METAPHASE KARYOTYPES OF FRUIT FLIES OF THAILAND II. FIVE SPECIES IN FOUR SUBGENERA OF *BACTROCERA*

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(Received May 2, 1996)

ABSTRACT

Five species of fruit flies of the genus Bactrocera (Diptera, Tephritidae) *collected from natural populations at different localities in Thailand were examined cytologically. They showed distinctive metaphase karyotypes with respect to the amount and distribution of constitutive heterochromatin in centromeric regions of autosomes and sex chromosomes.* Bactrocera (Hemigymnodacus) diversa, B. (Bactrocera) latifrons *and* B. (Asiadacus) modica, *three distinct species belonging to different subgenera, clearly exhibited different pericentric heterochromatin in all autosome pairs. The large metacentric X chromosome and conspicuous blocks of heterochromatin of* B. (B.) latifrons *makes it distinctive from* B. (A.) modica *and* B. (H.) diversa. *Further,* B. (Z.) rubella *and* B. (Z.) scutellaris *belong to the same subgenus* Zeugodacus, *but they have different metaphase karyotypes based on the amount of centromeric heterochromatin in all autosomes. The X and Y chromosomes of* B. scutellaris *are much longer than those of* B. rubella *due to accumulation of large quantities of heterochromatin. Such cytological differences among these distinct species are very useful as diagnostic characters in cytotaxonomic studies and may provide information concerning chromosomal evolution of these important pests in Thailand.*

INTRODUCTION

In recent years, interest in chromosomal evolution of the dipteran insects in Southeast Asia, particularly *Anopheles* and *Drosophila*, has focused on constitutive heterochromatin differentiation in mitotic karyotypes and its possible role in evolutionary change in these animals. The most extensive surveys of mitotic karyotype of dipteran insects of Thailand to date are those of Baimai and co-workers working on some 60 species of *Anopheles* and about 20 species of *Drosophila*.[1-7] The general knowledge gained from these studies is that these dipteran insects are, by and large, karyotypically uniform whereas variation in size and shape has been observed in sex chromosomes due to the presence or absence of constitutive heterochromatin. In fruit flies (*Diptera, Tephritidae*), a group of important agricultural pests in Thailand, little work has been done on karyotypic analysis in relation to chromosomal differentiation despite the extensive investigations on systematics and morphology.[8,9] Before the chromosomal variation among closely related species or species groups of fruit flies can be done, the normal or standard set of karyotypes must be known. Recently, metaphase karyotypes of several species of *Bactrocera* (previously recorded as *Dacus*), including five sibling species of the *B. dorsalis* complex, were reported.[10,11]

Our research project on the genetics of fruit flies has been undertaken to extend the observations on metaphase karyotypes and to look for general patterning of heterochromatin differences in the genome. Such findings might help explain the general significance of heterochromatin in chromosomal evolution and species differentiation.

In this report, we present the results of analysis and comparison of mitotic karyotypes of five species of *Bactrocera* which are found in Thailand with special emphasis on quantitative differentiation and distribution of constitutive heterochromatin in the genome.

MATERIALS AND METHODS

Larval samples of five species of fruit flies, namely *B. diversa, B. rubella, B. latifrons, B. scutellaris* and *B. modica* were collected from a variety of infested fruits and/or flowers of host plants at different localities

in Thailand (Table 1). Healthy third instar larvae were randomly sampled from infested host plants and were used for mitotic chromosome preparations and karyotypic analysis as described by Baimai *et al.*[11] Briefly, brain ganglia dissected from third instar larvae pretreated with 0.2% colchicine solution were prepared for metaphase karyotypes employing the air-dried technique.[12] Mitotic chromosomes from each larva were prepared in two sets. One set of the air-dried slides was stained with 3.3% Giemsa while the other set was stained with Hoechst 33258 using a modified method of Latt and Wohlleb.[13] Analysis of mitotic karyotype was based on a series of photomicrographs taken from the neuroblast cells under oil immersion (x670).

Table 1 Five species of *Bactrocera* obtained as larvae from infested fruits and/or flowers of various host plants collected in Thailand.

