A STUDY OF INTERSEXUALITY IN *DROSOPHILA VIRILIS*¹

G. A. LEBEDEFF*

Cornell University, Ithaca, N. Y.

Received February 24, 1939

I. GENETICS OF INTERSEXUALITY

IN THIS paper a detailed account of our study of intersexuality in *Drosophila virilis* will be given. The progress of this work has been reported elsewhere (LEBEDEFF 1934a and b, 1938a and b). These intersexes are the result of mutative changes within the normal diploid system, and they differ from intersexes in other species genetically, morphologically and embryologically. Intersexes in *Lymantria* usually appear from interracial crosses, presumably due to an unbalanced condition between the female and male determining tendencies (GOLDSCHMIDT 1934). Intersexes in *Lymantria* also have been reported from intraracial crosses (KOSMINSKY 1930). In *D. melanogaster* intersexes appear from the cross between triploid females and normal diploid males (BRIDGES 1932). The chromosomal constitution of these intersexes was found to be intermediate between that of the normal female and the normal male. Several intersexes of the same nature have been obtained from a cross between triploid *D. melanogaster* females and normal *D. simulans* males (SCHULTZ and DOBZHANSKY 1933). A single autosomal gene was found to be responsible for intersexuality in *D. simulans* (STURTEVANT 1921).

The intersexes described here are the result of the action of a single autosomal gene, called intersex (*ix*). The gene was called *ix* soon after it had been discovered, and before its nature had been studied. Since then it has been renamed *ix^m* to denote its true nature as a gene for maleness. These intersexes were observed first in 1933 in a short vein (*sv*) stock which originated in 1930-31 in one of the cultures received in Cold Spring Harbor from PROFESSOR T. KOMAI, Kyoto, Japan. By inbreeding normal flies from the stock it was observed that some cultures produced intersexes in abundance, while in other cultures intersexes were less numerous. Altogether 40 cultures of the first type were obtained, these producing 2088 males, 1191 females and 962 intersexes. The number of normal males, females, and intersexes approached that of a 2:1:1 ratio; in other words, in these sets of cultures one-half of the female population became intersexual. The 41 cultures in which intersexes appeared less frequently, pro-

¹ Paper No. 221, Department of Plant Breeding, Cornell University, Ithaca, N. Y. Based on the thesis presented to the Faculty of the Graduate School of Cornell University for the degree of Doctor of Philosophy.

* A part of the cost of the accompanying illustrations is defrayed by the GALTON and MENDEL Memorial Fund.

duced 1993 males, 1516 females and 489 intersexes, thus approaching a 4:3:1 ratio of males, females, and intersexes. In this case one-quarter of the female population became intersexual. These data indicate that females homozygous for the ix^m gene become intersexes, that this gene has no effect on males, and that ix^m/ix^m males are fertile. The slight departure from the 2:1:1 and 4:3:1 ratios is explained by assuming that at the beginning of the work the extreme female- and male-like intersexes were classified as females and males. The equal number of 2:1:1 and 4:3:1 segregating cultures is due to the fact that some selection was practiced and only cultures giving good segregation were recorded and propagated.

Many breeding data were accumulated during the work with the intersexes. The original stock was subjected to selection for the various morphological types. Three distinct morphological types of intersexes were isolated: female-like, male-like, and extremely male-like called respectively types 1, 3, and 4. The stock cultures of each line were kept and counts recorded for some time. The summaries of all these cultures are given in tables 1 and 2. Altogether 269 cultures producing different morphological

TABLE 1

Summary of cultures segregating in 2:1:1 ratio for different morphological types of intersexes.

TYPE OF INTERSEX	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES
Line 1	43	1412	790	669
Line 3	55	2127	1023	997
Line 4	76	2539	1354	1222
	174	6078	3167	2888

TABLE 2

Summary of cultures segregating in 4:3:1 ratio for different morphological types of intersexes.

TYPE OF INTERSEX	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES
Line 1	51	1543	1254	411
Line 3	15	479	355	113
Line 4	29	972	706	231
	95	2994	2315	755

types of intersexes were counted. Among these, 174 cultures segregated in a 2:1:1 ratio, giving 6078 males, 3167 females and 2888 intersexes. The other 95 cultures segregated in a 4:3:1 ratio, producing 2994 males, 2315 females and 735 intersexes.

Linkage. A set of experiments was performed to determine the chromosome in which the ix^m gene is located. The normal males² from stocks throwing intersexes were crossed with virgin females from stocks which carried the following dominant genes: clipped (Cl), in the fourth chromosome (lethal when homozygous); garnet (G), in the third chromosome; and rounded (R), in the second chromosome. The F_1 males from such crosses were outcrossed to virgin females from the intersex-throwing stocks. From the $+/+ ix^m/+$ female and $Cl/+ ix^m/+$ male cross, three cultures were obtained giving altogether 130 flies; of these 63 were males, 50 females and 17 intersexes. Of these 17 intersexes, 9 were clipped and 8 were of the wild type, which indicates that the ix^m gene is inherited independently of the fourth chromosome genes.

For the linkage test with the second and the third chromosomes, a compound rounded garnet stock was used. Virgin females from this stock were crossed to normal males from intersex producing stock. From the cross of F_1 males to $ix^m/+$ females seven cultures were obtained which segregated for intersexes. They gave 419 males, 392 females and 59 intersexes. Twenty-seven of these latter were rounded, 32 were of the wild type, and none of the rounded garnet and garnet types. This indicates that the ix^m gene is independent of R , the gene of the second chromosome, and is linked with the garnet (G) gene of the third chromosome.

From the reciprocal cross $G +/+ ix^m$ females with $+ ix^m/+ ix^m$ or $+ ix^m/+ +$ males (the rounded gene can now be disregarded), a number of cultures were obtained in which the ratio of females to intersexes was modified from the expected 1:1 and 3:1 into 3:1 and 7:1 respectively by the presence in the garnet stock of a dominant suppressor of the ix^m gene. Though these data could be used for calculation of the linkage between G and ix^m , garnet females were repeatedly mated to the wild type males from this cross in order to eliminate the ix^m suppressor. It was soon observed that, from the cross between $G +/+ ix^m$ females and $+ ix^m/+ ix^m$ or $+ +/+ ix^m$ males, cultures appeared giving 2:1:1 and 4:3:1 segregation for males, females and intersexes. In other words, the suppressor was eliminated from the stock. The 2:1:1 segregating cultures were recorded and have been used in connection with the linkage studies. Altogether sixteen cultures have been obtained (table 3). In these cases the progeny is the result of a backcross for both G and ix^m , and therefore both female and intersexual populations can be used to compute the linkage value between the G and ix^m genes. Disregarding the males, the population consisted of 332 garnet

² For these and for most crosses in this experiment flies were used preferably from cultures showing a 2:1:1 segregation. Males were used in preference to females, since one-half of the male population from such cultures was homozygous for the ix^m gene, the other half being heterozygous ($ix^m/+$). All of the females from such cultures were heterozygous ($ix^m/+$), the intersexes being homozygous (ix^m/ix^m).

females, 31 wild-type females, 19 garnet intersexes, and 278 wild-type intersexes. The percent of crossing over between G and ix^m is equal to $(50/660) \times 100 = 7.58$.

TABLE 3

Linkage study. The result of the cross of $G +/+ ix^m$ females and $+ ix^m/+ ix^m$ males.

NUMBER OF CULTURES	MALES		FEMALES		INTERSEXES	
	G	$+$	G	$+$	G	$+$
16	370	366	332	31	19	278

Three point test. In order to find the exact locus of the ix^m gene, a three point linkage test was conducted. A short vein (sv) stock³ carrying the ix^m gene was made, and the sv females from the stock throwing intersexes were crossed to garnet males.⁴ The F_1 generation in this, as well as in the other F_1 crosses, consisted of normal males and females. Then F_1 females ($++G/sv ix^m +$) were backcrossed to sv males from the cultures giving 2:1:1 segregation for males, females, and intersexes. The results of these crosses are given in table 4.

TABLE 4

The three-point test. Summary of the crosses between $++G/sv ix^m$ females and $sv ix^m +/sv ix^m +$ or $sv ix^m +/sv ++$ males. Females and intersexes only were classified in regard to phenotype.

