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METAPHASE KARYOTYPES OF *ANOPHELES* OF THAILAND AND SOUTHEAST ASIA: IV. THE BARBIROSTRIS AND UMBROSUS SPECIES GROUPS, SUBGENUS *ANOPHELES* (DIPTERA: CULICIDAE)

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ABSTRACT. Metaphase karyotypes of 2 and 3 species of the Umbrosus and the Barbirostris Groups, respectively, of the subgenus *Anopheles* occurring in Thailand and Indonesia show inter- and intraspecific differences with respect to the amount and distribution of constitutive heterochromatin in the sex chromosomes and/or autosomes. Four forms of metaphase karyotypes have been recognized in the wild samples of *Anopheles barbirostris* based on differences in size and shape of X and Y chromosomes. It is not known whether forms A, B, and C of the metaphase karyotype of *An. barbirostris* found in Thailand represent inter- or intraspecific differences. However, form D, which occurs only in Indonesia, may represent a mitotic karyotype of a distinct species closely related to *An. barbirostris. Anopheles campestris* and *An. barbumbrosus* are each readily separated from *An. barbirostris* by mitotic chromosomes. *Anopheles umbrosus* and *An. letifer* of the Umbrosus Group also exhibit heterochromatin variation in the X chromosomes. These 2 species can be readily distinguished by the gross morphology of mitotic karyotypes, particularly the X and Y chromosomes.

INTRODUCTION

The Barbirostris and Umbrosus species groups of the subgenus *Anopheles* comprise a large number of species occurring widely in Southeast Asia. However, only certain species of these groups are present in Thai mosquito populations (Harrison and Scanlon 1975, Harrison et al. 1991). Most of the cytological information, particularly on the metaphase chromosomes of Oriental *Anopheles,* comes from studies of human malaria vectors. Among the members of these 2 species groups, chromosomal data are known only in *Anopheles barbirostris* Van der Wulp from India (Avirachan et al. 1969, Chowdaiah et al. 1970). Our aim in this investigation is to provide cytological data of the anopheline mosquitoes of Southeast Asia for a better understanding of chromosomal evolution and its application in the cytotaxonomy of groups of closely related species (Baimai et al. 1993a, 1993b). This paper reports metaphase karyotypes of certain species belonging to the Barbirostris and Umbrosus Groups, and describes their mitotic chromosome morphology with special emphasis on the different amount and distribution of constitutive heterochromatin in sex chromosomes and autosomes.

MATERIALS AND METHODS

Wild female *Anopheles* mosquitoes used in this study were collected from bovine and/or human baits during the course of our cytogenetic investigations of anopheline mosquitoes of Southeast Asia (Table 1). They were identified to species based on morphological criteria alone. These species are *An. barbirostris* Van der Wulp, *An. campestris* Reid, *An. barbumbrosus* Stickland and Chowdhury, *An. umbrosus* Theobald, and *An. letifer* Sandosham. The first 3 species belong to the Barbirostris Group and the latter 2 are placed in the Umbrosus Group (Table 1). Isofemale lines were established from individual wild-caught females collected at different localities in Thailand, with the exception of 2 families of *An. barbirostris* from Java, Indonesia, which were kindly provided by the Department of Medical Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok. Air-dried mitotic chromosome preparations were made from neural ganglia of healthy 4th-instar larvae pretreated with colchicine using a modified method described by Baimai (1977) and Baimai et al. (1993a).

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RESULTS AND DISCUSSION

Thirty-four families of the 5 morphological species (Table 1) were examined cytologically. The typical karyotype of these species is essentially the same as that of the Hyrcanus Group, which was described previously (Baimai et al. 1993a). It consists of 2 pairs of autosomes (metacentric II and submetacentric III) and one pair of sex chromosomes of variable sizes and shapes, which is a general phenomenon of anopheline mosquitoes in Thailand and Southeast Asia. Metaphase chromosomes of these species, including forms, are described below.

