

NEW EVIDENCE FOR THE HOMOLGY OF THE SHORT EUCHROMATIC
ELEMENTS OF THE X AND Y CHROMOSOMES OF *DROSOPHILA*
BUSCKII WITH THE MICROCHROMOSOME OF
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THE metaphase X chromosome of the mitotic and meiotic cells of *Drosophila busckii* is rod-shaped and has a secondary constriction located $1/4$ – $1/3$ its length from the proximal end. In salivary gland cells this chromosome consists of two euchromatic elements: a long distal one, and a short, proximal piece approximately $1/20$ th as long. Both of these elements are connected with the nucleolus. It has also been proven that the nucleolus of the polytene X is located at a site corresponding to the secondary constriction of the metaphase chromosome, and the short euchromatic polytene element corresponds to the smaller block of the metaphase chromosome that lies proximal to the secondary constriction. In salivary gland cells the centromere of the X chromosome is located at the very proximal end of the short euchromatic element, and it is probably terminally located in the metaphase chromosome as well (KRIVSHENKO 1955).

The metaphase Y chromosome is also rod-shaped, but has a primary constriction near the proximal end which divides the chromosome into two arms: a long left arm and a short, knob-like, right arm. In the salivary gland cells the Y chromosome is usually observed as a short euchromatic element that is approximately equal to the short euchromatic element of the X chromosome (SIROTINA 1938; KRIVSHENKO 1939). It has been proven that this euchromatic part of the Y chromosome is genetically active and corresponds to the right arm of the metaphase chromosome, whereas the long left arm is heterochromatic (KRIVSHENKO 1950, 1952). It was also found that XO males of this species are not viable (KRIVSHENKO 1941a, b), and that viability factors are located in the small, euchromatic right arm of the Y chromosome (KRIVSHENKO 1952).

In previous investigations of *D. busckii*, cytological and genetical evidence was obtained pointing to the homology of the short euchromatic elements of the X and Y chromosomes with each other, and indeed also with the microchromosome of *D. melanogaster* (KRIVSHENKO 1952, 1955). The basis for this conclusion lies in: 1) the observed somatic pairing of X and Y by their proximal ends in ganglion cells, and the conjugation of the short euchromatic elements of these chromosomes at their centromeric regions in salivary gland cells; 2) the presence in the short Y chromosomal element of normal allelomorphs to four different

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mutant genes of the short X chromosomal element; and, 3) the presence in the short element of the X chromosome of a mutation, *Cubitus interruptus*, that is characteristic of the microchromosome of *D. melanogaster*. These findings are quite sufficient to prove at least partial homology among all three of the elements. Nevertheless, these elements have been subject to very different phyletic and genetic histories, and it would be of considerable evolutionary importance to know, in the greatest detail possible, the degree to which they have retained homology as well as in what ways they have undergone evolutionary divergence.

In the present paper new X chromosomal mutations of *D. busckii* are described which are homologous to well-known microchromosomal mutations of *D. melanogaster*. Cytogenetic study of these mutations provides an explanation of why XO males in *D. busckii* are not viable, and affords a basis for some general phylogenetic conclusions.

DESCRIPTION AND CYTOGENETIC STUDY OF THE NEW MUTATIONS

The X chromosomal mutation #321 was found in the progeny of X-rayed *D. busckii* males of a wild strain from the population of Columbia, Mo. (8-10, 1949). Phenotypically characteristic of the mutant males is a fusion of the medial (L-3) and cubital (L-4) veins of the wings in their basal parts up to the region of the I-st crossvein, although this is occasionally not complete. The ocellar region of the head is smooth, and the anterior ocellus and ocellar bristles are always absent; though the posterior ocelli are usually present, they are strongly reduced. The dark spot characteristic of the ocellar region is completely absent. At 23-25°C variations are insignificant in the modification of vein structure and of the ocellar region, the morphological peculiarities of the mutant are distinctly expressed, and the viability and fertility of males are quite high. In heterozygous females wing vein structure is usually normal, though in some crosses the basal parts of the medial and cubital veins are noticeably closer to each other. The dark ocellar spot is considerably diminished, and very often one or both ocellar bristles are absent. Females homozygous for this mutation are not viable.