RATIO	NO. OF CULTURES	MALES	FE-MALES	INTER-SEXES	O $sv ix^m$	G	1		2		1-2	
							$sv G$	ix^m	$sv ix^m$	$G +$	sv	$ix^m G$
4:3:1	2	80	80	27	19	45	5	6	2	5	23	—
8:7:1	23	894	763	112	98	346	52	4	9	45	320	1
16:15:1	16	707	590	35	29	273	40	5	1	33	247	—
32:31:1	15	596	556	15	11	234	47	4	—	37	233	—
	56	2277	1989	189	157	898	144	19	12	120	823	1

There are certain peculiarities involved in the results of this cross which need some explanation. First, out of 56 cultures obtained, not a single one showed a 2:1:1 segregation in spite of the fact that approximately one-half of the males used undoubtedly were homozygous for ix^m . Instead, the intersexual population was greatly deficient. The detailed discussion of this and the other cases of modification of the intersexual ratios will be presented in connection with the study of the modifying factors. It is evident, that in these data only the intersexual population can be considered

³ Short vein is a third chromosome recessive gene.

⁴ The garnet males were taken from $cn G$ stock, and not from RG stock used for the two point test.

for calculation of linkage relationships between the *sv*, *ix^m* and *G* genes. Since intersexes were found but once in each of the possible classes, namely, non-crossover, single and double crossovers, the data are unquestionably reliable. The only disadvantage in using only one representative of each possible class for linkage purposes is that the number of observations is reduced. The percent of crossing over between *sv-ix^m* is $(19+1)/189 \times 100 = 10.6$; between *ix^m-G*, $(12+1)/189 \times 100 = 6.88$; and between *sv-G* it is $(19+12)/189 \times 100 = 16.4$. The order of the genes is therefore *sv ix G*. The data in regard to *sv-G* genes are in fairly good agreement with those of the standard third chromosome map (CHINO 1936). The following is a map of the left end of the third chromosome:

<i>rl</i>	<i>G</i>	<i>ix^m</i>	<i>sv</i>
0	8	15	25

Sex-linked genes and intersexes

In order to demonstrate that intersexes of all morphological types (and they vary from female-like to male-like) are not gynandromorphs, which they often resemble (DOBZHANSKY 1931), and in order to prove that they are females (XX constitution), sex-linked genes were introduced into the intersex-producing stocks. Yellow, vermilion, forked, miniature, and singed stocks were obtained which segregated for intersexes. From the crosses between females from these stocks and the wild-type males from the stocks throwing intersexes were obtained 934 males, which carried one or another of the above mentioned sex-linked factors, 800 wild-type females, and 397 wild-type intersexes of all morphological types (table 5). The slight defi-

TABLE 5

Sex linked genes and intersexes. Result of crosses between females homozygous for sex linked genes and heterozygous for ix^m, and wild type males from intersex-throwing stocks.

FEMALE PARENT	NUMBER OF CULTURES	MALES	WILD TYPE FEMALES	WILD TYPE INTERSEXES
Forked	10	267 <i>f</i>	321	131
Vermilion singed	13	382 <i>v si</i>	314	164
Vermilion miniature	4	237 <i>v mt</i>	127	83
Yellow	2	48 <i>y</i>	38	19
	80	934	800	397

ciency in males is probably due to sex-linked semi-lethals, and the imperfect ratio of females to intersexes is due to the fact that some cultures segregated into 3:1 and the others into 1:1 ratios as to the number of females to intersexes. The fact that intersexes from these crosses were of the wild type like the normal females indicates that they are of XX con-

stitution. This is true for all morphological types, including extreme female- and male-like types.

II. MORPHOLOGY OF INTERSEXES

Morphologically intersexes exhibit a high degree of variability in respect of each separate feature of sexual organs, which may be like those of females, males, or both, and may be either reduced or absent. However, for genetical studies the intersexes were grouped into four morphological classes: female-like, hermaphrodites, male-like, and extremely male-like.

The female-like (type 1) intersexes differ from true females externally in that the anal tubercle, instead of having two simple dorso-ventral valves, shows valves divided laterally into four half valves. Often the lateral cleft is incomplete, so that the anal tubercle is composed of three parts. The vaginal plates of these specimens are often slightly displaced from their symmetrical lateral position. This change in the form of the anal tubercle is often accompanied or preceded by the presence of an outgrowth, which is a rudiment of the male external genitalia. It is found near the caudal end of the abdomen, between the anal and vaginal plates (figure 1, A-C). Internally these intersexes differ from the true females in that one, or both, of the ovaries is rudimentary and contains no growing eggs. Rarely ovaries are overdeveloped and contain growing eggs, which are sterile (Plate I, 2 and 4). Often gonads of these intersexes develop into ovotestes, which are usually attached by their ovarian part to the oviduct. The testicular part may be either attached or unattached to the rudimentary seminal vesicles (Plate I, 3 and 6).

In hermaphrodites (type 2 intersex) the male reproductive organs, both external and internal, are far more developed than in the previous type. The male external genitalia in hermaphrodites are developed to such an extent that the claspers and a penis are present. However, they are often incompletely formed. The female genitalia are still more displaced from their lateral position. The anal tubercle in these specimens usually has characteristics of both sexes, having both dorso-ventral and lateral clefts. Often the dorso-ventral cleft is incomplete or entirely absent, so that the anal tubercle is either composed of three parts, or assumes entirely a male-like aspect (figure 1D; figure 2A). The male sexual ducts in hermaphrodites are developed to various degrees. In the weak hermaphrodites seminal vesicles, and often paragonia are the only organs which are completely formed. In the more developed hermaphrodites the male sexual ducts are well developed, so that paragonia, sperm pump and vas deferens are easily seen upon dissection (Plate I, 5, 7 and 8; Plate II, 1). The gonads of hermaphrodites are ovotestes, and often are attached to both the female and the male sexual ducts.

PLATE I.—Internal reproductive organs of: 1, normal female; 2 and 4, most female-like intersex; 3, 5 and 6, weak hermaphrodite; 7-8, complete hermaphrodite. Microphotographs, magnification about 22-25.

od—oviduct; pa—paragonia; sm—spermathecae; sp—sperm pump; t—testes; tr—tubular receptacle; v—vagina; vd—vas deferens; vp—vaginal plates; vs—seminal vesicles.

The genitalia of normal males, as seen posteriorly, are rotated counter clockwise through 360° , so that the duct (ductus ejaculatorius) loops once about the intestines. This can easily be observed in the hermaphrodites. As soon as the male genitalia in these intersexes have reached a certain degree of development they begin to rotate around the duct to a considerable degree. During this process they are often drawn into the abdomen together with the female genital plates and the anal tubercle. In these cases specimens seem to lack any external genitalia, and often an anal

FIGURE 1.—Abdomen and external reproductive organs of: A, normal female; B-C, female-like intersex (type 1); D, weak hermaphrodite.

an—anal opening; cl—claspers; g—rudiments of male genitalia; mg—male genitalia; p—penis; vp—vaginal plates; 4S–6S—fourth–sixth sternites; 5t–7t—fifth–seventh tergites.

opening. However, dissection reveals their presence. In cases where the anal tubercle is involved, this process is frequently fatal to the individual as the feces are liberated into the body cavity, there being no opening to the exterior.

There is apparently some correlation between the degree of the development of external and internal sexual organs of the male in these hermaphrodites. However, instances were recorded when the male internal sexual organs were quite well developed, while the male external genitalia were not yet completely formed. The appearance and the development of the male system in these intersexes usually do not interfere with the complete development of the female system. However, in many cases it was observed that in intersexes of these types the female system begins to disappear before the male sexual organs are fully developed.

The complete development of the male system is accomplished in the

PLATE II.—Internal reproductive organs of: 1, hermaphrodite; 2-3, weak-male (type 3) intersex; 4-5, male-like intersex; 6, normal male. Magnification and lettering same as in the Plate I.

next two types of intersexes. It is usually accompanied by the gradual disappearance of the female sexual organs, leading to an almost complete sex reversal. In the type 3 intersexes both male external and internal sexual organs are almost completely formed. The male external genitalia, however, are located in an unsymmetrical position (figure 2, B). The gonads in these intersexes are ovotestes, which are usually attached to the male sexual ducts only; the oviduct lies free. In most cases the detachment of the oviduct from the ovotestis is accompanied by the reduction or the complete disappearance of the oviduct and often of the tubular receptacle (Plate II, 2-3). The last internal female organ (excluding the ovary) to

FIGURE 2.—Abdomen and external reproductive organs of: A, hermaphrodite (type 2); B, weak male-like (type 3) intersex; C-D, male-like intersex (type 4); D also of a normal male. Lettering as in the figure 1.

disappear is the spermatheca. In all the other types of intersexes the shape of the abdomen is that of a normal female. In this type, however, the abdominal characteristics for the first time show some signs of change; the seventh and the sixth tergites show some tendency to fuse.

