Chromosome Characterization of Three Cell Lines Derived from *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.)[1] (36409)

N. O. BIANCHI, B. H. SWEET, AND J. P. AYRES

Gulf South Research Institute, Public Health Sciences Division, New Orleans, Louisiana 70126

Although the chromosome complement of several mosquito species has been described in detail (1–3) similar studies in continuous cell lines derived from these insects are scanty or absent. Continuous mosquito cell cultures have been established from *Aedes* (4–9), *Anopheline* (10–11) and *Culicine* (12–13) species. In reports on *Aedes vexans* and *A. aegypti* (3, 8), *Culex quinquefasciatus* (12), and *Anopheles stephansi* (10), the number and general chromosome morphology has only been mentioned. Distinctive karyological traits generally used for cell identification such as the evidence of polyploidy, heteroploidy and chromosomal aberrations are not generally known or extensively defined for any of the mosquito lines presently available.

The present report presents the results of the karyology of lines developed by Singh (5) from *A. aegypti* and *A. albopictus* larvae, and by Peleg (6) from *A. aegypti* embryonated eggs.

Materials and Methods. The cell lines described by Singh (5) or Peleg (6) were obtained from the respective investigators in early passage and carried continuously in our laboratory using the methods and media employed by them. Morphologically and culturally the three lines are quite distinct and also can be characterized by differences in their host range for various arboviruses.

Chromosomal studies were performed in passages 25, 35 and 161 of the *A. albopictus*, in passage 87 of the *A. aegypti* (Singh) and in passage 117 of the *A. aegypti* (Peleg) lines. Cultures taken during the log phase of growth were treated with 0.12 μg/ml of Colcemid for 5 hr. Cells were removed from culture flasks by trypsinization or by shaking the flask. They were then hypotonically treated for 10 min in 3:1 distilled water:culture medium and fixed in two changes of 3:1 ethanol:acetic acid for a total of 40 min. Chromosome spreads were prepared by the air drying method and stained with carbol fuchsin. One to two hundred metaphases were analyzed in each sample and the incidence of diploidy, heteroploidy, polyploidy and chromosome aberrations was determined. Chromosome measurements were performed in five karyotypes from *A. albopictus* and in five karyotypes from *A. aegypti* (Singh) lines.

Results. A. aegypti (Singh) line. Cells from this line had three pairs of chromosomes. Following the usual nomenclature for mosquito chromosomes the smallest pair was designated No. 1 and the longest No. 3. There was little difference in size of the pairs 1 and 2; their identification was possible only in metaphases with well elongated chromosomes (Fig. 1). The nomenclature for centromeric position proposed by Levan *et al.* (13) defines metacentric or *m*-chromosomes as all those elements ranging from 1 to 1.7 in arm ratio. Accordingly, the three chromosome pairs in this line should be included in this group (Table I). Previous reports define pair 2 of *A. aegypti* as submetacentric (2). However, since chromosome measurements of the system proposed by Levan were not employed in these publications, we think that discrepancies between our and other reports are probably a matter of nomenclature.

Somatic pairing was observed as a common event in prophase and prometaphase cells (Fig. 2). Homologous chromosomes were associated at the centromere level and very often

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TABLE I. *Aedes aegypti* (Singh): Chromosome Length Expressed as Percent of the Haploid Set.

	Pair 1	Pair 2	Pair 3
Short arm	18.6 ± 0.06	17.9 ± 0.07	12.6 ± 0.09
Long arm	19.2 ± 0.09	18.1 ± 0.08	13.3 ± 0.05
Total	38.8 ± 0.05	36 0 ± 0.08	25.9 ± 0.07
Arm ratio	1.03	1.01	1.05

a process of centromere fusion seemed to occur.

