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METAPHASE KARYOTYPES OF *ANOPHELES* OF THAILAND AND SOUTHEAST ASIA. III. THE NEOCELLIA SERIES OF THE SUBGENUS *CELLIA* (DIPTERA: CULICIDAE)

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ABSTRACT. Metaphase karyotypes of eight species of the Neocellia Series (excluding the Maculatus Complex) showed inter- and intraspecific variation in the sex chromosomes and in the centromeric region of the autosomes based on the amount and distribution of heterochromatin. *Anopheles jamesii* Theobald and *An. karwari* (James) exhibited two and three forms, respectively, of metaphase karyotypes based on the different size and shape of the X and Y chromosomes. *Anopheles annularis* Van der Wulp, *An. pseudojamesi* Strickland and Choudhury, and *An. philippinensis* Ludlow also showed heterochromatin variation in the sex chromosomes. These kinds of sex chromosome variations have not been observed in limited samples of *An. stephensi* Liston, *An. nivipes* (Theobald), and *An. splendidus* Koidzumi. Two groups of X chromosomes have been recognized based on the amount of centromeric heterochromatin in the euchromatic arm. *Anopheles karwari, An. annularis, An. jamesii, An. pseudojamesi,* and *An. stephensi* form one group, while group two includes *An. nivipes* and *An. splendidus. Anopheles philippinensis* has both kinds of X chromosomes, which may reflect the common ancestral karyotype of the species in this series.

INTRODUCTION

The Neocellia Series of the subgenus *Cellia* is one of the largest groups of Oriental *Anopheles*. Reid (1986) listed a dozen species belonging to the Neocellia Series occurring in Southeast Asia. Recent studies of Green et al. (1985), Rattanarithikul and Green (1986), and Rattanarithikul and Harbach (1990) have led to the recognition of eight species within the previous concept of *An. maculatus,* including three species elevated from synonymy and four new species, thus bringing the total to 19 species. Recently, Baimai et al. (1993b) reported metaphase karyotypes of the eight closely related species of the Maculatus Complex. In this report we present metaphase karyotypes of eight of the remaining 11 species belonging to the Neocellia Series in Southeast Asia.

MATERIALS AND METHODS

Adult females of the eight species of the Neocellia Series reported in this study were collected from bovine and/or human bait in Thailand, the Philippines, and Bangladesh during 1981–1987 in the course of our research program. The species include *An. karwari* (James), *An. annularis* Van der Wulp, *An. jamesii* Theobald, *An. pseudojamesi* Strickland and Choudhury, *An. stephensi* Liston, *An. nivipes* (Theobald), *An. philippinensis* Ludlow, and *An. splendidus* Koidzumi (Table 1).

Brain ganglia of fourth-instar larvae from individual isofemale lines were used for mitotic chromosome preparations. Early prophase or metaphase chromosomes from neuroblast cells were photographed and analyzed using the method described by Baimai (1977) and Baimai et al. (1993a).

