

Karyotype Variation in *Drosophila birchii*

V. BAIMAI

Genetics Laboratory, Zoology Department, University of Queensland,
Brisbane, Australia

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Abstract. *Drosophila birchii*, a member of the *melanogaster* species group of the subgenus *Sophophora*, is common in the tropical rain forests of the Australia-New Guinea areas. Chromosome squashes are easily prepared from the larval ganglion cells and the sex chromosomes are readily recognizable. The species exhibits a remarkable karyotype variation. The metaphase plate figures, in general, show two pairs of V's, one pair of dots and one pair of sex chromosomes. Variations in metaphase chromosome morphology are found in the X (with four types), the Y (with three types) and chromosome IV (with two types). Chromosomal interchanges between X- and Y-chromosomes Type I are postulated to be involved in the differentiation of sex chromosome morphology while the modification of chromosome IV seems likely to be a result of the acquisition of extra heterochromatin. These chromosome types form seven distinct metaphase plate figures, all encountered in wild populations, thus giving *D. birchii* the most variable karyotype in the genus *Drosophila*.

Introduction

Structural changes of chromosomes which involve breakage of the chromosomes, followed by recombination of the broken ends and often accompanied by gain or loss of parts, generally fall in the categories of fusion, pericentric and paracentric inversions, and translocations of various kinds. These chromosomal rearrangements lead to reconstituted karyotypes and could result in extensive modification of chromosome morphology particularly as determined in metaphase plate configurations that may distinguish one species from another closely related form. Such phenomena are now known in many groups of animals. Wharton (1943) made the first extensive analysis of the role of such structural changes including variation in heterochromatin and species relationships within the genus *Drosophila*. This type of investigation has revealed that variations in metaphase chromosome morphology have been encountered not only in closely related species but also within a single species. In the latter case, the variations may involve a particular chromosome such as the Y chromosomes in *D. pseudoobscura* (Dobzhansky, 1935, 1937), and in *D. athabasca* (Miller and Stone, 1962; Miller and Roy, 1964).

Cytological studies in several strains of *D. birchii* from the Australia-New Guinea areas have shown extensive variations in metaphase plate configurations of the X, the Y and the 4th chromosomes. The results of observations on chromosomal variation in *D. birchii* are reported in this article.

Materials and Methods

The several strains of *D. birchii* employed in the present study were collected from fermenting banana baits placed in rain forests at Cairns, Northern Queensland, Australia; Port Moresby, Daru, Popondetta, Bulolo, Wewak, in the territory of Papua-New Guinea; and at Rabaul, New Britain.

Metaphase plate chromosomes were studied in lacto-aceto-orcein squash preparations of larval ganglion nuclei. The method adopted here is essentially based on the technique recently developed by Lewis and Riles (1960) with a slightly modified version of the staining period. The procedure consists of feeding third instar larvae with a mixture of equal parts of 0.1% colcemide and yeast-sugar suspension solutions (A and B) for about 2.10 hours before dissecting the larval ganglion in hypotonic sodium citrate solution (C). After 10 minutes in the citrate solution, the ganglion was transferred to a drop of acetic-alcohol solution (D) for about 30 seconds. It was then transferred into a drop of staining solution E. After 15 minutes a drop of staining solution F was added to it. The ganglion was then covered with a coverslip and squashed about 15 minutes later by applying thumb pressure over it. The slide was sealed with nail polish.

This technique seems to facilitate the separation of sister chromatids so that acrocentric chromosomes appear V-shaped while metacentric chromosomes appear more or less X-shaped. Heterochromatic chromosomes usually appear more deeply stained than euchromatic chromosomes.

The best metaphase chromosome configurations were recorded photographically on Kodak 35 mm microfilm under oil immersion using a green filter.