Species(subgenus)	Host plant(family)	Locality
B. (Hemigymnodacus) diversa (Coquillett)	*Cucurbita moschata* (Cucurbitaceae)	CM(T) 1, CM(U) 10, CM(O) 3, CR(M) 1, MS(C) 1, MS(F) 1, PE(U) 7, SE(B) 3, SK(H) 4, SU(C) 1, SU(B) 4, PH(M) 13, MS(D) 2, MS(D) 10, SU(A) 4, SU(B) 1,
	Lagenaria siceraria (Cucurbitaceae)	CM(T) 4, UT(F) 4, NR(F) 2
B. (Zeugodacus) rubella (Hardy) *n. comb.*	*Diplocyclos palmatus* (Cucurbitaceae)	PE(Q) 1, PB(B) 5
B. (Zeugodacus) scutellaris (Bezzi)	*Cucurbita moschata* (Cucurbitaceae)	CM(L) 6, CM(T) 7, CM(T) 1, CM(L) 17, CM(O) 3, CM(U) 10, CM(U) 9, MS(B) 1
B. (Bactrocera) latifrons(Hendel)	*Solanum torvum* (Solanaceae)	MS(D) 26, PE(O) 2, UT(F) 14, BR(A) 3, SK(H) 5, SK(D) 1
B. (*Asiadacus*) *modica* (Hardy) *n. comb.*	*Diplocyclos palmatus* (Cucurbitaceae)	SR(J) 2, NR(F) 1

Note: abbreviation for provinces: BR = Burirum, CM = Chiangmai, CR = Chiangrai, MS = Maehongsorn, NR = Nakhorn Ratchasima, PB = Prachinburi, PE = Phetchabun, PH = Petchaburi, SE = Srakaeo, SK = Sakonnakhon, SR = Saraburi, SU = Surin, UT = Uthaithani

RESULTS

Five species of *Bactrocera* here considered had similar mitotic karyotypes (2n=12) consisting of one pair of sex chromosomes (XX female and XY male, generally referred to as chromosome no. 1) and 5 pairs of autosomes (nos. 2-6). The sex chromosomes and autosomes of these species show variation in size and shape attributable to the amount and distribution of constitutive heterochromatin at the centromeric regions (Fig. 1-20). Specific differences in mitotic karyotypes of the five species are briefly described below.

Bactrocera diversa

Giemsa (G-) staining and Hoechst (H-) banding of mitotic chromosomes of *B. diversa* failed to reveal any conspicuous blocks of pericentric heterochromatin in all autosome pairs (Fig. 1, 2, 11, 12). The X is a small submetacentric chromosome. The short arm is euchromatic while the long arm is assumed to be entirely heterochromatic. The Y chromosome also shows a small submetacentric configuration somewhat similar to the X chromosome (Fig. 1, 11) but it is totally heterochromatic. The Y chromosome often exhibits a dot-shape in appearance due to its small size.

Bactrocera rubella

The mitotic karyotype of *B. rubella* is clearly different from that of *B. diversa* in having prominent blocks of pericentric heterochromatin in all autosome pairs (Fig. 3, 4, 13, 14). The presence of pericentric heterochromatin obviously stands out in the H-banding preparations (Fig. 13, 14). The small X chromosome is subtelocentric consisting of a short euchromatic arm and a long heterochromatic arm. The small Y chromosome is entirely heterochromatic of a dot-shape. In general chromosome preparations, the X and Y often appear as large dot-like chromosomes.

Bactrocera scutellaris

The metaphase karyotype of this species is strikingly different from *B. rubella* in the autosome pairs as well as in the sex chromosomes with respect to the amount and distribution of constitutive heterochromatin (Fig. 5, 6, 15, 16). All the autosomes consist of conspicuous blocks of pericentric heterochromatin. The X chromosome has a very large submetacentric shape. The long arm apparently contains two major blocks of centromeric heterochromatin and a considerable portion of euchromatin at the distal region. The short arm of chromosome X is totally heterochromatic. Interestingly, a small portion of lighter staining heterochromatin has been occasionally observed at the distal region of this short arm. The Y chromosome is submetacentric to almost metacentric in shape and is entirely heterochromatic.