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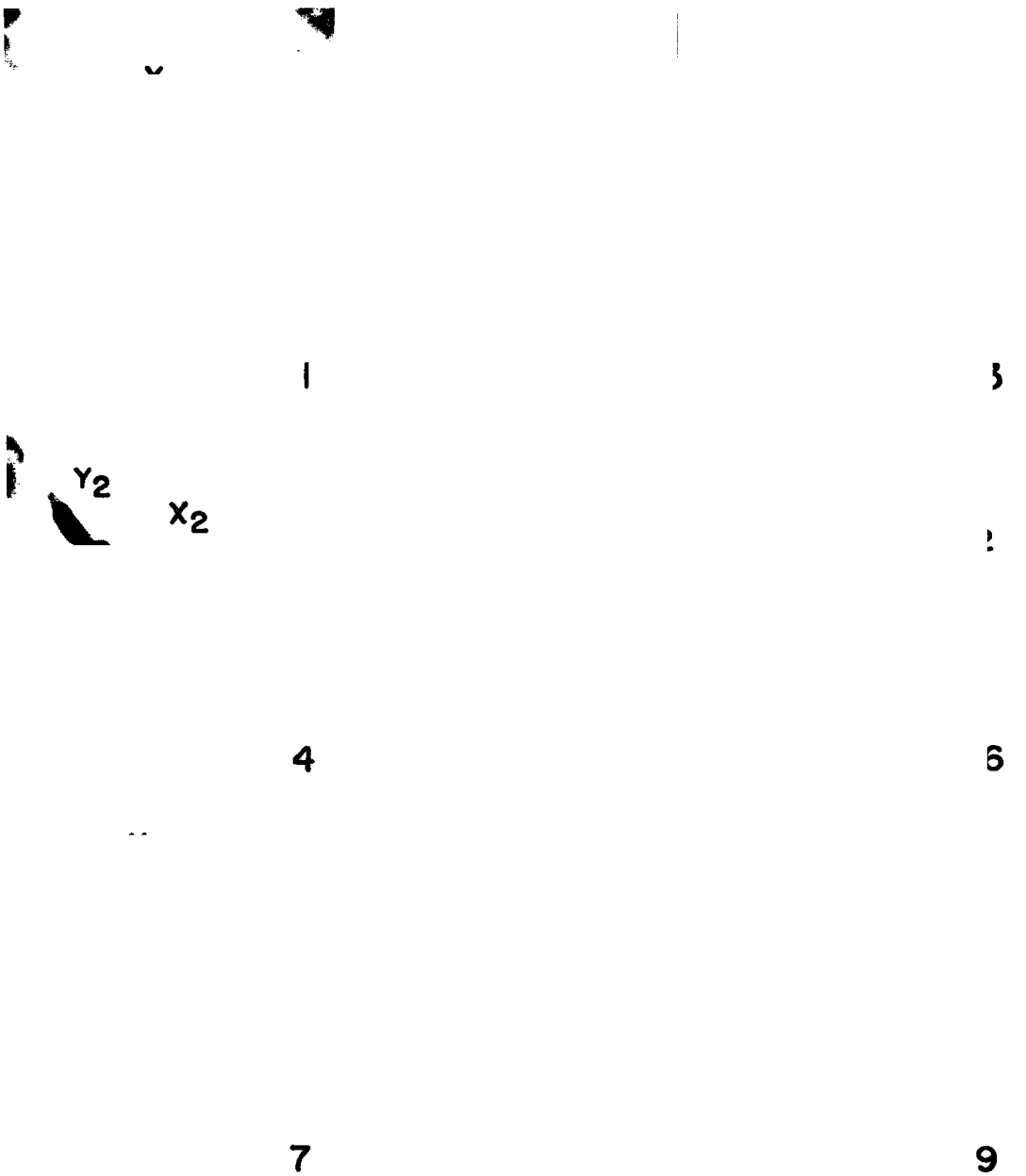
Table 1. Number of female isolines of eight species of the Neocellia Series of *Anopheles* (*Cellia*) collected from different wild populations in Thailand, the Philippines, and Bangladesh and examined cytologically.

Species/form	Locality	No. of isolines examined	Date of collection
karwari			
Form A	Ban Phu, Udonthani Province	3	October 1986
	Ban Pang, Nakhonphanom Province	2	August 1985
	Na Chaluai, Ubon Ratchathani Province	1	January 1987
	Makarm, Chanthaburi Province	3	October 1984
Form B	Ban Phu, Udonthani Province	3	October 1986
Form C	Kapong, Phangnga Province	2	August 1986
annularis	Maetang, Chiangmai Province	3	September 1982
		2	May 1983
		3	September 1984
jamesii			
Form A	Chumphon Province	1	June 1984
Form B	Phuphamarn, Khonkaen Province	2	March 1984
pseudojamesi	Phuphamarn, Khonkaen Province	1	March 1984
	Taknaf, Bangladesh	1	November 1984
stephensi	Maetang, Chiangmai Province	1	May 1983
nivipes	Khonburi, Nakhonratchasima Province	2	August 1984
	Na Chaluai, Ubon Ratchathani Province	2	October 1986
philippinensis	Rayong Province	2	January 1981
	Ban Na, Nakhon Nayok Province	2	September 1982
	Chumphon Province	1	June 1984
	Kapong, Phangnga Province	1	August 1986
	Luzon, Philippines	1	April 1984
splendidus	Maetang, Chiangmai Province	1	May 1983