The type 4 intersexes resemble true males externally and internally. The male external genitalia reach symmetrical or nearly symmetrical lateral positions. The number of abdominal segments (tergites and sternites) is gradually reduced to the number found in normal males (figure 2, C-D). These changes are accompanied by the disappearance of the ovarian part of the gonads and the development of testes (Plate II, 4-5). Often ovarian tissue can still be found attached to one or both of the testes in these intersexes. The converted males do not have sperms in their testes and are sterile, as are all the other types of intersexes.

To complete the list of various types of intersexes found in this study, some odd types should be mentioned. Among sex intergrades of the hermaphrodite types several cases were observed in which the number of spermathecae was increased from the usual two to three and four. Sometimes the number remained normal, but the shape was slightly altered from the oblique to slightly flattened, the size being also reduced in some cases. In several instances it was found that one of the testes, together with the seminal vesicle and one paragonium, was missing from the male part of the hermaphrodite. Several instances were recorded of converted males having only one testis and one set of ducts. In one case a converted male was found with two perfectly symmetrical sets of male external genitalia, to each of which one testis, together with a seminal vesicle and a single paragonium was attached. Finally several individuals resembling normal males were found which did not show any sign of a reproductive duct system nor any external genitalia, but which had in their body cavity two red ellipsoidal testes.

From this brief description of the morphology of intersexes it is evident that there is at once a striking similarity and a striking dissimilarity between intersexes of *D. virilis* and those of *Lymantria* and *D. melanogaster*. GOLDSCHMIDT found that *Lymantria* intersexes possess a mixture of male and female characteristics, and that a condition of intersexuality did not effect all the sexual characteristics equally in a given individual. Thus some organs of an intersex were normal male or female, while the others were intersexual. Further investigations have demonstrated that the mixing of the male and female characteristics in a given intersex is not accidental, but is a result of a very definite process in the embryonic development. The intersex develops for a time as the sex indicated by the genotype; then from a certain point, the turning point, it continues to develop under the control of the other sex. As a result, some of the characteristics of the initial sex of the individual are transformed or replaced by corresponding sexual characteristics of the other sex. The degree of intersexuality is determined by the time of occurrence of the turning point. DOBZHANSKY and BRIDGES (1928) concluded that the same principle holds for intersexuality in *D. melanogaster*. They found that there is a definite relation between the stability of a character in the intersex and the time of development of this character in normal sexes.

The conclusion that the principle of the turning point provides the mechanism for the formation of *D. virilis* intersexes is based on genetical, cytological, and morphological studies of intersexes. The genetical and cytological evidences indicate that intersexes of all morphological types, including converted males, are of 2X:2A constitution, or zygotic females. Morphological studies of *D. virilis* intersexes reveal that the first male

characteristic to appear in intersexes is the changed structure of the anal tubercle. This is followed by the appearance and development of the male sexual duct system and penis, together with the formation of the genital arch and the fusion of the sixth and seventh tergites. In other words, there is a definite relation between the order of the appearance of the male sexual characteristics in intersexes and the time they originate and differentiate in the pupal development. For the female characteristics the situation is exactly reversed. The late developing female characteristics, such as the organs of the external genitalia, tend to be the first to disappear in the intersexes. The early developing female characteristics, such as the vagina and tubular receptacle, are the last to disappear in the intersexes. Therefore, it can be assumed that *D. virilis* intersexes develop as females up to a certain point, after which they start to develop in a male direction.

However, morphological studies of these intersexes indicate that while the turning point is responsible for the intersexuality here, the embryology of those sexual organs which had not completed their development at the time of the occurrence of the turning point must be different in intersexes of *D. virilis* from that in *Lymantria* and *D. melanogaster* intersexes. In the case of *D. virilis* intersexes, all sexual organs, with the exception of the secondary ones, which have not completed their development at the time of reversal, do so after the occurrence of the turning point. The turning point does not interfere with the development of most of the sexual organs of the initial sex (female in the case of *D. virilis*) once the imaginal discs of these organs have been laid down, even though they have not sufficiently differentiated. However, the occurrence of the reversal reaction stimulates the development of the corresponding sexual organs of the other sex. The male organs develop from fresh outpushings (or else from new imaginal discs). As a result, the two systems, the initial female and the additional male, develop side by side in the same individual, resulting in the development of hermaphrodites of various degrees.

All these considerations on the nature of intersexuality in *D. virilis* have been based on genetical, cytological and morphological studies of intersexes. The embryological development of sexual organs in intersexes can be interpreted by the morphological studies of the adult intersexes, provided the embryology of the normal male and female is well known. The development of sexual organs in normal sexes of *D. melanogaster* was studied by DOBZHANSKY and BRIDGES (1928). The conclusions as to the nature of intersexuality in *D. virilis* have been based on their work, the assumption being that the sequence of appearance and development of imaginal discs of the sexual organs are basically the same in both species.

III. THE DEVELOPMENT OF THE GONADS IN INTERSEXES

Materials and methods

The intersexes from our lines 1, 3 and 4 were subjected to analysis. From three to five pairs of flies, preferably from cultures the male parents of which were homozygous for *ix^m*, were placed in clean half-pint milk bottles. Strips of blotting paper smeared with culture media and seeded with yeast suspension were placed on regular slide glasses and slipped into these culture bottles. Cultures were kept at 22–25°C. Every twelve hours the blotting paper with the media and the deposited eggs was removed and placed in fresh clean bottles, while new medium was introduced into the bottles containing the flies. The eggs were examined every six or twelve hours, and newly hatched larvae were removed to the regular culture bottles. Under these conditions the egg period lasted about 1½ days, larval period 6½–7 days and pupal period 6½–7 days. When the larvae and the pupae reached a certain age, they were removed from the culture and their sex determined. In most of the cases the gonads were dissected and then fixed. In several instances the tissues were fixed in situ with less satisfactory results, as has been the experience of all *Drosophila* cytologists. A strong Flemming solution was used for fixation and iron-alum haemataxylin for staining.

Gonads in intersexes of types 2 and 3

Most of our studies on the development of gonads of intersexes were made with these two types. There are about sixty slides available of gonads of these intersexes, from about four day old larvae up to the adult stage. Since the development of the gonads in these two types of intersexes is essentially similar, it will be considered together.

During the larval stages, the gonads of these intersexes resemble both in size and shape the gonads of normal male larvae of the same age (Plate III, 11–12, 16–17). The germinal cells, however, are distinctly female-like in aspect; they are oogonia and oocytes in various stages of development. These cells, however, are considerably larger than oogonia and oocytes found in the normal ovaries, as they are in the process of transformation into spermatocyte-like cells. The young unencysted oogonia are found in the most distal part of these gonads. Next to them are located cysts with the young oocytes, while those with oocytes in more advanced stages are found in the central and the proximal parts of the gonads. Often cysts with young oocytes are also found among those with oocytes in more advanced stages of development. There are usually 16 oocytes in the cysts. In several instances as many as 32 or more oocytes were found to be in the same cyst.

PLATE III.—Gonads of normal females; 1-3, larval stage; 4-6, pupal stage. Gonads of female-like intersex (type 1); 7-8, pupal stage; 9-10, adult stage. Gonads of hermaphrodites (type 2); 11-12, larval stage; 13-14, pupal stage; 15—adult stage. Gonads of male (type 3) intersex; 16-17, larval stage; 18-19, pupal stage; 20, ovotestis of the adult; 21, ovarian part of the ovotestis. Microphotographs from permanent preparations, magnification about 125 diameters.

Beginning with the late larval stage the gonads of these intersexes become smaller than those of the normal male of the same age, and therefore can be distinguished without difficulty from the gonads of normal males and females (Plate III, 13-14 and 18-19). By this time some of the oocytes in the most proximal part of the gonads have increased considerably in size and have undergone a transformation into spermatocyte-like cells. Some of these transformed spermatocytes show signs of disintegration.

