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Heterochromatin variation in chromosome X in a natural population of *Anopheles willmori* (Diptera: Culicidae) of Thailand

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Abstract

Among the eight families of *Anopheles willmori* derived from individual wild-caught females collected from Chiangmai Province (northern Thailand) and examined, four isofemale lines showed variation in the X chromosome, including the normal X_1 and three aberrant types (X_3, X_4 and X_L). It is postulated that these different types of X chromosomes could have arisen as a result of spontaneous chromosomal rearrangements involving tandem translocation and paracentric inversion followed by acquisition of constitutive heterochromatin. Such rare events of chromosomal changes have become established in the natural population of *An. willmori* in northern Thailand.

Introduction

Intraspecies variation in mitotic chromosomes involving constitutive heterochromatin is a general phenomenon in many groups of animals (White, 1973). Such structural changes cause gain or loss of a noticeable amount of heterochromatin in metaphase chromosomes but have little or no phenotypic and genetic effects (John & Miklos, 1979). We have been carrying out an extensive study on intra- and interspecies differences in heterochromatin in sex chromosomes and, to a lesser extent, in pericentric regions of autosomes of closely related species of anopheline mosquitoes in Thailand and in Southeast Asia. Our findings suggest that the sex chromosomes of the Oriental *Anopheles* are prone to accumulation of constitutive heterochromatin causing variation in size and shape of these mitotic chromosomes (Baimai & Traipakvasin, 1987; Baimai, Andre & Harrison, 1984; Baimai, Harbach & Kijchalao, 1988; Baimai, Rattanarithikul & Kijchalao, 1993; Baimai *et al.*, 1993).

Anopheles willmori (James, 1903), a member of the *An. maculatus* group of the Neocellia Series (*Cellia*), is common in the Indian subcontinent and Southeast Asia. This species has been incriminated as a malaria vector at high altitudes of Nepal (Pradhan, Shrestha & Vaidya, 1970). It has been found in Chiangmai and Mae Hong Son Provinces, northwestern Thailand. This species and other members of the maculatus group have been systematically reviewed and revised by Rattanarithikul and Green (1986). They were also examined cytogenetically (Green, 1982; Green & Baimai, 1984; Green *et al.*, 1985; Baimai *et al.*, 1993). Cytologically, *An. willmori* shows distinctive characteristics of sex chromosomes which differ remarkably from those of other closely related species within the maculatus group (Baimai *et al.*, 1993).

During our study on metaphase karyotypes of *Anopheles* of Thailand, an unusual heterochromatin variation was observed in sex chromosomes of wild samples of *An. willmori* collected from Chiangmai Province. This report presents the results of these investigations.

Materials and methods

Wild female samples of *An. willmori* were collected from human baits at Maerim, Chiangmai Province, northern Thailand, in October, 1985. A total of eight isofemale lines were set up for oviposition in our Genetics Laboratory at Mahidol University, and F_1

