THE OVARIAN POLYTENE CHROMOSOME OF THE MOSQUITO COMPLEX SPECIES *Anopheles barbirostris* VAN DER WULP.

Supratman Sukowati[1], Herri Andris[1], Shinta [1]

KROMOSOM POLITEN OVARIUM DARI SPESIES KOMPLEKS Anopheles barbirostris *VAN DER WULP*

Abstrak. *Malaria masih merupakan masalah kesehatan masyarakat di Indonesia. Salah satu upaya pemberantasan penyakit tersebut adalah dengan cara pengendalian vektornya. Sebagai dasar untuk menentukan strategi pengendalian vektor secara tepat guna adalah dengan pemahaman tentang spesies dan bioekologi serta habitatnya secara rinci. Untuk mengetahui adanya spesies sibling* Anopheles barbirostris *di Indonesia, telah dilakukan penelitian spesies sibling dengan teknik kromosom mitotik. Penelitian dilakukan di 5 daerah yaitu: (1) Ambarawa, Jawa Tengah, (2) Tara-Tara, Sulawesi Utara, (3) Boru-Boru, (4) Konga dan (5) Singaraja, tiga yang terakhir di Flores, Nusa Tenggara Timur. Analisis kromosom ovarium* An. barbirostris *Van der Wulp dilakukan dari ovarium nurse cell yang ditangkap dari 5 populasi alam yang berbeda daerah geografinya di Indonesia. Foto peta baku kromosom politen* An. barbirostris *telah dihasilkan dan didiskripsi dalam makalah. Spesimen-spesimen populasi tersebut menunjukan kariotipe kromosom mitotik yang serupa (2n=6) yang tersusun dari dua pasang autosom dan satu kromosom kelamin (X). Foto peta kromosom politen* An. barbirostris *yang berasal ovarian nurse cell telah dapat dihasilkan dan diidentifikasi sehingga dapat digunakan sebagai acuan untuk mempelajari adanya polimorfisme dan atau spesies komplek takson tersebut. Analisis pola penggelangan kromosom politen* An. barbirostris *dari 5 populasi alam dengan daerah geografi berbeda, tidak ditemukan perbedaan pola kromosom, semua sampel menunjukkan pola penggelangan yang homosekuensial. Foto peta kromosom politen baku* An. barbirostris *dapat digunakan sebagai acuan untuk mempelajari adanya spesies sibling takson tersebut.*

Kata kunci: Anopheles barbirostris, *malaria, kromosom politen, spesies sibling*

INTRODUCTION

Malaria continues to be a public health problem of high priority in the majority of malaria endemic countries throughout the Southeast Asia region, such as Indonesia. Vector control remains the most effective measure to prevent malaria transmission. The study of mosquito complexes and the understanding of their biology, evolution, epidemiology of malaria transmission, and the possibly of development of efficient control measures against malaria have been subjects of greatly increased interest in recent years.

The mosquito species *Anopheles barbirostris* is widely distributed in Indonesia, occurring in Sumatra, Java, Kalimantan, Sulawesi, Timor, Flores and Irian Jaya [(1)]. This species is the principal vector for human malaria in Sulawesi and Flores [(2,3)], and the filariasis vector in Sulawesi, Timor and Flores [(4,5)], even though in some

[1] Health Ecology Research & Development Center,NIHRD

places of its distribution, e.g. in Sumatra and Java, does not play an important role [6].

The *An. barbirostris* population differs in their tendency to feed on both human and animal blood; this characterristic has obvious relevance to the role of the species in the transmission of disease. In Java, the population of this species is primarily zoophilic, though in Sulawesi, Timor and Flores populations, it does appear to be more anthropophagic [7]. These observations seem to suggest that *An. barbirostris* could be a cluster of closely related species, each of which may play a vital role in malaria or filariasis transmission in its areas of distribution.