The analysis of more than 200 metaphases showed that 71% exhibited an image of chromatid discontinuity affecting one or more chromosomes from the same or different pairs. The analysis of 100 abnormal cells showed an incidence of 1.29 chromatid discontinuities per cell. Furthermore, 78% of these discontinuities were located in the proximal third of one arm in chromosomes from pair 1 (Fig. 2), 11% in the distal third of one arm in chromosomes from pair 2 (Fig. 3), 6% in the distal third of one arm in chromosomes from pair 3 (Fig. 3), and 5% were observed in variable positions in chromosomes from pair 2 or 3. Thus, it seems evident that most of these discontinuities were not randomly but selectively located in given chromosome regions. The different type of discontinuities observed were the following: secondary constrictions, chromosome gaps, chromosome breakages with fragment dislocation or deletion, structural rearrangements such as ring chromosomes, dicentrics and symmetric or asymmetric quadriradials (Figs. 2–5). In several instances it was possible to observe a secondary constriction in one chromosome and a chromosome breakage in the same region of the homologue. Consequently, images resembling secondary constrictions and gaps were considered in these cells to represent initial stages of chromosome breakage. The incidence of polyploidy in this line was 2%.

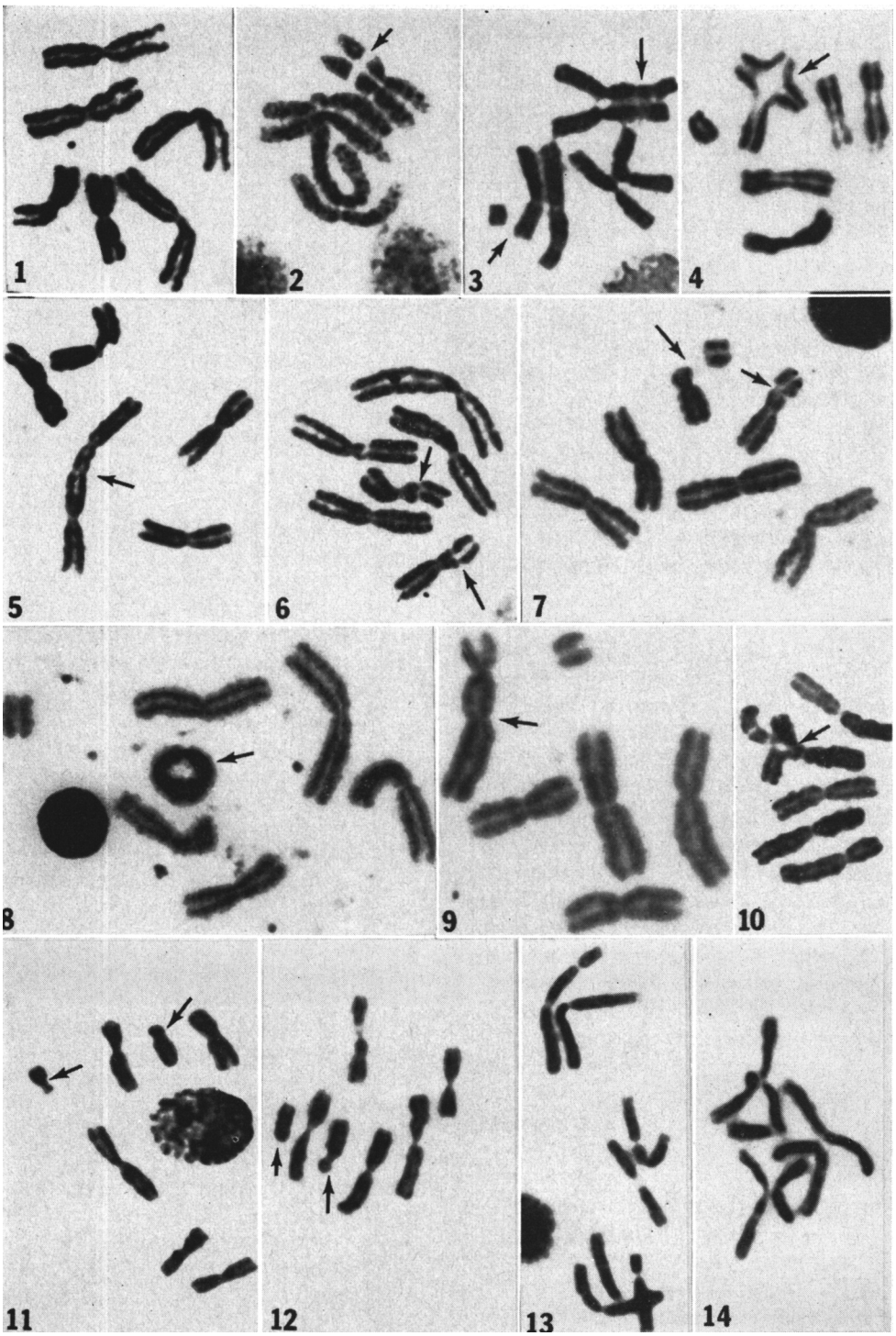
TABLE II. *Aedes albopictus:* Chromosome Length Expressed as Percent of the Haploid Set.