Bactrocera latifrons

This species exhibits conspicuous blocks of pericentric heterochromatin in all the autosome pairs similar to those of *B. scutellaris* (Fig. 7, 8, 17, 18). However, the sex chromosomes are quite distinct from those of *B. scutellaris* described above. The X chromosome has a large metacentric shape. One arm is almost totally euchromatic while the opposite arm consists of two major blocks of centromeric heterochromatin. Interestingly, a small block of heterochromatin has been observed at the distal region of the heterochromatic arm of the X in some chromosome preparations. The Y chromosome is medium-sized with a dot-like shape and is entirely heterochromatic.

Bactrocera modica

This species shows a distinctive type of mitotic karyotype in having very large blocks of pericentric heterochromatin in all the autosome pairs compared with those of other species here considered (Fig. 9, 10, 19, 20). Nevertheless, the X chromosome has a medium metacentric shape. One arm of the X is euchromatic while the opposite arm is entirely heterochromatic. The Y is a small dot-shaped chromosome and is totally heterochromatic.

A diagrammatic representation of mitotic karyotypes of the five species of the genus *Bactrocera* here described is presented in the form of haploid idiograms in Fig. 21.

DISCUSSION

Mitotic chromosomes serve as useful cytological markers for cytotaxonomic studies of closely related species of animals.[14] Interspecies differences in constitutive heterochromatin observed in metaphase karyotypes are often striking in *Anopheles*[3-7,15] and in *Drosophilla*.[1,16-18] Recently, Hunwattanakul and Baimai[10] and

