RADIATION-INDUCED INVERSIONS ON CHROMOSOME 3 OF *ANOPHELES ALBIMANUS*[1]

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ABSTRACT. Eight pericentric inversions on chromosome 3 of *Anopheles albimanus* were isolated and characterized. Two of these inversions are viable in homozygous condition and should prove useful in genetic crosses as crossover suppressors. An analysis of the inversion breakpoints, for the present inversions and others studied previously, showed that the regions near the free ends of chromosome 3 were more subject to breakage. A brief discussion is presented on the potential usefulness of inversions for the genetic control of *An. albimanus*.

In a previous report (Rabbani et al. 1977), 6 X-ray induced pericentric inversions were reported on chromosome 3 in *Anopheles albimanus* Wiedemann, the most important vector of human malaria over most of Central America. Studies on chromosomal aberrations have been continued in our laboratory with the intention to eventually use such aberrations in genetic control schemes (Curtis 1968, Robinson 1975).

In this present paper, we summarize the induction and characterization of 8 new, large, pericentric inversions, including 2 that are viable as homozygotes. Data from this study were combined with the earlier observations (Rabbani et al. 1977) for consideration of the properties of pericentric inversions in *An. albimanus*.

METHODS AND MATERIALS

Rearing procedures for larvae and adults followed those given by Rabbani and Seawright (1976). The stock used was homozygous for *reduced palmate (rp)*, a recessive trait on 3L (Seawright et al. 1979) and *stripe* (st^+), a dominant trait on 3R (Rabbani and Seawright 1976). Males, 0–24 hr old, were irradiated in a Faxitron X-ray machine at a dosage of 3180R (200 R/min) and were mated to virgin, untreated females. The F_1 males and females were crossed to a normal strain, SANTA TECLA, and the ensuing F_2 egg batches from individual females were checked for fertility. Those families exhibiting partial sterility of at least 30% were scored by cytological examination of the polytene chromosomes in salivary glands using the techniques and standard map of Keppler et al. (1973). Inversion stocks were established and maintained in heterozygous condition by crossing to an rp^+st stock with selection of the inversion type based on partial sterility and the st^+ marker for those inversions that included the st^+ locus.

Sterility percentages were estimated from at least 20 egg batches for each inversion. These percentages were corrected for the average sterility observed in control crosses of wild-type mosquitoes.

Each inversion was checked for viability as a homozygote by inbreeding heterozygotes, *In(3)rp/rp*$^+$. These matings were identified by checking egg hatchability, since a heterozygote X heterozygote will generally produce significantly fewer hatching eggs than heterozygote X wild. Fourth-stage larvae were scored for *rp* homozygotes, and polytene chromosomes of these *rp/rp* larvae were examined for confirmation of the viability of inversion

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homozygotes. The *rp* locus is ideally suited for this procedure because of its location adjacent to the centromere on 3L. For a fully viable homozygote, the ratio of wild:reduced palmate types will vary 3–5:1, respectively. The frequency of wild type will vary, depending on the rate of crossingover within the inverted region. Some of the aneuploid gametes produced by recombination in the heterozygotes will be complementary, thus producing viable zygotes, most of which will be marked with rp^+. One out of the six possible complementary zygotes will be *rp*.

RESULTS

Eight new pericentric inversions on chromosome 3 were established and studied. Table 1 contains a general description of these inversions in terms of fertility of heterozygotes, chromosomal breakpoints (according to the standard map of Keppler et al. 1973), length, and viability of homozygotes.

Inversion heterozygotes are partially sterile as a result of aneuploid gametes produced by crossingover within the inverted segment. Therefore, the amount of sterility is related to the frequency of crossingover, which is, in turn, inextricably related to the length of the inversion. The effect of double crossingover is important, because in longer pericentric inversions these events will more than likely occur. Two-strand double crossovers will produce viable gametes, but three-strand doubles should be twice as frequent and will result in 2 viable and 2 inviable gametes; four-strand doubles should occur as frequently as the two-strand type and will yield all inviable types. If single crossover events occur in all meiocytes, then there should be 50% sterility, and, providing that the 3 types of double crossovers occur in a 1:2:1 ratio there should be 50% sterility when double events occur in all meiocytes. Therefore, the theoretical expectation is that sterility will increase with inversion length up to 50% and level off at that point. Crossingover occurs in both sexes of *An. albimanus*; therefore, the fertility of heterozygotes was assayed for males and females. No differences were noted that could be attributed to sex so the sterility estimates in Table 1 were obtained by pooling the data for each inversion. The hatchability of eggs obtained from normal mosquitoes was 97.0%, and this value was used to correct the estimates of fertility of the inversion heterozygotes.

