

As is shown by the cytological examination of the parthenogenesis of the diploid *Artemia salina*, the development starts with irregular meiosis, in that initially 2 meiotic divisions take place and then follows the fusion of the pronucleus and the polocyte I⁶. Thereby a diploid nucleus is produced which starts the cleavage divisions as usual and the development leads to a pure diploidy. These results could be verified through the DNA measurements, because most of the nuclei possess the DNA content of 2c while DNA classes of c and 3c are absent. Because in the tetraploid form the maxima of extinction are shifted to-

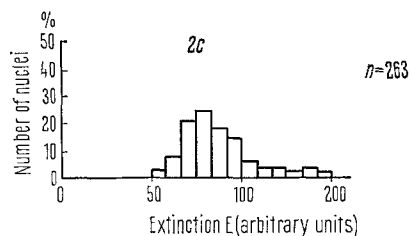


Fig. 4. Frequency distribution of DNA in the cells of the optical ganglia of *Artemia salina parthenogenetica* 2n. The DNA extinction mean is 0.78 ± 0.12 (arbitrary units).

wards 2c, and the composition of the cell material is similar to that of the diploid type, it can be concluded that in the obligatory parthenogenesis of diploid and tetraploid *Artemia* only one type of fusion of the descendants of the meiosis is present and a mosaic of haploid, diploid and polyploid cells is not established. In addition, the transition parthenogenetic 2n to parthenogenetic 4n is in agreement with the relation 1:2 in the DNA content.

Zusammenfassung. DNS-Messungen an Interphasekernen von *Artemia salina parthenogenetica* 2n und *Artemia salina anfigonica* zeigen ein Mengenverhältnis von 1.2:1, während zwischen *Artemia salina parthenogenetica* 4n und *Artemia salina anfigonica* DNS Werte im Verhältnis 2:1 gefunden wurden.

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⁶ R. STEFANI, Riv. Biol. 53, 463 (1960).

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Thalidomide as a Mutagenic Agent in the Mosquito (*Culex pipiens molestus*)

During the past decade several attempts have been made to detect radiation-induced, as well as spontaneously occurring, mutations in mosquitoes: the pioneer experiments of LAVEN¹ and KITZMILLER², with X-ray induced mutations in *Culex*, have played a useful part in the recognition of phenotypic expression of certain mutants in mosquitoes. Nevertheless, the application of inbreeding techniques of CRAIG³ and VANDEHEY^{4,5}, by single-pair matings for mutant isolation and other types of genetic research, imply that there are, perhaps, no proved cases of radiation-induced mutations, at least in *Aedes aegypti*, although a few such strains have been produced by irradiation techniques.

In view of previous findings related to the chromosome injuries produced by thalidomide (α -phthalimidoglutarimide)^{6,7}, it became of interest to test whether it could induce germinal mutations in *Culex*. The present communication presents part of the results of mutagenetic studies with *Culex*, where thalidomide has been used in the production of dominant lethal mutations, semilethals as well as induction of some phenotypic anomalies, the occurrence of which in repeated experiments has led to the presumption that the chemical might be useful in future mutagenesis studies and a possible means of inducing lethal genes in the control of mosquitoes.

Materials and methods. Egg-rafts of *Culex pipiens molestus* from our autogenous laboratory stock, were collected randomly. The larvae from each egg-raft were reared in separate enamel wash-basins. Half of the larvae from each basin were chosen for tests and the other half reared as controls. The newly emerged adult males, which were fed on the drug at prepupal stage, were starved in a less humid atmosphere for at least 24 h and further fed on the drug mixed with 10% sugar for 72 h. They were then crossed with normal virgin females from the same egg-raft, which were segregated according to

size (female pupae larger than male pupae). Each egg-raft obtained from a parental pair (P) was separated and the F₁ adults which emerged were mated among themselves. The F₂ rafts were isolated and adults examined for any phenotypic deviations from the normal. For each experiment 5 lines were maintained, which were reared up to F₃ generation. At F₃ only 5 rafts from each line were tested for possible phenotypic changes. The results are demonstrated in Table I. The variant phenotypes in the F₁, F₂ and F₃ generations were scored as genetic mutants if they were similar in appearance to the previously established mutants. To be certain that they were not phenocopies, whenever possible they were tested for heritability by sib-matings for subsequent generations.

