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METAPHASE KARYOTYPES OF *ANOPHELES* OF THAILAND AND SOUTHEAST ASIA. VI. THE PYRETOPHORUS AND THE NEOMYZOMYIA SERIES, SUBGENUS *CELLIA* (DIPTERA: CULICIDAE)

VISUT BAIMAI,[1] UDOM KIJCHALAO[1] AND RAMPA RATTANARITHIKUL[2]

ABSTRACT. A total of 6 species of the Pyretophorus (4 species) and Neomyzomyia (2 species) series of the subgenus *Cellia* of *Anopheles* were used for metaphase karyotype analysis. *Anopheles subpictus* and *An. vagus* exhibit 4 and 2 forms of mitotic karyotypes, respectively, which are attributable to different types of Y chromosomes. Such distinctive mitotic chromosomes may reflect interspecies differences within each of these 2 taxa. Two distinct species, *An. indefinitus* and *An. sundaicus,* show similar metaphase karyotypes, particularly with regard to the size and shape of the sex chromosomes. Likewise, *An. tessellatus* and *An. kochi,* which are distinct species of the Neomyzomyia Series, also have metaphase karyotypes that resemble each other. They exhibit a typical feature of telocentric sex chromosomes resembling those of the *An. dirus* complex and the other species of the Leucosphyrus Group. Like the other cases of the Oriental *Anopheles,* heterochromatin has played a significant role in chromosome evolution of the 6 species.

INTRODUCTION

The subgenus *Cellia* of the genus *Anopheles* comprises 4 series, namely, Neocellia, Myzomyia, Pyretophorus, and Neomyzomyia (Reid 1968). Metaphase karyotypes of many species belonging to the first 2 series have been reported previously (Baimai et al. 1993b, 1994, 1996). Since some of the species belonging to the Neomyzomyia Series, particularly the Leucosphyrus Group, which includes the *An. dirus* and the *An. leucosphyrus* complexes, are primary vectors of human malarial parasites in Thailand and neighboring countries, they have received *a priori* attention. The results of our studies on metaphase karyotypes of these species were reported previously (Baimai et al. 1987, 1988; Baimai 1988a for reviews). Our earlier reports have shown that analysis of mitotic karyotypes is a useful cytotaxonomic tool for separating some cryptic species of the Oriental *Anopheles.* In this final report of the series, we present the results of metaphase karyotype analysis of the remaining 2 species of the Neomyzomyia Series found in Thailand and 4 species of the Pyretophorus Series occurring in the Oriental Region.

MATERIALS AND METHODS

Totals of 4 and 2 species of the Pyretophorus and Neomyzomyia series, respectively, from Thailand, Indonesia, and Bangladesh were examined cytologically. They include *An. subpictus* Grassi, *An. indefinitus* Ludlow, *An. sundaicus* Rodenwaldt, *An. vagus* Doenitz, *An. tessellatus* Theobald, and *An. kochi* Doenitz. Adult females of these species were collected from bovine or human bait at different localities (Table 1). Fully bloodfed, wild-caught females were identified morphologically to species as far as possible. All females were individually set up to produce F_1 larval progeny for chromosome study. Brain ganglia of 4th-instar larvae from each isofemale line were used for mitotic chromosome preparations and analysis employing the techniques described by Baimai (1977) and Baimai et al. (1993a).

[1] Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.
[2] Department of Medical Entomology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok 10400, Thailand.

RESULTS

The 6 species (including forms) examined cytologically in this study exhibit uniformity in chromosome number ($2n = 6$) except for the existence of supernumerary (B) chromosomes in some samples of *An. indefinitus* from Kanchanaburi Province, western Thailand. Thus, the metaphase karyotype of these species consists of, as a general rule, two pairs of autosomes and a pair of heteromorphic sex chromosomes that may vary in size and shape depending on the amount and distribution of constitutive heterochromatin. Differences in mitotic chromosomes found in this study are briefly described below.

