A GENE FOR THE FOURTH CHROMOSOME OF DROSOPHILA

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Drosophila ampelophila contains two pairs of long 'autosomes' (chromosomes other than sex-chromosomes) and one pair of minute 'autosomes'; there are, in addition, a pair of long sex-chromosomes alike in the female ('X's) but unlike in the male ('X' and 'Y').[1] Correspondingly, breeding tests of the numerous mutants of Drosophila have revealed a great group of genes (containing over thirty members) which are sex-linked in such a way that they are distributed, in the reduction division, to exactly the same cells as is the X-chromosome, and two other great groups (with about twenty members each) which are not sex-linked and so have been considered to follow in their distribution two of the pairs of autosomes (presumably the long ones). The genes in these three groups are therefore said to lie in Chromosomes I, II and III, respectively. The members of any group of genes are linked with one another; in the female the linkage is partial and is of the linear type to be expected on the basis of the chiasmatype theory of Janssens and Morgan; in the male the linkage is complete (no crossing-over). All the members of one group assort independently of the members of either of the other groups, in both sexes, as would be expected on the generally accepted postulate of the random assortment of non-homologous chromosomes in the reduction division.

[1] Two lines of recent cytological investigation, the one followed by Mr. C. W. Metz, the other by Mr. C. B. Bridges, have given evidence (not yet fully published) which goes far towards refuting the view suggested by Stevens that the so-called 'X' chromosome of Drosophila actually consists of a short autosome with the real X-chromosome joined to it and that the 'Y' chromosome represents this short autosome without any 'X' attached.

It will be seen that the above grouping of genes leaves one pair of autosomes (presumably the small ones) and the Y-chromosome without any genes to correspond. As regards the Y, it seems at first sight surprising that no mutations have been found involving genes which follow it in their distribution (i.e., genes which are always transmitted from father to son), for it is of about the same length as the X, in which over thirty genes are known. This fact would force us to one of three conclusions: (1) Either the genes connected with the Y-chromosome for some reason do not mutate; or (2) these mutations are all recessive to dominant normal allelomorphs present in X (in spite of the fact that mutations in X are not dominated, conversely, by allelomorphs in Y); or (3), as Mr. C. W. Metz first suggested, genes are degenerate or entirely absent in the Y-chromosome. The first conclusion is *a priori* unlikely; the third may now be supported by a number of considerations, which it will be of interest to discuss briefly in this connection, before proceeding to our account of the fourth chromosome.

In the first place, the Y-chromosome is known to vary greatly in size and number in closely related species of animals. Secondly, as Mr. C. B. Bridges has recently shown, a female occasionally, owing to an abnormal reduction division ('non-disjunction'), receives a Y-chromosome in addition to the two X's, yet such a female is indistinguishable from the ordinary form, which contains no Y. The Y-chromosome therefore either contains no genes or else only genes which are allelomorphic to those in X, but never dominant to them. This is proved also by the fact that mosaic flies sometimes develop, of which a part of the body is female but another part is male, owing to the accidental loss of one of the X-chromosomes in an embryonic cell-division. These male parts must have an X, but no Y, and yet they are indistinguishable from corresponding parts on real males which contain a Y. Furthermore, Mr. C. B. Bridges has obtained (again by 'non-disjunction') males which must have two Y's, yet these also show no peculiarities. It is therefore certain that Y either contains no genes at all which have an effect upon the individual or it contains genes allelomorphic to those in X. It can be proved, however, that if it contains allelomorphs to the

genes in X, these are not normal allelomorphs (i.e., the same kind as those present in the X of the wild fly) for they are recessive to mutant genes in X to which the normal genes in X are dominant, i.e., a male always manifests all the genes, mutant or normal, recessive or dominant, that are present in its single X-chromosome. In other words, if there be genes in Y allelomorphic to those in X, they are abnormal allelomorphs of those in X, and are always recessive to all genes in X. It is difficult to conceive why genes in Y should be recessive, on the one hand, to normal genes in X, in those cases where the mutant genes in X are dominant to the normal, and, on the other hand, to mutant genes in X, in those more frequent cases where the normal genes in X are dominant to the mutant, unless the genes in Y are mere 'absences' or nearly so.[2]

There is an *a priori* explanation for this lack of genes, or lack of dominant genes, in the Y-chromosome, an explanation in the development of which Dr. A. H. Sturtevant has coöperated with me. Owing to the fact that crossing-over never occurs in the male Drosophila, any mutation which originally occurred in Y remained in that chromosome and was never exchanged for a normal gene from X. Furthermore, these mutations in Y which were recessive would not have been subject to the action of natural selection for, since the normal gene in the X-chromosome will dominate over them, individuals containing them will not be abnormal. In the course of time, therefore, recessive changes in the Y-chromosome will tend to accumulate. If, now, we assume that mutations sometimes consist in losses[3] of genes, a degeneration of the Y-chromosome (so far as its *genes* are concerned) would result.

