CORRELATION OF LINKAGE GROUPS WITH CHROMOSOMES IN THE MOSQUITO, *Aedes aegypti*

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Reciprocal translocations have recently become of special interest to geneticists working with insects of medical and veterinary importance. The possible use of the inherited semisterility associated with reciprocal translocations for genetic control (Serebrovskii 1940) has stimulated considerable interest in obtaining and studying these chromosomal aberrations, especially in insect pest species (Rai 1967; Rai and Asman 1968; Curtis 1968; Laven 1969; Wagoner, Nickel and Johnson 1969). In addition, reciprocal translocations have been employed for correlating linkage groups and chromosomes in such economically important species as the house fly, *Musca domestica* (Wagoner 1967) and the sheep blow fly, *Lucilia cuprina* (Childress 1969).

Basic research in *Aedes aegypti* has developed concomitantly with the demonstration of the role of this species in the transmission of diseases such as yellow fever, dengue fever, and Asian hemorrhagic fever. As a result, a considerable amount of research has been undertaken in the bionomics and systematics of this species. Indeed, this species has been more extensively studied than any other lower Dipteran. In the area of genetics, relatively little work had been done by 1960 when Christophers published his comprehensive monograph on this species. However, during the last decade, the genetics of *A. aegypti* has developed to the point where some ninety mutants have been described, and one-third of these have been mapped on three linkage groups. The genetic system has proved to be an extremely interesting one: In addition to the morphological mutants, physiological and biochemical mutants, gynandromorphs, intersexes, and segregation distorters have been uncovered (Craig and Hickey 1967).

Cytologically, three homomorphic pairs of chromosomes constitute the diploid set in this species. These chromosomes have been designated I, II and III in the order of increasing size (Rai 1963). Furthermore, the chromosomes can be distinguished by the position of the centromeres and a secondary constriction. The shortest chromosome is metacentric; the medium-length chromosome is submetacentric with a secondary constriction on the longer arm; and the longest chromosome is metacentric. As is the case in other Diptera, somatic pairing occurs in this species. Thus, this species is a very favorable one for cytological

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studies with rather large and easily distinguishable chromosomes. Furthermore, with a wealth of genetic information and extremely good cytological potential, the future of cytogenetic study in this species seems very promising.

Although genetic studies distributed the genes into three linkage groups and cytological analysis showed three pairs of chromosomes, until now no correlation between the linkage groups and chromosomes had been achieved. The same is true of all other mosquitoes, including species of Aedes and Culex, many of which are shown to have karyotypes similar to Aedes aegypti (RAI 1963, 1966; Mukherjee, Rees and Vickery 1966; Baker and Aslam Khan 1969). This paper correlates the linkage groups of A. aegypti with the individual chromosomes through the use of two radiation-induced reciprocal translocations. The first reciprocal translocation, \( T(1;2) \), involves linkage groups one and two and has breakpoints 0.3 crossover unit from the male-determining allele (\( M \)) on linkage group one and 1.6 crossover units from the wild allele for spot abdomen \( (s^+) \) on linkage group two (RAI, MCDONALD and ASMAN 1970). The second reciprocal translocation, \( T(1;3) \), involves breaks which occurred 0.4 crossover unit from the wild-type allele for red eye \( (re^+) \) on linkage group one and 0.6 crossover unit from the wild allele for black tarsi \( (blt^+) \) on linkage group three (RAI, MCDONALD and ASMAN 1970). The two translocations are diagrammatically represented in Figure 1. It may be pointed out that in Aedes aegypti, sex determination takes place by allelic genes \( M \) and \( m \) with \( Mm \) being male and \( mm \) female. (McClelland 1962.)

This is the first reported correlation of linkage groups with chromosomes among approximately 2400 species and subspecies of mosquitoes.

**MATERIALS AND METHODS**

All stocks of mosquitoes employed in the present studies were reared in an insectary maintained at a constant 26°C and 80% ± 10% relative humidity. Detailed procedures employed for the maintenance of mosquito stocks and making crosses have been described previously (Craig and Vandehey 1962). The original translocated lines were produced from and maintained with a wild-type strain designated ROCK. This strain is homozygous for the dominant, wild-type alleles of red eye \( (ref) \), spot abdomen \( (sf) \) and black tarsi \( (blt^+) \) on linkage groups one, two, and three, respectively.