Observations

Cytological Characteristics and Distributions of the Chromosome Types

Morphologically, chromosomes X and Y may have median or sub-median spindle attachments. One arm of the X chromosome is normally composed of euchromatin while the other arm is heterochromatic. Apparently, the Y is composed entirely of heterochromatin. The two arms of these chromosome types vary in their relative lengths according to the position of the centromere. These variations permit the identification of four types of X-chromosomes and three types of Y-chromosomes. Two types of 4th chromosomes have been recognised on the basis of the presence or absence of additional heterochromatic material to the normal dot chromosome.

Chromosome X. Type I (Fig. 1a and b) is slightly J-shaped. The short arm of the chromosome is apparently entirely heterochromatic while the long arm is euchromatic. It has been occasionally observed that the heterochromatic short arm varies slightly in length and thickness which may be due to variation in preparation procedure and stage

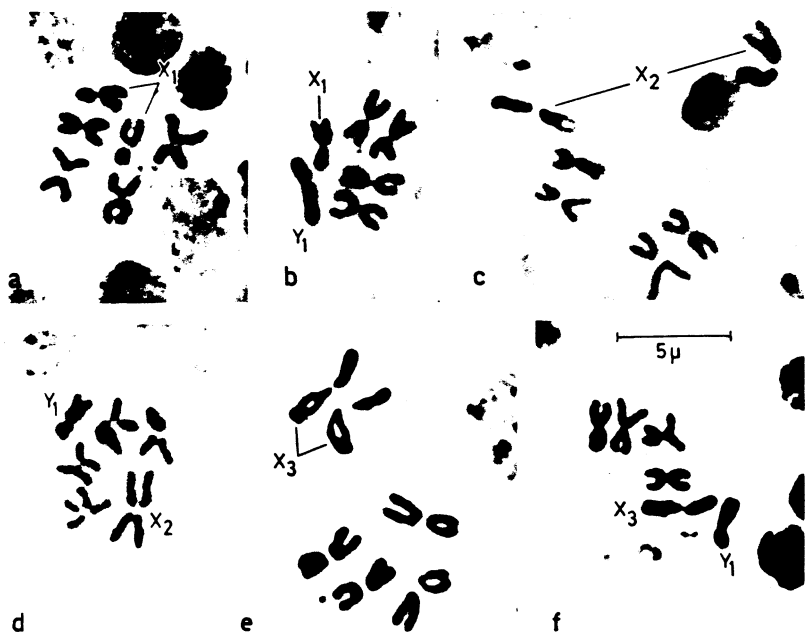


Fig. 1a—f. Photomicrographs of larval ganglion metaphase chromosome complements of *D. birchii*; X-chromosomes Type I (a and b), Type II (c and d) and Type III (e and f). The Y-chromosome Type I is shown in b, d and f

of mitosis. Generally, the ratio of the two arms of the chromosome is about 3:2. It is designated X_1 and has been found at Moresby, Popondetta, Bulolo, Daru and Wewak. It thus appears that Type I is widespread in the territory of Papua-New Guinea. Flies have been detected either homozygous for the X chromosome Type I or heterozygous for Types I and II or Types I and III. The former is common while the heterozygous conditions are less frequent.

Type II (Fig. 1c and d) is a metacentric chromosome, more or less V-shaped. One arm is entirely heterochromatic while the other arm is composed of euchromatin and heterochromatin in a 3:2 ratio. The euchromatic portion joins the distal end of the heterochromatic part which immediately links to the centromere. The heterochromatic parts of the chromosome, however, are morphologically homologous to the corresponding arms of the Y-chromosome Type I while the euchromatic portion appears to be a homologue of the corresponding arm of the Type I X-chromosome. It is referred to as X_2 and has been found at Moresby, Popondetta, and Bulolo in either the homozygous state or more frequently in the heterozygous condition with Type I (Fig. 2a).

