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

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ABSTRACT. Metaphase karyotypes of 9 species of the Myzomyia Series show intra- and interspecific differences based on quantitative variation and distribution of constitutive heterochromatin in the sex chromosomes or the centromeric regions of the autosome pairs or both. *Anopheles jeyporiensis* and *Anopheles aconitus* each exhibit 4 forms of mitotic karyotypes, which may reflect interspecific differences within each taxon. The well-defined genetic species within the *Anopheles minimus* and the *Anopheles culicifacies* complexes clearly exhibit distinctive metaphase karyotypes that can be used as diagnostic characters for separating these sibling species, which are difficult to identify by morphological criteria alone. Our analysis on metaphase karyotypes of *Anopheles pampanai, Anopheles varuna,* and *Anopheles flavirostris* also confirms their morphological identification based on heterochromatin differences in the sex chromosomes and autosome 2.

INTRODUCTION

The Myzomyia Series is one of the largest categories of the Oriental *Anopheles* and comprises at least 10 known morphological species (Reid 1968, Rao 1984). Some of these species are important transmitters of human malaria parasites or filarial worms in Southeast Asia, such as *Anopheles minimus* Theobald in Thailand and neighboring countries (Reid 1968, Prasittisuk 1985), *Anopheles aconitus* Dönitz in Indonesia (Kirnowardoyo 1988), and *Anopheles flavirostris* (Ludlow) in the Philippines (Salazar et al. 1988). In the Indian subcontinent, *Anopheles culicifacies* Giles is considered the primary vector of malaria (Rao 1984, Subbarao et al. 1988). Therefore, these members of the Myzomyia Series have been the object of a number of biological studies, including systematics (Harrison 1980) and population cytogenetics of the *An. culicifacies* complex (Subbarao et al. 1988 for reviews) and the *An. minimus* complex (Sucharit et al. 1988, Green et al. 1990). Cytogenetic investigations on the *An. culicifacies* complex in the Indian subregion have revealed some interesting differences in sex chromosome heterochromatin of these sibling species (Vasantha et al. 1982, 1983; Suguna et al. 1989). Otherwise little is known about mitotic chromosomes of other members of the Myzomyia Series occurring in Southeast Asia.

In this report we present metaphase karyotypes of 9 species, including various forms of this series that occur in Thailand and neighboring countries.

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MATERIALS AND METHODS

Nine species and 8 forms belonging to the Myzomyia Series occurring in Thailand and neighboring countries were examined cytologically. These are *Anopheles jeyporiensis* James, species A and C of the *An. minimus* complex, *An. aconitus, Anopheles pampanai* Büttiker and Beales, *Anopheles varuna* Iyengar, *An. flavirostris,* and species A and B of the *An. culicifacies* complex (Table 1). Adult female specimens of these species were collected from bovine or human bait at different localities during the past years of our research project.

Brain ganglia of 4th-instar larvae from each isofemale line were used for metaphase karyotype preparations and chromosome analysis employing the techniques previously described by Baimai (1977) and Baimai et al. (1993a).

RESULTS

The metaphase karyotypes of the 9 species are uniform in chromosome number ($2n = 6$), consisting of one pair of heteromorphic sex chromosomes and 2 pairs of autosomes similar to those of other known species of Oriental *Anopheles.* Intraspecies variation has been observed in the sex chromosomes mainly due to the different amount and distribution of heterochromatin. Interspecies differences have also been observed in the size and shape of sex chromosomes or in the pericentric heterochromatin of autosome(s) or both. These differences in the mitotic chromosomes of the 9 species and 8 forms are described briefly below.

