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METAPHASE KARYOTYPES OF CERTAIN SPECIES OF THE *DROSOPHILA MONTIUM* SUBGROUP

VISUT BAIMAI

Department of Biology, Faculty of Science, Mahidol University,
Rama 6 Road, Bangkok 4, Thailand

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A total of 20 species in the *Drosophila montium* subgroup has been cytologically examined. All species show similar basic pattern of metaphase karyotype. The most extensive variations in metaphase chromosome configurations have been observed in the Y and 4th (dot) chromosomes while the X chromosome is slightly variable. Interspecific karyotype differentiation is largely due to the acquisition of different amounts of heterochromatin.

INTRODUCTION

The *Drosophila montium* subgroup, belonging to the *melanogaster* species group of the genus *Drosophila*, comprises some 58 described species (Bock and Wheeler 1972; Bock, personal communication). Almost all species of this subgroup are characterised by a large sex-comb on the male foreleg. Most of the members of the subgroup are very similar in external morphology. Classification of the species is essentially based on the structure of male genitalia, which are quite distinct in most cases but indistinguishable in some sibling species complexes. Two such sibling species of the *D. kikkawai* complex from the Southeast Asian region have recently been described based on the evidence of cytogenetic studies (Tsacas and David 1977; Baimai 1979).

Some information on metaphase karyotypes of 22 species of the *montium* subgroup has been compiled in an extensive catalog recently prepared by Clayton and Wheeler (1975). There are only two species of this subgroup that have been extensively investigated cytologically, i.e. *D. birchii* and *D. kikkawai* (Baimai 1969a, 1969b, 1978; Baimai and Chumchong 1980). These two species strikingly exhibit intraspecific variation in metaphase chromosome configurations which is mainly due to the different amount of extra heterochromatin particularly involving the sex chromosomes and the small 4th chromosome. Although metaphases of members of the *montium* subgroup had been included in some cases in the species descriptions, detailed accounts and photographs of the metaphase chromosome configurations suitable for cytological comparisons have been largely lacking. The aim of this study is to reinvestigate metaphase karyotypes of certain species of the *montium* subgroup available in the laboratory to determine: (a) details of chromosome configurations, e. g. the amount of extra heterochromatin and secondary constrictions, (b) interspecific variations of metaphase karyotypes, and (c) possible intraspecific variations in addition to those of the two species already known. This is a report on recent findings and interpretations regarding metaphases of some members of this interesting subgroup of the genus *Drosophila*.

MATERIALS AND METHODS

Most of the laboratory culture stocks of the *montium* species subgroup available for this study were kindly provided by The Genetics Foundation, University of Texas at Austin. Two newly described species were collected from wild populations in Thailand by the author and his colleagues (Table 1).

Preparations of metaphase karyotypes were made from 3rd instar larval brain ganglions using the standard method of Lewis and Riles (1962) and the technique recently described by Baimai (1977), consisting essentially of pretreatment of the brain ganglion by 0.1% colchicine solution or 10 mcg/ml of colcemid in Hanks' Balanced Salt Solution (Gibco) before proceeding to the routine lactic-acetic-orcein squash preparations or heat-dry method. These procedures yielded a large number of well-spread metaphase chromosomes favourable for detailed analysis and photographic comparison. Photomicrographs of metaphase chromosomes were taken by Kodak High Contrast Copy Film under oil immersion (670 magnification) with green filter.

