

# KARYOTYPE VARIATION AND GEOGRAPHIC DISTRIBUTION OF THE THREE SIBLING SPECIES OF THE *DROSOPHILA* KIKKAWAI COMPLEX

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A total of 191 samples of the three sibling species of the *D. kikkawai* complex were examined cytologically. These are: 107 samples of *D. kikkawai* from 25 different localities, 67 samples of *D. leontia* from 7 localities in Thailand, and 17 samples of *D. bocki* from 5 locations in Thailand and Taiwan. *D. kikkawai* exhibits 7 types of metaphase karyotype recorded so far, and is one of the most cytologically variable species in the genus *Drosophila*. Four different types of metaphase chromosome configurations have been encountered in this study, two of which are new records. Differences in the amount of extra heterochromatin present in the 4th chromosome are the chief cause of the metaphase karyotype variation in *D. kikkawai*. Each type of chromosome 4 seems to manifest a distinct geographic distribution which apparently reflects the mode of chromosomal evolution via the continuing process of acquisition of extra heterochromatin. Only two types of Y chromosome have been detected while the X chromosome is surprisingly uniform in general appearance. *D. leontia* and *D. bocki*, however, show uniform metaphase figures of the typical karyotype of the *D. montium* species subgroup.

## Introduction

Metaphase chromosome variation due to different amounts of heterochromatin is not an unusual phenomenon in higher organisms in both plants (Swanson, 1957) and animals (White, 1973). In the genus *Drosophila*, karyotype variation has been encountered not only among closely related members of several species groups but also within single species (Patterson

& Stone, 1952; Miller & Stone, 1962; Wilson et al., 1969; Baimai, 1969a).

This is a report on recent findings regarding intra-specific and interspecific variation in metaphase karyotype in the *Drosophila kikkawai* species complex belonging to the *montium* subgroup of the *melanogaster* species group. *D. kikkawai* was described by Burla (1954) from a strain from Brasil, South America, as a different species from its close relative, *D. montium*, which had been described by de Meijere in 1916 from Java. The latter species has not been collected since the original description while the former is regarded as sub-cosmopolitan (Bock & Wheeler, 1972). It is believed that earlier reports on *D. montium* were misidentifications for *D. kikkawai* (Kikkawa, 1936; Hsu, 1949; Ward, 1949; Baimai, 1969b, 1970). Recently, Tsacas & David (1977) described two sibling species of *D. kikkawai*: *D. leontia* from Malaysia Peninsula and the neighbourhood of Singapore, and *D. diplacantha*, a species endemic to the African continent. Baimai (1979) has recently discovered a new sibling species, *D. bocki*, from Thailand, which is believed to be common in the South-east Asian areas since it has been found sympatrically both with *D. kikkawai* in Thailand and Taiwan and with *D. leontia* in certain populations in Thailand according to the records so far. However, *D. kikkawai* is widespread, ranging from the Asian regions through the Pacific islands to South America. These three species are not easily distinguishable morphologically. Nevertheless, *D. kikkawai* differs in metaphase chromosomes from its two sibling species; the latter share the same metaphase karyotype (David et al., 1978; Baimai, 1979).

Cytologically, *D. kikkawai* is particularly interest-

Table 1

Metaphase types of the 4th and Y chromosomes and number of strains of the three sibling species of the *D. kikkawai* complex used in this study. L, M and S = large, medium and small respectively; m = metacentric (nomenclature after Levan et al., 1964); sm = submetacentric; D = dot chromosome; t = rod chromosome.