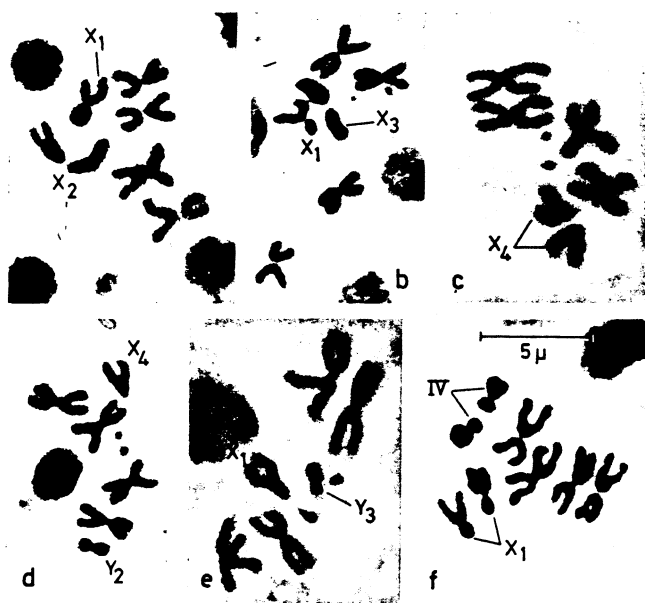


Fig. 2a—f. Photomicrographs of larval ganglion metaphase chromosome complements of *D. birchii*. a A female with X-chromosomes Types I and II; b a female with X-chromosomes Types I and III; c a female with X-chromosomes Type IV; d Type II Y-chromosome; e Type III Y-chromosome, and f the 4th chromosome Type II

Type III (Fig. 1e and f) is also a metacentric chromosome, slightly V-shaped. One arm of the chromosome is composed of heterochromatin while the other arm is a combination of euchromatin and heterochromatin similar to Type II. It differs from Type II in having the euchromatic portion directly attached to the centromere and the heterochromatic part is located distally to it. This chromosome is designated X_3 and has been recovered only once in the heterozygous state with Type I at Port Moresby (Fig. 2b). The homozygous stock for X_3X_3 obtained from inbreeding, however, shows somewhat less fecundity and viability than heterozygous or homozygous stocks for X_1X_1 or X_2X_2 . It thus appears that the homozygous condition for X_3X_3 is non-adaptive in nature.

Type IV (Fig. 2c and d) is a J-shaped chromosome similar to Type I except for its short arm which is entirely heterochromatic and is about half the length of the corresponding arm of the X_1 chromosome. It is designated X_4 and has been recorded only in the Rabaul and Cairns populations.

Hence X-chromosomes Types I, II and III are apparently confined to the territory of Papua-New Guinea while Type IV is restricted to Rabaul and Cairns populations. It is also noted that Type I is more common than Type II whereas Type III is rarely present in wild populations.

Chromosome Y. Type I (Fig. 1 b, d and f) was identified on the basis of its large J-shape with a submedian spindle attachment (referred to as Y_1). The ratio of the two arms is about 1:2. It is widely distributed within the territory of Papua-New Guinea but is absent at Daru.

Type II (Fig. 2d) is a small metacentric chromosome with a V-shaped appearance (referred to as Y_2). Each arm of this chromosome is apparently similarly homologous to the short arm of Type I Y-chromosome. Type II has been found only at Rabaul and Cairns. In fact the Y_2 and the X_4 are uniquely sex chromosomes in the Rabaul and Cairns populations. It is interesting to note that these two geographical regions are widely separated by water barriers, yet they exhibit uniformly these X and Y chromosome types.

Type III (Fig. 2e) is a more or less small J-shaped chromosome. The ratio of the two arms is approximately 1:2. The long arm of the chromosome appears to be about the same length as the short arm of the Type I Y-chromosome. It is designated Y_3 and so far has been recorded only at Daru.

