

Metaphase Karyotypes of Fruit Flies of Thailand (III): Six Members of the *Bactrocera dorsalis* Complex

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Visut Baimai, Jaros Phinchongsakuldit and Wachareeporn Trinachartvanit (1999) Metaphase karyotypes of fruit flies of Thailand (III): Six members of the *Bactrocera dorsalis* complex. *Zoological Studies* 38(1): 110-118. Six species of fruit flies belonging to the *Bactrocera dorsalis* complex used in this study include *B. propinqua*, *B. irvingiae*, *B. carambolae*, *B. pyrifoliae*, *B. arecae*, and *B. melastomatos*. Wild samples collected from infested fruits of various host plants were examined cytologically. Analysis of mitotic karyotypes of these species has revealed distinct patterns of metaphase chromosomes due to different amounts and distribution of constitutive heterochromatin in sex chromosomes and autosomes. The general pattern of mitotic karyotypes of *B. irvingiae* and *B. propinqua* resembles that of *B. dorsalis*, and they are classified in Group 1. Likewise, *B. carambolae* and *B. pyrifoliae* exhibit mitotic karyotypes of Group 4 showing specific patterns of heterochromatin in the centromeric regions of the X chromosome and autosomes. *Bactrocera arecae* exhibits distinctive patterns of pericentric heterochromatin in autosomes and sex chromosomes. Interestingly, metaphase chromosomes of *B. melastomatos* do not match with any groups of mitotic karyotype categorized earlier because of the very small size of the X and Y chromosomes. These findings suggest the significance of heterochromatin differentiation in karyotypic evolution of these species of fruit flies in Thailand. Such distinctive patterns of heterochromatin in mitotic karyotype are useful as diagnostic characters for separation of these closely related species of the *B. dorsalis* complex.

Key words: *Bactrocera dorsalis* complex, Mitotic chromosomes, Heterochromatin, Karyotypic evolution.

The *Bactrocera* group of the genus *Bactrocera* belonging to the subfamily Dacinae (Diptera: Tephritidae) is widely distributed in subtropical and tropical forests of Southeast Asia, Australia, and the Pacific region. This group of fruit flies includes some 234 known species (Hardy and Adachi 1954, Hardy 1973, Drew 1989, Drew and Hancock 1994). Drew and Hancock (1994) have described 40 new species and revised 12 species within the *B. dorsalis* complex based on external morphological characters. Morphologically, all members of the *B. dorsalis* complex exhibit a clear wing membrane with a narrow costal band. The scutum is almost black with lateral postsutural vittae, and the scutellum is mostly yellow. The abdomen shows a medial longitudinal dark band of variable size. Recently, Baimai et al. (1995) have described metaphase karyotypes of 5 sibling

species of the *B. dorsalis* complex with special emphasis on differences in the amount and distribution of constitutive heterochromatin in sex chromosomes and/or autosomes, i.e., *B. dorsalis* s.s. (species A), *B. kanchanaburi* (species B), *B. raiensis* (species C), *B. verbascifoliae* (species D), and species E, a new sibling species of this complex discovered in that study. Such a cytological difference in patterning of constitutive heterochromatin is a useful tool for cytotaxonomic study of cryptic or isomorphic species, at least in some dipteran insects of Southeast Asia (Baimai et al. 1981 1988 1996a). In our ongoing studies of the genetics of fruit flies in Thailand we maintain our interest in cytotaxonomic investigations of the *B. dorsalis* complex and other species groups of *Bactrocera* in order to gain a better understanding of chromosomal evolution of these important insect

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pests in the region.

In this report we present metaphase karyotypes of 6 additional species of the *B. dorsalis* complex occurring in Thai populations.

MATERIALS AND METHODS

The 6 species of the *B. dorsalis* complex used in this study include *B. (B.) propinqua* (Hardy and Adachi), *B. (B.) irvingiae* Drew and Hancock, *B. (B.) carambolae* Drew and Hancock, *B. (B.) pyrifoliae* Drew and Hancock, *B. (B.) arecae* (Hardy

and Adachi), and *B. (B.) melastomatos* Drew and Hancock. Wild samples of larvae of the 6 species were collected from a variety of infested fruits of various host plants at different localities in Thailand as previously described (Baimai et al. 1995 1996b) (Table 1). Metaphase chromosomes were prepared from the brain ganglia of healthy 3rd instar larvae, and karyotype analysis was performed on a series of photomicrographs of mitotic chromosomes, one set stained with Giemsa and another set with Hoechst 33258, using the techniques described by Baimai et al. (1995). Heterochromatin patterns of mitotic karyotypes

Table 1. Wild specimens of larvae of the 6 species of the *Bactrocera dorsalis* complex obtained from infested fruits of a variety of host plants collected in Thailand

