MULTIPLE SEX-GENES IN *DROSOPHILA*?—A CRITIQUE.

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(With One Text-figure.)

Sex is determined by a quantitative relation or balance between female and male sex-determining genes; the mechanism of the sex-chromosomes, which carry only one of these types of determiners, takes care of the proper balance in favour of one or the other. This theory which was developed by Goldschmidt between 1911 and 1920, and exhaustively proved by the analysis of intersexuality, has since been found to cover all cases as well in plants as in animals, and each new contribution shows its universal application. The theory was derived from the analysis of a phenomenon which in our first papers (1911–14) was considered as a special case of gynandromorphism. Later it was realised that a new and different phenomenon had been discovered for which I introduced in 1915 the terms intersexuality and intersexes. The analysis of the original *Lymantria* case pointed to these sexual determiners being single genes or completely linked genes, and all later work with this material has supported this view. Even in pre-Mendelian times another type of sexual abnormality following species crosses between moths had been discovered by Standfuss, who in 1914 reverted to these facts, realising that Goldschmidt's theory of the balance of sex-genes, together with Federley's proof of diploid gametes in F_1 of such crosses, furnished the clue to his experiments. Without using the term triploid intersexes he correctly interpreted his intersexual moths as such, and gave also the correct interpretation of the case by genic balance. This paper, published in a small entomological journal, remained unknown nor probably would it have been understood because Standfuss still used the cumbersome symbols which Goldschmidt had introduced in his first papers. Thus the phenomenon of triploid intersexuality only became widely known when Bridges rediscovered it in 1920 for *Drosophila*. Here a visible demonstration of the quantitative relation of the sex-genes was found instead of the same relation, which, in *Lymantria*, was accessible only through logical reasoning and therefore difficult to grasp. Bridges indeed accepted the then well-established idea of the balance of sex-genes in and outside of the *X*-chromosomes in explanation of normal sex determination, and

the upsetting of this balance in explanation of intersexuality, demonstrating its correctness by the visible relation of the numbers of autosomes and sex-chromosomes carrying these genes. But Bridges went further and, instead of accepting the view of single (or completely linked) sex-genes, assumed that throughout the chromosomes are distributed numerous genes which pull sex towards maleness or femaleness. Within the autosomes (in *Drosophila*) the majority of these modifiers are supposed to pull towards maleness; within the *X*-chromosomes, however, towards femaleness; and the net result is that expected on the principle of balance.

On *a priori* grounds this idea of the numerous plus and minus modifiers, which had grown out of the discovery of such modifiers for somatic characters, was not very attractive, since it constituted a rather poor mechanism for so constant a thing as sex determination. But it is of course experimental evidence which counts. Dobzhansky and Schultz set out to furnish this by means of very ingenious experiments using the highly developed technique of duplications and translocations in *Drosophila*. They claim to have succeeded in establishing experimentally the correctness of Bridges' view of the existence of many different sex modifiers, mostly female but some also male, within the *X*-chromosome. The question seems important enough to warrant a closer inspection of their data, which, to me, have a very different significance from that attributed to them by the authors.

Dobzhansky and Schultz offer two different main sets of facts. The first set gives the results of adding different pieces of *X*-chromosome, differing in length as well as in the loci involved, to triploid intersexes, *i.e.* individuals containing $3n$ autosomes and $2X$-chromosomes. Thus individuals are produced with an *X*-chromosome content intermediate between intersexes ($3A$, $2X$) and normal triploid females ($3A$, $3X$). If the pieces of the *X*-chromosome in question contain female determiners, and if their number corresponds roughly to the length of the fragment, a more and more marked shift towards femaleness is to be expected with the addition of more and more of the *X* fragments.

The second set of facts relates to diploid females ($2A$, $2X$) and males ($2A$, $1X$) which contain in addition the same fragments of *X*-chromosomes, or which, in the case of females, lack such a fragment in one of the chromosomes.