In recent years, new technologies have become available for the identification of malaria vector species complexes, which promise a much better understanding of malaria vectors. Polytene chromosome differences in *Anopheles,* as shown by a comparative study of closely related species, involve changes in banding sequence due to paracentric inversion of fixed inversions and differences in unique arrays of chromosomal polymorphism which have led to findings of sibling species of malaria vectors, e.g., *An. gambiae* complex [8,9], *An. culicifacies* complex [10,11], *An. subpictus* complex [12], *An. dirus* complex [13]. Many taxa of medical importance are known to be complexes of morphologically-cryptic species. *Anopheles gambiae*, the most important malaria vector in Africa, has been shown to be a complex of at least six sibling species, with marked differences in behavior and specific polytene chromosome patterns. Practical and reliable cytotaxonomic characters were established for identification of members of the *An. gambiae* complex by White and Davidson & Hunt [14,15]. A major malaria vector in India, *An. culicifacies*, has been identified as a complex of three sibling species [10,11]. The other vectors species which have been identified as species complexes are *An. balabacensis* (6 species) [16], *An. maculatus* (3 species) [17], *An. subpictus* (2 species) [12]; and *An. farauti* (3 species) [18,19]. From these examples, it may be concluded that there is a need for new research studies using new technologies such as cytogenetics, electrophoresis and DNA in the field of malaria vectors in Indonesia, aimed at a better understanding of malaria epidemiology and control strategies.

The application of cytogenetics in the study of sibling species and evolution of the malaria vectors in Indonesia is somewhat limited, despite serious problems with regards to controlling malaria vectors. We present a photomap of the ovarian polytene chromosomes of *An. barbirostris* that we have designated as "standard" for that taxon. We also report the results of survey on polytene chromosome arrangements relative to "standard" arrangement in a natural population of *An. barbirostris.*

MATERIALS AND METHODS

Natural populations of *An. barbirostris* Van der Wulp were sampled from 5 geographically isolated populations in Indonesia: (1) Ambarawa, Central Java, (2) Tara-Tara, North Sulawesi, (3) Boru-Boru, (4) Konga and (5) Singaraja. Numbers 3-5 are all located in Flores, East Nusa Tenggara.

The mosquitoes were kept alive and fed until fully engorged with guinea pig blood, and maintained in a cool, humid environment. The fully blood-fed females were maintained at about 20^0 C with re-

lative humidity of about 80%. About 24-36 hours after feeding (development time for production of half a gravid female), ovaries were dissected, put on labeled filter paper and placed in Carnoy's fluid and where they were kept at low temperature (4^0C) for at least 24 hours before making a polytene chromosome preparation.

The polytene chromosomes were spread and processed according to the methods of Green and Hunt and Sukowati & Baimai [20, 21]. The chromosome photographs were made using a phase contrast microscope and on Kodak Technical Pan Film 2415. The standard chromosome map was made using methods described by Hunt, and Sukowati and Baimai [22,21]. A series of photographs were selected to produce the map. Accumulated prints of each chromosome showing several of stretching were made for comparative purposes. The chromosome maps were then assembled by joining the photographs in such a way that the curvature of alternate segments compensated for that of the preceding segment.

The standard photographic map of *An. barbirostris* was constructed from a series of photographs taken from wild caught populations. Arbitrary divisions along the photomap basically followed the methods of Baimai *et al.*, Baimai *et al* and Sukowati & Baimai [23,24,21]. Determination of banding sequences for polytene chromosomes of wild specimens was accomplished by comparison with the standard photomap directly through a camera Lucida attached to the microscope, or by comparing photograph.

RESULTS

A standard photomap based on the ovarian nurse cell polytene chromosomes for the taxon *An. barbirostris* Van der Wulp collected from Jambu, Ambarawa, Central Java is presented in Figure 1. Polytene chromosome complements of *An. barbirostris* comprise three synapse elements: a short pair of sex chromosomes (chromosome 1/X chromosome) and two longer pairs of autosomes 2 and 3 that are labeled as chromosome 2R/2, 3L/3, 3R/4 and 3L/5 (Figure 1). The right and left arms are somewhat unequal in autosomes 2 and 3, the right being the longer element. The ovarian polytene chromosome complement of *An. barbirostris* has been arbitrarily divided into 50 zones, starting with the free end of an X-chromosome running through the free ends of arms 2R/2 and 3R/4 and finishing at the free end of arm 3L/5 as follows; X-chromosome covering zones 1-5, 2R/2 zones 6-16, 2L/3 zones 17-25, 3R/4 zones 26-39, and 3L/5 zones 40-50 as described below. There were no inversion polymorphisms detected in this study. All the wild samples examined showed similar chromosomal arrangements to the standard photomap.