Species	Locality	Host Plant Species (Family)	Number of Larvae	Date of Collection
<i>B. (B.) propinqua</i>	Ranong ^e	<i>Garcinia</i> sp. (Guttiferae)	7	May 96
			11	June 96
			6	Apr. 96
<i>B. (B.) irvingiae</i>	Sakonnakorn ^c	<i>Irvingia malayana</i> (Ixonanthaceae)	2	Sept. 96
	Prachinburi ^d	<i>Irvingia malayana</i> (Ixonanthaceae)	3	July 93
	Chumporn ^e	<i>Irvingia malayana</i> (Ixonanthaceae)	5	Sept. 97
<i>B. (B.) carambolae</i>	Ranong ^e	<i>Anacardium occidentale</i> (Anacardiaceae)	1	Feb. 96
		<i>Capparis micracantha</i> (Capparaceae)	15	Feb. 97
		<i>Diospyros wallichii</i> (Ebenaceae)	1	Mar. 96
		<i>Elaeocarpus</i> sp. (Elaeocarpaceae)	1	Jan. 97
		<i>Garcinia</i> sp. (Guttiferae)	3	June 96
		<i>Gnetum montanum</i> (Gnetaceae)	11	Apr. 96
		<i>Manilkara</i> sp. (Sapotaceae)	2	Apr. 96
		<i>Melientha suavis</i> (Opiliaceae)	2	June 96
		<i>Payena</i> sp. (Sapotaceae)	3	Apr. 96
		<i>Platea</i> sp. (Icacinaceae)	1	May 96
		<i>Polyalthia</i> sp. (Annonaceae)	10	Jan. 96
		<i>Psidium guajava</i> (Myrtaceae)	1	July 96
		<i>Syzygium</i> sp. (Myrtaceae)	2	Mar. 96
		<i>Zizyphus</i> sp. (Rhamnaceae)	1	May 96
		<i>B. (B.) pyrifoliae</i>	Ubon	<i>Elaeocarpus</i> sp. (Elaeocarpaceae)
Ratchathani ^c	<i>Gnetum macrostachyum</i> (Gnetaceae)		2	Mar. 97
Chiangmai ^a	<i>Psidium guajava</i> (Myrtaceae)		6	Aug. 93
		15	Nov. 95	
		3	Nov. 95	
<i>B. (B.) arecae</i>	Phetchaboon ^b	<i>Prunus persica</i> (Rosaceae)	10	Apr. 96
	Prachinburi ^d	<i>Psidium guajava</i> (Myrtaceae)	4	Sept. 93
	Chumporn ^e	<i>Areca triandra</i> (Palmae)	5	Mar. 93
<i>B. (B.) melastomatos</i>	Chumporn ^e	<i>Areca catechu</i> (Palmae)	8	Jan. 94
		<i>Melastoma malabathricum</i> (Melastomataceae)	4	May 96
<i>B. (B.) melastomatos</i>	Ranong ^e	<i>Melastoma malabathricum</i> (Melastomataceae)	9	Jan. 96
			7	Feb. 96
	Nan ^a	<i>Melastoma malabathricum</i> (Melastomataceae)	5	Dec. 96
	Loey ^c	<i>Melastoma</i> sp. (Melastomataceae)	4	Sept. 96

^aNorth; ^bCentral; ^cNortheast; ^dEast; ^eSouth.

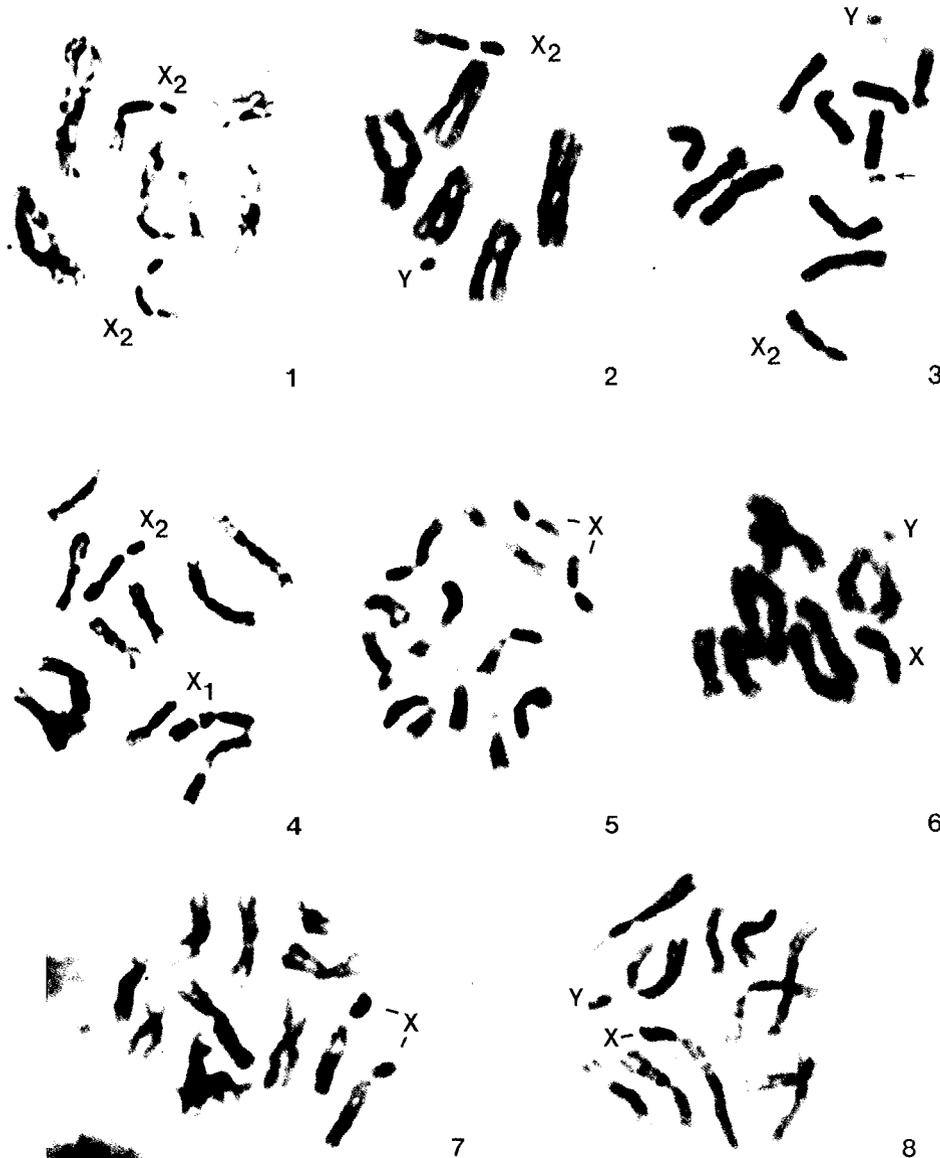
derived from larvae were correlated with the species identified by external morphology of adults that emerged from larvae from the same infested fruits.

