

GENETIC CONTROL OF MOSQUITOES: CHROMOSOMAL TRANSLOCATIONS AND INHERITED SEMISTERILITY IN *CULEX QUINQUEFASCIATUS*. A FILARIAL MOSQUITO

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SUMMARY

12 male-linked (T^M), 10 autosomal-autosomal (T^A) simple reciprocal and 8 double translocation heterozygotes $T(1;2;3)$ were artificially induced and isolated after exposure to gamma rays in the filarial vector *C. quinquefasciatus*. The presence of translocations were confirmed by genetic and cytological analyses. Translocation in heterozygous condition led to semisterility of the carriers and is inherited from generation to generation with small variation ($\pm 10\%$). The use of sex-linked translocations in the genetic control programme of *C. quinquefasciatus* in India is considered.

Key Words: *Culex quinquefasciatus*, translocations, genetic control.

INTRODUCTION

Culex quinquefasciatus Say is one of the members of *Culex pipiens* complex. This species is found throughout the tropics and extends into the temperate regions of the northern and southern hemispheres (Mattingly 1951). This is one of the ideal species for cytogenetic and genetic studies because of its amenability to laboratory condition, maintenance and manipulation.

C. quinquefasciatus is one of the important carriers of Bancroftian filariasis in Asian countries. Urbanization of this species has caused an alarming increase in the rate of filarial infection. The natural vigour of this species combined with its new tolerance, indeed resistance to insecticides has made it obligatory that we look for control methods involving genetic manipulation. Hence, there is an immediate need for a greater understanding of the genetics of this vector species. Considerable progress is being made on the genetics and cytogenetics of *C. quinquefasciatus* (Shetty 1974, 1987, 1989, Shetty & Chowdaiah 1975a, b, 1976a, b, 1977, 1983, 1985).

Translocation heterozygotes have important uses for the development and application of several genetic control mechanisms: sex-sorting system, translocation homozygotes, compound chromosomes and assign linkage groups to chromosomes. Inherited semisterility due to translocation has long been suggested as a possible means for control or eradication of insect pests (Serebrovskii 1940, Laven 1968, Curtis 1968, Rai & Asman 1968, Wagoner et al 1969). This paper reports induction, isolation, genetic and cytologic characterization of chromosomal translocations in *C. quinquefasciatus*.

MATERIAL AND METHODS

C. quinquefasciatus used in the present study was originally collected from Mangalore (Karnataka). The mosquitoes were reared at $25 \pm 1^\circ \text{C}$ with the photoperiod of 14 h light and relative humidity of 70%. The colonies were maintained in 30 x

30 x 30 cm cages. Mass matings were made in 30 x 15 x 30 cm cages. Females were blood-fed on pigeon. For digestion of blood, 70-80 h were allowed and the gravid females were isolated individually in 2 x 9.5 cm shell vials with water for egg deposition. The larvae were reared in white enamel pans (15 x 30 cm) containing tap water and were fed on liver powder. Adults were provided with 10% sucrose solution. Stocks with high fertility (99% hatchability) were selected from Mangalore strain according to the procedure of Shetty (1983) for the study.

For induction and isolation of translocations, 325 males of 2- or 3-day-old were irradiated with 400 rads of gamma rays at the rate of 140 rads/min from a Co^{60} source of Kidwai Memorial Institute of Oncology, Bangalore. To determine the incidence of induced translocation, the irradiated males were immediately mass-mated with the virgin females of the same age. Gravid females were individually isolated for oviposition. Most of the females laid eggs after 4 days of blood meal; those which laid eggs were reared as individual families in all subsequent generations. The families which showed less than 60% fertility were retained and the rest discarded. Total number of eggs in each of the raft and the number of hatched larvae were recorded and the percentage of induced sterility was calculated. Reduced fertility was used as marker for the recognition of translocation.