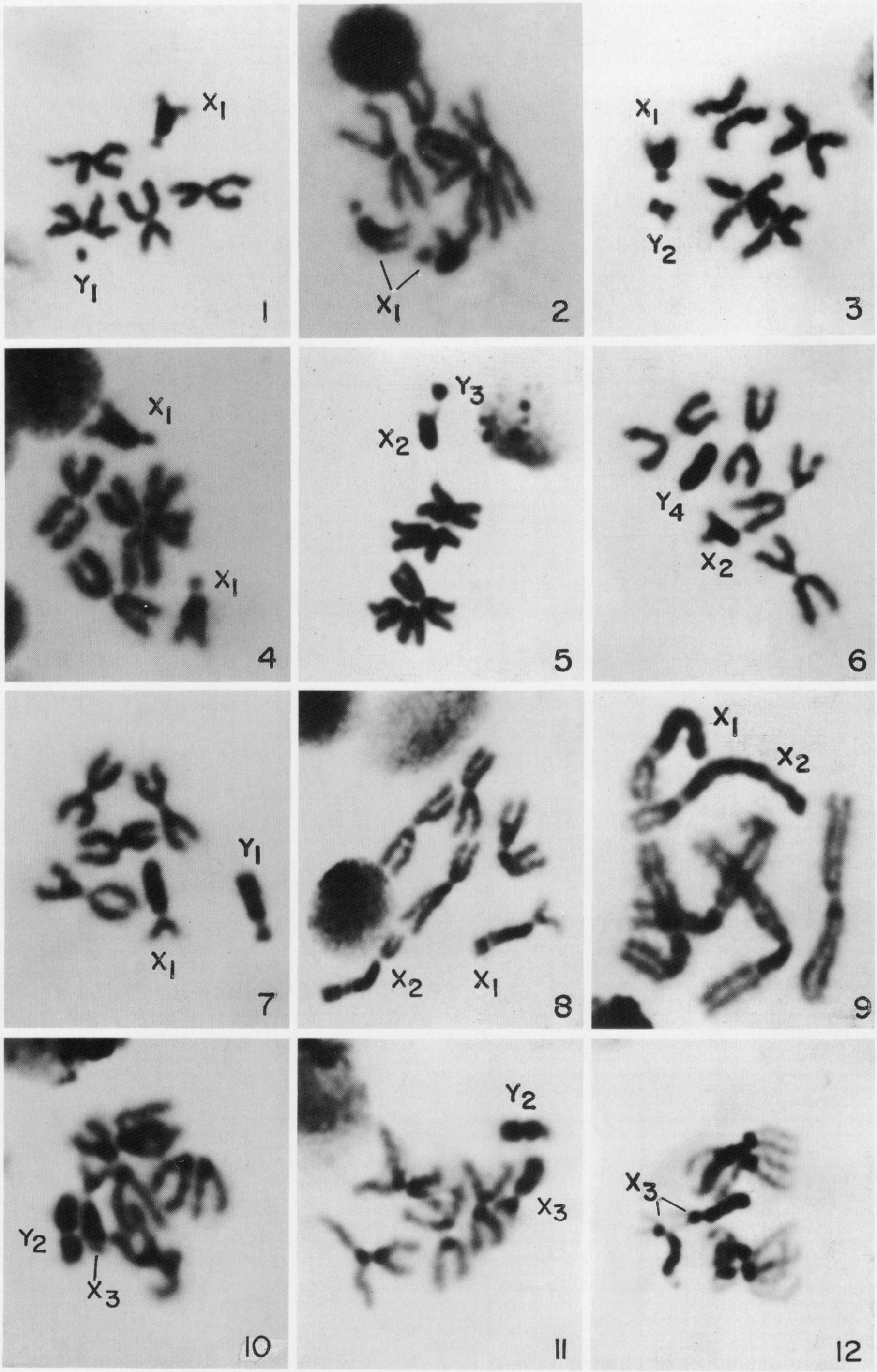
Anopheles jeyporiensis: Seven families of this species from Prae, Chiangmai, and Ubon

Table 1. The number of females (isolines) of 9 species of the Myzomyia Series, subgenus *Cellia*, collected and examined cytologically from different wild populations in Thailand, the Philippines, and Indonesia. All localities are villages or districts of provinces.