RESULTS

Metaphase karyotypes (2n = 6) of the eight species of the Neocellia Series are similar to those of the Maculatus Complex, consisting of metacentric and submetacentric autosomes II and III, respectively, and one pair of heteromorphic sex chromosomes. Variations in the X and Y chromosomes due to different amounts of heterochromatin have been observed in five species. Such heterochromatin variations were not detected in the limited sample sizes of the remaining three species. Specific differences in metaphase karyotypes of the eight species are described briefly below.

Anopheles karwari: Fourteen families of this species from eastern, northeastern, and southern Thailand were examined cytologically. Two types of X and three types of Y chromosomes (Figs. 1–7) were observed in these families. The X_1 is submetacentric, while the X_2 is metacentric (Figs. 2, 3, 5, 7). One arm of each type of X chromosome is euchromatic, whereas the opposite arm is heterochromatic. The Y_1 is a small subtelocentric (acrocentric) (Fig. 1) that is about one-half the length of the large subtelocentric Y_2 chromosome (Fig. 4). The Y_3 chromosome, on the other hand, has a small metacentric shape (Fig. 6). Three forms of metaphase karyotypes are shown by these sex chromosomes, *viz.*, form A (X_1, X_2, Y_1), form B (X_1, X_2, Y_2), and form C (X_1, X_2, Y_3). Form A is common in eastern and northeastern Thailand. Form B has been found in sympatry



Figs. 1–9. Metaphase karyotypes from larval neuroblast cells. *An. karwari*: 1, male and 2, 3, female of form A; 4, male and 5, female of form B; 6, male and 7, female of form C. *An. annularis*: 8, male and 9, female.

with form A in Udonthani Province. However, form C has been detected only in Phangnga Province, southern peninsular Thailand. The autosomes of these forms are similar, showing a limited amount of pericentric heterochromatin. The different types of Y chromosome may reflect interspecies and/or intraspecies differences within the current concept of *An. karwari.*

Anopheles annularis: Eight families of this species collected at different times from Maetang, Chiangmai Province, all show two types of X chromosome. Both X_1 and X_2 are submetacentric (Fig. 9). The short arm is eu-

chromatic, while the opposite arm is heterochromatic. The heterochromatic arm of X_2 is slightly longer than that of the X_1. This is likely to be due to the addition of an extra block of heterochromatin on the distal end of X_1, transforming it to X_2. The Y chromosome is almost totally heterochromatic and of a subtelocentric configuration (Fig. 8). The autosomes apparently are similar to those of *An. karwari* mentioned above.