	Pair 1	Pair 2	Pair 3
Short arm	18.8 ± 0.08	17.9 ± 0.04	12.2 ± 0.05
Long arm	19.3 ± 0.08	17.9 ± 0.07	13.8 ± 0.07
Total	38.1 ± 0.06	35.8 ± 0.06	26.0 ± 0.05
Arm ratio	1.02	1.00	1.13

Aedes albopictus line. The chromosome morphology and the incidence, type and location of chromosome breakages in passages 25, 35 and 161 of this line were similar to those described for *A. aegypti* (Singh) cells. The chromosome complement in *A. albopictus* cells was formed by three pairs of metacentric chromosomes; pair 3 being the longest and pair 1 the shortest of the set (Table II) (Fig. 6). Somatic pairing occurred in all prophases and prometaphases.

The incidence of cells with chromatid discontinuity was 48% in passage 25, 52% in passage 35 and 45% in passage 161. The discontinuities ranged from secondary constriction-like images to complete chromosomal breakages with occasional chromosomal rearrangement (Figures 6–10). In each passage sampled, a total of 100 abnormal metaphases were analyzed. The results obtained indicated that the incidence of breakage per cell was 1.20 in passage 25, 1.18 in passage 35 and 1.28 in passage 161. Breakages were located in the proxmal third of one arm in pair 1 in 76–85% of cells, in the distal third of one arm in pair 2 in 12–20% of cells, in the distal third of one arm in pair 3 in 6–8% of cells, and in other regions of the complement in 2–6% of cells. The incidence of polyploidy in *A. albopictus* cultures ranged between 10–18%.

Since the chromosome complements in *A. aegypti* (Singh) and *A. albopictus* cultures are alike, the possibility of cell contamination should be considered. Tests for immunospecificity by immunodiffusion indicated complete identity, but by immunoelectrophoresis slight differences between the two lines could be observed (15). Furthermore, chances of cell contamination could be ruled out due to the characteristic cell morphology of each of these two lines and their differences in sensitivity when infected with Group B arboviruses (16). Cells from *A. aegypti* (Singh) cultures were small and grew in clumps which progressively increased in size, became interconnected and formed a network configuration before reaching confluency. On the



FIGS. 1–14. (All figures are shown at 1100× magnification.) 1. Normal metaphase from *A. aegypti* (Singh) line. 2. *A. aegypti* (Singh) prometaphase showing somatic pairing and breaks in the two homologues of pair 1 (arrow). 3. *A. aegypti* (Singh) metaphase showing somatic pairing, a

other hand, cells from *A. albopictus* cultures were twice as large as those from *A. aegypti* (Singh) cultures and reached confluency without forming clumps. Moreover, approximately 48 hr after infection with certain Group B arboviruses, *A. albopictus* cells form a syncytium and die, whereas these seem to propagate in *A. aegypti* (Singh) cultures without any apparent harmful effect to the cells.