Anopheles subpictus: This morphological species is widely distributed in Southeast Asia. The 2 pairs of autosomes are quite uniform in all specimens examined. Differences in mitotic chromosomes were found in the X and Y chromosomes. Two types of X chromosome and 4 types of Y chromosome were observed among the 15 families examined. The X_1 is submetacentric. The short arm of the X_1 is entirely heterochromatic while the long arm consists of a euchromatic portion and a conspicuous block of centromeric heterochromatin (Figs. 1–9). The X_2 appears to be metacentric, differing from the X_1 in having a major block of heterochromatin at the distal end of the heterochromatic arm. This difference between the X_1 and the X_2 can be readily seen in a heterozygous female

Table 1. The number of females (isolines) of 4 and 2 species of the Pyretophorus and the Neomyzomyia series, respectively, collected and examined cytologically from different wild populations in Thailand, Indonesia, the Philippines, and Bangladesh. All localities are listed as districts and provinces.

Species/form	Locality	No. of isolines examined	Date of collection
Pyretophorus Series			
An. subpictus			
Form A	Flores, Indonesia	2	April 1984
	Luzon (Montalban), Philippines	2	August 1986
Form B	Pathiu, Chumphon	1	March 1986
	Flores, Indonesia	1	April 1984
	Luzon (Montalban), Philippines	3	August 1986
Form C	Pathiu, Chumphon	1	March 1986
Form D	Sawee, Chumphon	5	March 1986
An. indefinitus	Srinakarin Dam, Kanchanaburi	3	August 1982
	Taknaf, Bangladesh	6	November 1984
An. sundaicus	Takuapa, Phangnga	4	March 1990
An. vagus	Nangrong Fall, Nakhon Nayok	3	August 1982
Form A	Maetang, Chiangmai	2	August 1982
Form B	Sadao, Songkhla	2	January 1986
Neomyzomyia Series			
An. tessellatus	Tung Ka Ngok, Phangnga	3	June 1982
An. kochi	Tung Ka Ngok, Phangnga	2	June 1982
	Maetang, Chiangmai	4	September 1982

(Figs. 4, 6, and 9). The Y_1 is subtelocentric (acrocentric) (Fig. 1) while the Y_2 is metacentric (Fig. 3). The Y_1 and Y_2 are apparently similar in size. The Y_3 has a large submetacentric shape (Fig. 5) when compared with the large metacentric Y_4 chromosome (Fig. 7). Thus, these types of Y chromosome clearly differ from each other in the amount of heterochromatin and the position of the centromere. Based on the different types of Y chromosome, 4 forms of metaphase karyotypes are recognized in *An. subpictus,* i.e., form A (X_1, Y_1), form B (X_1, X_2, Y_2), form C (X_1, X_2, Y_3) and form D (X_1, X_2, Y_4). Of these forms, form B appears to be widely distributed since it was found in Thailand, Indonesia, and the Philippines. Form A appears to be common in Indonesia and the Philippines. In contrast, forms C and D were found only in Thailand, with form C occurring in sympatry with form B at Chumphon Province. Such distinctive forms of mitotic chromosomes may reflect interspecies differences within the taxon *An. subpictus.* Unfortunately, we did not have information on polytene chromosomes for comparison with the fixed inversion of the 4 species of the *An. subpictus* complex described by Suguna et al. (1994). This interesting possibility of cryptic species warrants further investigations on the population genetics of *An. subpictus,* especially in the areas where this taxon has played a major role in malaria transmission.

Anopheles indefinitus: Samples of this species from Thailand and Bangladesh show generally similar metaphase karyotypes. Interestingly, specimens from Thailand exhibit 2 supernumerary (B) chromosomes as previously reported (Baimai et al. 1984). Two types of X chromosome were observed. The X_1 is telocentric with a very large portion of centromeric heterochromatin occupying about one half of the chromosome length (Figs. 11 and 12). The X_2 is a larger telocentric chromosome due to the presence of extra block(s) of heterochromatin, possibly in the distal area of centromeric heterochromatin (Figs. 10 and 12). The Y chromosome is a telocentric chromosome of considerable size compared with the X_1 (Figs. 10 and 11). The autosomes II and III consist of prominent blocks of pericentric heterochromatin (Figs. 10–12).

