de Mazar Barnett, Beatriz K. Comisión Nacional de Energía Atomica, Argentina. Recessive lethals induced in sperm by X rays, alkylating agents and combinations of both.

A comparison was made of the frequencies of sexlinked recessive lethal mutations induced in mature sperm by (a) X rays, (b) two alkylating agents and (c) a post-irradiation 30 minutes after administration of the chemicals. The alkylating agents used, a nitrogen mustard (NITROMIN) and a polyethylene-imine (THIO TEPA)

were injected intra-abdominally in a 0.4% saline solution and the irradiation dose was 800r in all cases.

For the experiments, one day old Oregon-R males were treated and 24 hours later mated to "Basc" females, left for one day and then discarded. The females were allowed to oviposit for two additional days.

Standard recessive lethal tests were made with the  $F_1$  females. The results obtained in the  $F_2$  are shown in the table. Taking into consideration these results, as well as others from combined treatments carried out at different intervals, the effect of combined treatments with alkylating agents and X-rays is at least additive with the nitrogen mustard but not with the polyethylene-imine, in which case no combined treatment has ever induced a recessive lethal frequency as high as that induced by the chemical alone.

	Alkylating agent		X rays		Alk. a. X rays 30 m interval	
	No. chro	m. %let	No. chrom	. %let.	No. chro	m. %let.
NITROMIN (7×10 <sup>-2</sup> M)	788	1.14	1084	1.92	951	4.94
THIO TEPA (3x10 <sup>-2</sup> M)	983	12.00	1084	1.92	693	6.63

de Mazar Barnett, Beatriz K. Comisión Nacional de Energia Atomica, Argentina. Extreme variability in oviposition rate.

An attempt is being made to establish the frequency of dominant lethals induced in mature or nearly mature oocytes of females treated with various combinations of X-rays and chemical mutagens, in the hope of elucidating previous data

on recessive lethals induced by these agents. Individual females were mated in vials containing a special medium for egg counts (see note in this issue DIS) and were allowed to lay eggs for 36 hours (this time had to be used since it was the period used previously for the recessive lethal tests). It was found that a variable proportion of the females did not lay eggs at all during the 36 hour period (though most of them did finally lay eggs later). This was true of all groups including the controls.

In addition, there was an extreme variation in the number of eggs laid by the individual females, again in all groups. In some treatments, the range was from 1 to 100, in others 1 to 50, etc. and the distribution of egg number was rarely a normal one.

Schneider, Imogene. Yale University. Inadvisability of using the raft technique for Drosophila organ culture. In a recent review, the observation was made that although insect organs have been cultured in hanging drops and on solid media for in vitro differentiation studies, there has been no report in the literature of using the rayon raft

technique for this purpose, the latter being a very common practice in vertebrate organ culture (M. Martignoni in <u>Insect Physiology</u>, Oregon State Univ. Press, 1963).

This technique was attempted a number of times in culturing <u>D</u>. <u>melanogaster</u> organs (cephalic ganglia with attached eye-antennal discs, salivary glands, testes and ovaries) using not only rayon rafts but also rafts of nylon monofilament cloth, perforated cellophane and millipore filters. Regardless of the material which served as the raft, the end result was unsatisfactory. Except for testes and ovaries from young third instar larvae, all the above-mentioned

organs, whether from third instar larvae, prepupae, or pupae, immediately flattened out when placed on such rafts. After 24 hours in culture most organs were so distorted that it was difficult to visualize their original outlines. None of the organs survived as long as their controls in hanging drops, nor was there, with few exceptions, any significant differentiation. Millipore filter rafts had one further disadvantage in that it was extremely difficult to see the explants against the solid white background. Placement of Drosophila organs at an air interface within the culture chamber should therefore be avoided since such an arrangement promotes neither long survival nor extensive differentiation and as such is decidedly inferior to complete submersion of explants in a liquid medium.

 $\frac{\text{Ayala, Francisco J.}}{\text{Development of incipient sexual isolation}}.$  Development of incipient sexual isolation between laboratory populations.

Two strains of  $\underline{D}$ .  $\underline{birchii}$  from Cairns, Australia, and from Popondetta, New Guinea (Ayala, 1965a), were crossed in both directions. Two populations were started with 150 pairs of  $F_1$  hybrids each, one being maintained at  $25^{\circ}$  C and the second at

 $19^{\circ}$  C. The technique of maintaining the populations has been described elsewhere (Ayala, 1965b). After 48 weeks of "natural" selection (about 16 generations at  $25^{\circ}$  and 10 generations at  $19^{\circ}$  C) samples were taken from both populations, and crossed to each of the parental strains in the two possible directions. The test was made by placing  $10\phi\phi$  and  $10\phi\phi$  together in a 1/2 pint bottle for 10 days, and then the females were dissected and examined for presence of sperm. The results are given in table 1. (The control crosses give 90% or more insemination). The two hybrid

Table 1: Sexual preferences between two hybrid experimental populations and their parental strains.

Cross	çç tested	çç inseminated	Per cent inseminated
Hybrid population 25°x Cairns	64	14	22
Hybrid population 25°x Popondetta	57	26	46
Hybrid population 19°x Cairns	55	40	73
Hybrid population 19° x Popondetta	60	31	52

populations show a similar moderate degree of sexual isolation with the Popondetta parental strain. However their behavior with the Cairns parental strain is strikingly different, the  $25^{\circ}$  population showing fairly high isolation and the  $19^{\circ}$  very little. The difference in isolation of the  $25^{\circ}$  and  $19^{\circ}$  populations with Cairns is highly significant ( $\chi^2=30$ , P<.001), and the difference between the behavior with Cairns and with Popondetta is significant at P<.01 for the  $25^{\circ}$  hybrid population and at P<.05 for the  $19^{\circ}$  population. The two populations have, then, evolved different degrees of sexual isolation with respect to the parental strains. Since the populations were kept completely separated, sexual isolation has evidently arisen as a by-product of genetic divergence.

Literature: Ayala, F. J. 1965a. Sibling species of the <u>Drosophila serrata group</u>. Evolution, in press. Ayala, F. J. 1965b. Relative fitness of populations of <u>Drosophila serrata</u> and Drosophila birchii. Genetics, in press.

Ayala, Francisco J. Columbia University. Improvement of fitness in experimental populations.

Two experimental populations, one maintained at  $25^{\circ}$  C and the second at  $19^{\circ}$  C, were started with 150 pairs of founders taken from mass culture stocks of the Popondetta strain of <u>D</u>. <u>serrata</u>. The technique of maintaining the populations has

been described elsewhere (Ayala 1965), the relevant factor being that strong competition exists