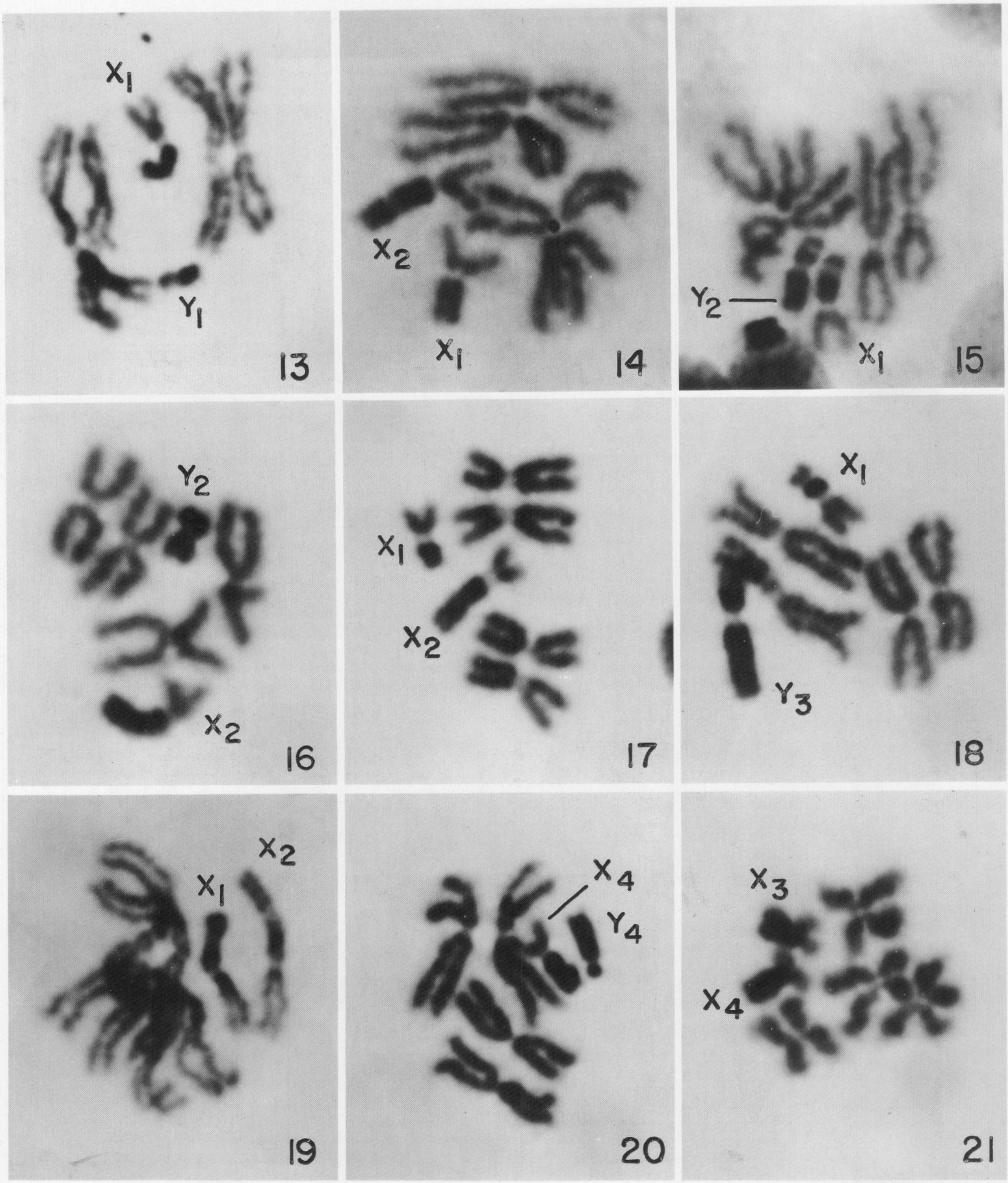
Species/Form	Locality	No. of isolines examined	Date of collection
Anopheles jeyporiensis			
Form A	Rongkwang, Phrae	2	February 1984
Form B	Maetang, Chiangmai	1	September 1984
Form C	Srimuangmai, Ubon Ratchathani	3	December 1987
Form D	Srimuangmai, Ubon Ratchathani	1	December 1987
An. minimus			
Species A	Wangnok Ann, Phitsanulok	5	September 1984
	Pakchong, Nakhonratchasima	4	January 1984
	Saiyok, Kanchanaburi	5	May 1989
Species C	Saiyok, Kanchanaburi	2	May 1989
An. aconitus			
Form A	Maetang, Chiangmai	2	October 1983
	Saodao, Songkhla	2	October 1983
Form B	Maetang, Chiangmai	5	October 1983
Form C	Maetang, Chiangmai	2	October 1983
Form D	Java, Indonesia	1	April 1984
An. pampanai	Srimuangmai, Ubon Ratchathani	1	December 1987
An. varuna	Hua Chang, Lumpun	1	October 1984
An. flavirostris	Antipolo Rizal, Philippines	3	April 1989
An. culicifacies			
Species A	Maetang, Chiangmai	1	February 1983
Species B	Maetang, Chiangmai	2	February 1983