Anopheles sundaicus: The metaphase karyotype of this species is, in general, similar to that of *An. indefinitus* mentioned above, although *An. sundaicus* is morphologically and ecologically quite a distinct species. Nevertheless, *An. sundaicus* exhibits large telocentric X and Y chromosomes (Figs. 13 and 14) compared with those of *An. indefinitus.* Two types of telocentric X chromosome were re-

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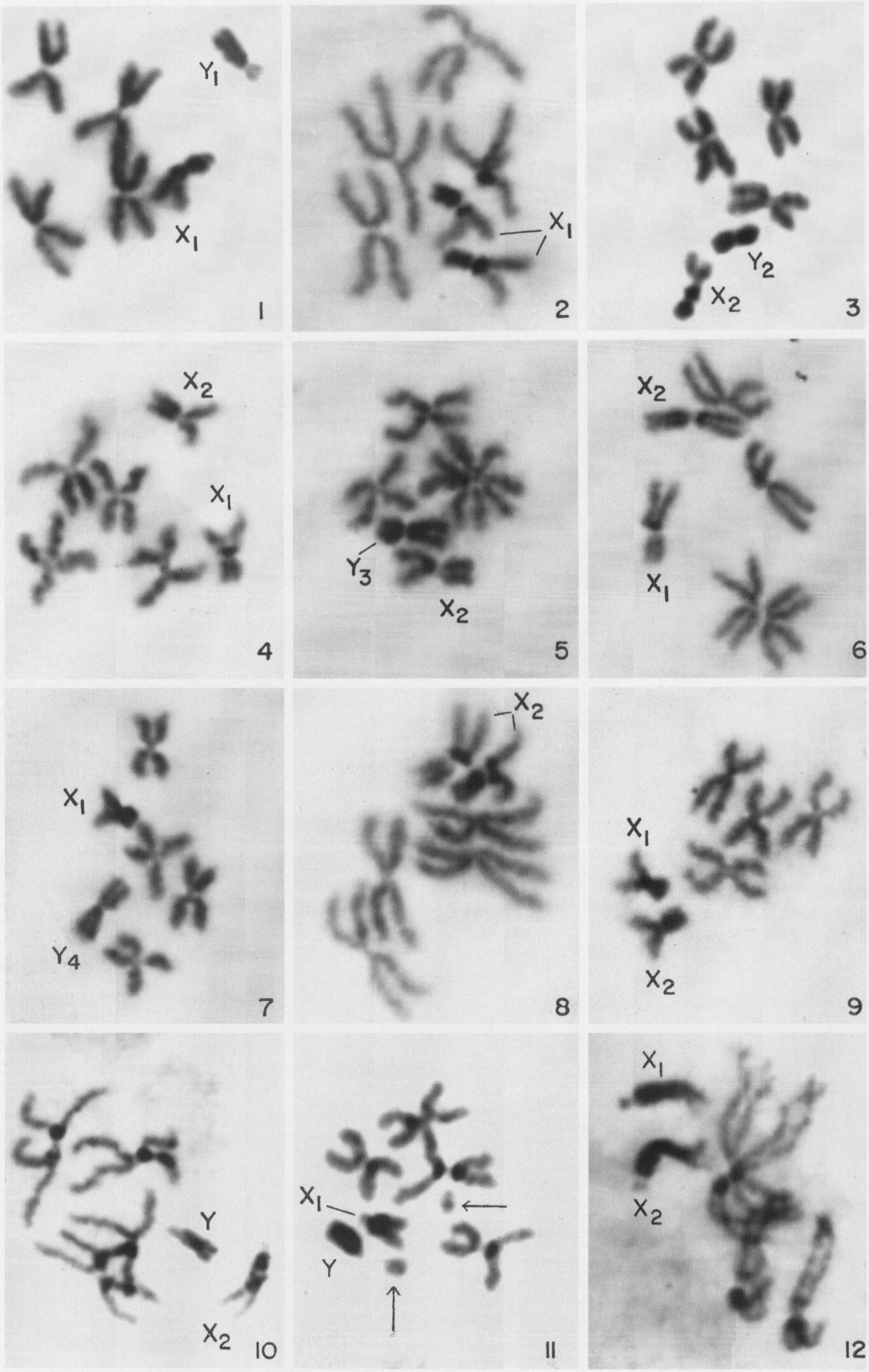
Figs. 1–12. Metaphase karyotypes from larval neuroblast cells. 1–9, *Anopheles subpictus.* 1, 2. Male and female, respectively, of form A; 3, 4. Male and female, respectively, of form B; 5, 6. Male and female, respectively, of form C; 7, 8–9. Male and female, respectively, of form D; 10–11, 12. Male and female, respectively, of *An. indefinitus* (arrows indicate supernumerary chromosomes).



