Genetics of speciation in the *Aedes (Stegomyia) scutellaris* group (Diptera: Culicidae)

V. Chromosomal relationships among five species

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Abstract

A comparison was made of karyotypes of 5 species in the *Aedes scutellaris* group and their hybrids. All species had 3 distinct pairs of metacentric chromosomes (2n = 6). These were of similar lengths in *Ae. malayensis* and *Ae. alcasidi*, and in *Ae. polynesiensis* and *Ae. pseudoscutellaris*. However, chromosome 1 in *Ae. polynesiensis* and *Ae. kesseli*, and chromosome 2 in *Ae. pseudoscutellaris* and *Ae. kesseli* were of unequal lengths. Meiotic analyses revealed that chromosome asynapsis was frequently seen in species hybrids. There was a significant variation in chiasma frequencies between species and their hybrids. However, the mean chiasma frequency was species specific. In addition, the mean chiasma frequency of species hybrids and the extent of chromosomal asynapsis provided a measure of genetic homology between species. Based on the assumption that a dicentric bridge and an acentric fragment were due to a single crossover within a paracentric inversion loop the following conclusions are made. *Ae. malayensis* and *Ae. alcasidi* are polymorphic for one paracentric inversion in chromosome 1. *Ae. polynesiensis* and *Ae. pseudoscutellaris*, and *Ae. pseudoscutellaris* and *Ae. kesseli* are fixed for one paracentric inversion in chromosome 2. Similarly, *Ae. polynesiensis* and *Ae. kesseli* are fixed for one paracentric inversion in chromosome 1. These chromosomal differences between species are discussed with respect to hybrid fertility data reported earlier.

Introduction

The *scutellaris* group of the genus *Aedes* is comprised of over 30 closely related species widely distributed in Southeast Asia and the South Pacific (Marks, 1954; Belkin, 1962; Huang, 1972; Huang & Hitchcock, 1980). Since many of its species are endemic to single islands isolated by natural geographical barriers, this complex provides an ideal system to study the process of speciation. There has been considerable interest in determining the crossing relationships among species of this group (Macdonald, 1976; Rai et al., 1982). Such studies were further extended by Dev (1983), Dev and Rai (1982, 1983), and Sherron and Rai (1983) by providing additional data on several interspecific hybrids and their backcross progenies.

Relatively few data are available on the types and extent of chromosomal differences among aedine species and their relevance to the speciation process. Polytene chromosomes in *Aedes* are unsuitable for comparative studies. Consequently, only the somatic and meiotic chromosomes have been subjected to analyses in elucidating the cytogenetic relationships among aedine species (Rai, 1980). Colluzzi and Sabatini (1968) found meiotic abnormalities indicative of paracentric inversions in species hybrids of the *Ae. mariae* complex. Motara (1978) recorded a fixation of a paracentric inversion between *Ae. aegypti* and *Ae. mascarensis*. Szymczak (1980) found that *Ae. atropalpus* and *Ae. epactius* differ by three paracentric inversions and a pericentric inversion. Taylor (1982) reported meiotic abnormalities in species hybrids of the *Ae. triseria*-
tus complex including paracentric inversions and chromosomal breakage. Cytological analyses in species of the *Ae. scutellaris* group have been limited to descriptions of karyotypes of few species (Rai, 1966; Rooney, 1977; Motara & Rai, 1978). Only preliminary observations have been made on meiotic analyses in species and their hybrids (Rooney, 1978; Dev, 1982).

Our previous study revealed that among five species under investigation viable hybrids are obtained reciprocally between *Ae. polynesiensis*, *Ae. pseudoscutellaris* and *Ae. kesseli*, and unidirectionally between *Ae. malayensis* and *Ae. alcasidi* (Dev & Rai, 1982). All other interspecific crosses were incompatible. The present investigation is in continuation and was designed to correlate hybrid fertility data with hybrid cytology, and to determine the extent of chromosomal divergence in species differentiation. This involved a comparison of species karyotypes, chiasma frequencies and chromosomal homologies among various species determined by meiotic analyses of species hybrids. In addition, variation in chiasma frequencies between species was analysed to evaluate whether these frequencies were species specific and of potential use in cytotaxonomy. The data included herein constitute the first detailed report on cytological analyses of species and their hybrids of this group. The relevance of these observations with regard to speciation in the *scutellaris* group of species is discussed.

**Material and methods**

Five species of the *scutellaris* group, *Ae. polynesiensis* Marks, *Ae. pseudoscutellaris* (Theobald), *Ae. kesseli* Huang and Hitchcock, *Ae. malayensis* Colless and *Ae. alcasidi* Huang were used. The history of the strains used and their natural geographical distribution are given in Dev and Rai (1982). These species were reared in an insectary kept at a temperature of 25 ± 2 °C and 80 ± 5% relative humidity. The larvae were fed aliquots of liver powder suspension. Colony maintenance and detailed procedures for matings were the same as described earlier (Dev & Rai, 1982).

For cytological analyses, chromosome preparations were made by techniques developed by Rai (1963, 1967). Testes of 16 to 20 h old male pupae were dissected in tap water and squashed after staining in 2% lacto-aceto-orcein for approximately 5 min. These preparations were ringed with nail polish and could be stored in the refrigerator for long periods of time. Photomicrographs were taken using Panatomic-X film and Zeiss phase contrast optics.

For chromosome measurements, photomicrographs of the stage and ocular micrometers were taken and prints were made at the same magnification as that of the chromosome preparations. These were used as the scale to measure the length of individual chromosomes from the photomicrographs. All measurements were taken from cells in spermatogonial metaphase stages.

Asynapsis and chiasma frequencies were scored from metaphase-I cells in primary spermatocytes in various species and their hybrids. The total number of pupae and the cells scored varied from species to species. Cells with at least one asynaptic chromosome pair were counted to calculate the percentage asynapsis. The numerical data collected on chiasma frequencies were analysed by statistical analysis system (SAS) for summary statistics, analysis of variance (ANOVA), and by Duncan's multiple range test for comparison of means.

Throughout the text, *Ae. polynesiensis*, *Ae. pseudoscutellaris*, *Ae. kesseli*, *Ae. malayensis* and *Ae. alcasidi* are abbreviated as PO, PS, KE, MA, and AL respectively. The first named species in all crosses was used as the female parent.

**Results**

**Somatic karyotypes and chromosome lengths**

Five species were examined cytologically. All had three homomorphic pairs of chromosomes (2n = 6). Each chromosome pair could be distinguished from each other based on its length (Table 1). In accordance with nomenclature proposed for *Ae. aegypti* (McDonald & Rai, 1970) the smallest chromosome was designated as chromosome 1, the largest as 2, and the intermediate as 3. The three pairs were metacentric in five species. The ratio of the lengths of chromosome 1 to 2 + 3 ranged from 0.34 to 0.39, which is typical of genus *Aedes* (Rai, 1963).

In order to examine heteromorphism of individual chromosome pairs particularly in species hy-