Cytological analysis shows a small inversion (microinversion) located at the proximal end of the salivary gland X chromosome. The left break of this inversion occurred at the very beginning of the 19th section, corresponding to the region where the nucleolus organizer is located. The right break occurred at the beginning of the 20th section, that is, in the most distal part of the short euchromatic element (see map of the X of *D. busckii* in KRIVSHENKO 1955).

Thus this semidominant, morphologically complex X chromosomal mutation is connected with a structural aberration. Whether it is caused by one gene with a pleiotropic effect, by two adjacent genes, or by a position effect brought about by the aberration is not known. However, two mutations are known in *D. melanogaster* in which a change in vein structure occurs that is very similar to that of the mutation described here. These mutations are fused and Cell. The first is X chromosomal, the second microchromosomal (BRIDGES and BREHME 1944). Now it is possible to suppose that either fused or Cell is homologous to the mu-

tation of *D. busckii*; but which one? Such an ambiguity very often arises in comparative genetical studies of species that do not cross with each other, and may present great difficulty in establishing the homologies of phenotypically similar mutations. Nevertheless, it is sometimes possible to find a convincing answer by taking into consideration the genetic surroundings of the locus under question.

Since the mutation described here is associated with an inversion, it is likely that the mutant factor is located near one of the breaks of the inversion. Should there be two independent factors, then one of them may be located at one break, the other at the other break. To check this hypothesis and to localize the responsible factor of this mutation, two experiments were performed in which special lines of flies were crossed.

In the first of the experiments *Ce/Bly* females were crossed with *Sms* males. In females of this cross one X chromosome carries the mutation under study, here designated as *Ce*. The other X chromosome is marked by the dominant mutation Blondy (*Bly*) connected with an inversion of the proximal half of the salivary gland X chromosome. Blondy flies lack pigmented abdominal bands and have a yellowish body. The males of this cross have a dominant X chromosomal mutation, Small spots (916) (the abdominal pigmented bands are very reduced), the factor for which is located in a small X chromosomal part inserted into IIIIL. The breaks in the X chromosome that gave rise to the small inserted part occurred to the left at the end of section 17, and to the right somewhere in the center of section 19. Thus the first break is located considerably to the left, the second a little to the right of the distal break of the inversion in the mutant strain. Because the X chromosomal part carrying the factor Small spots is inserted into IIIIL, Small spots is inherited as a dominant autosomal factor and is independent of the normal X chromosome.

The results of this cross are shown in Table 1. Since the mutation Blondy is epistatic to the mutation Small spots, six phenotypically different classes of flies appear in the progeny.

Of these flies, the males of class *Ce;Sms* are of special interest. These males

TABLE 1

Determination of the location of gene Ce by means of the cross

$$\text{♀ ♀ } \frac{\text{Ce } +}{\text{Bly } +} \times \text{♂ ♂ } \frac{+ \text{ Sms}}{+} :$$

Phenotypic classes	Number of flies
♀ ♀ <i>Ce; Sms</i>	91
♀ ♀ <i>Ce</i>	125
♀ ♀ <i>Bly</i>	198
♂ ♂ <i>Ce</i>	91
♂ ♂ <i>Ce; Sms</i>	83
♂ ♂ <i>Bly</i>	141
Total	729

carry the X chromosome of the investigated mutant strain #321, as well as the X chromosomal duplication phenotypically manifested by the characteristic Small spots. In spite of the fact that the *Sms* duplication covers the region of the distal break of the inversion in the *Ce* mutant line, the *Ce;Sms* males have the distinctive phenotypic complex of males of the *Ce* line expressed without any changes. Thus the appearance of these males in the progeny of this cross indicates that the hereditary factor causing the *Ce* phenotype is located neither at the region, nor close to the region, of the leftmost break in the X chromosome of the mutant line #321.