RESULTS

An analysis of metaphase karyotypes ($2n = 12$) based on the G- and H-banding of the 6 species of the *B. dorsalis* complex has revealed different patterns in the amount and distribution of constitutive heterochromatin in sex chromosomes

and autosomes. Such differences in mitotic chromosomes can be used as species-specific diagnostic characters for this species complex as described below.

Bactrocera propinqua: Two types of X chromosome have been observed in this species. Chromosome X_1 has a submetacentric shape; the short arm is basically euchromatic, while the long arm is comprised entirely of a few major blocks of heterochromatin. The X_2 chromosome is also submetacentric similar to the X_1 but it contains an extra-large block of heterochromatin at the distal end making the connecting point a conspicuous

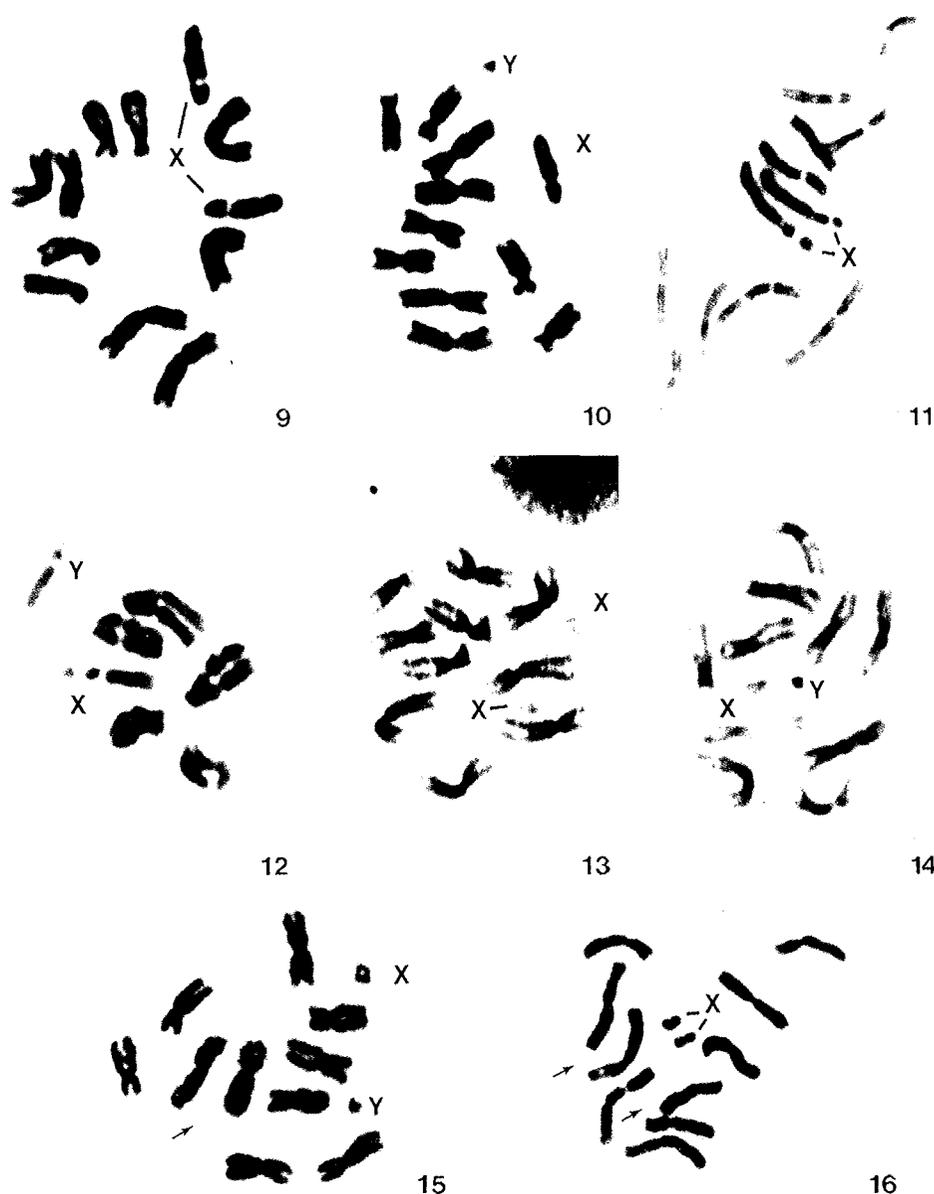


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secondary constriction (Figs. 1-2, 17-18). The difference between the X_1 and X_2 chromosomes can be readily observed in a heterozygous female larva (Figs. 4, 20). The Y chromosome has a small submetacentric shape and is totally heterochromatic. It may appear as a large dot-like chromosome (Figs. 2-3, 19). All autosome pairs show relatively small amounts of centromeric

heterochromatin except for no. 2 which contains a considerable amount of pericentric heterochromatin. Occasionally, the submetacentric autosome 6 exhibits a small heterochromatic portion at the tip of the long arm (Fig. 3).

Bactrocera irvingiae: In general, the mitotic chromosomes of this species look similar to those of *B. dorsalis* showing a medium metacentric X