Clearly the second set of facts is the more important one. It has been known for a very long time in the *Lymantria* work that the degree of intersexuality may be shifted by certain modifying factors, or by the

action of temperature. The diploid *Lymantria* intersexes are otherwise rather constant in degree; but it has been known for all triploid intersexes since Standfuss (Bridges-Dobzhansky, Goldschmidt-Pariser, Harrison, Meisenheimer, Seiler) that their degree is extremely variable. As a matter of fact Dobzhansky had shown for *Drosophila* intersexes that it is possible to shift the degree considerably by selection (of modifiers) or even by external agencies without altering the chromosome set. This makes us extremely sceptical towards experiments in which the degree of intersexuality within triploids is shifted. The addition of fragments may mean an addition of modifiers of the same type as those selected in the former experiments. A positive effect therefore in both cases allows only of the conclusion, that the degree of intersexuality in the unbalanced triploid intersex is genetically modifiable. But to conclude that sexual determiners are here at work of the same type as those primarily responsible for sex is in my (and if I understand correctly also Punnett's) opinion an unwarranted conclusion for which the burden of proof falls upon the authors. We shall presently return to this point, but may here emphasise that this part of the analysis is of most doubtful value, whatever the actual results. This conclusion is strongly supported by the results of a recent paper by Lebedeff on hermaphroditism in *Drosophila virilis*. Here it is proven beyond doubt that a single mutated recessive gene in the third chromosome changes the sexual balance in favour of maleness; but the amount of this change is controlled by additional modifiers. This forms an exact parallel to the *Lymantria* case, except that no different multiple allels of the main gene have yet appeared in *Drosophila*. Of course this case does not touch directly the question of a female gene in the *X*-chromosome; but it might concern the male determiner in an autosome, which may be identical with Lebedeff's mutant gene *ix*, which, in this case, would have to be called *M* weak. It may be added that the well-known work on dioecious maize leads to the same conclusion.

With the second set of facts the situation is different. Here we have the diploid male with one *X* and the female with two *X*, in neither of which can the sex be shifted by selection of modifiers or external agencies. If the one sex-gene theory is correct, addition of fragments to the male set can only leave a male or produce a female, though both may be abnormal or unviable as a consequence of hyper- or hypoploidy. If Bridges' view is correct, a series of regular intersexes must be produced by this method. Therefore let us review first critically this decisive second set of facts, which according to Dobzhansky and Schultz also gave

positive results. The following facts have been considered by the authors as favourable:

(1) Diploid females carrying an additional piece of *X*-chromosome are intermediate between females and superfemales, and the degree of change towards the type of superfemales is proportional to the size of the added fragment. Here the authors have fallen victims to their own terminology. $2A + 3X$ females have been called superfemales by Bridges. Of course such hyperfeminine females must exist if the balance view is correct. This holds as well for *Lymantria* (no chromosome change), where this has been stated a long time ago, as for *Drosophila* with chromosomal abnormality. In *Lymantria* such animals cannot be distinguished; in *Drosophila* this is possible, *because a hyperploid individual has certain abnormalities in different parts of the body. Such abnormalities, however, have nothing to do with the hyperfeminine sex constitution, but are the effect of hyperploidy.* Thus, the superfemales are sterile and have abnormal ovaries, which certainly are no hyperfeminine traits. The more hyperploid, the more abnormal are the females, but what has this to do with sex? Nothing but the term superfemale, in this connection unfortunate.

(2) A very similar argument is the following. Two fragments belonging to the translocation X–IV 1 were added to normal females, fragments which together form a whole *X*-chromosome. An eventual individual sex-gene therefore must be contained in one or the other fragment. Superfemales with $3X$ are sterile and therefore, conclude Dobzhansky and Schultz, the addition of one or the other of the pieces, namely the one with the supposed sex-gene, must cause sterility which is not the case. The starting point of this argumentation is the idea that superfemales are sterile because they are hyperfeminine! They are sterile of course, because they are hyperploid. (The same argument is also applied to intersexes plus these fragments. The answer is the same.)

(3) Two cases of females with a deficiency in one *X*-chromosome are mentioned. This is the yellow-scute deficiency, which in intersexes shifts the type towards maleness, but has no sex effect in diploid females, though it ought to make them slightly intersexual under the authors' conception. Females carrying a deficiency for *v-m* were again normal. But whereas both deficiencies are rather small no great importance may be attached to these two cases beyond their proving that no single sex-gene is found in the region in question.

