Table II. Centromeric index of 8 species studied

Pair of chromosomes	1	2	3	4	5	6	7	8	9	10	11
B. marinus	0.45	0.50	0.40	0.44	0.50	0.44	0.50	0.50	0.50	0.46	0.40
B. paracnemis	0.40	0.50	0.37	0.34	0.44	0.39	0.50	0.50	0.50	0.50	0.40
B. ictericus	0.50	0.45	0.38	0.33	0.40	0.40	0.44	0.50	0.42	0.50	0.50
B. arenarum ♂	0.48	0.36	0.41	0.48	0.45	0.50	0.47	0.45	0.45	0.45	0.42
B. arenarum ♀ (somatic chromosomes)	0.47	0.39	0.42	0.48	0.44	0.48	0.45	0.44	0.45	0.44	0.43
B. spinulosus spinulosus	0.41	0.45	0.50	0.33	0.40	0.39	0.46	0.44	0.45	0.50	0.48
B.g. d'orbigny	0.47	0.45	0.33	0.45	0.49	0.45	0.45	0.45	0.47	0.50	0.50
B. crucifer	0.46	0.45	0.40	0.45	0.47	0.40	0.48	0.50	0.48	0.48	0.50
B.g. fernandezae	0.46	0.49	0.33	0.46	0.40	0.46	0.45	0.45	0.45	0.50	0.45

Yosida[22] described an XY pair in the male *Hyla arborea*, Weiler and Ohno[23] found heteromorphism in the female *Xenopus laevis*, Morescalchi[8] described them in *Discoglosus pictus*, and Manna and Bhunya[24] reported heterogamety in the *B. melanostictus* female. Saez et al.[1-4] studied in detail the existence of the sex chromosomes in amphibia anura specially in the *B. arenarum* species. They concluded that the presumed sex chromosomes found in different species of amphibia by other authors were only bivalets that had a different behavior. In this work, it was pointed out that this bivalent element and behavior are only common chromosomes which can have different shapes, sizes and affect different chromosomes of the same individual. Therefore we postulate that there is not yet sufficient data available to prove the existence of the sex chromosomes, inspite of the presence of differences in size of the members in a somatic pair of homologues[25].

Resumen. Se estudiaron los cariotipos de ocho especies de Bufonidae sudamericanos: *B. arenarum, B. ictericus, B. paracnemis, B. marinus, B. crucifer, B. granulosus d'orbignyi, B. granulosus fernandezae* and *B. spinulosus*. En todas las especies se encontraron $2n = 22$ chromosomas. No se encontró en el macho, ningun par heteromórfico, ni bivalente con caracteristicas y comportamiento que indicara la presencia de cromosomas sexuales.

N. Brum-Zorrilla and F. A. Saez

Departamento de Citogenética,
Instituto de Investigación de Ciencias Biológicas,
Av. Italia 3318, Montevideo (Uruguay), 6 October 1970.

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Inherited Semisterility for Control of Harmful Insects. I. Productions of Semisterility due to Translocation in the Mosquito, *Culex pipiens* L., by X-Rays

Inherited semisterility, i.e. the inviability of approximately half of the gametes of an organism and as a consequence half of the zygotes, was observed for the first time already 65 years ago as a natural phenomenon in different plant species[1]. Cytological investigations on other plant species provided the clue to the cause of semisterility[2]. Semisterile individuals of plants or animals are heterozygotes for reciprocal chromosomal translocations or pericentric inversions.

Soon after the epoch-making discovery of the mutagenic activity of X-rays by Muller[3], it was recognized that ionizing irradiation produces to a great extent also chromosomal abberrations like translocations and inversions. Several authors have studied the production of translocations in *Drosophila* under quantitative and qualitative aspects[4-6]. A very interresting side line of these investigations on translocations was the artifical composition of a *Drosophila* strain with 2 different translocations, which was reproductively isolated from normal strains[7].

Already in 1940, Serebrovsky[8] suggested the release of individuals with translocations into a natural population as a new means for pest control. However his paper remained unknown and has not stimulated any efforts for pest control by the mechanism suggested. Without knowing the paper of Serebrovsky, the present author[9] and Curtis[10] have recently and independently again suggested translocations and the ensuing semisterility as a possibility for control of harmful insects. We were led to this new approach after the first successful eradication of the mosquito species *Culex fatigans* in a Burmese village through the preexisting mechanism of cytoplasmic incompatibility[11]. This is a very rare genetical mechanism, only known from not more than 3 different groups of insects (4 species). Therefore we started to explore the possibility of producing translocations and semisterility in different mosquito species.

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In this series of communications some basic data of these investigations will be presented. Part of these data have already been published in summary form in another paper[12]. However, a more detailed account seems to be appropriate for 2 reasons. First, the application of translocations in laboratory experiments[13] and recently for the first time in the field against a mosquito species has proved that this genetical mechanism can suppress a natural population to a very low level[14]. Second, in several laboratories attempts are made to produce translocation systems in other insect species and these have been partly successful[15-17]. The data presented here could probably promote research of the same type with other insects harmful for man, domestic animals or crops.