[2] This might be otherwise explained on the very improbable hypothesis that all of the mutations which occurred in X were in a restricted part (the end?) of the chromosome, and that the Y lacked only this part of the chromosome and so appeared to contain genes recessive to all those in X which underwent mutation, although it actually contained normal genes in the other part of the chromosome, which never mutated. This is not quite equivalent, although nearly so, to the idea that X is attached to an autosome (see footnote 1).

[3] By losses of genes are meant not necessarily their total bodily disappearance (losses of loci from the chromatin) but also any changes in them whereby they are rendered permanently inactive and incapable, under any circumstances, of exerting an influence on the organism.

This is because most losses of genes, if they occurred, would be recessive (i.e., one 'dose' of a factor usually has the same effect as two), as indicated by the fact that the one-X part of a mosaic is like the XX part in respect to all characters affected by X except the sex characters (provided the two X's of the XX part are alike). Now, if, as seems likely, recessive losses are more apt to occur than recessive additions of genes (since one 'dose' usually has the same effect as two) the Y-chromosome will gradually become functionless. On *a priori* grounds, we should expect such a fate, in any species, for the sex-chromosome peculiar to the heterozygous sex, if crossing-over never occurs between it and its homologue.[4]

These considerations will also explain the size difference which often exists between X and Y, the apparent unimportance of supernumerary Y-chromosomes in development, when these occur, and other irregularities of the Y-chromosome. Hitherto it has been a mystery why the difference merely in the sex factor or factors contained in the sex chromosomes should often be correlated with such a large difference between the two chromosomes, whereas differences in respect to other factors did not involve any visible size differences in the chromosomes concerned, which presumably contain hundreds of genes.

There is, therefore, all things considered, no cause for surprise in the fact that no mutations have occurred involving a group of genes transmitted only from father to son, and thus following in their distribution the Y-chromosome. The chief gap, if it may be so termed, then remaining in the parallelism between the configuration of the chromosomes and the distribution of genes in Drosophila has been due to the fact that no genes were found in a fourth independent group (a third independent non-sex-linked group) to correspond with the fact that there are three pairs of autosomes.

[4] Conversely, where this chromosome appears degenerate or different genetically from its homologue (in addition to the difference in the sex factors) we should expect to find no crossing-over between the two sex-chromosomes in the heterozygous sex, i.e., complete, not partial, sex-linkage. This argument applies to an known cases of sex-linkage, for in all these cases a recessive mutant factor call manifest itself in the heterozygous sex, proving that the sex-chromosome peculiar to the heterozygous sex is different from the other sex-chromosomes in that it contains no dominant normal allelomorph of the mutant factor.

Supposing, however, that mutations are equally likely to occur at any locus in the chromatin, it could be explained as a result of pure chance that no mutations had as yet happened to lie in the restricted space of the small autosome. It was expected, nevertheless, that mutations in a fourth group would eventually be found, and such a mutation has now in fact arisen.

The new character is a recessive wing and leg abnormality, the wings being held out from the body but bent backwards near the base, and the metatarsal joint of the legs being frequently greatly shortened and thickened. The wing is also apt to be curved, with the dorsal surface convex, and shortened. The character varies somewhat, but there is very rarely any difficulty in distinguishing it from the normal form, unless the flies have been raised in very dry bottles. Drought therefore hinders the manifestation of this character, as it may also, and to a greater degree, in some way hinder the development of the character 'abnormal abdomen,' a case reported by Morgan.

I found the mutation 'bent wing' in a race with bifid wings and vermilion barred eyes, all three of these characters being in Chromosome I (sex-linked). A cross of bifid vermilion barred bent male by a pink-eyed female (pink is in Chromosome III) gave in F_1 all the males normal, and the females also normal except in respect to barred, which is dominant; in the next generation, F_2, all combinations of the factors concerned appeared. If bent had been in Chromosome III, no crossing-over would have occurred between bent and pink in the F_1 *male*, consequently, no pink bent spermatozoa could have been formed, and thus (since pink and bent are both recessive) no pink bent F_2 individuals could have been produced. Since these were produced, bent did not lie in the *third* chromosome. Similarly, if bent had been in the first (X) chromosome or in the Y, no crossing-over between it and the sex-factor would have taken place in the P_1 or F_1 male, and consequently no bent females could have been produced in F_2. As bent females were produced, bent did not lie in the *first* (X) chromosome, or in the Y.