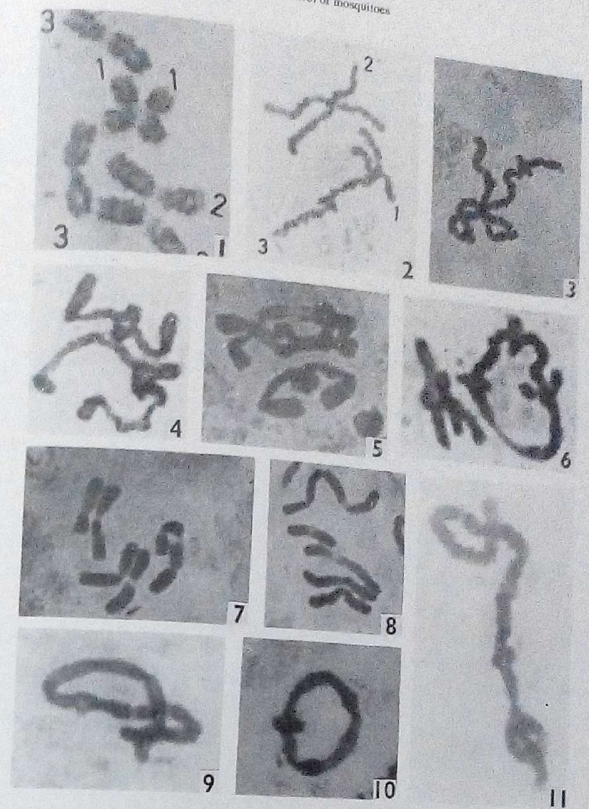
For induction and isolation of double translocation heterozygote, one of the translocation heterozygotes showing high viability and full penetrance was selected to induce double translocation heterozygote. For this purpose, a few males of the translocation lines (T^{31}) having a single reciprocal translocation ($53 \pm 10\%$) which showed constant percentage of semisterility from induction after generation was re-irradiated for the second time at the dose of 3000 rads of gamma rays (140 rads/min) in order to induce new multiple translocations. Cytology for confirmation of the translocations was done from squash of the testes of young pupae and ovaries of newly emerged adults, according to the method described by French et al. (1962).

Maintenance of male-linked translocation (T^M) was easy as no selection is necessary in such a stock because the semisterility is inherited only through the males and all sister females were normal. In this type of translocation, the males are all the time heterozygous for the sex chromosomes, the translocation remaining permanently in the heterozygous condition. In female linked translocation (T^F), a few females were selected from the line showing reduced hatchability (semisterility) and outcrossed with the wild type mosquitoes (pure line) in each generation. Males cannot, but females could, become homozygous for such translocations. In autosome-autosome translocation (T^A), the semisterility is inherited through both the sexes in almost equal proportions. This translocation could become homozygous in both the sexes. Either a few males or a few females were selected from the families showing reduced hatchability (semisterility) and outcrossed with the wild type mosquitoes (pure line) in each generation. The maintenance of male-linked double translocation heterozygotes were similar to that of T^M translocations. The semisterility was inherited only through males. All the translocation lines included in the present study were maintained for more than 30 generations. Mitotic chromosomes from normal testes are shown in Fig.1.

OBSERVATIONS

Altogether 22 single reciprocal translocations and 8 sex-linked double heterozygous translocations were isolated from irradiated males of *C. quinquefasciatus*. Cytological preparations of the mitotic chromosomes revealed the presence of translocations (Figs.2-11).

Table 1 lists the degree of percentage semisterility in each of the translocated families. It was found that in lines T^7 , T^{16} , T^{17} , T^{21} , T^{25} , T^{30} , T^{31} , T^{48} , T^{74} , T^{78} , T^{84} and T^{105} the semisterility was inherited only through the males whereas all the sister females were normal. In all T^M translocation lines, levels of semisterility were constant from generation to generation (Table 1). Because of this fact, these translocations can be considered suitable for field release study. In lines T^{12} , T^{14} , T^{39} , T^{63} , T^{72} , T^{79} , T^{92} , T^{102} , T^{167} and T^{174} the semisterility was inherited through a proportion of both the sexes



Figs. 1-11: Cytology of translocation in *Culex quinquefasciatus* 1, Metaphase chromosomes from normal pupal testis. 2, $T(1;3)$, 3, $T(1;2)$, 4, $T(1;2)$, 5, $T(1;3)$, 6, $T(1;2)$, 7, $T(1;2)$, 8, $T(1;2;3)$, 9, $T(1;2;3)$, 10, $T(1;2;3)$, 11, $T(1;2;3)$

indicating that translocation had occurred between the 2 autosomes (T^A). No female-linked translocations (T^F) were included in the present study.

Table 2 lists the degree of semisterility in each of the translocated families. Eight double heterozygous translocations were isolated from the second irradiated line (T^{31}) of *C. quinquefasciatus*.

TABLE 1: Showing average degree of semisterility in 22 lines of *C. quinquefasciatus*.

