Chemically-Induced Inheritable Semisterility in the Mosquito (*Culex pipiens molestus*)

As is apparent from the recent findings of investigators in different laboratories[1-3], the induction of inheritable semisterility associated with translocation by X-rays in *Culex*[4], has created a new field of interest for pure genetic control of mosquitoes.

In the last few years numerous experiments have been conducted on the chemical sterilisation of insect pests, mostly with the objective of producing sterile males for eradication purposes[5-7]. Yet relatively little attention has been given to the actual semisterilising effects of such chemicals, which might lead to the introduction of inheritable lethal genes in the population.

In an effort to evaluate the effectiveness of chemical mutagenes in the production of lethal genes in *Culex*, experiments were conducted with a difunctional alkylating mutagenic agent, PMS (1, 3-propanediol-dimethanesulfonate) and a terratogen, thalidomide (α-phthalimidoglutarimide) which was shown to have mutagenic effects in *Culex*[8].

Materials and methods. The autogenous strain of *Culex* from our laboratory strain was chosen for the experiment. Immediately after the emergence of the mosquitoes from the pupal stage, they were exposed to dehydration and starved for 24 h before offering them the chemicals mixed with 10% sugar meal. After feeding the males on the chemical (0.04% PMS and saturated solution of thalidomide) separately for about 24 h, they were crossed with normal virgin females. The controls were offered sugar meal without the chemicals under the same conditions.

Each egg raft recovered from the treated insects as well as the controls, was reared in a separate container. When the hatching of the larvae was completed after 48–90 h, counts were made to determine the number of embryonated (+), unembryonated (−) and hatched larvae (L) from each egg raft. Rafts with more than 15% dominant lethality (the total of (+) and (−) eggs in 100) were considered as semisterile, which were isolated and further studied for inheritability. The males from the semisterile F_1-rafts were outcrossed with normal virgin females. The F_2-rafts obtained from such crosses, with high degree of semisterility, were either inbred or outbred for subsequent generations to confirm the inheritability of the induced lethal genes. The data obtained from the experiments were statistically analysed, by comparing the percent of (+) and (−) eggs in each generation with the subsequent ones, as presented in Tables 1 and 2.

Results and discussions. As is apparent from the analysis of the data from both experiments, in most outbred series the degree of dominant lethality was significant. Yet, the inbred stocks maintained had almost a constant degree of semisterility (54% in PMS and 65% in thalidomide series) without significant differences between the subsequent generations, which itself is a proof for the inheritability of the lethal genes: The main difference observed between the PMS and thalidomide series were in the nature of semisterile rafts, as presented in the Figure. The reason for the higher percent of unembryonated eggs in the thalidomide series, as noted in a previous study[9], might be due to the nature of the lethal genes that affected the death of the embryos at an early blastoderm stage. A further cytological study on the stock maintained, might throw more light on the actual mechanism of this phenomenon.

The genetic basis of dominant lethality is due to induction of chromosomal damage and rearrangements, such as translocations or pericentric inversions, which has been shown in *Culex*[1] as well as other animals[10] to be associated with heritable semisterility.

Due to lack of morphological differences in the chromosomes of both sexes in *Culex*, genetic determination of

1 P. T. McDonald and K. S. Rai, Science *168*, 1229 (1970).
2 K. S. Rai, P. T. McDonald and Sr. M. Asman, Genetics *66*, 635 (1970).
3 R. H. Baker, R. K. Sakai and A. Mian, Science *171*, 585 (1971).
4 H. Laven, Nature *221*, 958 (1959).
5 L. E. LaChance and J. G. Riemann, Mutation Res. *1*, 318 (1964).
6 L. E. LaChance, D. T. North and W. Klassen, *Principles of Insect Chemosterilization* (Eds. C. LaBrecque and C. N. Smith; Appleton-Centu-Crafts, Meredith Corporation, New York 1968).
7 K. S. Rai, *Sterile-Male Technique for Eradication or Control of Harmful Insects* (International Atomic Agency, Vienna 1969), p. 107.
8 J. D. Amirkhanian, Experientia *26*, 798 (1970).
9 E. Jost and J. D. Amirkhanian, Mutation Res. *13*, 49 (1971).
10 G. D. Snell, E. Bodemann and W. Hollander, J. Expl Zool. *67*, 93 (1934).
11 B. M. Gilchrist and J. B. S. Haldane, Hereditas *33*, 175 (1947).