Anopheles barbirostris: Twenty-seven fami-

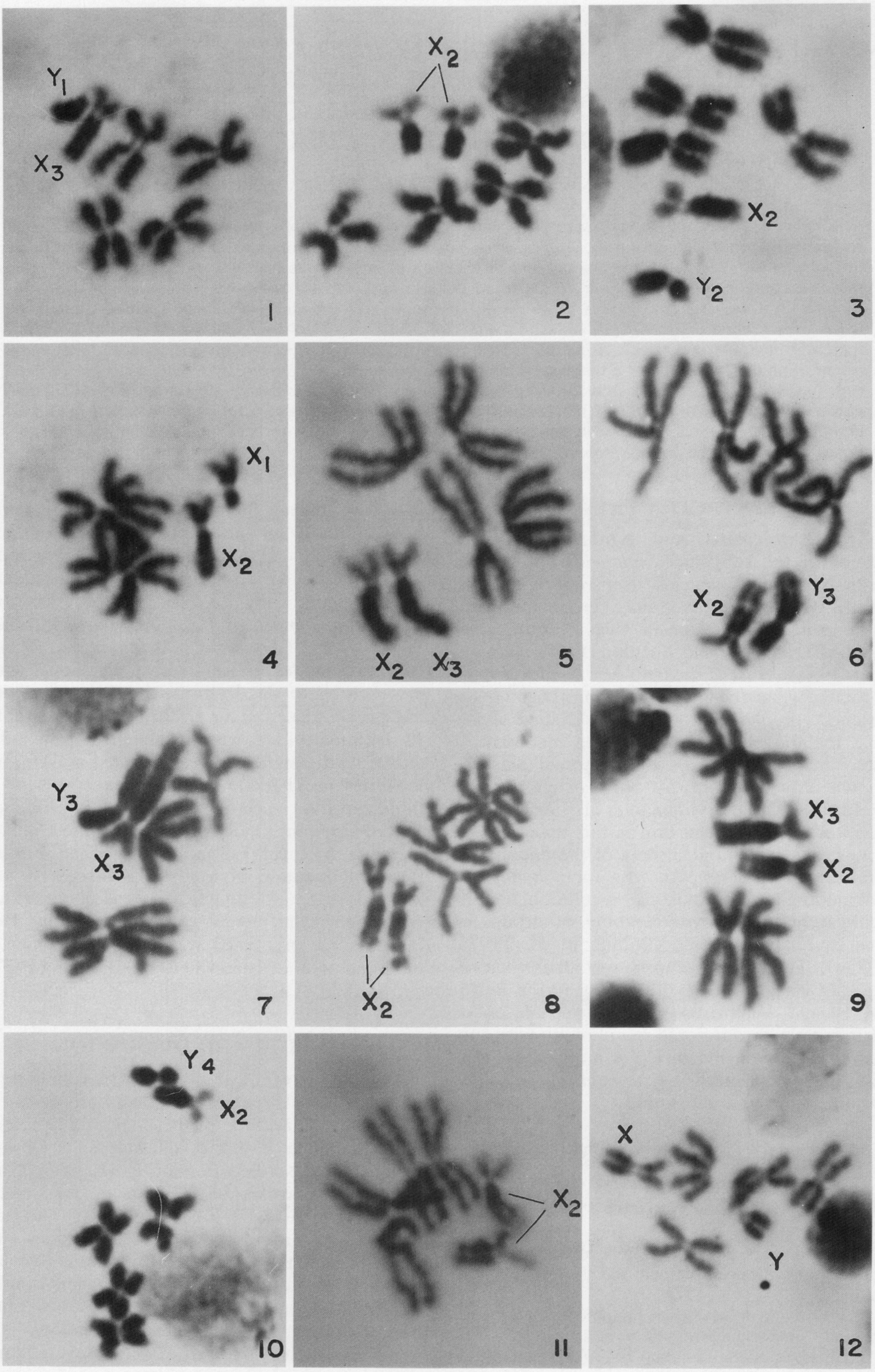
Y_1
X_3
1
X_2
2
X_2
Y_2
3
X_1
X_2
4
X_2
X_3
5
X_2
Y_3
6
Y_3
X_3
7
X_2
8
X_3
X_2
9
Y_4
X_2
10
X_2
11
X
Y
12

Table 1. The number of females (isolines) of 5 species of the *Anopheles barbirostris* and *Anopheles umbrosus* Groups collected and cytologically examined from wild populations in Thailand and Indonesia.