Ratchathani provinces were examined cytologically. Two types of X chromosome have been observed. The X_1 is a large subtelocentric (acrocentric) chromosome showing a very large amount of centromeric heterochromatin in the long arm whereas the short arm is totally heterochromatic (Figs. 1–4). It has been found in Prae and Chiangmai provinces. In contrast, the telocentric X_2 chromosome is relatively shorter than the X_1. The centromeric heterochromatin occupies approximately ½ of the chromosome length (Figs. 5 and 6). The X_2 has been found only in Ubon Ratchathani Province. Four types of Y chromosome were recognized in this study. The Y_1 is a small dotlike chromosome (Fig. 1) compared with the large dot chromosome of Y_3 (Fig. 5). Chromosome Y_2 has a small metacentric configuration (Fig. 3), whereas the Y_4 has a very large telocentric shape (Fig. 6). There was no difference in the pericentric heterochromatin of the autosomes in all specimens examined. Based on the different types of X and Y chromosomes, 4 forms of metaphase karyotypes have been recognized within the taxon *An. jeyporiensis*, i.e., form A (X_1 Y_1), form B (X_1 Y_2), form C (X_2 Y_3), and form D (X_2 Y_4). The first 2 forms have been found in the north, and they form group 1 of metaphase karyotype. The last 2 forms occur sympatrically in Ubon Ratchathani, northeastern Thailand, and they are placed together in group 2 of metaphase karyotype. Thus, these 2 groups may reflect interspecific differences within the taxon *An. jeyporiensis*. Nevertheless, it is not known whether forms A and B represent intra- or interspecific differences within group 1. Likewise, it is not possible to form a conclusion at this point about the species status of forms C and D.

Anopheles minimus: Two sibling species of the *An. minimus* complex included in this study are species A (*An. minimus s.s.*) and species C. Species A is widespread throughout Thailand, whereas species C has been found in sympatry with species A only in Kanchanaburi Province, western Thailand. Species C differs from species A in having a prominent landmark of pericentric



Figs. 1–12. Metaphase karyotypes from larval neuroblast cells. 1–6, *Anopheles jeyporiensis.* 1, 2. Male and female, respectively, of form A; 3, 4. Male and female, respectively, of form B; 5. Male of form C; 6. Male of form D. 7–12. The *An. minimus* complex. 7, 8-9. Male and female, respectively, of species A; 10–11, 12. Male and female, respectively, of species C.



Figs. 13–21. Metaphase karyotypes from larval neuroblast cells of *Anopheles aconitus*. 13, 14. Male and female, respectively, of form A; 15–16, 17. Male and female, respectively, of form B; 18, 19. Male and female, respectively, of form C; 20, 21. Male and female, respectively, of form D.

heterochromatin in autosomal pairs as well as in the short arm of the submetacentric X chromosome (X_3 in Figs. 10–12) when compared with those of species A (Figs. 7–9). Moreover, 2 types of X chromosome, X_1 and X_2, have been commonly found in species A (Fig. 9). Furthermore, the submetacentric Y chromosome of species A is obviously different from the Y of species C (compare Fig. 7 with Figs. 10 and 11).

Anopheles aconitus: This species also exhibits variation in the X and Y chromosomes based on the amount and distribution of constitutive heterochromatin (Figs. 13–21). Four types of X chromosome have been observed in this study.