The \( T(1;2) \) translocation, being closely sex linked, had been maintained from father to son by crossing with ROCK females. Translocated males were crossed with females of the RED strain homozygous for the recessive mutant alleles of red eye \( (re) \), of spot abdomen \( (s) \), and of black tarsi \( (blt) \), and the \( F_1 \) male progeny backcrossed. An \( F_2 \) male progeny carrying the translocation with the wild allele for spot abdomen and otherwise with the mutant alleles from the RED strain was obtained and a line established by crossing these males with RED strain females. In a similar fashion a line of \( T(1;3) \) carrying the translocation with wild alleles for red eye and for black tarsi and otherwise with the mutant alleles from the RED strain was obtained and a line established by crossing with the RED strain.

By recovery of crossovers between the \( M \) allele at the sex locus and the breakpoint of \( T(1;2) \) or \( T(1;3) \) on linkage group one, translocations linked with the female determining allele \( (m) \) were obtained. Thus both \( T(1;2) \) and \( T(1;3) \) translocation heterozygotes could be studied in the females as well as the males. Translocation carrying females were mated with males carrying the other translocation and progeny of appropriate phenotype for a double heterozygote were secured for cytological examination (Figure 1).
DOUBLE TRANSLOCATION HETEROZYGOTE

Figure 1.—Crossing scheme to obtain males heterozygous for two reciprocal translocations, T(1;2) and T(1;3).

Tissues used for cytological examination included brain and limb buds from early fourth instar larvae, testes from pupae, and ovaries from newly emerged adults. The tissues were dissected in distilled water, placed immediately into a drop of 2% aceto-lactate-orcein stain, and a squash preparation made. Pictures were taken with a Zeiss phase contrast microscope and a
Zeiss Ikon camera using Kodak Pan 135 film. Camera lucida drawings were made for measurements of chromosome lengths.

RESULTS

*Aedes aegypti* is characterized by the presence of three pairs of somatically paired chromosomes; the shortest pair is metacentric, the medium-length pair is submetacentric with a secondary constriction and the longest pair is metacentric. Although one fails to find the secondary constriction throughout mitotic and meiotic stages, it is often seen, either in one or in both homologs, during mitotic prophase and is associated with the medium-length chromosome (Figures 2, 2a, 3, 3a). Measurements of camera lucida drawings of normal as well as translocated chromosomes are given in Table 1. In primary spermatocytes, the chiasma frequency at metaphase I is relatively low, ordinarily ranging from three to six chiasmata per cell (Figure 4).

Study of the somatic cytology from the oogonial squashes demonstrated that $T(1;2)$ involves the shortest chromosome and the longest chromosome. When the homologs are paired, the heteromorphism in two of the pairs indicated inequality of the exchanged segments in the translocation heterozygotes. Of the four chromosomes involved in this translocation, $T(1;2)$, one is the normal shortest (1) and the other is the normal longest (2). Both of these are metacentric. One of the translocated chromosomes (2') has the centromere of the longest chromosome, is considerably shorter than it, and is acrocentric. The other translocated chromosome (1') has the centromere of the shortest chromosome, is longer than it, and is submetacentric (Figures 5, 5a, 6, 6a, 7, 7a).

An analysis of early metaphase stages from the spermatogonial cells illustrated that the chromosome not involved in the translocation is the medium-length pair with the secondary constriction (Figure 8a). The exchange between a part of the longest chromosome and that of the shortest is evidenced in the increase in the length of the submetacentric translocated chromosome (1') over the normal shortest chromosome (1). That the exchange was a reciprocal one was inferred from the pairing of the tip of the normal shortest chromosome with the tip of the acrocentric translocated chromosome (Figures 7, 7a). At this stage of mitosis only homologous tips are paired in the normals.

An analysis of meiotic chromosomes in primary spermatocytes also showed the involvement of the shortest and longest chromosomes in the translocation.