Species/form	Locality (village, province)	No. of isolines examined	Date of collection
An. barbirostris			
Form A	Maetang, Chiangmai	5	September 1984
	Sadao, Songkhla	2	October 1983
	Muang, Chumphon	1	April 1984
	Srimuangmai, Ubon Ratchathani	1	December 1987
Form B	Maetang, Chiangmai	1	September 1984
	Sadao, Songkhla	1	October 1983
	Lojunka, Phatthalung	3	February 1985
	Muang, Chumphon	1	April 1984
	Ban Phu, Udonthani	2	October 1986
	Ban Kang Rieng, Kanchanaburi	1	February 1986
Form C	Ban Thasalao, Phetchaburi	1	August 1983
	Tung Ka Ngok, Phangnga	2	June 1982
	Makarm, Chanthaburi	1	June 1983
	Muang, Chumphon	3	April 1984
Form D	Java, Indonesia	2	April 1984
An. campestris	Bangpa-in, Ayutthaya	1	March 1984
An. barbumbrosus	Ban Thasalao, Phetchaburi	1	August 1983
An. umbrosus	Muang, Chumphon	2	April 1984
An. letifer	Muang, Chumphon	3	April 1984

lies derived from individual wild-caught females identified as *An. barbirostris* were examined for mitotic chromosomes. Four forms of metaphase karyotypes are recognized based on the characteristics of X and Y chromosomes (Figs. 1–11).

Form A exhibits 2 types of X chromosome (X_2 and X_3) and one type of Y chromosome (Y_1). Both X_2 and X_3 are submetacentric with a euchromatic short arm and a heterochromatic long arm (Figs. 1 and 2), but the X_3 is slightly longer than the X_2. The X_3 could have been derived from the presumed ancestral X_2 simply through the addition of an extra block of heterochromatin, which is darkly stained compared with the rest of the chromosome arm. Chromosome Y_1 is obviously subtelocentric (acrocentric) with a considerable portion of heterochromatin present in the short arm (Fig. 1).

Form B shows 3 types of X chromosome (X_1, X_2, and X_3) and one type of Y chromosome (Y_2). The X_1 (Fig. 4) is apparently a small metacentric chromosome, whereas X_2 and X_3 (Fig. 5) are medium and large submetacentric chromosomes, respectively, similar to those of form A. Again the X_2 and X_3 could have arisen from the presumed ancestral X_1 chromosome via a gradual increase of heterochromatin. The Y_2 is a large submetacentric chromosome (Fig. 3).

Form C exhibits 2 types of X chromosome (X_2 and X_3) and one type of Y chromosome (Y_3). The X_2 and X_3 of form C (Figs. 8 and 9) are somewhat similar to those of forms A and B. However, the Y_3 is a large submetacentric or metacentric chromosome showing a large block of lightly staining heterochromatin at the distal end of the long arm (Figs. 6 and 7). It is not known at this stage whether chromosomal forms A, B, and C represent intra- or interspecific differences. Choochote et al. (1983) reported genetic incompatibility between 2 strains of *An. barbirostris* in Thailand, but it is unknown what forms of metaphase karyotype these strains represented.