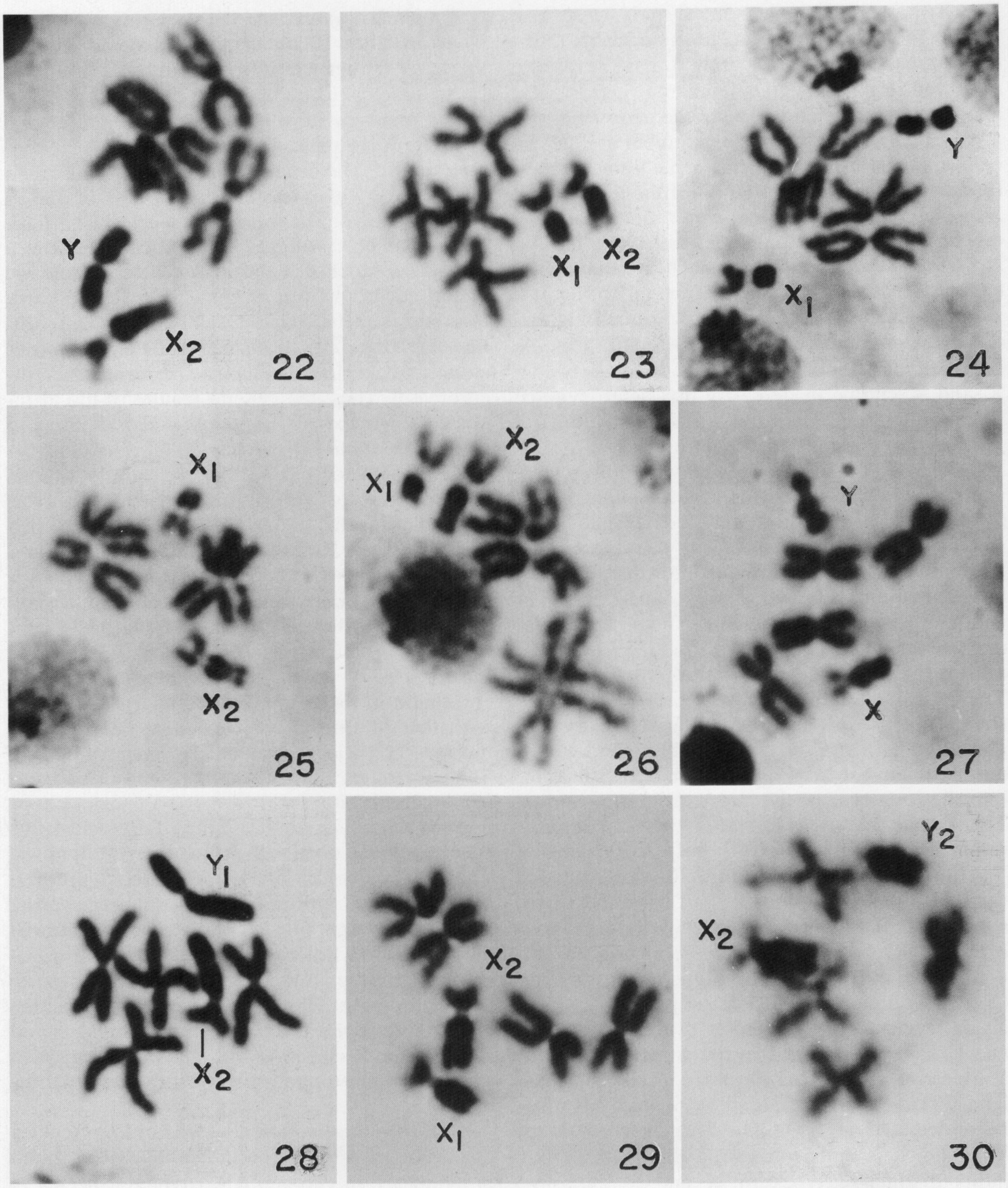
The X_1 has a metacentric shape and the X_2 has a large submetacentric configuration. Such a difference found in the X_1 and X_2 can be easily observed in heterozygous females (Figs. 14, 17, and 18), and it is likely due to the acquisition of a major block of heterochromatin in the heterochromatic arm. The X_3 has a medium submetacentric shape that is slightly different from the X_1 in having 2 equal, distinctive blocks of heterochromatin in the long arm. Likewise, the X_4 is a large submetacentric chromosome that could have arisen from the X_3 via a process of acquisition of an extra block of heterochromatin at the distal end of the long heterochromatic arm. A good comparison of the size and shape between X_3 and X_4 can be made easily in a heterozygous female (Fig. 21). Like the situation in the *An. jeyporiensis* complex, the Y chromosome of *An. aconitus* also exhibits extensive variation in size and shape due to the different amounts and distributions of heterochromatic blocks. Thus Y_1, a small submetacentric figure (Fig. 13), represents the simple form of the Y chromosome. The Y_2 has a medium submetacentric shape (Figs. 15 and 16) that differs from the Y_1 in having an extra block of heterochromatin added into each arm of the presumed ancestral Y_1 chromosome. The Y_3 is the largest submetacentric chromosome (Fig. 18). It could have arisen from the presumed ancestral Y_2 simply through the addition of an extra block(s) of heterochromatin into the long arm of the ancestral chromosome. The Y_4 has a somewhat subtelocentric configuration (Fig. 20) that is clearly different from the Y_2 chromosome (compare Fig. 20 with Figs. 15 and 16) although they appear to have the same size. The combinations of these different types of X and Y chromosomes can be classified into 4 forms of metaphase karyotype, *viz.*, form A (X_1, X_2, Y_1), form B (X_1, X_2, Y_2), form C (X_1, X_2, Y_3), and form D (X_3, X_4, Y_4). Forms A, B, and C have been found in the same population at Maetang, Chiangmai Province, northern Thailand. Form A is widespread because it has also been detected in Songkhla Province, southern Thailand. It is quite interesting that form D exhibits distinctive types of X and Y chromosomes, and it has been found only in Indonesia. This cytological evidence seems to suggest that form D may represent a separate species distinct from other closely related species of the *An. aconitus* complex occurring in Thailand. However, it is not possible to say whether heterochromatin variation in the X and Y chromosomes of forms A, B, and C represents intra- or interspecific differences. Further cytogenetic investigation is needed to clarify the sibling species problem within the taxon *An. aconitus* in Thai populations.

