

STUDIES OF SIBLING *Drosophila* SPECIES FROM LAGUNA VERDE, VERACRUZ, MEXICO. III. EFFECTS OF RADIATION ON VIABILITY

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ABSTRACT

The sibling species *Drosophila melanogaster* and *D. simulans* were collected from two sites at Laguna Verde, Veracruz, Mexico. Isofemale lines were established from each species from both sites. Eggs of each isofemale line were exposed to acute γ -radiation doses of 4.0, 6.0 or 8.0 Gy from an external source, and then placed in vials. Non-irradiated eggs served as a control. Each vial was scored for the number of adults produced. For the flies collected at Site-I, *D. melanogaster* was superior in viability to *D. simulans*. However, for the flies collected at Site-II, there was no significant difference in egg to adult survival between the species. A comparison of irradiated and control groups of each species from the various collections showed that in both collections made in July there was a significant reduced viability of irradiated groups, but this difference was completely absent in the collection made in January. A comparison of the control groups of each species from the various collections showed that Site-I *D. melanogaster* were superior to those from Site-II. For *D. simulans* collected in July, Site-I flies showed significantly greater viability than Site-II flies. However, this difference disappears when one compares collections made at opposite seasons of the year. This complex situation involving both populational and seasonal differences in viability is discussed.

RESUMEN

En la localidad de Laguna Verde, Veracruz, México, se colectaron las especies fraternas *Drosophila melanogaster* y *D. simulans* en dos sitios. A partir de las hembras colectadas se establecieron isolíneas, los huevos de cada una de ellas fueron expuestos a dosis agudas de radiación gamma 4.0, 6.0 y 8.0 Gy en una fuente externa. Se sembraron en frascos homeopáticos y posteriormente se contó el número de descendientes. De las moscas colectadas en el Sitio I la viabilidad fue

superior en *D. melanogaster*, mientras que en el II no hubo diferencia significativa entre las dos especies. Cuando se compararon los grupos irradiados de cada una de las especies con el testigo en las diferentes colectas, se encontró que en las realizadas en julio hubo una reducción significativa de la viabilidad en los grupos irradiados, mientras que en la muestra de enero no se observó esa diferencia. Al comparar los grupos testigo de cada especie de las diferentes muestras se observó que *D. melanogaster* del Sitio I presentó mayor viabilidad que la del II. Para *D. simulans*, colectada en julio la viabilidad fue significativamente más alta en el Sitio I. Sin embargo, esta diferencia desapareció cuando se compararon las colectas hechas en las estaciones opuestas del año. Se discute esta compleja situación que involucra diferencias poblacionales y estacionales en la viabilidad.

INTRODUCTION

Those species that are reproductively isolated from one another but are morphologically very similar, namely, sibling species, are of great interest to many population geneticists. A number of investigations have been conducted to determine whether they are also very similar in their biochemistry (Choudhary and Singh 1987), physiology (McKenzie and Parsons 1974), or behavior (Hoffman 1987). The findings of such studies often help explain differences in species' frequencies associated with particular localities and with seasonal or other environmental changes. One pair of sibling species *Drosophila melanogaster* and *D. simulans*, has been widely studied due, at least in part, to the fact that they are cosmopolitan in distribution and commensal with humans. These species are the focus of this project as well.

The present paper deals with the effects of radiation on viability, namely egg to adult survival. Our interest in radiation sensitivity stems from the fact that not only are the long term, multi-generation effects of radiation a consequence of the induction of mutations, but even short term, life-time effects are often the result of damage to the individual's genome. Viability is used as the criterion by which to measure radiation sensitivity because egg to adult survival represents the sum of challenges that must be overcome before sexual maturity and reproduction can be achieved.

We have collected and studied the *Drosophila* at two sites in the area of Laguna Verde, Veracruz, Mexico. Laguna Verde is on the Gulf of Mexico, at longitude 96° 24' 30" W and latitude 19° 43' 24" N, about 75 Km north of the city of Veracruz. It is the location of the country's first nuclear power plant. The prevailing wind flow in this area is from North to South. Collection Site-I is located 1350 meters, 292° WNW, from the center of the nuclear reactors. Site-II is located 350 meters, 215° SSW, from the reactors. Site-I is 1320 meters, 309° WNW, from Site-II. At the time of the collections, the power plant had not yet gone "on-line". In addition to supplying us with information of importance for an understanding of the radiation sensitivity of sibling species, it is expected that the data obtained through an extended study of these populations will

indicate what, if any, are the viability effects of the operation of the nuclear reactors on surrounding populations.

MATERIAL AND METHODS

The flies used in the present experiments came from isofemale lines, each line consisting of the descendants of either an individual *D. melanogaster* or *D. simulans* female that had mated in the wild. Flies were collected at Site-I in July, 1986 (hereafter referred to as collection I-8607) and tested in September, 1986. One group of flies was collected at Site-II in January, 1987 (II-8701) and tested in March, 1987, whereas a second group was collected in July, 1987 (II-8707) and tested in August, 1987.