In polytene preparations, the length of chromosome 3 is 218 μm. The longest inversion, *In(3)17*, listed in Table 1 was 190 μm, and the shortest, *In(3)23*, was only 28 μm. For some of the inversions of similar length, e.g., *In(3)17* and *In(3)21*, there was a marked difference in the amount of partial sterility exhibited by the heterozygotes. Indeed, the sterility for *In(3)21*, which is 187 μm in length, was just slightly higher than the sterility

Table 1. Summary of characterization of eight pericentric inversions in *An. albimanus*.

Inversion	Breakpoint[a] 3R	Breakpoint[a] 3L	Length (μm)	Partial sterility (%)	Viability of homozygote
In(3)17	28A	45A	190	48.0	+
In(3)18	31A	41A	120	43.0	–
In(3)19	33B	45B	138	52.5	–
In(3)20	28B	37B	95	39.1	–
In(3)21	27B	44B	187	24.9	–
In(3)22	34B	42A	90	41.9	+
In(3)23	35A	37A	28	19.5	–
In(3)24	33A	38A	63	31.2	–

[a] According to the standard map of Keppler et al. (1973).

for *In(3)23*, which is only 28μm long. Generally, the amount of sterility observed increased with inversion length and leveled off around 40%. Only 2 of the inversions, *In(3)17* and *In(3)19*, produced around 50% inviable gametes, and there was a significant difference in inversion length, 190 compared to 138 μm, for those stocks. A summary of the relationship between sterility and inversion length is shown in Figure 1; for this plot data from Rabbani et al. (1977) were included. Inversions that covered >40% of chromosome 3 caused the most sterility in heterozygotes. As depicted in Figure 1, some of the long inversions caused less sterility. Perhaps this was due to the survival, at least until the 1st larval stage, of duplication-deficiency zygotes, since for long inversions the missing or duplicated segments of chromosome 3 would be smaller. No duplication-deficiency types were noted in preparations of salivary gland chromosomes, but the glands are always prepared from the 4th (or last) larval stage. The rearing procedures for this species, and most other anophelines, are not adequate or consistent enough for a critical analysis of overall survival during the immature life stages. Therefore, it was impossible to check the mortality of duplication-deficiency types that might have hatched and died during subsequent development.

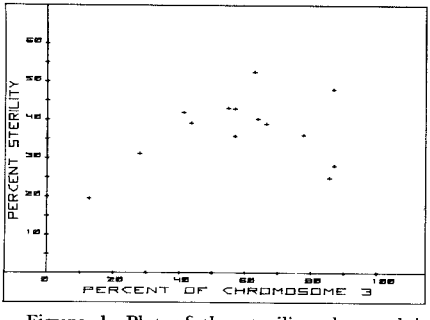

Figure 1. Plot of the sterility observed in heterozygotes of 14 inversion stocks and inversion lengths expressed as a percentage of chromosome 3.

In the analysis of the inversion breakpoints, we included the data presented earlier by Rabbani and Kitzmiller (1974) and Rabbani et al. (1977) for pericentric inversions. In Table 2, the right and left arms of chromosome 3 are divided into more or less equal regions and the number of breaks is shown for each of these segments. The majority of the breaks were in the regions at or near the free ends of each arm, regions 26–27 and 44–45. Breaks in the other combined regions were more or less equally distributed, but no breaks were recorded for region 30 on 3R and 36 and 40 on 3L. Region 36 is adjacent to the centromere, which would have made breaks in this segment difficult to study.

When all 8 of the inversions were tested for viability as homozygotes, *In(3)17* and *In(3)22* (Fig. 2) were viable; a detailed description of these stocks will appear elsewhere (Suguna et al. 1980). Amongst the progeny from crossing *In(3)17* heterozygotes (*In(3)17 rp/rp*$^+$), there were 228 *rp*$^+$ and 56 *rp*, a 4:1 ratio.*In(3)22* was inadvertently marked with *rp*$^+$; therefore, the viability of the homozygote was checked cytologically. For the other 6 inversions, no homozygotes were detected using *rp* or cytological examinations, an

Table 2. Distribution of breakpoints of pericentric inversion on chromosome 3 in *An. albimanus*. The nearly equal segments are numbered according to Keppler et al. (1973).

Regions of chromosome	No. of breaks	Percent of breaks
	Right arm	
26–27	8	22.2
28–29	3	8.3
30–31	1	2.8
32–33	3	8.3
34–35	3	8.3
	Left arm	
36–37	2	5.5
38–39	3	8.3
40–41	2	5.5
42–43	3	8.3
44–45	8	22.2

indication of the presence of recessive lethal factors within the inverted section.

