

GENETICS AND CYTOLOGY OF *DROSOPHILA* *SUBOBSCURA*

V. THE GENITAL ABNORMALITIES ASSOCIATED WITH THE SEX-LINKED RECESSIVE *CROSSVEINLESS*²

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(With Five Text-figures)

INTRODUCTION

In *Drosophila subobscura* the sex-linked recessive mutant *crossveinless*² (*cv*²) is associated with genital abnormalities in a certain percentage of males. The external expression is always the same, the complete removal of both the anterior and posterior cross-veins in the homozygous female and the hemizygous male. The internal effect varies, on an average only 23% of the males being affected.

Abnormalities are always present in the *cv*² laboratory stock, and it seems therefore that they are either not selected against, a very improbable situation, or that they are invariably associated with the wing effect and cannot be separated from it.

*cv*² and *cv*³ were both found by Dr Spurway (November 1944 and May 1946). *cv*² occurred in a single fly in a *scarlet maroon* stock, and proved to be heterozygous for *interrupted*. *cv*³ occurred in many males of the *grandchildless* line *F*₃ 4_{3i}.

RELATION BETWEEN *cv*² AND THE GENITAL ABNORMALITIES

To test whether the genital abnormalities are pleiotropic expressions of *cv*² or due to a separate locus, *cv*² females were crossed to *vermilion* (*v*), wild type (+) and *thin-maroon* (*th ma*) males. *cv*² and *v* are very closely linked, the recombination percentage being 0.13. *th* and *ma* are second chromosome (i.e. autosomal) recessives. The results are shown in Table 1.

Table 1

Cross	Generation	<i>cv</i> ² males		<i>v</i> or + males		<i>cv</i> ² type* of <i>v</i> or + males
		Dissected	Abnormal	Dissected	Abnormal	
$\frac{cv^2}{cv^2} \times v$	<i>F</i> ₂	292	36	331	0	— —
	<i>F</i> ₃	1936	253	714	2	39 53
$\frac{cv^2}{cv^2} \times +$	<i>F</i> ₂	675	383	653	4	53 53
	$\frac{cv^2}{cv^2} \times th\ ma$	<i>F</i> ₂	139	5	178	1

* See Fig. 4.

All seven genital abnormalities in the *v* or + males were either unique in type or similar to those found in certain small atypical groups of *cv*². They may be regarded as chance occurrences in both the + and *cv*² stocks and not typical *cv*² abnormalities. Thus no convincing cross-over was found between the genital and wing effect in the 1876 flies examined. This proves that if the genital abnormalities are due to a separate locus, it is extremely close to *cv*. *v* and *cv* are already known to be very closely linked, which seems to indicate that this section of the chromosome may be one in which crossing-over is very limited.

The genital abnormalities may represent a 3rd locus. If cross-overs occur, the recombination percentage with *cv* will be the smallest recombination percentage known in *D. subobscura*. Even with *cv* and *v* one cross-over is found in approximately 700 flies, giving a recombination percentage of 0.13.

Preliminary evidence shows that a similar genital effect is associated with *cv*³, although *cv* shows no genital abnormality. This strongly suggests that genital and wing effects are both due to a change at one locus, as a mutation of two loci at the same time on two separate occasions is highly unlikely.

VARIATION OF GENITAL ABNORMALITIES IN INBRED LINES

The *cv*² flies obtained from the $F_3 \frac{cv^2}{v} \times cv^2$ were inbred for five successive generations. The results are shown in Table 2.

Table 2

Generation	Total no. of males dissected	No. of abnormal flies	% of abnormal males
F_1	26	7	26.9
F_2	202	36	12.3
F_3	1936	253	13.1
F_4	2754	754	27.4
F_5	622	98	15.8
F_6	532	89	16.7
F_7	693	258	37.2
F_8	611	234	38.3

In the F_1 and F_2 only the first 20 males were dissected to leave sufficient paired matings for breeding purposes. It is conceivable that the first 20 males are not representative of the total number hatching in a culture, and therefore in the F_3 and F_4 all the males except those needed for breeding were dissected.

Table 3 shows that later hatching flies have about 16% more abnormalities on the average than the earlier ones. As they are few in number they do not greatly alter the combined total, and it was considered adequate to dissect only the first 20 males in the later cultures.