The late pupal stage of the gonads of these intersexes is the most difficult to study, as the gonads appear as having an indefinite shape and size, while in the stages preceding they were of considerable size and of a definite shape. Our preparations of the gonads in this stage are not very satisfactory. There is a considerable amount of pycnosis in them, and a number of germinal cells were found which seemed to be outside of the boundaries of the gonads. However, these preparations, when taken together with those of the gonads of freshly hatched intersexes of these types, suggest that during the late pupal stage the ovary gives rise to a testis, both organs then remaining attached to each other as an ovotestis. The process of transformation of these gonads is not yet clear. There is an indication that in some cases this might be accomplished by a simple budding (Plate III, 14, 19). The evidence from dissections of the gonads of these intersexes in the larval and pupal stages suggests that it is unlikely that the ovotestis starts to develop as two distinct organs. In no case was more than one pair of gonads found in the dissected larvae and the young pupae of these intersexes.

The adult intersexes emerge with two sets of gonads (Plate III, 15 and 20-21). The ovarian part is a well defined rounded body, especially in the type 3 intersex, and filled with oogonia and oocytes in the various stages of development. The testicular part is tubular in shape and is mostly filled with disintegrating cells. There is considerable variation in the degree of development of the ovotestes, especially in type 2 intersexes. The peritoneal sheath of the ovarian part of the ovotestis remains colorless, while that of the testicular part becomes red.

Gonads in the male-like (type 4) intersexes

The earlier stages of the development of gonads in these intersexes are essentially similar to those of the type 3 intersexes, as can be seen from the limited number of preparations (Plate IV, 1-4). The gonads of the male-like intersexes, however, are larger, reaching almost the size of the gonads of normal males. The other striking characteristic of the gonads of these intersexes at this stage is the absence of the giant spermatocyte-like cells. Apparently the process of transformation of the oocytes into spermatocyte-like cells in this case is much retarded.

PLATE IV.—Gonads of male-like (type 4) intersex; 1-2, larval stage; 3-5, pupal stage; 6-7; of the adult intersex (only part of the testis is shown). Gonads of normal male; 8-9, larval stage, 10-12, pupal stage; 13, of the adult (only part of the testis is shown). Magnification about 125 diameters.

The gonads of these intersexes continue to grow at almost the same rate as the normal testes. At the late pupal stage they elongate and assume a tubular form. Only at this time, or shortly before, can the giant cells be found in them (Plate IV, 5). The testes of the adult male-like intersex are mostly filled with spermatocyte-like cells, some of which are disintegrating. In several instances these testes were filled with small, spermatogonia-like cells (Plate IV, 6-7).

Gonads in type 1, female-like intersexes

An account of the development of gonads in intersexes would not be complete without some reference to the development of gonads in the female-like intersexes. At present our series of sections of the gonads of this type of intersex is far from complete. Considerable material is available in the sections of gonads of adult intersexes of this type (Plate III, 9-10). Also, several sections of these gonads in the larval stage were obtained (Plate III, 7-8). As may be seen from this material, the gonads of the intersexes of the female type during the larval stage approach in size and cell content the gonads of normal females of the same age. There is considerable variation in the condition of the gonads in the adult intersexes. Some of the gonads of the adult intersexes of this type are in a rather rudimentary state of development, comparable with the gonads of normal females at larval or pupal periods. They are filled with very small oogonia and oocytes in various stages of development. These cells are encysted, but there is no evidence of the formation of ovarioles. On the other hand, some of the gonads of the adult intersexes of the female type are quite well developed, containing ovarioles at different stages of development, and sometimes even a few growing eggs. The nuclear contents of these gonads have not been studied, and it is not known whether or not meiosis has occurred in the growing eggs.

The study of the development of gonads in various morphological types of intersexes indicates that in all of these morphological types the gonads start their development as ovaries. In the female type of intersex the ovaries continue their development, though it is retarded in various degrees. In the hermaphrodites, a part of the female germ cells in the ovaries are transformed into spermatocyte-like cells, while the ovary gives rise to a testis-like structure in which are found some of the oocytes now transformed into spermatocytes. The transformation of female germ cells into male-like ones is often accompanied by the disintegration of these cells. In the male type of intersexes the ovaries are gradually transformed into testes-like organs, while their cell contents undergo the process of transformation into spermatocyte-like cells, some of which eventually disintegrate. There is often a great deal of variation in the size and the structure

of gonads even in the same individual. The transformation of one of the two gonads is often more complete than that of the other. This is especially noticeable in the male type of intersexes. A number of cases were observed where in the same male intersex one gonad was testicular in character, while the other was an ovotestis.

In concluding this discussion of the development of gonads in *D. virilis* intersexes it may be pointed out that gonad development in *D. virilis* intersexes also differ from the intersexes of *Lymantria* and *D. melanogaster*. As has been shown by GOLDSCHMIDT and KERKIS (for *D. melanogaster*), gonads of the intersexes of these species either develop into those of the initial sex or into the gonads of an opposite sex, or are transformed into an organ which has the external and internal characteristics of both ovaries and testes.

IV. ABORTIVE GAMETOGENESIS IN INTERSEXES

The small cells found in the most distal part of the gonads of the intersexes during the larval stage are distinctly oogonial in various stages of development. In their nuclei about six heavily stained, small bodies can be seen, which probably represent five rod-shaped chromosomes and the nucleolus (Plate V, 1). The prochromosomes then become less distinct passing through the diffusion stage, while the nucleolus becomes a compact heavily staining body (Plate V, 2). After the oogonia undergo four mitotic divisions in succession, a group of sixteen young oocytes is formed from each. Several mitotic figures were observed in gonads of intersexes of all morphological types, which showed five pairs of rod-like and one pair of dot-like chromosomes (Plate V, 3). This comprises a typical female chromosome complement.

The young oocyte nucleus is smaller, and its prochromosomes are more compact than in the nucleus of the oogonium (Plate V, 4). As in the young oogonia, the prochromosomes of the young oocytes pass through the diffusion stage, while the nucleolus becomes spherical and tends to move away from the nuclear membrane (Plate V, 5). After the diffusion stage the chromosomes of the oocytes appear as fine chromatic threads, corresponding to the leptene stage (Plate V, 6). After this stage the nucleus grows quickly and the chromatic threads undergo a marked shortening and twisting, thus realizing the pachytene and, perhaps, the strepsitene stages (Plate V, 7). Soon after the nucleolus moves toward the nuclear membrane, while the chromatin aggregates and forms a small closely bound network, which has a tendency to lie close to the nucleolus (Plate V, 8). All these stages of the growth of the oocytes are very similar to those found in the oocytes of normal females (GUYENOT and NAVILLE 1933). However, the oocytes of the normal female are very small, and only two

PLATE V.—Abortive gametogenesis in intersexes. Simplified camera lucida drawings. Magnification about 700 diameters. 1-2, young oogonia; 3, oogonial chromosomes at metaphase; 4, young oocytes; 5-8, "premeiotic" stages of the oocytes during which they gradually assume spermatocyte-like aspect; 9-10, oocytes transformed into spermatocyte-like cells; 11-14, disintegration of the transformed spermatocytes.

in the cysts undergo growth stages, while the oocytes of the intersexes are of considerable size, and all 16 in the cysts pass through these stages.

In the oocytes of normal females at this stage of growth, both the nucleolus and the chromatin content undergo the diffusion stage which lasts until the egg is about ready to be laid. The behavior of the oocytes in the intersexual gonads is quite different and resembles that found in the normal spermatocytes (METZ 1926). Instead of undergoing diffusion, the nucleolus continues to grow and moves rapidly toward the nuclear membrane, and forms a deep pocket in it, while the chromatin tends to resolve itself into a thread-like form (Plate V, 9). By this time the cells further increase in size and closely resemble spermatocytes of normal males. The diffusion stage follows soon and the chromatin appears very lightly stainable (Plate V, 10). The stages leading to the meiotic divisions can be seen in some of these spermatocyte-like cells. Some of them, and particularly their nuclei, become oblong. However, instead of the meiotic divisions these cells undergo a process of disintegration. The nucleolus appears to sink into the cytoplasm, and then is lost to view. This is followed by the elongation and flattening of these cells. A number of vacuole-like masses then appear in the cytoplasm (Plate V, 11-14). Often the process of disintegration is different. The nucleolus, instead of passing through the diffusion stage, moves away from the nuclear membrane. The pseudo-chromatic material around the nucleolus opens up and forms heavy, ribbon-like, deeply staining bodies. Pycnosis often is observed in this type of cells.