RESULTS AND DISCUSSION

The results of this study revealed that there are no major changes in metaphase karyotypes of the 20 species of the *montium* subgroup studied. All species showed the basic pattern of metaphase karyotype of the *melanogaster* species group, viz., one pair of sex chromosomes, two pairs of large V-shaped autosomes and one pair of dot-like 4th chromosomes. Results reported by Clayton and Wheeler (1975), although 22 species could be compared, were basically consistent with the present study. Changes in metaphase chromosome configurations of these species are entirely due to the process of acquisition of extra heterochromatin particularly involving the 4th and/or Y chromosomes and to a lesser extent in the X chromosome. Interspecific variation in metaphase figure can be described as follows (see Table 1):

X chromosome

The general configuration of the X chromosome is submetacentric (J-shaped) comprising one short arm which is totally heterochromatic and one long arm which contains mainly euchromatin. Metaphase variation has been observed in the short arm of the X chromosome of *D. baimaii*, *D. mayri* and *D. pseudomayri* which generally show a large submetacentric (LJ) shape. Apparently, the short arm of the X chromosome found in the latter two sibling species is relatively longer than that of *D. baimaii* which in turn contains more heterochromatin than that of the normal type (Figs. 6, 7, 8). This feature of the X chromosome serves as a diagnostic cytological characteristic

Table 1. List of species of the *montium* subgroup used in the present study and the metaphase figures of X, Y and 4th chromosomes

Species	Stock no.*	Locality	Original descriptions***	X	Y	4th
1. *D. auraria*	T3040.15	Nappora, Japan	Peng 1937	J	SJ	D
2. *D. baimaii*	T3033.24	Khao Yai, Thailand	Bock and Wheeler 1972	LJ	LJ**	D
3. *D. barbarae*	T3033.21	Malaya (Malaysia)	Bock and Wheeler 1972	J	LJ	D
4. *D. birchii*	—	New Guinea	Dobzhansky and Mather 1961	J	LJ	D
5. *D. bocki*	A01B3	Khao Yai, Thailand	Baimai 1979	J	LV	D
6. *D. dominicana*	T3029.4	Madang, New Guinea	Ayala 1965	J	MV**	SV
7. *D. jambulina*	T3120.5	Cambodia	Parshad and Paika 1964	J	MV	D
8. *D. kikkawai*	A03B7	Bangkok, Thailand	Burla 1954	J	LV	MV
9. *D. lacteicornis*	—	Okinawa, Japan	Okada 1965	J	R	LD
10. *D. leontia*	A02B11	Chiangmai, Thailand	Tsacas and David 1977	J	LV	D
11. *D. lini*	T3146.1	Taiwan	Bock and Wheeler 1972	J	SJ	R
12. *D. mayri*	T3020.6	New Guinea	Mather and Dobzhansky 1962	LJ	LJ	D
13. *D. nikananu*	T2371.5	Ivory Coast, Africa	Burla 1954	J	MV	D
14. *D. orosa*	T3250.17	Khao Yai, Thailand	Bock and Wheeler 1972	J	LJ	D
15. *D. pennae*	T3028.1	New Guinea	Bock and Wheeler 1972	J	SV	LD
16. *D. pseudomayri*	—	Bulolo, New Guinea	Baimai 1970	LJ	LJ	D
17. *D. punjabiensis*	T3033.4	Malaya (Malaysia)	Parshad and Paika 1964	J	R	D
18. *D. rufa*	T3040.16	Japan	Kikkawa and Peng 1938	J	SJ**	MD
19. *D. seguyi*	T3254.1	Rhodesia, Africa	Smart 1945	J	LJ	D
20. *D. serrata*	T2404.6	Queensland, Australia	Malloch 1927	J	SV	LD

D=dot; MD and LD=medium and large dots; R=rod shape; J=J-shape; SJ=and LJ=small and large J-shape; SV, MV and LV= small, medium and large V-shape.

* Stock no. with T indicates catalog no. of The Genetics Foundation, University of Texas at Austin.

** The chromosome exhibits a secondary constriction.

*** Most of these references were quoted in Bock and Wheeler (1972).

for these species. It seems, therefore, that the X chromosome is more uniform with respect to the acquisition of the amount of heterochromatin than the Y and 4th chromosomes in the *montium* subgroup.

