

The different volume applied to achieve the dosage required appeared to have no effect on the results.

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### A PRELIMINARY STUDY ON INDUCING RECIPROCAL TRANSLOCATIONS AND OTHER CHROMOSOMAL ANOMALIES IN *CULEX TARSALIS*

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**ABSTRACT.** Following radiation treatment with approximately 2400 r., young *C. tarsalis* male adults fathered stocks which produced large numbers of inviable embryos over 15 generations. Cytological examinations of the primary spermatocytes of viable offspring indicated that in some cases this reduction in egg hatch was due to re-

ciprocal translocations or other inherited chromosomal aberrations. This preliminary investigation suggests that inherited chromosomal anomalies, with possible application in control programs, can be induced and successfully maintained in this species.

**INTRODUCTION.** Following the suggestions of Serebrovskii (1940) that chromosomal interchanges causing semi-sterility could theoretically serve as a control system for obnoxious insects, chromosomal interchanges have been successfully induced in several mosquito species in the last decade: *Aedes aegypti* (Asman 1966); *Culex pipiens* (Laven 1969); *Culex tritaeniorhynchus* (Sakai et al. 1971); *Anopheles albimanus* (Rabbani and Kitzmiller 1972). The growing interest in this phenomenon and the various roles

these aberrations as well as other genetic systems might play in integrated control programs is well documented in recent reviews by Pal and Whitten (1974) and Whitten and Foster (1975).

In light of the importance of *Culex tarsalis* as a vector of western equine encephalomyelitis virus, and the need to control this species to tolerable levels, genetic mechanisms leading to autocidal control are being investigated. This preliminary study describes the successful induction of reciprocal translocations as well as other chromosomal anomalies that can function as heritable control mechanisms contributing to zygote lethality in *C. tarsalis*.

Translocation heterozygotes result when 2 non-homologous chromosomes exchange genetic material after breakage—either

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naturally occurring or induced by some mutagenic agent. If gametes that arise from such cells fertilize normal gametes they frequently form zygotes with unbalanced sets of chromosomes, and this may lead to 50% or more zygotic deaths in the following generation. In addition, the surviving progeny can inherit the interchange which has the potential to continue as an autocidal system in subsequent generations. More importantly, heterozygous translocations are the basis of homozygous translocations, and specific ones are necessary for the construction of compound chromosomes. The homozygous types theoretically are fully fertile since the chromosomes form pairs which segregate in a normal manner. This is an advantage specifically in relationship to mass rearing, as large numbers can be produced for release projects. Homozygotes also rank high as potential transport systems to carry desirable genotypes into natural populations. Compound chromosomes, on the other hand, are theoretically even more desirable for introducing desired alleles into a population. Since all hybrids between individuals carrying compound chromosomes and wild-type chromosomes die as embryos, an introduced allele can be securely fixed in a population without allowing leakage from the chromosome pool of the wild strain (Foster et al. 1972). Thus for several reasons a routine method for inducing chromosomal interchanges is an important tool for exploring several possible autocidal mechanisms.

**MATERIALS AND METHODS.** A *C. tarsalis* laboratory strain originating from Kern County, California, was irradiated to produce chromosomal aberrations. The mutagenic agent was a Co-60 source at the Lawrence Radiation Laboratories on the U.C. Berkeley campus. Approximately 50 young adult males (0-24 hr) that had been exposed to approximately 2400 r (85 r per minute) were crossed with 3-day-old virgin females of the same strain. Mass mating of the F<sub>1</sub> progeny involving several egg rafts continued through a second generation, and 43 individual egg

rafts were isolated in the F<sub>3</sub> to observe percent hatch. If the hatch in the F<sub>3</sub> egg rafts was approximately 50% or lower, cytological studies to observe chromosome damage were made when this generation reached the pupal stage. A cytological method of identification was necessary because marker stocks for genetic analysis of interchanges were not yet available. Since salivary gland preparations of *C. tarsalis* are still inadequate for study, evidence of chromosomal interchanges and other aberrations were observed in the spermatogonia and primary spermatocytes of late pupae. Squash preparations were modified from techniques described by Breland (1961). Photomicrographs were taken under phase with an Olympus PM-6 camera mounted on a Tiyoda R21 microscope, using Kodak Panatomic-X film.

