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Homozygous translocations in *Anopheles albimanus*

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Summary. Four homozygous, autosomal translocation stocks were established in *Anopheles albimanus,* which is an important vector of malaria in Central America. When inbred, the fertility of these homozygous translocation stocks ranged from 87.4 to 92.5%, and similar fertilities were observed in outcrosses to a normal strain. The chromosomal breakpoints for these four translocations were located close to the centromeres. The sterility in heterozygous translocation males was consistently higher than that observed for females.

Key words: Homozygous translocations – *Anopheles albimanus* – Malaria vector

Introduction

The theoretical basis for the use of homozygous translocations for the control or replacement of insect pest populations was adequately discussed by Curtis (1968 a, b) and Robinson (1976). Matings between translocation homozygotes and normals yield hybrids with reduced fertility, which serves as the mechanism for the replacement of one chromosome type by the other in a mixed population. The efficiency of the replacement of a normal type by a translocation homozygote largely depends on the viability and competitiveness of the homozygote and the sterility of the hybrid. It is absolutely essential that the fitness of translocation homozygote exceeds that of the hybrid. Since the sterility of translocation heterozygotes increases with each additional translocation, chromosome number can be important in regard to the speed of population replacement (Whitten 1971). Except for *Chagasia* sp. (n = 4) (Kreutzer 1978), mosquitoes have a haploid set of 3 chromosomes, hence, there is the limitation of only one independent translocation. This curtails the amount of genetic lethal load that can be caused by this type of chromosome aberration. Therefore, it seems that the most reasonable method for using translocations as a tool for the control of these pests is for the replacement of the noxious native form with a benign translocation type (Curtis 1968 a).

The majority of translocations which have been tested in pest species of mosquitoes have not been viable when homozygous. However, homozygous translocations have been developed in *Aedes aegypti* (Lorimer et al. 1972; Rai et al. 1974); *Culex tarsalis* (McDonald et al. 1978); and *Anopheles culicifacies* (Sakai et al. 1979; Baker et al. 1980). The autosomal translocation, T-1 in *An. culicifacies,* has a fertility that approaches the normal type (Sakai et al. 1979). Replacement of a small, natural population of *Ae. aegypti* was attempted in Kenya, but unfortunately, the homozygotes of a sex-linked translocation were deficient in several parameters of fitness when compared with native mosquitoes (Lorimer et al. 1976).

Anopheles albimanus Wiedemann, a neotropical species in the subgenus Nyssorhynchus, is the most important vector of human malaria over most of Central America. Over part of its range the control of this species, and hence the suppression of malaria, is difficult because of the marked degree of resistance to insecticides. Our current research is aimed at the development of a genetic control system as an alternative or supplemental method for the suppression of natural populations of *An. albimanus.* Currently, twenty-five loci (mutant markers and enzymes) have been studied and assigned to chromosomes; a partial list is given in Narang et al. 1981. An accurate map of the polytene chromosomes in salivary glands was published by Kep-

pler et al. 1973. Sex determination in this species is an X-Y system, and the male is the heteromorphic sex (Keppler et al. 1973; Seawright et al. 1982). Crossing over occurs on the autosomes of both sexes (Kaiser et al. 1979).

In a recent report we described the use of X-rays for the induction of chromosome aberrations, of which 8 were whole-arm, autosomal translocations (Kaiser et al. 1982). Homozygous stocks have been established for 4 of these wholearm translocations, and these stocks are described herein.

Materials and methods

Except for minor, insignificant modifications, established procedures (Benedict et al. 1979) were employed for the rearing and maintenance of mosquitoes.

