No 212

Reprinted from

Canadian Journal of Genetics and Cytology

Réimpression du

Journal canadien de génétique et de cytologie

Heterochromatin variation in the sex chromosomes in Thailand populations of *Anopheles dirus* A (Diptera: Culicidae)

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Volume 26 • Number 5 • 1984

Pages 633–636

National Research Council Canada   Conseil national de recherches Canada

Printed in Canada by K.G. Campbell Corporation

NOTES

Heterochromatin variation in the sex chromosomes in Thailand populations of *Anopheles dirus* A (Diptera: Culicidae)

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Received May 10, 1984
Revised manuscript received June 5, 1984

BAIMAI, V., R. G. ANDRE, and B. A. HARRISON. 1984. Heterochromatin variation in the sex chromosomes in Thailand populations of *Anopheles dirus* A (Diptera:Culicidae). Can. J. Genet. Cytol. **26**: 633–636.

Analysis of larval ganglion mitotic chromosomes of 63 strains of *Anopheles dirus* A from six different localities in Thailand has revealed polymorphism in the sex chromosomes with respect to the amount of constitutive heterochromatin they contain. Three forms of X and two forms of Y have been detected in wild population samples.

Key words: *Anopheles*, chromosome polymorphism, constitutive heterochromatin, sex chromosomes, speciation.

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L'analyse des chromosomes ganglionnaires mitotiques des larves de 63 souches d'*Anopheles dirus* A, provenant de six localités différentes de la Thaïlande a révélé un polymorphisme des chromosomes du sexe par rapport à la quantité d'hétérochromatine qu'ils contiennent. Trois formes de X et deux formes de Y ont été détectées dans des échantillons de populations sauvages.

Mots clés: *Anopheles*, polymorphism chromosomique, hétérochromatine constitutive, chromosomes du sexe, spéciation.

[Traduit par le journal]

Introduction

Intra- and inter-specific variation in the amount and distribution of constitutive heterochromatin is a common phenomenon in eukaryotes. Differences in constitutive heterochromatin have proved useful in identifying sibling species of animals and plants (White 1973; Gatti et al. 1977; Baimai and Ahearn 1978; Baimai et al. 1983; Schweizer 1980; Wibowo et al. 1984).