The model insect, with which these investigations have been conducted, is the common mosquito *Culex pipiens*. It can easily be reared and maintained, has a rather high number of offspring per female and can be crossed in single pairs. In all experiments for the induction of chromosomal translocations, males 2–3 days after emergence from pupae (P generation) were irradiated with X-rays and mated to virgin females. Surviving offspring (F_1) from these crosses, males as well as females, were again outcrossed to normal animals, this time in single pairs. Any reduced F_2 offspring from these crosses (semisterility) indicates the production of a translocation or of a pericentric inversion in one sperm of the P males. In order to verify the nature of the chromosomal aberration, such offsprings were conducted through 2–3 more generations of outcrossing or backcrossing. In this way the kind of translocation, whether sex-linked or autosomal, can easily be confirmed. Part of the lines with inherited semisterility have also been investigated cytologically and in most cases the presence of translocations was confirmed.

The first effect of irradiation is the production of gross chromosomal aberrations which lead to lethality of part of the F_1 offspring. The extent of this so-called dominant lethality is correlated with the irradiation dosage. Figure 1 shows the degree of dominant lethality for the mosquito *Culex pipiens* for various X-ray dosage from 500–8000 R (curve A). Similar dose-response-curves have been found in other mosquitos[18] and other dipteran insects[19]. Inspection of the non-hatching eggs reveals that most of them are not embryonated (curve B). Obviously the dominant lethal factors produced by the irradiation express themself during the first cleavage divisions leading to the very early death of the embryo. The same event has been found in house flies after the application of chemosterilants[20].

Only about 60% of the normally hatching eggs develop to normal adults of both sexes. The death of part of the larvae, pupae or adults is very likely also due to dominant lethals having their phase of activity predominantly in the first (25% of death) or fourth (28%) larval instar and in the pupal stage (37%).

Every animal in this F_1 generation is derived from one of the irradiated sperms of the P males and a normal egg from the mother. If the sperm carried a chromosomal aberration like a translocation, the resulting animal should be heterozygous for this aberration. As a consequence it should be semisterile owing to the fact that translocation heterozygotes produce partly gametes with a full complement of genes and partly such with deficiencies for certain genes and duplications for other genes. As a consequence part of the F_2 offspring will die, when such translocation animals are outcrossed to normal partners. That is the phenomenon of semisterility.

In several experimental series with different dosages of irradiation between 500 and 5000 R we have tested all available F_1 animals of both sexes for semisterility. Figure 2 shows the percentage of semisterile individuals for the different dosages applied. With dosages between 500 and 3000 R, only between 10 and 20% of the surviving animals are semisterile. With higher dosages between 3500 and 5000 R, the percentage increases faster up to 50% with 5000 R. This increase with higher dosages can be expected as a result of the increase of simultaneous breaks in different chromosomes.

These values for total translocation production in *Culex pipiens* after irradiation of mature sperm appear extremely high in comparison with values obtained from other insects. Unfortunately the data of only very few experiments with *Drosophila* can be compared directly with our results, because in both cases total production of translocations has been measured. In other experiments with *Drosophila* the authors have screened only for certain types of translocations. There is also a difference between our experiments and the ones with *Drosophila* in the screening technique used. We have tested all F_1 offspring for semisterility, the *Drosophila* workers have used the change of linkage groups as indicator for translocations.

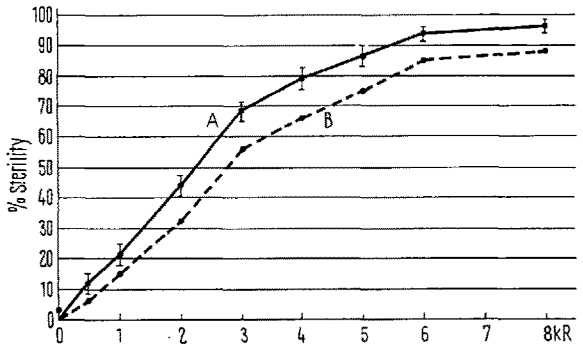

Fig. 1. Percentage of sterility in *Culex pipiens* due to dominant lethal factors produced by X-rays of different dosages. Curve A: Percentage of non-hatching eggs. Curve B: Percentage of eggs without visible embryonic development.

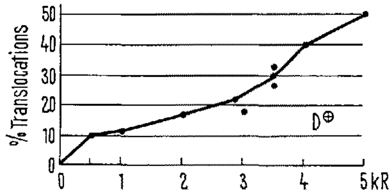

Fig. 2. Percentage of translocations in viable F_1 offsprings of *Culex pipiens* in correlation to dosage of irradiation. D = percentage of translocations in *Drosophila*.