Once an interchange in the meiotic process was cytologically detected, a new line was established by mass-mating the sibling progeny, or outcrossing them reciprocally to wild-type if the recovered number of adults was small. In crosses involving translocation heterozygotes with either siblings holding the same interchange or with a normal individual the translocation can be transmitted to the next generation, although to different degrees. Normal individuals are also produced in both crosses, again to different degrees. While a translocation homozygote could result in the former type of cross, no attempts were made to isolate these on cytological evidence alone. Thus in subsequent generations, individual egg masses representing single-pair matings (Asman 1975) were checked for percent hatch. Only those rafts showing 50% or less hatch were reared to maintain the lines. Every other generation male pupae were again cytologically checked for evidence of the inherited chromosome translocation. In this manner the lines carrying the interchanges were kept through successive generations.

Breland (1961) first described the chromosome number of *C. tarsalis* as  $2n=6$  with no difference in the karyotypes of

the two sexes. Sex determination in this species seems to depend on a single gene or small segment of a chromosome as in other *Culex* species (Barr and Myers 1966). The smallest chromosome is designated I and assumed to be the sex-deter-

mining chromosome as is the case in *Culex tritaeniorhynchus* (Baker, et al. 1971) and *Culex pipiens* (Dennhöfer 1975); the next in size, chromosome II, and the largest, III (Asman 1974).