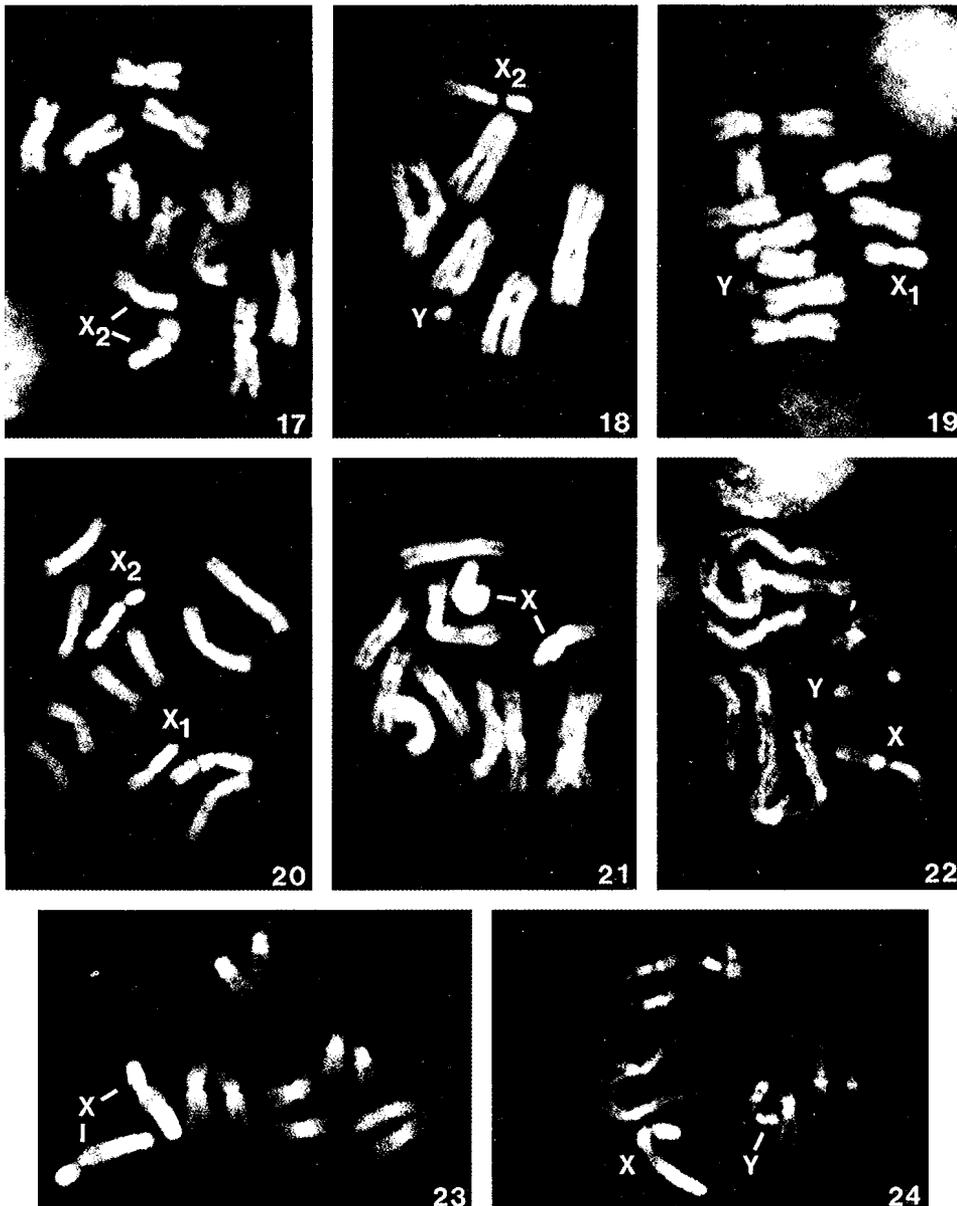


Figs. 1-16. Photomicrographs of metaphase karyotypes using Giemsa staining of 6 closely related species of the *B. dorsalis* complex. Figs. 1, 2-3, and 4. Female, male and heterozygous female for X_1 and X_2 , respectively, of *B. propinqua*. The arrow in Fig. 3 shows an extra heterochromatic portion in autosome 6. Figs. 5 and 6. Female and male, respectively, of *B. irvingiae*. Figs. 7 and 8. Female and male, respectively, of *B. carambolae*. Figs. 9 and 10. Female and male, respectively, of *B. pyrifuliae*. Figs. 11 and 12. Female and male, respectively, of *B. arecae*. Figs. 13 and 14. Female and male, respectively, of *B. melastomatos*. Figs. 15 and 16. Supernumerary B chromosomes of *B. melastomatos* as indicated by arrows.

shape, a dot-like Y chromosome, and noticeable centromeric heterochromatin in autosomes 5 and 6. However, a conspicuous block of centromeric heterochromatin in the euchromatic arm of the X chromosome makes it quite different from that of *B. dorsalis* (Figs. 5-6, 21-22). In addition, autosome 4 exhibits a large block of centromeric heterochromatin in the long arm. This feature of autosome 4 is lacking in *B. dorsalis*.