Cornmeal-agar medium plus yeast was used as food, and the flies were maintained at 24 °C. Twenty males and 20 females from each isofemale line were placed together in individual empty bottles. These were then inverted over petri dishes (4.8 cm diameter) into which a thin layer of food medium containing powdered charcoal had been poured. The females were allowed to oviposit for a period of two hours. At the end of that time, the petri dishes were removed, covered, and carried to the nearby radiation facility within the Nuclear Center of Mexico. There the eggs were exposed to 4.0, 6.0 or 8.0 Gy (1 Gy = 100 Rads). A fourth set of eggs from each isofemale line of both species was an unirradiated transportation control.

Radiation was delivered by a Vicks-Rad 2000 (Vickers Radiation Company, Swindon, England) at a rate of 0.06 Gy of gamma rays per second. In the Vicks-Rad 2000, specimens are placed in a central cylinder and lowered into a ring-shaped ^{60}Co source for uniform irradiation. At the time of irradiation, the activity of the ^{60}Co source was 2.785×10^{12} Bq (75 Ci).

After the radiation procedure was completed, the egg-containing petri dishes were returned to the laboratory, and 80 eggs were transferred from each petri dish to a separate vial (9 cm x 2.5 cm), containing 2 cm of food medium. Each test of each isofemale line was performed three times, and each vial was scored for the number of adults produced.

RESULTS

The data obtained for all three collections are shown in Table I and Figure 1. Also shown in Table I are the results of pairwise comparisons, using Student's t-test, of the two species for each experimental condition. It was found that in collection I-8607, *D. melanogaster* was significantly superior to *D. simulans* in viability in all four experimental situations. By contrast, there were no significant differences in egg to adult survival between the species of flies from collections II-8701 and II-8707.

Radiation sensitivity was measured by comparing the viability of the various ir-

TABLE I. EGG TO ADULT SURVIVAL

Collection	Treatment (Gy)	Proportion Eclosing ($\pm$ S.E.)				
		<i>D. Melanogaster</i>	<i>n</i>	<i>D. Simulans</i>	<i>n</i>	<i>P</i> (t - test)
I - 8607	0.0	0.607 $\pm$ 0.021	10	0.457 $\pm$ 0.026	10	< 0.001
	4.0	0.597 $\pm$ 0.023	10	0.346 $\pm$ 0.050	10	< 0.001
	6.0	0.520 $\pm$ 0.045	10	0.345 $\pm$ 0.044	10	< 0.02
	8.0	0.498 $\pm$ 0.034	10	0.278 $\pm$ 0.046	10	< 0.005
II - 8701	0.0	0.391 $\pm$ 0.061	8	0.390 $\pm$ 0.061	7	> 0.9
	4.0	0.392 $\pm$ 0.040	7	0.325 $\pm$ 0.072	6	> 0.4
	6.0	0.322 $\pm$ 0.046	8	0.250 $\pm$ 0.066	6	> 0.3
	8.0	0.391 $\pm$ 0.052	7	0.297 $\pm$ 0.037	6	> 0.1
II - 8707	0.0	0.310 $\pm$ 0.062	10	0.260 $\pm$ 0.064	9	> 0.5
	4.0	0.093 $\pm$ 0.017	10	0.073 $\pm$ 0.019	10	> 0.4
	6.0	0.077 $\pm$ 0.023	8	0.092 $\pm$ 0.023	10	> 0.6
	8.0	0.048 $\pm$ 0.017	8	0.069 $\pm$ 0.019	9	> 0.4

ⁿ number of isofemale lines

radiated groups with their respective control groups (0.0 Gy exposure). Using the t-test, it was found that, in collection I-8607, a significant reduction in egg to adult survival occurred in *D. melanogaster* at 8.0 Gy ($P < 0.02$), whereas *D. simulans* showed a significant reduction in viability at both 6.0 Gy ($P < 0.05$) and 8.0 Gy ($P < 0.005$). Collection II-8701 showed no significant reduction in viability at any of the levels of irradiation for either species. However, in collection II-8707, there was a significant reduction in egg to adult survival for both species in all experimental situations ($P < 0.005$ for *D. melanogaster*; $P < 0.05$ for *D. simulans*).

In order to further detect populational as well as seasonal differences within each of these sibling species, comparisons were made of the control groups from the three collections of each species. For *D. melanogaster* it was found that I-8607 control group flies demonstrated significantly greater viability than both II-8701 and II-8707 flies ($P < 0.005$). In the case of *D. simulans*, there was no significant difference in egg to adult survival between I-8607 and II-8701 control groups ($P > 0.3$), but I-8607 flies showed significantly greater viability than II-8707 flies ($P < 0.02$). For both species, there was no significant difference in egg to adult survival between the control groups of the two Site-II collections ($P > 0.1$).