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**Figures**

2 and 3.—Normal oogonial cells 2500×

4.—Normal spermatocyte 2500×

5, 6, and 7.—$T(1;2)$ oogonial cells 2500×

9.—$T(1;2)$ spermatocyte 1560×

10.—$T(1;3)$ oogonial cell 2500×

11.—$T(1;3)$ spermatogonial cell 2500×

12 and 13.—$T(1;3)$ limb bud cells 2500×

14.—$T(1;3)$ spermatocyte 2500×

15 and 16.—Double heterozygote $T(1;2) T(1;3)$ spermatogonial cells 2500×

17.—$T(1;2) T(1;3)$ spermatocyte 2500×
The total length of the four chromosomes involved in the interchange complex was approximately twice the length of the bivalent not involved in the translocation (Table 1). This confirmed that this particular bivalent is the medium-length chromosome pair.

Cytological analysis of $T(1;3)$ demonstrated that the chromosomes involved are the smallest metacentric chromosome and the medium-length submetacentric chromosome. The longest chromosome is not involved in this translocation and is found homologously paired while the other four chromosomes formed two heteromorphic pairs (Figures 10, 10a, 11, 11a, 12, 12a, 13). The translocated chromosome with the centromere of the shortest chromosome ($I^s$) is submetacentric. The translocated chromosome with the centromere of the normal medium-

Figures 2a, 3a, 5a, 6a, 7a, 10a, 11a, 12a, 15a, and 16a are camera lucida drawings of corresponding cells in Figures 2, ..., 16. Figures 8a and 18a are from spermatogonial cells. All drawings are at the same magnification. Chromosome number designations are according to the new system proposed (see Discussion) with chromosome $I^s$ the translocated chromosome with the centromere of $I$ and exchange piece from 2, etc.
TABLE 1

Lengths of the chromosomes in normal, T(1;2), T(1;3), and double heterozygote cells
Length is given as percent of the total chromosomal length in the cell

<table>
<thead>
<tr>
<th>Stock</th>
<th>Figure</th>
<th>Mitotic stage</th>
<th>i</th>
<th>j</th>
<th>k</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>3'</th>
<th>Translocation complex</th>
<th>Normal bivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2a</td>
<td>prophase</td>
<td>12</td>
<td>12</td>
<td>...</td>
<td>21</td>
<td>21</td>
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<td>17</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>3a</td>
<td>prophase</td>
<td>15</td>
<td>15</td>
<td>...</td>
<td>19</td>
<td>19</td>
<td>16</td>
<td>16</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>T(1;2)</td>
<td>5a</td>
<td>metaphase</td>
<td>13</td>
<td>16</td>
<td>...</td>
<td>20</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>6a</td>
<td>metaphase</td>
<td>13</td>
<td>16</td>
<td>...</td>
<td>21</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
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<td>7a</td>
<td>prophase</td>
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<td>14</td>
<td>...</td>
<td>21</td>
<td>16</td>
<td>19</td>
<td>19</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>8a</td>
<td>metaphase</td>
<td>14</td>
<td>15</td>
<td>...</td>
<td>19</td>
<td>17</td>
<td>17</td>
<td>18</td>
<td>...</td>
<td>...</td>
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<tr>
<td></td>
<td>9</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>68</td>
<td>32</td>
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<tr>
<td>T(1;3)</td>
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<td>14</td>
<td></td>
<td></td>
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<td>...</td>
</tr>
<tr>
<td></td>
<td>11a</td>
<td>prophase</td>
<td>14</td>
<td></td>
<td></td>
<td>17</td>
<td>19</td>
<td>19</td>
<td>17</td>
<td>14</td>
<td>...</td>
</tr>
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<td></td>
<td>12a</td>
<td>metaphase</td>
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<td>...</td>
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<tr>
<td></td>
<td>14</td>
<td>(meiotic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>59</td>
<td>41</td>
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<tr>
<td>Double heterozygote</td>
<td>15a</td>
<td>prophase</td>
<td>(14)*</td>
<td>18</td>
<td>15</td>
<td>18</td>
<td>16</td>
<td>17</td>
<td>15</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>16a</td>
<td>metaphase</td>
<td></td>
<td></td>
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<td>15</td>
<td>14</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>18a</td>
<td>metaphase</td>
<td></td>
<td></td>
<td></td>
<td>19</td>
<td>15</td>
<td>19</td>
<td>16</td>
<td>17</td>
<td>14</td>
</tr>
</tbody>
</table>