No. of isofemale lines examined	Localities and dates of collection (collectors)	Metaphase chromosome			
		4	Y	Type	
<i>D. kikkawai</i>					
8	Olimpia, Saõ Paulo, Brasil	Jan. 1978 (M. Belo)	Lm	m	VII
1	Londrina, Parana, Brasil	1974 (F. Sene)	Lm	m	
1	Saõ Paulo, Brasil	1974 (A. Malavasi)	Lm	m	
3	Belavista, Mato Grosso, Brasil	May 1977 (F. Sene et al.)	Lm	m	
2	Leticia, Colombia	Aug. 1960 (H.L. Carson)	Lm	m	
5	Oahu, Hawaii	Aug. 1975 (V. Baimai)	Lm	m	
3	Chungju, Seoul, Korea	1976 (O. Kitagawa)	Lm	m	
3	Naze, Amami-oshima Is., Japan	1976 (O. Kitagawa)	Lm	m	
1	Palau, Auluptagel Is.	Jan. 1968 (H.L. Carson)	Lm	m	
1	Kolonia, Ponape	Jul. 1959 (M. Wasserman)	Lm	m	
5	Puli, Nan-Tou, Taiwan	Feb. 1971 (Ting); Dec. 1977 (Lin)	Mm	m	III
5	Wulai, Taipei, Taiwan	Jul. 1972 (Lin, Tseng & Wang); Dec. 1977 (Lin)	Mm	m	
3	Mei-Shan, Chia-I, Taiwan	Jul. 1971 (Ting); Dec. 1977 (Lin)	Mm	m	
6	Ken-Ting, Pintung, Taiwan	Mar. 1971 (Lin); Dec. 1977 (Lin)	Mm	m	
1	Ma-Kong, Peng-Hu Is., Taiwan	Nov. 1971 (Teseng)	Mm	m	
1	Luzon, Philippines	May 1968 (L. Throckmorton)	Mm	m	
2	Townsville, Australia	1976 (I.R. Bock)	Mm	m	
1	Heron Is., Australia	1961 (H.L. Carson)	Mm	m	
5	Bangkok, Thailand	Jun. 1971 (V. Baimai)	Mm	m	
15	Samut Songkhram, Thailand	Aug. 1971 (V. Baimai)	Mm	m	
14	Songkhla, Thailand	Jan. 1976 (V. Baimai)	Mm	m	IV
18	Wangtakrai, Thailand	Nov. 1977 (V. Baimai)	Mm	m	
1	Madang, New Guinea	Aug. 1961 (M. Wasserman)	Sm	t	
1	Wau, New Guinea	Nov. 1977 (H.L. Carson)	Sm	t	VI
1	Goroka, New Guinea	Oct. 1961 (H.L. Carson)	sm	t	
<i>D. leontia</i>					
14	Chiangmai, Thailand	May 1971 (V. Baimai)	D	m	
5	Surathanee, Thailand	Sept. 1971 (T. Srikiow)	D	m	
20	Karnchanaburi, Thailand	Jul. 1978 (V. Baimai)	D	m	
2	Lamtakong, Thailand	Aug. 1977 (V. Baimai)	D	m	
14	Wangtakrai, Thailand	Nov. 1977 (V. Baimai)	D	m	
10	Songkhla, Thailand	Jan. 1976 (V. Baimai)	D	m	
2	Phuket, Thailand	Jan. 1976 (V. Baimai)	D	m	
<i>D. bocki</i>					
5	Khao Yai, Thailand	Mar. 1971 (V. Baimai)	D	m	
2	Lamtakong, Thailand	Aug. 1977 (V. Baimai)	D	m	
5	Wangtakrai, Thailand	Nov. 1977 (V. Baimai)	D	m	
2	Yun-Shui, Chia-I, Taiwan	Feb. 1974 (Lin & Wang); Dec. 1977 (Lin)	D	m	
3	Mei-Shan, Taiwan	Nov. 1967 (Lin); Dec. 1977 (Lin)	D	m	

ing since it displays karyotype variation among the strains from Sabah (Baimai, 1969b). The present article provides additional data on two new metaphase figures and the geographic distributions of the different metaphase karyotypes of *D. kikkawai* in comparison with those of its sibling species.

