Mosquito Systematics, 25(2):116–123, 1993

METAPHASE KARYOTYPES OF *ANOPHELES* OF THAILAND AND SOUTHEAST ASIA: II. MACULATUS GROUP, NEOCELLIA SERIES, SUBGENUS *CELLIA*

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ABSTRACT. Metaphase karyotypes of eight species of the Maculatus Group exhibit inter- and intraspecies variation based on differences in the amount and distribution of constitutive heterochromatin in the sex chromosomes and/or in the centromeric regions of the autosome(s). Analysis of heterochromatic variation has revealed three distinct groups of mitotic sex chromosomes. *Anopheles sawadwongporni, An. dravidicus,* and *An. pseudowillmori* form Group 1, which is characterized by telocentric or acrocentric sex chromosomes. The only representative of Group 2 is *An. notanandai,* which has a metaphase karyotype showing a unique chromosome X with a very small amount of centromeric heterochromatin. The remaining four species studied (*An. dispar, An. maculatus, An. willmori,* and *An. greeni*) exhibit a third group of sex chromosomes showing subtelocentric (acrocentric), submetacentric, or metacentric configurations.

INTRODUCTION

Members of the Maculatus Group of the Neocellia Series of the subgenus *Cellia* are widespread in the Oriental Region ranging from the Indian Subcontinent through Southeast Asia and Taiwan (Rattanarithikul and Green 1986). Cytogenetic study of wild samples of anopheline mosquitoes morphologically belonging to the Maculatus Group from Thailand and Southeast Asia (Green 1982, Green and Baimai 1984, Green et al. 1985, and unpublished data) has led to the recognition of eight closely related species, formally described and reviewed by Rattanarithikul and Green (1986) and Rattanarithikul and Harbach (1990). Two additional species (*An. maculatus* forms E and K) of *An. maculatus* have been recognized on the basis of chromosomal data (Green et al. 1985) and morphological observations (Rattanarithikul, unpublished data).

Anopheles maculatus s.l. has long been known as an important vector of human malarial parasites in peninsular Malaysia (Reid 1968). Recently, *An. maculatus* found in peninsular Malaysia has been designated as form E of the group based on polytene chromosome and morphological characteristics (Loong et al. 1988). This form also occurs in southern Thailand (Green et al. 1985). Members of the Maculatus Group had not, however, been considered as vectors of human malaria in Thailand (Upatham et al. 1988) until the recent incrimination (by microscopic detection of sporozoites in salivary glands and ELISA identifications) of *An. pseudowillmori* (Theobald, 1910) along the northwestern border with Burma (Green et al. 1991). Significantly, three other members of the Maculatus Group, *An. maculatus* Theobald, *An. dravidicus* Christophers, and *An. sawadwongporni* Rattanarithikul and Green, found biting man at the same site were consistently negative. Thus the Maculatus Group not only may play a widespread role in malaria transmission in Southeast Asia, but may include

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Table 1. The number of female isolines of eight species belonging to the Maculatus Group of the Neocellia Series collected and examined cytologically from different populations in Thailand and the Philippines. All localities are villages and towns of provinces in Thailand except for *An. dispar* and *An. greeni* as indicated.

Species/form	Locality	No. of isolines examined	Date of collection
sawadwongporni	Rongkwong, Phrae	3	October 1985
dravidicus	Nam Nao, Phetchabun	2	September 1986
pseudowillmori	Mae Sarieng, Mae Hong Son	5	September 1985
notanandai	Ban Thasalao, Phetchaburi	1	August 1986
dispar	Bataan, Luzon, Philippines	2	August 1986
maculatus			
form B	Nampat, Uttaradit	4	March 1987
	Makarm, Chantaburi	2	November 1983
	Ban Na, Nakhon Nayok	2	September 1982
	Ban Kang Rieng, Kanchanaburi	2	February 1986
form E	Tung Ka Ngok, Phangnga	3	June 1982
	Khao Toh, Krabi	4	November 1986
form K	Ban Phu, Udonthani	2	October 1986
	Srimuangmai, Ubon Ratchathani	2	December 1987
willmori	Maerim, Chiangmai	4	October 1985
greeni	Montalban, Luzon, Philippines	1	August 1986

species of significantly different vectorial capacity.

During the course of population cytogenetic studies of the anopheline fauna of Southeast Asia, analyses of mitotic chromosomes of members of the Maculatus Group were carried out along with morphological taxonomic observations. This paper presents the results of those investigations.