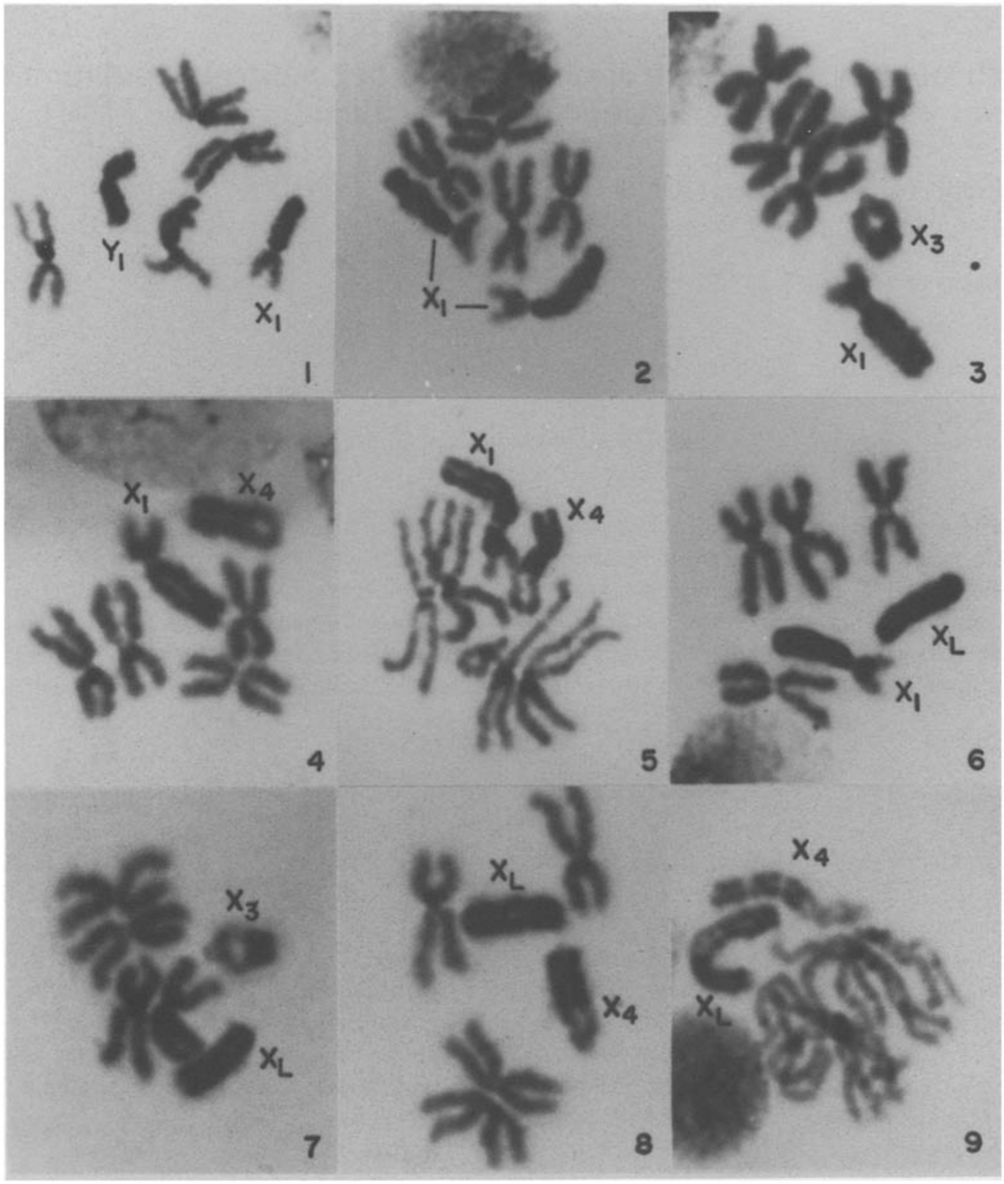

Fig. 1–9. Photomicrographs of mitotic chromosomes from larval neuroblast cells of *Anopheles willmori*. 1, 2, normal male and female, respectively; 3, X_1 X_3; 4, 5, X_1 X_4; 6, X_1 X_L; 7, X_3 X_L; 8, 9, X_4 X_L.

larval progeny were cytologically examined using the methods described previously (Baimai, Rattanarithikul & Kijchalao, 1993; Baimai *et al.*, 1993). Brain ganglia of healthy 4th stage larvae were used for mitotic chromosome preparations employing an air-dried Giemsa staining technique (Baimai, 1977). Well spread metaphase chromosomes were photographed on Kodak High Technical film under oil immersion (×670) with green filter.