Y chromosome

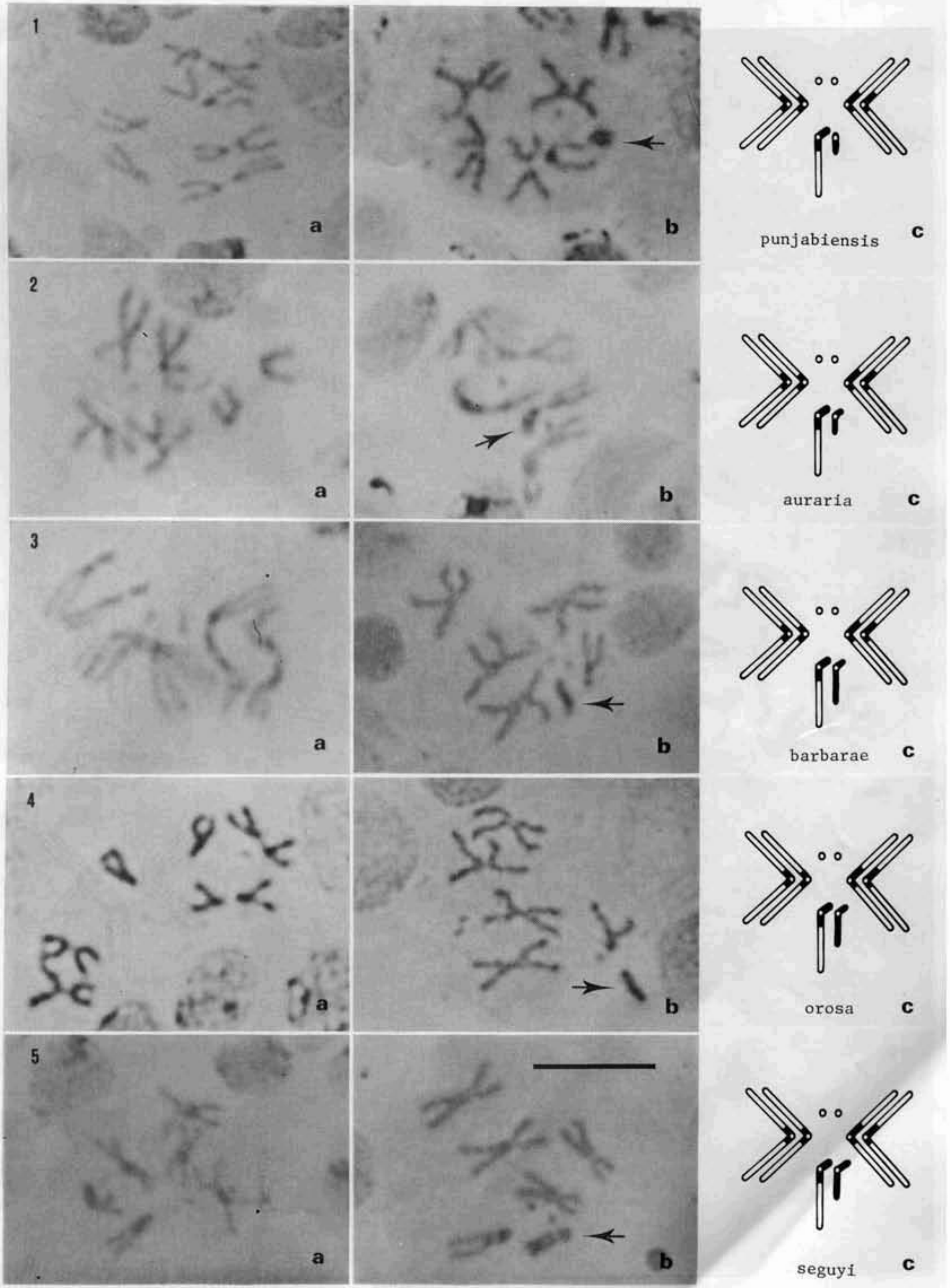
Among the 20 species of the *montium* subgroup in the present investigation, the most striking interspecific variation in metaphase has been encountered in the Y chromosome which is, as a general rule, almost entirely heterochromatic. The pattern of Y chromosome variation of both size and shape is, of course, due to the different amount of heterochromatin. Six types of Y chromosomes can be recognized and simply described as follows:

1. Rod shaped (acrocentric or R) chromosome is the smallest and hence the most simple form of Y chromosome configuration. This type has been detected in two species, i.e. *D. punjabiensis* and *D. lacteicornis* (Figs. 1, 17).

