USE OF DEFICIENCIES FOR MAPPING FOUR MUTANT LOCI ON THE SALIVARY GLAND CHROMOSOMES OF *ANOPHELES ALBIMANUS*

J. A. SEAWRIGHT, M. Q. BENEDICT AND S. NARANG

Insects Affecting Man and Animals Laboratory, Agricultural Research Service, USDA, P. O. Box 14565, Gainesville, FL 32604

ABSTRACT. Deficiencies, induced by radiation, were use to map four mutant loci on the salivary gland chromosomes of *Anopheles albimanus*. The loci for *red eye* and *ebony* were determined to be in Regions 10C and 20A, respectively, on Chromosome 2. *Reduced palmate* and *stripe* were assigned to 30B and 33B, respectively, on Chromosome 3. During the screening for deficiency types, a total of 272,233 F_1 progeny, from irradiated males, were examined, and 147 larvae had the genotype indicating a deficiency. Upon examination of the salivary gland chromosomes, visible deficiencies were recorded for 38 larvae.

INTRODUCTION

Anopheles albimanus Wiedemann is an important vector of human malaria in Central and the northern portion of South America. Until recently, there was a general lack of detailed knowledge about the genetics and cytogenetics of this species of anopheline. Cytogenetic studies were initiated by Frizzi and Ricciardi (1955), and Hobbs (1962) described the salivary gland chromosomes and a mitotic karyotype of 2 autosomes and a pair of heteromorphic sex chromosomes. Keppler et al. (1973) produced a detailed, and for the most part accurate, map of the polytene chromosomes of the salivary glands. This map was used subsequently in studies on the induction of translocations and inversions (Rabbani and Kitzmiller 1972, 1974; Kaiser et al. 1979).

Since about 1975, we have been engaged in studies on the establishment of a genetic map for *An. albimanus*. During this time, 28 mutant or isozyme loci have been assigned to their respective linkage groups. Although some of this information remains unpublished, most of it was summarized by Seawright et al. (1981a) or Narang and Seawright (1982).

The primary reason for conducting research on the genetics of *An. albimanus* is to develop new control methodologies based on genetic mechanisms, e.g., chromosomal aberrations (Curtis 1968, Foster et al. 1972), or hybrid sterility or dysgenesis. During the process of making specific types of chromosome aberrations or conducting other genetical studies, it is necessary to employ mutant markers in order to make the cross schemes manageable, simple and efficient. Therefore, it is highly desirable to combine the genetic (e.g., mapping of loci) and the cytogenetic (e.g., polytene chromosome map) information into a more usable format. This was accomplished years ago in research on *Drosophila melanogaster* (see summary by Roberts 1976) by inducing deficiencies (deletions) which could be observed on the salivary gland chromosomes. In this present paper, we have summarized our progress in determining the location of the loci for four mutants, viz. *red eye* (Seawright et al. 1982), *ebony* (Benedict et al. 1979), *stripe* (Rabbani and Seawright 1976), and *reduced palmate* (Seawright et al. 1979).

METHODS AND MATERIALS

Established procedures were employed for rearing and handling the mosquitoes (Rabbani and Seawright 1976, Benedict et al. 1979). Preparations of salivary gland chromosomes were made by the method of Kaiser et al. (1982).

Crossing-over occurs on the autosomes of both sexes of *An. albimanus*, but Y-linked translocations are holandric (Kaiser et al. 1979). These two attributes were used in determining the general location, within 3 or 4 regions on an autosome, for the loci of the four mutants. Crosses were made with translocations and the mutants, and the pseudolinkage data were used to estimate the degree of linkage between the translocation breakpoint and the mutant locus on the autosome. These crosses are listed in Table 1.

After determining the general location of a mutant locus, deficiencies were employed to ascertain the exact region containing the mutant gene. The cross scheme employed for the induction and detection of deficiencies was simple and straightforward. Wild type males, which were <48 hr old, were irradiated with 1500 R with either an X-ray machine (500 R/min; KVP = 90) or a ^{137}Cs source (1912 R/min). These males were crossed to virgin females which were homozygous for the recessive markers *re rp st* or to females heterozygous for *eb*. The F_1 progeny were examined and those larvae with the mutant phenotypes were held overnight in an incubator which was set at 22°C. (The cool

Table 1. Summary of crosses showing pseudolinkage of mutants of *Anopheles albimanus* to the breakpoints of translocations.