(4) The most important group of individuals are the diploid males with the addition of an *X* fragment. According to the authors' theory

they ought to show many types of intersexuality corresponding to the size of the extra piece. Let us then pass in review these cases. There are first five cases of addition of a fragment from the left end of the *X*-chromosome of different length. They are all without sex effect though the same duplications are supposed to have a definite effect on intersexes. The authors will of course claim that these fragments are too small to shift sex beyond the epistatic minimum in diploids. The cases then may be dismissed as neutral. There is another group of males containing a duplication of the left end to prune (*Pn*) and, in addition, the whole right end from fused. These considerably hyperploid males are but slightly viable and sterile, but not intersexual. The authors claim that the added piece does not contain sufficient female modifiers to pass the epistatic minimum; yet the same duplication contained enough female modifiers to shift the triploid intersexes to the extreme class VI (practically females, sometimes fertile). It is especially emphasised by the authors how strong the female influence of this duplication is; nevertheless, it has no sex effect in the male! Now follows the case of a duplication consisting of about half of an *X*-chromosome and containing the right end including rudimentary. These males, if they survive, are sterile and sexually normal, which was the case with "several hundreds inspected". Among these "several hundreds" two (!) were found with one of the anal plates broken transversely. Since this has never been seen in normal males (?) but is found in intersexes, it is considered to be a sign of intersexuality. From corresponding cases in *Lymantria* it is well known that comparable small abnormalities of the armature are to be considered as malformations due to inhibition and may be produced by action of low temperature (Kosminsky, Goldschmidt). I am therefore not able to accept these few males, less than 1 per cent., with a minute abnormality on one side of the armature as proof of the production of intersexuality, when so much depends theoretically upon the validity of the proof. Until better proofs are furnished the facts are that even this large addition (which again shifts sex in triploid intersexes towards femaleness) does not produce intersexes. It should be noted that, according to the authors, the female influence of this duplication in the triploid intersexes is less strong than in the former case. But in spite of the authors' idea that more female modifiers are needed to make a diploid intersexual than to shift the grade of a triploid intersex, the effect on the diploid is here interpreted as the stronger one!

Another fragment derived from Mr Bonnier's translocation contains the left end including ruby. Diploid males carrying this are almost

inviable, but those surviving were sexually completely normal! Here again a feminising effect upon triploid intersexes was found.

Finally we have the case of an addition of the *v-m* region to diploid males. In one experiment they were all sexually normal; in this case no *Y*-chromosome was present. In a second experiment out of 165, 55 were normal, 11 had abnormal genitalia which are described as similar to those of intersexes. In addition 99 are tabulated as class III intersexes. But the text explicitly states that class III intersexes are very different, and that the peculiarity of these individuals is what I should call a malformation due to inhibition, which, according to the authors, is also found in normal males. The case therefore stands and falls with the 11 aforementioned individuals. Would not a closer study reveal these also as non-intersexual monstrosities caused by hyperploidy?

I think that this decisive set of experiments, admirably conducted by the authors, gives indeed very meagre results: they are absolutely negative in regard to the multiple modifiers with the only exception of 2+11 very doubtful individuals among a majority of normals.

We may now ask what are the consequences of the assumption of one locus for the female gene in the *X*-chromosome of *Drosophila* for the outcome of such experiments? A diploid male plus a fragment containing this locus is a genetic female, but it will not be viable because of the extensive deficiency, excepting the case where almost a whole chromosome has been added. A diploid female plus the same fragment would not be distinguishable from another hyperploid female, because the superfemale phenotype has nothing to do with sex. A diploid female minus the fragment would be a hyperploid male, which is only viable if the fragment of the second *X*-chromosome is relatively small. A triploid intersex plus the section containing the sex-gene would be genetically a triploid female but one with a considerable deficiency, and therefore either not viable or in some respects abnormal, perhaps sterile. This enumeration shows how difficult an actual proof of the one-gene concept is in experiments using duplications, the decisive points being mostly negative.