The eight translocations, which were tested for their homoviability, are listed in Table 1. As shown in this table, all of the chromosomal breakpoints were close to the centromeres of the autosomes. Each translocation was marked with *stripe* (st^+), a dominant allele at a locus located close to the centromere on the right arm of chromosome 3 (Kaiser et al. 1981). By using this conveniently located marker, the heterozygous translocations were maintained by observing the sterility of those mosquitoes that had the phenotype of stripe. In the previous report on these autosomal translocations, we reported that four of the translocations were combined to make two double-heterozygous strains, *T(2R; 3R)8/T(2L; 3R)2* and *T(2R; 3R)7/T(2R; 3L)3*, for use in a "capture" scheme for the synthesis of compound chromosomes (Holm 1976). These double-heterozygote, translocation stocks were maintained by measuring the fertility of egg batches from individual females in each generation. This procedure also served as a preliminary test for the homoviability of the translocations. Since the fertility of a double-heterozygote stock should be low (< 40%), the presence of egg batches with higher fertility was taken as an indication of the possibility of homozygous translocation types. When egg batches with high fertility were encountered, the families were reared, and the salivary gland chromosomes of several 4th stage larvae were examined (Kaiser et al. 1982) in order to confirm whether the high fertility was indeed due to the homoviability of one of the two translocations. The families containing homozygotes were combined, and in the next generation, matings between homozygotes were identified by a fertility > 80%; the chromosomes of larvae of these families were examined for confirmation of the homozygous translocation. This procedure was continued in subsequent generations until pure homozygous stocks were obtained.

Since the breakpoint on chromosome 3 in each translocation was close to the *stripe* locus (i.e., little or no crossing over), the other four wohle-arm translocation strains (listed in Table 1) were tested by inbreeding, recording sterility, and discarding the recessive (*st/st*) individuals. Homoviability was detected by an increase in fertility as follows: the fertility of matings between heterozygotes should be low (< 40%); heterozygous to homozygous matings should be approximately 45–55% fertile (depending on the sex of the heterozygote); homozygous matings should produce little or no sterility. On that basis, heterozygotes were crossed, and their progeny were inbred by means of sib-matings. High-hatch families were identified in the F_2, and the salivary gland chromosomes of larvae in F_2 families were examined for the presence of the homozygous translocation.

The use of high fertility as a criterion for the identification of homozygous translocations should necessarily exclude any homozygous type that had low viability. Therefore, the *T(2R; 3L)3* stock was tested by another method because of the need in our research program for a homozygous stock, even one with low viability, for this particular type of translocation. Recombinants, which were heterozygous for *T(2R; 3L)3* and marked with the recessive trait, *non-stripe,* were outcrossed to *stripe*. The F_1 individuals were inbred, and F_2 families were observed for sterility and phenotype. In using this scheme, most of the homozygotes for the translocation are also expected to be homozygous for the recessive trait, *st*.

Table 1. List of autosomal, reciprocal translocations, in *An. albimanus* that were tested for viability when homozygous

Stock	Chromosomal breakpoints
T(2R; 3R)5	14A, 34B
T(2R; 3R)6	15A, 33B
T(2R; 3R)7	14B, 34B
T(2R; 3R)8	14E, 34A
T(2R; 3L)3	14B, 38B
T(2L; 3R)2	18C, 34A
T(2L; 3R)3	19B, 35A
T(2L; 3R)4	18A, 34A

Results

Of the four translocations that were used in the double-heterozygote stocks, homozygotes of *T(2R; 3R)7*, *T(2R; 3R)8* and *T(2R; 3R)2* were identified, and the families containing homozygotes for each translocation were combined and inbred. As noted earlier, matings between homozygotes were identified by high (> 80%) fertility, and homozygous stocks of *T(2R; 3R)7* (Fig. 1a) and *T(2R; 3R)8* (Fig. 1c) were established after two generations beyond the initial detection. The *T(2L; 3R)2* stock (Fig. 1e) was pure after four generations of inbreeding. *T(2L; 3R)2* is also homozygous for a paracentric inversion that appears to share one chromosomal breakpoint (at 34A) in common with the translocation on 3R.

Of the other four translocations which were tested, only *T(2R; 3R)6* was viable as a homozygote. Homozygous larvae were noted in the 2nd generation, and a pure stock was established after three generations. In the retesting of *T(2R; 3L)3*, homozygotes were detected and confirmed cytologically, but unfortunately the homozygous type invariably died during the pupal stage.

As shown in Table 2, the four homozygous stocks have high fertility when inbred or outcrossed to a normal type. A marked difference was observed in comparing the fertilities of heterozygous males and females. Invariably, the males were less fertile, and the difference was greatest for the *T(2R; 3R)8* translocation.