CROSS Female × Male	Translocation breakpoint	Number of families	Phenotype of progeny		Recombinant frequency
			Parental	Recombinant	
(1) *st* × F_1(*st* × *T(Y;3R)3 st*$^+$)	34A	13	583	6	0.010
(2) *rp* × F_1(*rp* × *T(Y;3R)2 rp*$^+$)	31A	—[a]	514	17	0.032
(3) *rp* × F_1(*rp* × *T(Y;3R)3 rp*$^+$)	34A	13	537	28	0.088
(4) *eb*$^+$ × F_1(*eb* × *T(Y;2R)3 eb*$^+$)	15A	17	849	108	0.219
(5) *eb*$^+$ *st* × F_1(*eb st* × *T(2L;3R)2 eb*$^+$ *st*$^+$)	18C[b]	14	988	28	0.027
(6) F_1(*eb st* × *T(2L;3R)2 eb*$^+$ st$^+$) × *eb*$^+$ *st*	18C[b]	10	1275	25	0.020

[a] Mass collection of eggs from 15 females.
[b] Breakpoint on 3R at 34A for translocation and breaks at 31A and 34A for paracentric inversion.

temperature enhances the preparation of readable polytene chromosomes). The salivary gland chromosomes of each larva were examined for the presence of visible deletions or other aberrations, e.g., inversions and translocations.

RESULTS

Keppler et al. (1973) published a schematic map of the salivary gland chromosomes in *An. albimanus,* and reference to this map should be very helpful in understanding the presentation of these results.

A summary of the results of the screening for deficiencies is given in Table 2. Most of the mosquitoes with a deficiency phenotype had no visible aberration on the chromosomal arms where the mutants were known to be located, but there were enough deletions identified for each mutant so that the locations of the four loci were ascertained. The frequency of presumed deletions was 0.00073 for *red eye, reduced palmate* and *nonstripe.* In the case of *ebony,* for which the homozygous state is lethal during the 4th larval stage, we had to cross irradiated males to heterozygotes; therefore the recorded frequency of 0.00032 should be doubled (=0.00064) because half of the deletions that possibly could occur would not be measured. For three of the mutants, we also detected either translocations or inversions that had chromosomal breaks in generally the same regions as those involved in the deficiencies (Table 2).

A list of the deficiencies with the corresponding map positions are given in Table 3. From those data, the location of the locus of each mutant was readily deduced. In the following, we have assembled a brief description of each mutant, the general location of each mutant, and the exact region where each mutant is located as determined from the deficiencies.

Stripe (*st*$^+$) is a variant characterized by the presence of a wide, dorsal, white stripe on the thorax and abdomen of larvae and on the cephalothorax and abdomen of pupae (Rabbani and Seawright 1976). *Stripe* occurs with a high (>50%) frequency in some field populations, and is the dominant gene of an allelic series composed of *stripe, white thorax (stW)* and nonstripe (*st*) (Kaiser et al. 1981).

Rabbani and Seawright (1976) reported a short distance of 3 map units between *st*$^+$ and *T(Y;3R)1* (breakpoint at 31B) on chromosome 3; however, the relative location of *stripe,* proximally or distally, to 31B was not known. A cross was performed with *T(Y;3R)3* (breakpoint at 34A), and a value of 1.02 map units was obtained for the distance between *st*$^+$ and 34A (Cross 1, Table 1). Therefore, we assumed that *st*$^+$ was between 31B and 34A. Supportive evidence of this was derived from an autosomal

Table 2. Summary of the number of deficiencies (Df) reciprocal translocations (T), and inversions (In) observed amongst the progeny of irradiated males of *Anopheles albimanus.*

Mutant marker	No. F_1 progeny screened	No. with Df phenotype	No. Df confirmed cytologically	No. other aberrations
stripe	78,446	47	11	4 In; 2T
reduced palmate	32,731	33	10	0
red eye	33,889	26	8	2T
ebony	127,167	41	9	2In

Table 3. List of deficiencies, induced with radiation and confirmed cytologically, in *Anopheles albimanus*.