V. GENETICAL RELATIONSHIP BETWEEN THE VARIOUS MORPHOLOGICAL TYPES OF INTERSEXES

In the beginning of this work, an attempt was made to isolate lines producing predominantly one type of intersex. After but a few generations of selection lines 1, 3, and 4 were isolated. Since that time these lines have been kept in stock cultures. At various times attempts were made to improve these stocks, selecting only those giving more uniform intersexual types. However, it was noticed that this selection did not bring much improvement in the uniformity of the respective types. The following table shows the degree of variability in each of the isolated lines.

As can be seen from table 6, line 1 is the most variable of all the three lines, segregating approximately in a 1:1 ratio of type 1 and type 2. It must be remembered that there is no clear cut morphological difference between these two types, and therefore, the classification was not always accurate. Line 3 is the easiest to classify, and is comparatively stable. Only 19.6 percent of the intersexual population belonged to the other types. Line 4 is the most uniform, giving less than ten percent of the other types. However, the differentiation of type 4 from type 3 is not always

easy. This is mainly due to the variability in the degree of the fusion of the abdominal segments, and the gonad development. The intermediate number of abdominal segments between that of a true female and that of a true male can be found in both types. The degree of gonad development also often overlaps in both types.

TABLE 6
Variations in the morphological types of intersexes in the lines 1, 3, and 4.

LINES	NUMBER OF INTERSEXES DISSECTED	MORPHOLOGICAL TYPES			
		1	2	3	4
		%	%	%	
Line 1	192	44.7	53.7	1.6	—
Line 3	189	1.5	18.1	80.4	—
Line 4	184	—	1.3	8.3	90.4

The study of genetical relationship between the different morphological types, lines 1, 3 and 4 were intercrossed in various ways and also crossed to various stocks. The F_1 , F_2 and the test cross generations from these crosses were raised and the intersexual population was studied. Very often the intersexes were dissected and the findings were recorded.

Quantitative inheritance

The study of the morphology of intersexes indicates that the various types of intersexes differ in the degree of development of the female and the male characteristics in them, the variations between such morphological types of intersexes being continuous. In other words, we are dealing with quantitative characteristics. It is well known that the study of the inheritance of such characteristics is extremely difficult due to many contributing factors. There usually are a larger number of genes involved, which contribute toward the expression and the degree of development of such characteristics (intersexuality in our case). On account of the lack of dominance of these genes, and their interaction, the division of the population into separate groups was often too artificial and did not represent the true genetical situation. If we add to this the consideration that sex is expressed in a number of complex organs, it is evident that the genetical analysis of intersexuality is very complicated. For these reasons this analysis is simplified by the separation of the intersexual population into four classes only. It is evident that by a classification restricted to a limited number of classes the error of sampling is increased, and the possibilities of detecting genes are lessened. However, by this method the complexity of genetical analysis due to incomplete dominance and gene interaction is also lessened. The purpose of this study, therefore, is rather to reveal the

minimum number and for that reason probably the most important genetical factors affecting the degree of intersexuality, than to find the possible maximum number of such factors, which is obviously beyond the scope of the present work.

The analysis of the data pertaining to the genetical relationship between the various morphological types of intersexes will be treated in three separate groups, due to the different types of crosses and somewhat different results obtained in each group.

A. Crosses between lines 4, 3 and 1

Cross between lines 4 and 3. In the F_1 from the cross between lines 4 and 3, 129 intersexes were obtained. Of this number 110 were classified as being intermediate between types 3 and 4 (designated as type $3\frac{1}{2}$), seventeen intersexes belonged to type 4, and two to type 2 (table 7a). The type $3\frac{1}{2}$

TABLE 7
Summary of crosses between lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE				
					1	2	3	$3\frac{1}{2}$	4
a $3 \times 4 F_1$	10	336	317	129		2		110	17
b $3 \times 4 F_2$	3	184	108	69			17	40	12
c $3 \times (3 \times 4)$	7	338	188	159*		5	19	27	
d $(3 \times 4) \times 4$	4	194	160	73				40	33
e $1 \times 3 F_1$	8	287	179	101*		77	12		
f $1 \times 3 F_2$	24	1231	893	343*	43	186	59		16
g $(1 \times 3) \times 1$	10	586	344	187*	57	84	14		
h $(1 \times 3) \times 3$	11	565	294	246	3	119	120		4
i $1 \times 4 F_1$	11	365	291	146		135	11		
j $1 \times 4 F_2$	8	299	242	202	23	97	36		46
k $(1 \times 4) \times 1$	2	87	52	29	9	17	5		
l $(1 \times 4) \times 4$	4	223	144	82		33	13		36

* Only part of the intersexual population from these crosses were recorded as to the morphological types.

intersexes were noticeably different from either type 3 or 4. The genitalia in the type $3\frac{1}{2}$ intersexes were more completely twisted than in type 3, but not reaching an almost symmetrical position as in type 4. Also the abdominal segments of type $3\frac{1}{2}$ were less completely fused than in type 4. However, the classification of the intermediate type $3\frac{1}{2}$ was attempted only in crosses where only the types 3 and 4 were involved. It was difficult to identify this intermediate type among the intersexual population from the crosses where type 1 was involved, because of the variability of types 3

and 4 themselves, and also because other genetical factors are involved in such crosses.

In the F_2 from the cross of lines 3×4 , 69 intersexes were obtained, of which 17 belonged to type 3, 40 to type $3\frac{1}{2}$, and 12 to type 4 (table 7b). The segregation of the intersexual population in the F_2 of this cross approximates that of 1:2:1 ratio, indicating that line 4 differs genetically from line 3 either by a single semi-dominant modifier of the ix^m gene, or by a different allele of ix^m . The segregation of the intersexual population in the test-cross is in agreement with either assumption. From the cross of line 3 females to F_1 (3×4) males, 159 intersexes were obtained, of which 51 were taken at random and dissected (table 7c). Of these 51 intersexes, five were classified as type 2, 19 type 3, and 27 type $3\frac{1}{2}$. Considering the five type 2 intersexes as variations of type 3, it can be assumed that 1:1 segregation of types 3 and $3\frac{1}{2}$ took place. Similarly from the test-cross of F_1 (3×4) females to males of type 4, 73 intersexes were obtained, 40 of which were of type $3\frac{1}{2}$ and 33 of type 4 (table 7d). Again a 1:1 segregation occurred, but this time of types $3\frac{1}{2}$ and 4.

Cross between lines 3 and 1. The segregation of the intersexual population in the crosses where line 1 was involved was very conflicting. Therefore no attempt will be made to interpret the genetical difference (if such exists) between types 1 and 2 and these two types will be considered together.

From the cross between lines 1 and 3, 101 intersexes were obtained in the F_1 (table 7e).⁵ Out of the 89 intersexes which were dissected and recorded, 77 were classified as belonging to type 2 and 12 to type 3. Considering the variations in the morphology of intersexes of the parental lines 1 and 3 (see table 6), the F_1 intersexual population from this cross can be considered as being intermediate between that of the parental lines. In other words, the dominance of one type of intersex over the other is lacking in the cross between lines 1 and 3, as is also true in the case of the cross between lines 3 and 4.

From the 24 F_2 cultures from this cross 343 intersexes were obtained (table 7f). Of these, only 304 were classified and 229 of them were either types 1 or 2, 59 of type 3, and 16 of type 4. The intersexual population from this cross segregated approximately in 12:3:1 ratio of the types 1 and 2 (taken together) and 3 and 4 respectively. This result is of considerable interest. The type 4 intersex is a new type in this cross, resembling neither of the parental types. Its appearance in 1/16 in the F_2 generation indicates that the various morphological types of intersexes are not due to various alleles of the ix^m gene, but are the result of ix^m modifiers. There

⁵ In the F_1 and test-crosses in these series of experiments the ratio of males, females and intersexes departs from both the 2:1:1 and the 4:3:1 ratios due to the fact that no attempt was made to group separately the cultures giving either of these two ratios.

are probably two modifying factors involved, type 4 being a double recessive (*ab*), and types 1 and 2 being either double dominant or dominant for at least one modifier (*AB* or *Ab*), and type 3 dominant for the other modifier (*aB*).