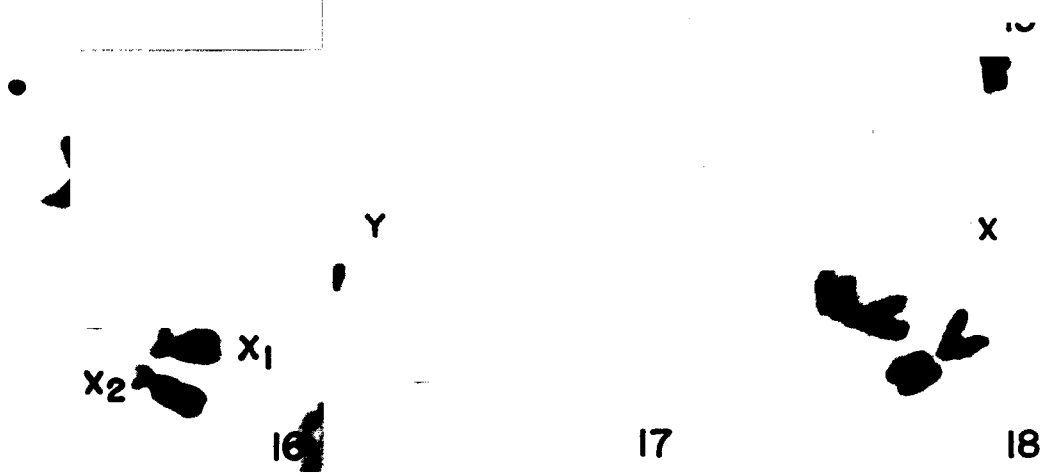
Anopheles jamesii: Two forms of metaphase karyotype have been found in this species. Form A shows sex chromosomes of X and Y_1 (Figs. 10, 11), while form B exhibits X and Y_2 (Figs. 12, 13). The most common X chromosome is submetacentric. The short arm is euchromatic, whereas the long arm is heterochromatic. The Y_1 is apparently telocentric, while the Y_2 is obviously submetacentric. The long arms of Y_1 and Y_2 are approximately equal in length. Thus, it is likely that the Y_2 could have arisen from the presumed ancestral Y_1 through the acquisition of a major block of heterochromatin near the centromeric region, transforming it to a submetacentric Y_2. The autosomes of these two forms are similar. Form A occurs in Chumphon Province, southern peninsular Thailand, while form B has been found in Khonkaen Province, northeastern Thailand. It is not known whether these allopatric populations represent interspecies differences within the taxon *An. jamesii.*

Anopheles pseudojamesi: One family from Khonkaen Province, northeastern Thailand, and one family from Taknaf, western Bangladesh, showed similar metaphase karyotypes. They consist of two types of submetacentric X chromosome (Figs. 14–16). The short arm of each type of X chromosome is euchromatic and the long arm is heterochromatic. The long arm of X_2 is slightly longer than that of X_1 (Fig. 16). This clearly is due to the presence of an extra block of heterochromatin in the distal end of X_2. The Y chromosome is subtelocentric (Figs. 14, 15). The autosomes are similar to those species described above.

Anopheles stephensi: The single family of *An. stephensi* from Chiangmai Province showed a metacentric configuration of both X and Y chromosomes (Figs. 17, 18). This metaphase karyotype apparently resembles that of *An. karwari* form C described above. However, a small amount of centromeric heterochromatin can be observed in the euchromatic arm of the X and in the autosomes in well-spread metaphase chromosomes. These metaphase chromosome characters of *An. stephensi* are somewhat different from those of other species of the group presented in this study.

Anopheles nivipes: This species showed distinct sex chromosomes with submetacentric X and subtelocentric Y (Figs. 19, 20). The short arm of the X consists of a very large block of centromeric heterochromatin and a normal euchromatic portion, while the long arm is entirely heterochromatic. The heterochromatic Y chromosome exhibited conspicuous secondary constrictions (Fig. 19). The autosomes showed a limited amount of pericentric heterochromatin. Recently, it has been demonstrated, based on cytogenetic evidence from natural populations (Hankaew 1986), that there are two sibling species of the *An. nivipes* complex in Thailand. Hankaew's data also suggested that form A is more common than form B. The karyotype we studied may correspond to form B of *An. nivipes* reported by Hankaew (1986).

Anopheles philippinensis: Samples from four localities in Thailand and one from the Philippines were examined cytologically. They showed remarkable variation in the sex chromosomes. Thus, three and two distinct types of the X and Y chromosomes, respectively, have been observed. The X_1 apparently is submetacentric (Figs. 21, 27). The short arm is almost totally euchromatic, while the long arm is heterochromatic. The X_2 differs from the X_1 in having extra blocks of heterochromatin in the distal end of the long arm (Figs. 22, 25). The euchromatic arm of these types of X chromosomes apparently is similar to all species studied here except for *An. nivipes* and *An. splendidus.* However, the submetacentric X_3 chromosome is quite different from the X_1 and X_2 in having an extensive block of centromeric heterochroma-



Figs. 10–18. Metaphase karyotypes from larval neuroblast cells. *An. jamesii*: 10, male and 11, female of form A; 12, male and 13, female of form B. *An. pseudojamesi*: 14, 15, male and 16, female. *An. stephensi*: 17, male and 18, female, respectively.

