

MOSQUITO CYTOGENETICS, ELECTROPHORESIS AND CROSS MATING STUDIES

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OBJECTIVES : To use the latest cytogenetic, cross mating and electrophoresis techniques to : (a) delineate the vector species and vector strains of mosquito species in Thailand and Southeast Asia as a check against current morphological species concepts; (b) identify rapid and accurate techniques and discriminating characters for differentiating sibling species in vector species complexes; and (c) accurately determine genetic variation in natural populations of vector species and correlate this variation with the susceptibility of the vector(s) to infection with human pathogens.

BACKGROUND : These studies are a continuation of projects initiated in 1978 and outlined in the 1977-78 annual report (1). The recognition of sibling or cryptic species in important vector species groups of mosquitoes is steadily increasing with each year, and is essential for developing effective control programs and understanding the epidemiology of vector-borne diseases (2). Cytogenetic and electrophoresis techniques have been shown to be effective in differentiating such cryptic species and in determining the genetic variability (including disease susceptibility) in natural populations of mosquitoes (3). These techniques, when coupled with morphological studies and hybridization experiments, provide the best basis for species and vector strain differentiation.

METHODS : Cytogenetic techniques employed include a modification of the standard chromosome squash technique (4) for salivary polytene chromosomes, the Coluzzi technique (5) for polytene ovarian nurse cell chromosomes, a modified technique (6) for larval brain metaphase preparations, and the standard technique (7) for pupal testis meiotic preparations. The electrophoresis techniques and the enzyme terminology and abbreviations are those of Steiner and Joslyn (8).

Chromosome maps and electrophoresis starch-gel esterase patterns are being made for selected laboratory colony strains or species. These maps or patterns will serve as "standards" for later comparisons with other strains and/or species. Once "standard" maps or esterase patterns have been established, wild

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collected populations will be sampled to survey the variations occurring in natural populations. Wild and/or colony strains of currently recognized morphological species that exhibit sufficient cytogenetic and/or electrophoretic differences will be studied further by hybridization experiments to determine if they are conspecific.

During this period 2 Thailand strains of *Anopheles maculatus* were firmly established as colonies. Both colonies, i.e., the Huai Kuum-Chon Buri strain and the Nakhon Nayok strain, require forced mating, however, large numbers are easily produced for other studies. In addition, a colony of *Anopheles philippinensis* (Rayong strain), was firmly established using the forced mating technique. Initially, the *philippinensis* females were reluctant to oviposit in ceramic bowls with water or in petri dishes with moist filter paper, and had to be confined over water in vials to obtain adequate eggs for the colony. However, after about 7 generations sufficient eggs were being deposited in the bowls and petri dishes to maintain the colony and to allow the discontinuation of the vial method. The 2 *maculatus* colonies readily oviposit on moist filter paper. All 3 colonies are being maintained in cages (21 cm x 21 cm x 21 cm), on a 5% multi-vitamin solution (includes 2% sucrose) and hamsters for a blood source. The larvae are fed a finely ground laboratory animal (mouse) chow twice daily. Although the *maculatus* immatures thrive in pans of clear water, the *philippinensis* immatures did not survive well until a small amount of sterilized mud slurry was added to each pan. A small colony of *nivipes* has recently been initiated but is still unstable. These colonies are essential for the malaria susceptibility, cytogenetic, crossing and taxonomic studies currently underway in the department.

Electrophoresis studies were discontinued during the past year to allow for the colonization of various taxa and the completion of certain crossing experiments (below). These studies will be initiated again in the next report period.

Attempts to utilize the ovarian nurse cells of members of the Leucosphyrus Group for polytene chromosome preparations have not been very successful. Although experimentation with this technique is continuing, the larval salivary glands remain the primary source of high quality polytene chromosome preparations for this group. On the other hand, good quality preparations using the larval salivary glands have been uncommon from *Anopheles maculatus* and *philippinensis*. Hopefully, the ovarian nurse cells will provide good chromosome preparations for these 2 species.