Since the test cross was made only to parental types 1 and 3, the hypothesis was tested only partially. From the cross of F_1 females to males from line 1, 187 intersexes were obtained most of which belonged either to types 1 or 2 (table 7g). While from the cross of F_1 males to females of line 3, 246 intersexes were obtained. Of these, three belonged to type 1, 119 to type 2, 120 to type 3 and four to type 4 (table 7h). This is an almost perfect 1:1 segregation of types 2 and 3. This test supports the assumption of one gene difference between lines 1 and 3.

Cross between lines 1 and 4. The result of crosses between lines 1 and 4 substantiates the assumption of two *ix^m* modifiers. However, further modification of the dihybrid ratio was found in this type of cross. In the F_1 from the cross between lines 1 and 4, 146 intersexes were obtained. Of this number 135 were of type 2, and 11 of type 3 (table 7i). This result is similar to that obtained in the F_1 from the cross between lines 1 and 3. This indicates that dominance of one morphological type over the other is lacking in the cross between lines 1 and 4, as was true also in the case of crosses between lines 1×3 and 3×4 .

The F_2 intersexual population from this cross segregated into 120 intersexes of types 1 and 2, 36 of type 3, and 46 of type 4 (table 7j). This segregation approximates a 9:3:4 ratio. The appearance of type 4 intersex in the 4/16 proportion among the intersexual population from this cross indicates that the modifier *A* alone has no effect on the *ix^m* gene.

The result of the test cross, and especially the cross to the parental line 4, substantiates this hypothesis. The cross of F_1 females to the males of line 4 resulted in 82 intersexes (table 7l). Of these 33 belonged to type 2, 13 to type 3, and 36 to type 4. Considering 13 intersexes of the type 3 as being variations of the type 4, a very good 1:1 segregation of types 2 and 4 occurred in this case. From the cross of the F_1 females to the males of the parental line 1 only 29 intersexes were obtained most of which belonged either to type 1 or 2, which is in agreement with expectation (table 7k).

Summing up the results of the crosses between lines producing various morphological types of intersexes it may be assumed that intersexes of types 1 and 2 are of *AB* constitution, of type 3 *aB*, and type 4 of *Ab* and *ab*.

However, the hypothesis does not meet the crucial test. If line 3 intersexes are of *aB* constitution, and line 4 intersexes are *Ab* or *ab*, then from the majority of the F_1 crosses between lines 3 and 4, the intersexes should be of types 1 and 2 (*AB*). However, from several of these crosses the intersexes were of type $3\frac{1}{2}$, as if line 4 intersexes were of *ab* constitution. At the

present time the author is not in a position to offer another explanation for the segregation of the morphological types of intersexes. The assumption of the presence of two modifying factors will be retained as a working hypothesis until additional experimental data are available. The results of the crosses of lines 1, 3 and 4 with rounded and puffed stocks, which will be discussed later, support this assumption. On the other hand, at the present there is no adequate explanation for the appearance of intersexes of type $3\frac{1}{2}$ in the F_1 from the cross between lines 3 and 4. There is one possibility, however, not very probable, which may explain this inconsistency, namely, that $aaBB$ and $aaBb$ males of line 4 are sterile, so that the line 4 males used in the cross were of $aabb$ constitution. Then from the cross between $aabb \times aaBB$ flies the F_1 intersexual population genotypically is $aaBb$ and phenotypically should be intermediate between intersexes of types 3 and 4.

B. Crosses between lines 4, 3 and 1 and
rounded and puffed stocks

The conflicting data as to the segregation of the different morphological types of intersexes in the crosses described above suggested further tests with different stocks not carrying the ix^m gene. Rounded and puffed, which were used for this purpose, are both dominant genes of the second chromosome. Since the result of the crosses between intersex producing lines and rounded (R) and puffed (Pu) stocks is similar, the data will be considered together.

From the cross between line 4 and R and Pu stocks, 322 intersexes were obtained in the F_2 (table 8a, b, and c). Of these 301 were classified as type

TABLE 8
Summary of the F_2 crosses between R and Pu stocks and lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a $R \text{ } \varnothing \times \text{line 4 } \sigma^7$	24	821	691	195		3	10	182
b $Pu \text{ } \varnothing \times \text{line 4 } \sigma^7$	12	504	425	127			8	119
c	37	1325	1116	322		3	18	301
d $R \text{ } \varnothing \times \text{line 3 } \sigma^7$	11	419	333	102		6	64	32
e $Pu \text{ } \varnothing \times \text{line 3 } \sigma^7$	29	1378	1048	335		25	174	82
f	40	1797	1381	437		31	238	114
g $R \text{ } \varnothing \times \text{line 1 } \sigma^7$	25	1157	960	313	29	149	74	61
h $Pu \text{ } \varnothing \times \text{line 1 } \sigma^7$	21	844	600	145	12	69	35	29
i	46	2001	1560	458	41	218	108	90

4, 18 as type 3, and three as type 2. Since the variability of intersexes in the F_2 of this cross does not exceed that of the line 4 itself, it can be assumed that the F_2 intersexual population morphologically is the same as the parental line 4.

From the cross between line 3 and rounded and puffed stocks 437 intersexes were obtained in F_2 . Of these 31 were classified as belonging to type 2, 238 to type 3, and 114 to type 4 (table 8d, e, and f). Assuming that 31 intersexes of type 2 are variations of the type 3 and grouping them together, there will be 269 intersexes of type 3 and 114 of type 4, which approximates a 3:1 ratio.

The F_2 from the cross between line 1 with *R* and *Pu* stocks gave 458 intersexes (table 8g, h, and i). Of these 41 belonged to type 1, 218 to type 2, 108 to type 3, and 90 to type 4. Considering types 1 and 2 altogether, the intersexes segregated into 259:108:90, which significantly deviated from the expected 9:3:4 ratio. However, since this segregation closely approximates a 9:7 ratio the deviation of the data from a 9:3:4 ratio might be due to faults in the classification of lines 3 and 4.

The result from these crosses seemed to be in agreement with that obtained from intercrosses between lines 1, 3 and 4. The most important point brought out by these crosses is the nature of the ix^m gene itself. At least one modifier is required to produce an intersex of other than the extreme male type (type 4). In other words, when the ix^m gene is not influenced by its specific modifying factors, females homozygous (ix^m/ix^m) become converted into sterile males. Therefore the ix^m gene is the gene for maleness. The masculinizing effect of this gene is so strong that it overrides the 2X:2A mechanism, the balance of which is on the female side.

C. Dominant suppressors of ix^m

In connection with linkage studies, in which the genes *sv*, ix^m , and *G* were involved, it was observed that the intersexual population in those crosses was deficient. The result of these and other similar crosses will now be discussed in detail.

The F_1 females, of $+G/sv\ ix^m+$ genotype, were crossed to *sv* males from the stock segregating for intersexes. It was expected that from these crosses cultures throwing intersexes would segregate either in a 1:1 or a 3:1 ratio of normal females and intersexes. However, out of 56 cultures from this cross, only in two, the ratio of females to intersexes approximated that of 3:1 (table 4). In the rest of the cultures the female to intersex ratio is modified into 7:1, 15:1 and even 31:1. This can be explained on the assumption that the *G* stock used in the cross carries perhaps two or more dominant suppressors of the ix^m gene, each of which alone is capable of nullifying the effect of the ix^m gene. As our observations suggest, the

ix^m/ix^m females homo- or heterozygous for these suppressors become normal females. However, no attempt was made to study the internal organs of these females, nor to test their fertility.

In order to find the linkage relation of these suppressors to the ix^m or to each other, a reciprocal cross was made. The G females were crossed to males from lines 1 and 3. Then F_1 males from these crosses were outcrossed to $v\ mt$ females of line 4. Line 4 females were used in preference to the females of lines 1 and 3 for the following reasons. While records were made for the three point test, it was observed that most of the intersexual population from this cross consisted of an extreme male type. Therefore it was considered desirable to use $v\ mt$ females of line 4 for the test cross so that the males and intersexes could be easily distinguished. From these crosses 609 $v\ mt$ males, 603 females, and 86 wild type intersexes were obtained (table 9). Both males and females from these crosses also segregated for

TABLE 9
Summary of the crosses between $v\ mt$ line 4 females and F_1 males from
the cross G females with males of lines 3 and 1.