Y1
X1
1
X1
2
Y2
X2
3
X2
X1
4
Y3
X2
5
X2
X1
6
X1
Y4
7
X2
8
X1
X2
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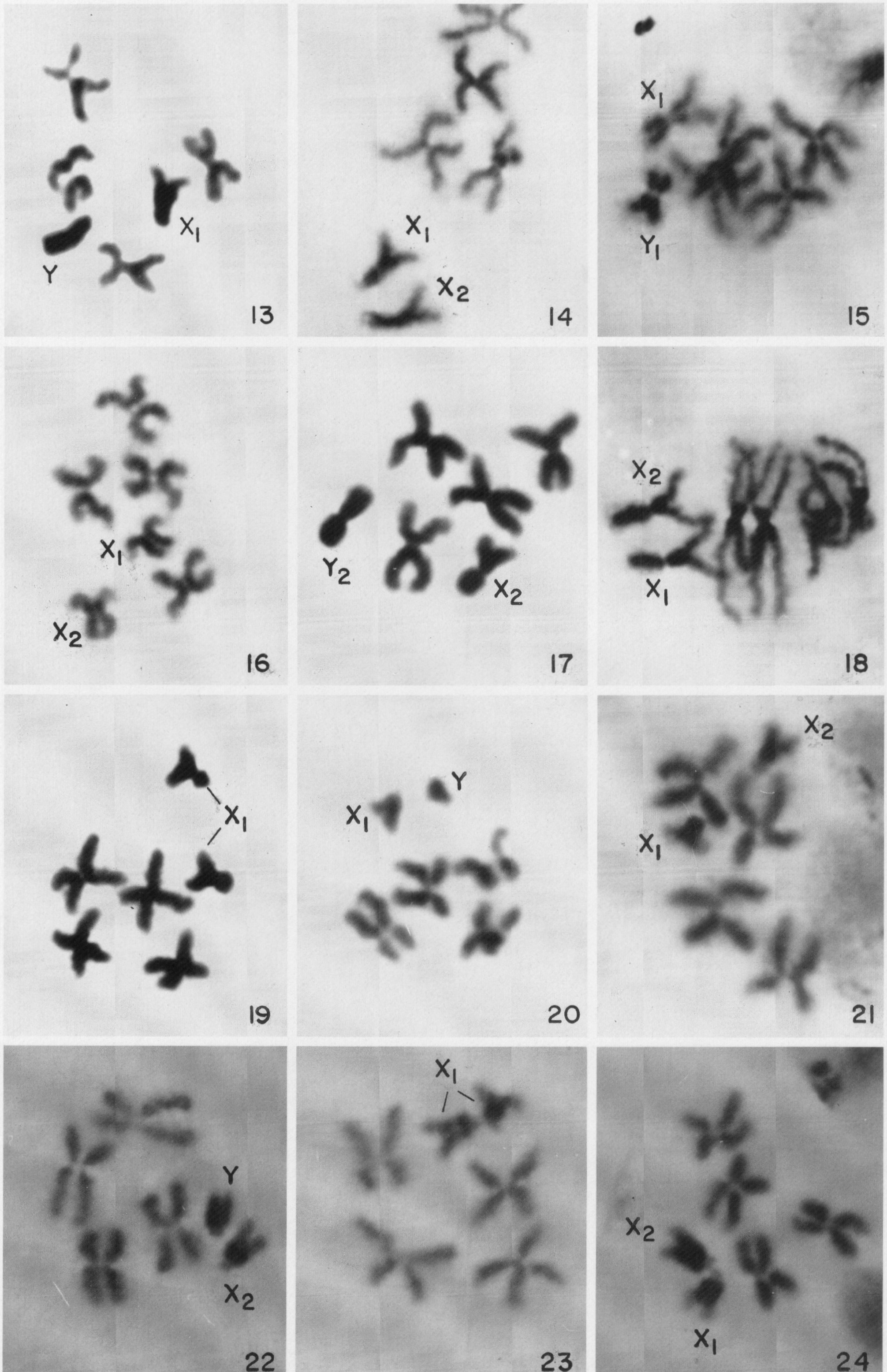
Y
X2
10
X1
Y
11
X1
X2
12