A map of the salivary polytene chromosomes for *An. dirus* was completed and published (9). This map is being used as a "standard" for comparing the chromosomes of other members of the leucosphyrus Group, as good quality salivary polytene chromosome preparations have also been made for *An. takasagoensis* and *An. balabacensis* (Perlis form). As indicated below and elsewhere in this report (10), *takasagoensis* was previously called *balabacensis* (Taiwan form), but recently was elevated to species status (11) as a result of these multidiscipline studies. The autosomes of *dirus*, *takasagoensis* and *balabacensis* (Perlis form), appear to have identical banding patterns, however, the sex chromosomes are different lengths, with different banding sequences in zone 6 of the X chromosomes. Further study of these differences is continuing.

Karyological studies of the mitotic and meiotic chromosomes of *Anopheles dirus*, *takasagoensis* and *balabacensis* (Perlis form) have revealed significant differences in the sex chromosomes. These differences are largely due to the position of the centromere, the different amounts of constitutive heterochromatin and euchromatin, and the respective lengths of the chromosomes (Fig. 1). The sex chromosomes of *dirus* and *takasagoensis* are telocentric, while those of *balabacensis* (Perlis form) are large acrocentric and very distinct. Also, all 3 taxa have a secondary constriction on the sex chromosomes. The X chromosome of *takasagoensis* is the shortest, while those of *dirus* and *balabacensis* (Perlis form) are nearly equal length. These karyological differences are very useful in differentiating these taxa, particularly the Perlis form, and support the concept of these taxa being elevated to species status. These data have been prepared for publication and will be submitted in the near future (12).

Karyotype studies from larval brain ganglia of *Anopheles philippinensis* have resulted in excellent preparations, several of triploid individuals. These studies are in preparation for comparative cytogenetic studies with *Anopheles nivipes*.

Anopheles maculatus is recognized as a primary vector of human malaria parasites in peninsular Malaysia and Sumatera, Indonesia, however, *maculatus* in the countries north of Malaysia, is normally considered a non-vector. In Thailand, *maculatus* is considered a primary vector, but, only in reference to southern Thailand adjacent to Malaysia. These differences in vector capabilities have caused several authors, e.g., Gould et al. (13) to suggest that *maculatus* in Southeast Asia may represent a sibling species complex. During this past period we attempted to resolve this question by crossing *maculatus* (IMR strain, Malaysia) with the *maculatus* (Nakhon Nayok strain, Thailand). However, our data from these reciprocal crosses and back crosses are inconclusive at present and suggest further crosses will be necessary. Additional crosses will be carried out during the coming year.

Forced mating crosses in both directions between *Anopheles dirus* (Bangkok colony) and *An. takasagoensis* (= *balabacensis* Taiwan form), yielded reduced numbers of F_1 hybrids of both sexes. Natural mating studies in both directions between *An. dirus* and *An. balabacensis* (Perlis form), produced very small numbers of viable F_1 hybrid offspring in only one direction (*dirus* female x Perlis male). The F_1 female hybrids of the *dirus* x *takasagoensis* crosses were fertile when they were backcrossed with males of the respective parents, however, male progeny of these backcrosses often exhibited morphological abnormalities. The F_1 males of this cross were completely sterile with abnormal reproductive systems. Only a very few F_1 hybrids of the *dirus* x Perlis cross were produced. Of these, one F_1 female was backcrossed to a Perlis male and failed to oviposit. F_1 hybrid males of the *dirus* x Perlis cross often died as pupae and those reaching the adult stage were sterile with abnormal reproductive systems.

Salivary gland chromosomes of F_1 hybrid larvae exhibited about 40-50% asynapsis in the *dirus* x *takasagoensis* crosses, and 70-80% asynapsis in the *dirus* x Perlis form crosses. Although considerable asynapsis occurred in all the F_1 hybrids, the banding patterns of most of the chromosome arms were very similar. The most obvious differences occurred in zone 6 of the X chromosomes.

These cytological and crossing data show that *dirus*, *takasagoensis* and *balabacensis* (Perlis form), represent full biological species. Furthermore, they support the recent recognition of *dirus* (14) and *takasagoensis* (11) as distinct species. The recognition of these sibling species makes it imperative that their roles or potential roles in the transmission of human pathogens be re-examined.