Anopheles pampanai: This species was found by us in Ubon Ratchathani Province adjacent to the Laos border. Only one family was available for cytological examination. It exhibits 2 types of X and one type of Y chromosome (Figs. 22 and 23). The X_1 is metacentric, whereas the X_2 is submetacentric. The heterochromatic long arms of X_1 and X_2 are somewhat similar to that of the X chromosome of *An. minimus* species C. Moreover, both types of the X chromosome show a distinctive block of centromeric heterochromatin in the euchromatic arm (Fig. 22). The Y chromosome is metacentric, resembling the X_1 chromosome (Fig. 22). Both pairs of the autosomes exhibit a conspicuous block of pericentric heterochromatin. Thus, the general feature of the metaphase karyotype of *An. pampanai* is similar to that of *An. minimus* species C described above.

Anopheles varuna: Only one family of this species collected from Lumpun Province was available in this study. This species exhibits quite different X chromosomes when compared with those of other species in the Myzomyia Series examined in this study. The X_1 is apparently metacentric, whereas the X_2 has obviously submetacentric shape (Figs. 25 and 26). Clearly, the X_2 consists of an extra large block of heterochromatin in the heterochromatic arm compared with that of the X_1 chromosome. The Y chromosome has a large metacentric shape (Fig. 24). The autosomes show a small amount of pericentric heterochromatin.

Anopheles flavirostris: Three families of this species were obtained from the Philippines. They all show uniformity in mitotic chromosomes. The X chromosome is submetacentric, showing 2 large blocks of heterochromatin in the long arm, whereas the short euchromatic arm exhibits a very limited amount of centromeric heterochromatin (Fig. 27). The Y chromosome is also metacentric and is similar in size and shape to the X chromosome. The autosomes also show a small amount of pericentric heterochromatin.

