INDUCTION AND ISOLATION OF TRANSLOCATIONS IN *ANOPHELES STEPHENSI*

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ABSTRACT. *Anopheles stephensi* males were irradiated with 3.5 and 5.0 krad of X-rays to induce chromosomal aberrations. Late embryonic lethality scored as dark brown eggs gives a good indication for the presence of translocations. Three reciprocal translocations were isolated, [T(Y;2)1, T(2;3)2 and T(Y;3)3], from meiotic analysis of 90 out of 129 families of backcrossed F_1 individuals with a sterility of above 30%.

INTRODUCTION

As part of a new research program, genetic studies on *Anopheles stephensi* Liston, a malaria vector in Pakistan and India, were initiated. One of the objectives is the development of a transport mechanism for the introduction of genes beneficial to man into a target population. Chromosomal rearrangements can play such a role. *Anopheles stephensi* males were irradiated and the progenies investigated for semi-sterility and the presence of chromosomal aberrations based on cytological analysis of meiosis in males.

In two previous publications (Aslamkhan and Aaqil 1970, Sharma et al. 1978), data and cytological evidence were given on translocations induced in *An. stephensi*. Cytological analysis of inversions was carried out by Coluzzi et al. (1970, 1973) and Sharma et al. (1977).

A preliminary report on γ-induced translocations (Aslamkhan and Aaqil 1970) presented data on semisterility. One sex-linked translocation was cytologically confirmed. Sharma et al. (1978) investigated visible alterations in the polytene chromosomes of the F_1 following irradiation of males.

MATERIALS AND METHODS

A limited number of *An. stephensi* mosquitoes was collected in Pakistan in the district of Kasur close to Lahore in the summer of 1980 (through the courtesy of M. Aslamkhan) and were fed in the laboratory. A wild type stock derived from this material was reared in Wageningen. The mosquitoes were reared and maintained in an insectary at a temperature of 28°C and a relative humidity of 80%. Photoperiodicity is maintained at 16 hr of light and 8 hr dark. Anaesthetised mice were put on top of a cage (25 × 25 × 25 cm) for blood meals. Larvae were reared in trays and fed a mixture of liver powder and dog food.

For irradiation in this experiment, young (1–4 days old) males were treated with either 3.5 or 5.0 krad (35 or 50 Gy) of X-rays. In one experiment, mature male pupae were irradiated. The dose rate was 239.7 rad/min (320 kV-10 mA, focus distance 50 cm, filters: 0.25 Cu + 1.0 Al, HVL 1.4 Cu, irradiation period 14.6 min). A relatively low dose was used to increase the chances for homozygosity in the translocations.

The irradiated males were mated in mass with virgin control females. Several bloodmeals were provided and the progeny of successive ovipositions reared. Reciprocal crosses between the F_1 and the Lahore control stock were carried out and females were separated in small cages (6 × 6 × 10 cm). The wet filter papers on which the eggs on an individual female were laid, were placed on a piece of plastic foam floating in a small cup with water. Liquifry No. 1 baby fish food (Interpet Ltd., Dorking, Surrey) was supplied in these cups as food for the hatching larvae. Later on it appeared that oviposition directly on water instead of wet filter paper gave a higher fecundity. After 3 days, reduction in fertility was measured and only the progeny from an egg hatch of less than 70% were kept. In addition to the empty (E) eggs and completely white (W) eggs (minority), two different types of brown eggs occur: 1) light (L) brown eggs which die in an early embryonic stage, without any visible development (comparable to unembryonated); 2) dark (D) brown eggs (embryonated unhatched) showing late embryonic lethality in which the embryo (young prelarvae) can be seen.

Cytological analysis was carried out with 4th instar male larvae or young male pupae. The method described by French et al. (1962) was slightly modified to improve it for *An. stephensi*. As a routine procedure testes preparations were made. No polytene analysis was carried out since salivary gland cells gave poor quality chromosomes.[1] Further meiotic analysis has the advantage that it can assess which arm of the Y-chromosome is involved in a transloca-

[1] Problems with polytene analysis in salivary glands of larvae are now solved.

tion. Due to a limited number (three) of morphological mutants and a high degree of recombination, the isolation of translocations was based on semisterility in combination with meiotic analysis.