Table. *Known distribution of D. birchii* X, Y and 4th chromosome types.
+ = presence, - = absence, ? = not known

Chromosome types	Moresby	Bulolo	Popondetta	We-wak	Daru	Rabaul	Cairns
X-chromosomes							
I	+	+	+	+	+	-	-
II	+	+	+	?	?	-	-
III	+	?	?	?	?	-	-
IV	-	-	-	-	?	+	+
Y-chromosomes							
I	+	+	+	+	-	-	-
II	-	-	-	-	-	+	+
III	-	-	-	-	+	-	-
4th chromosomes							
I (dot)	+	-	+	-	+	+	+
II (J-shape)	-	+	-	+	-	-	-
No. of strains tested	10	4	5	3	5	5	5

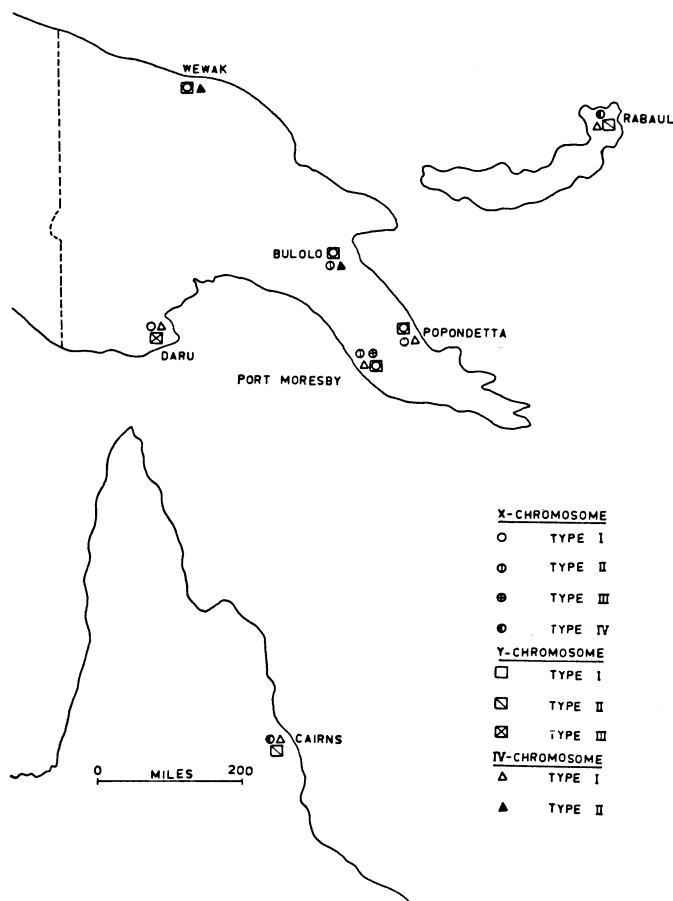


Fig. 3. Map illustrating known geographic distributions of *D. birchii* X, Y and 4th chromosome types in the Australia-New Guinea region

Chromosome IV. The 4th chromosome type found at Bulolo and Wewak differs from the normal dot chromosome (referred to as Type I) in being a small metacentric chromosome with one arm twice the length of the other (referred to as Type II) (Fig. 2f). Type II 4th chromosome is mainly composed of heterochromatic material and so far has been recorded only at Bulolo and Wewak. Thus Type II appears to be limited to the northern regions of the territory while Type I has an extensive range of distributions covering the southern and eastern parts of the species distribution including Cairns and Rabaul.

The information on the incidence of the X, Y, and 4th chromosome types is summarized in the Table. Fig. 3 presents a map showing the known distribution of *D. birchii* chromosome types.

In addition, hybrids between Types I and II or Types I and III X-chromosomes have been observed in wild populations and these are fertile in both sexes. Intercrosses between *D. birchii* strains of different geographic origins proceeded without difficulty and produced F_1 hybrids, under laboratory conditions (Baimai, 1969b). The Rabaul strain which is characterized by X_4Y_2 sex chromosomes yielded sterile F_1 male hybrids with all of the others while the Cairns strain (also with X_4Y_2 sex chromosomes) produced sterile F_1 male hybrids with those from Papua-New Guinea (except the Daru strain), when the Cairns males were involved in the crosses. On the other hand, the Daru strain which is characterized by X_1Y_3 sex chromosomes yielded fertile hybrids with both the Cairns and the Papua-New Guinea strains.

