INDUCED CHROMOSOMAL ABERRATIONS IN *ANOPHELES CULICIFACIES*

RICHARD H. BAKER, RICHARD K. SAKAI, UMAIMA T. SAIFUDDIN AND ALMAS PERVEEN

Pakistan Medical Research Center 6, Birdwood Rd., Lahore, Pakistan, and International Health Program, University of Maryland School of Medicine, 660 W. Redwood St., Baltimore, Maryland 21201

ABSTRACT. Gamma radiation-induced translocations and inversions in *Anopheles culicifacies* Giles involving all 3 chromosomes have been isolated and examined cytologically in both the mitotic and polytene chromosomes. These aberrations support the hypothesis that only the short arm of the mitotic X chromosome is present in the polytene chromosome complement, and that 2R and 2L and 3R and 3L of the polytene chromosomes are correlated with the longer sub-metacentric and metacentric mitotic chromosomes respectively. A homozygous pericentric inversion involving most of the long arm of the mitotic X chromosomes has been isolated. Individuals homozygous for an autosomal-autosomal translocation have been recovered, but a pure breeding homozygous line has not been established. There is no evidence for crossing over between the X and Y chromosomes.

Many induced chromosomal aberrations have been described in culicines (e.g., Baker and Sakai 1974, Baker et al. 1977, Rai et al. 1974) but only a few have been reported in anophelines. Most of the induced aberrations (inversions and translocations) in the anophelines have been isolated in *Anopheles albimanus* (Rabbani and Kitzmiller 1972, 1974, 1975), a few translocations in *An. gambiae* species A (Krafsur 1972, Akiyama 1973, Curtis et al. 1976) and one translocation in *An. stephensi* (Aslamkhan and Aaqil 1970). Recently with *An. culicifacies,* the major malaria vector in much of South Asia, colonization has been achieved (Ainsley 1976), the ovarian polytene chromosomes and mitotic chromosomes from ovaries and testes have been described (Saifuddin et al. 1978), and one morphological mutant has been isolated (Sakai et al. 1977). In this species no naturally occurring chromosomal aberrations have been found, although there is variation in the lengths of the long arm of the mitotic X chromosome (Saifuddin et al. 1978). This paper reports the isolation and characterization of radiation-induced aberrations in *An. culicifacies.*

MATERIALS AND METHODS

One hundred and forty-five 4-day old males of the Sattoki colony irradiated with 3500 r from a ^{60}Co source were mass-mated to 202 rose eye (*re*) females (a recessive sex-linked eye mutant, Sakai et al. 1977). The F_1 heterozygous normal-eyed females were backcrossed to rose males, blood fed on a mouse, and isolated into vials for egg laying. Three days after oviposition the hatched and unhatched eggs of each female were counted, and the larvae were reared as a family in enamel pans. Daily the larvae were fed a 1:1:1 mixture by weight of baker's yeast, Kellog's concentrate and wheat germ. After the 1st pupae developed, the entire family was transferred to a 1-liter cotton-plugged Erlenmeyer flask containing 150–200 ml tap water and fed daily until all adults emerged. The mitotic and ovarian polytene chromosomes of phenotypically wild type females from each family were examined by the methods previously described (Saifuddin et al. 1978). If a family showed either more than 20% sterility and/or exhibited a chromosomal aberration, the remaining wild type females were again backcrossed to rose males.

The F_1 males were crossed to rose females. Families that consistently showed sterilities over 20% were saved. From these families, mitotic chromosomes from ovaries and testes, ovarian polytene

chromosomes and male larval salivary gland chromosomes (French et al. 1962) were also examined.

RESULTS AND DISCUSSION

Aberrations involving all 3 chromosomes were found. Many paracentric inversions were seen, but most were discarded. Out of 101 lines isolated from the F_1 heterozygous female backcrosses, 4 lines with chromosomal aberrations have been maintained for nearly 1 year, and out of the 56 F_1 heterozygous male backcrosses, 3 lines are being maintained. All but 1 aberration are currently maintained by backcrosses and by noting the sterilities and/or through cytological examinations. Other translocations and pericentric inversions were found, but were lost before isolation and adequate cytological study could be made.