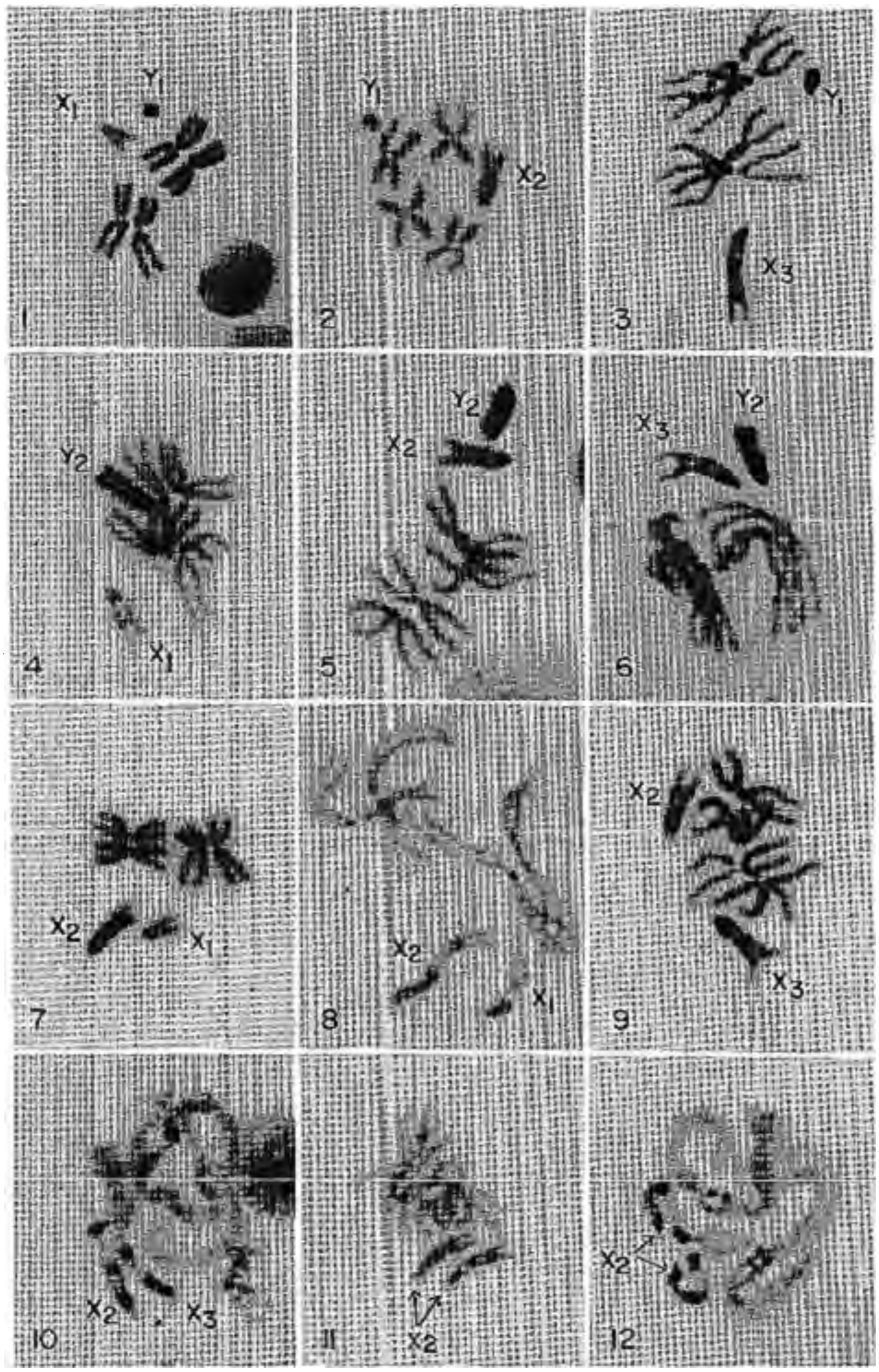
Anopheles dirus Peyton and Harrison (= *dirus* A) is considered to be one of the most important vectors of human malarial parasites in Thailand and other parts of the Oriental region. In Thailand, *A. dirus* A occurs in forested foothill areas. During our studies of this species we discovered differences in the metaphase sex chromosomes of *A. dirus* A from different geographic origins with respect to the amount of constitutive heterochromatin they contain and these observations are reported in this paper.

TABLE 1. Mitotic sex chromosomes of *A. dirus* A and their distribution in natural populations at six localities in Thailand

Locality	No. of isofemale lines examined	Y_1	Y_2
Chantaburi	16	7	9
Prachinburi	17	8	9
Nakhon Nayok	4	2	2
Kanchanaburi	7	3	4
Phetchaburi	17	8	9
Phrae	2	1	1

Materials and methods

Samples from Thailand populations of adult female *A. dirus* A were collected on human bait in the following areas: Kanchanaburi, Petchaburi, Chantaburi, Prachinburi, Nakhon Nayok, and Phrae (Table 1). Wild-caught females were placed individually into glass vials and allowed to lay eggs in



FIGS. 1–12. Mitotic chromosome of neuroblast cells from a larval ganglion of *A. dirus* A. Figs. 1–3. Male metaphase karyotype showing Y_1 and the three types of X chromosomes. Figs. 4–6. Y_2 with X_1, X_2, and X_3, respectively. Figs. 7–8. X_1X_2 female. Figs. 9–10. X_2X_3 female. Figs. 11–12. X_2X_2 female.

the laboratory. Early 4th-instar larvae were treated with a 0.1% colchicine solution for 3−4 h, after which their ganglia were removed and mitotic chromosome preparations were made following a modification of the method of Baimai (1975). Slides were stained with a 2% Giemsa solution and later examined under oil immersion using an Olympus light microscope. Photomicrographs of the mitotic karyotypes were taken with Kodak High Technical film under oil immersion (6.7 × 100) with a green filter.

Results and discussion

The metaphase karyotype of *A. dirus* A ($2n = 6$) consists of one pair of metacentric (V shaped) autosomes (II), one pair of submetacentric autosomes (III), and one pair of telocentric sex chromosomes. The term telocentric is used for both X and Y chromosomes in preference to acrocentric because the small portion of the extremely short arm of centromeric heterochromatin generally is not observable using conventional preparation techniques. The karyotypic variation found in the X and Y chromosomes is due mainly to differences in the amount and distribution of major blocks of heterochromatin. Three types of X and two types of Y chromosomes have been observed.