Form D is a metaphase karyotype distinctly

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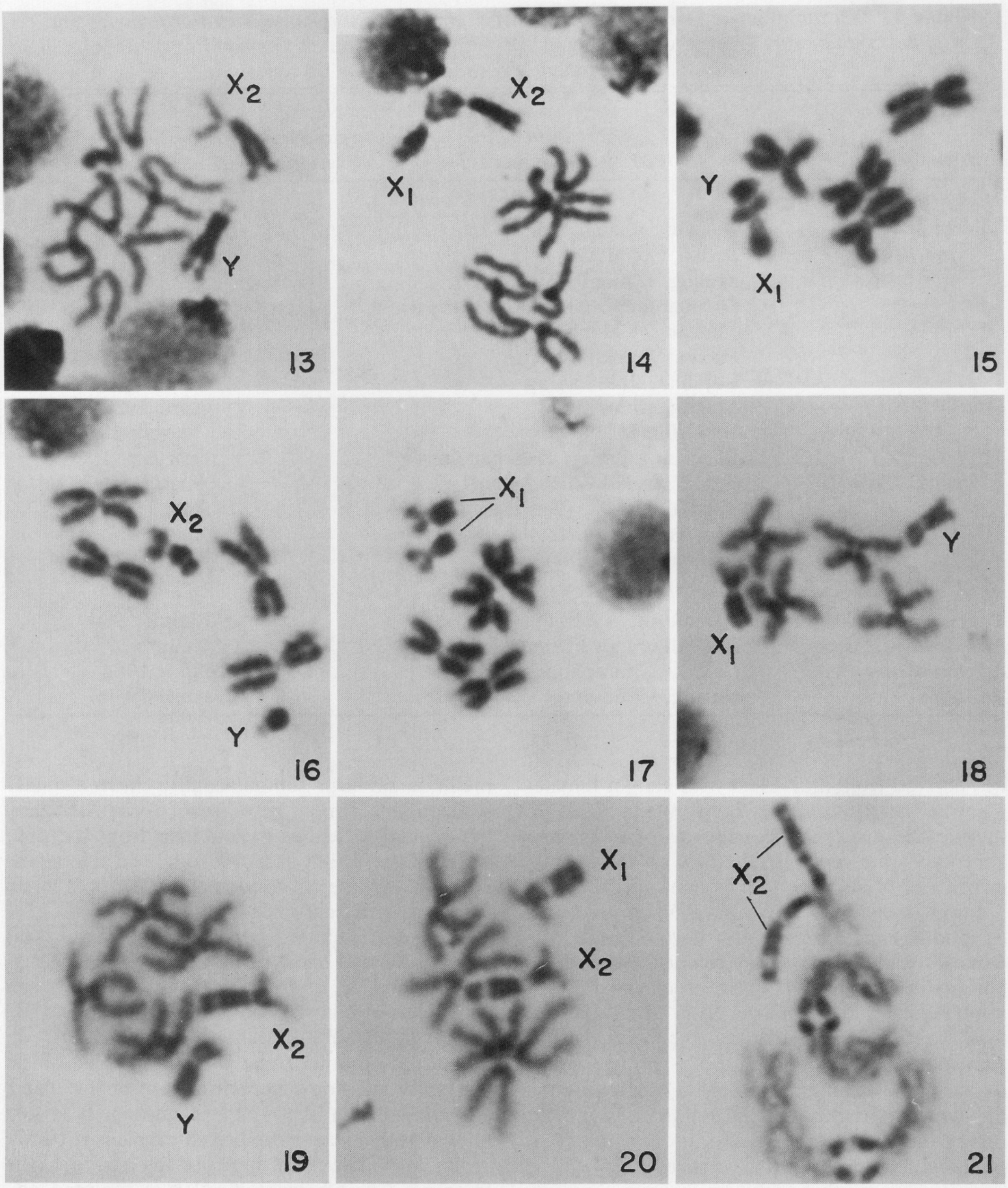
Figs. 1–12. Metaphase karyotypes from larval neuroblast cells. 1–11. *Anopheles barbirostris*; 1, 2. Male and female, respectively, of form A; 3, 4–5. Male and female, respectively, of form B; 6–7, 8–9. Male and female, respectively, of form C; 10, 11. Male and female, respectively, of form D; 12. Male of *Anopheles campestris.*



Figs. 13–21. Metaphase karyotypes from larval neuroblast cells. 13, 14. Male and female, respectively, of *Anopheles barbumbrosus*; 15–16, 17. Male and female, respectively, of *Anopheles umbrosus*; 18–19, 20–21. Male and female, respectively, of *Anopheles letifer*.

different from the other forms described above. The large submetacentric X chromosome consists of a euchromatic short arm and a heterochromatic long arm with a secondary constriction approximately at the middle of the arm (Fig. 11). It is somewhat similar to the X_2 of the other forms. Chromosome Y_4 is a medium metacentric (Fig. 10) that is obviously different from those of the other forms. Form D is from Java, Indonesia. It has not been detected in any popula-