The X Chromosome

As in the general rule for the genus *Anopheles*, the X chromosome of *An. barbirostris* is the shortest one [21,25,26,27]. In most preparation observed in this study, the X-chromosome has very distinct bands with a flared and large centromeric region. Zone 1 marks the free end of chromosome X. The occurrence of two largely diffuse and light dotted bands with the same curvature is a consistent feature at the tip, followed by two dark bands and a short dark band at the constriction area. Following the constriction area is a light broad dotted band, two light and two dark bands, and continues with four dotted bands respectively. Zone 2 begins with two dark bands followed by three dotted bands, a double dark band at the constriction, and three dotted bands. Zone 3 is marked by a closely spaced set of four bands (one dark, two dotted and one dark band, respective-

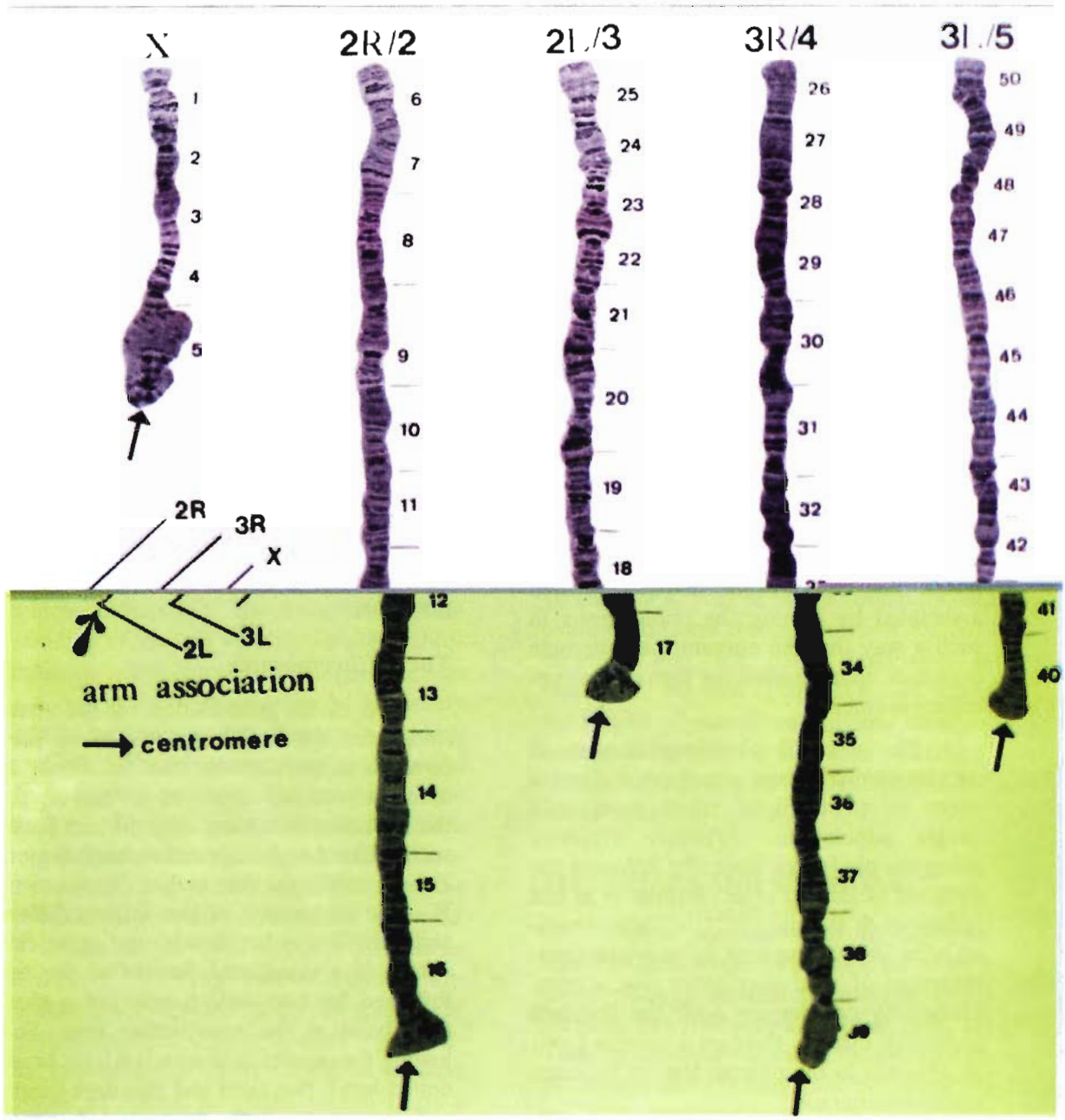

Figure 1. Standard Photomap of The Ovarium Nurse Cell Polytene Chromosomes of Anopheles barbirostris from Indonesia. Centromeric end of Each Chromosome Arm is Indicated by an Arrow

ly). Broad light areas containing four diffuse bands follow this region. The remaining part of zone 3 is composed of two dark bands and one dotted light band in between. Zone 4 has a prominent landmark of three dark bands; these are followed by one dark dotted band, a diffuse dotted band and ends with a doubled dark band at the constriction. The centromeric region is covered by zone 5 that has one prominent dark band, followed by a large puff that consists of 5 diffuse dotted bands and four dark dotted bands at the contromer.

Chromosome arm 2R/2

The free end, including zone 6, has four dotted bands at the tip followed by doubled dark bands, two dotted light bands, three dark bands, two light dark bands and ends with doubled dotted dark bands. Zone 7 begins with three light dotted bands, followed by three closely dotted dark bands, two pairs of light dotted bands, and one dark band, three light dotted bands, one prominent dark band, one dotted dark band, and one dark band respectively. Zone 8 begin with one light dotted band, followed by a doubled closed dotted dark band, three light dotted bands, two dark bands, two light dotted bands, one dark band, two dotted bands, and two light bands respectively. Zone 