## Material and methods

All strains of the *D. kikkawai* species complex from Thailand were derived from individual wild-caught females which had been inseminated in nature prior to capture. These stocks were from collections made by the author. Other strains of different geographic origins employed in this study were kindly provided by various persons (see Acknowledgements and Table 1).

Squash preparations of ganglion metaphase plates were made from third instar larvae after pretreatment with Colcemid in Hanks Balanced Salt Solution (Gibco) and Giemsa staining based on the technique described by Baimai (1977).

## Results and discussion

*D. bocki* and *D. leontia* share the same metaphase karyotype which is the basic configuration of the *montium* subgroup. It consists of 2 pairs of metacentric chromosomes (Nrs. 2 and 3), 1 pair of sex chromosomes, and 1 pair of dots (microchromosome 4). The metacentric Y chromosome is almost entirely heterochromatic and can be easily recognized (Fig. 1). No variation in metaphase chromosomes has been observed in these two species. Thus they are quite similar both in morphology and karyotype. The metaphase karyotype of *D. leontia* was described by David et al. (1978).

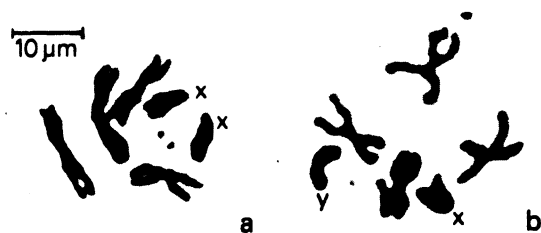


Fig. 1a-b. Metaphase karyotype of *Drosophila bocki*: (a) female; - (b) male.

Many strains from different geographic origins throughout the distribution range of *D. kikkawai* were available for cytological investigation in this study. Two more metaphase karyotypes, with respect to the 4th and/or Y-chromosomes, have been discovered in this study in addition to the 5 types of metaphase karyotypes previously described by many authors (Kikkawa, 1936; Ward, 1949; Baimai 1969b; David et al. 1978). This makes a total of 7 types of metaphase karyotype recorded so far. Thus *D. kikkawai* is one of the most cytologically variable species of *Drosophila*, resembling *D. birchii* in this respect (Baimai, 1969a). However, only 4 types of metaphase karyotypes have been found in this investigation. Since there is still confusion as to the morphology of metaphase chromosome figures existing in *D. kikkawai*, it is necessary to redescribe the possible 7 types of metaphase karyotype based on the original observations made by different authors with comments on interpretations as follows:

Type I was originally observed and described by Ward (1949) from the China strain, consisting of 2 pairs of metacentric chromosomes (Nrs. 2 and 3), 1 pair of acrocentric (rod-shaped) chromosomes (Nr. 4) and 1 pair of sex chromosomes. The X and Y chromosomes were described as acrocentric. This type of metaphase chromosome has not been detected since the original description.

Type II corresponds to that of type B as described by both Kikkawa (1936) and Ward (1949). It exhibits 2 pairs of metacentric chromosomes (Nrs. 2 and 3), 1 pair of acrocentric chromosomes (Nr. 4) similar to those of Type I, and 1 pair of heteromorphic sex chromosomes. The Y-chromosome was clearly described to be metacentric and heterochromatic. Kikkawa (1936) previously recorded this type of metaphase from Matsuyama (Sikoku), Japan. Type II has also not been observed in this study. If the interpretations of metaphase figures of both Types I and II are correct they appear to be confined only to certain parts of China and Japan respectively.