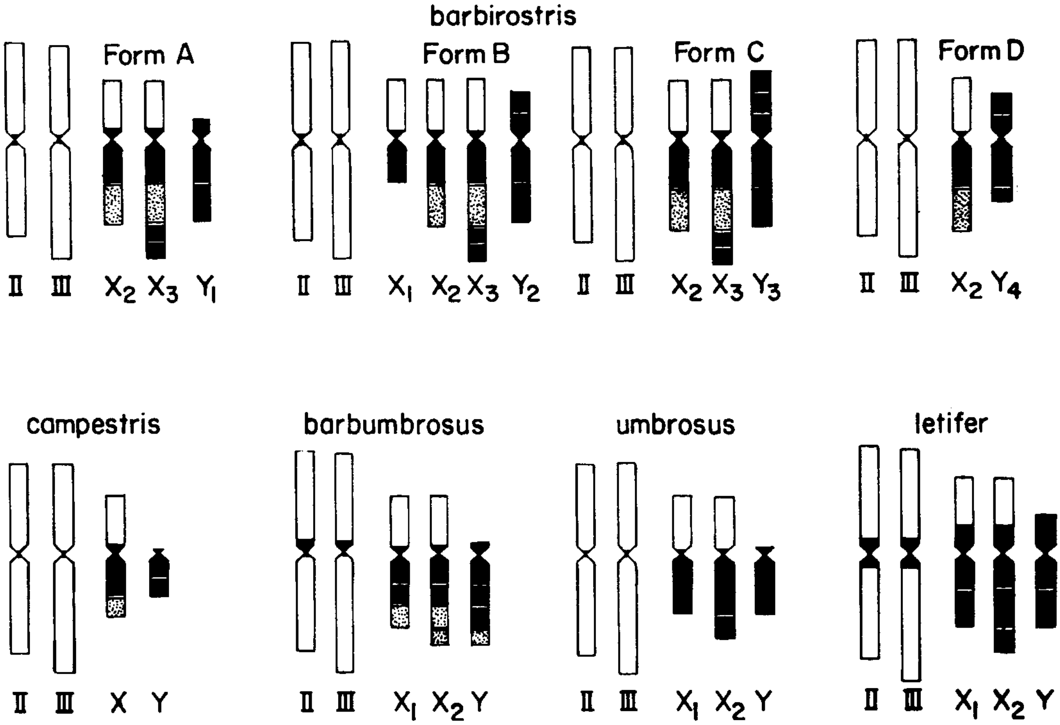


Fig. 22. Diagrammatic representation and comparison of metaphase karyotypes of 3 species (including 4 forms) and 2 species of the *Anopheles barbirostris* and the *Anopheles umbrosus* Groups, respectively. Only haploid idiograms are presented. Variable heterochromatin is indicated as black or shaded. Centromeric regions are indicated by constrictions of each chromosome. Chromosome lengths, arm ratios, and heterochromatic portions are shown in proportion.

tions in Thailand despite our extensive collections made throughout the country. On the basis of differences in mitotic chromosomes, morphology, and geographical distribution observed in this study, it seems probable that form D represents the metaphase karyotype of a distinct species closely related to *An. barbirostris.*

Anopheles campestris: Only one male larva from a single isofemale line of *An. campestris* was examined for mitotic chromosomes (Fig. 12). It showed metacentric X and telocentric Y chromosomes. Clearly, the sex chromosomes of *An. campestris* are quite different from those of *An. barbirostris* described above, despite the morphological similarity between these 2 species that often causes problems for identification.

Anopheles barbumbrosus: Only one family of this species was available for study. One side of each of the 2 pairs of autosomes shows a noticeable amount of pericentric heterochromatin (Figs. 13 and 14). This species also shows heterochromatin variation in the sex chromosomes. The X_1 and X_2 chromosomes are submetacentric, each with the euchromatic short arm and the heterochromatic long arm with a lightly staining block at the distal region. Chromosome Y is subtelocentric or acrocentric with a very small portion of heterochromatin present in the centromeric region. Like the X chromosome, the distal portion of the Y exhibits a lightly staining block of heterochromatin compared with the remaining darkly staining portion.

Anopheles umbrosus: This species shows a typical form of metaphase karyotype consisting of 2 types of X chromosome and a small subtelocentric or acrocentric Y chromosome (Figs. 15–17). The X_1 is clearly metacentric (Figs. 15 and 17); one arm is euchromatic and the opposite arm is heterochromatic and of approximately equal length. The X_2 is a larger submetacentric chromosome. The X_2 differs from the X_1 in having an extra block of heterochromatin at the distal end of the heterochromatic arm.

Anopheles letifer: This species shows a different metaphase karyotype (Figs. 18–21) from that of *An. umbrosus.* The 2 pairs of autosomes exhibit conspicuous pericentric heterochromatin compared with those of *An. umbrosus.* There are 2 types of X chromosome. The X_1 is apparently metacentric with a block of conspicuous centromeric heterochromatin in the euchromatic arm. The X_2 is submetacentric with a very long heterochromatic arm compared with that of the X_1. As a general phenomenon, the X_2 could have arisen from the presumed ancestral X_1 through the acquisition of extra block(s) of heterochromatin in the distal end of the chromosome arm.

A diagrammatic representation of metaphase karyotypes of the 5 species and forms of the 2 species groups is presented in Fig. 22. Heterochromatin variation in the sex chromosomes and, to lesser extent in the centromeric regions of the autosomes, appears to be common in the Barbirostris and Umbrosus Groups, similar to other species groups of Oriental *Anopheles* (Baimai et al. 1984, 1993a, 1993b; Baimai 1988). This kind of chromosome variation could simply occur by means of gain of major block(s) of constitutive heterochromatin, which is a general phenomenon among higher organisms (White 1973, John and Miklos 1979). The findings in this study lend support to our viewpoint that sex chromosomes of Oriental *Anopheles* and some other dipteran insects are prone to the accumulation of constitutive heterochromatin, which presumably has played an important role in chromosomal evolution of the Southeast Asian fauna.

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