DISCUSSION

Burnham (1962) summarized the value of inversions used as crossover suppressors for a variety of purposes ranging from the synthesis of special stocks to the use of naturally occurring inversions in evolutionary studies. Two of the most widely used attributes of crossover suppression are the assignment of mutants to positions on their respective chromosomes and the availability of an efficient means of exposing concealed variability. *In(3)22* was used by Seawright et al. (1979) in assigning the recessive mutant, *rp*, to the left arm of chromosome 3 by measuring the crossover suppression between *rp* and *st* and relating the linkage data to the inversion breakpoints. The *In(3)17* homozygote is marked with *rp* and st^+ and thus should prove useful for screening chromosomes marked with rp^+ and *st* for recessive concealed variability. Since *In(3)17* covers about 82% of chromosome 3, this strain should also prove valuable in the study of the inheritance of insecticide resistance, because, by taking advantage of the crossover suppression, chromosome 3 from natural populations can be studied more or less intact. The original genes and their order in the inverted segment will, for the most part, remain intact, which will enable one to better define the ancillary genes that usually confuse the analysis of resistance mechanisms. Using the inversion for suppressing crossingover will permit making most of chromosome 3 homozygous for that part of the genome as it occurs naturally.

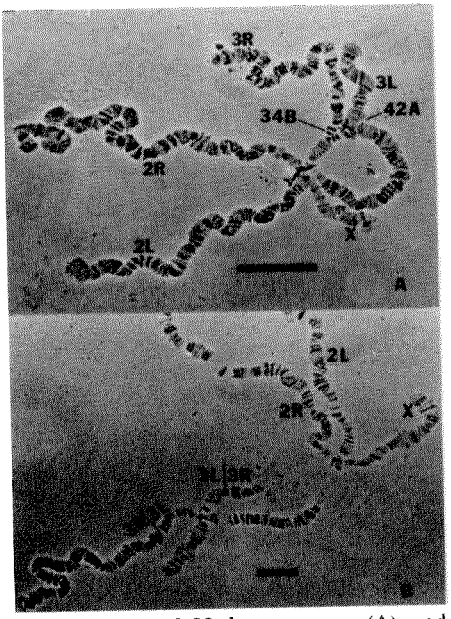

Figure 2. *In(3)22* heterozygote (A) and homozygote (B). The heavy dark line represents 20 μm.

Robinson (1975) suggested using long, pericentric inversions in genetic control programs for insect species in which crossingover occurs in both sexes. In other words, inversion homozygotes could be released into natural populations in sufficient numbers to cause a substantial genetic lethal load and at the same time the inversion should displace the native chromosome type. This occurs because in the heterozygotes the normal and inversion chromosomes are eliminated at the same rate via the production of duplication-deficiency gametes; therefore, if there is a numerical superiority of the inversion type and if the fitness of the inversion homozygote is sufficient, the normal type will be progressively eliminated from the mixed population. The speed of the displacement process would depend on the frequency of the inversion type and its fitness relative to the fitness of the heterozygote and normal types.

Calculation of an equilibrium frequency for a given inversion can be achieved by using the approach employed by Whitten (1971) for reciprocal translocations. From theoretical considerations and the data shown in Table 1, the magnitude of the genetic load in an

individual heterozygous for 3 pericentric inversions is limited to around 87.5% sterility. This projection is based on the use of inversions such as *In(3)17* and *In(3)19*, that show a sterility of around 50%. However, the sterility is dependent on crossingover, which is in turn partly dependent on length of inversion; one wonders if pericentric inversions on the short X chromosome in *An. albimanus* will cause much sterility. Also, all evidence (holandric male-linked translocations, Kaiser et al. 1978) until now indicates that crossingover does not occur between the X and Y; thus, sterility in males due to inversions may be limited to 75%. In a separate paper, Suguna et al. (1980) report that when the *In(3)17* inversion was combined with the *T(Y;3R)1* translocation, the resultant males were 77% sterile. Thus, the addition of a heterozygous inversion on chromosome 2 could cause a sterility of around the 87.5% expected from theoretical considerations. It is quite possible to produce males for release that would be heterozygous for 3 inversions and a male linked translocation by simply crossing females homozygous for the inversions to males of a male-linked translocation strain. To avoid releasing large numbers of females (and for efficiency in the mass production), one could employ a genetic sexing system such as the one reported by Kaiser et al. (1979) to facilitate the preferential killing of females during the egg stage. In matings with native females, the released males would pass the translocation to their sons and 2 of the inversions to their daughters; the other inversion would segregate randomly to both sexes. The genetic load caused by the released males should be high enough to effect a significant degree of population control, and at the same time a competitive displacement of the normal karyotype by the inversions should begin. Of course, initiation of such a scheme requires 2 homozygous pericentric inversions on the X and 2; therefore, a great deal of work remains to be done before a control scheme that combines inversions and a male-linked translocation can be attempted. Nevertheless, the potential usefulness of such a scheme is encouraging.

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