Type III is generally similar to Type II described above, except for the 4th chromosome which is a medium size metacentric (Fig. 2a and b). This type of metaphase corresponds to type A, as described by Kikkawa (1936) from materials from Shuri, (Okinawa) and Fukuoka, Japan. It may be noted that this type of metaphase chromosome is widely distributed in the Australian and the Asian regions, ranging from

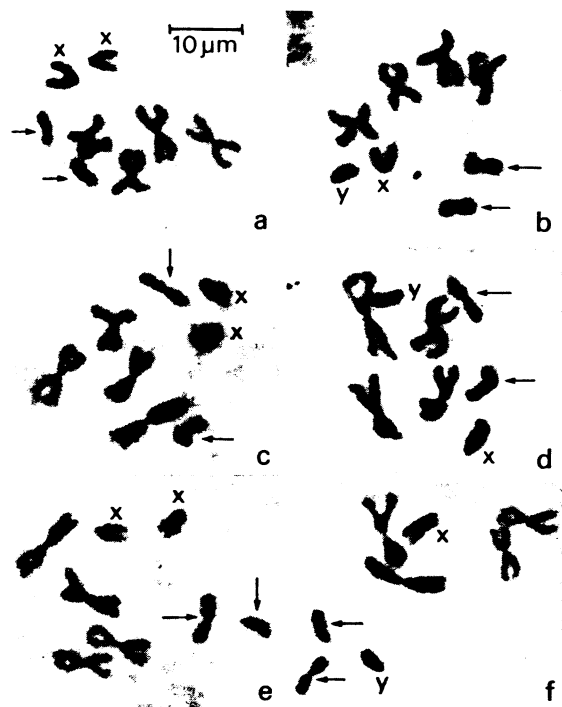


Fig. 2a-f. Photomicrographs of metaphase chromosomes of *Drosophila kikkawai*: (a-b) Type III, female and male respectively; - (c-d) Type VII, female and male respectively; (e-f) metaphase chromosomes of, respectively, female and male hybrid larvae between Types III and VII. The 4th chromosomes are indicated by arrows.

Heron Island and Townsville in Australia to Sabah (East Malaysia), Thailand, Philippines and Taiwan.

Type IV differs distinctively from Types II and III in the 4th and Y-chromosomes. The 4th chromosome is metacentric and smaller than that of Type III, which can be clearly seen in metaphases of hybrids between strains of these two types (Baimai, 1969b). The small metacentric chromosome 4 is clearly seen in general preparations (Fig. 3a and b). Occasionally, it appears as a large dot. The Y is an acrocentric chromosome. Type IV has been found only at Madang and Wau (New Guinea).

Type V was previously reported by Baimai (1969b) from the Kota Kinabulu strain, Sabah. It only differs from IV in the 4th chromosome which, in this case, is a very small metacentric (Baimai, 1969b). The 4th chromosome normally appears as a quite sizeable dot which could easily be misinterpreted as similar to the normal dot found in metaphase chromosome figures of *D. leontia* and *D. bocki*. Unfortunately,

there is no strain from Kota Kinabalu available for chromosome comparison in this study.

Type VI closely resembles Type IV in general appearance. It differs only slightly from Type IV in the 4th chromosome which, in this case, is a small submetacentric chromosome (Fig. 3c and d). In general preparations, it frequently appears as a large dot chromosome which could be misinterpreted as Type IV. The difference between Types IV and VI can be clearly seen in hybrids between them (Fig. 3e and f). This is a new metaphase karyotype discovered in this study from the Goroka strain (New Guinea). It is likely that both Types IV and VI are restricted only to the territory of New Guinea.

Type VII is also a new metaphase karyotype discovered in the present study. Generally, this type is very similar to Type III. The only difference is found in the 4th chromosome which has been transformed into a large metacentric configuration (Fig. 2c and d). This may be simply due to the addition of more extra