* By measurement of a normal chromosome one that would arise from a crossover between i2 and j2.

length chromosome (3') is shorter than it and is metacentric (Figure 10, 10a).

The breakpoint on the medium-length chromosome must have occurred proximal to the secondary constriction as the exchange piece included the latter. As a result, the translocated chromosome with the centromere of the shortest chromosome (1') carried the secondary constriction (Figures 10, 10a, 11, 11a). The pairing of homologous regions of translocated with normal chromosomes, as seen in mitotic cells from the limb buds, illustrated that this is a reciprocal translocation (Figures 12, 12a).

The meiotic chromosomes in the primary spermatocytes provided confirmatory evidence of the involvement of the shortest and medium-length chromosomes in the translocation (Figure 14). The pair with two chiasmata is the pair of longest chromosomes. The length of the ring bivalent in Figure 14 far exceeded half the total length of the four chromosomes involved in the translocation (Table 1).

In spermatogonial cells, the double heterozygote consists of two translocated chromosomes from T(1;2), two translocated chromosomes from T(1;3) and two normal chromosomes. Both chromosomes with the centromere of the normal shortest chromosome are translocated. Both are submetacentric. It was evident that the breakpoints on the shortest chromosome producing the two translocations must have occurred on either side of the centromere, for this pair is heteromorphic in both arms (Figures 15, 15a, 16, 16a, 18a). The heteromorphism in the other two pairs was the same as that found in T(1;2) with the longest and translocated longest and in T(1;3) with the medium-length and translocated medium-length...
chromosomes. Often the heteromorphism in the longest pair appeared to be present in both arms; this occurred in the double heterozygote (Figures 15, 15a, 16, 16a, 18a) as well as $T(1;2)$ alone (Figures 5, 5a, 6, 6a, 7, 7a). Figure 17 shows the involvement of all six chromosomes in an interchange complex in a primary spermatocyte in a double translocation heterozygote.

**DISCUSSION**

The cytological analyses of the two translocations $T(1;2)$ and $T(1;3)$ as well as the double heterozygote demonstrated that (1) the shortest chromosome, common to both translocations, is carrying the genes for linkage group one, (2) the medium-length chromosome involved in $T(1;3)$ and not in $T(1;2)$, is carrying the genes of linkage group three, and (3) the longest chromosome, involved in $T(1;2)$ and not in $(1;3)$, is carrying the genes of linkage group two.

As mentioned earlier, RAI (1963) arbitrarily numbered the chromosomes of *A. aegypti* from I to III in increasing order of their length with chromosome I being the smallest and III being the largest. However, with the demonstration that linkage group three corresponds to RAI's (1963) chromosome II (median length) and linkage group two corresponds to the longest chromosome or RAI's (1963) chromosome III, we propose that the chromosomes be renumbered as follows:

<table>
<thead>
<tr>
<th>Chromosome length</th>
<th>Old system (RAI 1963)</th>
<th>New system proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortest</td>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>Median</td>
<td>II</td>
<td>3</td>
</tr>
<tr>
<td>Longest</td>
<td>III</td>
<td>2</td>
</tr>
</tbody>
</table>

This proposed system would have the advantage that both the chromosomes and the linkage groups will have the same designations. The chromosome number designations appearing in the camera lucida drawings, in Table 1, and in the Results section are based on the new system.

During the course of cytological analysis of $T(1;2)$, it became apparent that the pair involving chromosome 2 and the translocated chromosome with the same centromere ($2'$) showed heteromorphism in both arms. The fact that the net difference in the length of these two chromosomes is nearly the same as the difference in the other two chromosomes involved in the translocation suggests that a pericentric inversion is associated with this translocation. Further genetic analysis with linkage group two markers and study of early prophase cytology is presently underway. For the present, it is assumed that the heteromorphism characteristic of the translocation alone is that seen in the pair including chromosome I.