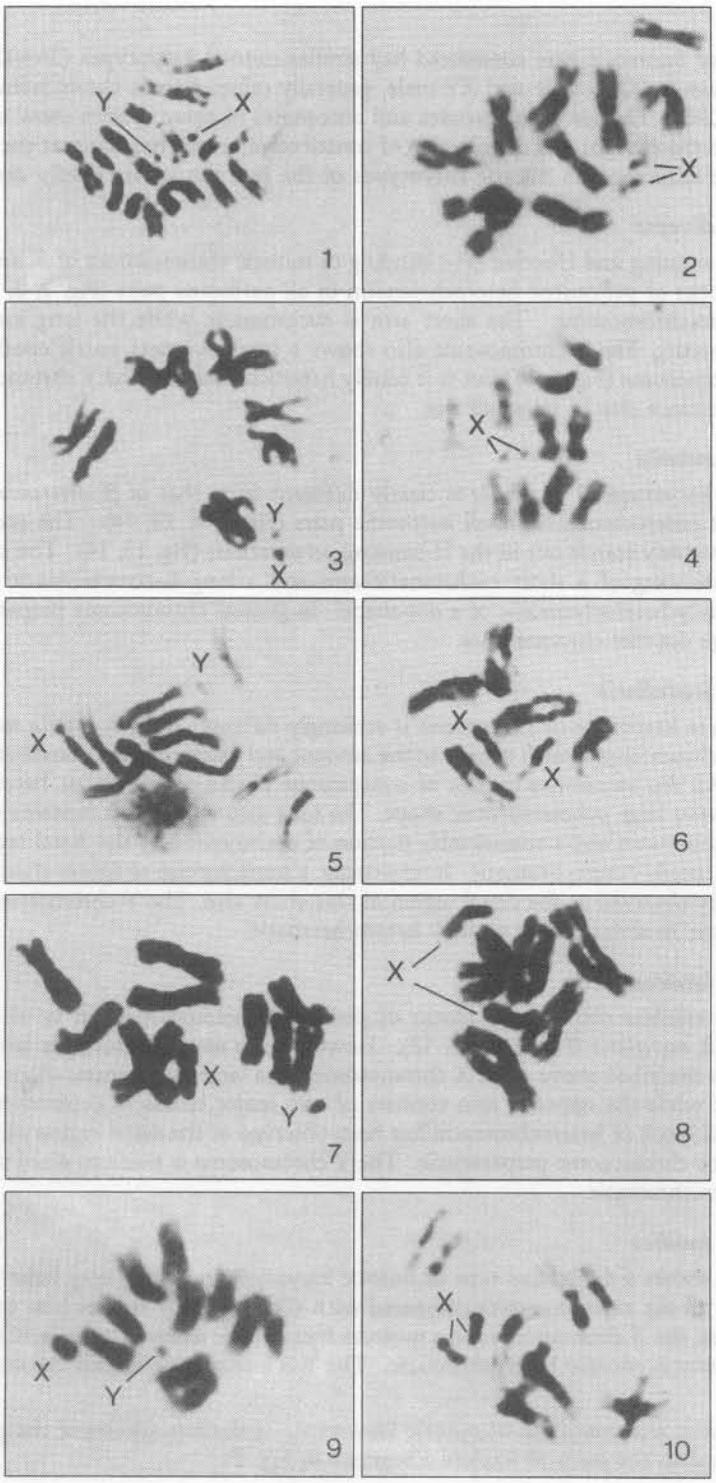

Fig. 1-10 Photomicrographs of metaphase karyotypes (Giemsa staining) of five species of *Bactrocera*: 1 and 2, male and female, respectively, of *B. diversa*; 3 and 4, male and female, respectively, of *B. rubella*; 5 and 6, male and female, respectively, of *B. scutellaris*; 7 and 8, male and female, respectively, of *B. latifrons*; 9 and 10, male and female, respectively, of *B. modica*.