Bactrocera carambolae: The X chromosome shows a striking submetacentric configuration. The short arm is totally heterochromatic. The long arm, by contrast, contains a combination of euchromatin

near the proximal region and a series of major blocks of heterochromatin toward the distal end (Figs. 7-8, 23-24). Such a large quantity of heterochromatin makes this unique X the largest chromosome in the mitotic karyotype. The Y chromosome has a considerable size with a submetacentric configuration (Figs. 8, 24). However, the most remarkable feature of the mitotic karyotype of *B. carambolae* is the presence of conspicuous pericentric heterochromatin in all autosome pairs. The presence of large amounts of heterochromatin in the X and the autosomes can be used as a diagnostic character for *B.*



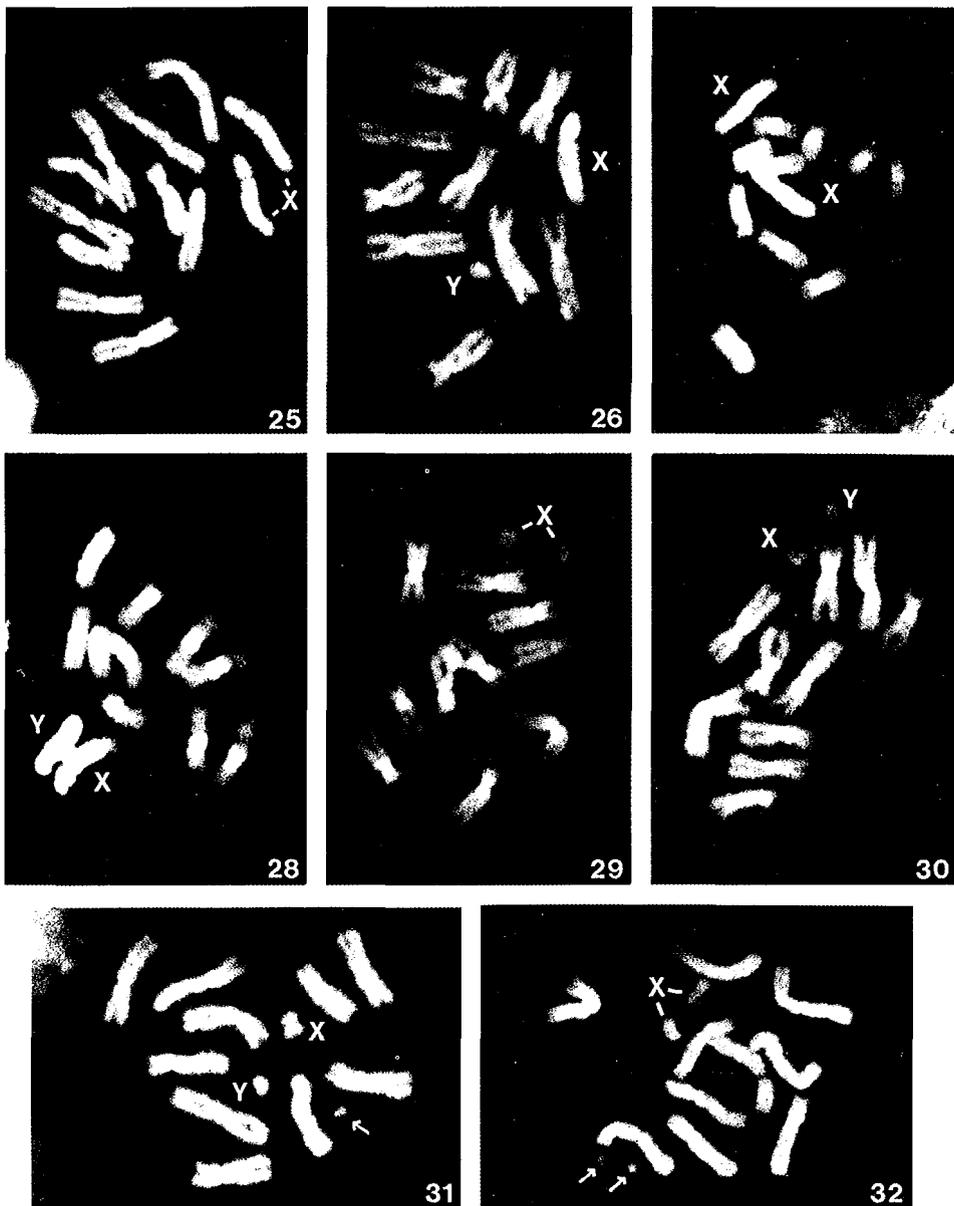
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carambolae.