A bent barred eyed F_2 male was then crossed to a female containing three mutant genes in the *second* chromosome, namely,

the genes for black body color, purple eyes, and curved wings. Here, too, some flies were obtained in F_2 which showed the characters of both grandparents at once (i.e., were both bent, and black, purple, and curved, and sometimes also barred). This proved that bent did not lie in Chromosome II. The details of the count are shown in table 1.

TABLE 1

Not bent

WILD TYPE	BLACK PURPLE CURVED	BLACK	PURPLE CURVED	CURVED	BLACK PURPLE
84	30	1	3	6	5
		Bent			
43	9	1	1		

The number of barred and non-barred, also of males and females, in each class of moderately large size, were approximately equal. The linkage manifested between black, purple, and curved corresponds as closely with expectation based on the previous linkage results of Bridges, as could be demanded for the small numbers involved. The determination of curved was at times uncertain, owing to the tendency of bent to curve too, and the determination of purple in eyes which were barred was also sometimes uncertain. But as far as the results go, they show that bent is independent of black, purple, curved, barred, and sex.

As no counts had been made in the cross with pink, and few were obtained in the cross with black purple curved, it was still conceivable that although bent was not absolutely linked, in the male, with the members of one of the three previously known groups, as has always been found to be the case with other genes, still it might perhaps be partially linked, in either or both sexes. New crosses were therefore made with the object of securing accurate counts. Some bent males descended from F_2 of the cross with pink were mated to black pink females. These males were found to be heterozygous for pink, as half of the F_1 flies were pink (although none were black or bent). The red-eyed F_1 flies were

mated together, to show the distribution of bent with respect to all three previously known chromosomes. The pinks were also mated together. The latter cross should show the distribution of bent with respect to Chromosomes I and II only, as all the flies were homozygous for pink, the mutant gene in Chromosome III.

The composition of the pink flies used as parents, with the paternal and maternal genes which they contained, was as follows:

F_1 male	Paternal genes —	Gray	Pink	Bent
	Maternal genes sex	Black	Pink	Straight
F_1 female	Paternal genes sex	Gray	Pink	Bent
	Maternal genes sex	Black	Pink	Straight

The count of offspring of this cross of pink flies resulted as shown in table 2.

TABLE 2

All individuals pink

		WILD TYPE	BLACK	BENT	BLACK BENT
F^2 numbers observed	females..	83	30	24	8
	males....	77	24	31	9
	Total....	160	54	55	17
Total numbers expected on independence		162	54	54	18

Numbers of females expected = numbers of males expected.

The results conform with the theoretical expectation on the assumption of independent segregation between black, sex and bent.

The composition of the parents in the cross of red flies was as follows:

F_1 male*		Gray	Red	Bent
	sex	Black	Pink	Straight
F_1 female	sex	Gray	Red	Bent
	sex	Black	Pink	Straight

* The paternally derived allelomorphs are on the upper line, the maternal on the lower.

The offspring were as seen in table 3.

TABLE 3

		WILD TYPE	BLACK	PINK	BENT	BLACK PINK	BLACK BENT	PINK BENT	BLACK PINK BENT
F^2 numbers observed	females..	87	40	36	17	5	6	12	3
	males....	98	27	39	29	17	10	10	3
	Total....	185	67	75	46	22	16	22	6
Total numbers expected on independence		185	62	62	62	20.6	20.6	20.6	6.9

Numbers of females expected = numbers of males expected.

A considerable differential viability came into play in these bottles, as is proved by that fact that certain 'contrary' or opposite classes which were necessarily produced in equal numbers at fertilization (no matter whether linkage was involved or not), gave rather different counts of adult flies (e.g., 206 females; 233 males; 97 pink straight, 62 red bent; which were the worst discrepancies). On allowing for these differences obviously due to viability, we find that there are no discrepancies due to linkage of bent with black or pink, for the average between black straights and gray bents (pink plus red), is to the black bents as 3:1, and the average between pink straights and red bents (gray plus black) is to the pink bents as 3:1, as expected on independence. These results therefore confirm those previously obtained in regard to the independence of black (Chromosome II) and bent; they show in addition, however, that bent is independent of pink (Chromosome III).

The distribution of bent with regard to black and pink was next determined separately for each sex, by means of back-crosses. A normal male was mated to black pink bent females. The F_1 males, which were heterozygous for all three factors, as well as for sex, were then mated to triply recessive black pink bent females. If the factors were independent, all classes in the next generation should be equal in number. The actual result was as shown in table 4.