Mutants and region(s) deleted			
stripe	*reduced palmate*	*red eye*	*ebony*
Df(3R)st-1; 33A–34A	*Df(3R)rp-1;* 30B	*Df(2R)re-1;* 10C	*Df(2L)eb-1;* 20A
Df(3R)st-2; 33A–34A	*Df(3R)rp-2;* 30A–30B	*Df(2R)re-2;* 10B–10C	*Df(2L)eb-2;* 20A–21B
Df(3R)st-3; 33A	*Df(3R)rp-3;* 30B	*Df(2R)re-3;* 10B–10C	*Df(2L)eb-3;* 20A–20B
Df(3R)st-4; 32C–33B	*Df(3R)rp-4;* 30A–30B	*Df(2R)re-4;* 10C	*Df(2L)eb-4;* 20A–20B
Df(3R)st-5; 33A–33B	*Df(3R)rp-5;* 30B	*Df(2R)re-5;* 10C	*Df(2L)eb-5;* 20A
Df(3R)st-6; 32C–33B	*Df(3R)rp-6;* 30A–30B	*Df(2R)re-6;* 10C	*Df(2L)eb-6;* 20A
Df(3R)st-7; 32C–33B	*Df(3R)rp-7;* 30B	*Df(2R)re-7;* 10B–10C	*Df(2L)eb-7;* 20A–21B
Df(3R)st-8; 33A–33C	*Df(3R)rp-8;* 30B–31A	*Df(2R)re-8;* 10C	*Df(2L)eb-8;* 20A–21B
Df(3R)st-9; 33A–33B	*Df(3R)rp-9;* 29B–30B		
Df(3R)st-10; 33A–33B	*Df(3R)rp-10;* 30A–31A		
Df(3R)st-11; 33A–35A			

translocation, *T(2L;3R)2* a stock that also had a induced inversion covering Regions 31A to 34A. There was no crossing over between *stripe* and this inversion-translocation complex (Kaiser et al. 1982). Eleven deficiencies (Table 3) that included the *stripe* locus were observed, and this mutant was assigned to the edge of Region 33B. One of the larger deletions, *Df(3R)st-1;* 33A–34A is shown in Fig. 1C.

Reduced palmate is a temperature-sensitive, recessive trait that affects the size and shape of the palmate hairs of the 4th stage larva (Seawright et al. 1979). Crosses 2 and 3 in Table 1 were used to assign *rp* to the middle of 3R. Map distances of 8.83 and 3.20 were recorded for the linkage between *rp* and the translocation breakpoints at 34A and 31A, respectively. Ten deficiencies (Table 3) were observed for *rp,* and its location was determined to be in Region 30B.

Ebony (eb) is a semi-dominant, melanotic mutant that is lethal when homozygous (Benedict et al. 1979). A loose linkage of 21.9 map units was measured between *eb* and *T(Y;2R)3* (Cross 4, Table 1), and this coupled with a map distance of 45 units between *eb* and pr^r, which is on 2R (Kaiser et al. 1979), led to the assignment of *eb* on 2L. The location of *eb* was better defined by a cross that employed the translocation, *T(2L;3R)2* (crosses 5 and 6, Table 1). As noted earlier st^+ was a marker for this particular translocation, so a cross was set up to measure the distance between *eb* and the translocation breakpoint at 18c on 2L. A short distance of 2.35 map units was obtained. Eight deficiencies (Table 3) were confirmed cytologically, and *eb* was assigned to Region 20A. *Df(2L)eb-4;* 20A–20B is shown in Fig. 1B.

Red Eye is a recessive trait that is tightly linked to *propoxur resistance* (pr^r) on 2R (Seawright et al. 1982). The general location of pr^r was known from earlier studies on the assembly of genetic-sexing strains composed of translocations and inversions (Kaiser et al. 1979, Seawright et al. 1981b). Eight deficiencies (Table 3) were used to assign *re* to Region 10C. *Df(2R)re-3;* 10B–10C is shown in Fig. 1A.

A list of translocations and inversions, that were present in larvae with a deficiency phenotype, is given in Table 4. The breakpoints for these aberrations were either very close to or in the same regions as the deficiencies. The breakpoints in 33B, which was common to most of the aberrations for the st^+ locus, were at the very edge of 33B and 33C. It is difficult, at best, to read the exact breakpoints for a translocation or inversion heterozygote, because of the lack of complete pairing at the point of interchange.

DISCUSSION

The work reported in this paper on the use of deficiencies for mapping visible mutants of *An. albimanus* is part of a more comprehensive, basic research project, in which we are conducting studies on population structure, sex determination, recombinant DNA and chromosomal aberrations. In working toward the development of a genetic control system that can be used to suppress natural populations of *An. albimanus,* we have found that regardless of the type of work in which we become involved, there is always a need for more basic information or genetic tools, e.g., a more detailed ge-

Table 4. List of reciprocal translocations and inversions in *Anopheles albimanus* that conferred the deficiency phenotype.