Table I. Comparison of the degree of semisterility induced in different generations, when 1,3-propanediol, dimethanesulphonate (PMS) was used as mutagenic agent

Generations compared	Degree of freedom	Embryonated eggs (+) (p)	Unembryonated eggs (−) (p)	(+) and (−) or semisterility (%)
Control (%)				8.42
F_1 and F_2[a]	15	<0.05	<0.01	F_1 76.59
F_2[a] and F_3	22	n. s.[b]	n. s.	F_2 43.47
F_3 and F_4	23	<0.01	n. s.	F_3 47.03
F_4[a] and F_5	12	0.05	n. s.	F_4[a] 33.78
F_5 and F_6	13		n. s.	F_5 68.43
F_6 and F_7	16	n. s.	n. s.	F_6 43.90
F_7 and F_8	13	n. s.	n. s.	F_7 52.30
F_8				F_8 56.74

[a] Outbred generations (male progenies of the treated groups outcrossed with normal virgin females). [b] n. s., not significant.

Table II. Comparison of the degree of semisterility induced in different generations, when α-phtalimidoglutarimide was used as mutagenic agent

Generations compared	Degree of freedom	Embryonated eggs (+) (p)	Unembryonated eggs (−) (p)	(+) and (−) or semisterility (%)
Control and F_1	54	< 0.05	< 0.01	F_1 36.6
F_1 and F_2^a	29	< 0.01	n.s.	F_2^a 23.51
F_2^a and F_3	19	n.s.	0.02	F_3 67.41
F_3 and F_3^a	27	< 0.02	n.s.	F_3^a 80.06
F_3^a and F_4^a	33	< 0.01	0.05	F_4^a 65.24
F_4^a and F_4^b	33	n.s.	n.s.	F_4^b 67.38
F_4^b and F_5	33	n.s.	n.s.	F_5 69.57
F_5 and F_6	19	n.s.	n.s.	F_6 67.72
F_6 and F_7	26	n.s.	n.s.	F_7 68.01

[a] Outbread generations. [b] Another outbred generation.

sex is by a single pair of genes: maleness (M/m) being dominant over femaleness (m/m)[11], where M signifies the dominant gene for maleness and m its allelomorph. Thus the reason why in the present experiment only the male mosquitoes from the F_1 progenies of the treated males were tested for semisterility associated with the M/m sex determining factor involved in the translocations. These males with t^M translocations were able to transmit the sterility associated with it to the subsequent generations of the inbred or outbred stocks.

The localized chromosomal anomalies induced in different biological systems by chemical mutagenes[12–14] is, indeed, an encouraging fact for the future mutagenesis studies in mosquitoes, which could be applied for the introduction of specific genetic changes in a species. As suggested by Serebrovsky[15], the population of insect pests could be controlled specifically, by the introduction of specific translocations in the population. With the use of chemical mutagenes, perhaps, such results could be achieved more effectively than by irradiation techniques, which have more random biological effects[16].

The main advantage of introducing semisterile genes in the population lies in the fact that dissemination of the genetic abnormalities would be accomplished further into the population by the survivors of the treated progenies. Thus, the reproductive potential of the insect pests will be reduced without additional release of mosquitoes, or the contamination of the environment by potentially dangerous chemosterilants[17].

Zusammenfassung. Mit dem dominanten Männlichkeitsfaktor M assoziierte Semisterilität wurde chemisch induziert (1, 3-Propandiol-dimethansulfonat und Thalidomid). Männchen mit t^M-Translokationen vererbten die Semisterilität fast konstant über 8 Generationen weiter.

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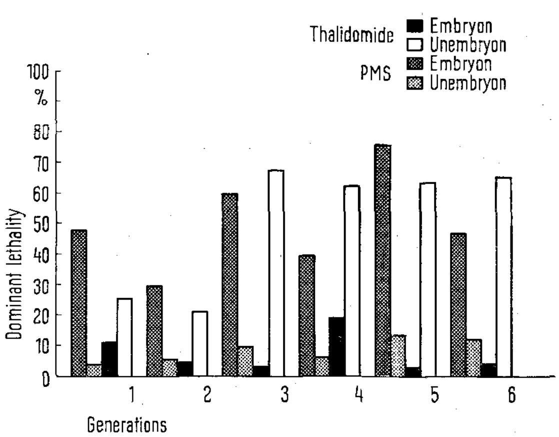

The mean of embryonated and unembryonated eggs in each generation of thalidomide and PMS stock of *Culex pipiens* as calculated from the second generation up to seventh generation.

[12] A. T. Natarajan and M. D. Upadhya, Chromosoma (Berl) *15*, 156 (1964).
[13] O. G. Fahmy, M. J. Fahmy, J. Massasso and M. Ondrej, Mutation Res. *3*, 201 (1966).
[14] E. Bautz and E. B. Freese, Proc. natn. Acad. Sci., USA *47*, 845 (1962).
[15] A. S. Serebrovsky, Zool. Anz. *19*, 618 (1940).
[16] O. G. Fahmy and M. J. Fahmy, Proc. Symp. on the induction of mutations and the mutation process in Prague 1963 (Czechoslovak Academy of Sciences 1965).
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