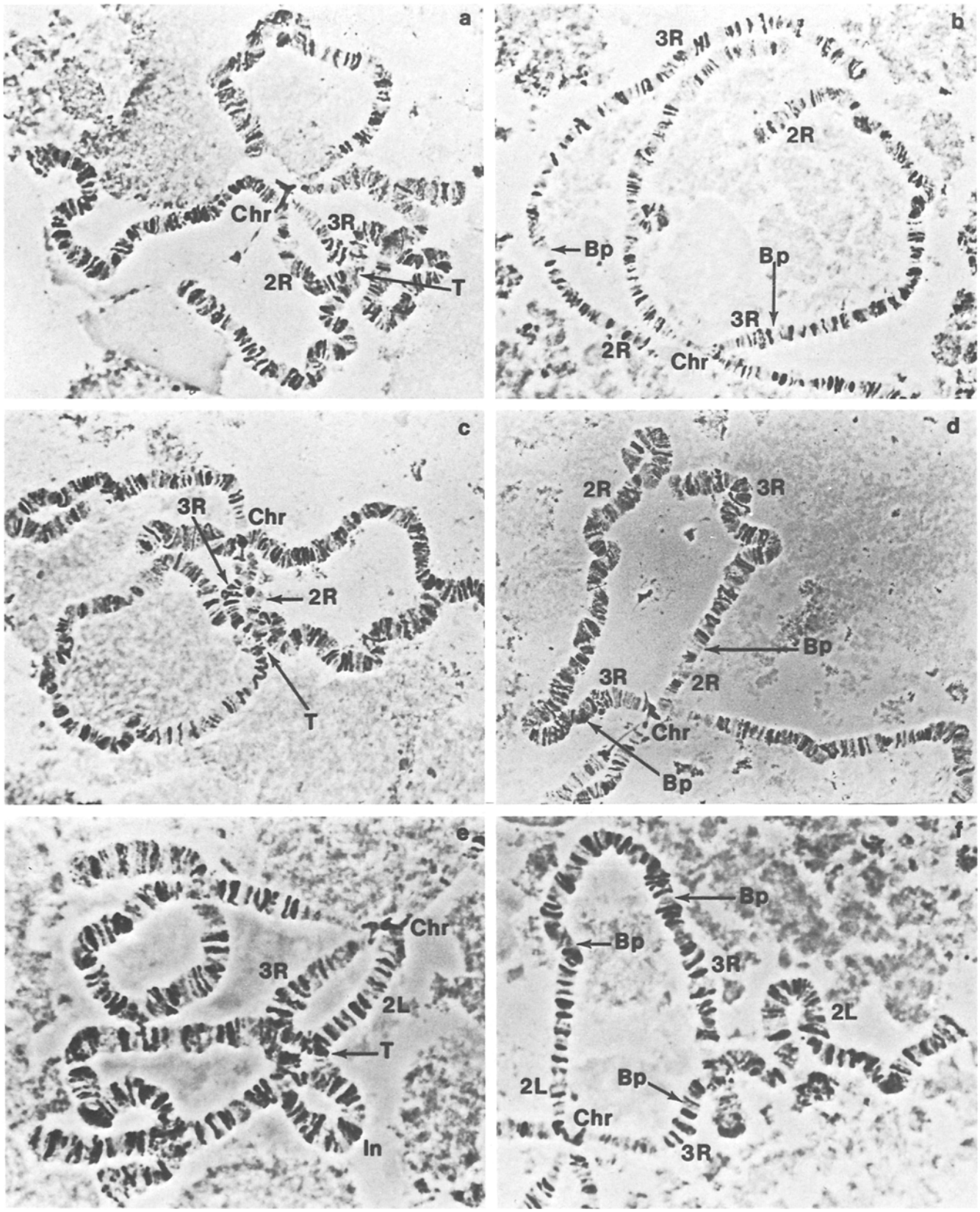

Fig. 1 a – f. Salivary gland chromosomes of reciprocal translocations in *Anopheles albimanus:* **a** *T(2R; 3R)7* heterozygote; **b** *T(2R; 3R)7* homozygote; **c** *T(2R; 3R)8* heterozygote; **d** *T(2R; 3R)8* homozygote; **e** *T(2L; 3R)2* heterozygote; **f** *T(2L; 3R)2* homozygote. Chr – chromocenter, Bp – breakpoint, In – inversion, T – translocation