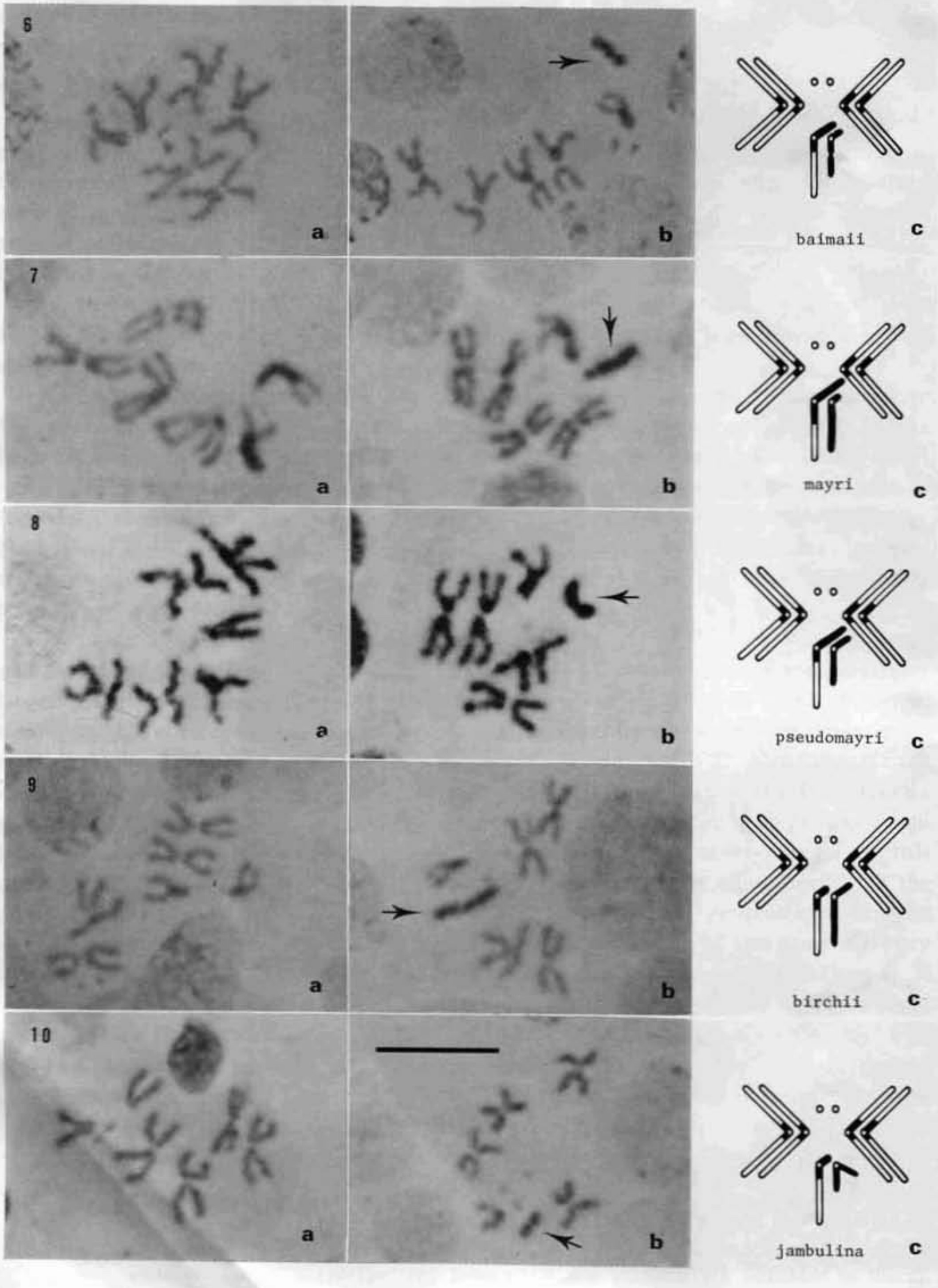
2. Small J-shaped (submetacentric or SJ) Y chromosome has been encountered in *D. auraria* and *D. lini* (Figs. 2, 18). This type of Y chromosome obviously differs from the rod shape by the acquisition of an extra heterochromatic portion in the short arm. The Y chromosome of *D. rufa* is also regarded as a small J-shape but with a secondary constriction occurring at the middle of the long heterochromatic arm (Fig. 14). Hence the Y chromosome in this case appears to consist of 3 approximately equal segments of heterochromatin. It can be easily recognized and has been consistently observed in most preparations.