Anopheles culicifacies: Two sibling species of the *An. culicifacies* complex have been found in sympatry in Chiangmai Province. Species A shows 2 types of X chromosome (Figs. 28 and 29). The X_1 is submetacentric, consisting of euchromatic short arm and heterochromatic long arm. A prominent block of centromeric heterochromatin is present in the euchromatic arm. The X_2 is also submetacentric, similar to the X_1 chromosome, but the heterochromatic arm is relatively much longer due to the acquisition of a major block of heterochromatin in the distal region (Fig. 29). The Y chromosome has a very large submetacentric shape (Fig. 28). Again, the autosomes show a limited amount of pericentric



Figs. 22–30. Metaphase karyotypes from larval neuroblast cells. 22, 23. Male and female, respectively, of *Anopheles pampanai*; 24, 25–26. Male and female, respectively, of *An. varuna*; 27. Male of *An. flavirostris*. 28–30. The *An. culicifacies* complex. 28, 29. Male and female, respectively, of species A; 30. Male of species B.

heterochromatin. The general appearance of the metaphase karyotype of species B is somewhat similar to that of species A except for the Y_2 chromosome, which has a telocentric shape (Fig. 30) in contrast to the submetacentric Y_1 chromosome of species A. Only the X_2 chromosome has been encountered in species B in this study. Thus metaphase karyotypes of *An. culicifacies* species A and B correspond well to those described by Vasantha et al. (1982).

Figure 31 shows a diagrammatic representation of mitotic karyotypes of the 9 species and forms within the Myzomyia Series observed in this study.