Table 3

	Up to first 20 males (A)		Beyond first 20 males (B)		A % abnormal	B % abnormal	A + B
	Dissected	Abnormal	Dissected	Abnormal			
F_3	1173	143	763	110	12.2	14.4	13.1
F_4	1981	522	773	232	26.4	30.0	27.4
			F_3 B/A	1.18	F_4 B/A	1.14	

Sets of paired brother-sister matings (producing at least 100 males) showed a greater range in the percentage of the abnormal flies (i.e. from 8.4 to 46.3) than the individual generations. The percentage in each generation has been influenced by selection and does not represent the figure obtained by straightforward inbreeding.

Table 4 shows the correlation between the number of abnormalities found in the first 20 males of a culture (F_1) and the number of abnormalities produced in the first 20 males of the brother \times sister progeny (F_2). The means and the standard errors of the F_2 have been calculated. It can be seen that there is a marked tendency for the number of abnormalities

to increase in the progeny of flies whose brothers were frequently abnormal, and the calculation of the correlation gave $r=0.30 \pm 0.05$ (see Fig. 1).

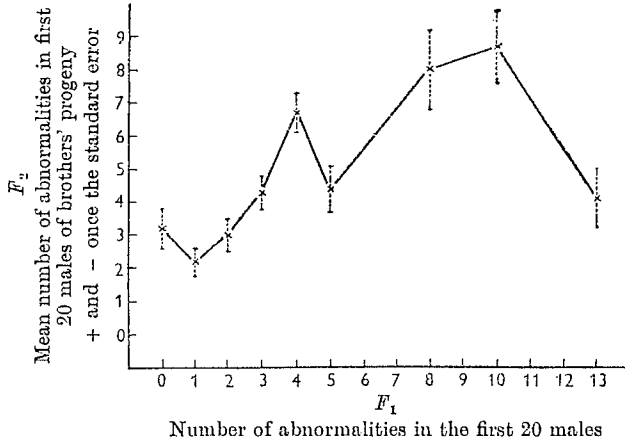


Fig. 1. Abscissa: number of abnormal males in first 20 from a brother-sister mating. Ordinate: mean number of abnormal males in the first 20 of their nephews from brother-sister matings.

It therefore seems probable that the percentage of abnormal flies is at least partly genetically controlled. Other factors, such as environmental conditions, may influence the number of abnormal males per culture, but they could not be responsible for producing the entire effect.

Selection for the different types of abnormality proved unsuccessful, and their distribution was at random throughout individual cultures and generations.

Table 4

No. of abnormalities in the first 20 males of the brothers' progeny (F_2)	17	1	.
	16
	15
	14	1	1	.	.	.
	13	1	.	.	3	1	1	1	1	.
	12	.	.	.	1	1	.	.	2	.
	11	2	.	.	1	4	1	1	2	.
	10	1	.	.	.	2	1	1	2	.
	9	1	.	.	4	3	.	1	1	.
	8	.	.	1	.	3	1	.	.	.
	7	2	.	1	2	2	.	2	1	.
	6	.	1	1	3	3	.	.	.	2
	5	2	1	.	7	2	5	1	2	3
	4	4	3	3	3	8	2	.	2	2
	3	3	3	3	7	1	4	.	.	3
	2	10	3	4	4	2	8	1	.	2
	1	7	4	3	6	.	1	.	1	2
	0	11	4	2	8	1	3	.	.	1
F_1 no. of abnormalities in first 20 males of a culture	0	1	2	3	4	5	8	10	13	
F_2 mean of abnormalities	3.20	2.21	3.00	4.33	6.70	4.39	8.00	8.73	4.13	
F_2 standard error	0.56	0.42	0.53	0.54	0.58	0.65	1.24	1.11	0.91	
F_2 average	3.97						6.95			

THE EFFECT OF OUTCROSSING ON THE NUMBER OF ABNORMAL MALES PRODUCED

Two outcrossing experiments were carried out to ascertain whether different genetical environments influenced the expression of the genetal effect.

*cv*² females were crossed to *th ma* males. The F_2 figures (previously given) show that *cv*²