Bactrocera pyrifoliae: The general appearance of the X chromosome of this species is in some ways similar to that of *B. carambolae*, exhibiting a short portion of euchromatin adjacent to the centromere (Figs. 9-10, 25-26). However, a relatively smaller amount of heterochromatin can be observed at the centromeric and the distal regions of the X chromosome of *B. pyrifoliae*

compared with that of *B. carambolae*. The Y chromosome exhibits a dot-like shape in most preparations (Figs. 10, 26). All autosome pairs of this species show a small amount of pericentric heterochromatin, particularly autosome 3.

Bactrocera arecae: This species has large submetacentric X and Y chromosomes which are quite distinctive from those of other related species within the *B. dorsalis* complex (Figs. 11-12, 27-28).



Figs. 17-32. Photomicrographs of metaphase karyotypes using Hoechst staining of 6 species of the *B. dorsalis* complex. Figs. 17, 18-19, and 20. Female, male, and heterozygous female for X_1 and X_2 , respectively, of *B. propinqua*. Figs. 21 and 22. Female and male, respectively, of *B. irvingiae*. Figs. 23 and 24. Female and male, respectively, of *B. carambolae*. Figs. 25 and 26. Female and male, respectively, of *B. pyrifoliae*. Figs. 27 and 28. Female and male, respectively, of *B. arecae*. Figs. 29 and 30. Female and male, respectively, of *B. melastomatos*. Figs. 31 and 32. Supernumerary B chromosomes of *B. melastomatos* as indicated by arrows.

The X chromosome has a relatively small euchromatic portion at the distal end of the long arm while the short arm is totally heterochromatic. The Y chromosome is totally heterochromatic. Each autosome contains a very large block of pericentric heterochromatin which is a good cytological character of *B. arecae*.

Bactrocera melastomatos: Contrary to the 5 species mentioned above, *B. melastomatos* exhibits small metacentric sex chromosomes. Thus, the X chromosome consists of a very short euchromatic arm while the opposite arm is totally heterochromatic (Figs. 13-14, 29-30). The metacentric Y chromosome is slightly smaller than the X chromosome (Figs. 14, 30). Such a small size of the sex chromosomes is unique for *B. melastomatos* and presents a good cytological character for this species. Each autosome pair contains a considerable amount of pericentric heterochromatin, particularly those of nos. 2, 5, and 6. Even more surprising is the presence of supernumerary B chromosomes in some samples from Ranong and Nan Provinces (Figs. 15-16, 31-

32).

A diagrammatic representation of metaphase karyotypes of the 6 species of the *B. dorsalis* complex described here are summarized and presented in the form of haploid idiograms in Fig. 33.

DISCUSSION

In our previous report, we described mitotic karyotypes of 5 closely related species of the *B. dorsalis* complex (Baimai et al. 1995). Four of those species, i.e., *B. dorsalis*, *B. kanchanaburi*, *B. raiensis*, and *B. verbascifoliae*, are morphologically distinguishable from each other (Drew and Hancock 1994). We also demonstrated that these 4 closely related species are chromosomally quite distinct on the basis of quantitative differences in constitutive heterochromatin in centromeric regions of autosomes and sex chromosomes. However, our cytological data suggested that the mitotic karyotype of form E is different from those of the known species morphologically described by Drew and Hancock (1994). In the present study, we have observed and described mitotic karyotypes of wild specimens which morphologically belong to *B. carambolae* but are chromosomally somewhat similar to species E. Nevertheless, heterochromatic patterns in the mitotic karyotype of *B. carambolae* are slightly different from those of species E, particularly in the centromeric regions of autosomes 5 and 6. These results confirm our previous findings suggesting the existence of species E as a new member of the *B. dorsalis* complex. Although the general appearance of sex chromosomes of *B. pyrifoliae* is in some ways similar to that of *B. carambolae* and species E, its autosomes are remarkably different from those of the other 2 species based on the amount and distribution of heterochromatin in the centromeric regions. Thus on the basis of the heterochromatic pattern in the X chromosome, the mitotic karyotypes of *B. carambolae* and *B. pyrifoliae* belong to group 4, like species E as previously described (Baimai et al. 1995). Geographically, *B. carambolae* and *B. pyrifoliae* have been found in infested fruits of a wide variety of host plants (Table 1). Our collection records indicate that *B. carambolae* has a disjunct distribution, being found only in the south (Ranong Province) and in the northeast (Ubon Ratchathani Province) of Thailand, and not in between these 2 areas. It may be noted that Drew and Hancock (1994) found *B. carambolae* only in southern and