9 is marked by one thick bands, three dotted bands, and light area, following the constriction area are three short thick dark bands, three thin dark bands, two wide diffuse bands, one dotted dark band and one thick curvature dark band. Zones 10 and 11 consist of several dark bands. Zone 10 start with three medium dotted bands, continued by two dotted bands, a pair of curvature dark bands, two light bands, one small thick band, a light area, two dotted bands, three conspicuous dotted dark bands and ends with two light dotted bands. Zone 11 comprises one dotted band, four thin dotted bands, one curvature small dark band, two dotted light bands, three pairs of dotted bands, two dark bands, one thin dark band, and a pair of dotted band res-pectively. Zone 12 has one long puffing area with a series of two small black dotted bands, two light dotted bands, one thick black band on the constriction, two pairs of light dotted bands, two black dotted bands at the middle of puffing, a pair of black bands, two light dotted bands, two pairs of dotted bands and ends with a pair of prominent black bands. Zone 13 starts with one pair of dotted bands, followed by two pairs of diffused dotted bands, one curved black band, one dotted band, two thick prominent dark bands, three pairs of light dotted bands, and one doubled small dark band respectively. Zone 14 is characterized by two prominent dark bands with space in between which consist of two pairs of dotted light bands, followed by several light dotted bands, two pairs of curvature black bands with space in between, followed by three light dotted bands, four black dotted bands and four dotted light bands respectively. Zone 9 represents another characteristic, consisting of several dark bands, starting with one thick dark band, followed by three dotted bands with curvature, three thin black bands, a pair of dotted bands, one doubled dark band at the constriction, and five dotted bands with curvature respectively. Zone 16, covering the centromeric region, is characterized by five prominent dark bands. Zone 16, begins with a doubled dark band, one light band, five prominent dark band, several wavy bands, a pair of prominent dark bands with one dotted band in between, several diffuse light bands, and a pair of small dark bands at the centromeric region.