Chromosome Evolution in *D. birchii*

The available evidence indicates the relationships between given chromosome types in *D. birchii*. A probable cytological phylogeny of the *D. birchii* strains is illustrated in Fig. 4.

Considering the X_1 and Y_1 chromosomes as starting points the following schemes are postulated to account for the origins of the remaining X-chromosome types.

Type II X-chromosome may be simply derived from Type I if a chromosomal interchange between the X_1 and the Y_1 is postulated (Fig. 5). If the translocation occurs in a male possibly by simple heterologous crossing over, two possibilities have to be considered. As a rule, at least two breaks are involved in a reciprocal translocation. Thus, on the one hand, one break could have occurred at the proximal region close to the centromere of the long arm of the X_1 and the other one could be at the free end region presumably distal to the appropriate fertility factors of the short arm of the Y_1 . As a result, the euchromatic arm of the X_1 was transferred distal to the short arm of the Y_1 which has essentially remained intact to give rise to a more or less V-shaped chromosome X_2 (Fig. 5a). On the other hand while one break could have occurred at the free end region of the short arm of the X_1 , the other one would be at the proximal region close to the centromere of the long arm of the Y_1 . Accordingly, only the long arm of the Y_1 had been translocated to link distally to the short arm of the X_1 to produce a hypothetical chromosome which subsequently underwent a pericentric inversion converting it into a more or less V-shaped chromosome X_2 (Fig. 5b).

Interestingly enough, the resulting chromosome which is composed entirely of heterochromatin of either the short arm of the X_1 and the distal portion of the short arm of the Y_1 (Fig. 5a) or vice versa (Fig. 5b) morphologically shows some resemblance to chromosome Y_3 found at

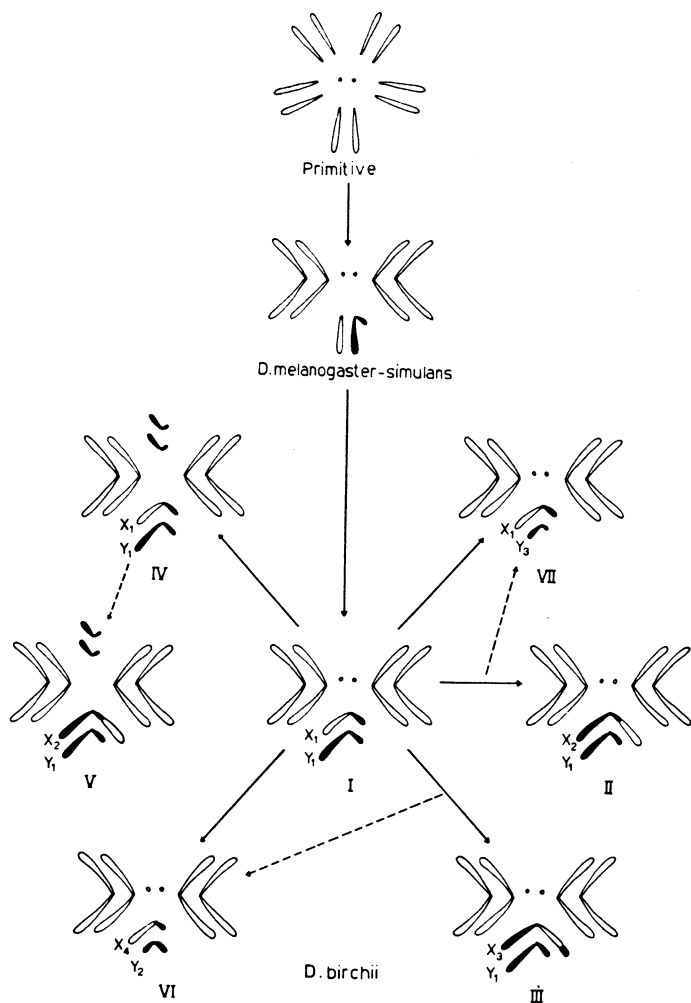


Fig. 4. Diagrammatic scheme for the probable cytological phylogeny of the *D. birchii* strains. Heterochromatin is shown in black

Daru. However, there is no available conclusive evidence as yet that they are the same elements.