Results and discussion

The normal metaphase karyotype of *An. willmori* ($2n = 6$) consists of two pairs of autosomes and one pair of heteromorphic sex chromosomes (XY male, XX female, Figs. 1 and 2, respectively). The normal X_1 and Y_1 chromosomes have a submetacentric configuration. The euchromatic short arm (X_S) of the X_1 comprises a prominent block of centromeric heterochromatin while the long arm (X_L) is totally hete-

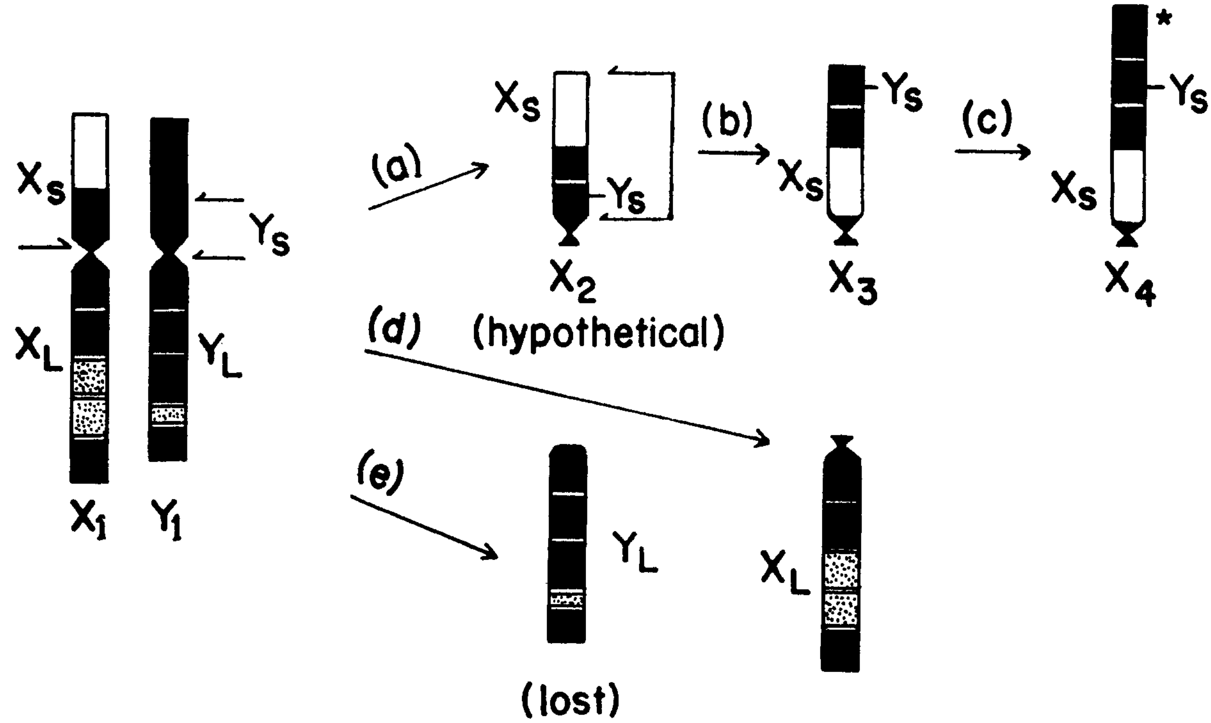

Fig. 10. Diagrammatic representation of proposed events of chromosomal changes involving the X_1 and Y_1 chromosomes giving rise to the existing types of X_3, X_4 and X_L. The hypothetical X_2 has not been detected in this study.

rochromatic. The Y_1 is, as a general rule, almost entirely heterochromatic comprising the short (Y_S) and the long (Y_L) arms (Figure 10). Among the eight families examined in this study, four isofemale lines exhibited variation in size and shape of the X chromosomes depending on the amount and distribution of major block(s) of constitutive heterochromatin. These varied shapes of X chromosomes have been interpreted and classified into four types designated X_1, X_3, X_4 and X_L (Figures 3–10). The hypothetical form of the X chromosome, X_2, has not been encountered in this study. In contrast, only the normal Y_1 chromosome has been detected in these wild samples.

These types of X chromosomes could have arisen through a series of events of chromosomal rearrangements including a translocation, paracentric inversion and acquisition of heterochromatin as proposed below (Figure 10).