Sl. No.	No. of lines	Types of translocations tested	No. of eggs	No. of larvae	Semisterility (%)
1.	T ⁷	T ^M	2210	1127	49.1
2.	T ²⁶	T ^M	13957	4239	69.0
3.	T ¹⁸	T ^M	11616	4063	65.0
4.	T ²¹	T ^M	11477	4652	59.0
5.	T ²³	T ^M	1919	921	52.1
6.	T ³⁰	T ^M	1957	1193	39.1
7.	T ³¹	T ^M	1075	505	53.1
8.	T ³⁸	T ^M	16370	3508	78.0
9.	T ⁷⁴	T ^M	11311	2617	76.0
10.	T ⁷⁸	T ^M	8260	2496	69.0
11.	T ⁸⁴	T ^M	15244	4055	73.0
12.	T ⁰⁵	T ^M	960	568	40.9
13.	T ¹²	T ^a	1018	366	64.1
14.	T ¹⁴	T ^a	5316	1747	67.0
15.	T ³⁹	T ^a	4797	2686	44.1
16.	T ⁶³	T ^a	1863	1173	37.1
17.	T ⁷²	T ^a	1650	844	48.9
18.	T ⁷⁹	T ^a	2663	1358	49.1
19.	T ⁹²	T ^a	2195	1042	52.6
20.	T ¹⁰²	T ^a	2163	1038	52.1
21.	T ¹⁶⁷	T ^a	7775	2926	62.0
22.	T ¹⁷⁴	T ^a	7744	2108	72.0

TABLE 2: Showing average degree of semisterility in 8 lines of translocation stocks of *C. quinquefasciatus*.

Sl. No.	Stock	No. of eggs	No. of larvae	Semisterility (%)
1.	MPL - A (1;2;3)	12,657	2,961	76.61
2.	MPL - C (1;2;3)	10,314	2,388	76.85
3.	MPL - D (1;2;3)	8,598	2,187	74.57
4.	MPL - E (1;2;3)	16,404	3,192	80.55
5.	MPL - G (1;2;3)	8,628	1,737	79.87
6.	MPL - K (1;2;3)	13,167	2,322	82.37
7.	MPL - N (1;2;3)	17,106	3,663	78.59
8.	MPL - T (1;2;3)	29,403	5,217	82.26

It was found that in lines MPL-A, MPL-C, MPL-D, MPL-E, MPL-G, MPL-K, MPL-N and MPL-T the semisterility was inherited only through the males whereas all the sister females were normal. Cytological preparations revealed that exchanges had occurred between the sex chromosomes and the autosomes. These translocations are considered most suitable for the field release study.

DISCUSSION

C. quinquefasciatus has almost 3 metacentric chromosome pairs. One of these pairs is the one which carries sex-determining factors, M for maleness and m for femaleness. Males are heterozygous M/m, females are homozygous m/m. According to this chromosome complement, 3 different kinds of chromosome translocations are possible viz., (1) a translocation between M chromosome and an autosome, (2) a translocation between the m-chromosome and an autosome and (3) a translocation between the 2 autosomes. Out of 30 translocations analysed, 12 were of the first kind i.e., T^M (1;2;3). In the double translocation heterozygotes, autosomes are tightly linked to the male determining factor. Because of this fact, in all the double translocation heterozygote lines included in the present study semisterility is inherited only through males.

Attempts have been made to make the autosomal translocations homozygous. Only one out of the 10 has become homozygous. In this line the semisterility disappears and the line continues with full fertility. However, outcrossing with any normal line, again translocation heterozygotes are produced and lethality turns up again in the following generations (Shetty unpubl.)

A large number of radiation-induced chromosomal translocations and cytological evidence for the same have been studied in *C. pipiens* complex (Laven 1969, Laven et al. 1972; Bhalla et al. 1974, Shetty 1982, 1987). Field evaluation studies by using such translocations in the genetic control programme of a few species of mosquitoes have been reported (Laven 1969, Laven et al. 1971, 1972, Rai et al. 1973, Seawright et al. 1976, 1977, Terewedon et al. 1977).

As indicated earlier, all the translocations included in the present investigation have passed more than 30 generations. The male-linked translocations (T^M) included in the present study could be considered for use in genetic control programme of *C. quinquefasciatus*. For the same reason, a sex-linked double translocation heterozygote, MPL-K (1;2;3) was selected for this purpose (82.37 ± 10% sterility). This line showed a higher mating competitiveness than the normal males both in the laboratory and field cages (Shetty 1984). Hence, the line MPL-K (1;2;3) established in the present study would be of value in the genetic control of *C. quinquefasciatus* in India. Similar studies have been reported in *Anopheles stephensi*, one of the important urban malaria vectors in Indian subcontinent from our laboratory (Shetty & Gayathri Devi 1989, Gayathri Devi & Shetty 1992).