In the other experiment *Ce/Bly* females of the same origin as in the first experiment, were crossed with *mo/Ri* males. The males of this cross have complex X and Y structural changes of independent origin; that is, their chromosomal complement consists of two aberrant haploid sets of euploid constitution. The X chromosomal recessive mutation mosaic (*mo*, 188) (black spotted eyes and other effects) is connected with an aberration that involves X and IIIR. The X chromosome was broken in two regions, namely at the very beginning of the 2nd section and in the distal half of the 19th section (but to the left of the rightmost break of the inversion associated with the mutant *Ce*). The break in IIIR is located at approximately one fifth the length from the distal end. The long, middle part of the X chromosome, to the proximal end of which the short, distal portion of the X was joined, was translocated in an inverted position onto the proximal part of IIIR. The distal part of IIIR united with the proximal or centromeric part of the X chromosome. As a result of this last union, an aberrant short X chromosome was formed of which the X chromosomal part consists only of the basal part of the 19th (a heterochromatic, nucleolar region) and the whole of the 20th section (a short, euchromatic element). The dominant mutation Radius incompletus (*Ri*) is connected with a complex aberration involving Y and IIR. In its inheritance this autosomal mutation cannot be separated from the Y chromosome. The results of this cross are given in Table 2.

The first four of six classes of flies obtained in the progeny of this cross are regular, having derived from segregation that produce haploid euploid complements from the aberrant sets of chromosomes present in the parental males. However, the two last classes, namely ♂♂ *Ce/?* and ♂♂ *Bly/?*, are males of an exceptional type. The striking peculiarity of these males consists in the absence of Radius incompletus from their phenotype. Because Radius incompletus is inseparable in its inheritance from the Y chromosome, its absence shows the absence of the Y chromosome.

The occurrence of 143 XO males would at first seem absolutely incompatible with earlier well-established observations and experimental data. Our studies of primary and secondary nondisjunction of the sex chromosomes in both males and females of *D. busckii* have shown that adult exceptional XO males are not produced in this species. Although 73 exceptional females have been found among 85469 flies obtained from special crosses carried out for the study of primary nondisjunction in males and females, no exceptional males have been found

TABLE 2

Determination of the location of gene Ce by means of the cross

$$\text{♀ ♀ } \frac{\text{Ce}}{\text{Bly}} \frac{+}{+} \frac{+}{+} \times \text{♂ ♂ } \frac{\text{mo}}{\text{Ri}} \frac{+}{*} \frac{*}{+} :$$

Phenotypic classes	Number of flies
♀ ♀ type Ce (heteroz.)	154
♀ ♀ type Bly	216
♂ ♂ Ce/Ri	38
♂ ♂ Bly/Ri	137
♂ ♂ Ce/?	58
♂ ♂ Bly/?	85
Total	688

* The asterisks indicate the autosomes involved in the translocations that mark X(mo) and Y(Ri).

(KRIVSHENKO 1941a,b,c). On the basis of these results the conclusion was drawn that the Y chromosome of *D. busckii* is a genetically active chromosomal element necessary for the normal development of males. This conclusion has since been repeatedly confirmed by numerous observations and experiments (unpublished data).

Nevertheless, the occurrence of males without a Y chromosome in the experiment described above is indubitable. Evidently in this experiment, therefore, specific conditions exist that permit the appearance of such males. These conditions are expressed in the phenotype of males of *Ce/?* class, and cytologically in males of both these exceptional classes.

Although the males of *Ce/?* class have the maternal X chromosome of the mutant *Ce* strain, the change in wing vein structure is not expressed at all. The phenotypic changes in head characteristics are expressed by the reduction of the dark ocellar spot and by the absence of one or two ocellar bristles. The phenotypical expression of the *Ce* characteristics in *Ce/?* males is, therefore, exactly similar to that of ordinary females heterozygous for the new mutation. Such a modified expression of the phenotype in *Ce* mutant males had never been observed before nor do the *Ce/Ri* males of this experiment show the slightest reduction in the *Ce* phenotype.