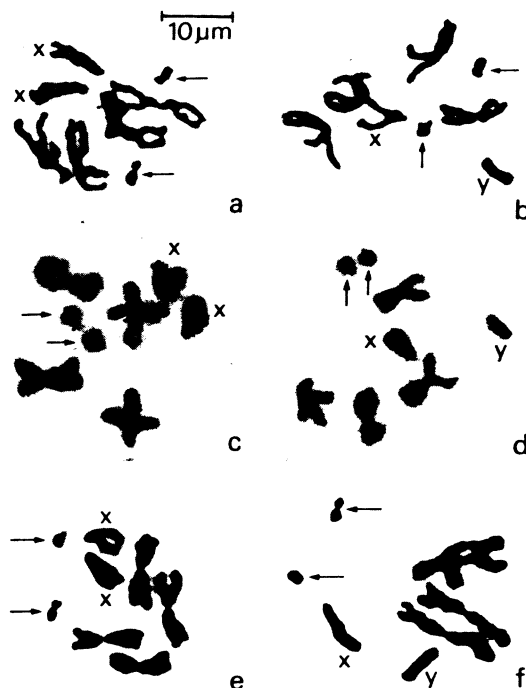


Fig. 3a-f. Photomicrographs of metaphase chromosomes of *Drosophila kikkawai*: (a-b) Type IV of female and male respectively; - (c-d) Type VI of female and male respectively; (e-f) metaphase chromosomes from respectively, female and male hybrid larvae between Types IV and VI. The 4th chromosomes are indicated by arrows.

heterochromatic material to the ancestral form of Type III. It is very difficult, if not impossible, to tell these two types apart if one just looks at each metaphase at a time. However, the difference can be easily seen in hybrids between these two types (Fig. 2e and f). Type VII is recorded in the strains from Korea and Naze (Amami-oshima island, Japan) and is widespread throughout the islands in the Pacific ocean e.g. Palau, Ponape and Hawaii Archipelagoes to Colombia and Brasil in South America.

Variation in metaphase karyotype in *Drosophila* due to the acquisition of extra heterochromatic portions generally involves the dot microchromosomes as well as the sex chromosomes (Patterson & Stone, 1952; Clayton & Wheeler, 1975). Miller & Roy (1964) described distinct geographic distributions of

different forms of Y chromosome in *D. athabasca*. Four different kinds of X, three forms of the Y and two forms of chromosome 4 have been found in *D. birchii* from Northern Australia and the Papua-New Guinea regions (Baimai 1969a). These chromosome types form seven distinct metaphase figures found in natural populations. Karyotype variation concerned with the Y and the 4th chromosomes has been reported in certain strains of *D. albomicans* (Wilson et al., 1969). A similar situation has been encountered in *D. kikkawai*. Regarding the metaphase chromosome types, the present data reveal a pattern which suggests that the karyotypes have distinct geographic distributions.

Figure 4 is a diagram showing the distributions of different metaphase karyotypes found in *D. kikkawai*.