The success of genetic manipulation involving sterile-male-release can be enhanced by developing a method by which males can easily be separated from the females during the mass production. Since the females of the species are potential vectors and cause biting nuisance they should be eliminated during early developmental stages by genetic methods. This will also help to lower the cost of mass production of males for release purpose. To achieve this objective, a genetic sexing strain T (1^M; 2) 1 of *C. quinquefasciatus* was synthesized for preferential elimination of females during

larval stage from our laboratory (Shetty 1987). The T(1^M,2) 1 strain males showed higher mating competitiveness than the normal laboratory males and field collected males in the laboratory cages (Shetty unpubl.).

ACKNOWLEDGEMENT

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SOCGI PLANT CHROMOSOME NUMBER REPORTS XI

Presented by S.S. BIR
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These reports are primarily intended to ensure quick recording of chromosome numbers of plants studied from unexplored regions/areas particularly of Tropical Asia, Africa and America which otherwise may remain unpublished. While the importance of chromosomal surveys of the Flora and the critical cytotaxonomical identification hardly needs any emphasis, it is but essential for each chromosome number report to be documented by an authenticated voucher specimen with specific accession number and preserved in a recognised herbarium. Two copies of contributions regarding hitherto unrecorded chromosome number with family, genera and species arranged in alphabetic order (separate lists required for algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms) and following the style of the recent report should reach Dr. S.S. Bir, Emeritus Professor of Botany, Punjabi University, Patiala 147 002, India or Prof. B.H.M. Nijalingappa, Department of Botany, Bangalore University, Bangalore 560 056 India. Requirements of reprints (to be supplied of the consolidated report only) may be intimated while communicating the chromosome reports.

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VOUCHERS IN PUN

Poaceae

- Alloteropsis cimicina* (Linn.) Stapf. n=18. Central India: Pachmarhi, Mahadev 550 m. (39495).
Andropogon pumilus Roxb. n=21. Central India: Pachmarhi, Contonment area 1650 m. (388730).
Arthraxon lancifolius (Trin.) Hochst. n=9. Central India: Pachmarhi, Singhanama 450 m. (39482).
Brachiaria distachya (Linn.) Stapf. n=18. Central India: Pachmarhi, Singhanama 450 m. (39484).
Brachiaria ramosa (Linn.) Stapf. n=18. Central India: Pachmarhi, Singhanama 450 m. (38968).
Chloris dolichostachya Lagasca. n=9. Central India: Pachmarhi, B.Fall 750 m. (36069) n=9, 2n=18. Pachmarhi, Dhuggarh 1050 m. (39109).
Cymbopogon martini (Roxb.) Watts. n=10. Central India: Pachmarhi, Shanti Sadan 1050 m. (39153).
Dactyloctenium aegyptium (Linn.) P. Beauv. n=18. Central India: Pachmarhi, Singhanama 450 m. (39480).
Dichanthium annulatum (Forsk.) Stapf. n=20. Central India: Pachmarhi, Maikuli 400 m. (38887).
Dichanthium aristatum (Poir.) C.E. Hubb. n=20. Central India: Pachmarhi, Barriaam 1150 m. (38935).
Ischaemum indicum (Houtt.) Merrill. n=18. Central India: Pachmarhi, Panarpani 750 m. (39493); n=18. Pachmarhi, Barri aam 1150 m. (38915).
Oplismenus burmannii (Retz.) P. Beauv. n=13. Central India: Pachmarhi, Singhanama 450 m. (39465).
Oplismenus compositus (Linn.) P. Beauv. n=18. Central India: Pachmarhi, B.Fall. 750 m. (39049).
Paspalum paspaloides (Michx.) Scriben. n=26. Central India: Pachmarhi, Lake side 1150 m. (39478) n=30, Pachmarhi, Panarpani 750 m. (38840); n=30. Pachmarhi, Little Fall 750 m. (38873).
Paspalum scrobiculatum Linn. n=30. Central India Pachmarhi, Dutches Fall 900 m. (37310) n=20. Pachmarhi, Lake side 1150 m. (39476); n=30. Dhuggarh road 1050 m. (38831).