The similarity in phenotype of the *Ce/?* males with that of heterozygous females gives reason to suppose that these males are also heterozygous for the factor (or factors) determining this X chromosomal mutation. This may be caused by the presence of a duplication for the region on which *Ce* is localized. The paternal short X chromosome of the mutant mosaic, consisting of the small distal part of IIIR and of the short proximal part of the X chromosome, is exactly such a duplication. It undoubtedly represents the chromosomal part containing the genetic factor (or factors) causing heterozygosity for *Ce* and compensating for the viability factors normally present in the Y chromosome.

There is no doubt that the same conditions are also responsible for the appear-

ance of the Blondy males of the *Bly*/? class. In them, however, heterozygosity for the proximal part of the X chromosome is not expressed because of the absence of an appropriate marker in this part of the chromosome.

A parallel cytological analysis of the salivary and ganglion cells of male progeny in the given cross completely corroborates the above conclusions. Thus the males of both exceptional classes mentioned above have the short paternal X-IIIR translocation instead of a Y chromosome; hence, these exceptional males are X/dp-X, and not truly XO.

Thus, from the data of this cross, it follows that the hereditary factor of the new mutation *Ce* is located wholly in the region of the proximal break of the inversion. It is therefore located at the beginning of the 20th section, that is, in the distal part of the short, proximal, euchromatic element of the polytene X chromosome. Because the factor *Cubitus interruptus* is also located in the same part of the X chromosome, and of course has its homologue in the microchromosome of *D. melanogaster*, the mutation described here undoubtedly is a homologue of the phenotypically similar microchromosomal mutation *Cell*.

The other X chromosomal mutations #960 (10-7, 1958) and #969 (10-9, 1958) were found in the progeny of X-rayed males of a wild strain from the population at Princeton, N. J. The morphological peculiarities of the mutant males of both stocks are expressed by (1) a reduction in the abdominal bristles (of both sternites and tergites), the degree of reduction varying from a complete disappearance to a differential reduction in the size of these bristles; (2) in the absence of one or both ocellar bristles, and some of the orbital bristles. The viability and the fertility of the mutant males are high. Neither mutation produces viable females when homozygous. Compound females having both mutant X chromosomes (that is, 960-969) are also unviable. However, heterozygous females are phenotypically normal and have both high viability and fertility. Cytological analysis of the salivary gland chromosomes disclosed no macrochromosomal aberrations. Thus we have here recessive mutations, very likely homologous and connected with a homologous recessive lethal.

No mutations among the recorded X chromosomal mutations of *D. melanogaster* (BRIDGES and BREHME 1944) are morphologically similar to those just described for *D. busckii*. Of the autosomal mutations which are phenotypically expressed by the reduction of abdominal bristles, the microchromosomal mutation *shaven* has the greatest resemblance to them. Nevertheless, morphological similarity alone is, of course, insufficient to draw conclusions about homology. In this case, as for *Cell*, the important criterion must be the positions of the loci of the mutations under investigation to those of other mutations which are already known and typical for the given chromosome or chromosomal part.

Several different crosses were carried out to locate the hereditary factors of the mutations described here, one of which was most demonstrative and its results are given below. In this cross *sv/Bly* females were crossed with *mo/Ri* males. Structurally this cross is similar to that used in the second experiment localizing the mutant *Cell*. The only difference is that the factor of mutation #969 is locat-

ed in one of the X chromosomes of the females, and is designated as *sv*; all else is the same as in the Cell experiment.

The structural peculiarities of the parents again determined the appearance of six phenotypic classes of flies as shown in Table 3.