After the authors' experiments we may, however, exclude certain regions as possible seats of a sex-gene, namely all those which added to a diploid male leave it a viable male. These are the regions *y-pn r-bb*. Further, regions which as deficiencies in diploid females leave them viable females may be excluded. As such the *v-m* region has to be added. In looking for a possible locus for one sex-gene we have to exclude these regions and to look for cases in which diploid males plus the fragment

do not occur, and intersexes plus the same fragment are either not viable or normal females with hypoploid phenotype. Five such types of males have been registered by Dobzhansky and Schultz: (1) *X+cv-bb* Dupl., (2) *X+y-lz* Dupl., (3) *X+v-bb* Dupl., (4) *X+y-m* Dupl., (5) *X+s-car* Dupl. Triploid "intersexes" plus the same fragments are in case (1) like triploid females, many fertile, but with some abnormal somatic characters. They are classified as class VI intersexes. But there is nothing in the description of this class which would explain why these animals could not also be considered as real females with some somatic abnormalities caused by hypoploidy. In case (2) they belong again to this same class VI, are like females with insufficiently developed ovaries and somatically very abnormal. But we know that so-called superfemales, *i.e.* abnormal hyperploids, also have rudimentary ovaries and are sterile; yet they are certainly not to be classed as intersexes. In case (3) again, we have the same class VI and less abnormal. One of 65 individuals has sex-combs and this one individual alone may possibly be regarded as an intersex. All the others of this class VI are more or less underdeveloped females which are considered as very female-like intersexes, but without proof that their characteristics have anything to do with intersexuality. In case (4) also occur class VI "intersexes" to which the foregoing remarks may be applied. Case (5), mostly inviable, though a few weak individuals of the same type.

It is worth while to look at these facts with the aid of a standard map of the first chromosome. This shows that three of the fragments have in common the region *cv-lz*, while three others have in common the region *s-r*. All of these five so different fragments have practically the same effect when added to $3A$, $2X$. Is it then not safer to conclude that the facts disprove the multiple modifier concept and suggest that the regions *s-cr* or *cv-lz* contain the sex-gene?

We conclude that: (1) Dobzhansky and Schultz have in their laborious and ingenious work not succeeded in proving the theory of multiple sex modifiers. Shifting of grade of intersexuality by whatever means does not prove anything. (2) The argument derived from hyperploid females is based on a misconception of the phenotype of so-called superfemales. (3) The argument based upon hyperploid males rests on extremely indecisive evidence, in fact on a few abnormal individuals among a large number of normals. (4) The facts do not only not afford a proof of the theory but they tell definitely against it on critical scrutiny.

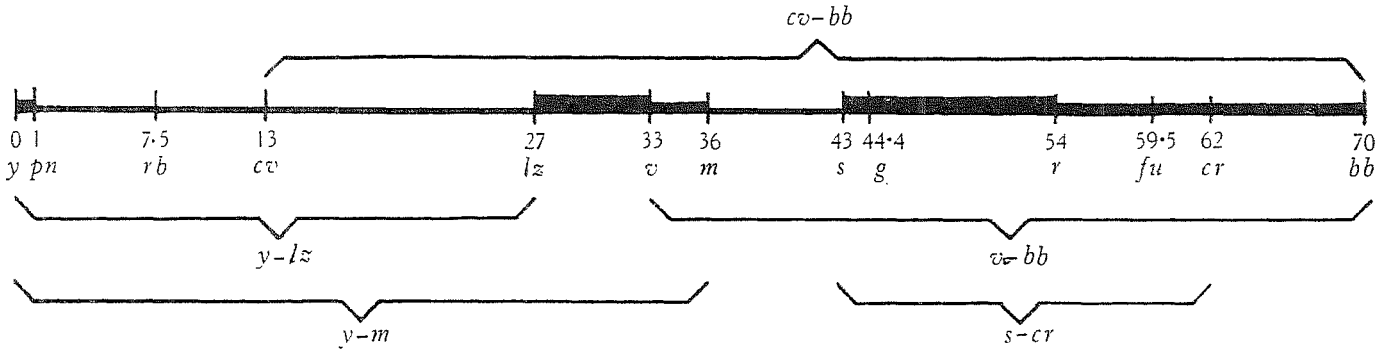

Fig. 1. Standard map of the X-chromosome of *Drosophila melanogaster*. Sections shown in double thickness are those from which single sex-genes are excluded; those shown in treble thickness are suspect sections (cf. text). *bb* = bobbed, *cr* = carnation, *cv* = crossveinless, *fu* = fused, *g* = garnet, *lz* = lozenge, *m* = miniature, *pn* = prune, *r* = rudimentary, *rb* = ruby, *s* = sable, *v* = vermilion, *y* = yellow.

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