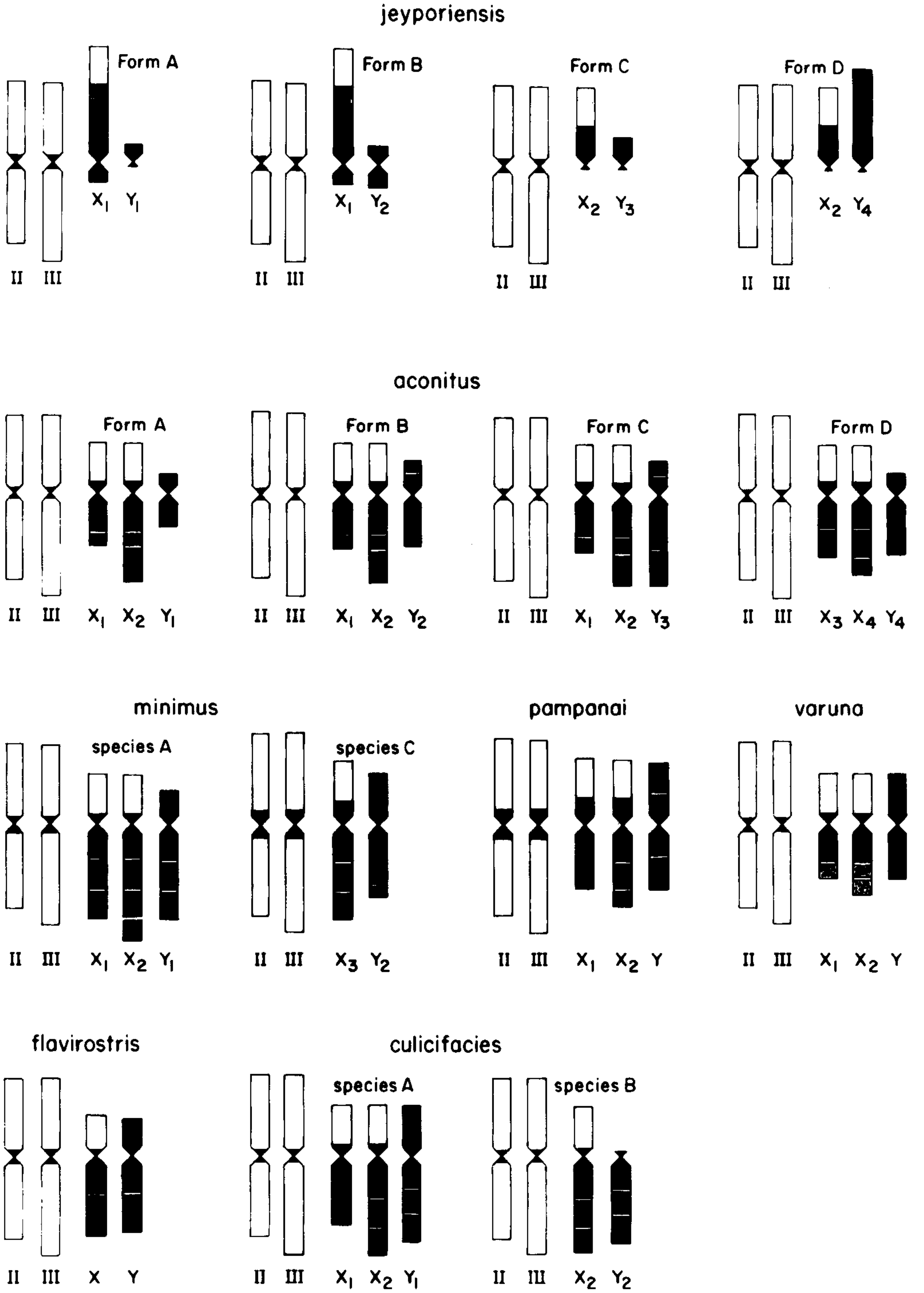


Fig. 31. Diagrammatic representation and comparison of metaphase karyotypes of 9 species (including forms) of Myzomyia Series. Only one set of autosomes II and III is presented. Variable heterochromatic portion is depicted in black or shaded. The centromeres are indicated by constrictions of each chromosome. Chromosome lengths, arm ratios, and heterochromatic portions are shown in proportion.

DISCUSSION

Although some closely related species or even sibling species of *Anopheles* resemble one another in external morphology, they are obviously different cytologically, primarily concerning the mitotic sex chromosomes or the amount of pericentric heterochromatin or both as exemplified in some sibling species of the Leucosphyrus and the Maculatus Groups and other members of the Neocellia Series (Baimai et al. 1987, 1988, 1993a, 1993b). In the Myzomyia Series, homosequential species within the *An. minimus* complex (Sucharit et al. 1988, Green et al. 1990) and the *An. culicifacies* complex (Green and Miles 1980; Subbarao et al. 1983, 1988; Vasantha et al. 1991) have been observed. Vasantha et al. (1982, 1983) reported distinctive mitotic karyotypes, especially with respect to the sex chromosomes of the sibling species within the taxon *An. culicifacies*. Our findings in this study are in accordance with the reports of Vasantha and coworkers. Metaphase karyotype analysis of the wild specimens genetically identified as *An. minimus* species A and C revealed that these sibling species were remarkably different in mitotic sex chromosomes as well as in the amount of pericentric heterochromatin in both pairs of autosomes (Fig. 31). The metaphase karyotypes of *An. minimus* species A and C appear to be different from that of species B from China (Xu and Qu 1991) and from north Vietnam (Lien et al. 1992). Our findings on mitotic karyotype differences involving the amount and distribution of heterochromatin between these sibling species of the *An. minimus* complex support population genetic and morphological studies (Green et al. 1990, Rattanarithikul, unpublished data).

Anopheles varuna and *An. pampanai* are closely related species. Yet they exhibit distinctive mitotic karyotypes with respect to the amount and distribution of heterochromatin in the X and Y chromosomes as well as in the centromeric region of the autosome pairs. *Anopheles flavirostris* from the Philippines has its own characteristics of the sex chromosomes, which can be readily distinguished from those of its close relatives such as the *An. minimus* complex described above. Interesting observations have been made in this study on the remarkable differences of mitotic karyotypes in natural populations of *An. jeyporiensis* and *An. aconitus,* each of which exhibited 4 karyotypic forms primarily involved with the sex chromosomes. Such distinctive mitotic karyotype could well represent interspecific differences, as evidently shown in other groups of closely related species reported in this study and elsewhere (Baimai et al. 1993a, 1993b, 1994, 1995). Further cytogenetic investigations in natural populations of the *An. jeyporiensis* and the *An. aconitus* groups are particularly important because these species are vectors of human malaria parasites in some areas of their distribution in the Southeast Asian region.

Cytotaxonomic studies of the Southeast Asian *Anopheles* mainly involve quantitative variation of constitutive heterochromatin in the sex chromosomes or the autosome(s) or both. The phenomenon of accumulation of heterochromatin in the genome is becoming more and more intriguing although its functional role is still unclear at present (John and Miklos 1979). In any event, gain of heterochromatin seems to play a vital role in the chromosomal evolution and possibly in the processes of speciation of the Oriental *Anopheles,* as clearly demonstrated in our studies.

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