In(X)1. This aberration was isolated from an F_1 heterozygous female and has been tentatively identified as a pericentric inversion involving no recognizable part of the polytene banded X chromosome but most of the long arm of the mitotic X chromosome (Figure 1A, B, C). Female heterozygotes have normal banding patterns in the polytene preparations, but the mitotic inverted X chromosomes are very distinctive. Homozygous females have been isolated and a pure line with females showing both aberrant mitotic X chromosomes has been established. Little sterility is found in heterozygous or homozygous females or hemizygous males. Approximately 8% crossing over occurs between this aberration and the *re* locus in heterozygous females.

T(X;2L)1. This translocation, which was isolated from F_1 heterozygous females, cannot be detected in the mitotic chromosomes (Figure 2A) as it appears to be the result of an equal exchange of chromosomal material between the X chromosome and chromosome 2. Polytene preparations indicate that the break points occur at 4B in the X and 26C on 2L (Figure 2B). Sterility in females is approximately 50% and slightly higher in males (59.5%). Little or no crossing over is found between this aberration and *re*. No homozygotes have been found.

T(X;3L)1. This translocation was isolated from an F_1 heterozygous female and can be detected in both the mitotic and polytene chromosomes; thus, correlation between the mitotic and polytene chromosome can be made (Figure 3 A,B). In the mitotic chromosomes a large piece of the metacentric chromosome (3) has apparently been exchanged with a

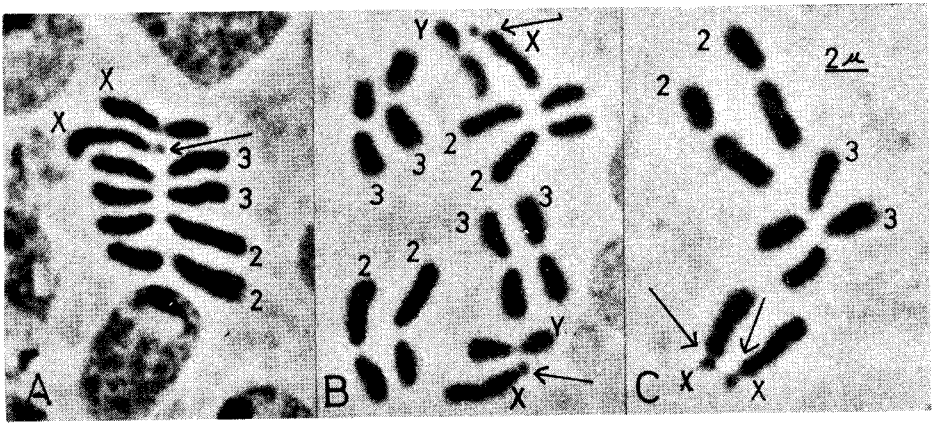

Fig. 1. Karyotypes of I(X)1, a pericentric inversion on the X chromosomes involving most of the long arm. Arrows point to aberrant chromosomes. A-heterozygous female (ovary); B—two complements from hemizygous male (testis); C—homozygous female (ovary).

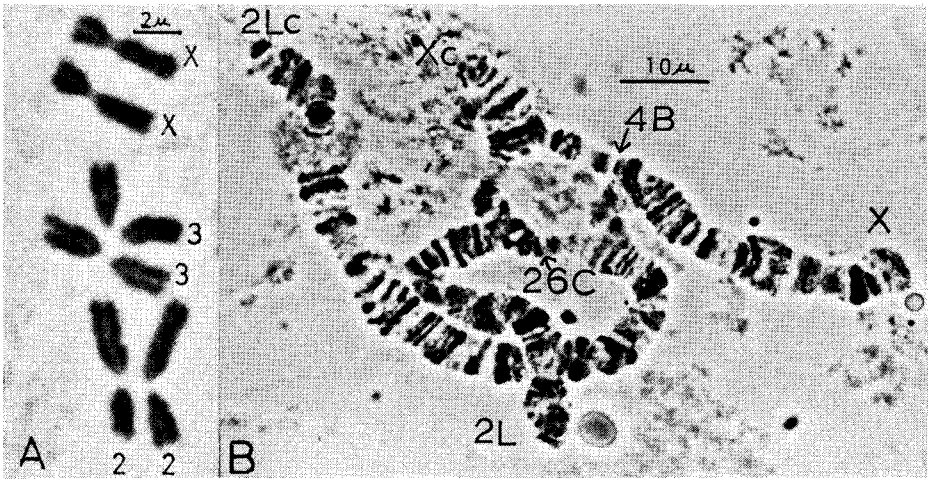

Fig. 2. Chromosomes of T(X,2L)1 heterozygous females (ovary). A—mitotic chromosomes showing no detectable aberration. B—polytene chromosomes, arrows point to approximate break points. c = centromere end of the X and 2L arms.