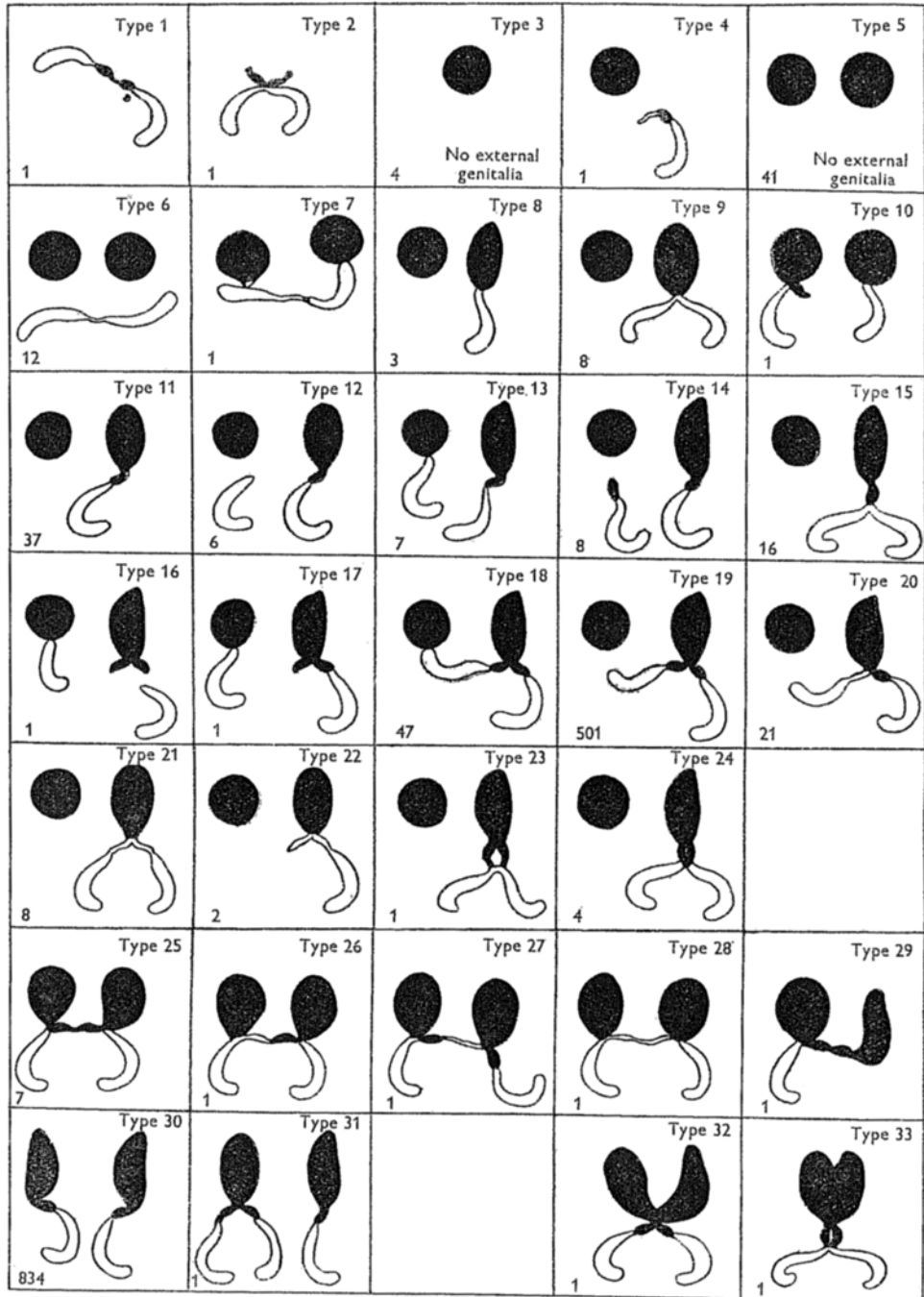


Fig. 3. *crossveinless*² genital abnormalities (diagrammatic). Types 1-33 without a ductus ejaculatorius. In all 60 types the number found of each is given in the lower left-hand corner of the square, and all structures which were pigmented (i.e. orange) are shown in black.