- Pennisetum alopecuroides* Desv. n=14. Central India: Pachmarhi, Contonment area 1050 m (39592); n=14. Pachmarhi, Lake side 1150 m. (39474).
- Pennisetum pedicellatum* Trin. n=21. Central India: Pachmarhi, Lake side 1150 m. (39472); n=18. Pachmarhi, B.Fall, 750 m. (39047); n=18. Pachmarhi, Dhupgarh 1150 m. (39124).
- Saccharum spontaneum* Linn. n=30. Central India: Pachmarhi, Lake side 1150 m. (38894); n=30. Pachmarhi, onway to Dhupgarh 1050m. (38835).
- Setaria glauca* (Linn.) P.Beauv. n=9. Central India Pachmarhi, Amsamai mandir 1050 m. (37318).

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Poaceae

- Bothriochloa perusa* (L.) A. Camus
- CPI** 104672A n=20. North India: Kuluva, 18 km from Bhopal-Raisen 23.15°N 77.34°E, 460 m (JCT 10757).
- CPI 104876 n=20. North India: 6 km SE Tamia-Chhindwara 22.18°N 78.42°E, 975 m (JCT 10752).
- CPI 106082A n=20. North India: 2 km W Nevasa 19.33°N 74.55°E, 420 m (JCT 10755).
- CPI 106350 n=20. South India: 9 km N Bellary Railway x Siraguppa 15.13°N 76.55°E, 440 m (JCT 10754).
- CPI 106426 n=20. South India: 19km SE Davangere-Bangalore 14.22°N 76.04°E, 700 m (JCT 10753).
- CPI 106491 n=20. South India: 14 km WSW Hassan - Mangalore 12.58°N 75.58°E, 940 m (JCT 10760).
- CPI 106819 n=20. South India: 10 km NW Pondicherry-Tindivanam 12.00°N 79.46°E, 50 m (JCT 10758).
- Bowen** n=20. Australian ecotype (JCT 10759).
- Medway** n=30. Australian ecotype (JCT 10756).

** The CPI numbered introductions were collected by Dr. I.B. Staples, Queensland Department of Primary Industries.

++ Australian lines. Seed collected from <100 m. The original introductions which may have given rise to these lines are not known. They may have arisen from crosses.

ANNOUNCEMENT

The V All India Conference on Cytology and Genetics will be held in October/November 1994 at Kurukshetra University Campus, Kurukshetra.

The first Circular in this connection will be sent to members in January 1994.

SUGGESTIONS TO CONTRIBUTORS

Research articles encompassing cytology and genetics are accepted for publication in The Journal of Cytology and Genetics. All authors shall be the members of the Society of Cytologists and Geneticists, India before the paper is submitted for publication. The maximum length of the research paper is usually restricted to 5 printed pages (including one page of illustrations) unless the author is willing to pay the excess charges for additional pagination. Short communications presenting interesting findings of current interest and Review articles on significant topics will be published out of turn at the discretion of the editor in the first available issue. All manuscripts should be addressed to Prof. B.H.M. Nijalingappa, Editor, The Journal of Cytology and Genetics, Department of Botany, Bangalore University, Jnanabharathi, Bangalore 560 056.

All papers will be first assessed by a Reviewing Editor. Papers found unsuitable in terms of overall requirements of the journal will be returned to the authors. Returned papers cannot be of acceptance, rejection, or need for revision on the basis of referee's opinion. It is a basic condition that the papers submitted have not been, and will not be published elsewhere, either simultaneously or at a later date. The papers should be written in a concise form and in clear, grammatically correct language. The style of the manuscript should conform to the format followed in the latest issue of The Journal of Cytology and Genetics.

Preparation of manuscript

Manuscripts should be typed double-spaced with ample margins allround on one side of white bond paper and submitted in duplicate. The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The title should be brief, specific and contain words useful for indexing. Each paper should be preceded by a short SUMMARY which should be a lucid digest of the whole paper, complete in one paragraph with no numbered parts and not exceeding 100 words. This is followed by 3-5 KEY WORDS which should be chosen carefully in a form that can be fed into a data bank.

Text: The paper must be set out under the following headings: INTRODUCTION, MATERIALS AND METHODS, OBSERVATIONS and DISCUSSION. Main headings are in full capitals and bold face. Subheadings are in lower case and bold face. Bold prints should be indicated by double underlining. ACKNOWLEDGEMENTS should be placed between the Text and literature REFERENCES.

Scientific names should conform to the International Rules of Nomenclature. Complete scientific names should be given when organisms are first mentioned. The generic name may subsequently be abbreviated to the initial. Authors of names of taxa should be cited both in the Summary and at the first mention of a taxon in the text, but not elsewhere. The scientific names of organisms will be printed in italics and should be underlined.

Citations in the text of works listed in the References should be set out as follows: Nagl & Ehrendorfer (1974), more than 2 authors: Evans et al. (1972), indication of page or figure Teppner

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