Using conventional Giemsa staining methods, the X chromosome clearly exhibits two to three major blocks of constitutive heterochromatin in comparison with the lightly staining euchromatic portions. The three types of X chromosomes, designated X_1, X_2, and X_3, show quantitative differences in heterochromatin content (Figs. 1−6) which are especially evident in heterozygous females (Figs. 7−10), particularly during the late prophase or premetaphase stage (Figs. 8−12). These three types of X chromosomes have been detected in all natural populations of this species that we have examined. It appears that X_3 may have arisen from the X_2, which in turn is presumably derived from X_1, through the acquisition of extra heterochromatin specifically in the vicinity of centromeric region. Intraspecific heterochromatin polymorphism of the X chromosome has also been reported in other species of *Anopheles* (Bonaccorsi et al. 1980; Wibowo et al. 1984).

The Y chromosome is almost totally heterochromatic. Two distinct types of Y, designated as Y_1 and Y_2, have been observed in all of our wild population samples. The Y_1 is a small rod-shaped chromosome (Figs. 1−3) which may occasionally appear as a very large dot in some preparations. The photograph of the Y presented by Aslamkhan and Baker (1969) for the *A. balabacensis* Bangkok colony strain (= *A. dirus* A) looks similar to our Y_1 chromosome.

The Y_2 is a long rod-shaped chromosome approximately four times the length of Y_1. The Y_2 is slightly longer than the X_1 chromosome (Fig. 4) but shorter than the X_2 (Fig. 5). This type of Y chromosome corre-

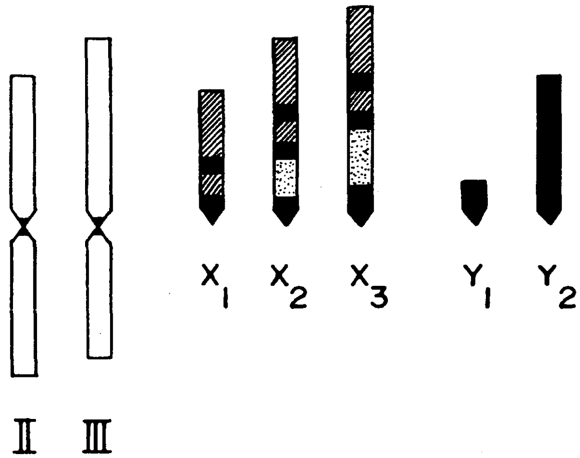


FIG. 13. Diagrammatic representation of the heterochromatin variation of the sex chromosomes of *A. dirus* A compared with the uniform autosomes.

sponds with that of *A. dirus* A reported by Baimai et al. (1981) and Zhu Hong-kan et al. (1981). The Y_2 apparently consists of three major blocks of heterochromatin. Thus, two intercalary constrictions can be seen along the chromosome arm in some preparations. The Y_2 could have arisen from Y_1 via the addition of extra heterochromatin in a manner paralleling the differentiation of the X chromosome types. These two types of Y chromosomes are common in all populations studied except from the southern peninsula of Thailand where *A. dirus* A is apparently absent. A comparison of the sizes of mitotic chromosomes of *A. dirus* A is presented in Fig. 13.

Our observations suggest that the evolution of sex chromosomes in *A. dirus* A results from quantitative changes in constitutive heterochromatin. The gain of constitutive heterochromatin in chromosomal evolution is a common phenomenon in eukaryotes (White 1973; John and Miklos 1979; John and King 1977) and in insects often involves the sex chromosomes (e.g., Dobzhansky and Epling 1944; Miller and Roy 1964; Baimai et al. 1983; Gatti et al. 1977; Bonaccorsi et al. 1980). The type of quantitative heterochromatin variation of sex chromosomes found in natural populations of *A. dirus* A is, however, not common in anophelines.

It is not known whether this variation in sex chromosome heterochromatin involves differences in susceptibility to the human malarial parasite. This approach warrants further investigation.

Acknowledgements

This investigation received the financial support of the Malaria Applied Field Research component of the United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and

Training in Tropical Diseases. We thank Professor Bernard John for comments on the manuscript. Technical assistance of U. Kijchalaow and L. Somchit is greatly appreciated.

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