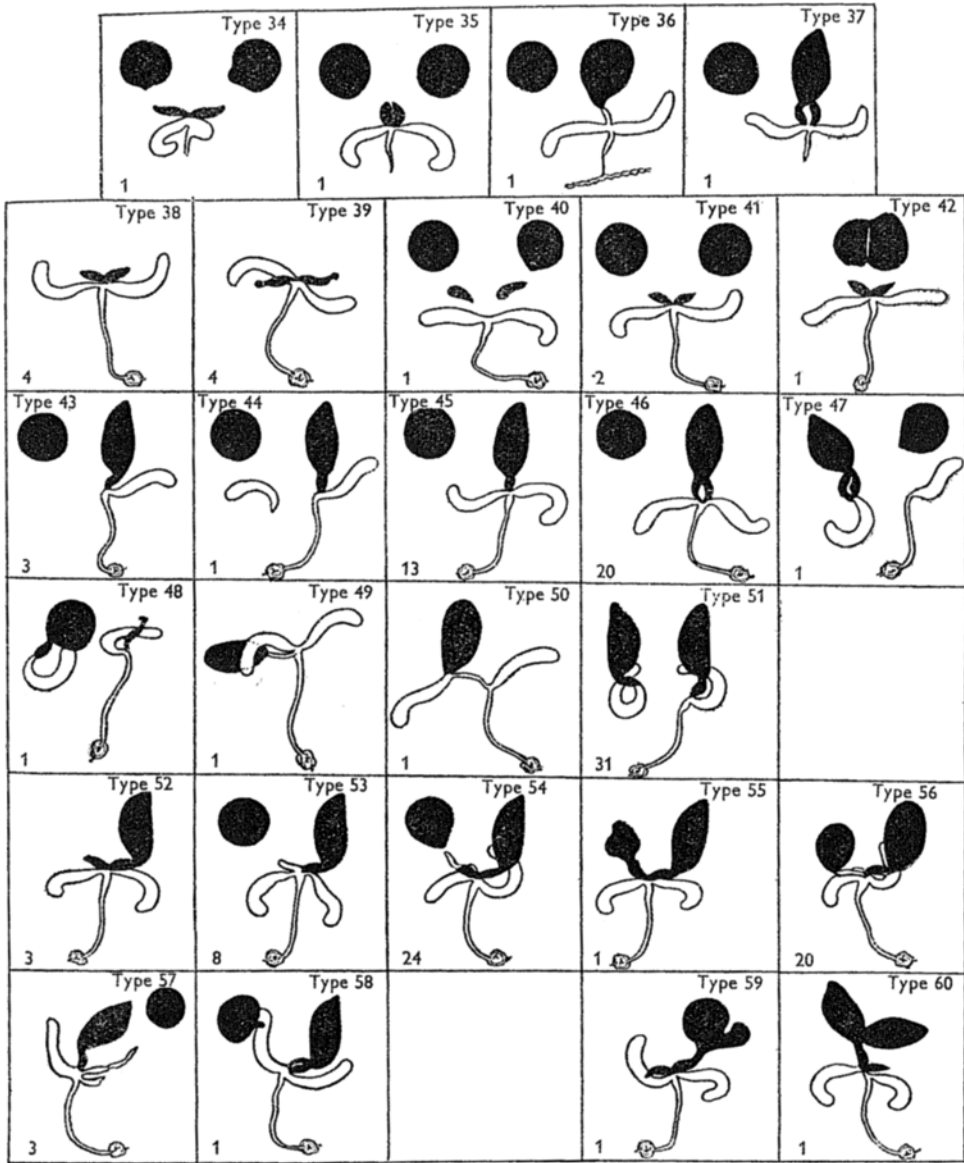


Fig. 4. *crossveinless*² genital abnormalities (diagrammatic). Types 34-37 with a degenerate ductus ejaculatorius. Types 38-60 with a normal ductus ejaculatorius.

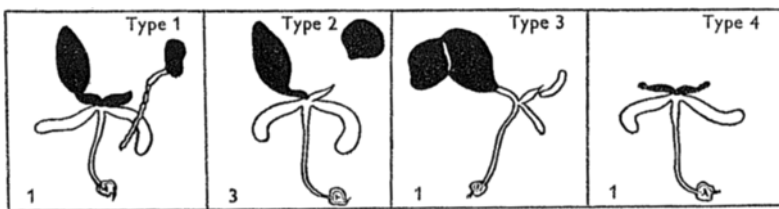


Fig. 5. Abnormalities found in the + or v males dissected.

The relative frequency of the various types in *sz* is not stated, nor does Stern mention whether the external genitalia are absent in group 5.

Normal development

The normal course of development of the genitalia is known but not that of *cv*² or *sz*, therefore any suggestion concerning the development of genital abnormalities in *cv*² must be based on the normal development and descriptions of abnormal adults.

A short account of the normal development as described by Stern (1941*a*) will be given before the *cv*² abnormalities are considered. The testes develop from the testes rudiments which are situated in the anterior third of the larva. The rest of the male duct system develops from the genital anlage which lies at the posterior end of the larva. This system consists of the vasa deferentia, the accessory glands (paragonia), the ductus ejaculatorius, the ejaculatory bulb and the external genitalia.