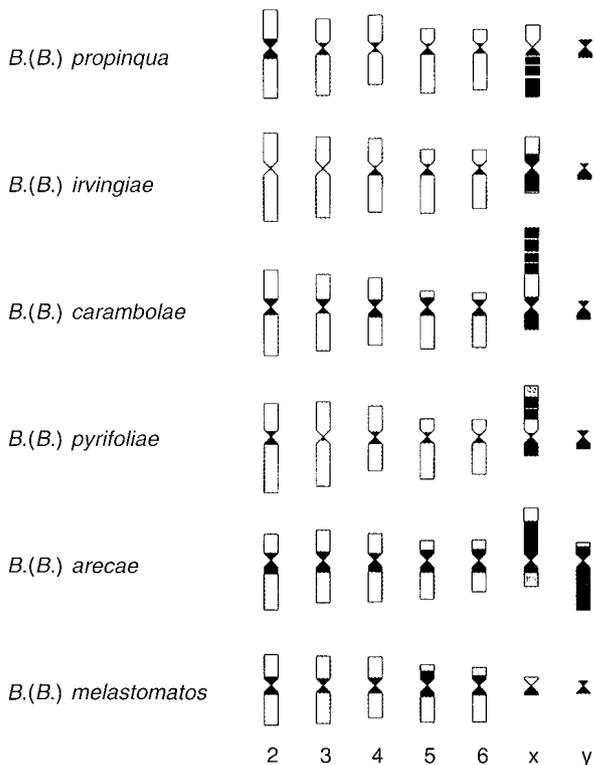


Fig. 33. Diagrammatic representation of haploid idiograms of the metaphase karyotype of 6 species of the *B. dorsalis* complex. Black and shaded areas represent heterochromatic portions. The centromeres are depicted by constrictions of each chromosome. Chromosome lengths, arm ratios, and heterochromatic portions are shown in proportion.

peninsular Thailand.

It is interesting to note that the mitotic karyotype of *B. irvingiae* is somewhat similar to that of *B. dorsalis*. Nevertheless, the presence of conspicuous blocks of centromeric heterochromatin in the euchromatic arm of the X and in the long arm of autosome 4 of *B. irvingiae* makes it cytologically distinct from *B. dorsalis*, although they are morphologically similar. However, *B. irvingiae* has been found specifically in *Irvingia malayana* (Ixonanthaceae), whereas *B. dorsalis* is known to infest a wide variety of host plants. Likewise, the X₁ chromosomes and pericentric heterochromatin patterns in all autosome pairs except for no. 2 occurring in *B. propinqua* are in some ways similar to those of *B. dorsalis*. On the other hand, sex chromosomes of *B. propinqua* are clearly different from those of *B. irvingiae*. In general, mitotic karyotypes of these 3 species can be placed into group 1 as previously described by Baimai et al. (1995). Geographically, *B. irvingiae* is more widespread than *B. propinqua* which has been found only in Ranong Province (Table 1).

Mitotic chromosomes of *B. arecae* are the most strikingly different from those of other members of the *B. dorsalis* complex studied thus far mainly due to the presence of a large quantity of pericentric heterochromatin in all autosome pairs and in the X chromosome. The extremely large size of the Y chromosome is a unique cytological character for *B. arecae*. It is clear, however, that *B. arecae* is systematically a member of the *B. dorsalis* complex (Drew and Hancock 1994).

Even more interestingly, the sex chromosomes of *B. melastomatos* are unique in having a very small-sized metacentric configuration compared with those of other species under investigation. Yet all autosomes of *B. melastomatos* contain a considerable amount of pericentric heterochromatin, notably in no. 6. Hence, the overall feature of metaphase chromosomes of *B. melastomatos* does not match with any groups of mitotic karyotypes of the *B. dorsalis* complex described previously (Baimai et al. 1995). Moreover, 1 or 2 small supernumerary B chromosomes have been observed in some larval samples collected from Ranong and Nan Provinces. This is the first record of supernumerary B chromosomes found in *Bactrocera* from Thai populations, although its significance is unclear. *Bactrocera melastomatos* is widely distributed throughout Thailand (Table 1).