Table 2. Observations on sterility in four strains of *An. albimanus* that are both homozygous (HT) or heterozygous (T) for reciprocal translocations

Stock	No. of families observed	No. of eggs observed	No. of eggs hatched	Fertility %	Chromosomal breakpoints
HT *(2R; 3R)7*	28	2800	2590	92.5 ± 1.4	14B, 34B
HT ♀ × N ♂	14	1400	1276	91.1 ± 3.2	
N ♀ × HT ♂	14	1400	1286	91.9 ± 3.1	
T ♀ × N ♂	39	3900	2363	60.6 ± 2.1	
N ♀ × T ♂	39	3900	1860	47.7 ± 1.6	
HT *(2R; 3R)8*	49	4900	4454	90.9 ± 1.1	14E, 34A
HT ♀ × N ♂	40	4000	3772	94.3 ± 0.8	
N ♀ × HT ♂	31	3100	3040	98.1 ± 0.4	
T ♀ × N ♂	28	2800	1706	60.9 ± 2.6	
N ♀ × T ♂	21	2100	898	42.8 ± 2.0	
HT *(2L; 3R)2*	44	4400	3844	87.4 ± 1.3	18C, 34B
HT ♀ × N ♂	19	1900	1656	87.2 ± 2.5	
N ♀ × HT ♂	23	2300	2178	94.7 ± 1.6	
T ♀ × N ♂	27	2700	1744	64.6 ± 2.3	
N ♀ × T ♂	24	2400	1250	52.1 ± 2.1	
HT *(2R; 3R)6*	9	450	360	80.0 ± 3.78	15A, 33B
HT ♀ × N ♂	34	1700	1473	86.6 ± 2.32	
N ♀ × HT ♂	29	1450	1279	88.2 ± 2.85	
T ♀ × N ♂	31	1550	1044	67.4 ± 2.43	
N ♀ × T ♂	38	1900	1034	54.4 ± 1.97	
N ♀ × N ♂	35	3500	3342	95.5 ± 1.6	

Discussion

As mentioned earlier, homozygous translocations have been suggested as a mechanism for modifying the noxious nature of a pest species of mosquito. In the case of *An. albimanus,* the critical factor is the elimination or curtailment of the transmission of malaria. Although a genetic mechanism, e.g. refractoriness to malaria transmission or some trait that modifies behavior, is not available at this time, the synthesis of homozygous translocations which can serve as a transport mechanism is an encouraging step toward the assembly of a genetic control system.

As noted by Curtis (1968 a), it will be necessary to control genetic recombination, through the use of inversions, for a genetic replacement of the noxious traits, otherwise these genes would become part of the gene pool of the translocation type. This requirement of breaking a chromosome several times to produce the proper translocation-inversion complex has been a matter of some concern, because most chromosome damage of this sort tends to behave a recessive lethal. The *T(2L; 3R)2* stock also has a rather large paracentric inversion as a part of a chromosomal aberration complex; thus, it is possible to make such a rearrangement of the chromosomes without having an adverse affect on the fertility and viability of a laboratory stock. It seems reasonable that selective breeding can be employed to adapt fully-fertile laboratory stocks bearing aberration complexes for survival under the more rigorous constraints of a natural setting.

The fact that heterozygous males are less fertile than females is interesting, but not completely explainable. Kaiser et al. (1982) reported similar differences for seven additional whole-arm translocations in *An. albimanus,* and Sakai et al. (1979) noted this phenomenon for a whole-arem translocation in *An. culicifacies.* In discussing similar observations on translocations in *Drosophila,* Roberts (1976) postulated a decrease in adjacent II segregation because of the formation of chiasmata in females as a possible explanation. This is not a plausible reason for *An. albimanus,* because crossing over occurs for the autosomes of both sexes. However, there is definitely some fundamental difference controlling the segregation of the chromosomes during the meiotic process in males and females of *An. albimanus.* Dennhofer (1975) suggested that differences in the amount of sterility caused by translocations in mosquitoes may be due to simple Mendelian factors, but this hypothesis cannot be tested fully at this time for *An. albimanus* because of an insufficient knowledge of the genetics of this species. We did try to select for high fertility of heterozygotes with whole-arm translocations through five generations without success.