MATERIALS AND METHODS

A total of eight species and two forms of the Maculatus Group occurring in Thailand and neighboring countries were examined cytologically: *An. sawadwongporni* (= species A), *An. dravidicus* (= species C), *An. pseudowillmori* (= species I), *An. notanandai* Rattanarithikul and Green (= species G), *An. dispar* Rattanarithikul and Harbach (= species J), *An. maculatus* (forms B, E, and K), *An. willmori* (James) (= species H), and *An. greeni* Rattanarithikul and Harbach (= species D) (Table 1). Adult female specimens of these species were collected from animal or human bait at different localities and intervals during the past several years.

Brain ganglia of fourth-instar larvae from each isofemale line were used for mitotic chromosome preparations and analysis using the techniques described by Baimai (1977) and Baimai et al. (1993).

RESULTS

The metaphase karyotype (2n = 6) of the Maculatus Group consists of one pair each of metacentric (II) and submetacentric (III) autosomes and one pair of heteromorphic sex chromosomes similar to that of other species of the Oriental *Anopheles*. The X and Y chromosomes vary in size and shape depending on the amount and distribution of heterochromatin. Interspecies differences have also been observed in the pericentric heterochromatin of autosomes. Species differences in the mitotic chromosomes of the eight members of the Maculatus Group are briefly described below.

Anopheles sawadwongporni. This species is widely distributed in central, northern, and northeastern Thailand but seems to be absent in southern, peninsular Thailand. The X chromosome is telocentric, consisting of a

Figs. 1–9. Metaphase karyotypes from larval neuroblast cells. 1,2, Male and female, respectively, of *An. sawadwongporni*; 3,4, male and female, respectively, of *An. dravidicus*; 5,6–7, male and female, respectively, of *An. pseudowillmori*; 8,9, male and female, respectively, of *An. notanandai.*

very large block of centromeric heterochromatin (Figs. 1, 2). The heterochromatic Y chromosome is also telocentric and approximately equal in length to the heterochromatic block of the X chromosome (Fig. 1). Major blocks of pericentric heterochromatin of the autosomes are clearly discerned in this species.

Anopheles dravidicus. The mitotic chromosomes of this species are generally similar to those of *An. sawadwongporni* except that they clearly show larger blocks of pericentric heterochromatin (Figs. 3, 4). This distinctive feature of the mitotic chromosomes can be used as a diagnostic character for separating these two closely related species. Furthermore, two types of X chromosomes with differing amounts of centromeric heterochromatin have been observed.

Anopheles pseudowillmori. This species has metaphase chromosomes similar to those of *An. sawadwongporni* and *An. dravidicus.* The X and Y chromosomes each exhibit a considerable portion of centromeric heterochromatin appearing as a short arm of the chromosome (Figs. 5–7). This is a good diagnostic character for this species. The X chromosome shows intercalary heterochromatic bandings in the region adjacent to the euchromatic portion. The almost totally heterochromatic Y chromosome is considerably shorter than the X. Pericentric heterochromatin of the autosomes is of normal size (Fig. 6).

Anopheles notanandai. This species shows quite different X chromosomes compared to those of other species of the group. They are submetacentric. The short arm is almost entirely euchromatic while the long arm is presumably totally heterochromatic (Figs. 8, 9). This is a unique X chromosome feature in the Maculatus Group. *Anopheles notanandai* exhibits two types of X chromosomes. The X_2 differs from the X_1 in having additional blocks of heterochromatin in the distal end of the long arm (Fig. 9). The Y chromosome is clearly submetacentric. The autosomes show a very limited amount of pericentric heterochromatin.

Anopheles dispar. This species has two types of X chromosomes. The X_1 is metacentric; one arm consists of a large block of centromeric heterochromatin and normal euchromatin while the opposite arm is entirely heterochromatic (Fig. 10). The X_2 is a large submetacentric; its short arm is similar to that of X_1. The heterochromatic long arm, however, consists of at least one additional major block of heterochromatin in the distal region of the chromosome arm (Fig. 11). The Y chromosome is a large submetacentric approximately equal in length to the heterochromatic portions of the X_2 chromosome (Fig. 10). The autosomes show relatively inconspicuous pericentric heterochromatin.

Anopheles maculatus. Forms B, E, and K have generally similar patterns of mitotic chromosomes. Forms B and E are recognized from inversion frequencies and geographical distribution (Green and Baimai 1984, Green et al. 1985), while form K is differentiated morphologically (Rattanarithikul, unpublished data). Three types of Xs and two types of Ys have been found in different combinations in these forms. Thus, form B shows X_3, Y_1, and Y_2; form E exhibits X_1, X_3, and Y_1; and form K shows X_2, X_3, and Y_2. The X_1 has been found only in form E, where it is a small submetacentric; the short arm is heterochromatic while the long arm consists of a euchromatic portion and a small block of centromeric heterochromatin (Fig. 16). Chromosome X_2, found only in form K, is metacentric; one arm is entirely heterochromatic whereas the opposite arm consists of a euchromatic portion and centromeric heterochromatin of approximately equal length (Fig. 18). The X_3, which is common to all three forms, is a large submetacentric. The short arm of X_3 consists of a euchromatic portion and a large block of centromeric heterochromatin similar to that of the X_2. The heterochromatic long arm is obviously larger than that of the X_2, possibly because of the accumulation of an extra block of heterochromatin (Fig. 14). Chromosome Y_1 is acrocentric or a small submetacentric (Figs. 12, 15), while Y_2 is a very large submetacentric (Figs. 13, 17). There is no difference in pericentric heterochromatin of the autosomes among the three forms.