X_1
Y
13
X_1
X_2
14
X_1
Y_1
15
X_1
X_2
16
Y_2
X_2
17
X_2
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18
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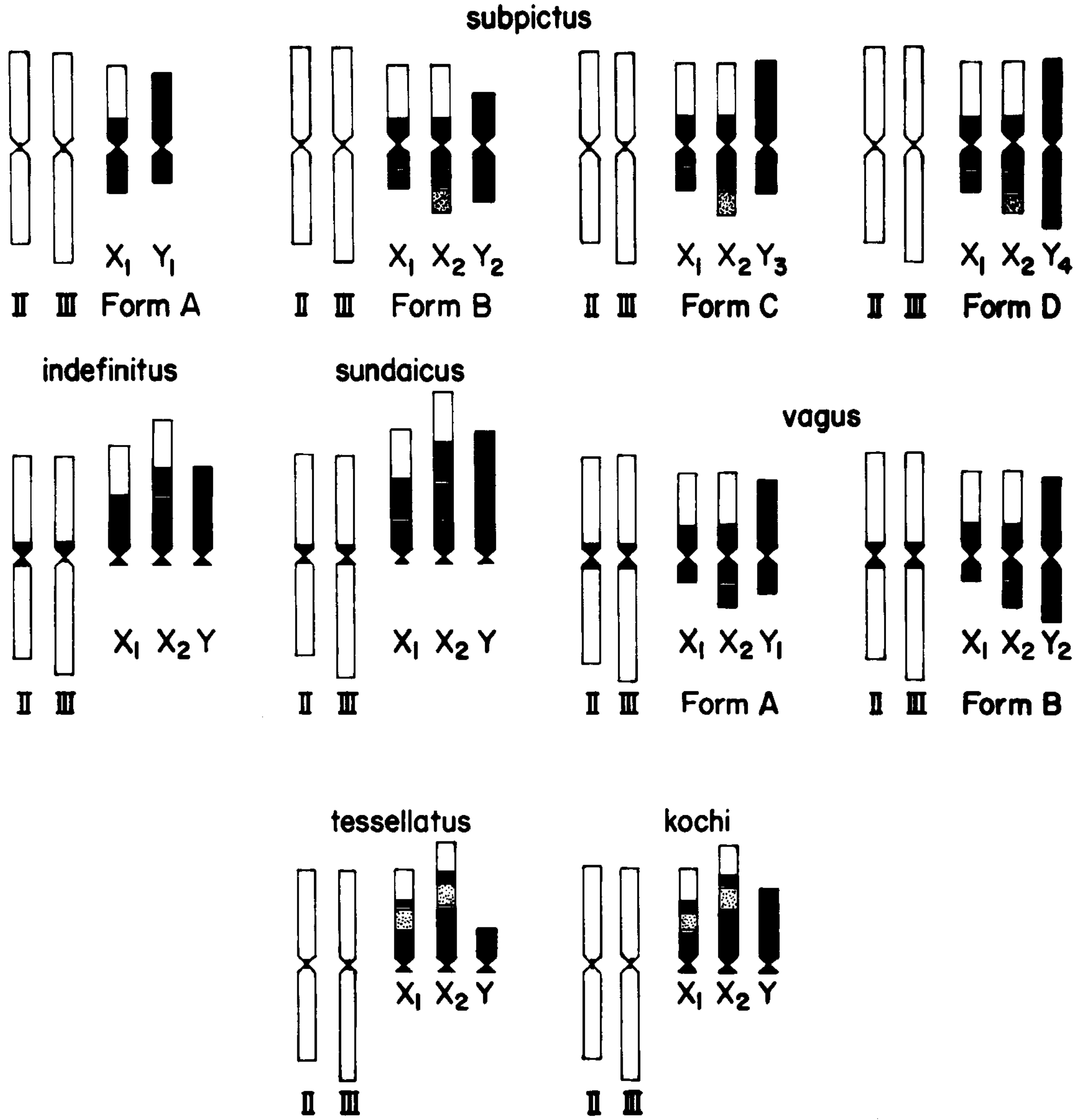