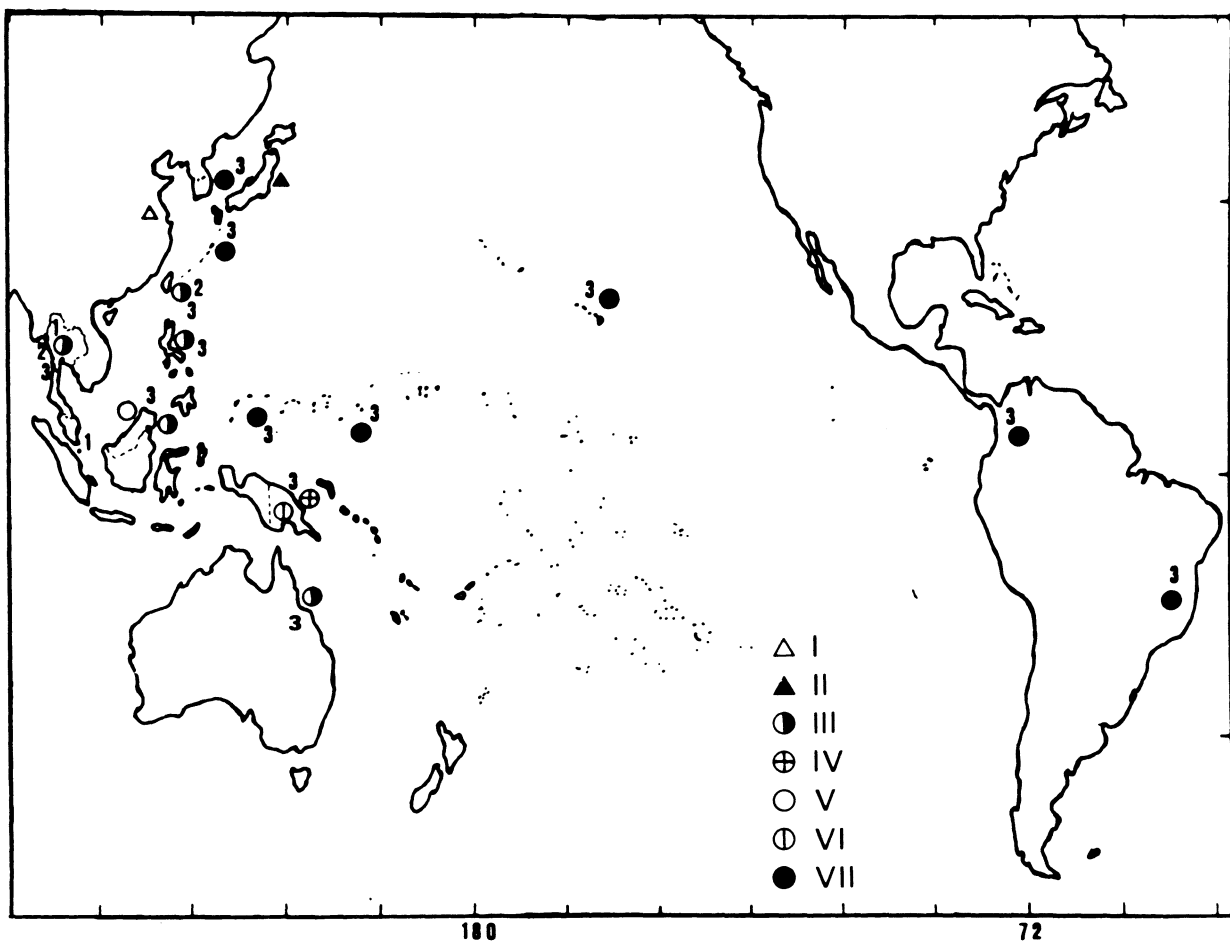


Fig. 4. Geographic distributions of the 7 karyotypes found in *Drosophila kikkawai* and ranges of the three sibling species of the *D. kikkawai* complex: 1 = *D. leontia*; 2 = *D. bocki*; 3 = *D. kikkawai*

Types IV and VI appear to be limited to New Guinea, while Type III is common in the Australian and the Asian regions. Type VII is widely distributed across the Pacific areas to South America. A reasonable assumption at this stage is that the chromosomal evolution of the *D. kikkawai* species complex could have occurred in the Asian regions.

Cytological evidence seems to suggest that acquisition of extra heterochromatin is not an unusual phenomenon in chromosomal evolution in animals. It is interesting to note that the normal dot-shaped 4th chromosome has never been observed in any of the strains of *D. kikkawai* available for this study. It is possible that this type of chromosome-4 configuration is very rare or not even existing in *D. kikkawai*. If this is the case, it may be suggested that either *D.*

*leontia* or *D. bocki* could be an ancestor of *D. kikkawai* since the former two species have maintained a metaphase karyotype typical of the *montium* species subgroup viz. 2 V's, 1R and 1D. The Kota Kinabalu strain is the one which manifests a metaphase karyotype most closely resembling the typical one as seen in the two sibling species. Thus the metaphase karyotype found in Kota Kinabalu could represent the transitional form in chromosomal evolution in *D. kikkawai* by the continuing process of acquisition of heterochromatin (Fig. 5).

In addition, Baimai (1969b) demonstrated that the Kota Kinabalu strain showed strong sexual isolation from the Tawau and Sandakan strains (Type III) also from Sabah, but was nevertheless cross-fertile with the strains from Madang and the Philippines.