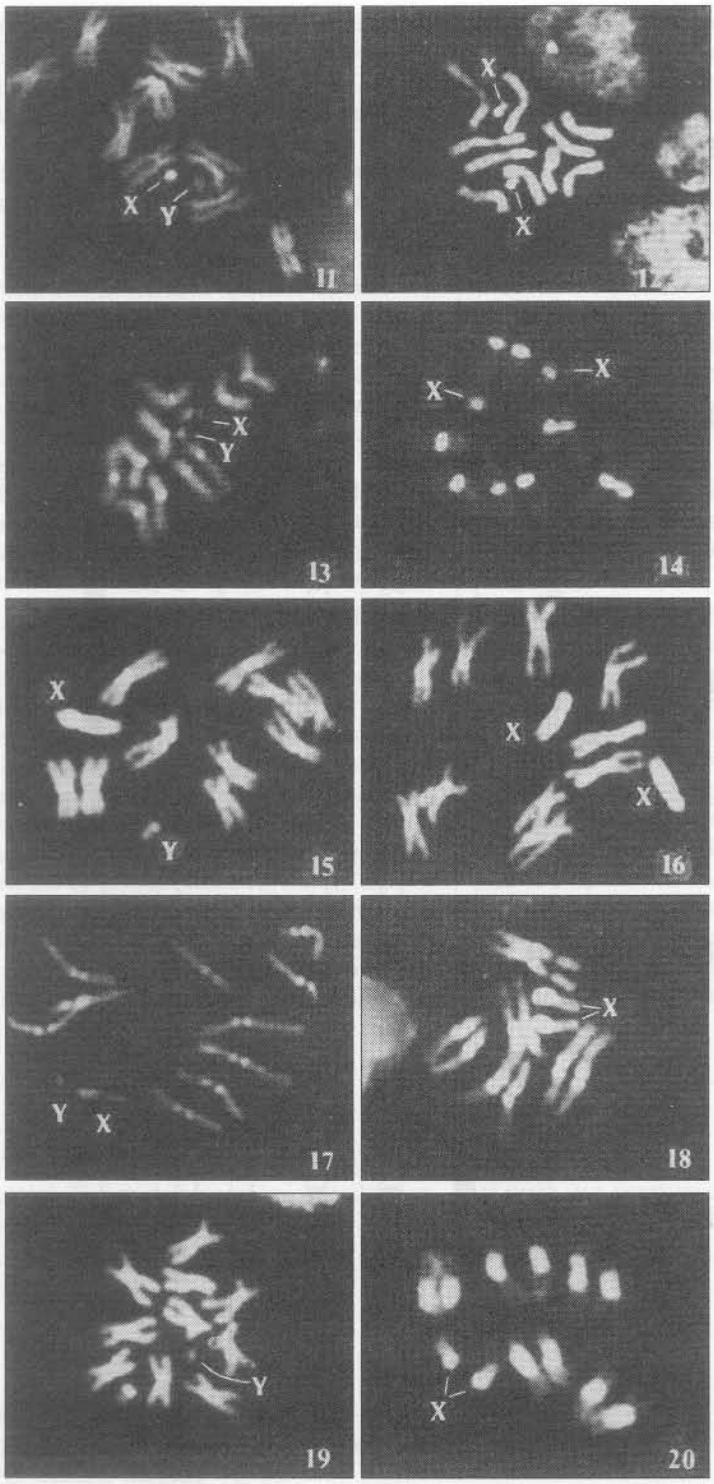

Fig. 11-20 Photomicrographs of metaphase karyotypes (H-banding) of the five species of *Bactrocera*: 11 and 12, male and female, respectively, of *B. diversa*; 13 and 14, male and female, respectively, of *B. rubella*; 15 and 16, male and female, respectively, of *B. scutellaris*; 17 and 18, male and female, respectively, of *B. latifrons*; 19 and 20, male and female, respectively, of *B. modica*.