In the F₃ generation of the treated group, phenotypically female mosquitoes with partially transformed male appendages, together with gynandromorphs (sex mosaics), also mosaics with morphological traits not related to sex such as (Kuf/+) were obtained in high frequencies, which is in agreement with the results obtained with chemical mutagens as demonstrated by AUERBACH⁸.

The occurrence of fused (fu) with knobbed protrusions on antennal segments and (SpW) in high frequencies of

¹ H. LAVEN, Proc. R. ent. Soc., Lond. 31, 17 (1956).

² J. B. KITZMILLER, Expl. Parasitol. 7, 439 (1958).

³ G. B. CRAIG JR. and R. C. VANDEHEY, Ann. ent. Soc. Am. 55, 47 (1962).

⁴ R. C. VANDEHEY, Ann. ent. Soc. Am. 57, 488 (1964).

⁵ R. C. VANDEHEY, Mosquito News 29, 183 (1969).

⁶ G. GIACIMELLO, P. MALATESTA and G. QUAGLIA, Nature 201, 940 (1964).

⁷ J. D. AMIRKHANIAN, Proc. R. microsc. Soc., Lond. 1, 153 (1966).

⁸ C. AUERBACH, J. M. ROBSON and J. G. CARR, Science 105, 243 (1947).

different series of experiments repeated with thalidomide, is a probable indication of an apparent directional tendency of these mutations. The reproduction of some of the mutants by thalidomide, similar to those produced by other investigators, when X-ray had been used as the mutagenic agent^{1,2,9}, is an evidence for the radiomimetic



Fig. 1. A semisterile female mutant (patch-winged) (pW), with extremely reduced wing patches, without halteres, usually with asymmetric legs.



Fig. 2. Male antenna with fused (fu) and compressed antennal segments and a knobbed protrusion at distal end of the antenna.

property of the chemical. Furthermore, the disturbances in the normal sex ratio of the progenies of the treated groups, also semisterility and non-viability observed in the sex distorted families, led us to assume that they were caused by translocations, as reported recently by LAVEN¹⁰ on the probable effect of translocations in the distortion of the normal sex ratio in *Culex*. Although it would be premature to conclude on direct relationship of sex distortion with translocations in *Culex*, anomalous chromosomal associations were detected in the reproductive cells of the treated groups, prepared by the usual staining methods as well as the method described previously¹¹. A detailed study of the cytological findings will be reported elsewhere.

To determine dominant lethal mutations induced by thalidomide, at F₁ on the mosquitoes of the same stock, the adult males were treated by feeding for about 72 h before being crossed with normal virgin females from the same raft. The eggs obtained at F₁ were tested for hatchability, embryonation, etc. as shown in Table II.

Since thalidomide is sparingly soluble in water, a suspension of excess chemical in water (saturated solution) was used. Due to the fact that thalidomide reacts with water, undergoing hydrolysis, and is equally converted to its metabolites at pH 7 and 37°C in 24 h¹², freshly prepared suspensions were used at 12 h intervals for all treatments. The control and the treated larvae were fed

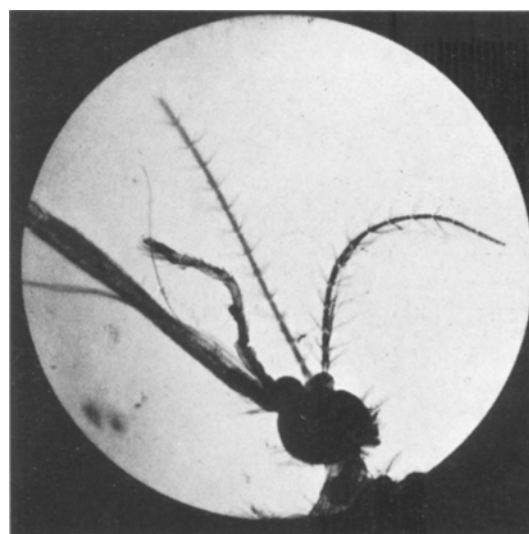


Fig. 3. Morphologically female, with left male antenna. The left palpus 5-jointed (5-j).



Fig. 4. Female head with dumbbell-shaped palpi (db) and with only one antenna.

⁹ R. PAL and B. S. KRISHNAMURTHY, *Nature* 184, 658 (1959).

¹⁰ H. LAVEN, *Nature* 221, 958 (1969).

¹¹ J. D. AMIRKHANIAN, *Stain Technol.* 43, 3 (1968).