Chromosome arm 2L/3

Zone 25 marks the free end of arm 2L showing a typical funnel shape at the free end with dotted and flared bands at the very tip. It is followed by one puff consisting of several diffuse dotted bands, and continued by a doubled dark band, two dotted light bands, and one dark band respectively. Zone 24 comprises one dark band, two pairs of dotted bands, three light bands, a pair of black bands in the constriction, and three pairs of light dotted bands. Zone 23 is characteristically marked by two pairs of dark bands located at the beginning of this zone and continued by a wavy diffuse band, and three pairs of curvature dotted bands which form a moderate swelling. Zone 22 begins with a pair of doubled dark bands, followed by one dark band in the constriction, a pair of dotted bands, two dark bands, and three dotted bands. Zone 21 is composed of one black band, followed by two black bands in the constriction, six dotted bands, one thin black band, a pair of prominent dark bands in the constriction, one light band, one thin light band and one dark band with a light area in between and three curvature dotted bands. Zone 20 begins with one black dotted band, followed by a light dotted band, one dark band, two thin dark bands with one light dotted band in bet-ween, a light area consisting of four thin bands, five dark bands, diffuse band, one dark band, diffuse band and three dark bands respectively. Zone 19 is composed of two light dotted bands, two close dark bands, one dark dotted band, 5 thin dark bands, a pair of prominent dark bands, two pairs of light bands and ends with three dotted bands. Zone 18 contains one dotted band, two prominent dark bands, one curved dark band, two dotted bands, one dark band, a pair of small dark bands, one light band, a pair of close dark bands, two dark bands with a light dotted band in between, and a pair of light dotted bands. Zone 17 is located at the centromeric end of arm 2L, beginning with two constricted dark bands followed by two dotted bands, four light bands, two dotted bands, two close dark bands, and several diffuse light bands contributing lighter area toward the centromeric region.

Chromosome arm 3R/4

The free end in zone 26 has a diffuse character tip followed by a pair of dotted bands and moderate swelling area with four dotted bands. Zone 27 starts with one dotted band, followed by two dark bands with a light area in between, a series sequence of 10 dotted bands, two close dark bands, and one dotted band. Zone 28 begins with a pairs of dark bands, one dotted band, one dark band, one short dark band in the constriction, and three prominent dark bands. Zone 29 has a pair of light bands, followed by one dark band, two diffuse bands, one dotted band, two dark bands, one light band, one prominent dark band, two light bands, and a pair of dark bands in the constriction. Having several pairs of prominent dark bands characteristically marks zone 30 and zone 31. Zone 32 and zone 33 are lighter than zone 30 and 31; most of the bands are composed of many dotted and light bands, and less dark bands. Zone 34 consists of a series of dark bands and dotted dark bands, which starts with two dark bands, followed by six dotted dark bands, two dotted bands, a pair of dark bands, one light band, and two pairs of dark bands respectively. Zone 35 starts with one light dotted band, three close prominent dark bands, one dark dotted band, two pairs of dotted light bands, one thin band, a pair of dotted bands, and ends with a pair of small band in the constriction. Zone 36 starts with a

pair of curvature dotted bands, continued by three light dotted bands, a pair of black bands, three light bands, a pair of dotted bands, three thin bands, two black bands, one black dotted band, and two light bands. Zone 37 consists of several black bands, starts with one prominent dark band, followed by 5 black bands, two close prominent dark bands, a pair of dotted bands and one light band. Zone 38 has one small puffing area with a series of dark, dotted bands and diffuse bands, consisting of one black band, three dotted bands, two thin bands, and several diffuse bands which form a light area in the puff, two prominent dark bands, and ends with four dotted bands. Zone 39 is located at the centromeric end of arm 3R/4 beginning with two constricted prominent dark bands, followed by four pairs of light dotted bands, one dotted band, and two pairs of light dotted bands contributing to a lighter area toward the centromeric region.