Measurements of camera lucida drawings of prophase of normal, $T(1;2)$, $T(1;3)$ and double heterozygote cells (Table 1) indicate that chromosome 1 is 27%, chromosome 2, 39%, and chromosome 3, 35% of the total chromosomal length. The heteromorphism of $T(1;2)$ is eight percent and of $T(1;3)$ is six percent of the total chromosomal length. The length of the larger exchanged piece from $T(1;2)$ and from $T(1;3)$ can be estimated from the probable position of the breakpoints (Figure 15a, arrows). The larger exchange piece for $T(1;2)$ from
chromosome 2 is 15%, so the smaller exchange piece must be seven percent to give a net heteromorphism of eight percent of the total chromosomal length. The larger exchange piece of T(1;3) from chromosome 3 is seven percent, so the smaller exchange piece must be one percent to give a net heteromorphism of six percent of the total chromosomal length. Figure 19 shows the position of the breaks and associates the genetic location and break location where possible.

The present genetic lengths do not parallel the lengths of the chromosomes at early prophase. The most noticeable difference is with the first chromosome. Although this chromosome is 27% of the total chromosomal length, it represents 36% of the presently known genetic length. Either the other genetic linkage groups will be found to be longer (thus the dashed lines in Figure 19) or the exchange per unit length is more in the first chromosome. From Figure 19 it can be seen that the exchange per unit length of chromosome 1 is less in the area of the centromere (and therefore, the sex locus also) than in the distal regions of the arms. Differential exchange in the centromere regions versus the distal arms has been reported in other species (Mather 1939).

The knowledge of the location of the genes on the mitotic and meiotic chromosomes through the use of translocations may be valuable for gene alterations by

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**Figure 19.**—Correlation of lengths of chromosomes and of present linkage maps, including position of genes at the translocation breakpoints on the chromosomes. Dashed lines reflect uncertainty that all linkage map distances have been found. Distances are in percent of total linkage map distance or percent of total chromosomal length.
genetic surgery when these techniques have developed sufficiently. Alternative alleles for vectorial capacity or insecticide tolerance might thus be developed. NAKANISHI and MARINO (1964) have reported microbeam irradiation of particular areas of chromosomes from isolated grasshopper tissue.

This correlation between linkage groups and chromosomes is the first that has been done in any mosquito species with the total number of mosquito species and subspecies in the neighborhood of 2400. It is quite likely that most members of the genus Aedes, with some 700 species, are karyotypically similar, having a smaller pair and two larger pairs. Most species studied are similar. Another large genus, Culex, with some 500 species, similarly has a smaller pair and two larger pairs. As in Aedes, most species studied are similar. No heteromorphism in any pair has been reported in any Aedes or Culex species. It is probable therefore, that as in Aedes aegypti, the smaller pair is the sex-determining pair in those other mosquito species with similar karyotypes. This is contrary to the suggestion of MUKHERJEE, REES and VICKERY (1966) who, based on late replication of a part of the longest chromosome, suggested that it may be sex determining.

The translocation systems that are described here in determining the correlation of linkage groups with chromosomes have also been investigated for their potential for population control, and in preliminary experiments appear to be quite promising. In addition, a very interesting apparent enhancement of crossing over has been reported for the double heterozygote (McDONALD and RAI 1970).

SUMMARY

Two radiation-induced reciprocal translocations were used to determine the correlation between the three pairs of chromosomes and three linkage groups of the yellow fever mosquito, Aedes aegypti. Linkage group one, containing the sex locus, is the smallest chromosome and is designated chromosome 1. The two autosomes, carrying genes corresponding to linkage groups two and three are designated chromosome 2 and chromosome 3, respectively, with chromosome 2 being the longer autosome.—The available data indicate that recombination may not be uniform throughout the lengths of the chromosomes in this species.

LITERATURE CITED