Our cytological data clearly show distinct patterns of heterochromatin differentiation in mitotic

karyotype. Yet, on the basis of such gross cytological differences, it is not possible to draw definite conclusions pertaining to phylogenetic relationships among the 11 species of the *B. dorsalis* complex studied thus far. However, our findings in the ongoing project lend support to the general trend showing a gain of heterochromatin in the process of karyotypic evolution (John and Miklos 1979). Although the functional role of heterochromatin is an unsolved problem (Pardue and Hennig 1990, Irick 1994), it is obviously involved in karyotypic evolution in many groups of eukaryotes (John 1988, White 1973, for a review) including some dipteran insects in Southeast Asia (Baimai 1988 1998). Whether heterochromatin accumulation in the eukaryotic genome is functionally involved in the speciation process remains an intriguing and challenging problem in evolutionary biology.

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REFERENCES

- Baimai V. 1988. Constitutive heterochromatin differentiation and evolutionary divergence of karyotype in Oriental *Anopheles (Cellia)*. *Pacif. Sci.* **42**: 13-27.
- Baimai V. 1998. Heterochromatin accumulation in karyotypic evolution in some dipteran insects. *Zool. Stud.* **37**: 75-88.
- Baimai V, RE Harbach, S Sukowati. 1988. Cytogenetic evidence for two species within the current concept of the malaria vector *Anopheles leucosphyrus* in Southeast Asia. *J. Am. Mosq. Control Assoc.* **4**: 44-50.
- Baimai V, BA Harrison, L Somchit. 1981. Karyotype differentiation of 3 anopheline taxa in the *Balabacensis* complex of Southeast Asia (Diptera: Culicidae). *Genetica* **57**: 81-86.
- Baimai V, U Kijchalao, R Rattanarithikul. 1996a. Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. VI. The Pyrethophorus and the Neomyzomyia Series, subgenus *Cellia* (Diptera: Culicidae). *J. Am. Mosq. Control Assoc.* **12**: 664-675.
- Baimai V, W Trinachartvanit, S Tigvattananont, PJ Grote. 1996b. Metaphase karyotype of fruit flies of Thailand. II. Five species of four subgenera of *Bactrocera*. *J. Sci. Soc. Thailand* **22**: 97-104.
- Baimai V, W Trinachartvanit, S Tigvattananont, PJ Grote, R Poramacom, U Kijchalao. 1995. Metaphase karyotype of fruit flies of Thailand. I. Five sibling species of the *Bactrocera dorsalis* complex. *Genome* **38**: 1015-1022.
- Drew RAI. 1989. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. *Mem. Queensl. Mus.* **26**: 1-521.

- Drew RAI, DL Hancock. 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae) in Asia. Bull. Entomol. Res. (Suppl. Ser.) Suppl. 2: 1-68.
- Hardy DE. 1973. The fruit flies (Tephritidae-Diptera) of Thailand and bordering countries. Pacif. Insects Monogr. 31: 1-353.
- Hardy DE, MS Adachi. 1954. Studies in the fruit flies of the Philippine Islands, Indonesia, and Malaya. Part I. Dacini (Tephritidae-Diptera). Pacif. Sci. 8: 147-204.
- Irick H. 1994. A new function for heterochromatin. Chromosoma 103: 1-3.
- John B. 1988. The biology of heterochromatin. In RS Verma, ed. Heterochromatin: molecular and structural aspects. London: Cambridge Univ. Press, pp. 1-128.
- John B, GLG Miklos. 1979. Functional aspects of satellite DNA and heterochromatin. Int. Rev. Cytol. 58: 1-114.
- Pardue ML, W Hennig. 1990. Heterochromatin: junk or collector's item. Chromosoma 100: 3-7.
- White MJD. 1973. Animal cytology and evolution. 3rd ed. London: Cambridge Univ. Press.

泰國之果實蠅核型 (III): 東方果實蠅群之六個種類

Visut Baimai¹ Jaros Phinchongsakuldit¹ Wachareeporn Trinachartvanit¹

檢查從被感染為害的果樹採集到的六種屬於東方果實蠅群的果實蠅：*Bactrocera propinqua*, *B. irvingiae*, *B. carambolae*, *B. pyrifoliae*, *B. arecae*, 以及 *B. melastomatos* 的核型發現，染色體上異染色質的比例和分布截然不同。最常見的型式為 *B. irvingiae* 和 *B. propinqua*，和東方果實蠅 (*D. dorsalis*) 的型式很相似，同樣被歸為第一類核型。*B. carambolae* 和 *B. pyrifoliae* 的 X 染色體和體染色體著絲點附近的異染色質型式很相似，可歸為第四類核型。*B. arecae* 的體染色體和 X 染色體的異染色質型式不同於其他種類。*B. melastomatos* 的 X 和 Y 染色體都很小，無法歸入任何一類早先畫分的核型中。這些發現顯示異染質分化在泰國的果實蠅種類的核型進化是很重要的。這種不同的異染質型式可作為鑑定這些東方果實蠅群近緣種的依據。

關鍵詞：東方果實蠅群，有絲分裂染色體，異染色體，核型進化。

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