The starting point of such spontaneous events could have involved three simultaneous chromosomal breaks in the sex chromosomes. One break could have occurred in the vicinity of centromeric heterochromatin in the short arm (X_S) of the X chromosome resulting in an acentric X_S and a telocentric X_L. Simultaneously, two chromosomal breaks could have occurred in the Y_1 chromosome: one break in the vicinity of centromeric heterochromatin in the long arm (Y_L) and another break near the middle of the short arm (Figure 10). Such chromosomal break events could have produced three disintegrated portions of the original Y_1 chromosome, i.e. a short telocentric Y_S, a relatively longer acentric Y_L, and the remaining heterochromatic portion of the short arm of the Y_1 chromosome. The last two heterochromatic portions without centromere could have been lost during these chromosome interchanging events (Figure 10e). Such events of simultaneous chromosomal breaks in the X_1 and Y_1 chromosomes followed by a translocation of the X_S portion into the distal end of the Y_S portion could have given rise to a hypothetical X_2 (= X_S + Y_S) (Figure 10a) and the presumed existing X_L (Figure 10d), which has been found in this study (Figures 6–9). Subsequently, the hypothetical X_2 could have undergone a process of paracentric inversion involving almost the whole arm, resulting in the existing X_3 chromosome (Figures 3, 7, 10b). The X_2 could have been lost or become very rare in natural populations because it has not been detected in the limited sample size of this study. Further chromosomal changes involving an addition of a large block of heterochromatin in the distal region of the X_3 could have given rise to the X_4 chromosome (Figure 10c), which still exists in the natural population at Maerim, Chiangmai Province (Figures 4, 5, 8, 9).

Chromosomal rearrangements due to paracentric or pericentric inversions and various kinds of translocations may lead to an extensive modification of chromosome morphology, particularly by appearing in the metaphase karyotype. Such structural changes of chromosomes resulting in gain or loss of heterochromatin in chromosome complements may occur spontaneously in natural populations without causing severe phenotypic or genetic effects. These phenomena have been found not only among closely related species but also within a single species of *Drosophila* (Wharton, 1943; Miller & Roy, 1964; Baimai, 1969; Baimai, Traipakvasin & Kitagawa, 1986).

The different types of X chromosomes found in this particular natural population of *An. willmori* have been postulated to have arisen as a result of the X–Y translocation followed by a paracentric inversion and the acquisition of extra block(s) of constitutive heterochromatin. These kinds of variation in the X chromosome which have been established in a natural population, however, rarely occur in *Anopheles* studied so far, at least in the Southeast Asian region. Thus the X chromosome variation in *An. willmori* from northern Thailand presented in this report would be a demonstration of the establishment of a translocation in a wild population and possibly represents a rare event of chromosomal rearrangement involving the X and Y chromosomes observed in the genus *Anopheles*.

Intraspecific variation in sex chromosomes due to the acquisition of constitutive heterochromatin is not an uncommon phenomenon in anopheline mosquitoes of Southeast Asia (Baimai & Traipakvasin, 1987; Baimai, Rattanarithikul & Kijchalao, 1993; Baimai *et al.*, 1993). The X_1 and X_4 found in *An. willmori* may continue to survive and become floating in the natural population. Accumulation of heterochromatin in the mitotic karyotype may be an important contribution in chromosomal evolution in the Oriental *Anopheles* (Baimai, 1988). However, the functional role of heterochromatin in sex chromosomes is an unsolved problem at this stage. It has been suggested that heterochromatin variation in the sex chromosomes may play a significant role in reproductive isolation and species differentiation in some anopheline mosquitoes (Bonaccorsi *et al.*, 1980; Fraccaro *et al.*, 1977). There is also a possibility that heterochromatin differentiation in sex chromosomes of *Anopheles* may exert some effect on the genetic susceptibility of malarial parasites (Baimai, 1988). Thus the situation in *An. willmori* warrants further studies in relation to the evolutionary role of sex chromosome heterochromatin in species differentiation.

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