3. Large J-shaped Y chromosome (LJ) has been found in *D. barbarae*, *D. orosa* and *D. seguyi* (Figs. 3, 4, 5). Obviously, this type of Y chromosome is comparatively larger than the small J-shape particularly in the long heterochromatic arm. However, the J-shaped Y chromosome of *D. baimaii* consistently exhibits a secondary constriction at the middle of the long arm (Fig. 6). Moreover, *D. mayri* and *D. pseudomayri* each manifests a considerably larger J-shaped Y chromosome than that of the four species mentioned above. Apparently, the long arm of Y chromosome found in *D. mayri* contains relatively more heterochromatic material than that of the other species of this category (Fig. 7) while the short arm remains similar to that of the others. On the other hand, the short arm of the Y chromosome observed in *D. pseudomayri* consists of relatively more heterochromatin than that of other species of the same category (Fig. 8) while the long arm is apparently similar to that of the others. Thus it is interesting to note that these two sibling species which are sympatric in certain populations in New Guinea are recognizably different in the Y chromosome. This difference could be the result of a pericentric inversion or simply the acquisition of extra heterochromatin in the ancestral Y chromosome. This is not an uncommon phenomenon in animals especially in insects (White 1973).

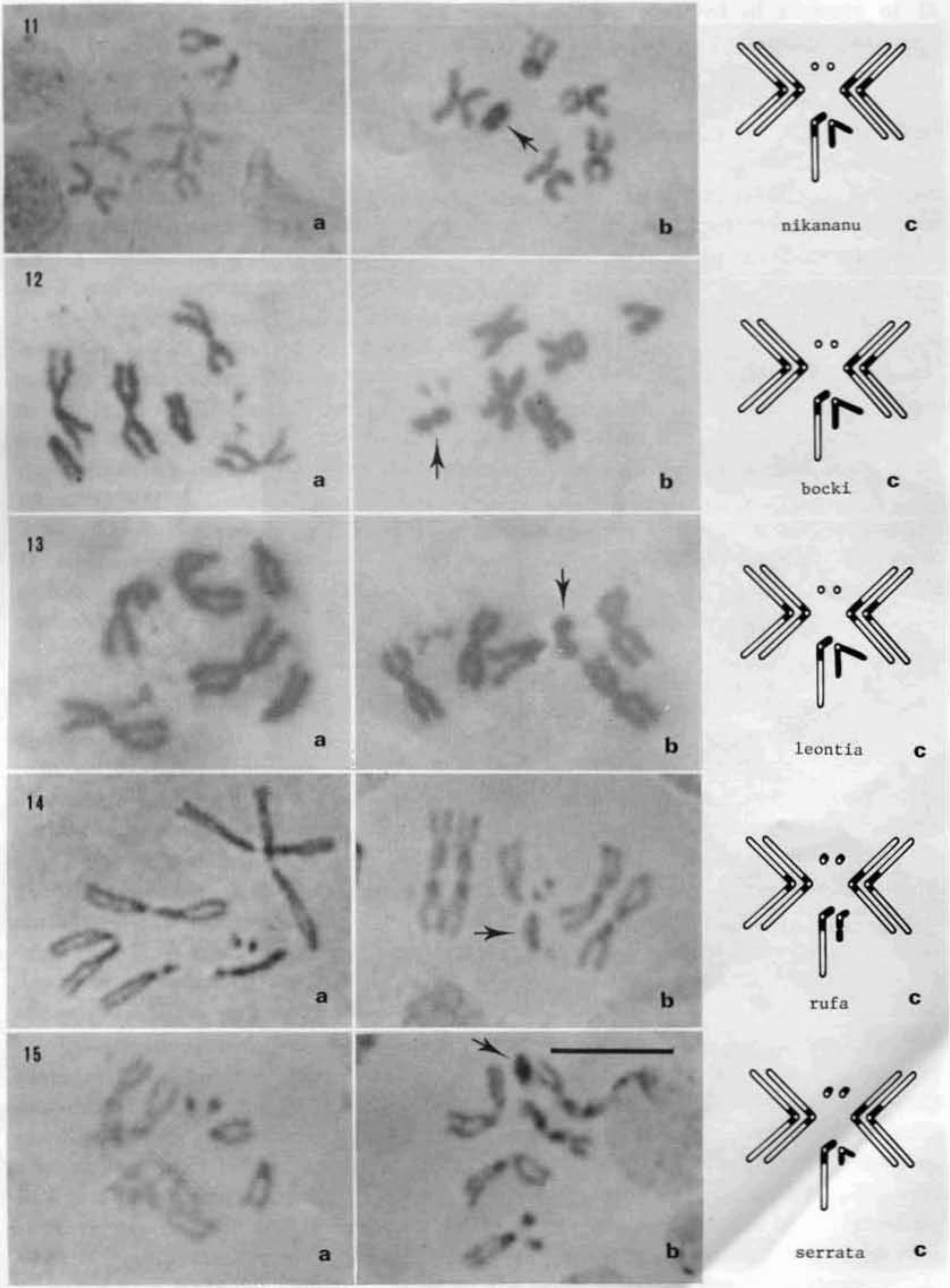
Figs. 1-20. Photomicrographs depict larval metaphase chromosome configurations of the 20 species examined in this study. Each figure shows (a) female, (b) male (the Y-chromosome is indicated by an arrow) and (c) diagramatic representation of the male metaphase karyotype (the heterochromatic portions are indicated in black). Scale: 5 μm.


1
a
b
punjabiensis
c
2
a
b
auraria
c
3
a
b
barbarae
c
4
a
b
orosa
c
5
a
b
seguyi
c