Fig. 25. Diagrammatic representation and comparison of metaphase karyotypes of 4 species (including forms) of the Pyretophorus Series and 2 species of the Neomyzomyia Series. Only one set of autosomes II and III is presented. Variable heterochromatin is indicated in black or shaded. The centromeres are indicated by constrictions of each chromosome. Chromosome lengths, arm ratios, and heterochromatic portions are shown in proportion.

corded in this study. The X_2 is clearly larger than the X_1 primarily due to the acquisition of heterochromatin in the centromeric region (Fig. 14). This type of metaphase karyotype is referred to as form A to distinguish it from 2 additional forms, B and C, which were discovered recently from the Indonesian populations (Sukowati and Baimai 1996).

Anopheles vagus: This species exhibits conspicuous blocks of pericentric heterochromatin in both pairs of autosomes. There are 2 types of X chro-

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Figs. 13–24. Metaphase karyotypes from larval neuroblast cells. 13, 14. Male and female, respectively, of *Anopheles sundaicus*; 15–19, *An. vagus*. 15, 16. Male and female, respectively, of form A; 17, 18–19. Male and female, respectively, of form B; 20, 21. Male and female, respectively, of *An. tessellatus*; 22, 23–24. Male and female, respectively, of *An. kochi*.

mosome. The X_1 is submetacentric. Its long arm consists of a euchromotic portion and a large block of centromeric heterochromatin, while the short arm is totally heterochromatic (Figs. 15–19). The X_2 is also submetacentric but its heterochromatic arm is slightly longer than that of the X_1 due to the presence of an extra block of heterochromatin (Figs. 16–18). The general figure of these types of X chromosome corresponds with that of *An. vagus* from the Indian subcontinent (Avirachan et al. 1969). Two types of Y chromosome were also observed. The Y_1 has a normal submetacentric shape (Fig. 15), while the Y_2 is a larger submetacentric chromosome due to the presence of an extra block of heterochromatin in the short arm (Fig. 17). Thus, 2 forms of metaphase karyotype are recognized in *An. vagus* based on the two types of Y chromosome, *viz.*, form A (X_1, X_2, Y_1) and form B (X_1, X_2, Y_2). Form B seems to be widespread since it was found in Chiangmai and Songkhla provinces in northern and southern Thailand, respectively, while form A was detected in Nakhon Nayok Province. It is not known at this stage whether these 2 forms of metaphase karyotype represent intra- or interspecies differences.