Anopheles willmori. The sex chromosomes of this species are exceptionally large compared with those of other species of the Maculatus Group. The X chromosome is submetacentric and the short arm consists of euchromatic and heterochromatic portions of approximately equal length, while the very long arm is totally heterochromatic (Figs. 19, 20). The Y is also a very large submetacentric

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11 12

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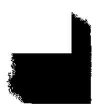

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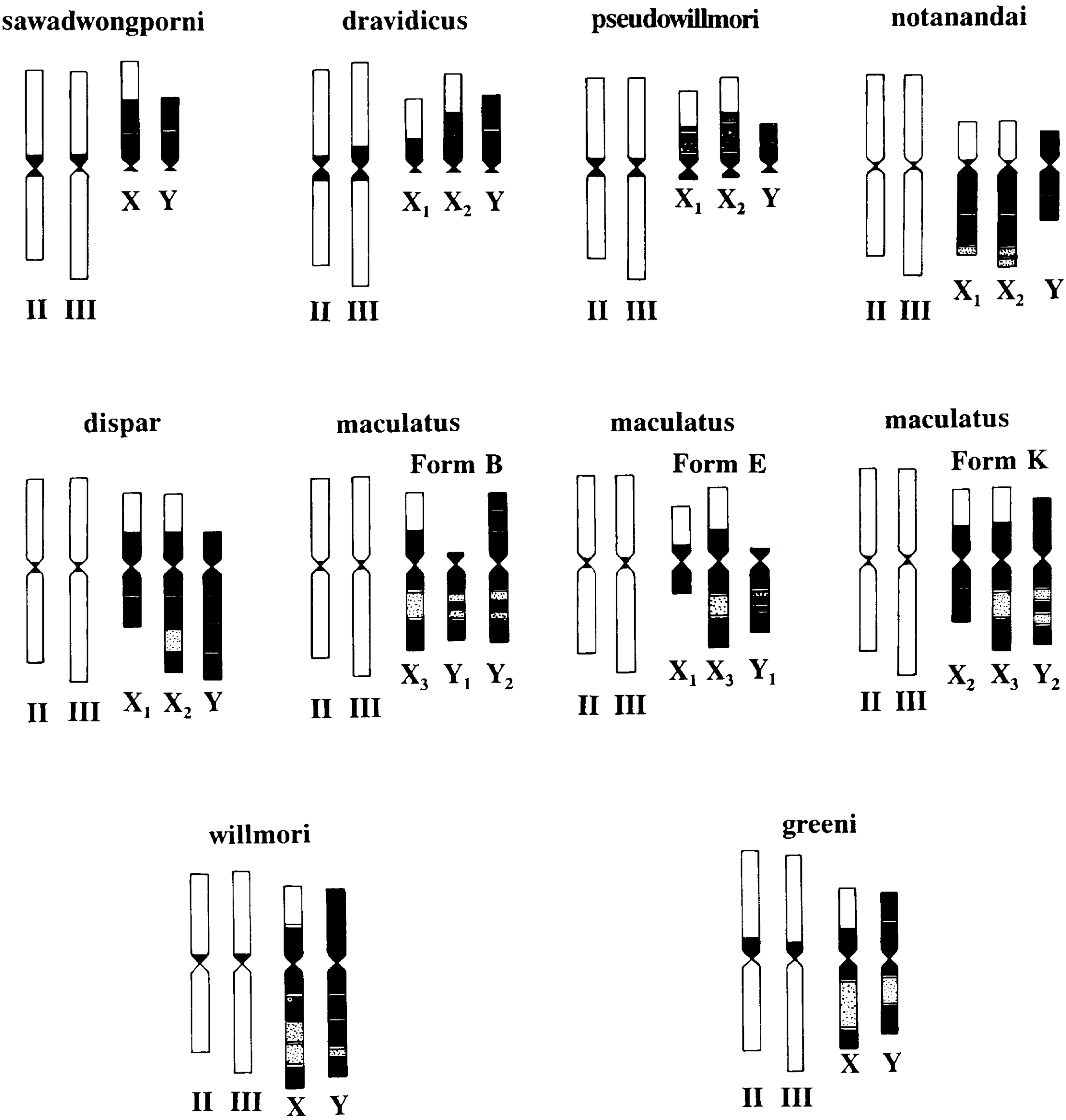

Fig. 22. Diagrammatic representation and comparison of metaphase karyotypes of the eight species (including forms) of the Maculatus Group. Only one set of autosomes II and III is presented. Variable heterochromatic portions are indicated in black or shaded. The centromeres are indicated by constrictions of each chromosome. Chromosome lengths, arm ratios, and heterochromatic portions are shown in proportion.

