevant occurring as mutants within a species may be of considerable importance as raw material for the construction of specific barriers.

We have assumed that in insects genes such as *gs* with delayed zygotic or maternal effects must have acted on the cytoplasm of the egg before fertilization, but such genes in viviparous animals, and especially in mammals have, as Haldane (1932) has pointed out, the possibility of acting on the embryo through the uterus, such as the recessive foetal atrophy described by Hammond (1934) in the rabbit. For this condition Hammond was able to demonstrate both that the father had no influence and also that the condition is transmitted by the male.

In man it will be difficult to distinguish between abnormalities due to delayed zygotic genes of this nature and abnormalities due to rare recessive genes producing incompatibilities between mother and father resulting in incompatibilities between mother and foetus. Both these conditions would be recognized by the increased frequency of consanguinity between the parents of the mothers of the affected individuals (Penrose, 1940, 1946), but whereas the father is irrelevant to the offspring of a female manifesting a delayed zygotic gene, a woman homozygous for a rare gene producing an incompatibility might avoid disastrous pregnancies by marrying a close relative, who would thus have a high chance to be heterozygous or even homozygous for the gene concerned.

SUMMARY

An autosomal recessive gene, *grandchildless*, causes no change in the phenotype of flies homozygous for it. But in the progeny of homozygous females, no matter by what father, all females have rudimentary ovaries, and are sterile, while 98·4% of the males have no

testes. The gene has been assigned to a linkage group. Whilst it segregated normally on outcrossing, it was possible to build up a balanced line in which all or almost all individuals were heterozygous or homozygous for it.

I wish to thank several colleagues and pupils for considerable contributions to this work. Dr A. U. Philip first recognized that the anatomical condition was different from that found as a pleiotropic effect of *cv*^{2} and that it was common to females and males. Dr O. Fahmy made and interpreted to me the various preparations of the female gonads. Misses J. M. Lumly, J. Bathgate and M. J. Bird jointly prepared and drew the three figures of the male genitalia. It is obvious that the preservation of such a mutant would be impossible unless a colleague will deputize during holidays and illness. For this arduous and essential work I am deeply and appreciatively indebted to Dr M. J. Bird.

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