The high fertility of females, which are heterozygous for a translocation could have an adverse impact on the

speed of the replacement of a normal type by the aberration type, because the "driving" mechanism for the replacement process is the sterility of the heterozygote (Whitten 1971). In effect, there is an inverse relationship, i.e. as the fertility of the heterozygote decreases, the speed of replacement of the numerically deficient type increases.

References

Baker RH, Sakai RK, Perveen A, Raana K (1980) Isolation of a homozygous translocation in *Anopheles culicifacies.* J Hered 71:25–28

Benedict MQ, Seawright JA, Anthony DW, Avery SW (1979) Ebony, a semidominant lethal mutant in the mosquito, *Anopheles albimanus.* Can J Genet Cytol 21:193–200

Curtis CF (1968 a) Possible use of translocations to fix desirable genes in insect pest populations. Nature 218:368–369

Curtis CF (1968 b) A possible genetic method for the control of insect pests with special reference to tsetse flies (*Glossina* spp.). Bull Entomol Res 57:509–523

Dennhofer L (1975) Inherited preferential segregation in translocation heterozygotes of the mosquito, *Culex pipiens* L. Theor Appl Genet 45:250–253

Holm DG (1976) Compound autosomes. In: Ashburner M, Novitshki E (eds) The genetics and biology of *Drosophila,* vol 1 b. Academic Press, London

Kaiser PE, Seawright JA, Joslyn DJ (1979) Cytology of a genetic sexing system in *Anopheles albimanus.* Can J Genet Cytol 21:201–211

Kaiser PE, Benedict MQ, Seawright JA (1981) A *white thorax* mutant and its relationship in an allelic series in *Anopheles albimanus* Wiedemann. Mosq News 41:455–458

Kaiser PE, Seawright JA, Benedict MQ, Suguna SG (1982) Radiation induced reciprocal translocations and inversions in *Anopheles albimanus.* Can J Genet Cytol (in press)

Keppler WJ, Kitzmiller JB, Rabbani MG (1973) The salivary gland chromosomes of *Anopheles albimanus.* Mosq News 33:42–52

Kreutzer RD (1978) A mosquito with right chromosomes: *Chagasia bathana* Dyar. Mosq News 38:554–558

Lorimer N, Hallinan E, Rai KS (1972) Translocation homozygotes in the yellow fever mosquito, *Aedes aegypti.* J Hered 63:158–166

Lorimer N, Lounibos LP, Petersen JL (1976) Field trials with a translocation homozygote in *Aedes aegypti* for population replacement. J Econ Entomol 69:405–409

McDonald PT, Asman SM, Terwedow Jr HA (1978) An alternative method for isolating homozygotes of autosomal translocations in the mosquito *Culex tarsalis.* Can J Genet Cytol 20:581–588

Narang S, Seawright JA, Kitzmiller JB (1981) Linkage relationships and assignment of esterase-4 and esterase-8 loci to chromosome 3 in *Anopheles albimanus.* J Hered 72:157–160

Rai KS, Lorimer N, Hallinan E (1974) The current status of genetic methods for controlling *Aedes aegypti.* In: Pal R, Whitten M (eds) The use of genetics in insect control. Elsevier/North Holland, Amsterdam, pp 119–132

Roberts Paul A (1976) The genetics of chromosome aberrations. In: Ashburner M, Novitshki E (eds) The genetics and biology of *Drosophila,* vol 1 a. Academic Press, New York, pp 68–184

Robinson AS (1976) Progress in the use of chromosomal translocations for the control of insect pests. Biol Rev 51:1–24

Sakai RK, Baker RH, Raana K, Saifuddin UT (1979) Isolation of a viable homozygous translocation strain in *Anopheles culicifacies.* Mosq News 39:631–633

Seawright JA, Benedict MQ, Narang S, Kaisser PE (1982) *White eye* and *curled,* recessive mutants on the X chromosome of *Anopheles albimanus.* Can J Genet Cytol (in press)

Whitten MJ (1971) Insect control by genetic manipulation of natural populations. Science 171:682–684