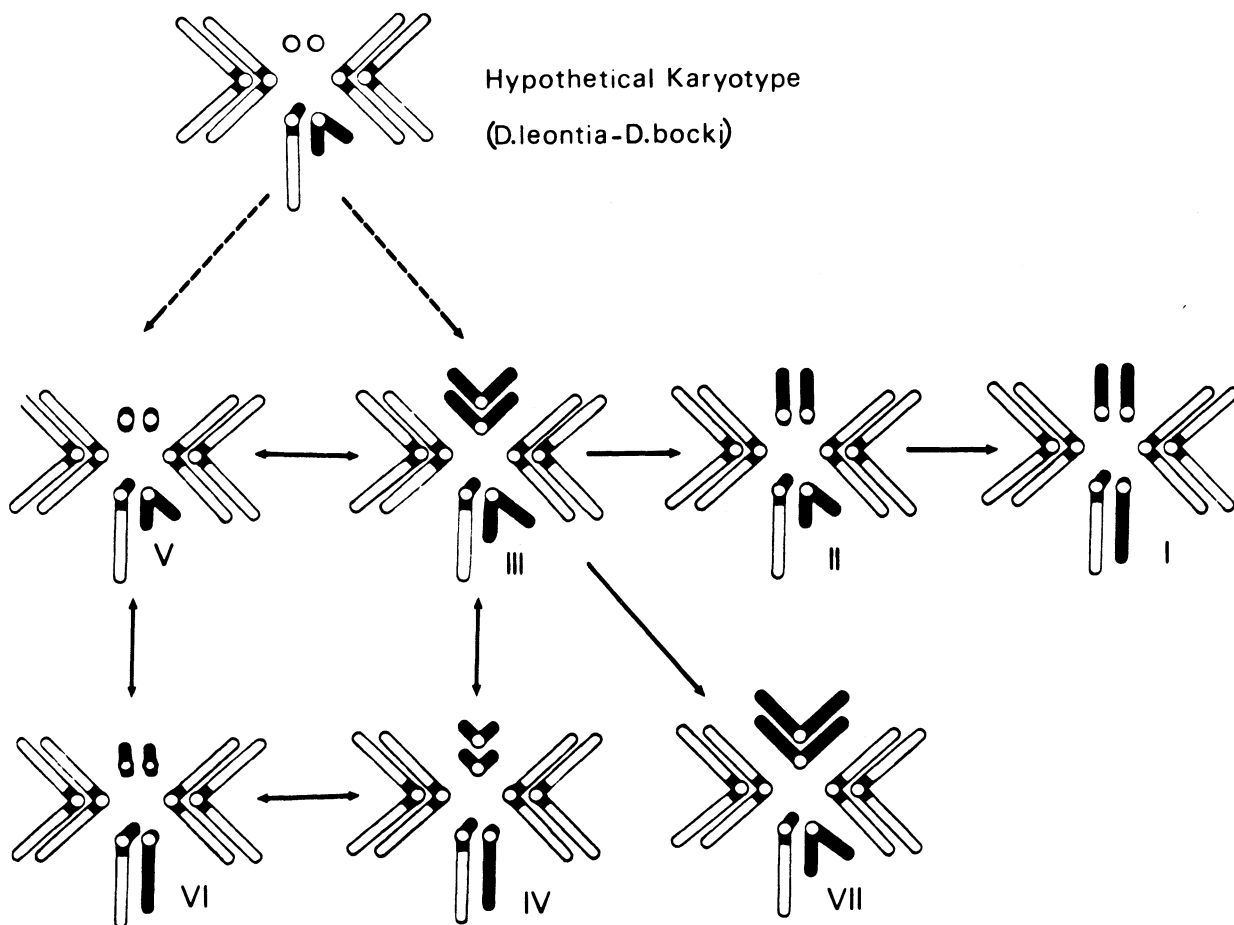


Fig. 5. Diagram showing the metaphase karyotypes and the possible pathways of chromosomal evolution via the process of gain or loss of extra heterochromatin and/or inversion involving heterochromatic blocks in the 4th and Y chromosomes of *Drosophila kikkawai*.