DISCUSSION AND CONCLUSIONS

The flies from the three collections reported here have also been studied for other characteristics (Rosa *et al.* 1989; unpublished data). One characteristic closely as-

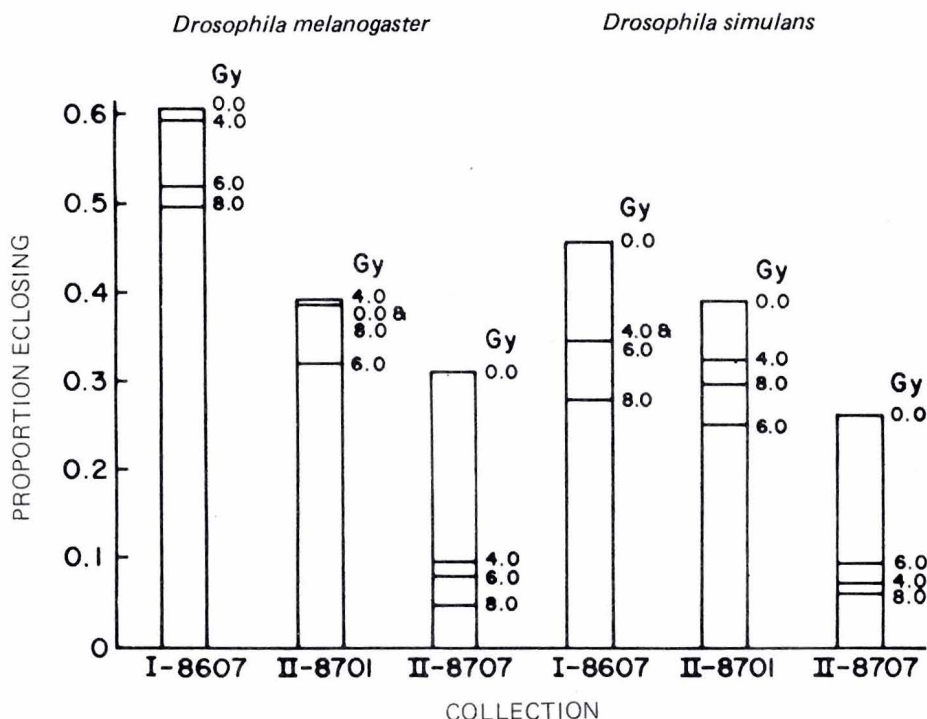


Fig. 1. Egg to adult survival of irradiated and non-irradiated *Drosophila melanogaster* and *D. simulans* collected at Laguna Verde, Veracruz, Mexico

sociated with viability is species frequencies. The frequencies of *D. melanogaster* and *D. simulans* males at Site-I, relative to one another, were respectively 83 % and 17 %. However, at Site-II there was a complete reversal in the relative frequencies of *D. melanogaster* and *D. simulans* males: 16 % vs 84 % (8701) and 13 % vs 87 % (8707).

The superior viability of the non-irradiated *D. melanogaster* from the Site-I collection is in concordance with its significantly greater relative frequency in the population. Although one would have liked to see a superior viability of the non-irradiated *D. simulans* from the Site-II collections, corresponding to its significantly greater relative frequency in that population, it did not occur. However, the absence of any superiority of *D. melanogaster* in egg to adult survival indicates the important contribution that viability makes in determining species' frequency but does leave unanswered the question of what other factors in the life cycles of the two species accounts for the significantly greater relative frequency of *D. simulans* in the Site-II collections.

In examining the results for populational as well as seasonal differences, one finds a complex situation. If one compares the viabilities of the sibling species in all four

experimental conditions, it is clear that there is a populational difference between flies from Site-I and those from Site-II. However, there is no seasonal difference between the results from the two Site-II collections. If one examines radiation sensitivity by comparing the viability of the various irradiated groups with their respective control groups, one does not find a distinct populational difference, but one does find a seasonal effect for the Site-II collections.

Finally, if one compares control groups within each species, one finds that *D. melanogaster* shows a populational but no seasonal difference. By contrast, *D. simulans* does not show a populational difference but does show a seasonal difference when comparing Site-I flies with the two collections of Site-II flies. However, there is no seasonal difference between Site-II flies. It is clear that the types and degrees of differences in the viability results obtained for these sibling species varies with the focus of the particular analysis. We plan to continue collecting and testing the flies from both sites at opposite seasons of the year in order to develop a body of information on the populational and seasonal characteristics of these species and, simultaneously, to monitor the effects, if any, of the operation of the nuclear power plant on surrounding populations.

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