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In *Drosophila melanogaster* translocations of all possible kinds were observed in 17.92% of the F_1 cultures ($N = 1000$) after irradiation of males with 4452 R of X-rays[21]. The majority of these translocations (12.36%) were such ones between the chromosomes 2 and 3. This last figure compares well with more recent data for 2–3 translocations. With a dosage of 4000 R of X-rays 14.39% were recovered[22] and with 3360 R X-rays 14.3%[23].

These values for total translocation production in *Drosophila* are only half or less of the values we have observed with *Culex*. It might be mentioned here that also in 2 other mosquito species, i.e. *Culex tritaeniorhynchus* and *Aedes albopictus*, the figures were of the same order of magnitude.

It is difficult to give an explanation for the exceptionally high values for translocation production in mosquitos. One possibility could be that the chromosomes in the sperm of mosquitos are in such a peculiar spatial position to each other that translocations can occur much more easily than in *Drosophila*. The very long and slender sperm head in mosquitos seems to indicate a position of the chromosomes parallel to each other. But this idea has to be discarded because the actual exchange of chromosomal segments occurs probably during karyogamy when the chromosomes have the possibility of free movement.

Another explanations seems to be more likely and is offered here as a working hypothesis. The mitotic chromosomes of *Drosophila* have a total length of 7.6 µm (I = 1.8 µm; II = 2.6 µm; III = 3.2 µm; IV, the dotlike chromosome, not taken into account), but the total length for *Culex* is 21.1 µm (I = 5.6 µm; II = 7.4 µm; III = 8.1 µm). Accordingly the total chromosome length of *Culex* is almost 3 times (2.8) that of *Drosophila*. The observed values of translocations show almost the same numerical correlation. Therefore the number of translocations which can be produced in an animal with a certain dosage of irradiation possibly depends on the total length of the chromosomes. This can only be taken as a very crude and approximate statement of a hypothesis, as it does not take into consideration other factors like volume and density of the chromosomes. Confirmation or rejection of the hypothesis needs comparative data from other objects besides *Drosophila* and *Culex*. It can be expected that such data become available in due time with the extension of the semisterility principle for control of other harmful insects[24].

Zusammenfassung. In Anbetracht der theoretischen und in einem Freilandexperiment bereits erwiesenen Möglichkeit der Bekämpfung von Schadinsekten durch Freilassung semisteriler Tiere wurde die Produktion von Translokationen und damit verbundener Semisterilität bei der Stechmücke *Culex pipiens* untersucht. Die Totalrate der erzeugten Translokationen liegt bei Stechmücken im Vergleich zu *Drosophila* auffallend hoch. Es besteht offenbar eine Korrelation zwischen der Gesamtlänge der Chromosomen und der Translokationsrate.

H. LAVEN and E. JOST

Institut für Genetik, Johannes-Gutenberg-Universität,
D-65 Mainz (Germany), 6 January 1971.

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The Specific Localization of Diethyl Sulphate-Induced Second Chromosome Recessive Lethal Mutations in *Drosophila melanogaster*

The current interest in the specificity for gene mutation shown by chemical and physical mutagens on eukaryotic microbial systems[1], has encouraged us to look for specificity in the distribution of recessive lethal mutations along the second chromosome of *Drosophila* after treatment with diethyl sulphate.

Material and methods. Newly-hatched wild type (Oregon-K) *Drosophila* males were treated with 0.5% diethyl sulphate for 48 h by an adult feeding method[2]; they were then, individually mated to 2 CyL^4/Pm females, for 3 days, as the first mating for the detection of second chromosome recessive lethal mutations[3]. The sampling procedure used allows the recovery of germ cells which were present as spermatozoa over the period of the treatment, and consequently lethals arising in the brood sample from individual males, do so independently of one another, i.e. they do not occur by clonal origin. However, in order to be sure of the independent origin of the lethals, only 1 lethal was taken from anyone male. Moreover, to eliminate the presence of spontaneous lethals which might have been present before treatment, males which gave approximately equal or greater numbers of lethal to non-lethal cultures in the F_3, were not used.

Results and discussion. The treatment induced 225 lethals in 884 chromosomes tested from 91 males, i.e. 25.4% second chromosome recessive lethal mutations. 47 of the 91 males yielded 1 or 2 lethals, but it was possible to examine only 38 out of the 47 lethals chosen owing to loss during maintenance. Semi-lethal mutations were excluded.

Tests of the 38 lethals for allelism in all possible pairwise combinations (703 separate crosses) showed that 18 of the lethals were non-identical (non-allelic); all these crosses showed the usual 2:1 phenotypic ratio expected from non-allelic crosses[3]. The majority of the remainder of the lethals behaved, when crossed with certain others, in a manner expected from a series of overlapping deficiencies, i.e. a *X* b and b *X* c show allelism, whilst a *X* c show non-allelism. 4 of the lethals showed identity by the allelism tests. These latter 20 lethal stocks were again tested for allelism 3 more times with the same results, but now including also reciprocal crosses to exclude the type of synthetic lethal reported by BATTEN and THODAY[4]. Thus, the lethals

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