This seems to suggest that the Kota Kinabalu strain could be a different species.

The metaphase karyotype of *D. kikkawai* that is next close to the typical form has been observed in the strain from Goroka (New Guinea), which contains considerably more heterochromatic material transforming the 4th chromosome into a small submetacentric chromosome. However, the Y chromosome is an acrocentric which is markedly different from the typical metacentric form. Further, differentiation of the 4th chromosome in *D. kikkawai* has been found in the strains from Wau and Madang, which clearly exhibit a small metacentric (Sm) chromosome. This type of chromosome-4 morphology could have occurred either by a simple pericentric inversion or by addition of an extra heterochromatic portion onto the short arm of the ancestral submetacentric chromosome. The latter interpretation is more likely because the small metacentric found in the Wau and Madang strains contains relatively more heterochromatic material than those shown in the Goroka strain. A similar process of acquisition of extra heterochromatin in the 4th chromosome of *D. kikkawai* could have continued producing two more distinctive types of metaphase chromosomes. The medium metacentric (Type III metaphase karyotype) is quite common in the Asian regions whereas the large metacentric (Type VII) is widely distributed throughout the Pacific islands and is apparently the only type of metaphase chromosome that occurs in the South American continent. It is suggested that some of these metaphase karyotypes may really represent different species which have yet to be described when supporting evidence becomes available.

In addition, the submetacentric X chromosome consisting of a long euchromatic and a short heterochromatic arm is uniform in appearance in all three sibling species. Nevertheless, the acrocentric Y chromosome of *D. kikkawai* is less common than the V-shaped one. Such difference in Y chromosome could have occurred by addition of extra heterochromatin or simply by pericentric inversion. It is well documented that the process of acquisition of extra heterochromatin plays an important role in metaphase chromosome variation in animals (White, 1973) and in particular in *Drosophila* species that manifest intra-specific metaphase variation (Patterson & Stone, 1952; Clayton & Wheeler, 1975). The evidence demonstrated in *D. kikkawai* is another interesting

example of this general phenomenon commonly found in insects and perhaps in other higher organisms as well.

A somewhat similar situation has been encountered in the picture-winged group of Hawaiian *Drosophila*. Three closely related species, *D. bostrycha*, *D. disjuncta* and *D. affinidisjuncta*, are morphologically indistinguishable. Yet they exhibit strikingly different metaphase karyotypes which are mainly due to the differences in the amount of heterochromatin present in the major chromosomes (Baimai & Ahearn, 1978; Hardy 1978). This interesting phenomenon of the presence or absence of heterochromatic portions correlated with the process of species differentiation has been found also in the well-defined species of the Hawaiiensis subgroup of Hawaiian *Drosophila* (Baimai, unpublished data).

It is believed that heterochromatin plays a significant role in chromosomal evolution and is possibly involved to some extent in species differentiation in higher organisms (Yunis & Yasmineh, 1971). Nevertheless, little is known about the circumstances by which heterochromatin arises. What might be the possible reason for the origin and subsequent maintenance of heterochromatin in a chromosome? This important problem remains unanswered. In this regard, Baimai (1975 and 1977) has recently discovered a clear-cut relationship between extra heterochromatin and certain inversions involving long segments of a chromosome in Hawaiian *Drosophila*. It has been suggested that a chromosome break occurring in the neighbourhood of centromeric heterochromatin may have an important cytological effect by provoking the production of heterochromatic material in the affected region of the chromosome.

The results present in this study show that *D. kikkawai* is certainly a good candidate for the study of the process of chromosomal evolution involving the gain or loss of heterochromatic material. Further detailed investigation into this aspect may throw some light on the role of heterochromatin in species differentiation in higher organisms.

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