shorter segment of the short arm of the X chromosome. This hypothesis is supported by observation of polytene chromosome preparations in which the break points are at 5B on the X chromosome and 38A on 3L. The sterility in heterozygous females (19.1%) is considerably lower than that of heterozygous males (61.8%). In heterozygous females this aberration crosses over frequently

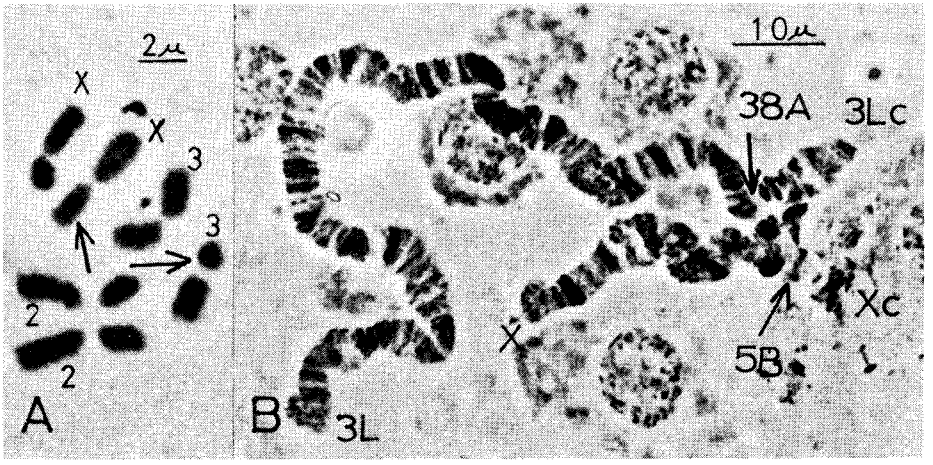

Fig. 3. Chromosomes of T(X,3L)1 heterozygous females (ovary). A—mitotic chromosomes showing an exchange between the X chromosome and chromosome 3, larger piece of chromosome 3 added to short arm of the X chromosome. Arrows point to affected chromosomes. B—polytene chromosomes, arrows point to approximate break points. c = centromere end of X and 3L arms.

(10–20%) with *re.* Attempts to produce homozygous lines have been unsuccessful. Heterozygous females produce only a few heterozygous sons, suggesting that the heterozygous males are lethal or of reduced viability.

T(Y;2L;3)1. This aberration was isolated from F_1 heterozygous males, and without exception all future generations of males showed a characteristic sterility of about 50%. The mitotic chromosomes from testes suggest that all three chromosomes are involved, but the exact exchange of segments has not been determined in larval salivary glands (Figure 4). Daughters do not show any characteristic sterility or chromosomal aberrations in mitotic or polytene chromosome preparations. Males carrying the aberration show delayed development and possibly lower viability than their normal sisters.

T(Y;?)1. This aberration, which has not yet been fully characterized, was isolated from F_1 heterozygous males, and appears to be Y-linked as a characteristic sterility of around 37.6% is found in all sons crossed to normal females. The mitotic chromosomes from testes suggest that a small piece of an undetermined chromosome has been added to the short arm of the Y chromosome (Figure 5A, B). This aberration has not been detected in the salivary gland chromosomes of male larvae. This is not surprising as the Y chromosomes probably do not exist as a banded element in agreement with the observation of Rabbani and Kitzmiller (1975) for *An. albimanus.*

In(31)T(2R;3L)1. This complex aberration was isolated from F_1 heterozygous males and involves both a translocation and a paracentric inversion. The translocation can be detected in both the mitotic (Figure 6A) and polytene chromosomes