Anopheles tessellatus: This species shows distinctive X and Y chromosomes which are typical of the Neomyzomyia Series as demonstrated in the Leucosphyrus Group. The X chromosome is telocentric, consisting of large blocks of centromeric heterochromatin occupying approximately two thirds of the chromosome length. Two distinctive types of X chromosome were found among our samples. The X_1 is shorter than the X_2, which obviously contains a larger amount of centromeric heterochromatin (Fig. 21). The Y chromosome has a short telocentric shape compared with that of the X_1 (Fig. 20). The autosome pairs show a limited amount of pericentric heterochromatin.

Anopheles kochi: The metaphase karyotype of this species resembles that of *An. tessellatus*, described above. It consists of 2 types of X chromosome. The telocentric X_2 is slightly longer than the X_1 chromosome due to the presence of centromeric heterochromatin (Figs. 23 and 24). The Y chromosome is also telocentric and is only slightly shorter than the X_1 (Fig. 22); the Y chromosome of this species is longer than that of *An. tessellatus*. A limited amount of pericentric heterochromatin is present in the autosome pairs.

Diagrammatic representations of the metaphase karyotypes of the 6 species, including forms, found in this study are shown in Fig. 25.

DISCUSSION

Analysis of metaphase karyotypes of the 15 isofemale lines derived from the wild-caught females of *An. subpictus* collected from different localities in Thailand, Indonesia, and the Philippines has revealed remarkable differences in the Y chromosome with respect to the amount of constitutive heterochromatin. Four forms of mitotic karyotypes are recognized based on the different types of Y chromosome. However, none of these forms corresponds with the mitotic karyotype of "*An. subpictus*" reported by Avirachan et al. (1969). Furthermore, Suguna et al. (1994) described a paracentric fixed inversion on the X chromosome of 4 species (A, B, C, and D) in the *An. subpictus* complex in India. We are unable to say what species of the *An. subpictus* complex occurs in Thailand because we did not have polytene chromosome data from our material for comparison with that of Suguna et al. (1994). In our earlier work, we reported some groups of closely related species which are known to have different types of the Y chromosome, for example, the *An. dirus* complex (Baimai 1988a), the *An. maculatus* complex (Baimai et al. 1993b), and the *An. minimus* and the *An. culicifacies* complexes (Baimai et al. 1996). It is possible that some of these forms of metaphase karyotypes of *An. subpictus* might represent distinctive characters at an interspecific level. Further cytogenetic study of this possible species complex is required to clarify this species problem since *An. subpictus* plays an important role in malaria transmission in certain areas of Southeast Asia, including Indonesia (Kirnowardoyo 1988) and Malaysia (Loong 1988). A similar situation is found in the 2 forms of *An. vagus* which exhibit distinctive Y chromosomes.

There has always been some taxonomic confusion in separating *An. subpictus* and *An. indefinitus* based on morphological criteria alone (Reid 1968). The evidence from metaphase karyotype analysis shown in this study clearly demonstrates that these 2 closely related species are quite distinct cytologically, particularly in the size and shape of heteromorphic sex chromosomes (Fig. 25). On the contrary, *An. indefinitus* and *An. sundaicus* exhibit similar metaphase chromosomes despite their distinct morphologies. The metaphase karyotypes of *An. tessellatus* and *An. kochi* generally resemble those of the other species of the Neomyzomyia Series described previously (Baimai 1988b) despite their distinctiveness in morphology.