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Figs. 19–30. Metaphase karyotypes from larval neuroblast cells. *An. nivipes*: 19, male and 20, female. *An. philippinensis*: 21–24, male and 25–27, female; 28, triploid condition found in a female larva. *An. splendidus*: 29, male and 30, female.

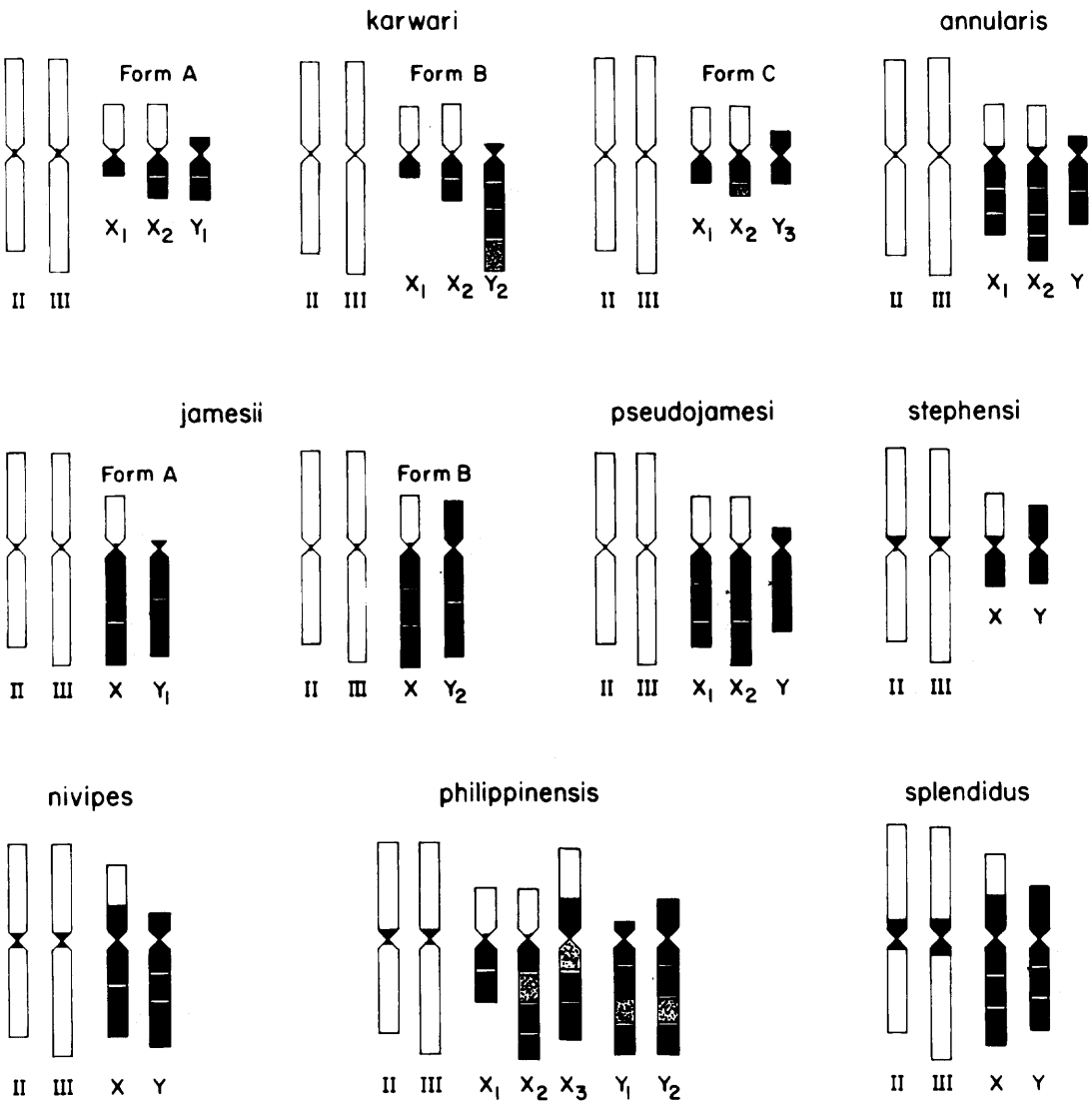

Fig. 31. Diagrammatic representation and comparison of metaphase karyotypes of eight species (including forms) of the Neocellia Series of *Anopheles* (*Cellia*). Only one set of autosomes II and III is presented. Variable heterochromatic portions are indicated in black or shaded. The centromeres are indicated by constrictions of each chromosome. The gaps indicate secondary constrictions. Chromosome lengths, arm ratios, and heterochromatic portions are shown in proportion.

tin in the short arm (Figs. 23, 24, 26–28). A conspicuous secondary constriction can be observed in the heterochromatic long arm. The X_3 of *An. philippinensis* thus is similar to the X chromosome of *An. nivipes*, its close relative. Interestingly, a triploid metaphase karyotype (6 + X_3 X_3 X_3) (Fig. 28) was detected in an F_1 female larva of one family of *An. philippinensis* collected from Ranong Province. The Y_1 chromosome is subtelocentric (Figs. 21–23), whereas Y_2 is submetacentric (Fig. 24). The autosomes again are similar to the other species mentioned above.

Anopheles splendidus: The metaphase karyotype of *An. splendidus* from Chiangmai Province is strikingly different from the other species presented here. The autosomes consist of conspicuous pericentric heterochro-

matin (Figs. 29, 30). This is a diagnostic character for mitotic chromosomes of *An. splendidus* that could be used to distinguish this species from the others. The X chromosome is submetacentric, consisting of the euchromatic portion and a large block of centromeric heterochromatin in the short arm, while the long arm is totally heterochromatic. The heterochromatic Y chromosome is also a large submetacentric (Fig. 29). Our observations on mitotic chromosomes of this species correspond with the metaphase karyotype of *An. splendidus* from Yunnan Province, China, recently reported by Xu and Qu (1991).