A. aegypti (Peleg) line. The outstanding feature of this cell line was the existence of 7 chromosomes in all metaphases analyzed. The complement of these cells was characterized by the presence of 5 large metacentric chromosomes and 2 medium size subterminal chromosomes (Figs. 11, 12). However, a series of morphological variables made it difficult to compare the chromosome complement from different cells, to obtain chromosome measurements, or even in some cases, to identify the homologue chromosomes. The more frequent deviations from the general complement described were: a) difference in size of the two apparent homologues; b) presence of submetacentric chromosomes; c) presence of one or two acrocentric chromosomes replacing the subterminal ones; and d) great variability in the size of the subterminal chromosomes. Secondary constrictions (or secondary constriction-like images), chromatid gaps and chromosome breakages were found in approximately 60% of metaphases. The predominant location of these aberrations was the distal third of a large metacentric chromosome resembling chromosome 3 of *A. aegypti* (Singh) cells (Fig. 13). The analysis of somatic pairing showed that it also varied from cell to cell and in many cases the association of three or more chromosomes was found. This multiple association was very often at the centromere level; however, in some other cases it involved areas other than the centromeric ones (Fig. 13, 14).

Since *A. aegypti* (Peleg) cultures were derived from cells of a mosquito species having three pairs of biarm chromosomes (2), it is possible to surmise that the number of seven chromosomes and the chromosome polymorphism arose by breakages and translocations involving variable chromosome pairs.

Discussion. The common fact for the three lines analyzed in this report is the presence of a high percentage of cells showing one or more chromosome breakages. In *A. albopictus* and *A. aegypti* (Singh) cells most of these breakages remain open; while in *A. aegypti* (Peleg) cells, they were in most instances closed giving rise to different types of chromosomal rearrangements. The causes for this difference in behavior are presently not clear. However, it may be speculated that some impairment in the process of healing may prevent the cells of *A. albopictus* and *A. aegypti* (Singh) cultures from closing their breakages.

In *A. albopictus* cultures, different samples separated by more than ten passages exhibited the same incidence and the same type of breakages. Since open breaks are unstable and often accompanied by cell death, it seems reasonable to assume that breakages are produced at a steady rate in every cell generation of this line. Preliminary studies seem to suggest that this is also true for *A. aegypti* (Singh) and *A. aegypti* (Peleg) lines. The origin of these chromosome breakages is at present not fully understood. However, any hypothesis aimed at explaining these aberrations should consider the fact

chromosome breakage with fragment dislocation and secondary constrictions (arrows). 4. *A. aegypti* (Singh) metaphase showing an asymmetric quadriradial (arrow). 5. *A. aegypti* (Singh) metaphase showing a dicentric (arrow). 6. *A. albopictus* metaphase showing chromatid gaps in the same region of both homologues in pair 1 (arrows). 7. *A. albopictus* metaphase showing a secondary constriction in one chromosome 1 and a break with fragment dislocation in the same region of the homologue (arrows). 8. *A. albopictus* metaphase showing a ring chromosome (arrow). 9. *A. albopictus* metaphase showing a dicentric chromosome (arrow). 10. *A. albopictus* metaphase showing a symmetric quadriradial (arrow). 11. *A. aegypti* (Peleg) metaphase showing two subterminal chromosomes (arrows). 12. *A. aegypti* (Peleg) metaphase showing one subterminal and one acrocentric chromosome (arrows). 13 and 14. *A. aegypti* (Peleg) prometaphases showing somatic pairing with different types of multiple chromosome association.

that they are selectively located on given chromosome regions. The most common causes producing nonrandom chromosome breakages are some drugs and viruses. The former possibility can be easily excluded since no drug was added to our cultures. On the other hand, although far from being proved, the presence of virus received from primary cells or by contamination during the culture handling cannot be ruled out. Karyological information from other laboratories will be extremely helpful to determine whether the chromosome complements described in this report are general for all the lines originated from the same source.

Summary. The chromosome analysis of three continuous cell lines derived from mosquitos showed that *A. aegypti* (Singh) and *A. albopictus* lines had a complement formed by three pairs of metacentric chromosomes. Moreover, both lines exhibited chromatid discontinuities ranging from secondary constrictions to chromosome breakages with occcasional chromosomal rearrangements. These discontinuities were nonrandomly located and probably produced at a steady rate in every cell generation. On the other hand, the *A. aegypti* (Peleg) line had a complement formed by 7 chromosomes in all the metaphases analyzed. Chromosome morphology frequently varied from cell to cell and occasionally the homologue identification was difficult. The analysis of somatic pairing figures showed that the chromosome complement of this line is probably the result of chromosomal breakages and rearrangements involving different chromosomes.

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