RESULTS AND DISCUSSION. Seven lines

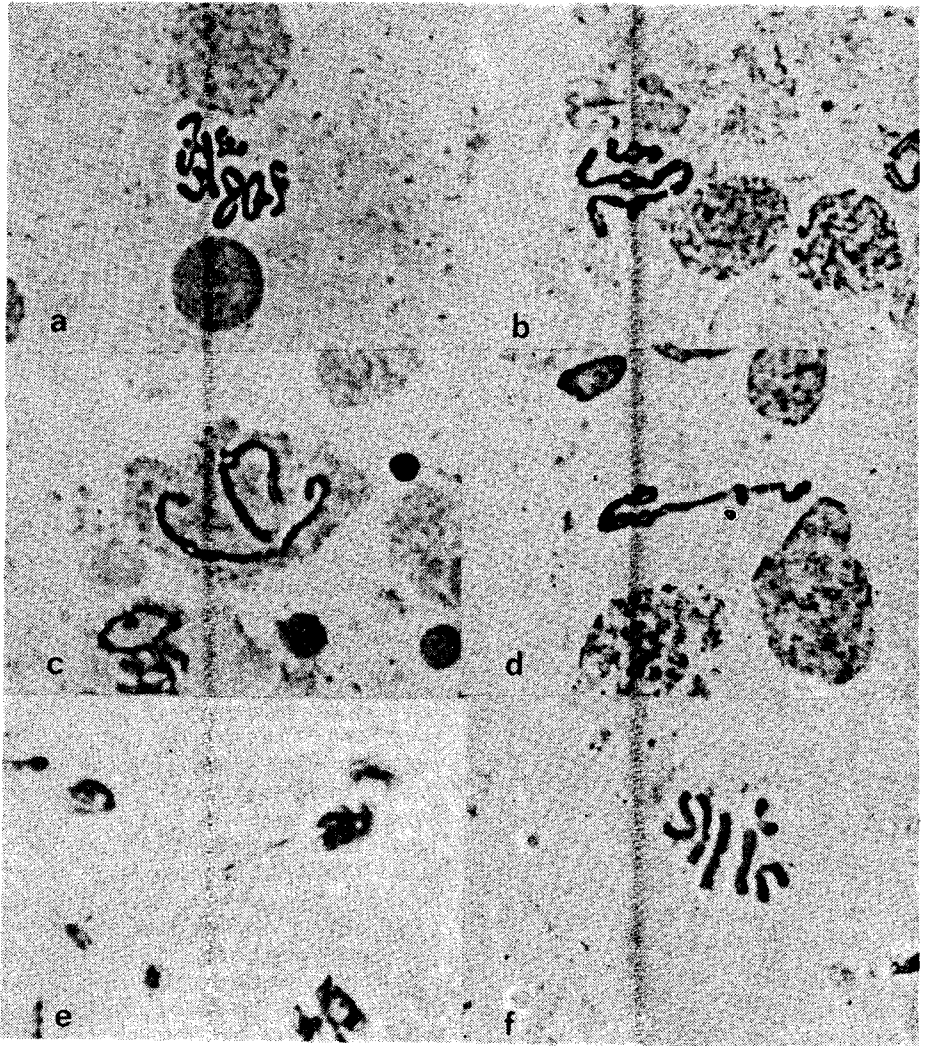


Fig. 1.—a. Cytological evidence of an interchange between 2 large chromosomes—alternate disjunction. b. Interchange between autosomes—adjacent disjunction. c. Chain of 4 involving chromosomes I and II. d. Chain of 6 indicating interchanges between II and III and one of these with I. e. Dicentric bridge and acentric fragment of paracentric inversion. f. Uneven chromosome pairs resulting from interchange or deletion.

carrying interchanges were started from the  $F_3$  generation egg rafts that gave 50% or lower hatch. With the mass-mating system used, more than one line could have stemmed originally from the same irradiated male and therefore have carried the same interchange; however, in this study the number of translocations was

not the major factor. Rather, mass rearing and mass-mating seemed adequate to see if laboratory-reared stocks of *C. tarsalis*, which in themselves are often difficult to maintain, would sustain radiation treatment and subsequent inherited chromosomal abnormalities. Now that genetic analysis is possible with multiple marker



Fig. 2.—a. Aneuploidy of  $2n+2$  with duplication of chromosomes I (mitotic metaphase). b. Trivalent of same tetrasomic at M1. c. Euploidy of  $4n$  with possible inversion and abnormal segregation. d.  $4n$ —all bivalents—at M1. e. Trisomy with extra chromosome forming a trivalent. f. Late AII of possible  $4n$ .

lines, future irradiated males can be mated with genetically marked females as single pairs, and only the isolated low-hatch egg rafts of the F<sub>1</sub> generation will be reared for back-crosses and genetic analysis to identify possible heterozygous translocations.

Interchanges were cytologically observed in 2 lines involving the sex-determining chromosome (I) with one of the larger pairs; 5 lines had translocations that involved the 2 autosomes, II and III. Chains of 4 at metaphase I showed disjunctions of both the alternate and adjacent types (Fig. 1-a,b,c). Observed chains of 6 suggested that 2 heterozygous interchanges involving all 3 pairs were present in one line (Fig. 1-d). In addition to reciprocal translocations, several stocks in earlier generations carried various other aberrations. Among these were paracentric inversions (Fig. 1-e), aneuploidy (Fig. 2-a,b), euploidy (Fig. 2-c,d) and combinations of polyploidy with other aberrations (Fig. 2-e,f).

In the first few generations percent hatch of egg rafts varied in the different lines from 14% to 55%, depending on the number of intrinsic autocidal factors that affected the dividing chromosomes. With succeeding generations the multiple aberrations inherent in a single line segregated or were lost to a great extent, and cytological observations indicated that reciprocal translocation systems alone were functioning. The hatch seemed to stabilize between 30% and 40% in all lines. The 7 stocks with interchanges were maintained for over 15 generations by either inbreeding or outbreeding to wild-type, and subsequently picking up the interchanges again by selecting low egg-hatch rafts and cytologically checking the dividing chromosomes.

As various multiple marker lines become available, newly-induced interchanges can be genetically screened for their potential to become homozygous systems or for the construction of compound chromosomes,

and possibly as contributing factors to an integrated control program for this species. Meanwhile it has been demonstrated that chromosomal translocations can be induced in this species and can be successfully maintained for many generations. It has also been shown that the radiation dosage used in this experiment can induce other heritable chromosome abnormalities that could be isolated for genetic purposes.

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