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Figs. 10–21. Metaphase karyotypes from larval neuroblast cells. 10,11, Male and female, respectively, of *An. dispar*; 12–18, *An. maculatus* (12–13,14, male and female, respectively, of form B; 15,16, male and female, respectively, of form E; 17,18, male and female, respectively, of form K); 19,20, male and female, respectively, of *An. willmori*; 21, male of *An. greeni*.

that is entirely heterochromatic (Fig. 19). One arm of each autosome shows a conspicuous block of centromeric heterochromatin compared with the opposite arm of the respective autosome.

Anopheles greeni. Both the X and Y chromosomes of this species are large submetacentrics; the X is slightly larger than the Y chromosome (Fig. 21). Like other species of the Maculatus Group, the short arm of the X chromosome consists of a euchromatic portion and centromeric heterochromatin, whereas the long arm is entirely heterochromatic. One arm of each of the autosomes exhibits a conspicuous block of centromeric heterochromatin.

A diagrammatic representation of mitotic karyotypes of the eight species and forms within the Maculatus Group is depicted in Fig. 22.

DISCUSSION

Metaphase karyotype analysis of eight species of the Maculatus Group has revealed interspecific differences in mitotic chromosomes with respect to the amount and distribution of constitutive heterochromatin in the sex chromosomes and in the centromeric region of the autosomes. This has led to the recognition of three distinct groups of sex chromosomes in the Maculatus Group. In Group 1, both X and Y chromosomes are telocentric or acrocentric, and we assume them to be ancestral. Group 1 includes *An. sawadwongporni, An. dravidicus,* and *An. pseudowillmori.* The mitotic chromosomes of *An. maculatus s.l.* from Sichuan, China, reported by Xu Shubi and Qu Fengyi (1991), also seem to belong to this group. Based on a comparison of mitotic chromosomes, they appear to be the chromosomes of *An. dravidicus.*

Group 2 is represented by only *An. notanandai.* Its submetacentric X chromosome consists of the almost entirely euchromatic short arm and the heterochromatic long arm. These are diagnostic for this species.

Sex chromosomes of Group 3 have a very common anopheline pattern showing meta- or submetacentric configurations. One arm of the X chromosome consists of a euchromatic portion and a large block of centromeric heterochromatin. However, the almost totally heterochromatic Y chromosome may be acrocentric, submetacentric, or metacentric; the remaining members of the Maculatus Group examined in this study (*An. dispar, An. maculatus, An. willmori,* and *An. greeni*) display these types. Among these species, *An. dispar, An. willmori,* and *An. greeni* can be separated from each other on the basis of autosomes and sex chromosomes (Fig. 22). The mitotic chromosomes of *An. maculatus s.l.* from Yunnan, China, illustrated by Xu Shubi and Qu Fengyi (1991), correspond well to the *An. willmori* metaphase karyotype obtained from northern Thailand.

The three forms of *An. maculatus* are difficult to differentiate using mitotic chromosomes alone. Forms B and E can be interbred to produce healthy and fertile hybrids through a number of generations (Chabpunnarat 1988). These two forms appear to represent geographic and (at least cytologically) polymorphic races of the taxon *An. maculatus.* Hybridization experiments between form K and others have not been done. Upatham et al. (1988) and Kittayapong et al. (1990) referred to form F of the Maculatus Group. We judge their form F to be form B as defined cytologically here (Rattanarithikul and Green 1986).

Intra- and interspecies differences in constitutive heterochromatin in the X and, to a lesser extent, in the Y are not uncommon in the Maculatus Group and are similar to differences found in other groups of Oriental anophelines (Baimai et al. 1984, 1993). Moreover, an interspecies difference in pericentric heterochromatin of the autosomes with an apparent trend toward gaining heterochromatin in the centromeric region has also been observed in some members of this group. The present data seem to support the hypothesis that heterochromatin acquisition played an important role in the chromosomal evolution of the Oriental *Anopheles.*

ACKNOWLEDGMENTS

We thank B.A. Harrison for valuable comments on the manuscript. We also thank K. Vejsanit and P. Panthusiri for preparing the illustrations. This work has been supported in part by UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases and by the Armed Forces Research Institute of Medical Sciences, Bangkok.

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