Type III X-chromosome superficially appears to have been derived from chromosome X_2 by a paracentric inversion of the entire euchromatic arm including its heterochromatic part. Studies of salivary gland preparations of type III stock (obtained by inbreeding of the stock heterozygous for X_1X_3) and of the hybrid larvae between type III stock

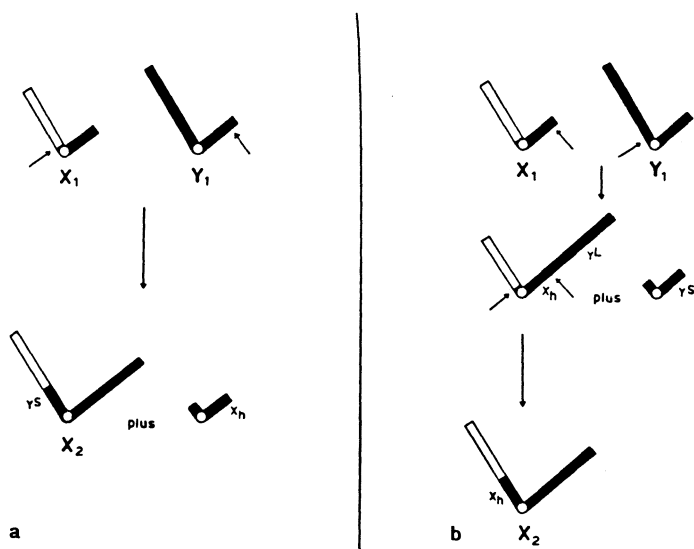


Fig. 5. Possible modes of origin of Type II X chromosome from the X₁ and the Y₁

and the standard strain reveal that this assumption proves to be deceiving since the gene order of chromosome X₃ is somewhat similar to that of other strains from the same locality (Moresby). Hence, the possibility of the occurrence of a whole arm paracentric inversion is excluded. Thus possible alternative schemes involving chromosomal interchanges between the X₁ and the Y₁ are proposed to account for the origin of the X₃.

If a structural change of the X—Y translocation type occurs, two alternative schemes are possible (Fig. 6a and b). Firstly, it is postulated that the points of breakage could have occurred at the proximal regions close to the centromeres of X₁ and Y₁ chromosomes. Recombinations of the chromosome parts would result in two metacentric chromosomes: small and large V-shaped chromosomes. The former would be entirely heterochromatic while the latter would be composed of one euchromatic and another heterochromatic arm (the intermediate X chromosome). A subsequent acquisition of heterochromatic material distal to the euchromatic arm of this chromosome would give rise to the chromosome X₃ configuration. It is probable that the additional heterochromatin could have been derived from a heterochromatic arm of either the X₁ or Y₁ chromosomes by means of a further chromosomal interchange (Fig. 6a).

Secondly, chromosome X₃ could have arisen through a series of simply crossovers between X₁ and Y₁ chromosomes (Fig. 6b), similar