TABLE 4

CLASSES	WILD TYPE	BLACK	BENT	BLACK BENT	PINK	BLACK PINK	PINK BENT	BLACK PINK BENT
females	29	28	23	30	16	14	15	9
males	25	22	22	17	14	18	16	15
Total	54	50	45	47	30	32	31	24

Here practically the only irregularity not due to chance is obviously caused by a deficiency (low viability) of pink flies of all classes, and by a lower viability of males than of females. The independent assortment of bent with respect to the other factors, and of the other factors with respect to each other, is best brought out (especially in a case involving differential viability) by a tabulation of the percent of cases in which any two pairs of factors, considered by themselves, underwent recombination in the formation of the germ cells of the heterozygous parent. Thus, in the case of sex and bent, the factors for sex and for bent in the heterozygous male parent were both derived from the mother, the Y-chromosome and the normal allelomorph of bent ('straight') both coming from the father. Yet in the segregation division by which the sperm were formed, a recombination occurred as frequently as a persistence of the old combination, so that as many eggs were fertilized by sperm bearing the sex factor and straight, or the Y-chromosome and bent, as were fertilized by sperm with sex and bent or Y and straight. As a result, straight females and bent males formed 50 per cent of the total number, as we should expect on the basis that the factors were in non-homologous chromosomes which were assorted independently. The other per cents of recombination were as follows:

	per cent
Sex-black	50.0
sex-pink	55.0
black-pink	50.5
black-bent	50.5
pink-bent	49.0

In a reciprocal cross some of the triply heterozygous F_1 females were back-crossed to triply recessive males. Here, too, we should

expect all classes equal, barring differential viability, on the basis of independent assortment, and approximately 50 per cent of recombination between any two factors. The result was similar to that obtained in the back-cross of the males (table 5).

TABLE 5

CLASSES	WILD TYPE	BLACK	BENT	BLACK BENT	PINK	BLACK PINK	PINK BENT	BLACK PINK BENT
females	23	14	16	22	19	11	15	10
males	22	23	17	22	15	12	9	8
Total	45	37	33	44	34	23	24	18

The per cents of recombinations were as follows:

	per cent
Black-pink	54.0
black-bent	45.5
pink-bent	52.0

Here we cannot obtain the per cents of recombinations between sex and the other factors, since the parent which was heterozygous for the other factors was not the one which was heterozygous for sex.

CONCLUSIONS

The foregoing experiments prove that the gene for bent wings segregates independently of the sex-linked group of genes and of the two hitherto known non-sex-linked groups; accordingly, the genes of Drosophila now fall into four divisions, one sex-linked, corresponding to the X-chromosome, and three non-sex-linked, corresponding to the three pairs of autosomes. Thus the chief gap yet remaining in the series of genetic phenomena that form a parallel to the known cytological facts in Drosophila ampelophila has now been filled. It may therefore be predicted that no genes undergoing independent assortment of those at present known can hereafter be discovered in individuals of Drosophila ampelophila that show the chromosome configuration normal to the species, and it also seems probable that when other mutations are discovered in the fourth group, the genes in which they occur will be found to be linked strongly to the gene for bent wings, since

the fourth chromosome is probably the small one, and so any genes in it must lie near together. The close parallel existing between the number and relative *sizes* of the groups of genes and of the chromosomes leaves little doubt that it must be the pair of small chromosomes with which the factor for bent wings is connected, and that mutations occur more frequently in larger groups of genes, which are connected with larger chromosomes, than in smaller groups; mutation therefore would happen pretty much at random, in that it would usually take place about as often in one group as in another of equal size.[5] This, too, makes it probable that the mutations in the larger chromosomes have occurred at various points scattered throughout their whole length, and are not confined, *as a group*, to a particular region or regions. The exceptional case of no mutations having been observed in the Y-chromosome, as we have seen, does not really form an argument against this view, which other facts support. That more mutations have been found in the X-chromosome than in either of the two long autosomes, which are nevertheless about the same size as X, is also to be expected, because a larger proportion of the mutations occurring in X would be noticed, since the male flies manifest all genes present in their single X, whereas in the case of other chromosomes, any mutant gene that is recessive to normal cannot manifest itself unless it be present in duplex. Granting, then, the correspondence between size and number of chromosomes and of groups of genes, it is difficult to see why larger groups of genes should follow the distribution of the larger chromosomes unless we conceive the connection between the genes and the chromosomes to be that the genes are material particles actually lying in and forming a part of the chromosomes with which they go. In any case, we must admit that the occurrence of a mutation in a fourth independent group of genes in Drosophila forms a further argument, if any more still be needed, in favor of the chromosome theory of heredity.

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[5] This should not be taken to mean that any particular gene mutates as often as any other; it is definitely known that, both in Drosophila and in other forms (corn, *Marabilis*, etc.), some genes are more likely to mutate than others.

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