A diagrammatic representation of metaphase karyotypes of the eight species reported here is summarized in Fig. 31.

DISCUSSION

Based on the amount and distribution of constitutive heterochromatin present in the X chromosome, two groups can be recognized among the eight species studied. Group 1 includes *An. karwari, An. annularis, An. jamesii, An. pseudojamesi,* and *An. stephensi.* This group is characterized by the X chromosome having a limited amount of centromeric heterochromatin and the normal euchromatic portion in one arm, while the opposite arm may vary in size and shape due to the presence or absence of major blocks of heterochromatic material. Thus, such morphology of the X chromosome is a conserved feature in these five species. Group 2 includes *An. nivipes* and *An. splendidus,* which clearly show a submetacentric X chromosome consisting of a very large block of centromeric heterochromatin and the euchromatic portion in the short arm. These two species can be distinguished on the basis of the morphology of the autosomes, which contain a large amount of pericentric heterochromatin in *An. splendidus* but much less in *An. nivipes.* Interestingly, *An. philippinensis* shares both kinds of X chromosomes. It may be possible that the mitotic chromosomes of *An. philippinensis* represent the ancentral condition in the Neocellia Series. The X chromosome of group 1 may be an ancestral form, which could have given rise to the X of group 2 via the acquisition of a large block of centromeric heterochromatin or through a pericentric inversion involving major portions of centromeric heterochromatin. Our current data on mitotic chromosome analysis support the view (Baimai 1988) that heterochromatin accumulation in the sex chromosomes and the centromeric regions of the autosomes plays an important role in the karyotype evolution of *Anopheles* in Southeast Asia.

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