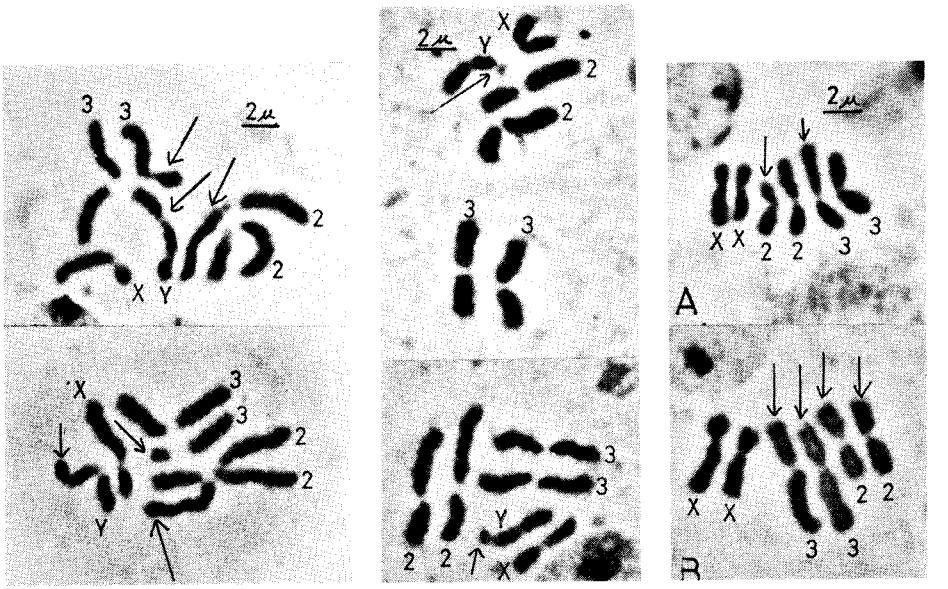

Fig. 4. Two complements of mitotic chromosomes from males (testis) of T(Y;2L;3)1. Arrows point to affected chromosomes.

Fig. 5. A,B—two complements of mitotic chromosomes from males (testis) of T(Y,?)1. Arrow points to a small chromosomal segment attached to the small arm of the Y chromosome.

Fig. 6. Mitotic chromosomes of In(3L)T(2R;3L)1 from females (ovary). A—heterozygous female. B—homozygous female. Arrows denote affected chromosomes 2 and 3.

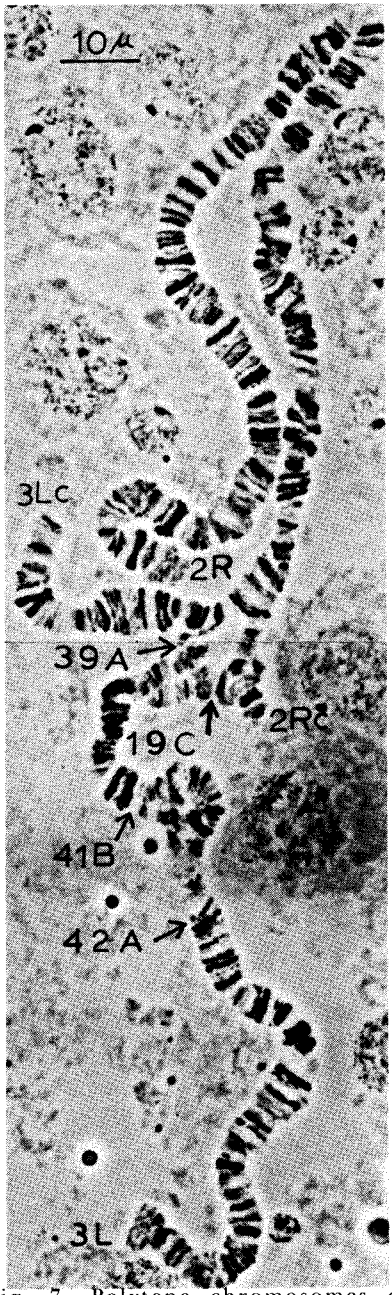

Fig. 7. Polytene chromosomes of In(3L)T(2R;3L)1 from a heterozygous female (ovary). Arrows denote approximate break points (inversion 42B and 42A; translocation 19C and 39A). c = centromere.