RESULTS AND DISCUSSION

Three irradiation experiments were carried out on male pupae or adult males. The data are summarized in Tables 1 and 2. Two different criteria were used to measure the effect of the irradiation of the male parent in the egg stage: 1) late embryonic lethality in which only the late dominant lethality (dark brown eggs) is considered and not the white and light brown eggs; 2) total embryonic lethality in which all nonhatching eggs (W + L + D) are considered. For the three different radiations the percentage of late embryonic lethality was roughly half of the total embryonic lethality. If the control data are considered it can be concluded that the criterion based on dark brown eggs and empty eggs is the most suitable for an accurate measurement of the reduction in fertility. Differences between the three treatments indicate the increase in parental sterility with dose. Further it is noted (Table 1) that old pupae receiving 5.0 krad are less sensitive than young adults receiving the same dose.

Table 1. Sterility levels in the parental generation following different irradiations as based on late embryonic lethality or total sterility.

Irradiation	% Late embryonic lethality (D×100/D+E)	% Total sterility (D+L+W) ×100/total
3.5 krad on adult ♂♂	22.46 (7.95)*	54.51 (18.65)
5.0 krad on adult ♂♂	40.48 (8.41)	78.97 (15.47)
5.0 krad on pupal ♂♂	33.76 (8.41)	68.24 (15.47)

* Control data in parentheses.

Explanation of symbols: D = dark brown eggs, E = empty eggs, L = light brown eggs and W = white eggs.

In these experiments, 395 males were irradiated and crossed with 460 virgin Lahore females. The 1526 F_1 males were backcrossed with 1929 control females and 137 F_1 females with 182 control males. A total of 1466 F_1 females were separated and 620 produced eggs. Of these, 129 families had a semisterility of over 30%, based on late embryonic lethality. Of the 90 families cytologically analyzed, three reciprocal translocations were established based on meiotic analysis. Compared to the results from *An. albimanus* Wiedemann (Kaiser et al. 1982), this is a low score. Here from a total of 1669 irradiated sperms, 175 new aberrations were identified which were mostly (102) reciprocal translocations. These data were based on the analysis of polytene chromosomes and therefore the comparison is not valid, since we kept only the translocations with a clear exchange recognizable in meiosis.

Classification of F_1 families from irradiated fathers (3.5 or 5.0 krad) according to the level of sterility, shows a skewed distribution (Table 2). Most F_1 families had a reduction in fertility up to 40%. It can be concluded that F_1 sterility based on late embryonic lethality occurs much more often following a higher dose.

Three translocations were observed in families with a sterility in the 40–70% class and each had an average sterility of 50%. Two of these descended from 5 krad irradiated males and the other one from a 3.5 krad male. No conclusions may be drawn as if a lower number of translocations can be isolated following a lower dose. The rearing conditions for the larvae were not always optimal and probably several F_1 families with sterility which could have possessed a translocation were lost.

In contrast to the studies in *An. albimanus* where only identification of new aberrations was involved, our work selected for viable translocations which could be reared for several generations. Aslamkhan and Aaqil (1970) suggested that they had a rather high number of probable translocations in *An. stephensi*, but only one sex-linked translocation was cytologically determined.

A normal (Fig. 1a) and a male linked

Table 2. Classification of F_1-families from 3.5 and 5.0 krad irradiated male parents, according to the sterility level (% D/D+E).

Irradiation	0–20%	20–40%	40–70%	70–90%	90–100%	Total ovipositing F_1 ♀♀ tested
3.5 krad on adult ♂♂	80.7	11.5	6.1*	.7	1.0	410
5.0 krad on adult ♂♂	52.5	19.1	19.7**	6.4	3.5	173
5.0 krad on ♂ pupae	62.2	16.2	16.2	2.7	2.7	37

* T(Y;2)1 ± 55% sterility.