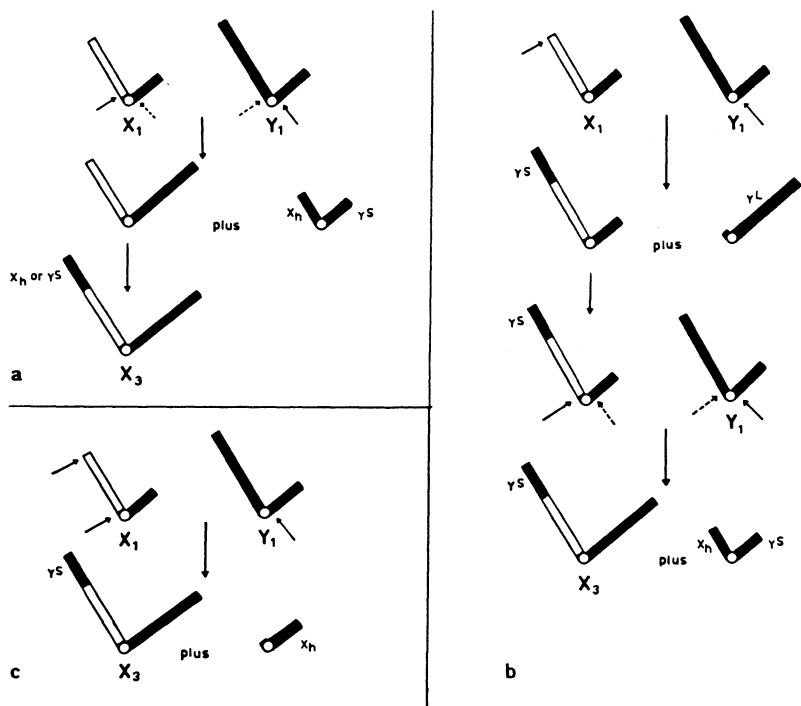


Fig. 6. Possible modes of origin of Type III X chromosome from the X_1 and the Y_1

to a model that has been proposed by Lindsley and Novitski (1959) to account for the results obtained in the induced attached XY chromosome in *D. melanogaster*.

It may be noted that the V-shaped heterochromatic chromosome resulting from the X—Y translocations proposed above appears to be similar to the Type II Y chromosome configuration. Nevertheless, it remains uncertain whether they are actually the same elements since there has been no available evidence as yet concerning the existence of chromosome Y_2 in the territory of Papua-New Guinea to give an indication of the trend of its general distribution. Additional samples from further collections for *D. birchii* will undoubtedly help to clarify this problem.

An alternative but much less probable interpretation would be to suppose that chromosome X_3 was derived from a male fly with X_1 and Y_1 sex chromosomes as a result of a direct insertion of the whole euchromatic arm of the X_1 into the Y_1 chromosome in between the proximal region of the short arm and the centromere (Fig. 6c). However, such

non-homologous chromosome displacement involving the shift of a whole chromosome arm is an unusual phenomenon in the genus.

Type IV X chromosome could have been simply derived from the X_1 chromosome if a deficiency for the distal part of the heterochromatic arm is postulated. If this occurs the lost part of the chromosome must be a genetically inert region so that the event could have happened without having a lethal effect. Such a deficiency in heterochromatic portions in the X of *D. melanogaster* has been demonstrated by Ger-shenson (1933). It is probable that the X_4 in *D. birchii* could have come about in this manner.

The modifications of chromosome Y morphology appear to be less complicated than those of the X chromosome types. Types II and III Y-chromosomes could simply derive from the Y_1 chromosome through the loss of part of the heterochromatic material to produce more or less V-shaped and J-shaped chromosomes respectively. Nevertheless, the lost heterochromatic portions must be devoid of Y-borne genes which are essentially fertility factors otherwise it would be a great disadvantage in terms of survival. Alternatively, these Y-chromosome types could have come about as the results of chromosomal interchanges between the X_1 and the Y_1 mentioned above. At present little can yet be said about the relationships of the different *D. birchii* Y-chromosome types.

It is obvious, of course, that the schemes of the derivation of various chromosome types in *D. birchii* X- and Y-chromosomes proposed are purely hypothetical, but they are the simplest explanations.

Finally, the 4th chromosome Type II (J-shaped) may be simply derived from Type I (a dot) if the acquisition of extra heterochromatic portions is postulated. This postulate is supported by the fact that the appearance of the short arm of Type II 4th chromosome in salivary gland chromosomes is the same as those of other strains of Type I since heterochromatin is not involved in increase in length and thickness in the polytene chromosomes. Thus it seems likely that Type II arose from Type I in this manner.