TABLE 3

Determination of the location of the gene sv by means of the cross

$$\text{♀ ♀ } \frac{\text{sv}}{\text{Bly}} \frac{+}{+} \frac{+}{+} \times \text{♂ ♂ } \frac{\text{mo}}{\text{Ri}} \frac{+}{*} \frac{*}{+} :$$

Phenotypic classes	Number of flies
♀ ♀ type normal	106
♀ ♀ type Bly	100
♂ ♂ <i>sv</i> /Ri	80
♂ ♂ Bly/Ri	81
♂ ♂ type normal	94
♂ ♂ type Bly/?	59
Total	520

* See Table 2.

The first four of the classes shown in Table 3 are regular and expected under ordinary conditions of chromosomal segregation (see the second experiment), but the males of the last two classes are exceptional. Their peculiarity consists in the absence of the dominant marker *Ri* which, as was indicated above, cannot be separated from the Y chromosome in its inheritance.

Since males of the XO constitution of *D. busckii* are not viable, the appearance of the exceptional classes of males is again due to the presence of a genetically adequate substitute for the Y chromosome carrying factors for male viability. Cytologically it was again proved that this substitute is the short duplication derived from the parental males which consists of the telomeric part of IIIIR and the proximal part of X representing the short euchromatic element of X. Undoubtedly the genetic factors which substitute for the Y chromosomal male viability factors are located in the X chromosomal euchromatic part as is also the normal allelomorph of the hereditary factor of the mutation *sv* here investigated. The presence of this normal allelomorph explains the fact that males of the 5th class are phenotypically normal. The data for mutation #960 are completely similar.

Since the common hereditary factor of mutations #960 and #969 is located in the short euchromatic element of the X chromosome in which the mutation *Cubitus interruptus* is located, and since these mutations are identical and phenotypically similar to the microchromosomal mutation *shaven* of *D. melanogaster*, it may be concluded that they are in fact homologous with the mutation *shaven*. Thus both of the two new mutations of *D. busckii*, Cell and *shaven*, present new

genetical evidence for the homology of the short euchromatic element of the X chromosome of *D. busckii* with the microchromosome of *D. melanogaster*.

DISCUSSION

Our previous investigations proved that the Y chromosomal factors for male viability are located in the right arm, which is observed as a short euchromatic element in the salivary gland cells. When this arm or element is absent, males are not viable (KRIVSHENKO 1952). In the experiments summarized in Tables 2 and 3 exceptional males appeared in which the Y chromosome was substituted by the short euchromatic element of the X chromosome. The fact that these males were perfectly viable and without any phenotypic abnormalities shows that factors for male viability are not unique to the Y but they can be substituted by X chromosomal allelomorphs. The localization of these allelomorphs in the short euchromatic element of the X chromosome represents a new and additional genetic proof of homology of this element with the euchromatic part of the Y chromosome. Nevertheless, the X chromosomal part cannot be a complete substitute for the Y chromosome because the exceptional males obtained in these experiments are completely sterile.

Earlier data had indicated the existence of an homology of the X and Y chromosomes with each other as well as homology of X with the IV chromosome of *D. melanogaster*, allowing these elements to be placed in an order, Y-X-IV, suggesting their genetic relationship. The lines connecting the adjacent elements indicate direct evidences of relationship between these elements. It was not possible to draw such lines of homology between Y and IV because of the absence of any direct confirmatory data. However, it is now possible to demonstrate this homology through an analysis of the genetic behavior of the mutants Cell, shaven and Cubitus interruptus.

The behavior of both the semidominant factor Cell and the recessive factor shaven differs in males and in heterozygous females in a manner typical for X chromosomal mutations that do not have normal allelomorphs in the Y chromosome. Even though the homozygous females are not viable and hence we do not have a complete picture of the phenotypic expression of these mutations, nevertheless, the phenotypic expression in hemizygous individuals gives sufficient ground to conclude that the process of dosage compensation is already complete. As a result of dosage compensation the original autosomal factors now express themselves in a typically X chromosomal manner. It is hard to say what has happened to the Y chromosomal allelomorphs of the factors for the mutations Cell and shaven if, in fact, a part homologous to that of X which bears *Ce* and *sv* is present in the short euchromatic element of the Y chromosome. The immediate connection of these mutations with lethals, of which normal allelomorphs are present in the Y chromosomal element, gives some reason to assume that such a part is still present.