** T(2;3)2 ± 53% sterility and T(Y;3)3 ± 56% sterility.

translocation heterozygous karyotype (Fig. 1b) of *An. stephensi* in meiosis is shown. An explanation of the configuration of the translocation is given in Fig. 2 with meiotic synapsis between chromosomes Y^2, X,2 and 2^Y and the translocation breakpoints are in the long arm of the Y chromosome and in the short arm of chromosome 2. The chromatid diagram of the multivalent is given in the lower part of Fig. 2. Since only chains of four were observed, synapsis between the long arm of X and the distal part of the long arm of Y, now connected to chromosome 2, apparently disappears due to the absence of chiasmata in this area of the complex. In contrast to Rishikesh (1959), there is little evidence that chiasmata occur in the long arms of X and Y. In Fig. 1c and 1d the chromosomal pictures of the autosomal translocation T(2;3)2 and the Y-linked T(Y;3)3 are presented.

Further cytological studies have to be carried out to establish the breakpoint positions and the positions of the centromeres in the quadrivalent. Polytene chromosome analysis is now underway and C- and G- banding will be done to increase the knowledge of the cytogenetics of this species.

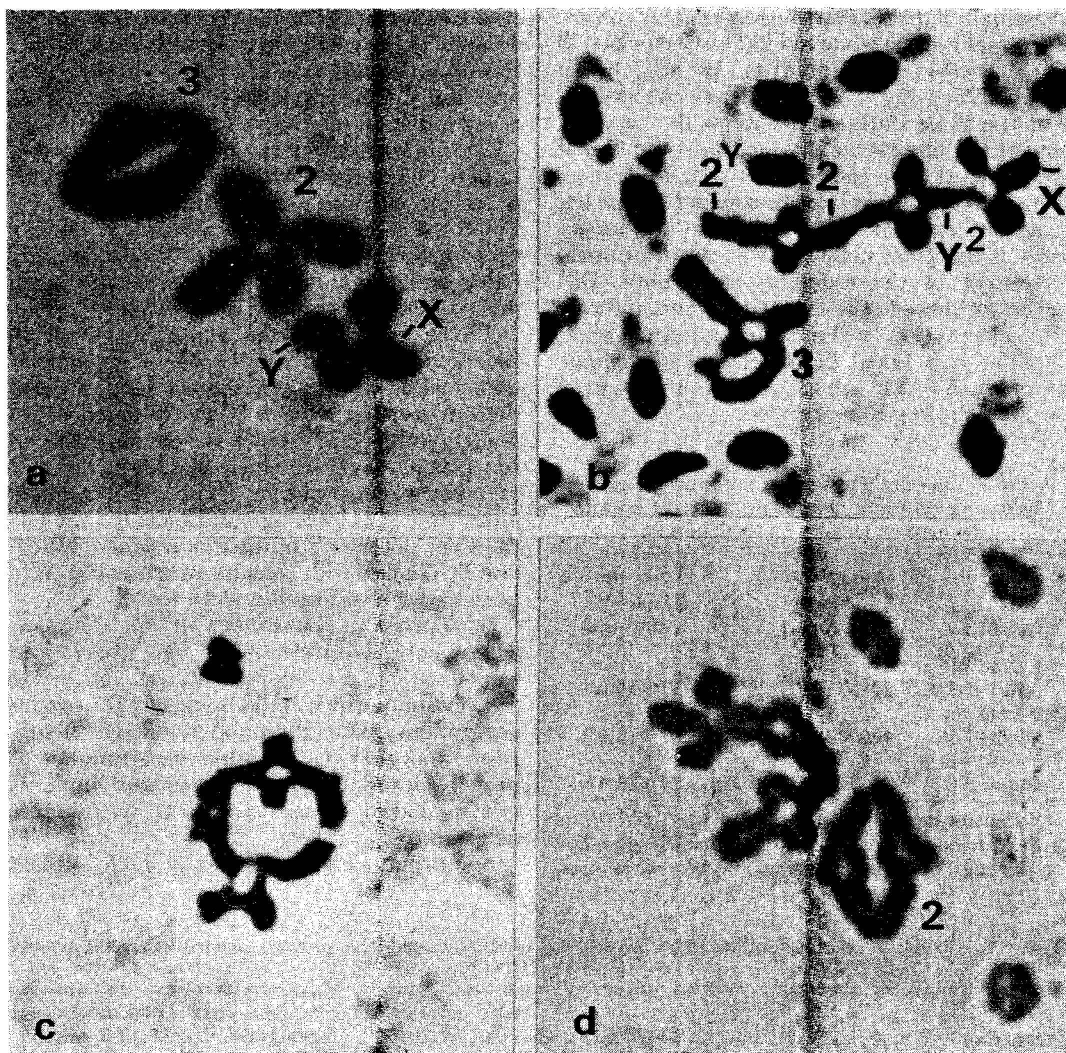

Fig. 1. a. Normal karyotype of *Anopheles stephensi*. Diakinesis in male meiosis.
b. Translocation heterozygote T(YL;2S)1. One quadrivalent and one bivalent can be seen.
c. Translocation heterozygote T(2;3)2, cell incomplete.
d. Idem for T(Y;3)3.

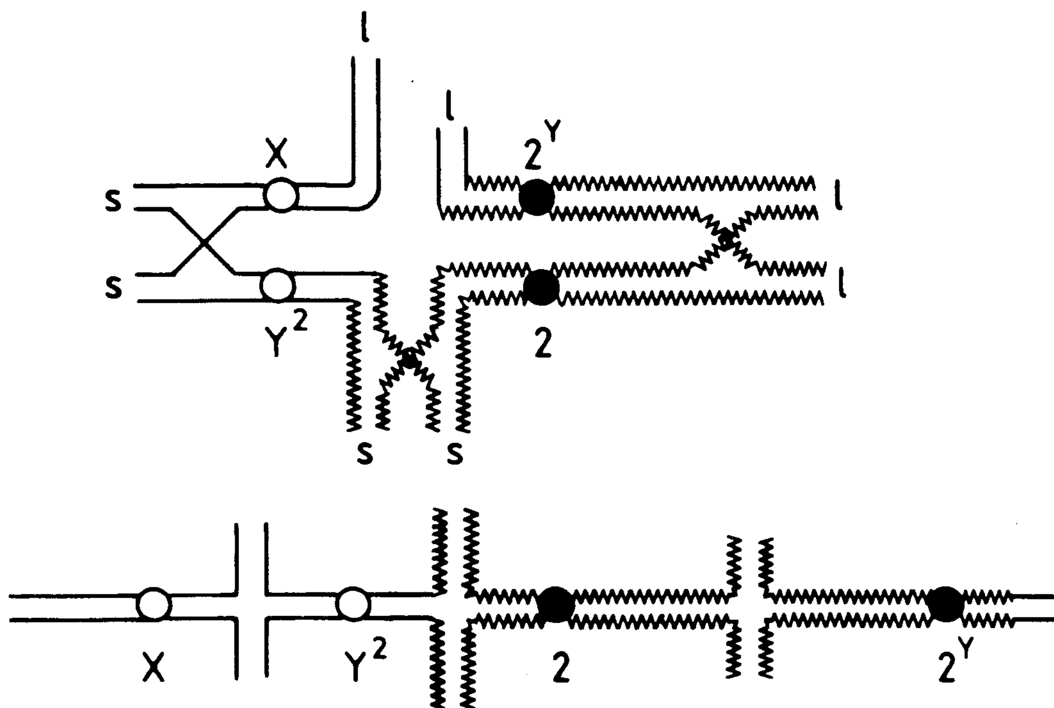

Fig. 2. Meiotic synapsis in males of T(YL;2S)1 and the chromatid diagram of the multivalent configuration (s = short arm; l = long arm).

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