¹² H. KEBERLE, J. W. FAIGLE, H. FRITZ, F. KNUSEL, P. LOUSTALOT and K. SCHMID, *Biol. Council Symp. on Embryopathic Activity of Drugs* (Eds. J. M. ROBSON, F. SULLIVAN and R. L. SMITH; Churchill Ltd., London 1965), p. 210.

Table I. Description of the morphological anomalies, numerical data and other characteristics related to the effect of thalidomide in *Culex pipiens molestus*

Symbol for parental pairs (P) ^a	No. of F ₁ larvae and pupae died up to emergence	No. of semilethal adults recovered in F ₁ ^b	No. of adults recovered in F ₁		No. of F ₂ rafts		No. of F ₂ adults examined	No. and description of abnormalities in F ₂ and F ₃	No. of proven mutants per mosquito
			Male	Female	Obtained	Examined	Examined		
A ₁	10	2	53	9	8	8	212	<u>fu</u> , 6♀; <u>SpW</u> , 1♂; kuf, gyn, 3	
A ₂	12	2	72	1	No rafts recovered			—	
A ₃	9	6	24	20	8	7	492	<u>fu</u> , 3♀, 2♂; <u>SpW</u> , 2♀; wa, 1♂; 5j, 2♀	
A ₄	4	2	59	2	2	2	72	<u>fu</u> , 1♀; <u>fu</u> , with knobbed protrusions, 2♂, 1♀	2.1/mosquito
A ₅	6	3	52	4	4	3	169	<u>fu</u> , 1♂; <u>fu</u> , 2♀; hg, 1♂; zwi, 1; min, 4♀	
C ₁	3	—	26	39	12	8	295	ba, 1; Ant, 1♂	
C ₂	1	—	36	41	10	9	499	<u>pfl</u> , 1♀; Wa, 1♂	
C ₃	—	—	34	37	10	10	571	ba, 1♀; Rap, 1♂	
C ₄	4	—	49	38	9	9	396	—	0.4/mosquito
C ₅	7	—	31	35	13	12	623	<u>Rap</u> , 1♂; Ant, 1♂	

^a A, adult males treated 24 h after emergence; C, controls. ^b (pW), patch winged, adult females with extremely reduced black wings with shallow and occasionally asymmetric legs and without halteres (Figure 1). The designated mutants underlined have been tested for heritability. The semilethals and lethals observed in F₁ generations also anomalies observed in the larval or pupal stage were not counted in the final analysis of mutants per mosquito.

Table II. Comparative study of dominant lethal mutations induced in F₁ egg rafts of the treated parental males (P) with the controls

	No. of eggs examined	No. of eggs embryonated, unhatched	% Dominant lethality	No. of eggs unfertilized	% Unfertilized eggs	No. of eggs hatched	% Hatchability
Test	4196	937	22.33	503	11.98	2756	65.72
Control	6700	308	4.59	167	2.49	6225	92.91

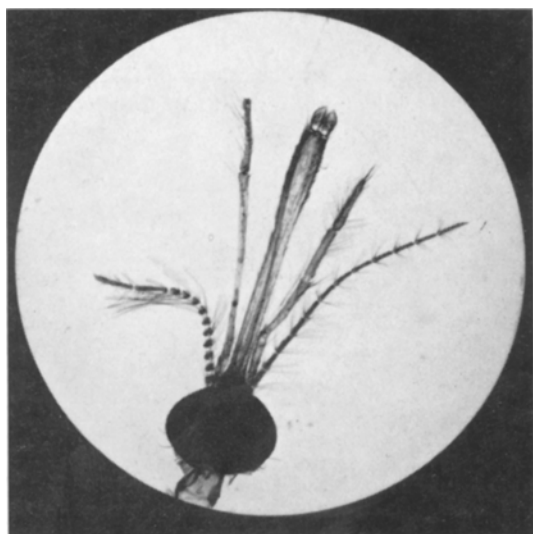


Fig. 5. Morphologically female, with left male antenna, right female antenna and right palpus 5-jointed (5-j).

on 'Bemax' cereal with yeast and 'Bemax' with yeast in the suspension of the chemical, respectively. The control and the treated adults were fed on 10% sugar and 10% sugar with chemical, respectively. The adults were maintained in the laboratory temperature of $27 \pm 2^\circ\text{C}$ and 80% relative humidity.

At molecular level, certain chemical reactions are responsible for the production of mutant genes. It has been shown that embryotoxic and teratogenic properties of thalidomide are determined by the phthalimide moiety of the molecule¹³. Also the evidence in favour of the concept of direct binding effect of thalidomide through acylation mechanism with DNA, RNA, histones and phospholipids^{14,15}, suggests that it is perhaps an acylating radio-mimetic mutagenic agent.