F ₁ MALES FROM THE CROSS	NUMBER OF CULTURES	$v\ mt$ AND $v\ mt\ G$ MALES	+ AND G FEMALES	+ INTER- SEXES	MORPHOLOGICAL TYPES			
					1	2	3	4
$G\ \varnothing \times \text{line } 3\ \sigma^7$	9	261	272	38			16	22
$G\ \varnothing \times \text{line } 1\ \sigma^7$	11	348	331	48		5	7	36
	20	609	603	86				

G , but no record was kept of this. However all intersexes were examined for eye color and were found to be of the wild type. Though the number of cultures is small, the results indicate that the female and intersexual population from these crosses segregate mostly into 7:1, and perhaps 15:1 ratios. This would indicate that some of the suppressors present in the G stock might be linked with the third chromosome, or linked together.

Further tests were made between intersex-producing lines and non-related stocks in order to reveal the presence of the ix^m suppressors. It was found that in a number of crosses the ratio between female and intersexual populations was altered from that of 3:1 and 1:1. Only two other crosses, with American wild stock and mt^4 , the data of which are more complete, will be considered here.

The American wild type females were crossed to males from intersex producing lines 4, 3 and 1. Then F_1 males from these crosses were crossed to $v\ mt$ females of line 4. From these crosses altogether 57 cultures were obtained, which gave 1735 $v\ mt$ males, 1675 wild type females and 211 wild type intersexes (table 10). The ratio of males, females and intersexes from

TABLE 10

Summary of the test crosses of F_1 males (from the crosses between American wild type females and the males from intersexual lines 4, 3, and 1) to v mt line 4 females.

F ₁ MALES FROM CROSSES	NUMBER OF CULTURES	v mt MALES	WILD TYPE FEMALES	WILD TYPE INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (+ ♀ × line 4 ♂)	7	232	202	27			2	25
b (+ ♀ × line 3 ♂)	27	815	796	106		5	44	57
c (+ ♀ × line 1 ♂)	23	688	677	78	8	8	21	78
	57	1735	1675	211				

these crosses approximates that of an 8:7:1 ratio, indicating that the wild stock is homozygous for at least one suppressor. From the F_2 cross altogether 45 cultures were obtained which gave 1738 males, 1814 females and 77 intersexes (table 11). The ratio of females to intersexes in these crosses

TABLE 11

Summary of the F_2 crosses between American wild type females and the males of intersexual lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (+ ♀ × line 4 ♂)	8	250	268	14			3	11
b (+ ♀ × line 3 ♂)	17	619	684	23		2	16	5
c (+ ♀ × line 1 ♂)	20	869	862	40	4	9	11	16
	45	1738	1814	77				

on the average is 25:1, though in individual cultures it appears to be modified still further. The result of these crosses indicates that the wild stock probably carried more than one suppressor of ix^m . The exact number cannot be estimated from the data.

The data in regard to the mt^4 stock are less complex and indicate the presence of only a single ix^m suppressor in this stock. The mt^4 females were crossed to males from lines 4, 3 and 1. The F_1 males or females from these crosses were back crossed to line 4 stock. From these crosses altogether 64 cultures were obtained, which gave 2678 males, 2519 females and 319 intersexes (tables 12 and 13). The ratio of females to intersexes approximates that of 7:1. The result of F_2 crosses substantiates the assumption of the presence of only one ix^m suppressor in the mt^4 stock. The 78 F_2 cultures from these crosses gave 3600 males, 3972 females, and 245 intersexes (table 14). The ratio of females and intersexes approaches closely that of 15:1

TABLE 12

Summary of test crosses of F_1 males (from the cross between mt^4 females and the males from intersexual lines 4, 3, and 1) to v mt line 4 females.

F_1 MALES FROM CROSSES	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (mt^4 ♀ × line 4 ♂)	4	113	101	10				10
b (mt^4 ♀ × line 3 ♂)	19	505	526	78		6	40	32
c (mt^4 ♀ × line 1 ♂)	19	553	558	50	2	5	8	35
	42	1171	1185	138				
From Table 13	22	1501	1334	181				
	64	2678	2519	319				

TABLE 13

Summary of test crosses of F_1 females (from the cross between mt^4 females and males from intersexual lines 4, 3, and 1) to v mt line 4 males.

F_1 FEMALES FROM CROSSES	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (mt^4 ♀ × line 4 ♂)	12	941	786	98			13	85
b (mt^4 ♀ × line 3 ♂)	4	168	157	21		1	8	12
c (mt^4 ♀ × line 1 ♂)	6	392	391	62	1		13	48
	22	1501	1334	181				

TABLE 14

Summary of the F_2 crosses between mt^4 females and males of the intersexual lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (mt^4 ♀ × line 4 ♂)	29	1481	1576	91	1		11	79
b (mt^4 ♀ × line 3 ♂)	15	515	634	43			10	33
c (mt^4 ♀ × line 1 ♂)	34	1604	1762	113	5	6	27	75
	78	3600	3972	245				

*Morphology of intersexes in crosses with stocks
homozygous for suppressors*

In the discussion of results of crosses in which suppressors were involved, the morphology of intersexes has not been considered. This merits special consideration. In table 15 the results of crosses between lines 4, 3 and 1 with wild and mt^4 stocks are summarized. The F_2 intersexual population

from the cross between line 4 and wild and mt^4 stocks is mostly of the extreme male type 4 (table 15a and b). This is in agreement with the results of crosses between line 4 and the other stocks free of ix^m suppressors, as it has been described above. The F_2 intersexes from the cross between

TABLE 15
Summary of the F_2 cross between lines 4, 3, and 1 and wild and mt^4 stocks.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
Line 4								
a $\times$ wild	8	250	268	14			3	11
b $\times mt^4$	29	1481	1576	91	1		11	79
					1		14	90
Line 3								
c $\times$ wild	17	619	684	23		2	16	5
d $\times mt^4$	15	515	634	43			10	33
						2	26	38
Line 1								
e $\times$ wild	20	869	862	40	4	9	11	16
f $\times mt^4$	34	1604	1762	113	5	6	27	75
					9	15	38	91

line 3 and wild and mt^4 stocks consisted of two intersexes of type 2, 26 of type 3, and 38 of type 4 (table 15c and d). The predominance of type 4 intersexes over those of type 3 can be easily accounted for, as it has been shown, there is probably one gene difference between lines 3 and 4; dominance of one type over the other is lacking. The intersexes heterozygous for this modifier are morphologically intermediate between types 3 and 4. In some crosses these intersexes morphologically are closer to type 3 and are classified with them. In these crosses some of the intermediate types morphologically are closer to type 4 and are classified together with this type. But the F_2 intersexual population from the crosses between line 1 and wild and mt^4 stocks is quite different from that when line 1 was crossed to stocks free from suppressors. The F_2 intersexual population from the cross between line 1 and R and Pu stocks segregated into a 9:3:4 ratio of types 1 and 2 (taken collectively), 3 and 4, respectively. Whereas in the F_2 from the crosses of line 1 with wild and mt^4 stocks the situation is quite different. Out of 153 intersexes from these crosses only nine belonged to type 1, 15 to type 2, and the majority of the intersexes were of types 3 and 4 (38 of type 3 and 91 of type 4), (table 15e and f). The segre-

gation of F_2 intersexes from these crosses is almost in reverse order from that found in the F_2 from the cross between line 1 and R and Pu stocks.

The segregation of the intersexual population in the test cross from the matings in which suppressors were involved correspond to the result obtained in the F_2 from these matings. As was stated before, F_1 males from the cross between lines 4, 3 and 1 with wild and mt^4 stocks were outcrossed to $v\ mt$ females from the line 4. This was considered desirable in view of the fact that a majority of the intersexes from such crosses were of the extreme male type. The introduction of the sex-linked genes permitted unmistakable separation of normal males from male-like intersexes. The summary of the results of these and reciprocal crosses are given in table 16. The test

TABLE 16

Summary of the test crosses. The wild and mt^4 stocks were crosses to intersexual lines 4, 3, and 1. The F_1 flies from these crosses were outcrossed to line 4.