	Chromosome aberration and breakpoints	
Mutant	Translocation	Inversion
stripe	*T(2R;3R);* 8B, 32C	*In(3R);* 32A, 33B
	T(Y;3R); Y, 33B	*In(3R);* 32A, 33B
		In(3); 33B, 39B
		In(3R); 29B, 33B
ebony		*In(2);* 9B; 20A
		In(2L); 18A; 20A
red eye	*T(X;2R);* X, 10C	
	T(2R;3L); 10B, 44B	

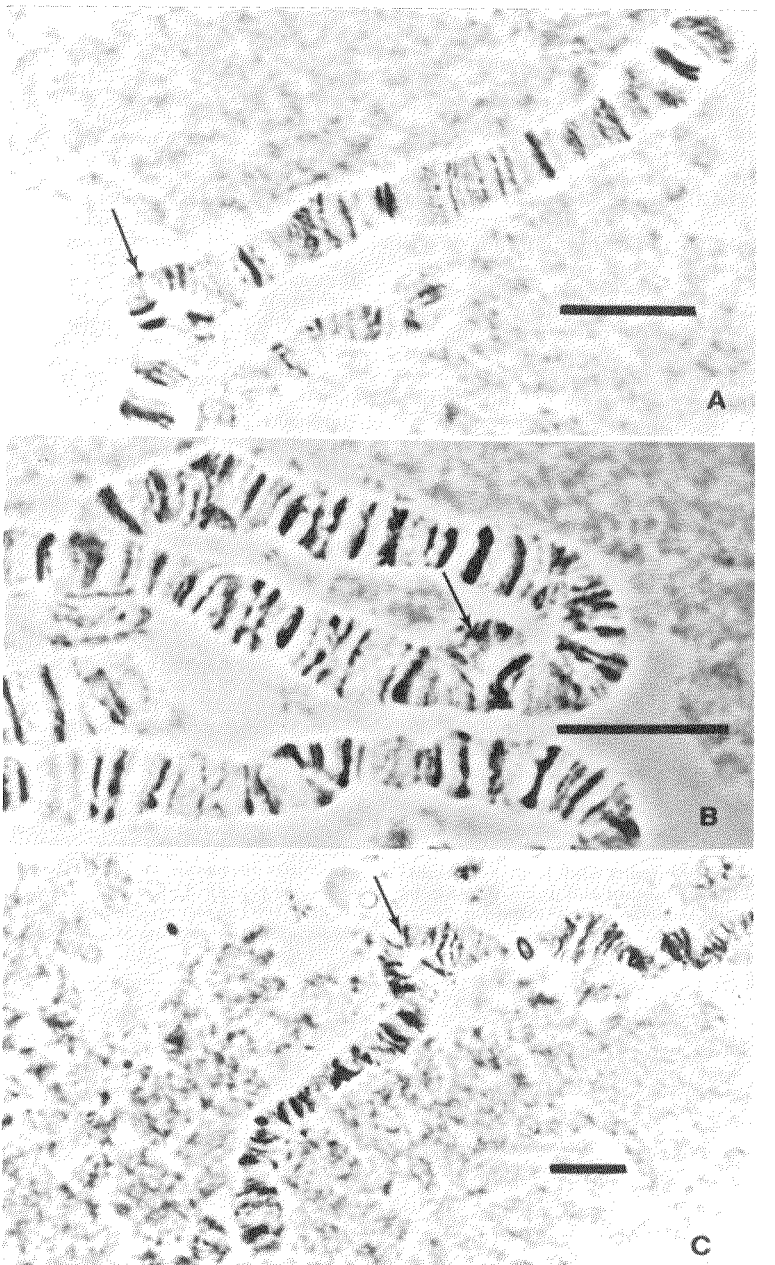

Fig. 1. Examples of the deficiencies (indicated by arrows) used to assign *stripe, reduced palmate, ebony* and *red eye* to specific regions on the autosomes of *Anopheles albimanus;* A – *Df(2R)re-3;* 10B – 10C, B – *Df(2L)eb-4;* 20A – 20B, C – *Df(3R)st-1;* 33A – 34A. The bar is equal to 10μm.