The dominant mutation Cubitus interruptus (585) behaves differently. The phenotypical expression of *Ci* is the same both in mutant males (possessing an

aberrant X and a normal Y) and in heterozygous females (possessing an aberrant and a normal X chromosome). In mutant males in which the Y chromosome is substituted by short X chromosomal duplication from the stock of mosaic (188) (as in exceptional males of second and third experiments), the phenotypic expression is also the same.

As was indicated in my previous work (KRIVSHENKO 1955), the occurrence of the mutation *Cubitus interruptus* was connected with a complicated series of chromosomal aberrations that involved breaks in X, IIR, IIIL and IIIR. As a result of the rearrangement of the parts of the chromosomes, the aberrant X chromosome fragment bearing this mutation is now a short (dot-like) chromosome consisting of the telomere part of IIIR and the proximal part of the short euchromatic element of the X chromosome; it is in the latter element in which the locus of *Cubitus interruptus* is located. Due to the small size of this chromosome and its independent behavior at the reduction division, it is easily extracted from the aberrant euploid set and may then be introduced as a duplication into chromosomal complements of flies of wholly different chromosomal constitutions. It is, therefore, possible to study the phenotypical expression of the factor *Cubitus interruptus* in different genetic environments. In this way it was established that males and females which, in addition to their normal chromosomal complements, have the short X duplication carrying *Ci* are phenotypically identical with the flies of the original, complexly rearranged *Ci* stock.

Thus the phenotypic expression of the mutation *Cubitus interruptus* is the same in both females and males of different chromosomal structure and does not seem to depend upon the number of the normal allelomorphs that are possibly present in their sex chromosomes (at least in the X). Whether this is due to an absolute dominance of *Ci*, or to the presence of a normal allelomorph in the Y chromosome creating equivalent genetic conditions in both sexes, cannot be decided for it is impossible at present to obtain males of XO structure.

Nevertheless, the genetical behavior of this mutation indicates that it should be placed in the group of so-called neomorphic mutations, for the phenotypical expression basically depends upon the gene dosage (MULLER 1932a). The mutation *Cubitus interruptus* displays the same peculiarities in males as well as females possessing a double dose of the factor (in presence of a normal X or Y), both sexes exhibiting a very strong, peculiar, and identical expression of the phenotype.

The genetic nature of neomorphic mutations is not always clear, but it is certain that many of them represent some form of position effect. The possibility that the mutation *Cubitus interruptus* of *D. busckii* also represents a position effect cannot be excluded. However, independently of the nature of this mutation, it is important to know whether in the short euchromatic element of the Y chromosome there is a part homologous to that of the X chromosome in which the mutation *Cubitus interruptus* arose. This problem is very difficult and an experimental solution seems impossible. But the fact that this mutation is connected with a recessive lethal for which the normal allelomorph is present in the short

euchromatic element of the Y chromosome gives a reasonable basis for concluding that the short euchromatic element of Y does possess a section homologous with that of X in the *Ci* region.

Of course, it is impossible to draw a definite conclusion as to whether the homologous part of the Y chromosome still preserves genetic potentialities similar to those of the corresponding part of the short euchromatic element of the X chromosome, and is in fact still capable of producing a change of the *Cubitus interruptus* type. Undoubtedly processes of inactivation or losses of genic material (as evidently has occurred in the cases of the mutations *Cell* and *shaven*), as well, perhaps, as processes of genic redifferentiation (BERG 1937a,b) leading to changes in the genetic material have taken place. Proof of this last process can be seen in the appearance of factors for male fertility in the short euchromatic element of the Y chromosome (KRIVSHENKO 1952). These factors do not have normal allelomorphs in the short euchromatic element of the X chromosome.