These studies are continuing.

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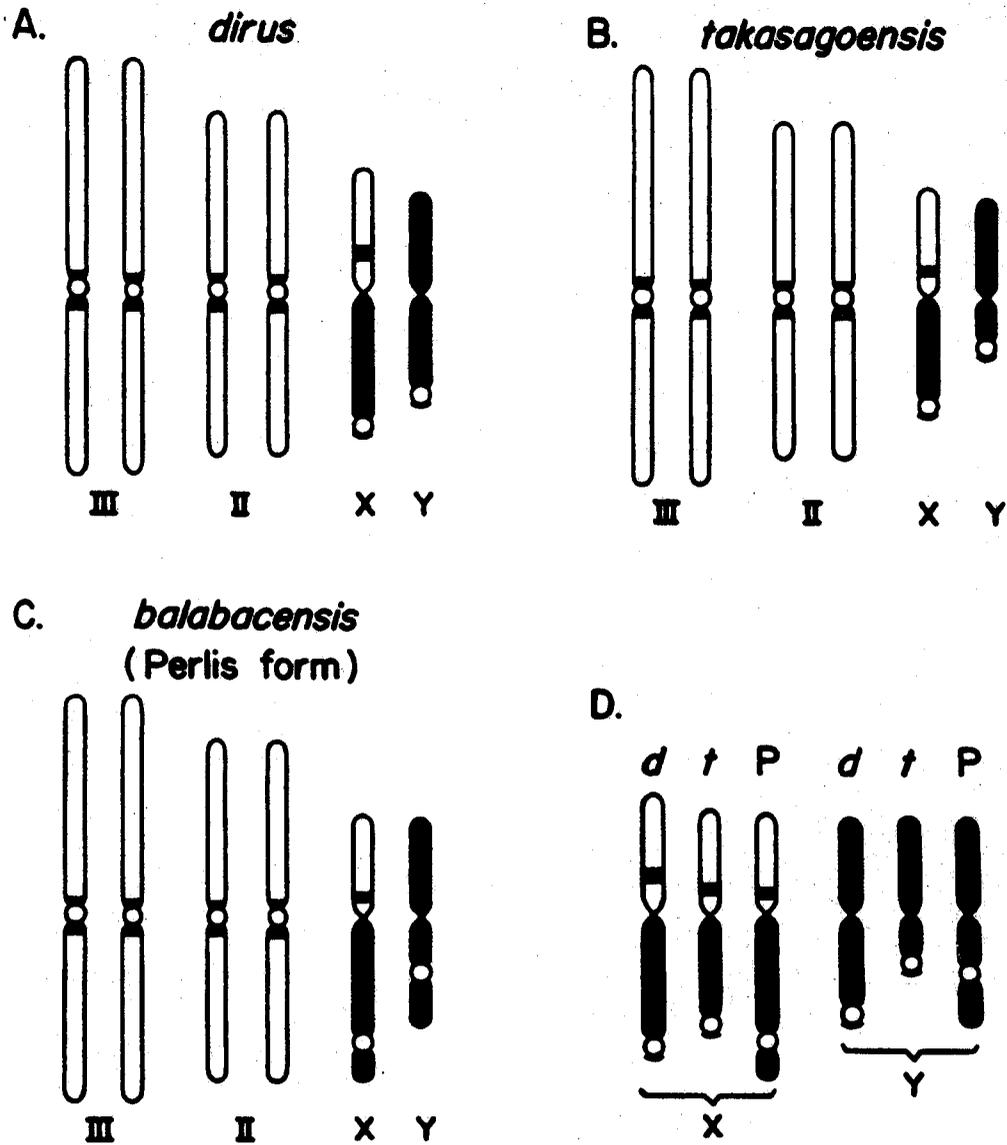


Fig. 1. Diagrams of metaphase karyotypes: A. An. dirus, B. An. takasagoensis, C. An. balabacensis, Perlis form, and D. Sex chromosomes for the 3 species. Dark areas represent constitutive heterochromatin.