During the development of the genital anlage, the median duct, which will become the ductus ejaculatorius, appears first. Then on each side of this median duct a lateral duct is formed. This differentiates into anterior and posterior regions, the former becoming the vas deferens, the latter the paragonium. It is only after the vasa deferentia and paragonia are clearly formed and the former have made contact with the testes that the connexion between the median and lateral systems is established. As has already been mentioned the ejaculatory bulb and external genitalia are also formed from the anlage and are associated with the ductus ejaculatorius.

Origin of the vasa deferentia

Stalker (quoted by Stern & Hadorn, 1939) found that the genital anlage gives two hollow tubes which develop into the vasa deferentia, but that the outer covering of these in the adult is composed of large flat irregular cells which have migrated to them from the testis sheaths. Transplantation experiments involving several species appear to confirm the work on pigment cell migration.

In several *cv*² abnormalities genitalia were found in which a vas deferens was unattached to its testis. The testis was spherical and showed no signs of having been attached, yet the vas deferens was pigmented. This appears to contradict Stalker's work and the transplantation experiments, but as it occurred in so small a number of males, and the actual development is unknown, it would be premature to suggest that migration of the pigmented cells does not take place in *D. subobscura*.

Elongation of the testis

It was found that if a testis remains unattached to a vas deferens it fails to elongate and spiralize. This was noted both by Stern and others during transplantation experiments (Stern, 1941*a*), and by Dobzhansky in work on gynandromorphs. Stern confirmed this in *sz* where testes were frequently free and remained spherical. He also noted that if the testis lay very close to the end of the vas deferens slight elongation, without curving, might occur, leading to the formation of a pear-shaped testis with its point towards the vas deferens.

The data on *cv*² confirm the work of previous authors. Fig. 2 shows that a *D. subobscura* testis is a slightly curved elongated structure when attached to a vas deferens. When free it usually remains spherical, but occasionally when lying very near to the vas deferens it is

pear-shaped. In some types such as 7, 10, 25, 26 and 27, as is seen in Fig. 3, even when attached to the vas deferens or a paragonium, the testis remains pear-shaped. However, the relative position of the testis, vas deferens and paragonium are abnormal in these instances. cv^2 work confirms that the vas deferens is responsible for the change in growth and form of the testis during development. Exactly how it exerts its influence is unknown, but it appears to do so only over a very short distance. If for any reason this distance is lengthened the testis remains spherical.

cv² abnormalities

The abnormalities found in cv^2 show that the testes rudiments themselves are rarely affected. The testes were absent or rudimentary in only 17 cv^2 males, and rudimentary testes were found in one of the cv^+ abnormalities. We may therefore assume that the cv^2 mutant affects the genital anlage to a greater or lesser extent.

In some abnormalities all the normal organs are present, but attached to each other in an unusual manner. In others individual organs may be absent or degenerate. The vast majority of abnormalities involve the absence of at least one organ (1605); the ductus ejaculatorius is the most frequently affected, 1399 abnormalities being due entirely to the absence of this duct.

We know that the establishment of the connexions between the lateral and median ducts is the last step in the development of the genitalia. If this step were prevented we would expect to find a normal testis attached in the usual manner to a vas deferens and paragonium, on each side, and an unattached median duct with an ejaculatory bulb and external genitalia. Actually the most frequent abnormality found (type 30) is very nearly this. The only difference is the complete absence of the anterior ductus ejaculatorius. This suggests either that the last stage in development is prevented and the median duct degenerates, or that the anterior section of the median duct is never formed.

All the most frequent abnormalities appear to be due to comparatively small alterations in the genital anlage, leading to the degeneration or absence of one organ (or more), or an unusual method of attachment (see types 19, 11, 50, 54, 56 and 20).

The most extreme disturbance found in more than 1% of the abnormal flies is type 5. It accounts for 2.4% of the total number of abnormalities, and appears to be caused by a very extreme or early disturbance in development. No products of the genital anlage are present, even the external genitalia found in all other types (except type 3) are missing. It may therefore be assumed that the entire genital anlage either failed to develop or degenerated before further differentiation.

cv^2 females appear to have normal genitalia; the duct system was complete in the 50 dissected.

SUMMARY

The allelomorphs cv^2 and cv^3 , but not cv , give rise to abnormalities of the male genitalia. Sixty different types are described. The ductus ejaculatorius was generally absent, the testis and external genitalia rarely so. The average frequency of abnormalities was 23%, but it was shown that the frequency of abnormalities in a family, though not the types found, depends both on genetical and environmental factors. Vasa deferentia were pigmented even when unattached to a testis; unattached testes were spherical or pear-shaped. cv^2 appears to act on the genital anlage. cv^2 females are normal.

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