¹³ H. SCHUMACHER, D. A. BLAKE and J. R. GILLETTE, Fedn. Proc. 26, 730 (1967).

¹⁴ H. SCHUMACHER, D. A. BLAKE and J. R. GILLETTE, J. Pharmac. exp. Ther. 160, 201 (1968).

¹⁵ C. LUTWAK-Mann, K. SCHMID and H. KEBERLE, Nature 214, 1018 (1967).

Results. It could be concluded that the occurrence in higher frequencies of some of proved mutants in the treated groups (2.1 per mosquito) in comparison with the controls (0.4 per mosquito); the distortion in the normal sex ratio; the reversion in the normal hatching sequence; the higher frequency of dominant lethal mutations; the lethals and semilethals obtained in the F_1 generation of the treated parental progenies; also the mosaic phenotypes with gross morphological aberrations induced in head appendages, proved to be the most important criteria for the mutagenicity of thalidomide in *Culex*¹⁶.

Zusammenfassung. Nach Behandlung von Männchen von *Culex pipiens molestus* mit Thalidomid konnten im Vergleich zur Kontrolle höhere Raten an phänotypischen Anomalien, Letalmutationen, Semiletalmutationen und

einige geprüfte Mutationen in der F_1 -, F_2 - und F_3 -Nachkommenschaft nachgewiesen werden.

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An Attempt to Induce Crossing-Over in Males of *Drosophila melanogaster* with Ovarian Extract

Crossing-over occurs with a very low frequency in *Drosophila* males but occurs freely in females. This observation encouraged REDDY, REDDY and RAO¹ investigate if there is any substance in the female germinal tissue, i.e. ovaries, which might induce crossing-over in males. They prepared an extract by homogenizing several *Drosophila* ovaries and tested this for the induction of crossing-over in the males. These experiments were performed both with fresh and boiled extract. The fresh extract proved to induce a significant number of cross-overs.

An attempt to confirm these interesting observations was made by SINGER et al.² Here, the fresh extract induced only 8 cross-overs in 68,974 flies, while the controls yielded 7 cross-overs in 19,170. These authors thus failed to confirm earlier results where 103 cross-overs were recovered in 29,147 flies. Therefore, it was thought worthwhile to repeat these experiments to support one or other of the observations.

A 3rd chromosome double recessive stock, *se cu*, and a wild stock Oregon-R, were used. Females heterozygous

for these 2 strains were mated to similar males and were dissected in saline. Nearly 400–500 ovaries were dissected and were homogenized with sand. Saline was added to make the volume of the homogenate to 1 ml. The homogenate was centrifuged twice at 4000 rpm for 5 min. The supernatant was injected into the heterozygous males with an AGLA brand micrometer syringe using a fine glass capillary. Fresh extract was injected into males in 2 sets at a rate of 0.2 μ l and 0.4 μ l per male. The extract was then boiled and similar injections were performed. The injected males were individually mated to 5–7 *se cu* virgins for 2 days per brood. 7 such broods were cultured. Scoring was not made for the first 2 broods. The results are given in the Table.

No cross-overs were observed in these experiments except in the one with boiled extract where only 2 recombinants appeared in 6252 flies. The conclusion is that the *Drosophila* ovarian extract does not induce crossing-over in the males.

Zusammenfassung. Von zwei früheren, sich widersprechenden Befunden wird einer gestützt: Ovarial-extrakte von *Drosophila* lösen in Männchen keine «crossing-over» aus.

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Induction of crossing-over in *Drosophila melanogaster* males treated with ovarian extract

Treatment	Dose per male	No. of males test-crossed	Total progeny	Total cross-overs
Saline	0.2 μ l	26	16,280	0
Fresh extract	0.2 μ l	16	8,482	0
Fresh extract	0.4 μ l	19	8,168	0
Boiled extract	0.2 μ l	20	7,726	0
Boiled extract	0.4 μ l	17	6,252	2

¹ O. S. REDDY, G. M. REDDY and M. S. RAO, *Nature* 208, 203 (1965).

² K. M. SINGER, A. CHOVNICK, D. T. SUZUKI, D. BAILLIE and D. HOAR, *Nature* 214, 503 (1967).

³ The author wishes to express her gratitude to Dr. P. G. KALE for guidance and to Prof. S. P. RAY-CHAUDHURI for the facilities provided.