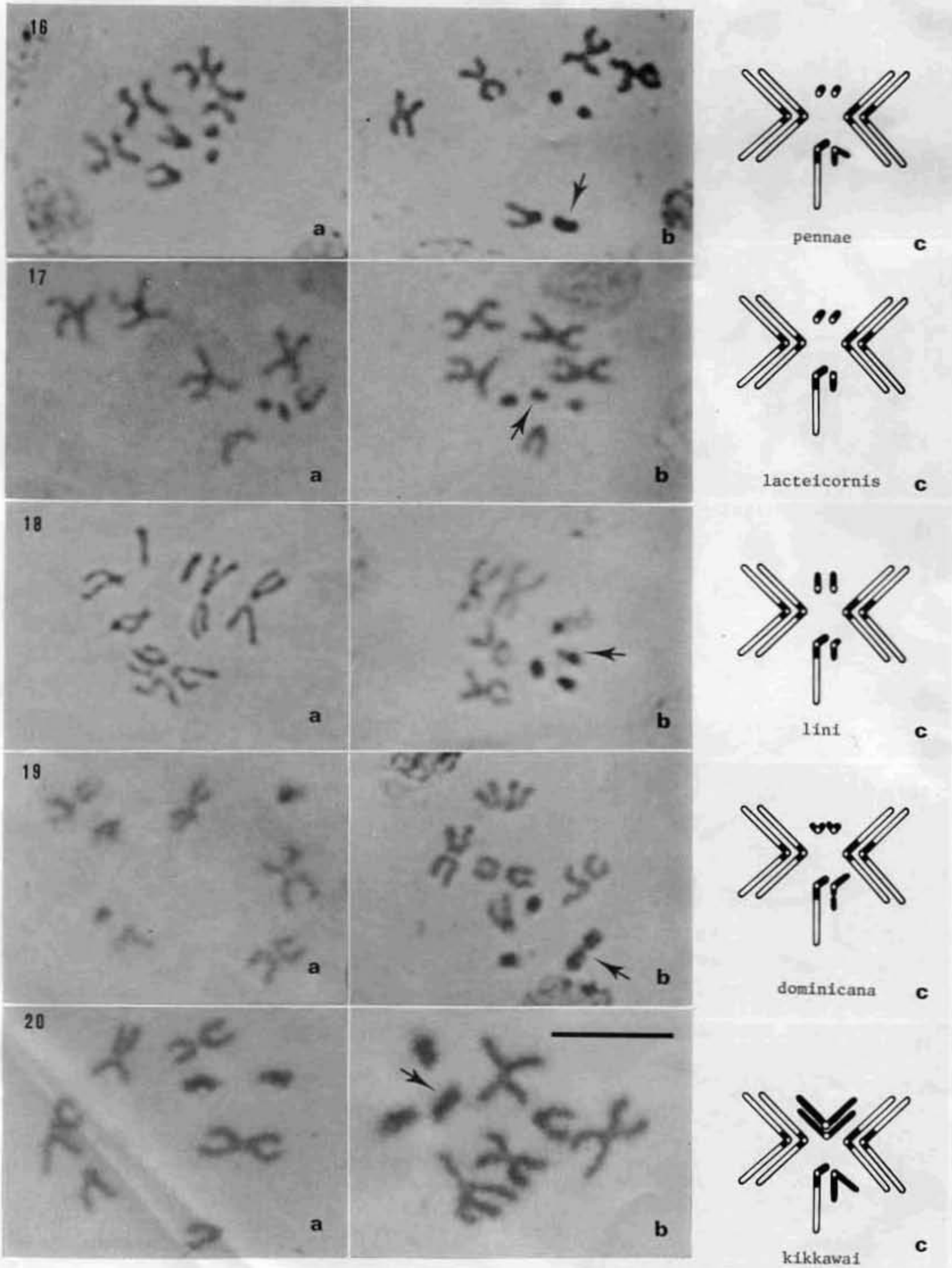
Figs. 1-5.


6
a
b
baimaii
c
7
a
b
mayri
c
8
a
b
pseudomayri
c
9
a
b
birchii
c
10
a
b
jambulina
c

Figs. 6–10.


11
a
b
nikananu
c
12
a
b
bocki
c
13
a
b
leontia
c
14
a
b
rufa
c
15
a
b
serrata
c

Figs. 11–15.


16
a
b
pennae
c
17
a
b
lacteicornis
c
18
a
b
lini
c
19
a
b
dominicana
c
20
a
b
kikkawai
c

Figs. 16–20.

A relatively larger J-shaped Y chromosome has been observed in a strain of *D. birchii* from New Guinea as shown in Fig. 9. Three types of Y chromosome have been previously reported by Baimai (Baimai 1969a).

4. Small V-shape (metacentric or SV). This type of Y chromosome has been observed in two species viz. *D. serrata* and *D. pennae* (Figs. 15, 16). Because of its inconspicuous centromere and short length, this type of Y chromosome frequently appeared as a short rod in most metaphase preparations. However, the small V-shaped configuration was occasionally observed in some preparations. Furthermore, these two species also exhibit a large dot-shaped 4th chromosome. Thus in some preparations, the Y and 4th chromosomes could not be distinguished.