F ₁ PARENT USED IN CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER- SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a F_1 (wild $\times$ line 4)	7	232	202	27			2	25
b F_1 (mt^4 $\times$ line 4)	16	1045	887	108			13	95
							15	120
c F_1 (wild $\times$ line 3)	27	815	796	106		5	44	57
d F_1 (mt^4 $\times$ line 3)	23	673	683	99		7	48	44
						12	92	101
e F_1 (wild $\times$ line 1)	23	688	677	78		8	21	49
f F_1 (mt^4 $\times$ line 1)	25	945	919	112	3	5	21	83
					3	13	42	132

cross population from the crosses in which lines 4 and 3 were involved segregated in a manner similar to that found from the crosses of these lines and stocks free from suppressors (table 16 a-d). The test cross intersexual population in which line 1 was involved again is exceptional, as it was in the case of the F_2 intersexes. Out of 190 intersexes obtained from the test-cross, only three belonged to type 1, 13 to type 2, 42 to type 3, and 132 to type 4 (table 16 e and f). Again type 4 predominates, which is contrary to expectation.

The predominance of the type 4 intersexes has also been observed in the progeny of crosses between intersexual lines and other stocks carrying ix^m suppressors. However, accurate records as to the morphology of the intersexual population from these crosses have not been taken. At the present the author has no adequate explanation for the predominance of

the male type of intersexes over those of the female-like and hermaphrodites in the progeny from these crosses. Further research undoubtedly would be desirable.

VI. DISCUSSION

The intersexes in *Drosophila virilis* have an entirely normal chromosomal constitution, and therefore may be called diploid intersexes. The intersexuality in this species is the result of the interaction of the mutant autosomal gene ix^m and its modifiers with the rest of the 2X:2A chromosomal complex. The females homozygous for ix^m and lacking modifiers become sterile males. The presence of one or more specific modifiers in the ix^m/ix^m females results in incomplete reversion and various degrees of hermaphroditism. The addition to the genotype of these individuals of one or more suppressors inhibits the action of the ix^m gene and its modifiers, resulting in the development of normal females. Also, 2X:2A individuals develop into normal females when they are heterozygous for ix^m ($ix^m/+$) irrespective of their constitution in regard to the ix^m modifiers. The 1X:2A individuals are not affected either by the ix^m gene or by its modifiers.

The nature of the ix^m gene and its normal allele, and their possible significance in the sex-determining mechanism has been discussed (LEBEDEF 1938b) and need not be repeated here. It should be pointed out that these considerations are purely hypothetical. It is hoped that further research with these intersexes may contribute to our understanding of the mechanism of sex determination. However, even at the present stage of our knowledge of these intersexes it might be assumed that in the sex-determining mechanism of *Drosophila* the main male (*M*) sex gene is present. The indirect evidence from *D. melanogaster* suggests the presence of the main female (*F*) sex gene (PATTERSON, STONE and BEDICHEK 1937). The interaction or the balance of these genes in the sex determination is subjected to the effect of their specific modifiers, the number of which is probably large.

ACKNOWLEDGMENTS

The present work was started while the author was an associate in the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York. To that Institution, and to DR. M. DEMEREC, in whose laboratory this work started, the author is greatly obliged. The author also wishes to acknowledge his obligations to PROFESSORS R. A. EMERSON, O. A. JOHANNSEN, and L. W. SHARP of Cornell University for facilities provided, and for helpful suggestions and criticism. At various times the author has had the privilege of discussing this problem with the late DR. C. B. BRIDGES and PROFESSOR R. GOLDSCHMIDT. To them the author is thankful for their kindly interest and advice. The author also is very

grateful to DR. C. W. METZ of the Carnegie Institution, who has read the manuscript and made valuable suggestions.

SUMMARY

Diploid intersexuality in *Drosophila virilis* is described in detail.

The third chromosome gene (ix^m), whose locus on the chromosome is known, converts homozygous females (ix^m/ix^m) into sterile males. The gene has no effect on males, and the cross between $ix^m/+ \text{♀}$ and $ix^m/ix^m \text{♂}$ produces males, females and intersexes in the ratio of 2:1:1. The cross of $ix^m/+ \text{♀} \times ix^m/+ \text{♂}$ produces males, females and intersexes in the ratio of 4:3:1.

However, these ratios are often modified, when intersex producing stocks are outcrossed to unrelated stocks, by the presence in these stocks of one or more dominant suppressors of the ix^m gene.

Two or more modifiers delay the transformation of the ix^m/ix^m females into males, resulting in various degrees of intersexuality. The genetical evidence suggests that these modifiers are incompletely dominant.

Morphologically intersexes vary from female-like to nearly male-like. The specimens intermediate between these two extremes possess well developed female and male sexual characteristics, including external and internal sexual organs, and gonads.

Genetical and morphological studies suggest that intersexes of all morphological types start their development as females and then from a certain time, known as the turning-point, continue to develop in a male direction. The development of the male sexual system, however, does not interfere with that of the female, resulting in the development of hermaphrodites of various degrees.

The gonads in all morphological types of intersexes start their development as ovaries. In the female-like intersexes ovaries continue their development, though it is retarded in various degrees. In hermaphrodites after the occurrence of the "turning-point" ovaries are transformed into ovotestes. In the male-like type of intersex ovaries are gradually transformed into testis-like organs.

During the transformation of ovaries into ovotestes or into testis-like organs, the female germinal cells are gradually transformed into spermatocyte-like cells, some of which eventually disintegrate.

The intersexes of all morphological types are sterile since gametogenesis is abortive. Meiosis has not been observed in the gonads of intersexes.

LITERATURE CITED

- BRIDGES, C. B., 1932 The genetics of sex in *Drosophila*. Sex and Internal Secretions, pp. 55-93.
(Contains references to all earlier papers on intersexuality.)
CHINO, M., 1936 The genetics of *Drosophila virilis*. Jap. Jour Genetics 12: 189-210; 257-277.

- DOBZHANSKY, Th., 1931 Interaction between female and male parts in gynandromorphs of *Drosophila simulans*. Arch. EntwMech. Org. **123**: 719.
- DOBZHANSKY, Th., and BRIDGES, C. B., 1928 The reproductive system of triploid intersexes in *Drosophila melanogaster*. Am. Nat. **62**: 425-434.
- GOLDSCHMIDT, R., 1934 Lymantria. Bibliogr. Genet. **11**: 1-185. (Contains references to all earlier papers.)
- GUYENOT, E., and NAVILLE, A., 1933 Les premiers phases de l'ovogénèse de *Drosophila melanogaster*. Cellule **42**: 213-230.
- KERKIS, J., 1934 On the mechanism of development of triploid intersexuality in *Drosophila melanogaster*. C. R. Acad. Sci. U.S.S.R. **3**: 288-294.
- KOSMINSKY, P., 1930 Untersuchungen über Intersexualität bei *Lymantria dispar*. L. Russ. Zool. Jour. **10**: 1-50.
- LEBEDEFF, G. A., 1934a A gene for intersexuality in *Drosophila virilis*. Am. Nat. **68**: 68-69 (abstract).
- 1934b Genetics of hermaphroditism in *Drosophila virilis*. Proc. Nat. Acad. Sci. **20**: 613-616.
- 1938a The nature of intersexuality in *Drosophila virilis*. Genetics **23**: 156 (abstract).
- 1938b Intersexuality in *Drosophila virilis* and its bearing on sex determination. Proc. Nat. Acad. Sci. **24**: 165-172.
- METZ, C. W., 1926 Observations on spermatogenesis in *Drosophila*. Z. Zellf. mik. Anat. **4**: 1-28.
- PATTERSON, J. T., STONE, W., and BEDICHEK, S., 1937 Further studies on X-chromosome balance in *Drosophila*. Genetics **20**: 259-279.
- SCHULTZ, J., and DOBZHANSKY, Th., 1933 Triploid hybrids between *Drosophila melanogaster* and *Drosophila simulans*. Jour. Exp. Zool. **65**: 73-82.
- STURTEVANT, A. H., 1921 Genetic studies on *Drosophila simulans*. III. Autosomal genes. General discussion. Genetics **6**: 179-207.