Analysis of metaphase karyotypes has proved to be useful as a cytotaxonomic tool in separating some cryptic species of anopheline mosquitoes, at least in the Southeast Asian region, as demonstrated in this series of publications (Baimai et al. 1993a, 1993b, 1994, 1996). Most, if not all, differences in mitotic chromosomes observed in this study and in the previous reports are due to the acquisition of extra blocks of constitutive heterochromatin. Thus the accumulation of heterochromatin in the sex chromosomes and, to a lesser extent, in the centromeric region of the autosomes, may play a significant role during the processes of species divergence, although the functional aspect of heterochromatin remains an unsolved problem (John and Miklos 1979). Further studies of mitotic chromosome variation attributable to gain of heterochromatin in the genome of a large number of species of *Anopheles* would contribute to a better understanding of the processes of speciation and possibly coevolution between the *Plasmodium* parasites and their mosquito vectors (Baimai 1988a).

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REFERENCES CITED

Avirachan, T. T., P. L. Seetharam and B. N. Chowdaiah. 1969. Karyotype studies in Oriental anophelines I. Cytologia 34:418–422.

Baimai, V. 1977. Chromosomal polymorphisms of constitutive heterochromatin and inversions in *Drosophila.* Genetics 85:85–93.

Baimai, V. 1988a. Constitutive heterochromatin differentiation and evolutionary divergence of karyotype in Oriental *Anopheles* (*Cellia*). Pacific Sci. 42:13–27.

Baimai, V. 1988b. Population cytogenetics of the malaria vector *Anopheles leucosphyrus* group. Southeast Asian J. Trop. Med. Public Health 19:667–680.

Baimai, V., R. E. Harbach and S. Sukowati. 1988. Cytogenetic evidence for two species within the current concept of the malaria vector, *Anopheles leucosphyrus* (Diptera: Culicidae), in Southeast Asia. J. Am. Mosq. Control Assoc. 4:44–50.

Baimai, V., U. Kijchalao and R. Rattanarithikul. 1996. Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. V. The Myzomyia Series, subgenus *Cellia.* J. Am. Mosq. Control Assoc. 12:97–105.

Baimai, V., R. Rattanarithikul and U. Kijchalao. 1993a. Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. I. The Hyrcanus group. J. Am. Mosq. Control Assoc. 9:59–67.

Baimai, V., A. Treesucon and R. Rattanarithikul. 1994. Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. III. The Neocellia Series of the subgenus *Cellia* (Diptera: Culicidae). Mosq. Syst. 26:116–124.

Baimai, V., S. Wibowo and R. G. Andre. 1984. Supernumerary (B) chromosome in *Anopheles indefinitus* (Diptera: Culicidae). Experientia 40:749–750.

Baimai, V., R. Rattanarithikul, U. Kijchalao and C. A. Green. 1993b. Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia. II. The Maculatus Group, Neocellia Series, subgenus *Cellia.* Mosq. Syst. 25:116–123.

Baimai, V., R. G. Andre, B. A. Harrison, U. Kijchalao and L. Panthusiri. 1987. Crossing and chromosomal evidence for two additional sibling species within the taxon *Anopheles dirus* Peyton and Harrison (Diptera: Culicidae) in Thailand. Proc. Entomol. Soc. Wash. 89: 157–166.

John, B. and G. L. G. Miklos. 1979. Functional aspects of satellite DNA and heterochromatin. Int. Rev. Cytol. 58:1–114.

Kirnowardoyo, S. 1988. *Anopheles* malaria vector and control measures applied in Indonesia. Southeast Asian J. Trop. Med. Public Health 19:713–716.

Loong, K. P. 1988. Current status of the vectors of human malaria in Malaysia. Southeast Asian J. Trop. Med. Public Health 19:725–729.

Reid, J. A. 1968. Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. Med. Res., Malaysia 31:1–520.

Suguna, S. G., K. G. Rathinam, A. R. Rajavel and V. Dhanda. 1994. Morphological and chromosomal descriptions of new species in the *Anopheles subpictus* complex. Med. Vet. Entomol. 8:88–94.

Sukowati, S. and V. Baimai. 1996. A standard cytogenetic map for *Anopheles sundaicus* (Diptera: Culicidae) and evidence for chromosomal differentiation in populations from Thailand and Indonesia. Genome 39:165–173.