netic map or a specific chromosomal aberration. The information on the more exact location of four mutant loci on the cytogenetic map of *An. albimanus* should be very helpful in our work on induced chromosomal aberrations, analysis of natural variability, and as a basis for determining the locations of new mutants on the genetic map. There are eight additional visible mutants of *An. albimanus* that are suitable for mapping by using induced deficiencies. Our current work also includes the mapping of enzyme loci by using in situ hybridization and restriction enzymes, but the old reliable technique of using deficiencies for mapping visible mutants is still an essential part of studying the genetics of any species. The accumulation of basic information through classical studies of the genetics and cytogenetics of mosquitoes complements and supports our other studies.

References Cited

Benedict, M. Q., J. A. Seawright, D. W. Anthony and S. W. Avery. 1979. *Ebony,* a semidominant mutant in the mosquito, *Anopheles albimanus.* Can. J. Genet. Cytol. 21:193–200.

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

Foster, G. G., M. J. Whitten, T. Prout and R. Gill. 1972. Chromosome rearrangements for the control of insect pests. Science 176:875–880.

Frizzi, G. and I. Ricciardi. 1955. Introduzione allo studio citogenetico della fauna anofelica del Brasile. Rev. Bras. Malar. Doencas. Trop. 7:399–407.

Hobbs, J. H. 1962. Cytogenetics of *Anopheles albimanus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 55:245–251.

Kaiser, P. E., M. Q. Benedict and J. A. Seawright. 1981. A white thorax mutant and its relationship in an allelic series in *Anopheles albimanus.* Mosq. News 41:455–58.

Kaiser, P. E., J. A. Seawright and D. J. Joslyn. 1979. Cytology of a genetic sexing system in *Anopheles albimanus.* Can. J. Genet. Cytol. 21:201–211.

Kaiser, P. E., J. A. Seawright, M. Q. Benedict, S. Narang and S. G. Suguna. 1982. Radiation induced reciprocal translocations and inversions in *Anopheles albimanus.* Can. J. Genet. Cytol. 24:177–188.

Keppler, W. J., J. B. Kitzmiler and M. G. Rabbani 1973. The salivary gland chromosomes of *Anopheles albimanus.* Mosq. News 33:42–49.

Narang, S. and J. A. Seawright. 1982. Linkage relationships and genetic mapping in *Culex* and *Anopheles,* pp. 231–290. *In:* Recent developments in the genetics of insect disease vectors (eds. W. W. M. Steiner, W. J. Tabachnik, K. S. Rai and S. Narang) Stipes Pub. Comp., Champaign, IL.

Rabbani, M. G. and J. B. Kitzmiller. 1972. Chromosomal translocations in *Anopheles albimanus* Wiedemann. Mosq. News 32:421–432.

Rabbani, M. G. and J. B. Kitzmiller. 1974. X-ray induced inversions in *Anopheles albimanus* W. Mosq. News 34:472–474.

Rabbani, M. G. and J. A. Seawright. 1976. Use of Y-autosome translocations in assigning the *Stripe* locus to chromosome 3 in the mosquito *Anopheles albimanus.* Ann. Entomol. Soc. Am. 69:266–268.

Roberts, P. A. 1976. The genetics of chromosome aberrations, pp. 68–189. *In:* The genetics and biology of Drosophila Vol. 1a (eds. M. Ashburner and E. Novitski). Academic Press, New York.

Seawright, J. A., M. Q. Benedict and S. G. Suguna. 1979. *Reduced palmate,* a recessive mutant on chromosome 3 in the mosquito, *Anopheles albimanus.* J. Heredity 70:321–324.

Seawright, J. A., P. E. Kaiser and S. Narang. 1981a. Chromosome manipulation studies of *Anopheles albimanus* for genetic control, pp. 249–261. *In:* Cytogenetics and genetic of vectors (eds. R. Pal, J. B. Kitzmiller and T. Kanda). Elsevier, New York.

Seawright, J. A., M. Q. Benedict, S. G. Suguna and S. Narang. 1982. *Red eye* and *vermillion eye,* recessive mutants on the right arm of chromosome 2 in *Anopneles albimanus.* Mosq. News 42:590–593.

Seawright, J. A., P. E. Kaiser, S. G. Suguna and D. A. Focks. 1981b. Genetic sexing strains of *Anopheles albimanus* Wiedemann. Mosq. News 41:107–114.