(Figure 7). In the mitotic chromosomes there is an unequal exchange between chromosomes 2 (long arm) and 3, with the longer segment from chromosome 2 translocated to 3. In the polytene chromosomes nearly the entire arm of 2R (break point at 19C) has been exchanged with a large part of 3L (break point at 38A). Thus, centromere 3 with the long segment from 2R is the longest chromosome in the new arrangement. Included in the translocated segment of 3L is a paracentric inversion with the break points at 41A and 42A. Homozygous adult females for this complex aberration have been detected cytologically (Figure 6B) but no pure stock has yet been established. These homozygotes show the expected karyotypes both in the mitotic and polytene chromosomes with the new chromosome 3 centromere having most of 2R, part of 3L and all of 3R. The homozygous females are fertile as the polytene chromosomes of 1 female carrying the homozygous translocated and paracentric inverted segments was examined after she laid a batch of eggs that hatched (she had been given a 2nd blood meal to stimulate polytene chromosome development after laying the 1st egg batch). Homozygous females probably have reduced viability as fewer homozygous individuals are found than would be expected in a heterozygous ♀ X heterozygous ♂ mating. The sterility in heterozygous females is 51.63% and in heterozygous males is 50.50% when mated to the normal Sattoki stock.

In(3R,3L)1. This chromosome 3 pericentric inversion was isolated from the F_1 heterozygous females. The break points are 35A and 42B in the polytene chromosomes (Figure 8), but the inversion is not detectable in the mitotic chromosomes. This stock has been maintained by interbreeding sibs of individuals carrying the aberration. Another paracentric inversion on chromosome 2R is also present in some individuals in this stock. Sterility is very variable, and this line needs further study to determine if lethality is due to the pericentric inver-

sion. Unfortunately no morphological mutants are yet available for this chromosome to assist in its maintenance.

The aberrations described above support the hypothesis that the short banded polytene X chromosome represents the short arm of the mitotic X chromosomes as described by Saifuddin et al. 1978. An apparent large aberration of In(X)1 involving most of the long arm of the mitotic X chromosome is not detectable in polytene chromosome preparations. In T(X,3L)1 a larger piece of the mitotic chromosome 3 has been added to the short arm of the X chromosome which appears in polytene preparations. These exchanges support the suggestion that the longer submetacentric chromosome 2 and metacentric chromosome 3 in mitotic configurations are represented by 2R and 2L and 3R and 3L respectively in the polytene chromosome map.

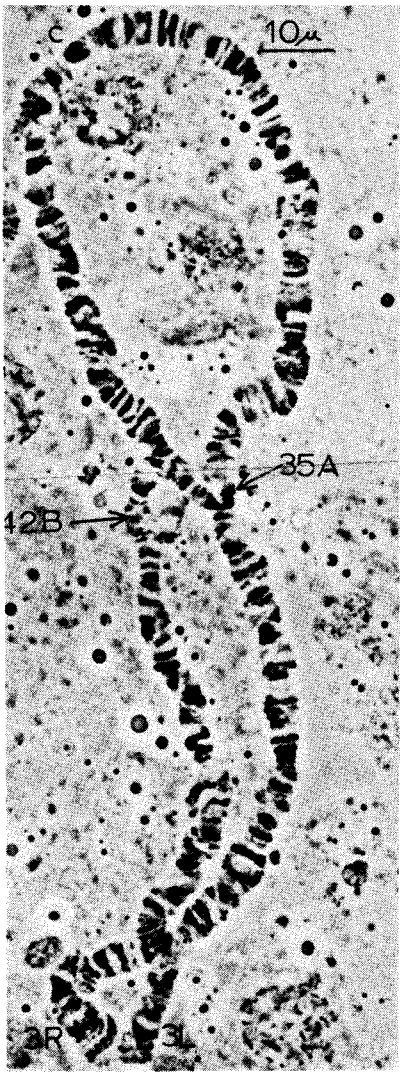

Fig. 8. Polytene chromosomes of In(3R;3L)1 heterozygous female (ovary). Arrows denote approximate break points. c = centromere.

There is no evidence in these studies to indicate that genetic recombination occurs between the X and Y chromosomes in either the X-linked inversion, In(X)1, translocations, T(X;2L)1, T(X;3L)1 or the Y-linked abberations T(Y;2L;3)1 and T(Y:?)1 which agrees with the results of Rabbani and Kitzmiller (1975) in *An. albimanus*.

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