Chromosome arm 3L/5

The telomere in zone 50 shows several light bands followed by one black band, then a small swelling part comprised of two black bands, a pair of curved black bands and one prominent dark band at the constriction. Zone 49 consists of four pairs of dark bands. Zone 48 starts with a pair of short black bands at the constriction, followed by one dotted band, a pair of thin black bands, two pairs of dotted black bands, one black band, one dotted black band, a pair of dotted black bands and a pair of light dotted bands. Zone 47 starts with one dotted band, followed by two dotted bands, eight black bands in series, and a pair of dotted bands. Zone 46 is composed of three pairs of dotted black bands, one light band, three dark bands, several diffuse bands, one dark band, and three dotted bands. Zone 45 starts with a pair of contracted black bands at the constriction, followed by one curved black band, three thin dark bands, three wavy bands, and one dotted band respectively. Zone 44 consists of two black bands at the constriction, three black bands, one dotted wavy band, one light dotted band, a pair of dotted bands, two dotted bands, one black band, four dotted light bands, and a pair of light bands. Zone 43 is characteristically marked by several dark bands, starting with one light band, and continued by three dark bands, a pair of constricted dark bands, one dark band, three pair of dotted dark bands, and two pairs of dark bands. Zone 42 is composed of a pair light dotted bands, one dark band, three pairs of wavy dotted bands, a pair of dotted bands, one black band, several diffuse bands, a pair of black bands, one light band, and two dotted bands. Zone 41 starts with two black constricted black bands, followed by three light dotted bands, one black band, two light bands, one light band at the constriction, and two dotted bands. At the centromeric region is zone 40; this zone is darker in appearance, composed of one dark band, a pair of prominent dark bands, three pairs of dotted dark bands, five dotted dark bands, and diffuses flare dark bands at end.

DISCUSSION

As in other dipterans insects, one of the essential requirements for cytogenetic investigations of anopheline mosquitoes is a standard polytene chromosome map; for example, in *An. subpictus*, *An. vagus* [26], *An. albimanus* [28], *An. nili* [27], *An. aconitus* [28], *An. culicifacies* [25], *An. dirus* [13,23] and *An. sundaicus* [21]. The standard polytene photomap of *An. barbirostris* has been prepared and is presented in this article for comparative investigations into differen-

ces, if any, in different wild populations. Differences in the banding patterns and the presence of chromosomal polymorphisms within and between populations of *An. barbirostris s.l.* could be useful information as to the genetic differentiation and possible speciation within this taxon. Such differences in polytene chromosome banding patterns have been reported in the homosequential species of *An. dirus* complex from Southeast Asia [13,29] and of *An. sundaicus* complex from Indonesia and Thailand [21].

In our study, all the wild of *An. barbirostris s.l* specimens obtained from different geographical areas in Indonesia appear to share most of the standard chromosome arrangements in the polytene complement. The absence of chromosomal polymorphism in these five populations from difference geographical areas might be due to no detectable differences in the banding patterns of polytenes (homosequential) in this species. The homosequential banding patterns of polytene chromosomes have been reported from some species: e.g. *An. atroparvus* and [17] *An. labranchiae*, *An. funestus and An. aruni*, and seven species from the *An. hyrcanus* group [30]. Alternatively, this species might have inversion or polymorphism in the polytene chromosome; however, this has not been found yet because only 5 populations in Indonesia are being studied. Therefore, from the mitotic chromosomes studied 4 forms of the mitotic chromosome in *An. barbirostris* complex in Indonesia were identified[31]. Nevertheless, this standard photomap of polytene chromosome provide a reference point for further studies, where more analysis of natural populations would be carried out together with the necessary taxonomic studies of chromosomally identified materials.

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