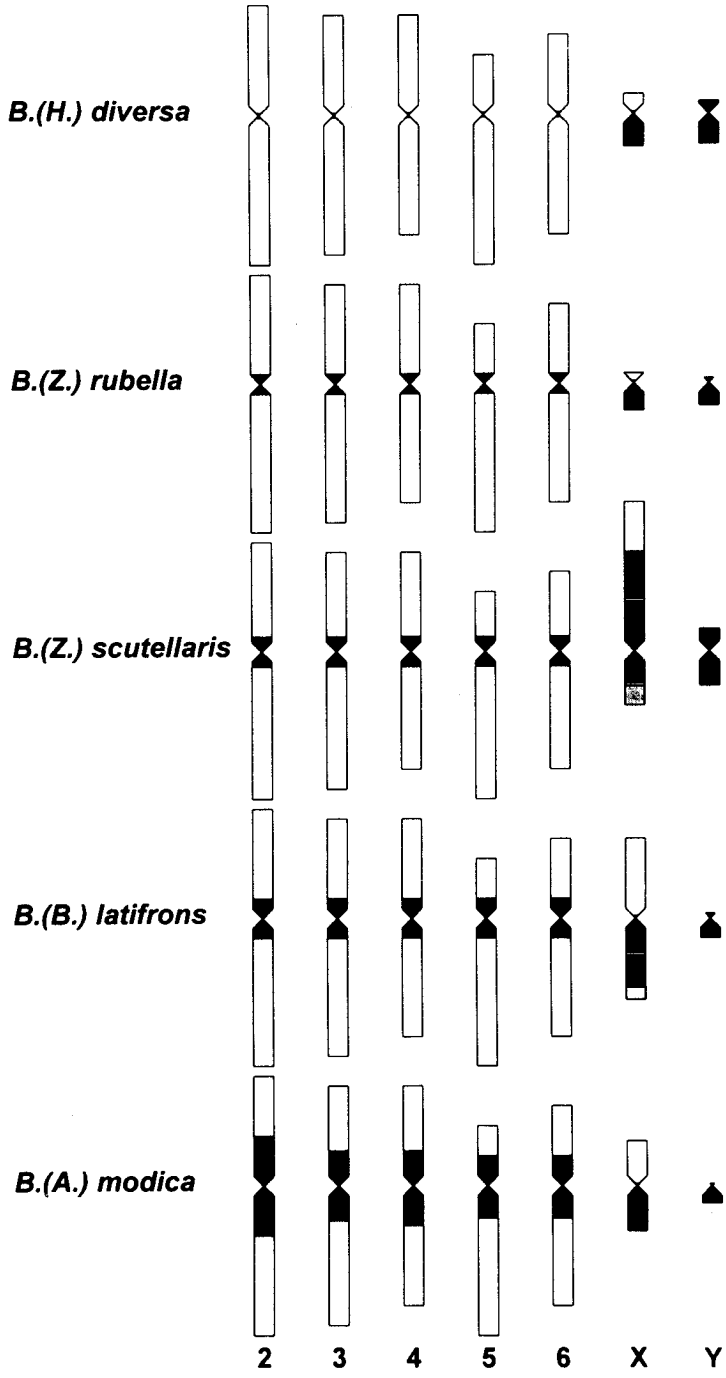

Fig. 21 Diagrammatic representation of metaphase karyotypes of the five species of *Bactrocera* shown as haploid idiograms for each species. Black areas represent heterochromatic portions. The centromeres are indicated by constrictions of each chromosome. Chromosome lengths, arm ratios and heterochromatic portions are depicted in proportion.

Baimai *et al.*[11] reported metaphase karyotypes of several species of *Bactrocera* in Thailand using G- and H-banding approaches to demonstrate chromosomal evolution based on the amount and distribution of constitutive heterochromatin in the genome. We found that differences in heterochromatin in sex chromosomes and autosomes taken together can be used as a diagnostic character for separation of species. In this study, our observation on heterochromatin differences in mitotic karyotypes among the five morphologically distinct species belonging to 4 subgenera of fruit flies of Thailand seems to be in accordance with the previous findings. All five species investigated in the present study have basic mitotic karyotypes of the Oriental fruit flies (2n=12). However, a striking chromosomal differentiation among these species has been found in the pericentric heterochromatin region of all the autosome pairs as well as in the sex chromosomes. *Bactrocera diversa* contains the least amount of pericentric heterochromatin. Although *B. rubella* and *B. scutellaris* are taxonomically classed in the same subgenus *Zeugodacus*, the latter species contains larger amounts of pericentric heterochromatin, especially in the sex chromosomes, than the former one. *Bactrocera latifrons*, of the subgenus *Bactrocera*, has a considerable amount of pericentric heterochromatin in the autosomes somewhat similar to those of *B. scutellaris,* but to a lesser extent in the sex chromosomes. Moreover, metaphase karyotype of *B. latifrons* is quite different from members of the *B. dorsalis* complex with respect to the amount and distribution of pericentric heterochromatin in all chromosome pairs.[11] Of the five species investigated in this study, *B. modica* contains extremely large quantities of pericentric heterochromatin in the autosomes. Thus interspecies comparison of quantity of heterochromatin as appearing in mitotic karyotypes is a useful approach not only for identifying distinct morphological species as demonstrated in this study but also for separating some cryptic or isomorphic species of fruit flies.[11]

The striking differences in pericentric heterochromatin in autosomes and sex chromosomes clearly show evolutionary divergence in mitotic karyotypes. Our cytological evidence seems to support John's suggestion[19] that chromosome change in karyotypes has played an important role in evolutionary divergence in certain phylogenetically and morphologically closely related species. Thus comparison of the metaphase karyotype of the five species of fruit flies here considered provides additional evidence for chromosomal evolution involving notable differences in the amount and distribution of constitutive heterochromatin. Moreover, Baimai[3] feels that heterochromatin differentiation in mitotic chromosomes has played and continues to play a significant role in the speciation process of the dipteran insects, at least, in the Southeast Asian region. Although heterochromation has frequently been considered as having no significance in evolution, it has been demonstrated to contain some regulatory elements[20] and to help chromatin organization of the genome.[21] Since heterochromation contains tandemly repeated sequences of a non-coding nature, its functional role is still an unsolved problem.[22] More information on heterochromatin differentiation in the genome of sibling species complexes and other closely related species of the dipteran insects in Thailand and Southeast Asia may contribute to an understanding of chromosomal evolution and species differentiation and its vital role in evolutionary processes.

ACKNOWLEDGEMENTS

We thank Somsak Tiangtrong for technical assistance. This work was supported by National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA).

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