5. Medium V-shape (MV). This type of Y chromosome has been found in *D. jambulina* and *D. nikananu* (Figs. 10, 11). The centromere position is normally apparent in most preparations. *D. dominicana* also manifests the medium V-shaped Y chromosome. However, it contains a unique secondary constriction in one arm. This feature together with the characteristic small V-shaped 4th chromosome make *D. dominicana* cytologically distinguishable from other species of the subgroup studied thus far (Fig. 19).

6. Large V-shape (LV). This type of Y chromosome is predominant among the *D. kikkawai* complex species. Thus it has been encountered in *D. bocki*, *D. leontia* and *D. kikkawai* (Figs. 12, 13, 20). *D. kikkawai* is one of the most remarkably variable species with respect to metaphase chromosomes (Baimai and Chumchong 1980).

The 4th chromosome

The normal dot-shape (D) is a common feature of the 4th chromosome (microchromosome) in most species of the *montium* subgroup. However, several species manifest deviation from the usual dot due to the acquisition of different amounts of extra heterochromatin at either side of the centromere. Thus *D. rufa* shows considerably more extra heterochromatin than the normal dot, transforming it into a medium size dot (designated as MD) (Fig. 14). Moreover, the 4th chromosomes of *D. serrata*, *D. pennae* and *D. lacteicornis* contain considerably larger amounts of extra heterochromatin, transforming them into conspicuously large dots (Figs. 15, 16, 17). It is very difficult, if not impossible, to determine definitely the amount and location of extra heterochromatin added to the centromere. Therefore it is best to interpret such 4th chromosomes as a large dot-shape (LD).

Even more remarkably, *D. lini* clearly exhibits an acrocentric 4th chromosome (rod shape or R). This is a unique feature of *D. lini* that can be used as a cytologically diagnostic character for the species (Fig. 18). Furthermore, *D. dominicana* apparently manifests a very small metacentric 4th chromosome (small V-shape, SV) which contains a small portion of heterochromatin on each side of the centromere (Fig. 19). However, such a V-shaped configuration has been occasionally observed. Generally, it appears as a very large dot 4th chromosome in most preparations because of its relatively short heterochromatic arms. In such a case, the 4th chromosome of *D. dominicana* appeared as a large dot similar to those of the other 3 species described above.

D. birchii has been shown to have two types of 4th chromosome (Baimai 1969a).

The normal dot shape as figured in this paper is the most common form while the J-shape is restricted to certain populations in New Guinea.

With respect to the 4th chromosome configuration, *D. kikkawai* is the most variable species recorded thus far in this subgroup or even in the genus *Drosophila* (Baimai and Chumchong 1980). This species exhibits at least six types of 4th chromosome each of which seems to have a definite pattern of geographic distribution. Fig. 20 shows the medium V-shaped 4th chromosome of *D. kikkawai* of the Bangkok strain which is apparently very common in the Oriental region. Such variation in the 4th chromosome due entirely to different amounts of heterochromatin makes *D. kikkawai* one of the most interesting species with respect to metaphase karyotype.

Although phylogenetic relationships within the *montium* subgroup are poorly known, the few groups of closely related species that have been investigated thus far exhibit two cytotaxonomic trends. In one direction, certain groups of species, for example, *mayri-pseudomayri* and *bocki-leontia,* show similarity in both morphology (especially male genitalia) and metaphase karyotype. On the contrary, certain groups of taxonomically most closely related species appear to be karyotypically quite distinct, viz. *birchii-dominicana-serrata,* and *leontia-kikkawai.* The same situation have been observed in certain groups of homosequential species of Hawaiian *Drosophila* (Carson *et al.* 1970; Carson and Kaneshiro 1976; Baimai and Ahearn 1978). The evidence seems to suggest that the process of acquisition of heterochromatin has, in part, played a role in the evolution of some groups of these taxa. The degree of metaphase variation due mainly to different amounts of heterochromatin may remain to be discovered in this large species subgroup. It is hoped that future investigations of cytotaxonomy may be useful in certain groups of these taxa.

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