Discussion

Structural changes causing differentiation in metaphase chromosome morphology but having little or no effect on the giant chromosomes are of importance in chromosome evolution in the genus *Drosophila* (Patterson and Stone, 1952). Intraspecific karyotype variation, however, is not uncommon in the genus. For instance, at least four and possibly five karyotypes have been reported in *D. montium* (Kikkawa, 1936; Ward, 1949; Baimai, 1969a). Four different karyotypes have also been described in *D. micromelanica* (Ward, 1949).

In *D. birchii*, cytological evidence suggests that there are at least four types of X's, three types of Y's and two types of 4th chromosomes. Seven types of metaphase plate figures have been observed in wild populations as a result of combinations of these chromosome types (Fig. 4). Five of these figures have been recorded in the territory of Papua-New Guinea while the Rabaul and Cairns populations show a uniform metaphase plate figure, the sixth combination (X_4Y_2). The Daru population displays the seventh metaphase plate figure characterized by X_1Y_3 sex chromosomes. Furthermore, there is some indication that different chromosome types occurring in the wild populations exhibit a distinct geographic distribution. Thus Types I, II and III X-chromosomes and Type I Y-chromosome are confined to the territory of Papua-New Guinea while X_4 - and Y_2 -chromosomes are unique to Rabaul and Cairns populations. Type III Y-chromosome has been found only at Daru. Likewise, Type II 4th chromosome is apparently found to be the northern form whereas Type I is widespread throughout the southern and eastern regions of the species range. Hence the geographical variation in the chromosome types in *D. birchii* resembles the situation in *D. pseudoobscura* and *D. athabasca*.

Variation in the X-chromosome, however, is rarely present in *Drosophila*. Wharton (1943) reported a strain of *D. bifurca* differing from the other strains by a pericentric inversion in the heterochromatic region of the X-chromosome. According to Sturtevant (1942), the configuration with the slightly J-shaped X chromosome without the dot-like chromosome observed in *D. takahashii* is due to fusion of the X and the dot chromosomes. In some cases, for example in *D. miranda*, *D. americana* and *D. prosaltans*, the structural changes of chromosomes involving Y-autosome and/or X-autosome fusions or translocations do occur causing an evolutionary transformation from the XY sex chromosome mechanism into another (Patterson and Stone, 1952).

In *D. birchii*, the different types of X- and Y-chromosomes have been postulated to come about as the results of X-Y translocation or loss of a part of heterochromatin. It has been suggested, nevertheless, that crossing over between the X and the Y occurs spontaneously in *D. melanogaster* (Stern, 1929; Philip, 1934). Moreover, it has been demonstrated that crossing over between the X- and Y-chromosomes causing changes in the chromosome morphology can be induced by X-radiation in both males and females of *D. melanogaster* (Philip, 1935; Neuhaus, 1937; Stern and Doan, 1936). It is not known whether the naturally occurring chromosomal interchanges as proposed in *D. birchii* derived from a similar process as in the laboratory origins described in *D. melanogaster*.

The acquisition of extra heterochromatic material is common in the *Drosophila* species that exhibit either interspecific or intraspecific

karyotype variation, e.g. in the *D. melanica* group (Ward, 1949), in the *D. immigrans* group (Mather, 1962), and in the *D. melanogaster* group (see Patterson and Stone, 1952). Hence the postulate concerning the addition of heterochromatin to the normal dot chromosome in *D. birchii* seems not unreasonable in the light of similar events demonstrated in the genus.

None of the karyotype variations observed in *D. birchii* manifests relative gain or loss of a centromere as shown in the metaphase plate figures. In fact the species does exhibit the unusual naturally occurring X-Y chromosomal interchanges which have presumably become established in the wild populations. Such chromosomal rearrangements involving fusions or translocations, however, are known in several species of animals (White, 1954), but are relatively rare in the genus *Drosophila*. Thus the chromosomal variations in *D. birchii* indicated above would be another demonstration of the fixation of translocations in natural populations of *Drosophila* and possibly the only case of X-Y chromosomal interchanges encountered in the genus.

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Dr. V. Baimai
Department of Biology
Faculty of Science
Mahidol University
Rama 6 Road, Bangkok, Thailand