It may be concluded that the factors for viability in the Y chromosome that are homologous to corresponding factors in the X chromosome, and which are immediately connected with the hereditary factors of the mutations *Cell*, *shaven* and *Cubitus interruptus*, represent an indirect proof of the homology of a Y chromosomal part of *D. busckii* with the microchromosome of *D. melanogaster*. We may at last represent the pattern of homology not in the form of a line (Y-X-IV) but in the form of a triangle at the apexes of which the chromosomal elements under discussion are situated (or, represented linearly, Y-X-IV-Y).

Inasmuch as homology of the short euchromatic elements of the X and Y chromosomes of *D. busckii* with the microchromosome of *D. melanogaster* has now been established, one might expect additional correspondence of the genic contents of these elements that determine morphogenetic and other functions in these two species of *Drosophila*. However, the presence of the viability factors in the Y chromosome which have their allelomorphs in the X chromosome do not fit this pattern. Indeed, a double dose of these factors is needed for the normal development of the male individuals of *D. busckii*. When one element (or even part of one) is absent, males do not appear, and it is likely the situation is the same for females as well. In *D. melanogaster* it is otherwise, for males and females of this species having but one microchromosome (so-called "haplo-IV" progeny) are viable, albeit poorly so, their fertility is low, and in addition they have characteristic phenotypical abnormalities (BRIDGES 1921; STURTEVANT and BEADLE 1939; and others).

Before drawing a direct homology between the short euchromatic elements of the X and Y chromosomes of *D. busckii* with the microchromosome of *D. melanogaster*, it is necessary to answer the question as to how, where, and from what the viability factors came into being which must now be present in double dose in the sex chromosomes. It is not known fully how many such factors are present in the parts of the sex chromosomes under investigation, but there are at least seven of them. It is implausible to assume that they appeared *de novo*, or as a

result of redifferentiation after microchromosomes of the *D. melanogaster* type became part of the X and Y chromosomes of *D. busckii*, for there is no crossing over between the heterochromosomes. It is quite evident that these factors are of an autosomal origin, for the fact that they are needed in a double dose, unlike normal X chromosomal factors, indicates they have not acquired dosage compensation. It is accordingly reasonable to assume that the ancestral form of the short euchromatic elements did in fact carry and supply these factors.

It is likewise possible to suppose that originally the microchromosome of *D. melanogaster* also possessed these factors but that in the evolution of this species they were either lost or underwent definite changes. The appearance of haplo-IV individuals would then be explained simply by the small size of this chromosome. But the euchromatic part of this chromosome in the salivary gland cells has a comparatively large size comprising more than 135 bands (SLIZYNSKI 1944) and autosomal deficiencies comparable in size and extent to the microchromosome are generally lethal. It is, therefore, hardly reasonable to suppose that this chromosome has less significance in the development of an individual as compared with comparable parts of other autosomal chromosomes. Indeed this cannot be correct if only for the reason that the microchromosome is present in the chromosomal complements of all species of *Drosophila* that have been cytologically investigated to date, and can be made out in salivary gland preparations.

It is well known that structural changes of the karyotype, as well as changes in the genic composition of separate chromosomal elements, represent continuous processes in the evolution of organisms. It is also known that conditions which determine at least some of these processes are different for sex and autosomal chromosomes. This viability of haplo-IV individuals may be explained by assuming that there was a time when the microchromosome was subjected to the same conditions which determined the nature of the X chromosomal factors. In other words, the microchromosome of *D. melanogaster* was in the past evidently a part of the X chromosome and dosage compensation was brought about for many hereditary factors that are located in it. Some of the synaptical peculiarities of this chromosome expressed by non-random segregation seem to be in accordance with this assumption (STURTEVANT 1934, 1936; GERSHENSON 1941; LINDSLEY and SANDLER 1956).

Evidently such an hypothesized original form, with the IV chromosome element as a part of the X, was ancestral to *D. melanogaster* as well as other closely related species (STURTEVANT 1946). *D. ananassae*, it seems, may represent one of the descendant species. But the line of separation of the proximal end which gave origin to the IV chromosome of this species must have passed to the left of the nucleolus organizer. As a result the IV chromosome of *D. ananassae* includes both the microchromosomal material and the heterochromatic part of the X chromosome in which the nucleolus organizer and the bobbed locus is located (KIKKAWA 1938; KAUFMANN 1937).

If this assumption is valid, the conclusion follows that the present condition of the microchromosome of *D. melanogaster* is not primary, nor is that of the

short euchromatic elements of the X and Y chromosomes of *D. busckii*. For these reasons we think it is necessary to look for the precursor of the short euchromatic elements of *D. busckii* in the microchromosomal elements of another species of *Drosophila*, in which they possess greater genetic potentialities than the microchromosome of *D. melanogaster*, or, put otherwise, in which the ancestral element had not departed so far from a typically autosomal nature.

The inclusion of the autosomal elements into the sex chromosomes is a widespread phenomenon in different groups of organisms (WHITE 1954) and, undoubtedly, is one of the general processes of karyotypic change. The species of *Drosophila* represent no exception in this respect (PATTERSON and STONE 1952). The mechanism of this process in all observed cases is similar and comparatively simple, namely translocation or so-called "centric fusion" of whole chromosomal elements. As a result, a complicated reconstruction of the genic balance of the newly formed karyotype may occur in many cases. Without question this reconstruction is a protracted process with a definite sequence.

Thus, as the results of this study of *D. busckii* suggest, the gene complex of the autosomal element that is now a part of the X chromosome still preserves at least some of its morphogenetic functions, despite the fact that some of the components of this complex now show X chromosomal peculiarities. The homologue, which has become part of the Y chromosome, no longer appears to possess normal allelomorphs to the morphogenetic factors located in the X chromosome. Nevertheless, comparatively numerous homologous viability factors are still preserved in both these elements and their autosomal nature is unchanged. Thus, whereas in the X chromosomal element processes of adaptation to new conditions took place, in the Y chromosomal element different processes occurred which led to the loss of the normal allelomorphs of these factors.

The origin of the Y chromosome in *Drosophila* as well as in other organisms is the subject of many discussions in the genetic literature and represents one of the more enigmatic problems of biology. MULLER (1914, 1918, 1932a,b) and NEUHAUS (1938, 1939) suggest that the reduction of this element to a nearly inert chromosome is due to inactivation, or simply to the loss of genes, of a formerly genetically active element initially homologous to the X chromosome. The condition which favored the accumulation of the products of inactivation was an absence of crossing over between X and Y. The loss of morphogenetic factors in the Y chromosome of *D. busckii* is consistent with such an hypothesis, and, furthermore, represents the first experimental evidence of the correctness of this hypothesis. But in the evolution of this Y chromosomal element there occurred not only processes of inactivation and loss, but undoubtedly also processes of redifferentiation leading to the appearance of the factors for male fertility.

SUMMARY

1. Two new X chromosomal mutations of *D. busckii* homologous to the microchromosomal mutations Cell and shaven of *D. melanogaster* are described. The fact that the genes involved in these mutations are located in the short euchro-

matic element of the polytene X chromosome, close to the locus of the previously described *Cubitus interruptus*, serves as new proof of the homology of this chromosomal element with the microchromosome of *D. melanogaster*.

2. For the first time males of *D. busckii* were obtained that do not have a Y chromosome. Such XO males appear only when the Y chromosome is substituted by the short, proximal euchromatic element of the X chromosome. The Y chromosomal factors for male viability are, therefore, not specific to the Y but have homologs or allelomorphs in the proximal part of the X chromosome.

3. In a previous paper it was proved that the Y chromosomal factors for male viability are located in the right arm of this chromosome which is observed as a short euchromatic element in the salivary gland cells. The presence of homologous factors for viability in the short euchromatic elements of both the Y and X chromosomes provides new evidence for the homology of these chromosomal elements, and must derive from their common evolutionary origin.

4. On the basis of comparative analyses of the genetical data, some general phylogenetical conclusions are drawn.

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